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DÉVELOPPEMENT DE NOUVEAUX OUTILS INFORMATIQUES DE SURVEILLANCE EN TEMPS RÉEL DES PHÉNOMÈNES ANORMAUX BASÉS SUR LES DONNÉES DE MICROBIOLOGIE CLINIQUE DU LABORATOIRE DE LA TIMONE

Pour obtenir le grade de Doctorat d'Aix-Marseille Université

Spécialité Pathologie Humaine : Maladies infectieuses

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AVANT PROPOS :

Le format de présentation de cette thèse correspond à une recommandation de la spécialité Maladies Infectieuses et Microbiologie, à l'intérieur du Master des Sciences de la Vie et de la Santé qui dépend de l'Ecole Doctorale des Sciences de la Vie de Marseille.

Le candidat est amené à respecter des règles qui lui sont imposées et qui comportent un format de thèse utilisé dans le Nord de l'Europe et qui permet un meilleur rangement que les thèses traditionnelles. Par ailleurs, la partie introduction et bibliographie est remplacée par une revue envoyée dans un journal afin de permettre une évaluation extérieure de la qualité de la revue et de permettre à l'étudiant de commencer le plus tôt possible une bibliographie exhaustive sur le domaine de cette thèse. Par ailleurs, la thèse est présentée sur article publié, accepté ou soumis associé d'un bref commentaire donnant le sens général du travail. Cette forme de présentation a paru plus en adéquation avec les exigences de la compétition internationale et permet de se concentrer sur des travaux qui bénéficieront d'une diffusion internationale.

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RESUME

Bien que considérées comme étant sous contrôle durant la seconde partie du 20ième siècle avec la découverte des antimicrobiens, les maladies infectieuses demeurent une menace bien réelle pour l'humanité. En effet, qu'il s'agisse d'agents infectieux connus depuis de nombreuses années, de nouveaux agents pathogènes ou de pathogènes ré-émergents, leur impact sur le plan démographique et économique est considérable, avec notamment près de 15 millions de décès humains leur étant imputables chaque année (estimation 2004 de l'OMS).

Quelque soit l'état de connaissance que nous avons sur ces maladies, toutes demeurent imprédictibles. Afin de lutter contre ce phénomène, de nombreuses stratégies de surveillance ont été développées amenant à la mise en place de divers outils informatiques de surveillance épidémiologique visant à détecter et identifier, le plus précocement possible, des événements anormaux incluant des phénomènes épidémiques, l'objectif ultime de cette approche étant l'information rapide des principales institutions de santé publiques pour prendre des mesures appropriées à la situation observée à l'échelle nationale et internationale.

L'objectif initial de notre travail a consisté à mettre en place, au sein de l'Institut Hospitalo-Universitaire Méditerranée Infection (IHU) et à partir du logiciel Microsoft Excel, deux nouveaux outils informatiques de surveillance épidémiologique visant à identifier, de façon hebdomadaire et automatisée, des

événements anormaux sur la base des données de microbiologie clinique issues du laboratoire du Centre Hospitalo-Universitaire Timone à l'Assistance Publique-Hôpitaux de Marseille (AP-HM). Une fois cette étape achevée, nous avons par la suite travaillé au développement d'une structure de surveillance complète intégrant l'investigation et la validation des alarmes émises par les systèmes de surveillance créés, l'émission d'alertes à l'Agence Régionale de Santé (ARS) de la région Provence-Alpes Côte d'Azur (PACA), la valorisation des cas d'événements anormaux confirmés par des publications scientifiques, ainsi que la mise en place de rétro-informations et de bulletins épidémiologiques hebdomadaires visant à informer les acteurs locaux de la surveillance épidémiologique des maladies infectieuses.

Le développement de l'activité de surveillance initiée au cours de ce travail se poursuit et a d'ores et déjà donné lieu au développement d'une plateforme informatique regroupant toute l'activité de surveillance développée au sein de l'IHU, ainsi qu'au développement d'un réseau de surveillance de laboratoires privés et publics de microbiologie clinique dans la région PACA.

Mots clés: maladies infectieuses, surveillance épidémiologique, données de microbiologie clinique, épidémie, information.

ABSTRACT

Although considered under control in the second half of the 20th century with the discovery of antimicrobials, infectious diseases remain a serious threat to humanity. Indeed, whether infectious agents known for many years, new pathogens or re-emerging pathogens, the scale of their demographical and economical impact is significant especially with 15 million attributable human deaths each year (2004 WHO estimation).

Regardless of the state of knowledge we possess on these diseases, all remained unpredictable. To fight this phenomenon, many monitoring strategies have been developed leading to the implementation of various epidemiological surveillance computer programs to detect and identify, as soon as possible, abnormal events including epidemic phenomena. The ultimate goal of this approach being the rapid dissemination of information to the main public health institutions in order to take appropriate measures against the observed situation at the national and international scales.

The initial objective of our work was to implement, within the Hospitalo-Universitaire Méditerranée Infection (IHU) and based on the Microsoft Excel software, two new automated computer-based programs for the weekly automated epidemiological surveillance of abnormal epidemic events using clinical microbiological data from the Timone teaching hospital of of Assistance Publique-Hôpitaux de Marseille (AP-HM). Once completed, we then worked to develop a

comprehensive monitoring structure incorporating the investigation and the validation of alarms emitted by the established surveillance systems, the transmission of alerts to the Regional Health Agency (ARS) of the Provence-Alpes Côte d'Azur (PACA), the public dissemination of confirmed abnormal events by publishing scientific articles, and the implementation of feedback and weekly epidemiological bulletins to inform local infectious diseases epidemiological surveillance actors.

The development of monitoring activity initiated during this work continues through the development of a computer platform bringing together all the monitoring systems developed in the IHU, and the development of a clinical microbiology laboratory surveillance network including several private and public laboratories in the PACA region.

Keywords: infectious diseases, epidemiological surveillance, clinical microbiology data, outbreak, information.

Introduction

Avec près de 1740 publications dans PubMed traitant de ce sujet entre 2000 et 2014, la surveillance épidémiologique, définie comme « un processus continu de collecte, d'analyse et d'interprétation de données de santé permettant la planification, la mise en place, et l'évaluation de pratiques de santé publique, intégré à une diffusion rapide de ces données vers les décideurs » (1), demeure un enjeu global majeur pour l'humanité.

Ainsi, depuis la fin du 16ième siècle et la mise en place du premier vrai registre comptabilisant le nombre de décès hebdomadaires ayant lieu dans la ville de Londres (2), de nombreuses approches de surveillance ont été et continuent d'être développées sous l'impulsion des avancées technologiques des secteurs informatiques (augmentation des capacités de stockage et de traitement des ordinateurs, accélération des échanges d'informations de santé grâce au développement d'internet…) et microbiologiques (amélioration de la qualité et du délai d'identification des pathogènes).

Toutes ces approches de surveillance peuvent être classées en deux groupes de stratégie de surveillance prédominants: la surveillance spécifique, aussi appelée surveillance traditionnelle, et la surveillance syndromique.

La surveillance spécifique permet la surveillance de pathogènes, maladies ou syndromes précis dans une population cible d'intérêt (3;4). A ce titre, elle permet de surveiller les maladies à déclarations obligatoires en se basant sur des rapports

envoyés par des structures ou médecins sentinelles, mais également sur les résultats issus de laboratoires de microbiologie clinique (3;4). Ces systèmes de surveillance peuvent être déployés à l'échelle nationale, tel que le National Tuberculosis Surveillance System aux États-Unis (3;4), spécifiquement dédié à la surveillance du nombre de cas de *Mycobacterium tuberculosis* depuis sa création en 1953. En France, divers réseaux de surveillance spécifique ont été développés sous la tutelle de l'INVS (Institut National de Veille Sanitaire) pour assurer la surveillance de pathogènes d'intérêt particulier. L'un d'entre eux est le réseau LaboVIH. Il s'agit d'un réseau de surveillance développé en 2001 spécifiquement déployé pour évaluer l'épidémiologie nationale du VIH. Selon les chiffres de 2014, ce réseau se base sur les données de près de 4300 laboratoires, incluant des laboratoires d'hôpitaux (5;6). A l'échelle internationale, l'European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) (7), développé en 2004 sous l'impulsion de l'European Surveillance of Sexually Transmitted Infections Project (ESSTI), est un programme de surveillance dédié à la surveillance de la résistance de souches de gonocoque provenant des pays membres de l'Union Européenne et de l'Aire Économique Européenne (EU/EEA) (7).

La surveillance syndromique, quant à elle, est définie par Sala Soler et al.comme une surveillance basée sur « des indicateurs non spécifiques, incluant des signes, des symptômes et autres mesures […] normalement collectés pour des besoins autres que la surveillance et, lorsque cela est possible, automatiquement produits »

afin de permettre « une collection, une analyse, une interprétation et une dissémination en temps réel (ou quasi-réel) des données de santé pour permettre l'identification précoce de l'impact (ou de l'absence d'impact) de potentielles menaces humaines ou vétérinaires de santé publique » (8). Ainsi, les systèmes de surveillance syndromique collectent et analysent par exemple des indicateurs de santé tels que le taux d'absentéisme au travail ou les consommations de médicaments des commerçants habilités à vendre ces produits (8-10). Cette stratégie de surveillance ne tenant pas compte des confirmations de présence ou d'absence de pathogène de la part des laboratoires (11), elle est connue pour être non spécifique mais sensible et rapide (8;9;11). Par ailleurs, comme ces systèmes de surveillance peuvent utiliser diverses sources de données, ils permettent une interconnectivité entre les différents participants de ces systèmes, augmentant ainsi les capacités des autorités de santé publique à comprendre et à gérer de possibles situations épidémiques (9). Parmi les exemples les plus connus de système de surveillance syndromique se trouve le National Retail Data Monitor (NRDM) aux États-Unis (12;13). Ce système de surveillance syndromique a été développé en 2002 par l'université de Pittsburgh. Il est équipé de plusieurs algorithmes de détection d'événements anormaux et permettait, selon les chiffres de 2009, de collecter et de surveiller les données quotidiennes de vente de divers produits de santé de plus de 29 000 détaillants des États-Unis (9). Un autre système de surveillance syndromique des États-Unis s'appelle ESSENCE (the Electronic

Surveillance System for the Early Notification of Community-Based Epidemics) (14). Il s'agit d'un système de surveillance développé il y a plus de 10 ans par le laboratoire de physique appliqué de l'université Johns Hopkins en collaboration avec le département de la défense des États-Unis (14;15). La première version du système, ESSENCE I, est actuellement utilisée pour la surveillance épidémiologique mondiale des troupes américaines (14;16). La dernière version, ESSENCE II, est quand à elle utilisée pour analyser les données anonymisées provenant de la région de la capitale des États-Unis (16). En France, un système de surveillance syndromique appelé ASTER (le système d'Alerte et Surveillance en Temps réel) (17) a été récemment développé pour la collecte et l'analyse en temps réel de données transmises par internet par des docteurs et autres professionnels de santé vivant avec les troupes armées françaises déployées dans le monde, incluant la Guyane française et Djibouti. Une fois transmises et analysées par le système, un tableau de bord résumant la situation épidémiologique sur le terrain des forces déployées et récapitulant les alarmes émises par le système est présenté au service de santé des forces armées de Marseille, France, tandis qu'une rétro-information indiquant l'état de santé des troupes déployées est envoyée aux professionnels de santé déployés sur le terrain.

Dans ce contexte, 12 types de données (Figure 1, extraite de l'article 1 «Traditional and Syndromic Surveillance of Infectious Diseases and Pathogens»)

sont actuellement utilisables pour la surveillance épidémiologique dans le monde, qu'elle soit syndromique ou spécifique.

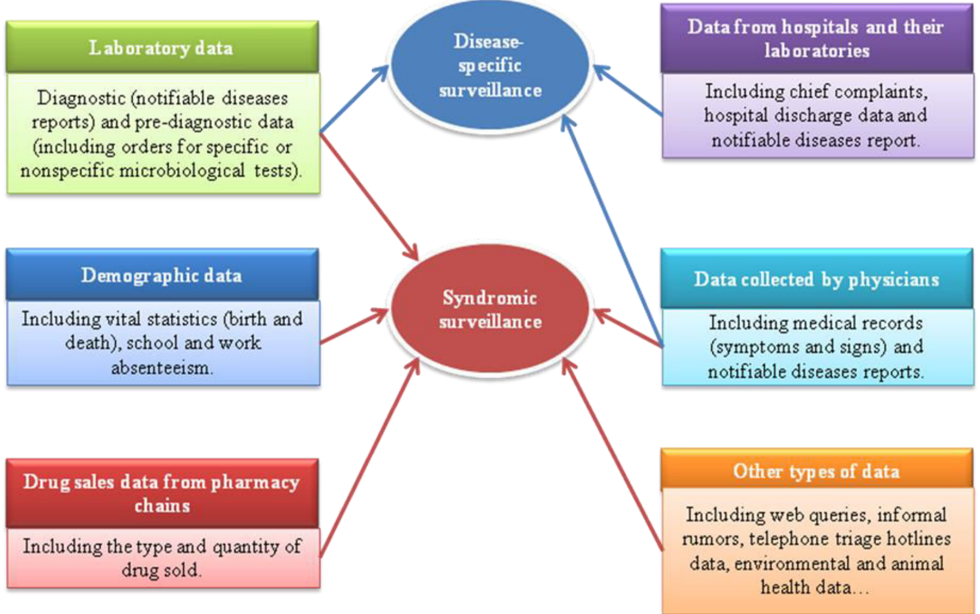

Figure 1. Les différentes sources et catégories de données utilisés par les principales stratégies de surveillance développées de part le monde.

Parmi les structures produisant des données exploitables par les systèmes de surveillance syndromiques ou spécifiques se retrouvent les laboratoires de microbiologie clinique privés ou publics. En effet, ces derniers représentent une source importante de données directement accessibles et utilisables pour les systèmes de surveillance. Une estimation de l'impact des données produites par les laboratoires de microbiologie clinique sur le fonctionnement de systèmes de surveillance syndromiques et spécifiques développés dans le monde entre Janvier

2009 et mi-Juin 2014 par l'intermédiaire d'une recherche effectuée dans PubMed a permis d'identifier que 262 systèmes de surveillance décris lors de la période d'étude utilisaient des données issues de laboratoires, démontrant ainsi la place centrale des laboratoires dans la surveillance épidémiologique actuelle (Figure 2, extraite de l'article 1 «Traditional and Syndromic Surveillance of Infectious Diseases and Pathogens»).

Figure 2. Les systèmes de surveillance des maladies infectieuses développés et bien décris dans le monde entre 2009 et mi-Juin 2014.

Dans ce contexte, notre premier travail a consisté à inventorier les données, stratégies et systèmes de surveillance développés de part le monde pour la surveillance des maladies infectieuses et des pathogènes, ainsi qu'à définir le rôle central des laboratoires de microbiologie dans la surveillance épidémiologique des

maladies infectieuses (**Partie I**). En parallèle, nous avons développé au sein de l'IHU Méditerranée Infection deux nouveaux systèmes de surveillance syndromique permettant la surveillance hebdomadaire d'événements épidémiques anormaux fondés sur les données de microbiologie clinique issues du laboratoire de la Timone (**Partie II**). Enfin, nous avons procédé à l'investigation, la description et à la publication d'événements épidémiologiques confirmés identifiés sur la base d'alarmes émises par les systèmes de surveillance syndromique développés (**Partie III**).

Partie I: Surveillance spécifique et syndromique des maladies infectieuses et des pathogènes.

Avant propos

L'objectif de cette partie consiste à faire un état des connaissances de la surveillance épidémiologique telle qu'elle est actuellement développée dans le monde (article 1). Ce travail a permis d'identifier 12 classes majeures de données pouvant être utilisées pour la surveillance des maladies infectieuses et des pathogènes. L'utilité de chacune de ces classes est illustrée par des exemples concrets d'épidémies observées en les utilisant. Ce travail permet également de caractériser les deux principales stratégies de surveillance utilisées à l'heure actuelle, d'une part la surveillance traditionnelle ou spécifique, d'autre part la surveillance syndromique. Chacune de ces deux stratégies est explicitée en précisant des exemples nationaux ou internationaux de systèmes de surveillance développés dans le monde. La place centrale des laboratoires de microbiologie est par la suite traitée avec, dans un premier temps, une liste des principales catégories de laboratoires disponibles réalisée sur la base de leurs rôles respectifs, et, dans un second temps, une revue de la littérature décrivant des systèmes de surveillance utilisant des données de laboratoire de microbiologie pour la surveillance des maladies infectieuses et des pathogènes dans le monde après recherche dans PubMed d'articles publiés entre le 1er Janvier 2009 et le 13 Juin 2014 (262 systèmes de surveillance finalement répertoriés). Enfin, l'avenir des systèmes de surveillance des maladies infectieuses durant le 21ième siècle est brièvement discuté.

Article 1: Review. Traditional and syndromic surveillance of infectious diseases and pathogens.

Cédric Abat, Hervé Chaudet, Jean-Marc Rolain, Philippe Colson, Didier Raoult

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ABSTRACT

21 Infectious diseases remain major public health problems worldwide. Hence, their
22 surveillance is critical. We reviewed data, strategies and systems used around the
23 world for the surveillance of infectious diseases and pathogens along with current
24 issues and trends. Twelve major classes of data were identified, according to their
25 timing relative to infections, available resources and type of surveillance. Two
26 primary strategies have been compared; disease specific surveillance and
27 syndromic surveillance. We finally registered and briefly described 262 systems
28 implemented worldwide for the surveillance of infections, with a focus on those
29 based on microbiological data from laboratories. Currently, a wealth of data on
30 infections is available and is growing with the recent emergence of new
31 technologies. Concurrently with the expansion of computer resources and
32 networks, these data will allow for the optimization of real-time detection and
33 notification of infections.

34 **Key words:** surveillance; infection; epidemiology

35 **TEXT**

36 **Introduction**

37 Classified as the second leading cause of deaths in humans by the World
38 Health Organization with approximately 15 million deaths worldwide every year
39 [1], infectious diseases remain a serious public health problem in the 21st century.
40 Among them, HIV/AIDS, tuberculosis and malaria have been nicknamed the "big
41 three" because of their important impact on global human health. In 2011,
42 tuberculosis, malaria and HIV infected 2 billion, 207 million and 35.3 million
43 people, and killed 1.3 million, 62,700 and 1.6 million people, respectively [2].

44 To take adequate measures for detecting and fighting infectious diseases,
45 their surveillance is essential. Surveillance consist in "the ongoing systematic
46 collection, analysis, and interpretation of health data essential to the planning,
47 implementation, and evaluation of public health practice, closely integrated with
48 the timely dissemination of these data to those who need to know" [3]. Attempts to
49 survey infectious diseases are not recent. One of the best known examples is the
50 use of the London Bills of Mortality by the clerks to establish from 1603 a weekly
51 monitoring of the number of deaths in London [4]. Later, in 1854, John Snow
52 performed a topographic study in London by systematically recording the
53 addresses of people infected with cholera, to identify the source of the pathogen
54 [5]. Currently, many surveillance strategies and systems are available around the
55 world. Computer resources have expanded considerably, but infectious diseases

56 surveillance remains challenging. Recent examples including the
57 2009 H1N1 influenza pandemic [6] and the current West African Ebola outbreak
58 [7] indicate that infectious diseases cannot be predicted and modelled reliably.
59 Nevertheless, the detection and investigation of abnormal health-related events
60 effectively allow for the identification of true epidemic events. The abnormal
61 increase in the number of young homosexual men infected by *Pneumocystis carinii*
62 in the city between 1980 and 1981 [8] allowed to discover the HIV virus in 1983
63 [9]. Similarly, the outbreak of severe respiratory illness of unknown origin that
64 affected 180 people who had attended a state American Legion convention in
65 Philadelphia in July 1976 allowed the identification of *Legionella pneumophila*
66 [10]. In late 2002, an unknown respiratory disease with no identifiable cause was
67 diagnosed and reported in several people living in the Guangdong Province of
68 China. The syndrome, designated as "severe acute respiratory syndrome" (SARS),
69 rapidly crossed borders and became a worldwide threat [11]. The Sars-CoV virus
70 was finally identified as the causative agent of the syndrome [12].

71 Considering all these aspects, we herein made an inventory of the data,
72 surveillance strategies and surveillance systems developed worldwide for the
73 surveillance of infectious diseases and to emphasize the role of microbiological
74 laboratories in surveillance.

75 **I. Infectious disease surveillance**

76 **1- Data used for surveillance**

77 Figure 1 summarizes the main types of data available for surveillance.
78 They were classified according the outbreak detection continuum published by
79 Texier et al. [13].

80 **a- Human environment**

81 ***Environmental data***

82 Environmental data includes water pollution, weather, or air pollution. For
83 example, water quality testing from samples collected at water treatment facilities
84 could be used to explain an increase in the number of patients presenting to
85 emergency departments because of gastrointestinal disorders, as it was done in the
86 case of ice made during the massive outbreak of cryptosporidium in Milwaukee
87 [14].

88 ***Animal health***

89 Animal health data come directly from wild or domestic animals and are
90 particularly valuable for the surveillance of zoonotic diseases such as plague,
91 rabies or monkeypox. For example, Chaintoutis et al. successfully used serum
92 collected from sentinel juvenile domestic pigeons for the early detection of West
93 Nile virus in Central Macedonia, Greece [15].

94 **b- Human behaviour**

95 ***Internet***

96 The internet can be used for infectious disease surveillance [16]. Studies
97 on influenza have proven the efficiency of using web queries to complement

98 existing surveillance methods. For example, the anticipation of the results of the
99 Canadian FluWatch was done successfully and at a low cost, which inspired
100 Google to develop Google Flu Trends, a free forecasting tool allowing the real-
101 time surveillance of influenza activity in the USA [17]. However, such approach
102 should not be used to replace traditional epidemiological surveillance networks as
103 flu-tracking techniques based on web data are more likely to be affected by
104 changes in people's search behaviour [18].

105 ***Telephone triage hotlines***

106 Telephone triage hotlines receive numerous phone calls from people
107 requiring immediate health care assistance. Electronic data extracted from these
108 hotlines can be a valuable source of data for surveillance. Although hotlines are
109 inherently non-specific, data can be produced regardless of the day of the week,
110 weather conditions, or holidays if the triage hotline is operated around the clock.
111 These data normally include the precise time of call, basic information about the
112 caller, their residence and some description of the symptoms [19].

113 ***Drug sales***

114 When people fall sick, they either go to see the doctor, treat themselves
115 with home remedies, or practice self-medication by purchasing non-prescription
116 remedies from a drugstore. In the last case, sales data are entered electronically in
117 store databases. These data are interesting for infectious disease surveillance
118 because they reflect customer behaviour. Indeed, the class of drug sold, the

quantity and the date of purchase can provide significant information on the age distribution, size and the level of access to health care of a given population [19].

Absenteeism

Absenteeism data includes work and school absenteeism declarations. They can be used for the early detection of outbreaks as has been demonstrated with influenza in France [16]. Absenteeism data can also give critical information about an outbreak such as the place where people have been infected [16]. Therefore, if several children or students are unable to go to school within a short span of time because of stomach pain, and if they all ate in the same place the day before, it is reasonable to suspect that they are all affected by the same foodborne pathogen.

c- Health care

Sentinel surveillance

Sentinel physicians are physicians who at regular intervals agree to notify public health authorities about their patients presenting some specific symptoms (i.e. influenza-like-illness). Public health authorities analyze that data and assess the activity and strains of diseases circulating in the population of interest [16].

Chief complaints

Chief complaints consist of short sentences or codes summarizing the reason of the emergency department admission (for example “headache” or “abdominal pain”) [16, 19]. Multiple chief complaints can be registered for the

140 same patient, the first one being the most important for infectious disease
141 surveillance [16]. Once registered, chief complaints can be classified into
142 syndromic surveillance categories manually or by using algorithms [19].

143 ***Medical records***

144 When patients go for medical examinations, physicians ask them
145 questions to collect useful information on their health status including the date of
146 appearance of the symptoms and their progression over time. These data help
147 physicians define the symptoms affecting the patients. Next, physicians physically
148 examine the patients to collect data on the signs of the disease. Together, signs and
149 symptoms help the physicians to assign a differential diagnosis and create a list of
150 probable diseases that may be affecting the patient. These records are translated
151 into codes according to the International Classification of Diseases, ninth revision
152 (ICD-9). These codes can then be grouped into syndromic categories [16, 19, 20].

153 ***Hospital discharge data***

154 Hospital discharge data include ICD-9 codes, hospital zip code, home zip
155 code, patient 's age, and patient's date of admission and discharge [16]. These data
156 can be useful for the surveillance of infectious diseases.

157 ***Microbiological orders***

158 Physicians and hospitals may ask laboratories to perform microbiological
159 tests to confirm or invalidate the presence of pathogens in patients. These tests can
160 be used for the pre-diagnostic surveillance of pathogens. The number of a

161 particular specific or nonspecific test performed per unit of time can be a good
162 indicator of the presence of a particular pathogen in a given population [19].
163 However, as the results of these tests are either positive or negative, they allow
164 only low specificity surveillance, which can lead to the investigation of wrong
165 epidemiological events.

166 ***Notifiable disease reports***

167 Notifiable disease reports consist of mandatory reporting by mail, phone,
168 fax, or using a computer, of diseases defined by health departments to be a threat
169 for a community of interest [16]. The list of diseases may be defined at the national
170 level or at the state level, and is flexible according to fluctuations in the incidence
171 and prevalence of pathogens over time [16, 19].

172 **d- Demographics**

173 Vital statistics include data on birth, death, and marital status [16]. Data
174 on birth can provide information on the cause of premature delivery or birth
175 anomalies, but equally for the surveillance of infant mortality [16]. Death
176 certificates are also valuable in surveillance because they include the cause, age
177 and the place of death [16].

178 **2- Surveillance strategies**

179 Diseases surveillance can be broadly divided into specific surveillance
180 and syndromic surveillance (Table 1).

181 **Table 1.** Advantages and limits of the main kinds of surveillance systems developed to follow infectious diseases around the world.

Kind of surveillance system	Principle	Advantage(s)	Limit(s)
Disease-specific surveillance system	Surveillance of specific pathogens, diseases, or syndromes in a target population	Surveillance of a wide range of pathogens Useful to follow global trends of surveyed pathogens Can be used to monitor public-health measures taken to fight precise pathogens	Standardization of data used is necessary Limited capacities can lead to underestimated prevalence of the surveyed event Targets (pathogens, diseases, syndromes and populations) must be clearly identified before starting the surveillance
Syndromic surveillance system	Real-time or near real-time collection, analysis, interpretation, and dissemination of health-related data for the early identification of potential health threats	Can be used in emergency cases High sensitivity because laboratory confirmation is not needed Possible deployment in low-incomes countries Rapid to implement	Efficiency depends on pathogens and patients characteristics Lacks of human and technological resources can affect data collection, management timeliness and share Low specificity

182 **a- Specific surveillance**

183 Specific surveillance consists in the surveillance of a selection of diseases, syndromes or risk exposures
184 considered as public health threats for the population of interest [16, 21]. It is the traditional surveillance based
185 on notifiable disease reporting using clinical case reports sent by sentinel structures or general practitioners and
186 positive results reported from clinical laboratories [16, 21]. These systems can be deployed at the national level.
187 A good example is the National Tuberculosis Surveillance System (NTSS) [22], which was first implemented in
188 1953 in the USA for the collection of data on tuberculosis cases. Briefly, if a patient is positive to
189 *Mycobacterium tuberculosis*, state health departments send anonymous reports to the NTSS. Reports
190 summarizing the data are then published on the CDC (Centers for Disease Control and Prevention) website
191 (http://www.cdc.gov/tb/topic/default.htm). In France, various specific surveillance networks have been
192 developed under the leadership of the INVS (Institut National de Veille Sanitaire), the French National Institute
193 for Public Health Surveillance. Among them, special mention can be made of LaboVIH, a laboratory-based
194 surveillance system implemented in 2001 for the national specific surveillance of the HIV activity [23]. Twice a
195 year, the INVS contacts all of the French biomedical laboratories (approximately 4,300 laboratories in 2014) to
196 collect data on the number of people tested for the HIV and the number of people found to be positive for the
197 first time in each laboratory in the network [23]. The analysis is then shared through the weekly epidemiological
198 report of INVS (http://www.invs.sante.fr). Such surveillance can be implemented internationally, as has been
199 done for the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) [24]. This surveillance
200 system was implemented in 2004 by the European Surveillance of Sexually Transmitted Infections Project
201 (ESSTI) to provide susceptibility data on gonococci for various antibiotics by studying the evolution of
202 gonococcal antibiotic resistance in the European Union and the European Economic Area [24]. All the states
203 included in the ESSTI were asked to participate in the Euro-GASP to contribute to the collection and the
204 antibiotic susceptibility testing of gonococcal strains in their laboratories [24].

205 **b- Syndromic surveillance**

206 According to Sala Soler et al., syndromic surveillance is based on data that are “non-specific health
207 indicators including clinical signs, symptoms as well as proxy measures”, which “are usually collected for
208 purposes other than surveillance and, where possible, are automatically generated” for allowing “a real-time (or
209 near real-time) collection, analysis, interpretation, and dissemination of health-related data to enable the early
210 identification of the impact (or absence of impact) of potential human or veterinary public health threats” [25].
211 Syndromic surveillance systems collect and analyze health indicators such as nurse calls, school or work

212 absenteeism rates [19, 20, 25]. These systems are known to be non-specific but are sensitive and timely because
213 data can be automatically collected without extra work [20, 25, 26]. Moreover, as data sources can be varied,
214 these systems allow interconnectivity among participants, increasing the capacity of public health authorities to
215 manage possible epidemic situations [20]. Finally, such surveillance systems can assist public health leaders in
216 their decision-making on the guidance, implementation, and evaluation of programs and policies for the
217 prevention and control of infectious diseases [27]. A good example of syndromic surveillance system is
218 ESSENCE (the Electronic Surveillance System for the Early Notification of Community-Based Epidemics) [28].
219 ESSENCE implementation started as a collaboration between the USA Department of Defense and the Johns
220 Hopkins University Applied Physics Laboratory more than a decade ago [28]. The first version of ESSENCE,
221 ESSENCE I, is currently used to perform worldwide monitoring of the army personnel in all USA military
222 treatment facilities [29]. The latest version of ESSENCE, ESSENCE II, performs an integrated surveillance by
223 analyzing de-identified data from the National Capital Region military and civilian health department data [29].
224 The data collected by ESSENCE II contain information on military ambulatory visits and prescription
225 medications, and various data from civilian databases including chief complaint data from civilian emergency
226 departments [29]. Once received, the data are archived and analyzed. ESSENCE II transfers information to its
227 users using secure websites [29]. To summarize, users can see data and results through different format including
228 a map of the geographic distribution of data sent by users and clusters obtained by scan statistics or lists of alerts
229 emitted after the detection processes [29]. ESSENCE II normally analyses data every 4 hours but can also alter
230 the processing period if real-time data are available [29]. The French Armed Forces developed a real-time
231 syndromic surveillance system, "le système d'Alerte et Surveillance en Temps réel" or ASTER [30-32]. Briefly,
232 every ten minutes, the system collects medical data routinely transmitted via secure Internet connections by
233 doctors, paramedics, and nurses who live with the French Armed Forces deployed outside of the country. The
234 data include the numbers of military personnel suffering from various symptoms, including cardiovascular,
235 gastrointestinal, and respiratory symptoms. Data are routinely analyzed using the Current Past Graph method and
236 the mean more or less 2 or 3 standard deviations method. At the end of the process, a dashboard summarizing the
237 epidemiological situation is presented to the Health Service of the Armed Forces based in Marseille, France, and
238 doctors, paramedics, and nurses deployed with the Armed Forces obtain real-time feedback on the health status
239 of the military personnel with whom they live.

240 **II. Role of laboratories in the surveillance of infectious diseases**

241 Laboratories produce some data currently usable for infectious diseases surveillance.

242 **1- Different kinds of laboratories**

243 According to Wagner et al., laboratories can be classified as follow [16]:

244 ➢ Clinical laboratories, which provide a wide range of services from rapid screening tests to confirmatory
245 analyses usable for the diagnosis and treatment of patients.

246 ➢ Environmental laboratories, which perform analysis on environmental samples to determine their physical,
247 chemical and microbiological characteristics.

248 ➢ Commercial laboratories, which are large, independent laboratories that can perform tests on clinical and/or
249 environmental samples. As these laboratories produce a large quantity of data, they can be central
250 structures for surveillance systems.

251 ➢ Governmental laboratories, which include federal laboratories that perform reference laboratory testing and
252 participate in the development of new laboratory technologies, state laboratories that are involved in the
253 surveillance of various diseases, including communicable diseases but also in public health programs, local
254 public health laboratories that play a role in the screening of diseases including tuberculosis or sexually
255 transmitted infections, and other state or local laboratories that can provide valuable test results.

256 Their functions generate a wide variety of information, making them significant source of data on infectious
257 diseases. Indeed, they can confirm the presence of target pathogens, diseases, or syndromes in a population.
258 Moreover, their activity can be used for syndromic monitoring system (i.e. the weekly number of tests
259 performed...). Finally, their data can be used to investigate epidemiological events (Figure 1).

260 **2- Current impact of laboratories on infectious disease surveillance**

261 To evaluate the importance of laboratories in the worldwide surveillance of infectious diseases, we
262 conducted a PubMed search of papers in English published between 2009 and June 13, 2014. The following
263 keywords were used always followed by “infectious diseases”: "surveillance system", “laboratory-based
264 surveillance”, "syndromic surveillance", "sentinel surveillance", "integrated surveillance", and “population-based
265 surveillance”. Only surveillance systems using laboratory data were registered. If more than one article described
266 the same surveillance system, the surveillance system was mentioned only once. The systems were then tagged
267 according to what they monitor (bacteria, viruses, fungus, parasites, or others), whether they are recognized
268 nationally or internationally, and whether they performed syndromic or disease-specific surveillance
269 (Supplementary file).

270 Analysis of the characteristics of the 262 surveillance systems is summarized Figure 3. Briefly, most of
271 the surveillance systems are recognized internationally and nationally, and perform disease-specific surveillance.

They were mostly implemented for the surveillance of viruses (84 surveillance systems) and bacteria (72). Thus, amongst the 76 pathogens monitored by the surveillance systems, influenza was found to be the most surveyed virus (monitored in 31 countries/group of countries), and *Listeria* spp. and *Salmonella* spp. the most surveyed bacterial species (14 for each).

III. The future of infectious disease surveillance

Developing surveillance systems to fight infectious diseases is a fast-growing and evolving field (Figure 4) engaging more and more countries and resources. Disease-specific surveillance has allowed effective management of numerous epidemics and infectious diseases. Thus, smallpox was successfully eradicated in 1978 after the World Health Organization initiated a smallpox-specific mass vaccination program that included laboratory investigations [33]. Similar results were seen in the case of rinderpest, a disease directly infecting the artiodactyls species (cattle, eland, buffalo...) causing famines. This disease was declared eradicated on 25 May 2011 after a long-term effort to fight it through the development of the disease-specific Global Rinderpest Eradication Programme [34]. Nevertheless, thanks to the global spread of internet use and the unprecedented interconnectivity of people it allows, we can speculate that, in the future, improved syndromic surveillance systems will be coupled with disease-specific surveillance systems like it was already done for influenza surveillance [17].

Many surveillance systems survey a limited number of pathogens and not necessarily throughout the year. The surveillance of infections needs to be more global and instantaneous. This is a prerequisite for the detection and notification of abnormal events related to infections, appropriate prioritization of public health threats, and the implementation of optimal strategies and policies. Such approaches appear increasingly feasible with the tremendous expansion of computer resources, networks, and the real-time acquisition and sharing of data worldwide.

What is already known on this subject

Infectious diseases are classified as the second leading cause of deaths in humans with approximately 15 million deaths worldwide every year. As they cannot be predicted and modelled reliably, their surveillance is crucial.

What this study adds

- This study is the first article to make a global overview of the main data and surveillance strategies that can be used for the surveillance of infectious diseases. It is also the first article to underline the major role of clinical microbiology laboratories in the surveillance of infectious diseases.

302 - The study is also the first article to present the wide variety of surveillance system developed around the
303 world for infectious diseases surveillance based on clinical microbiology data by describing 262
304 surveillance systems published between 2009 and June 13, 2014. We hope that this overview will help
305 worldwide public health workers to improve their knowledge in the wide field of infectious diseases
306 surveillance systems.

307

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312

313 REFERENCES
314

315 1 Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases.
316 *Nature* 2004;430(6996):242-9.

317 2 Bourzac K. Infectious disease: Beating the big three. *Nature* 2014;507(7490):S4-S7.

318 3 Thacker SB, Berkelman RL. Public health surveillance in the United States. *Epidemiol Rev* 1988;10:164-
319 90.

320 4 Graunt J. Natural and Political Observations made upon the bills of mortality. London: Roycroft and
321 Dicas; 1652.

322 5 Newsom SW. Pioneers in infection control: John Snow, Henry Whitehead, the Broad Street pump, and the
323 beginnings of geographical epidemiology. *J Hosp Infect* 2006;64(3):210-6.

324 6 Viboud C, Simonsen L. Global mortality of 2009 pandemic influenza A H1N1. *Lancet Infect Dis*
325 2012;12(9):651-3.

326 7 Piot P, Muyembe JJ, Edmunds WJ. Ebola in west Africa: from disease outbreak to humanitarian crisis.
327 *Lancet Infect Dis* 2014;14:1034-5.

328 8 Curran JW, Jaffe HW. AIDS: the early years and CDC's response. *MMWR Surveill Summ* 2011;60 Suppl
329 4:64-9.

330 9 Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-
331 lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*
332 1983;220(4599):868-71.

333 10 Centers for Disease Control and Prevention (CDC). Follow-up on respiratory illness--Philadelphia. 1977.
334 *MMWR Morb Mortal Wkly Rep* 1997;46(3):50-6.

335 11 Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated
336 with severe acute respiratory syndrome. *N Engl J Med* 2003;348(20):1953-66.

337 12 Gerberding JL. Faster... but fast enough? Responding to the epidemic of severe acute respiratory
338 syndrome. *N Engl J Med* 2003;348(20):2030-1.

339 13 Texier G, Buisson Y. From outbreak detection to anticipation. *Rev Epidemiol Sante Publique*
340 2010;58(6):425-33.

341 14 Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, et al. A massive outbreak
342 in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med*
343 1994;331(3):161-7.

344 15 Chaintoutis SC, Dovas CI, Papanastassopoulou M, Gewehr S, Danis K, Beck C, et al. Evaluation of a
345 West Nile virus surveillance and early warning system in Greece, based on domestic pigeons. *Comp*
346 *Immunol Microbiol Infect Dis* 2014;37(2):131-41.
347 16 Wagner MM, Moore AW, Aryel RM. Handbook of Biosurveillance. 1st ed. USA: Academic Press; 2006.
348 17 Butler D. Web data predict flu. *Nature* 2008;456(7220):287-8.
349 18 Butler D. When Google got flu wrong. *Nature* 2013;494(7436):155-6.
350 19 Babin S, Magruder S, Hakre S, et al. Part I: System Design and Implementation. In: Lombardo JS and
351 Buckeridge DL, editors. Disease Surveillance: A Public Health Informatics Approach. Hoboken: John
352 Wiley & Sons; 2006. pp. 43-91.
353 20 Chen H, Zeng D, and Yan P. Infectious Disease Informatics: Syndromic Surveillance for Public Health
354 and Bio-Defense. New York: Springer; 2010.
355 21 Koski E. Clinical laboratory data for biosurveillance. In: Zeng D, Chen H, Castillo-Chavez C, and
356 Thurmond M, editors. Clinical laboratory data for biosurveillance. Springer; 2011. pp. 67-87.
357 22 Vinnard C, Winston CA, Wileyto EP, Macgregor RR, Bisson GP. Isoniazid resistance and death in
358 patients with tuberculous meningitis: retrospective cohort study. *BMJ* 2010;341:c4451.
359 23 Cazein F, Le Strat Y, Sarr A, Ramus C, Bouche N, Le Vu S, et al. Dépistage de l'infection par le VIH en
360 France, 2003-2013. *Bull Epidémiol Hebd* 2014;32-33:534-40.
361 24 Cole MJ, Unemo M, Hoffmann S, Chisholm SA, Ison CA, van de Laar MJ. The European gonococcal
362 antimicrobial surveillance programme, 2009. *Euro Surveill* 2011;16(42).
363 25 Sala Soler M, Fouillet A, Viso AC, Josseran L, Smith GE, Elliot AJ, et al. Assessment of syndromic
364 surveillance in Europe. *Lancet* 2011;378(9806):1833-4.
365 26 M'ikanatha NO, Lynfield R, Van Beneden CA, et al. Infectious Disease Surveillance. 2nd ed. Wiley-
366 Blackwell; 2013.
367 27 Mostashari F, Hartman J. Syndromic surveillance: a local perspective. *J Urban Health* 2003;80(2 Suppl
368 1):i1-i7.
369 28 Lewis SL, Feighner BH, Loschen WA, Wojcik RA, Skora JF, Coberly JS, et al. SAGES: a suite of freely-
370 available software tools for electronic disease surveillance in resource-limited settings. *PLoS One*
371 2011;6(5):e19750.
372 29 Lombardo JS, Burkom H, Pavlin J. ESSENCE II and the framework for evaluating syndromic
373 surveillance systems. *MMWR Morb Mortal Wkly Rep* 2004;53 Suppl:159-65.

374 30 Meynard JB, Chaudet H, Texier G, Queyriaux B, Deparis X, Boutin JP. [Real time epidemiological
375 surveillance within the armed forces: concepts, realities and prospects in France]. *Rev Epidemiol Sante*
376 *Publique* 2008;56(1):11-20.
377 31 Chaudet H, Pellegrin L, Meynard JB, Texier G, Tournebize O, Queyriaux B, et al. Web services based
378 syndromic surveillance for early warning within French Forces. *Stud Health Technol Inform*
379 2006;124:666-71.
380 32 Jefferson H, Dupuy B, Chaudet H, Texier G, Green A, Barnish G, et al. Evaluation of a syndromic
381 surveillance for the early detection of outbreaks among military personnel in a tropical country. *J Public*
382 *Health (Oxf)* 2008;30(4):375-83.
383 33 Arita I. Virological evidence for the success of the smallpox eradication programme. *Nature*
384 1979;279(5711):293-8.
385 34 Morens DM, Holmes EC, Davis AS, Taubenberger JK. Global rinderpest eradication: lessons learned and
386 why humans should celebrate too. *J Infect Dis* 2011;204(4):502-5.
387

FIGURE LEGENDS

Figure 1. The different data sources and kinds used by the main surveillance strategies developed worldwide.

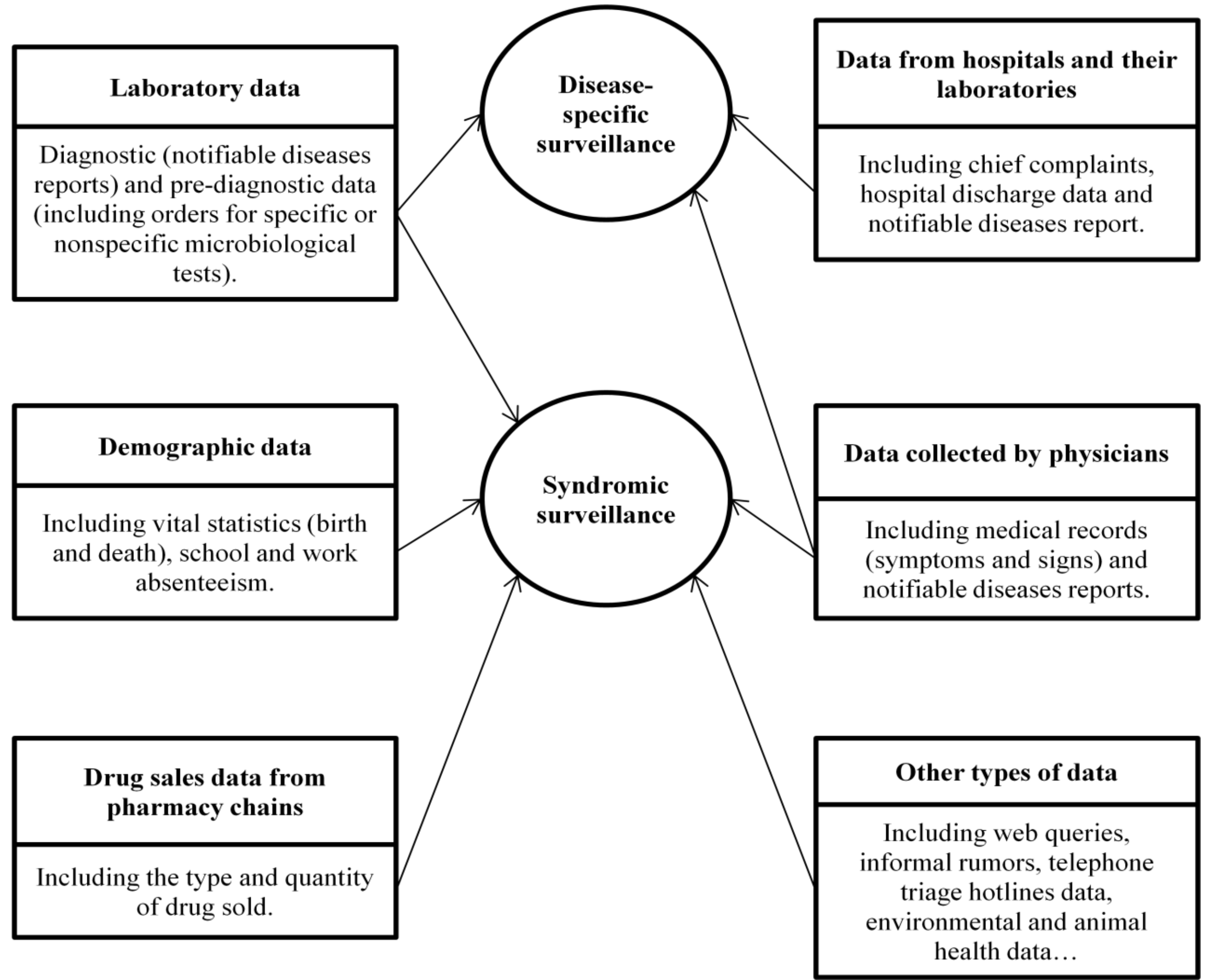


Figure 2. Infectious diseases surveillance systems described around the world from January 2009 to June 13, 2014. The map is available at https://www.google.com/maps/d/edit?mid=z4TNutoSpTfw.k7NzPhL00pmc. Virus picture is used to target surveillance system focused on viruses, bacteria picture is used to target surveillance system focused on bacteria, fungus picture is used to target surveillance system focused on fungi, and polymicrobial picture is used to target surveillance system monitoring various different pathogens.

Figure 3. Summary of the main characteristics of the 262 surveillance systems registered from January 2009 to June 13, 2014. A) Number of international or national surveillance systems, or neither one nor the other. B) Number of surveillance systems that are disease-specific (traditional surveillance) or syndromic. C) Classification of the surveillance systems according what they monitored.

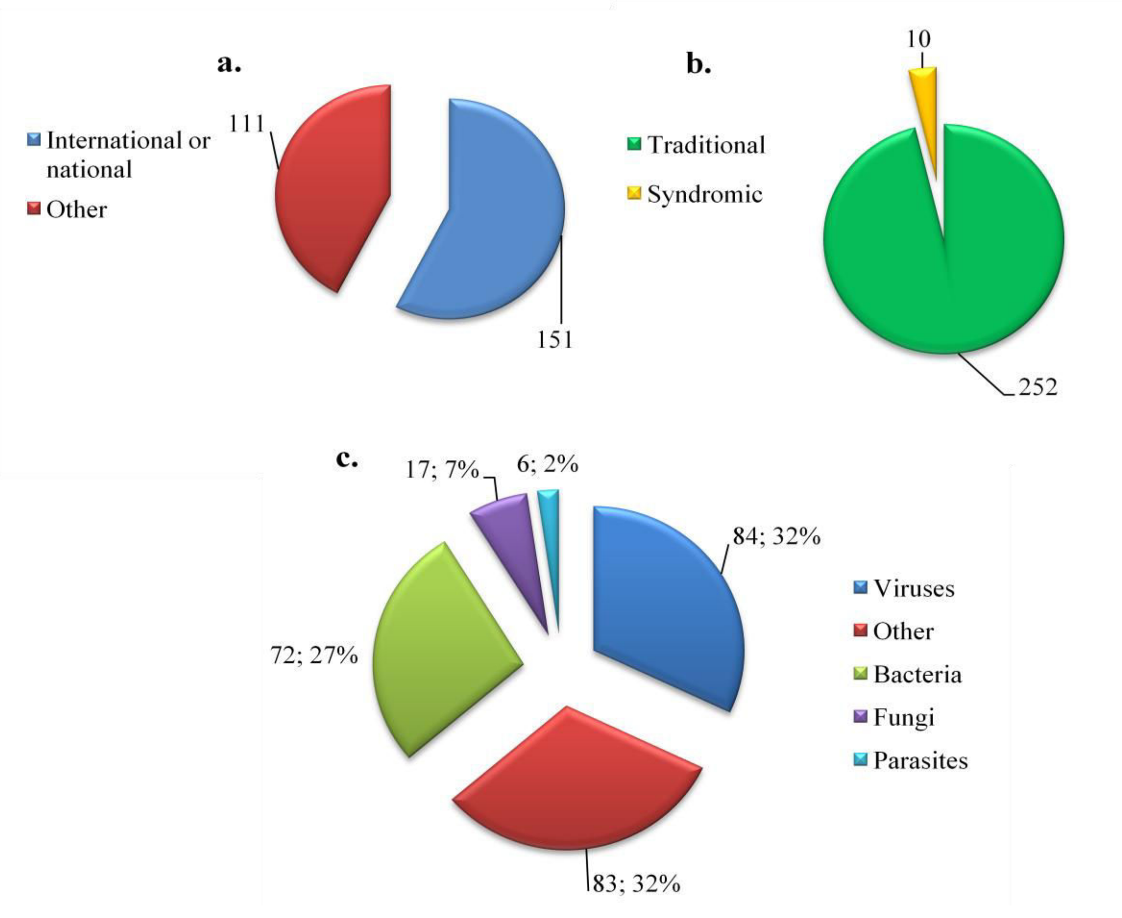
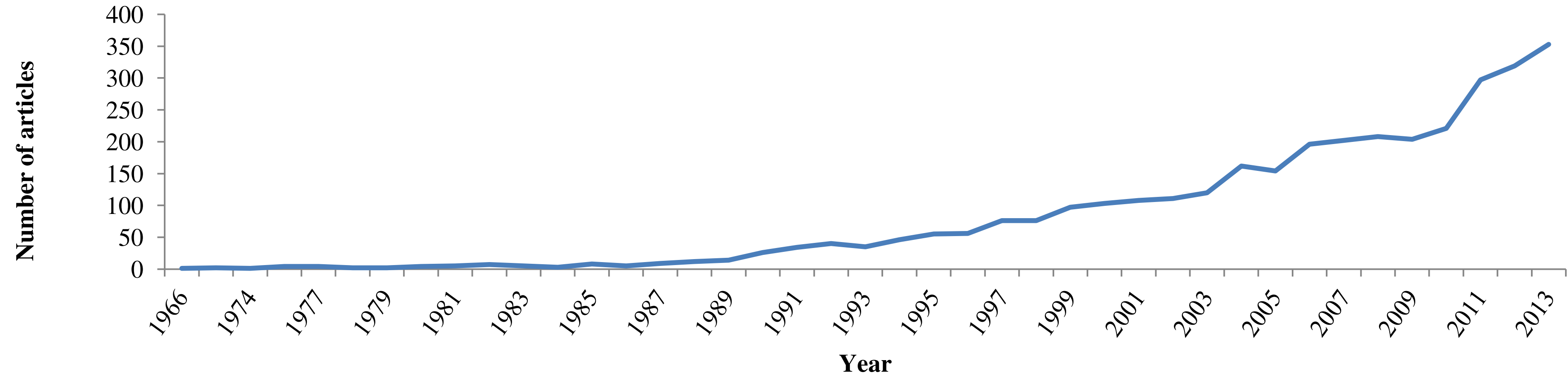


Figure 4. Global evolution of the number of publications dealing with "surveillance system" AND infect* from 1966 to 2013.



Supplementary file. Name and characteristics of the 262 infectious diseases surveillance systems using laboratory data published in PubMed between January 1, 2009 to June 13, 2014.

Short name	Full name	Goal	Country(ies)	Date	What is surveyed (*)	Reference(s)	Traditional or syndromic surveillance (†)
		To monitor emerging *K.pneumoniae* antibiotic-susceptibility profiles using the MultiExperiment Viewer (MeV) software	France	2013	*K. pneumoniae* **(b)**	[1]	Traditional **(i)**
ABCs	Active bacterial core surveillance	To evaluate the incidence and the epidemiological characteristics of the invasive diseases caused by the followed pathogens in various US populations	USA	1995	*Streptococcus pneumoniae*, group A and group B *Streptococcus*, *Neisseria meningitidis*, and *Haemophilus influenza* **(b)**	[2, 3]	Traditional **(i)**
		To increase the outbreak detection methods used at the national and regional level, mainly by identifying geographically distributed outbreaks	England and Wales	1990s	More than 3,300 infectious pathogens **(o)**	[4]	Traditional **(i)**
ESSENCE	Electronic Surveillance System for the Early Notification of Community-Based Epidemics	To observe abnormal behavior of health indicators across states and view their geographical evolution over time combining both military and civilian health care data from the national capital area	USA	In the late 1990s	Death, gastrointestinal, neurologic, rash, respiratory, sepsis, unspecified, and other **(o)**	[5-7]	Syndromic **(o)**
BMR-RAISIN	Bactéries MultiRésistantes-Réseau d'alerte d'investigation et de surveillance des infections nosocomiales	To evaluate the impact of the Infection Control Programme at the national level	France	2002	All the bacteria species which can have ESBLE or MRSA profiles **(b)**	[8]	Traditional **(i)**
PediSurv	Belgian Paediatric Surveillance system	To survey infectious diseases in children by collecting, compiling and analyzing data on various infectious diseases from children under 15 years	Belgium		Acute flaccid paralysis, measles, mumps, Invasive Pneumococcal Disease, the congenital rubella syndrome, the hemolytic uremic syndrome and Influenza A or B **(o)**	[9]	Traditional **(i)**

CHIF-NET	China Hospital Invasive Fungal Surveillance Net	To prospectively survey trends in yeast infections epidemiology and determine susceptibility to antifungal drugs	China	2009	All yeasts species **(f)**	[10]	Traditional **(i)**
MIS	Malaria Indicator Surveys	To evaluate the extent of transmission of malaria and explore the potential for elimination in numerous African countries	Madagascar, Rwanda, Sierra Leone, Sudan, Burundi, Eritrea, Malawi, Zambia, Zimbabwe, Angola, Ethiopia, Liberia, Tanzania, Cambodia, Kenya, Nigeria, Swaziland, Afghanistan, Namibia, Senegal, Botswana, Mozambique, Gambia, Djibouti, Uganda	First countries in 2006	Malaria **(p)**	[11]	Traditional **(i)**
KIzSS		To monitor day care-related infectious diseases and associated disease	The Netherlands	2010	All pathogens causing enteric diseases **(o)**	[12]	Traditional **(i)**
Euro-GASP	European gonococcal antimicrobial surveillance programme	To monitor *Neisseria gonorrhoeae* antimicrobial susceptibility in the countries included in the surveillance system	21 european member states	2004	Antimicrobial resistance of *Neisseria gonorrhoeae* **(b)**	[13, 14]	Traditional **(i)**
FoodNet	Foodborne Diseases Active Surveillance Network	To determine the impact of foodborne illness in the USA, monitor their evolution over time, evaluate the impact of specific foods and settings in the identified burden and to transfer information to improve public health practice and interventions.	USA	1996	*Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin-producing *Escherichia coli* O157 and non-O157, *Shigella*, *Vibrio* and *Yersinia* **(o)**	[15]	Traditional **(i)**
FluCAN	Influenza Complications Alert Network	To provide reliable, comprehensive, consistent and rapidly available data from sentinel hospitals on adult acute respiratory hospitalisations, including intensive care units admissions.	Australia	2009	H1N1 **(v)**	[16, 17]	Traditional **(i)**
	Measles/rubella integrated system	To monitor and investigate patients with fever and rash illness	Caribbean subregion	January 2000	Measles and rubella **(v)**	[18]	Traditional **(i)**

		To determine the rate of health care-associated infection and device-associated health care-associated infections, and distribution of causative microorganisms and etiologic factors responsible for these infections in a neonatal intensive care unit in one hospital of southeastern Turkey	Turkey	January 2008	Agents of nosocomial infections **(o)**	[19]	Traditional **(o)**
		To evaluate trends in *C. difficile* incidence at the national level	Finland	January 2008	*Clostridium difficile* **(b)**	[20, 21]	Traditional **(i)**
NARMS	National Antimicrobial Resistance Monitoring System	To track patterns of emerging resistance and facilitate outbreak investigations in foodborne and other enteric bacteria	USA	1996	Non-Typhi *Salmonella* isolate, *Salmonella* Typhi, *Shigella* isolate, and *E. coli* O157 and *Campylobacter* **(b)**	[22]	Traditional **(i)**
PulseNet	National molecular subtyping network	To connect foodborne ilness cases in order to identify and define national and international outbreaks	83 countries	1996	*E. coli* O157 and other Shiga toxin-producing *E. coli*, *Campylobacter jejuni*, *Clostridium botulinum*, *Listeria monocytogenes*, *Salmonella*, *Shigella*, *Vibrio cholerae* and *Vibrio parahaemolyticus* **(b)**	[23]	Traditional **(i)**
		To detect geographic and temporal clusters of patients presenting acute illness that might represent the initial manifestations of a bioterrorism event in California, Massachusetts, Minnesota, and Texas	USA	2007	Respiratory syndrome, influenza-like illness, upper gastrointestinal infection, lower gastrointestinal infection, hemorrhagic, lesions, lymphadenopathy, neurologic, and rash **(o)**	[24]	Syndromic **(o)**
	United Kingdom Severe Influenza Surveillance System	To monitor hospitalisations due to confirmed seasonal influenza in England	England	October 2010	Influenza **(v)**	[25]	Traditional **(i)**
		To assess the epidemiology and seasonality of influenza in Uganda based on data from 5 hospitals and 5 outpatient clinics in 4 geographically distinct regions	Uganda	April 2007	Influenza A and B viruses **(v)**	[26]	Traditional **(i)**

		To better understand the epidemiology, seasonality and impact of influenza in this country, and identify the influenza viruses that circulate at the national level based on data from 5 hospitals across the country	Tanzania	May 2008	Influenza A and B viruses **(v)**	[27]	Traditional **(i)**
		To better understand the epidemiology, seasonality and impact of influenza in this country, and identify the influenza viruses that circulate at the national level using 4 sentinel facilities	Nigeria	April 2009	Influenza **(v)**	[28]	Traditional **(i)**
ISS	Influenza sentinel surveillance system	To follow the epidemiology of seasonal influenza and track the emergence of a novel influenza strain with pandemic potential using two referral and four district hospitals	Rwanda	July 2008	Influenza A and B **(v)**	[29]	Traditional **(i)**
		To have an idea of the disease burden due to neurological infection in children	Cambodia	2006	Japanese encephalitis **(v)**	[30]	Traditional **(i)**
SIREVA II	Sistema de Redes de Vigilancia de Agentes Bacterianos Causantes de Meningitis y Neumonias	To determine antibiotic susceptibility profiles and epidemiological information on the followed pathogens in 19 Latin American countries	19 Latin American countries	1993	*S. pneumoniae*, *H. influenzae* and *N. meningitidis* **(b)**	[31]	Traditional **(i)**
NESP	National Enteric Surveillance Program	To detect outbreaks and report national trends of the followed pathogens	Canada	April 1997	*Salmonella*, *Campylobacter, Shigella, Vibrio,* Verotoxigenic *E. coli, Yersinia*; intestinal parasitic organisms such as *Giardia*, *Cryptosporidium*, *Entamoeba* and *Cyclospora*; and enteric viruses such as Norovirus and Rotavirus **(o)**	[32]	Traditional **(i)**
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance	To monitor antimicrobial use and antimicrobial resistance in selected species of enteric bacteria from humans, animals and animal-derived food sources	Canada	1997	Selected enteric bacterial species **(b)**	[32]	Traditional **(i)**
	Micronet	To monitor the antimicrobial suceptibility of various pathogens	Italy	2008	Various, including *K. pneumoniae* **(b)**	[33]	Traditional **(i)**
	EPIMIC	To identify abnormal events occuring in the clinical microbiology laboratory of university hospitals of Marseille	France	2005	Various criteria monitored, from the number of samples tested for specific pathogens to the number of tests performed globally for a specific specimen **(o)**	[34]	Syndromic **(o)**

PPHSN	The Pacific Public Health Surveillance Network	PPHSN's goal is to improve public health surveillance and response in the Pacific Islands	22 Pacific Island countries and territories	1996	Dengue, measles/rubella, influenza, leptospirosis, typhoid fever, cholera, SARS and HIV and STI **(o)**	[35]	Traditional **(i)**
MBDS	Mekong Basin Disease Surveillance	To share information on infectious diseases which are followed by the surveillance system and to cooperate in outbreak response and pandemic influenza preparedness	Cambodia, China, Lao People's Democratic Republic, Myanmar, Thailand and Vietnam	1999	H1N1/ H5N1, Acute Flaccid Paralysis, Severe acute respiratory syndrome, Cholera /Severe Diarrhea Encephalitis, Tetanus, Meningitis, Diphtheria, Leptospirosis, Chikungunya, Dengue fever, Typhoid fever, Measles, Malaria, Pneumonia, HIV/AIDs and Tuberculosis **(o)**	[35]	Traditional **(i)**
GeoSentinel	Clinic-based global surveillance system	To create a worldwide communication and data collection network of travel medicine clinics	Comprise 57 travel/tropical medicine clinics worldwide and 235 additional clinics	1995	All travel related illnesses observed in participating clinics **(o)**	[36]	Traditional **(i)**
GERMS-SA	The Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa	To survey bacterial and fungal pathogens of public health importance using data from about 200 South African clinical microbiology laboratories	South Africa	2003	Cryptococcosis, Pneumocystis pneumonia, salmonellosis, invasive pneumococcal disease, Cholera, typhoid fever, meningococcal disease, shigellosis, diarrhoeal disease due to diarrhoeagenic *E. coli*, *Haemophilus influenzae* type b disease and nosocomial infections **(o)**	[37]	Traditional **(i)**
GRSN	Global Rotavirus Surveillance Network	To collect data on rotavirus vaccine introduction and use; to survey disease trends; to develop a network capable to support vaccine effectiveness studies and to promote the importance of collecting surveillance data	Worldwide	2008	Rotavirus **(v)**	[38]	Traditional **(i)**

		To determine the prevalence of anorectal *Chlamydia trachomatis* serovars in a group of men who have sex with men with high risk sexual behaviour, attendees at a sexually transmitted infection unit from 8 regions in Northwest Spain	Spain		*C. trachomatis* **(b)**	[39]	Traditional **(o)**
CNISN	The Chinese National Influenza-Like Illness Surveillance Network	To survey influenza-like illness in the 31 Chinese provinces of interest	China		Influenza-like illness **(o)**	[40, 41]	Traditional **(i)**
		To monitor the global evolution of the followed disease in the country from 2005 to 2007	Bulgaria	2005	Brucellosis **(b)**	[42]	Traditional **(o)**
The Korean NNDSS	The Korean National Notifiable Disease Surveillance System	To follow the notifiable infectious diseases choosen for mandatory reporting	Korea	1955	50 infectious diseases **(o)**	[43, 44]	Traditional **(i)**
NNDSS	National Notifiable Diseases Surveillance System	To collect and publish data concerning nationally notifiable diseases	USA	1951	More than 60 infectious diseases **(o)**	[45-47]	Traditional **(i)**
The Australian NNDSS	The Australian National Notifiable Disease Surveillance System	To coordinate the national surveillance of communicable diseases or disease groups	Australia	1990	More than 50 infectious diseases **(o)**	[48]	Traditional **(i)**
CanNAISS	The Canadian Notifiable Avian Influenza Surveillance System	To survey avian influenza viruses at the national level	Canada	2008	Avian influenza viruses **(v)**	[49]	Traditional **(i)**

GPR surveillance system	Connecticut's Gram-positive rod surveillance system	To identify as soon as possible inhalational anthrax or unusual *Clostridium* spp. infections, and to establish round-the-clock laboratory reporting of potential indicators of bioterrorism, Connecticut state	USA	2003	Gram-positive rods **(b)**	[50, 51]	Traditional **(o)**
NHSN	National Healthcare Safety Network	To track healthcare-associated infections	USA	2005	Healthcare-associated infections **(o)**	[52, 53]	Traditional **(i)**
SINAN	Sistema de Informação de Agravos de Notifição / Information System for Notifiable Diseases	To merge and analyze data from notifiable diseases	Brazil	1993	All the nationally notifiable diseases **(o)**	[54]	Traditional **(i)**
NESID	National Epidemiological Surveillance of Infectious Diseases	To evaluate the occurrence of infectious diseases and agents followed by the system	Japan	1981	27 kinds of infectious diseases **(o)**	[55]	Traditional **(i)**
NARST	National Antimicrobial Resistance Surveillance Thailand	To improve the antimicrobial-resistant pathogens surveillance using data from 33 hospitals in Thailand and to standardize the laboratory practices all around the country	Thailand	1998	Various pathogens **(o)**	[56]	Traditional **(i)**
FERN	Food Emergency Response Network	To respond to emergencies contamination of food, including natural or voluntary biological contaminations	USA	2004	All those that can be found in food **(o)**	[57, 58]	Traditional **(i)**
	The BioWatch Program	To identify followed agents within 36 hours of release to organize rapid response	USA	2001	Various biological agents **(o)**	[58]	Traditional **(i)**
	National Syndromic Surveillance System (formerly BioSense 2.0)	To monitor all hazards and health outcomes threatening american people	USA	2003	Various parameters **(o)**	[58]	Syndromic **(i)**
	EuroFlu	To analyse and present epidemiological and virological data from the European Region Member States on flu	53 member states	1996	Influenza **(v)**	[58]	Traditional **(i)**

Global Avian Influenza Network for Surveillance	GAINS	To improve global monitoring capacity, strengthen the knowledges of viral strains and transmission of influenza viruses in wild birds, and transmit information on avian influenza viruses to all levels (governments, international organizations, the private sector and the general public)	23 countries around the world	2006	Avian influenza viruses **(v)**	[58]	Traditional **(i)**
	FluNet	To survey influenza viruses around the world	Worldwide	1995	Influenza **(v)**	[58]	Traditional **(i)**
	DengueNet	To create a platform to exchange surveillance data to improve detection and monitoring incidence and trends of dengue and dengue haemorrhagic fever	Worldwide	1995	Dengue **(v)**	[58]	Traditional **(i)**
	RabNet	To survey rabies around the world based on interactive surveillance maps and graphs using both human and animal data on rabies	Worldwide	End of 1990s	Rabies **(v)**	[58, 59]	Traditional **(i)**
	Global Malaria Programme	To coordinate World Health Organization's global efforts to survey and fight malaria (prevention, management, surveillance and evaluation)	Worldwide		Malaria **(p)**	[58]	Traditional **(i)**
VICNISS	Victorian Hospital Acquired Infection Surveillance System	To decrease the number of hospital-acquired infections in the participating hospitals of the Victoria region, Australia	Australia	2002	Hospital-acquired infections **(o)**	[60]	Traditional **(o)**
KISS	Korea Influenza Surveillance Scheme	To study influenza evolution over time and to track influenza epidemics, to determine predominant circulating influenza virus strains, to contribute, based on retrieved data, to the formulation of influenza control measures and to evaluate the efficacy of the	Korea	2000	Influenza **(v)**	[61]	Traditional **(i)**

	influenza vaccine					
Notifiable disease surveillance	To survey all the infectious diseases at the national level	Italy	2001	All the infectious diseases **(o)**	[62, 63]	Traditional **(i)**
	To survey invasive diseases in the Piedmont region, Italy	Italy	2001	Invasive infectious diseases **(o)**	[62, 63]	Traditional **(o)**
	To survey the nationally notifiable infectious diseases	France		31 nationally notifiable infectious diseases **(o)**	[62, 64]	Traditional **(i)**
	To improve infectious diseases surveillance during the 2006 FIFA World Cup event in 12 German cities	Germany	June 2006	Various infectious diseases **(o)**	[62, 65]	Traditional **(o)**
	To identify and analyze infectious events which necessitate immediate action	Belgium	2000	Mainly legionellosis, foodborne diseases, measles, pertussis, diphtheria, meningococcal meningitis, and rare imported diseases **(o)**	[62, 66]	Traditional **(i)**
	To allow rapid alert and appropriate response to targeted health event in the Northern region of Portugal	Portugal		Various diseases and syndromes, including foodborne outbreaks, legionnaires' disease, meningococcal disease, acute flaccid paralysis, diphtheria and measles and unexpected adverse health events **(o)**	[62, 67]	Traditional **(o)**
Acute flaccid paralysis surveillance	To conduct national surveillance of acute flaccid paralysis at the national level	Australia	1995	Acute flaccid paralysis **(o)**	[68]	Traditional **(i)**

		To implement an early warning system for West Nile Virus Activity in New York city	USA	2000	West Nile Virus **(v)**	[69, 70]	Traditional **(o)**
		To survey the number of birds death and show how these information can be used to follow West Nile virus in New York State, New Jersey and Connecticut	USA	1999	West Nile Virus **(v)**	[69, 71]	Traditional **(o)**
TRANSNET	Transplant-Associated Infection Surveillance Network	To survey all transplant recipients in 23 United States transplant centers to understand the burden and epidemiology of invasive fungal infections	USA	2001	Invasive fungal infections **(f)**	[72]	Traditional **(o)**
		To classify and monitor all surgical site infections observed at Mayo Clinic in Rochester, Minnesota	USA		All surgical site infections **(o)**	[73]	Traditional **(o)**
ArboNET		To follow Arboviral diseases in humans, mosquitoes and other animals	USA	2000	Arboviral diseases **(v)**	[74]	Traditional **(i)**
		To monitor possible West Nile Virus introduction in Germany.	Germany		West Nile Virus **(v)**	[75]	Traditional **(i)**
	Danish national surveillance system	To monitor of infectious diseases, microorganisms and vaccination coverage among Danish population	Denmark		Various infectious diseases **(o)**	[76]	Traditional **(i)**
SurvNet@rki or SurvNet	German national electronic surveillance system	To survey the nationally notifiable diseases using electronically notifications	Germany	2001	All the national notifiable diseases **(o)**	[77]	Traditional **(i)**
NHSS	National HIV Surveillance System	To follow HIV trends among the US population over the years	USA		HIV **(v)**	[78]	Traditional **(i)**
		To track the presence of the followed virus in the state of Montana	USA	2009	West Nile Virus **(v)**	[79]	Traditional **(o)**
	The *Campylobacter* surveillance system	To study the epidemiology of *Campylobacter* and its outbreaks, to establish and evaluate mesures to control and prevent it, to teach the public on what they have to do to prevent the disease and to plan services and priority setting in the Victoria region, Australia	Australia		*Campylobacter* spp. **(b)**	[80]	Traditional **(o)**

	GermWatcher	To identify possible nosocomial infections using results from culture from the Barnes-Jewish Hospital, Saint-Louis, Missouri	USA	February 1993	Nosocomial infections **(o)**	[81, 82]	Traditional **(o)**
		To monitor nosocomial blood-stream infections at the scale of the university hospital of Lausanne	Switzerland		Nosocomial blood-stream infections **(o)**	[81, 83]	Traditional **(o)**
	The Iranian notifiable infectious diseases surveillance system	To track notifiable diseases in Iran	Iran	the 1990s	National notifiable diseases **(o)**	[84]	Traditional **(i)**
	National Tuberculosis Surveillance System	To monitor the overall trend of tuberculosis in the USA over time	USA	1953	*Mycobacterium tuberculosis* **(b)**	[85]	Traditional **(i)**
	ARICABA	To identify and anticipate infectious diseases threats	Martinique, St. Lucia, and Dominica	2010	Infectious diseases **(o)**	[86]	Syndromic **(i)**
GEIS	Global Emerging Infections Surveillance and Response System	In general, to survey and provide responses in case of outbreaks for the followed pathogens and diseases	More than 35 partner laboratories around the world	1997	Respiratory infections, febrile and vector-borne infections, gastrointestinal infections, antimicrobial resistant organisms, sexually-transmitted infections **(o)**	[87]	Traditional **(i)**
		To survey bacterial meningitis and describe their epidemiology at the national level, to propose ideas on the composition of potential vaccine and to identify and share antibiotic susceptibility data of collected isolates	The Netherlands	1975	*Haemophilus influenza*, *Neisseria meningitidis* and *Streptococcus pneumoniae* **(b)**	[88]	Traditional **(i)**

		To survey patients positive to *M. tuberculosis* by sending real-time notification alerts to doctors and nurses who carried patients at the Kaohsiung Hospital	Taiwan	June 14 2005	*M. tuberculosis* **(b)**	[89]	Traditional **(o)**
	The Departement of Health and Mental Hygiene routine surveillance systems for influenza	To routinely survey the evolution of influenza in New York city	USA	April 26 2009	Influenza **(v)**	[90]	Traditional **(o)**
		To identify and notify all Shiga toxin/verotoxin-producing *Escherichia coli* cases	Germany	May 2011	Shiga toxin/verotoxin-producing *Escherichia coli* **(b)**	[91]	Traditional **(o)**
EWRS	Early Warning and Response System	To enhance the prevention and control of communicable diseases	European Community Member States		Communicable diseases **(o)**	[91]	Traditional **(i)**
		To enhance national surveillance of the followed pathogens (timeliness, data quality and investigation of confirmed cases including laboratory confirmation of diagnosis)	Italy	2007	Measles, mumps and rubella viruses **(v)**	[92]	Traditional **(i)**
		To survey antimicrobial resistance among Enterobacteriaceae and glucose non-fermenting bacteria using data from 15 hospitals nationwide	China	1994	Antimicrobial resistance in Enterobacteriaceae and glucose non-fermenting bacteria **(b)**	[93]	Traditional **(i)**

	CHINET	To investigate the resistance of bacteria species routinely identified from clinical isolates	China	Started in 1998 with 20 tertiary hospitals nationwide and became CHINET in 2005	Antimicrobial resistance of various bacterial species **(b)**	[93]	Traditional **(i)**
Monharin	MOH National Antibacterial Resistance Investigation Net	To collect and determine resistance profiles of selected bacterial species	China	Started in 1999 with 15–17 member hospitals nationwide and became Monharin in 2004 with more than 80 member hospitals	Antimicrobial resistance of various bacterial species **(b)**	[93]	Traditional **(i)**
COVIS	Cholera and Other Vibrio Illness Surveillance System	To retrieve and gather clinical data on illnesses associated with *Vibrio* species and investigate the probable source of contamination	USA	1988	*Vibrio* species **(b)**	[94]	Traditional **(i)**
WBDOSS	Waterborne Disease and Outbreak Surveillance System	To track outbreaks caused by drinking water and other water exposures	USA	1971	Various pathogens **(o)**	[94]	Traditional **(i)**
		To monitor influenza trends at the national level using sentinel clinics	Japan		Influenza-like illness **(v)**	[95]	Traditional **(i)**
	National Avian Influenza Surveillance System	To survey the evolution of H5N1 avian influenza virus	Thailand	1997	H5N1 **(v)**	[96]	Traditional **(i)**
SIMI	the Italian National Surveillance System of Infectious Diseases	To survey communicable disease outbreaks	Italy	1994	Communicable diseases **(o)**	[97, 98]	Traditional **(i)**

		To track and control nosocomial outbreaks of H1N1 (2009) influenza at the Kaohsiung Chang Gung Memorial Hospital	Taiwan	August 1, 2009	H1N1(2009) influenza **(v)**	[99]	Traditional **(o)**
		To identify and survey avian influenza viruses carried by various species of wild ducks in Alberta, Canada	Canada	1976	Avian influenza viruses **(v)**	[100]	Traditional **(o)**
		To evaluate if the closure of the Three Gorges Dam have an impact on the schistosome incidence of the Hunan, Jiangxi, Hubei and Anhui provinces populations over time	China	2002	*S. japonicum* **(p)**	[101]	Traditional **(o)**
	The FIFA Women's World Cup infectious disease surveillance system.	To survey infectious diseases trends during the FIFA Women's World Cup in 9 German cities	Germany	June 26, 2011	Various routine infectious diseases **(o)**	[102]	Traditional **(o)**
NOIDs	Notifications of Infectious Diseases	To follow and report nationally notifiable diseases	England	Start in 1891 in London	About 30 notifiable infectious diseases **(o)**	[103]	Traditional **(i)**
		To supply microbiological testing, risk evaluation and specialist analysis	England		Various infectious diseases **(o)**	[103]	Traditional **(i)**
		To survey *Neisseria meningitidis* serogroup C throughout the country	China	2000	Invasive meningococcal disease **(b)**	[104]	Traditional **(i)**
VIRGIL	European Vigilance Network for the Management of Antiviral Drug Resistance	To monitor current and emerging antiviral drugs resistance for influenza and viral hepatitis	Europe	May 1st, 2004	Antiviral resistance in influenza and viral hepatitis **(v)**	[105]	Traditional **(i)**
EISN	European Influenza Surveillance Network	To follow influenza seasonal activity in European countries	Europe	1996	Influenza **(v)**	[105]	Traditional **(i)**
VINCat	Vigilància de les Infeccions Nosocomials a Catalunya	To implement and support a standardized hospital-acquired infections surveillance system	Spain	2006	Hospital-acquired infections **(o)**	[106]	Traditional **(o)**

		To look for positive *Candida* blood cultures in 12 cities located in the South, Southeast, Central and Northeast regions of Brazil	Brazil	March 2003	*Candida* spp. **(f)**	[107]	Traditional **(o)**
EARS-Net	European Antimicrobial Resistance Surveillance Network	To survey and provide European reference data on antimicrobial resistance for public health purposes	Europe	January 1999	Antimicrobial resistance in *Streptococcus pneumoniae*, *Acinetobacter* spp., *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeuruginosa*. **(b)**	[108]	Traditional **(i)**
ANRESIS	The Swiss Antibiotic Resistance Surveillance database	To follow antibiotic resistance and consumption at the national level	Switzerland		Antimicrobial resistance and consumption **(b)**	[108]	Traditional **(i)**
	The Foodborne Disease Outbreak Surveillance System	To collect data on foodborne disease outbreaks in order to monitor them at the national level	USA		Foodborne diseases **(o)**	[109]	Traditional **(i)**
		To follow precise public health events in the state of Tamil Nadu	India	1984	Acute flaccid paralysis, measles, pertussis, diphtheria, tetanus neonatorum, tetanus, rabies, encephalitis, meningitis, and hepatitis **(o)**	[110, 111]	Traditional **(o)**
		To track and detect outbreaks of diarrheal illness in New York city	USA	1995	Diarrheal illness, particularly those caused by *Cryptosporidium* and *Giardia* **(o)**	[110, 112]	Syndromic **(o)**
CNISP	Canadian Nosocomial Infection Surveillance Program	To describe the impact of nosocomial inections in canadian hospitals included in the surveillance network	Canada	1995	Nosocomial infections **(o)**	[113, 114]	Traditional **(i)**
SARI	Surveillance System of Antibiotic Use and Bacterial Resistance in Intensive Care Units	To collect and analyze antimicrobial resistance data from 53 German intensive care units	Germany	2000	Antimicrobial resistance for 13 bacterial species **(b)**	[115]	Traditional **(i)**
NREVSS	National Respiratory and Enteric Virus Surveillance System	To monitor temporal and geographic trends of respiratory syncytial virus, human parainfluenza viruses, respiratory and enteric adenoviruses and rotavirus	USA	2007	Respiratory syncytial virus, human parainfluenza viruses, respiratory and enteric adenoviruses and rotavirus **(v)**	[116]	Traditional **(i)**

NESS	The National Enterovirus Surveillance System	To monitor trends in circulating enteroviruses	USA	1961	Enteroviruses **(v)**	[116, 117]	Traditional **(i)**
	FluWatch	To detect flu outbreaks across the country as soon as possible, to give rapid information on flu activity, to monitor circulating strains of the flu virus and test their sensitivity to antiviral medications and to provide information to the World Health Organization	Canada	1998	Influenza viruses **(v)**	[118]	Traditional **(i)**
		To survey nosocomial infections at the European Institute of Oncology in Milan	Italy	May 2006	*Acinetobacter baumannii*, *Aspergillus* sp., *Mycobacteria* in culture, *Mycobacteria* by microscope, strain extended-spectrum beta lactamase producing, *Clostridium difficile* (toxin A), *Enterococcus faecalis* resistant to ampicillin, *Enterococci* resistant to vancomycin, *Haemophilus influenzae* resistant to ampicillin, urinary antigen *Legionella* positive, spinal fluid positive culture, *Listeria monocytogenes*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa* multi-drug resistant, *S. aureus* resistant to meticillin, *S. aureus* intermediate to vancomycin, *S. aureus* resistant to vancomycin, *S. pneumoniae* resistant to cephalosporin, *S. pneumoniae* resistant to penicillin, *Salmonella* sp. in faeces, *Shigella* sp. in faeces, *S. maltophilia* and *Streptococcus* not susceptible to vancomycin **(o)**	[119]	Traditional **(o)**
		To evaluate the prevalence of drug resistance in HIV patients co-infected with *Mycobacterium tuberculosis* in Phnom Penh, Cambodia	Cambodia	March 2003	*M. tuberculosis* **(b)**	[120]	Traditional **(o)**
		To identify the antifungal drug resistances of *Candida* bloodstream isolates isolated in Andalusia, Spain	Spain	Octobre 2005	*Candida* spp. **(f)**	[121]	Traditional **(o)**
		To monitor community-onset *S.aureus* in the Illinois US state	USA	January 2005	*S. aureus* **(b)**	[122]	Traditional **(o)**

		To survey adult invasive pneumococcal disease in North-Rhine Westphalia, Germany	Germany	2003	Invasive pneumococcal disease **(b)**	[123, 124]	Traditional **(o)**
		To monitor foodborne diseases at the Middle East level	Jordan, the Palestinian authority and Israel	July 2005	Foodborne diseases **(o)**	[125]	Traditional **(i)**
		To monitor the epidemiology of candidaemia occurring in regional public healthcare facilities in Queensland	Australia	1999	*Candida* spp. **(f)**	[126]	Traditional **(o)**
		To monitor and investigate the dengue virus serotypes currently circulating at the national level	Singapore	2005	Dengue virus **(v)**	[127]	Traditional **(i)**
		To survey cholera at the national level	Nepal	June 2008	*Vibrio cholerae* **(b)**	[128]	Traditional **(i)**
	ToxoSurv	To optimise surveillance of congenital toxoplasmosis at the national level	France	June 2007	Congenital toxoplasmosis **(p)**	[129]	Traditional **(i)**
	The Viriato study	To survey antimicrobial susceptibility of the followed bacterial species	Portugal	1999	*S. pneumoniae*, *H. influenzae, M. catarrhalis* and *Streptococcus pyogenes* **(b)**	[130]	Traditional **(i)**
ACNN	The ANOFEL Cryptosporidium National Network	To estimate the incidence and epidemiology of human cryptosporidiosis at the national level	France	2004	Cryptosporidiosis **(f)**	[131]	Traditional **(i)**
		To estimate the incidence, species distribution, frequency of resistance, and risk factors associated with *Candida* infections in 40 tertiary hospitals	Spain	June 2008	*Candida* spp. **(f)**	[132]	Traditional **(i)**
		To evaluate the bacterial species-specific incidence of enteric fever in the Guangxi province	China		*Salmonella* spp. **(b)**	[133]	Traditional **(o)**
		To understand the epidemiology and risk factors associated with candidaemia in critically ill trauma patients from New Dehli, India	India	April 2008	*Candida* spp. **(f)**	[134]	Traditional **(o)**

		To describe the clonal and clinical profile of invasive pneumococcal disease caused by serotype 19A in Madrid, Spain	Spain	May 2007	Invasive pneumococcal disease **(b)**	[135]	Traditional **(o)**
		To monitor antifungal resistance in yeast species isolated from blood cultures	Argentina	June 2007	Yeasts species **(f)**	[136]	Traditional **(i)**
		To monitor the national epidemiology of invasive meningococcal disease	Austria	1995	*N. meningitidis* **(b)**	[137]	Traditional **(i)**
		To measure the impact of rotavirus vaccination	Belgium	1983	Various infectious diseases **(o)**	[138]	Traditional **(i)**
		To determine host and *Mycobacterium tuberculosis* strain-related factors associated with the development of extrapulmonary forms of tuberculosis in the Espirito Santo state of Brazil	Brazil	1998	*M. tuberculosis* **(b)**	[139]	Traditional **(o)**
		To evaluate the prevalence of extensively drug-resistant tuberculosis at the Shandong province level	China	November 2004	*M. tuberculosis* **(b)**	[140]	Traditional **(o)**
		To monitor invasive beta-haemolytic streptococci trends in Denmark	Denmark	2005	Beta-haemolytic streptococci **(b)**	[141]	Traditional **(i)**
		To monitor *M. pneumoniae* at the national level	Finland	1995	*M. pneumoniae* **(b)**	[142]	Traditional **(i)**
LaboVIH	Laboratory-based surveillance of HIV	To monitor HIV at the national level	France		HIV **(v)**	[143]	Traditional **(i)**
		To monitor resistance data of all clinical pathogens and sample types from hospitals and ambulatory care	Germany	2008	Antimicrobial resistance for all clinical pathogens and sample types **(o)**	[144]	Traditional **(i)**
		To study the epidemiology of varicella-associated invasive group A streptococcal infections at the national level	Germany	January 1996	Varicella-associated invasive group A streptococcus infections **(o)**	[145]	Traditional **(i)**

		To monitor the molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in the five intensive care units of the San Martino Tertiary Referral Hospital of Genoa	Italy	January 2007	Healthcare-associated infections **(o)**	[146]	Traditional **(o)**
		To monitor invasive listeriosis at the Lombardy region level	Italy	2005	Invasive listeriosis **(b)**	[147]	Traditional **(o)**
		To evaluate the epidemiology of candidemia and antifungal susceptibility profiles of *Candida* isolates using data from 34 departments of clinical microbiology	Italy	January 2009	*Candida* spp. **(f)**	[147]	Traditional **(o)**
		To monitor cryptococcal disease at the national level	South Africa	January 2005	Cryptococcal disease **(f)**	[148]	Traditional **(i)**
		To estimate the potential coverage of serotype-specific *S. agalactiae* vaccines	South Africa	January 2004	*S. agalactiae* **(b)**	[149]	Traditional **(o)**
		To survey infants with culture-confirmed *M. tuberculosis* in Cap Town, South Africa	South Africa	January 2004	*M. tuberculosis* **(b)**	[150]	Traditional **(o)**
		To survey *W. bancrofti* at the national level	Togo	2006	*W. bancrofti* **(b)**	[151]	Traditional **(i)**
DIAL	Data Integration for Alberta Laboratories	To monitor any disease tested within the laboratory of interest	Canada	2009	Various parameters **(o)**	[152]	Syndromic **(o)**
WMLN	The Wisconsin Mycobacteriology Laboratory Network	To monitor mycobacteries species at the Wisconsin state level	USA	1998	Mycobacteries **(b)**	[153]	Traditional **(o)**
CIDR	Computerised Infectious Disease Reporting	To operate all the surveillance and control of infectious diseases at the national level. Antimicrobial resistance amongst various organisms is also surveyed by the surveillance system	Ireland	2005	Over 80 notifiable diseases **(o)**	[154]	Traditional **(i)**
ICS	International Circumpolar Surveillance network	To monitor infectious disease throughout the Arctic countries	Arctic countries	1999	The first priorities were invasive bacterial diseases caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and groups A and B S*treptococcus* **(b)**	[155]	Traditional **(i)**

HIMM	Hospital-based Influenza Morbidity and Mortality	To survey morbidity and mortality due to influenza based on data from seven South Korea hospitals	South Korea	2011	Influenza viruses **(v)**	[156]	Traditional **(o)**
KINRESS	Korea Influenza and Respiratory Viruses Surveillance System	To monitor acute respiratory infections at the national level	South Korea	May 2009	Acute respiratory infections **(o)**	[157]	Traditional **(o)**
		To survey unexplained pneumonia at the national level	China	2004	Pneumonia of unexplained origin **(o)**	[158]	Traditional **(i)**
		To estimate the incidence of leptospirosis in two districts of interest of the Kilimanjaro region	Tanzania	June 13, 2011	Leptospirosis **(b)**	[159]	Traditional **(o)**
GUARDIAN	Geographic Utilization of Artificial Intelligence in Real-Time for Disease Identification and Alert Notification	To develop an automated surveillance system able to detect infectious agents and provide help for diagnosis in the Chicago metropolitan area	USA		Various infectious diseases **(o)**	[160]	Syndromic **(o)**
	Acute Meningitis and Encephalitis Syndrome Project	To evaluate the incidence and epidemiology of preventable causes of meningitis and encephalitis using already marketed vaccines and to increase the laboratory capacity to identify these diseases in 4 Chinese prefectures (Jinan, Yichang, Shijiazhuang and Guigang)	China	September 2006	Bacterial meningitidis pathogens including *S. pneumoniae*, *N. meningitidis* and *H. influenzae* type b **(b)**	[161]	Traditional **(o)**
		To determine the geolocation of West Nile virus, to evaluate the link between the seroprevalence in pigeons and the incidence of human infected by the virus, and to assess the possibility of using pigeons as a marker for a West Nile virus surveillance system	Greece	2010	West Nile virus **(v)**	[162]	Traditional **(o)**
		To survey malaria incidence and prevalence in the Oromia regional state of Ethiopia using data from 10 sentinel facilities	Ethiopia	Since 2010	Malaria **(p)**	[163]	Traditional **(o)**

TESSy	The European Surveillance System	To collect, analyze and disseminate data on 49 communicable diseases using data from all the European Union Member States and the European Economic Area countries	Europe	January 2008	49 communicable diseases **(o)**	[164]	Traditional **(i)**
		To survey avian influenza using sentinel geese and ducks in the Danube Delta	Romania	September 2008	Avian influenza **(v)**	[165]	Traditional **(o)**
ESS	The Electronic Surveillance System	To implement a bloodstream infections surveillance system based on data coming from the Calgary Health Region, Canada	Canada	2005	Bloodstream infections **(o)**	[166, 167]	Traditional **(o)**
DMSS	Defense Medical Surveillance System	It consists in a USA central repository of medical surveillance data for all illnesses and injuries of public health or military operational importance	USA	1990	All illnesses and injuries of public health or military operational importance **(o)**	[168, 169]	Syndromic **(i)**
		To register and survey invasive meningococcal diseases at the national level	Poland		Invasive meningococcal diseases **(b)**	[170]	Traditional **(i)**
		To identify severe influenza infections, observe their epidemiology over the time and their virological characteristics, and evaluate their impact on the healthcare system	Tunisia		Influenza **(v)**	[171]	Traditional **(i)**
RDMS	The Respiratory DataMart System	To detect and follow the incidence trends of various viruses	England	2009	Various viruses, including influenza A(H1N1), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), rhinovirus, parainfluenza viruses, and adenovirus **(v)**	[172]	Traditional **(i)**
		To survey *Neisseria gonorrhoeae* infection in Tainan to assess underreporting in the National Gonorrhea Notifiable Disease System (NGNDS), and to better understand why physicians do not report all the cases they observe	Taiwan		*N. gonorrhoeae* **(b)**	[173]	Traditional **(o)**
		To implement a sentinel surveillance system able to monitor respiratory syncytial virus at the European level	16 European countries		Respiratory syncytial virus **(v)**	[174]	Traditional **(i)**

		To establish a surveillance system to better understand influenza evolution in the Cambodian cities of Takeo, Kampong Cham, Battambang, Siem Reap and Phnom Penh	Cambodia		Influenza **(v)**	[175]	Traditional **(o)**
SISSS	Spanish Influenza Sentinel Surveillance System	To survey influenza at the national level and collect valuable data on this disease	Spain	1996	Influenza **(v)**	[176, 177]	Traditional **(i)**
		To implement a national enhance surveillance system for pandemic influenza A (H1N1)	Greece		Influenza A (H1N1) **(v)**	[178]	Traditional **(i)**
ACCESS	The Australian Collaboration for Coordinated Enhanced Sentinel Surveillance of sexually transmitted infections and blood borne viruses	To evaluate the impact of national control programs on the trends of sexually transmitted infections and blood borne viruses at the national level	Australia	May 2007	Sexually transmitted infections and blood borne viruses **(o)**	[179]	Traditional **(i)**
		To follow enteroviruses across Hong Kong, China	China		Enteroviruses **(v)**	[180]	Traditional **(o)**
		To survey influenza and monitor vaccine effectiveness at the national level based on data from the British Columbia, Alberta, Quebec, and Ontario states	Canada		Influenza **(v)**	[181]	Traditional **(i)**
		To deploy a surveillance system able to monitor in near real-time respiratory viruses circulating within the community of the Houston metropolitan area	USA		Respiratory viruses **(v)**	[182]	Traditional **(o)**
		To follow respiratory diseases among the Singapore military	Singapore	11 May 2009	Respiratory diseases **(o)**	[183]	Traditional **(i)**
		To implement a sentinel surveillance system for the monitoring of viral hepatitis in five large public hospitals of the country	Pakistan	August 2009	Viral hepatitis **(v)**	[184]	Traditional **(i)**
		To establish a surveillance system for the identification of the different serotypes of human enteroviruses circulating across the French city of Clermont-Ferrand, and to develop procedures for future national survey studies	France	1 April 2010	Hand, foot, and mouth disease **(v)**	[185]	Traditional **(o)**

		To implement a sentinel surveillance system for the routine reporting of the disease based on data from 52 sentinel hospitals	Sri Lanka	2004	Leptospirosis **(b)**	[186]	Traditional **(i)**
		To survey trends of syphilis and HIV in female sex workers in Jinan, China, and identify risk behaviors leading to these infections	China	Three consecutive surveys: one since 2003 and two others in 2008 and 2009	Syphilis and HIV **(o)**	[187]	Traditional **(o)**
		To implement a hospital-based sentinel surveillance system for the monitoring of patients with flu-like symptoms in Guangzhou city	China	2008	Influenza **(v)**	[188]	Traditional **(o)**
HSS	HIV sentinel surveillance system	This surveillance system has been implemented for the continuous collection of data on HIV, including behavioral characteristics of people who have HIV	China	1995	HIV **(v)**	[189, 190]	Traditional **(i)**
		To implement a surveillance system to monitor the seasonality and characteristics of influenza using data from the Lusaka University Teaching Hospital	Zambia	June 2008	Influenza **(v)**	[191]	Traditional **(o)**
		To deploy a monitoring system for the surveillance of influenza in the city of Vojvodina	Serbia	2004	Influenza **(v)**	[192]	Traditional **(o)**
		This surveillance system has been specifically implemented to evaluate the economic impact of influenza hospitalization per age groups in 3 Chinese hospitals located in Sichuan, Hunan, and Shandong	China	January 2011	Influenza **(v)**	[193]	Traditional **(o)**

To implement a surveillance system able to monitor influenza at the national level, identify risk factors for severe disease, and identify the etiology of influenza virus circulating nationally	China	2009	Influenza **(v)**	[193]	Traditional **(i)**
To implement an influenza surveillance system in a limited resource country like Sierra Leone	Sierra Leone	2011	Influenza **(v)**	[194]	Traditional **(i)**
To deploy a sentinel dengue surveillance system specifically implemented for the French armed force deployed overseas	France		Dengue **(v)**	[195]	Traditional **(i)**
To implement an enhance national Pertussis surveillance system in the Korean Jeonnam Province	Korea	2012	Pertussis **(b)**	[196]	Traditional **(i)**
To implement a monitoring system able to identify circulating influenza strains, to understand their changes over time, and to collect data valuable for the global surveillance of influenza	Kenya	2007	Influenza **(v)**	[197]	Traditional **(i)**
To perform an integrated surveillance in the New Zealand Manawatu region to better understand transmission routes and the impact of human activities on the circultation of zoonotic agents from animals to humans	New Zealand	March 1st 2005	*C. jejuni* **(b)**	[198]	Traditional **(o)**
This surveillance system has been designed to follow West Nile virus circulation in Emilia Romagna region in both animals (horses and wild birds) and humans	Italy	2009	West Nile virus **(v)**	[199]	Traditional **(o)**

		To implement an integrated Measles/Rubella Surveillance in Chile	Chile		Measles and rubella **(v)**	[200]	Traditional **(i)**
		To implement a surveillance system able to survey measles in Nepal	Nepal	2003	Measles **(v)**	[201]	Traditional **(i)**
	The French integrated surveillance system for Salmonella	To survey *Samonella* over the whole food-chain, from farms to humans	France	1947 for human data and 1980s for the animals	*Salmonella* **(b)**	[202]	Traditional **(i)**
PBM	Pediatric Bacterial Meningitis surveillance	To recover data on laboratory-confirmed bacterial meningitis occuring among children aged under 5 years in 23 African countries throughout the WHO African Region	Africa	2001	Pediatric bacterial meningitis **(b)**	[203, 204]	Traditional **(i)**
Dengue-GIS	Dengue integral surveillance system	Dengue-GIS was implemented for the collection, analysis and reporting of geo-referenced dengue-related data at the national level	Mexico		Dengue **(v)**	[205]	Syndromic **(i)**
		To survey West Nile virus at the level of the region Emilia-Romagna	Italy		West Nile virus **(v)**	[206]	Traditional **(o)**
		To implement a surveillance system for West Nile virus in the Veneto region, Italy	Italy	2010	West Nile virus **(v)**	[207]	Traditional **(o)**
		To implement a surveillance system to collect valuable data (including data on climatic changes, but also on the virus activity through the mosquito, humans, birds, squirrels or equine) on the circulation of West Nile virus at the Californian state level	USA	2000	West Nile virus **(v)**	[208]	Traditional **(o)**
		To develop a surveillance system to collect data on West Nile virus from humans, birds, mosquito and equine	Canada		West Nile virus **(v)**	[208]	Traditional **(i)**

		To develop a surveillance system to collect data on West Nile virus from humans, chicken, and mosquito	Romania	1997	West Nile virus **(v)**	[208]	Traditional **(i)**
FoodNet Canada	The Canadian integrated enteric pathogen surveillance system	This surveillance system collect data from passive sampling of human cases and active sampling of three exposure sources (food, water, and animal manure) on various enteric pathogens	Canada	2005	Various enteric pathogens, including *Salmonella, E. coli, Campylobacter, Yersinia, Listeria, Shigella, Vibrio, Cryptosporidium, Cyclospora, Giardia,* noroviruses and rotaviruses **(o)**	[209]	Traditional **(i)**
MARAN	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands	This surveillance system has been designed to survey antibiotic resistance in animals and food	The Netherlands		Antimicrobial resistance in animals and food **(o)**	[210]	Traditional **(i)**
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme	The objectives of this surveillance system are to survey antimicrobial consumption and resistance at each step of the meat production (form food animals to humans)	Denmark	1995	Antimicrobial resistance in humans, animals and food **(o)**	[210]	Traditional **(i)**
NORM	Norwegian Surveillance System for Antimicrobial Drug Resistance	This surveillance system has been implement to detect, analyze and evaluate evolution of resistance in Norway	Norway	2000	Antimicrobial resistance in humans, animals and food **(o)**	[210]	Traditional **(i)**
ITAVARM	Italian Veterinary Antimicrobial Resistance Monitoring	To survey antimicrobial resistance in animals and humans at the national level	Italy		Antimicrobial resistance in animals and humans **(o)**	[210]	Traditional **(i)**
FINRES-VET	The Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents report	FINRES-VET has been implemented to survey antimicrobial agents' consumption used in animal health, to survey antimicrobial agents resistance in major food-producing animals and in pets, and to determine trends in resistance prevalence, emergence of resistant clones and appearance of new resistance phenotypes	Finland		Antimicrobial resistance in animals and food **(o)**	[210]	Traditional **(i)**

Pilot Surveillance Program for Antimicrobial Resistance in Bacteria of Animal Origin	The aim of this pilot surveillance system was to evaluate the prevalence of antimicrobial resistance of key organisms found in the gut food-producing animals	Australia	November 2003	Antimicrobial resistance in food-producing animals **(o)**	[210]	Traditional **(i)**
WHONET-Argentina	To establish a national surveillance system for the monitoring of antimicrobial resistance of bacterial species isolated from human acute routine bacterial infections and respiratory tract bacterial infections	Argentina		Antimicrobial resistance of all acute routine bacterial infections and respiratory tract pathogens (*S. pneumoniae, S. aureus, Haemophilus influenzae, Moraxella catarrhalis*) **(b)**	[211]	Traditional **(i)**
	To identify the adult population at risk for bacteremic pneumococcal pneumonia in the five-county region surrounding Philadelphia, Pennsylvania	USA	31 March 2002	Bacteremic pneumococcal pneumonia **(b)**	[212]	Traditional **(o)**
	To evaluate the prevalence and incidence of disease associated with pneumonia, implement to evaluate interventions (for example new vaccine strategies), and study pneumonia etiology	Rural Thailand	2002	Pneumonia **(o)**	[213, 214]	Traditional **(o)**
	To establish a hospital-based surveillance for bacterial meningitis in the state infectious reference hospital of Salvador, Brazil	Brazil	1996	Bacterial meningitis **(b)**	[215]	Traditional **(o)**
	To survey invasive group B streptococci frequency in neonates and young children at the Alberta region level	Canada		Invasive group B streptococci **(b)**	[216]	Traditional **(o)**
	To implement a population-based surveillance system in the Mirzapur region to collect information on invasive penumococcal disease, including incidence, seasonality, antibiotic-resistance patterns, and serotype composition in children presenting community-acquired invasive pneumococcal disease	Bangladesh	2004	Invasive pneumococcal disease **(b)**	[217]	Traditional **(o)**

		To survey beta-hemolytic streptococcal bacteremia in Pirkanmaa Health District, Finland	Finland	1995	Beta-hemolytic streptococcal bacteremia **(b)**	[218]	Traditional **(o)**
		To implement a national scedosporiosis surveillance system based on 49 laboratories across Australia to try to identify species-specific characteristics that can impact the management and outcome of scedosporiosis	Australia	2003	Scedosporiosis **(f)**	[219]	Traditional **(o)**
HARS	HIV/AIDS Reporting System	To collect data on people infected by HIV in the USA	USA	1981	HIV **(v)**	[220, 221]	Traditional **(i)**
		To implement a laboratory surveillance system to survey *C. difficile* infections in Manitoba, Canada	Canada	April 18, 2005	*Clostridium difficile* **(b)**	[222]	Traditional **(o)**
		To implement a surveillance system to evaluate the economic burden of diarrhea in children under 5 years of age in 15 villages in rural Zhengding, China	China	14 October 2004	Diarrhea **(o)**	[223]	Traditional **(o)**
TIBDN	The Toronto Invasive Bacterial Diseases Network	To evaluate the impact of invasive disease due to various pathogens in a defined population in Toronto, Canada, and to provide an infrastructure for further research	Canada	1 January 1995	*Neisseria meningitidis*, group A streptococcus, group B streptococcus, *Streptococcus pneumoniae* and influenza **(o)**	[224]	Traditional **(o)**
		To implement an enhance population-based surveillance system to monitor Hepatitis C Virus in 6 US state or county health departments (Colorado, Connecticut, Minnesota, New York, Oregon, and Pinellas County, Florida)	USA	2006	Hepatitis C Virus **(v)**	[225]	Traditional **(o)**
		To establish a population-based monitoring system to survey infectious endocarditis based on 29 clinical centers of the italian Friuli-Venezia Giulia region	Italy	2004	Infectious endocarditis **(o)**	[226]	Traditional **(o)**

To implement a population-based surveillance system to monitor severe rotavirus gastroenteritis and the different rotavirus strains circulating in children under 5 years in Karachi, Pakistan	Pakistan	2005	Severe rotavirus gastroenteritis **(v)**	[227]	Traditional **(o)**
To identify epidemiological trends and hospital mortality associated influenza acquired influenza pneumonia in two provinces in Thailand	Thailand	January 2005	Influenza **(v)**	[228]	Traditional **(o)**
To evaluate the possibility to implement a population-based surveillance for invasive pneumococcal disease in children under five years based on data from three pediatric referral hospitals (Indira Gandhi Institute of Child Health, Kempegowda Institute of Child Health and Vani Vilas Hospital)	India		Invasive pneumococcal disease **(b)**	[229]	Traditional **(o)**
To perform a population-based surveillance for *Candida* blood stream infections to study their species distributions and the antifungal resistance rate of the isolates collected in Connecticut and Baltimore City/Baltimore County	USA	1 October 1998	*Candida* spp. blood stream infections **(f)**	[230, 231]	Traditional **(o)**
To survey candidemia in the San Francisco Bay Area in California and the metropolitan Atlanta area to better understand their public health importance, their epidemiology and the incidence of antifungal drug resistance of isolates	USA	1 January 1992	*Candida* spp. blood stream infections **(f)**	[230, 232]	Traditional **(o)**

	To evaluate the incidence, seasonal variations, diversity of strains and clinical symptoms of influenza infections in children under 5 years in Dhaka, Bangladesh	Bangladesh		Influenza **(v)**	[233]	Traditional **(o)**
	To identify the risk factors associated with blood stream infections due to non-*albicans Candida* species compared to *Candida albicans* in Barcelona, Spain	Spain	1 January 2002	Non-*albicans Candida* species blood stream infections **(f)**	[234]	Traditional **(o)**
	To survey invasive pneumococcal disease on and around the Navajo Nation	USA	1988	Invasive pneumococcal disease **(b)**	[235]	Traditional **(o)**
	To implement a population-based surveillance able to exhaustively include all age groups and cases of patients with influenza-like illness	Guatemala	November 2007	Influenza **(v)**	[236]	Traditional **(o)**
	To monitor the number of children with possible invasive pneumococcal disease in Goiânia, Brazil	Brazil	2007	Invasive pneumococcal disease **(b)**	[237]	Traditional **(o)**
	To implement an active disease surveillance program to evaluate the current risk of human monkeypox infection in endemic places in the Democratic Republic of the Congo	Democratic Republic of the Congo	2005	Monkeypox **(v)**	[238]	Traditional **(o)**
	To evaluate the disease burden due to shigellosis in the Zhengding County	China	1st January 2002	*Shigella* spp. **(b)**	[239]	Traditional **(o)**
	To supplement information collected from the National Notifiable Disease Surveillance System on the virus and to buid a collection of hepatitis strains isolated in the Colorado, Connecticut, Minnesota, New York, New York City, and Oregon states	USA	2005	Hepatitis A virus **(v)**	[240]	Traditional **(o)**
Population-based surveillance for Haemophilus influenzae bacteremia	To better understand the epidemiology of *H. influenzae* bacteremia based on data collected from three different countries	Australia, Canada, and Denmark	2000	*Haemophilus influenzae* bacteremia **(b)**	[241]	Traditional **(i)**

		To implement a nationwide population-based surveillance for acute hepatitis C using data from Colorado, Alabama, Washington, Florida, Oregon and California	USA	1982	Hepatitis C Virus **(v)**	[242]	Traditional **(i)**
		To help Murmansk region local public health authorities to combat *M. tuberculosis*	Finland and Russia	1997	*M. tuberculosis* **(b)**	[243]	Traditional **(i)**
		To implement an active population-based surveillance for cryptococcosis	South Africa	1 March 2002	*Cryptococcus* species **(f)**	[244]	Traditional **(i)**
		To perform an invasive pneumococcal disease population-based surveillance in persons 65 years or older from the region of Tarragona to evaluate their impact on this population and the prevalence of infections caused by serotypes used in pneumococcal conjugate vaccines and the 23-valent pneumococcal polysaccharide vaccine	Spain	2002	Invasive pneumococcal disease **(b)**	[245]	Traditional **(o)**
		To perform a population-based surveillance to evaluate the impact of herpes simplex virus infections in neonates and establish prevention strategies in New York City	USA	April 2006	Neonatal herpes simplex virus infection **(v)**	[246]	Traditional **(o)**
		To determine the impact of influenza infections using the number of patients hospitalized with community-acquired influenza infections in 239 hospitals in 10 US states	USA	2005	Influenza **(v)**	[247]	Traditional **(o)**
ANSORP	Asian Network for Surveillance of Resistant Pathogens	To monitor antimicrobial resistance in selected Asian countries	13 Asian countries in 2006	1996	Antimicrobial resistance **(o)**	[248, 249]	Traditional **(i)**

		The surveillance was set up to determine the incidence of invasive pneumococcal disease due to serotypes used for vaccine or not and the incidence of radiological pneumonia, but equally to survey the antimicrobial resistance profiles of the pneumococcal strains collected during the surveillance and to determine their impact on child mortality in the Upper River Region, Gambia	Gambia	July 2007	Invasive pneumococcal disease **(b)**	[250]	Traditional **(o)**
		To capture all patients with clinical suspicion of invasive pneumococcal disease and/or pneumonia in all health sectors in 5 counties in San José, Costa Rica	Costa Rica	20 April 2007	Invasive pneumococcal disease and pneumonia **(b)**	[251]	Traditional **(o)**
Flu-VE	Influenza Vaccine Effectiveness Network	To validate laboratory-confirmed influenza and determine the effectiveness of annual influenza vaccine	USA		Influenza **(v)**	[252]	Traditional **(o)**
		To establish a population-based study in children in Bogota to evaluate the incidence of invasive pneumococcal diseases and pneumonia in this population, and to determine the serotypes of the *Streptococcus pneumoniae* strains isolated from this population and their antimicrobial susceptibility	Colombia	November 16 2006	Invasive pneumococcal disease and pneumonia in children between 28 days and 36 months of age **(b)**	[253]	Traditional **(o)**
		To set up a population-based surveillance to determine the impact of pneumococcal conjugate vaccine on the incidence of consolidated pneumonia hospitalization in young children	Uruguay	January 1, 2009	Invasive pneumococcal disease and pneumonia in young children **(b)**	[254]	Traditional **(o)**
AHDRA	Aggregate Hospitalizations and Deaths Reporting Activity reporting system	To collect aggregate data on hospitalizations and deaths due to influenza at the national level to determine progression and trends of influenza through the USA	USA	August 2009	Influenza **(v)**	[255]	Traditional **(i)**

		To set up a surveillance of cases of meningococcal disease in Asia to better understand its epidemiology in China (Nanning), South Korea (the Jeonbuk Province) and Vietnam (Hanoi)	China, South Korea and Vietnam	January 2001 in China, September 1999 in the South Korea, and March 2000 in Vietnam	Meningococcal disease **(b)**	[256]	Traditional **(i)**
SEIEVA	Sistema Epidemiologico Integrato dell'Epatite Virale Acuta	To establish a surveillance system able to favor investigation and control of acute viral hepatitis	Italy	1985	Acute viral hepatitis **(v)**	[257, 258]	Traditional **(i)**
FluSurv-NET	Influenza Hospitalization Surveillance Network	To survey influenza related hospitalizations in children (persons under 18 years) and adults in 16 selected states	USA		Influenza **(v)**	[259, 260]	Traditional **(i)**
The NCCD surveillance system	The National Center of Maternal and Child Health surveillance system	To evaluate the impact the combined Diphtheria-Tetanus-Pertussis-Hepatitis B-Hib conjugate vaccine on childhood bacterial meningitis using data from the National Center of Maternal and Child Health, Khan-Uul District Hospital, Songinkhairhan District Hospital, Sukhbaatar District Hospital, and Bayanzurkh District Hospital, Mongolia	Mongolia	February 2002	Bacterial meningitis **(b)**	[261, 262]	Traditional **(i)**
	CANDIPOP	To evaluate the impact of *Candida* spp. blood stream infections in five of the largest municipal areas of Spain (Barcelona, Bilbao, Madrid, Seville and Valencia), their susceptibility patterns, and to determine risk factors for mortality	Spain	May 2010	*Candida* spp. blood stream infections **(f)**	[263]	Traditional **(o)**
		To determine and compare the prevalence of intestinal parasites in children from three ethnic populations of the southern part of Israel	Southern Israel	January 2007	Intestinal parasites **(p)**	[264]	Traditional **(o)**
		To evaluate the incidence of severe respiratory virus infections in children under 5 years after their hospitalization in the Indian Haryana State	India		Respiratory viruses **(v)**	[265]	Traditional **(o)**

		To determine the impact of respiratory pathogens in the Damanhour district, Egypt	Egypt	May 2009	Respiratory pathogens **(o)**	[266]	Traditional **(o)**
		To implement an enhance population-based monitoring system for the surveillance and the study of Hepatitis B virus in 6 US sites (Colorado, Connecticut, Minnesota, New York, Oregon, and San Francisco)	USA		Hepatitis B virus **(v)**	[267]	Traditional **(o)**
NYC-HANES	New York City Health and Nutrition Examination Survey	To collect various health data including obesity, hypertension, infectious diseases, and environmental exposures in New York	USA		Various health data **(o)**	[268]	Traditional **(o)**

* : b, bacteria; f, fungi; v, viruses; o, other; p, parasite.

† : i, surveillance systems internationally or nationally recognized; o, other surveillance systems.

1 REFERENCES
2

3 1 Berrazeg M, Drissi M, Medjahed L, Rolain JM. Hierarchical clustering as a rapid tool for surveillance
4 of emerging antibiotic-resistance phenotypes in *Klebsiella pneumoniae* strains. *J Med Microbiol*
5 2013;62(Pt 6):864-74.

6 2 Schuchat A, Hilger T, Zell E, Farley MM, Reingold A, Harrison L, et al. Active bacterial core
7 surveillance of the emerging infections program network. *Emerg Infect Dis* 2001;7(1):92-9.

8 3 Link-Gelles R, Taylor T, Moore MR. Forecasting invasive pneumococcal disease trends after the
9 introduction of 13-valent pneumococcal conjugate vaccine in the United States, 2010-2020. *Vaccine*
10 2013;31(22):2572-7.

11 4 Enki DG, Noufaily A, Garthwaite PH, Andrews NJ, Charlett A, Lane C, et al. Automated
12 biosurveillance data from England and Wales, 1991-2011. *Emerg Infect Dis* 2013;19(1):35-42.

13 5 Lombardo JS, Burkom H, Pavlin J. ESSENCE II and the framework for evaluating syndromic
14 surveillance systems. *MMWR Morb Mortal Wkly Rep* 2004;53 Suppl:159-65.

15 6 Lombardo J, Burkom H, Elbert E, Magruder S, Lewis SH, Loschen W, et al. A systems overview of the
16 Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE
17 II). *J Urban Health* 2003;80(2 Suppl 1):i32-i42.

18 7 Centers for Disease Control and Prevention. Assessment of ESSENCE performance for influenza-like
19 illness surveillance after an influenza outbreak--U.S. Air Force Academy, Colorado, 2009. *MMWR*
20 *Morb Mortal Wkly Rep* 2011;60(13):406-9.

21 8 Carbonne A, Arnaud I, Maugat S, Marty N, Dumartin C, Bertrand X, et al. National multidrug-resistant
22 bacteria (MDRB) surveillance in France through the RAISIN network: a 9 year experience. *J*
23 *Antimicrob Chemother* 2013;68(4):954-9.

24 9 Sabbe M, Hue D, Antoine J, Dupont Y, Van Eldere J, Van Ranst M, et al. Influenza surveillance in
25 children: first experiences with the Belgian Paediatric Surveillance system 'PediSurv'. *Arch Public*
26 *Health* 2010;68(3):94-9.

27 10 Wang H, Xiao M, Chen SC, Kong F, Sun ZY, Liao K, et al. In vitro susceptibilities of yeast species to
28 fluconazole and voriconazole as determined by the 2010 National China Hospital Invasive Fungal
29 Surveillance Net (CHIF-NET) study. *J Clin Microbiol* 2012;50(12):3952-9.

30 11 Noor AM, Mohamed MB, Mugyenyi CK, Osman MA, Guessod HH, Kabaria CW, et al. Establishing
31 the extent of malaria transmission and challenges facing pre-elimination in the Republic of Djibouti.
32 *BMC Infect Dis* 2011;11:121.
33 12 Enserink R, Noel H, Friesema IH, de Jager CM, Kooistra-Smid AM, Kortbeek LM, et al. The KIzSS
34 network, a sentinel surveillance system for infectious diseases in day care centers: study protocol. *BMC*
35 *Infect Dis* 2012;12:259.
36 13 Cole MJ, Unemo M, Hoffmann S, Chisholm SA, Ison CA, van de Laar MJ. The European gonococcal
37 antimicrobial surveillance programme, 2009. *Euro Surveill* 2011;16(42).
38 14 Spiteri G, Cole M, Unemo M, Hoffmann S, Ison C, van de Laar M. The European Gonococcal
39 Antimicrobial Surveillance Programme (Euro-GASP)--a sentinel approach in the European Union
40 (EU)/European Economic Area (EEA). *Sex Transm Infect* 2013;89 Suppl 4:iv16-iv18.
41 15 Centers for Disease Control and Prevention. Incidence and trends of infection with pathogens
42 transmitted commonly through food - foodborne diseases active surveillance network, 10 U.S. sites,
43 1996-2012. *MMWR Morb Mortal Wkly Rep* 2013;62(15):283-7.
44 16 Kelly PM, Kotsimbos T, Reynolds A, Wood-Baker R, Hancox B, Brown SG, et al. FluCAN 2009:
45 initial results from sentinel surveillance for adult influenza and pneumonia in eight Australian hospitals.
46 *Med J Aust* 2011;194(4):169-74.
47 17 Macesic N, Kotsimbos TC, Kelly P, Cheng AC. Hospital-acquired influenza in an Australian sentinel
48 surveillance system. *Med J Aust* 2013;198(7):370-2.
49 18 Irons B, Morris-Glasgow V, Andrus JK, Castillo-Solorzano C, Dobbins JG. Lessons learned from
50 integrated surveillance of measles and rubella in the Caribbean. *J Infect Dis* 2011;204 Suppl 2:S622-
51 S626.
52 19 Tekin R, Dal T, Pirinccioglu H, Erisir OS. A 4-Year Surveillance of Device-associated Nosocomial
53 Infections in a Neonatal Intensive Care Unit. *Pediatr Neonatol* 2013;54:303-11.
54 20 Kanerva M, Mentula S, Virolainen-Julkunen A, Karki T, Mottonen T, Lyytikainen O. Reduction in
55 *Clostridium difficile* infections in Finland, 2008-2010. *J Hosp Infect* 2013;83(2):127-31.
56 21 Kotila SM, Virolainen A, Snellman M, Ibrahem S, Jalava J, Lyytikainen O. Incidence, case fatality and
57 genotypes causing *Clostridium difficile* infections, Finland, 2008. *Clin Microbiol Infect* 2011;17(6):888-
58 93.

59 22 Crump JA, Medalla FM, Joyce KW, Krueger AL, Hoekstra RM, Whichard JM, et al. Antimicrobial
60 resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National
61 Antimicrobial Resistance Monitoring System, 1996 to 2007. *Antimicrob Agents Chemother*
62 2011;55(3):1148-54.
63 23 Swaminathan B, Barrett TJ, Hunter SB, Tauxe RV. PulseNet: the molecular subtyping network for
64 foodborne bacterial disease surveillance, United States. *Emerg Infect Dis* 2001;7(3):382-9.
65 24 Yih WK, Deshpande S, Fuller C, Heisey-Grove D, Hsu J, Kruskal BA, et al. Evaluating real-time
66 syndromic surveillance signals from ambulatory care data in four states. *Public Health Rep*
67 2010;125(1):111-20.
68 25 Bolotin S, Pebody R, White PJ, McMenamin J, Perera L, Nguyen-Van-Tam JS, et al. A new sentinel
69 surveillance system for severe influenza in England shows a shift in age distribution of hospitalised
70 cases in the post-pandemic period. *PLoS One* 2012;7(1):e30279.
71 26 Lutwama JJ, Bakamutumaho B, Kayiwa JT, Chiiza R, Namagambo B, Katz MA, et al. Clinic- and
72 hospital-based sentinel influenza surveillance, Uganda 2007-2010. *J Infect Dis* 2012;206 Suppl 1:S87-
73 S93.
74 27 Mmbaga VM, Mwasekaga MJ, Mmbuji P, Matonya M, Mwafulango A, Moshi S, et al. Results from the
75 first 30 months of national sentinel surveillance for influenza in Tanzania, 2008-2010. *J Infect Dis*
76 2012;206 Suppl 1:S80-S86.
77 28 Dalhatu IT, Medina-Marino A, Olsen SJ, Hwang I, Gubio AB, Ekanem EE, et al. Influenza viruses in
78 Nigeria, 2009-2010: results from the first 17 months of a national influenza sentinel surveillance
79 system. *J Infect Dis* 2012;206 Suppl 1:S121-S128.
80 29 Wane J, Nyatanyi T, Nkunda R, Rukelibuga J, Ahmed Z, Biedron C, et al. 2009 pandemic influenza A
81 (H1N1) virus outbreak and response--Rwanda, October, 2009-May, 2010. *PLoS One* 2012;7(6):e31572.
82 30 Touch S, Grundy J, Hills S, Rani M, Samnang C, Khalakdina A, et al. The rationale for integrated
83 childhood meningoencephalitis surveillance: a case study from Cambodia. *Bull World Health Organ*
84 2009;87(4):320-4.
85 31 Ibarz-Pavon AB, Lemos AP, Gorla MC, Regueira M, Gabastou JM. Laboratory-based surveillance of
86 *Neisseria meningitidis* isolates from disease cases in Latin American and Caribbean countries, SIREVA
87 II 2006-2010. *PLoS One* 2012;7(8):e44102.

88 32 Nesbitt A, Ravel A, Murray R, McCormick R, Savelli C, Finley R, et al. Integrated surveillance and
89 potential sources of *Salmonella enteritidis* in human cases in Canada from 2003 to 2009. *Epidemiol*
90 *Infect* 2012;140(10):1757-72.
91 33 Sisto A, D'Ancona F, Meledandri M, Pantosti A, Rossolini GM, Raglio A, et al. Carbapenem non-
92 susceptible *Klebsiella pneumoniae* from Micronet network hospitals, Italy, 2009 to 2012. *Euro Surveill*
93 2012;17(33).
94 34 Parola P, Colson P, Dubourg G, Million M, Charrel R, Minodier P, et al. Letter to the editor. Group A
95 streptococcal infections during the seasonal influenza outbreak 2010/11 in South East England. *Euro*
96 *Surveill* 2011;16(11).
97 35 Bond KC, Macfarlane SB, Burke C, Ungchusak K, Wibulpolprasert S. The evolution and expansion of
98 regional disease surveillance networks and their role in mitigating the threat of infectious disease
99 outbreaks. *Emerg Health Threats J* 2013;6.
100 36 Harvey K, Esposito DH, Han P, Kozarsky P, Freedman DO, Plier DA, et al. Surveillance for travel-
101 related disease--GeoSentinel Surveillance System, United States, 1997-2011. *MMWR Surveill Summ*
102 2013;62:1-23.
103 37 Keddy KH, Sooka A, Crowther-Gibson P, Quan V, Meiring S, Cohen C, et al. Systemic shigellosis in
104 South Africa. *Clin Infect Dis* 2012;54(10):1448-54.
105 38 Centers for Disease Control and Prevention. Building laboratory capacity to support the global rotavirus
106 surveillance network. *MMWR Morb Mortal Wkly Rep* 2013;62(20):409-12.
107 39 Mejuto P, Boga JA, Junquera M, Torreblanca A, Leiva PS. Genotyping *Chlamydia trachomatis* strains
108 among men who have sex with men from a Northern Spain region: a cohort study. *BMJ Open*
109 2013;3(6).
110 40 Xu C, Havers F, Wang L, Chen T, Shi J, Wang D, et al. Monitoring avian influenza A(H7N9) virus
111 through national influenza-like illness surveillance, China. *Emerg Infect Dis* 2013;19(8):1289-92.
112 41 Ip DK, Liao Q, Wu P, Gao Z, Cao B, Feng L, et al. Detection of mild to moderate influenza A/H7N9
113 infection by China's national sentinel surveillance system for influenza-like illness: case series. *BMJ*
114 2013;346:f3693.
115 42 Russo G, Pasquali P, Nenova R, Alexandrov T, Ralchev S, Vullo V, et al. Reemergence of human and
116 animal brucellosis, bulgaria. *Emerg Infect Dis* 2009;15(2):314-6.

117 43 Choe YJ, Bae GR. Current status of measles in the Republic of Korea: an overview of case-based and
118 seroepidemiological surveillance scheme. *Korean J Pediatr* 2012;55(12):455-61.
119 44 Yoo HS, Park O, Park HK, Lee EG, Jeong EK, Lee JK, et al. Timeliness of national notifiable diseases
120 surveillance system in Korea: a cross-sectional study. *BMC Public Health* 2009;9:93.
121 45 Yoder JS, Gargano JW, Wallace RM, Beach MJ. Giardiasis surveillance--United States, 2009-2010.
122 *MMWR Surveill Summ* 2012;61(5):13-23.
123 46 Yoder JS, Wallace RM, Collier SA, Beach MJ, Hlavsa MC. Cryptosporidiosis surveillance--United
124 States, 2009-2010. *MMWR Surveill Summ* 2012;61(5):1-12.
125 47 Centers for Disease Control and Prevention. Evolution of varicella surveillance--selected states, 2000-
126 2010. *MMWR Morb Mortal Wkly Rep* 2012;61(32):609-12.
127 48 Lahra MM, Enriquez RP. Annual report of the Australian Meningococcal Surveillance Programme,
128 2011. *Commun Dis Intell Q Rep* 2012;36(3):E251-E262.
129 49 Christensen J, Stryhn H, Vallieres A, El AF. A scenario tree model for the Canadian Notifiable Avian
130 Influenza Surveillance System and its application to estimation of probability of freedom and sample
131 size determination. *Prev Vet Med* 2011;99(2-4):161-75.
132 50 Mlynarski D, Rabatsky-Ehr T, Petit S, Purviance K, Mshar PA, Begier EM, et al. Evaluation of Gram-
133 positive rod surveillance for early anthrax detection. *Epidemiol Infect* 2009;137(11):1623-30.
134 51 Begier EM, Barrett NL, Mshar PA, Johnson DG, Hadler JL. Gram-positive rod surveillance for early
135 anthrax detection. *Emerg Infect Dis* 2005;11(9):1483-6.
136 52 Hellinger WC, Crook JE, Heckman MG, Diehl NN, Shalev JA, Zubair AC, et al. Surgical site infection
137 after liver transplantation: risk factors and association with graft loss or death. *Transplantation*
138 2009;87(9):1387-93.
139 53 Edwards JR, Peterson KD, Andrus ML, Tolson JS, Goulding JS, Dudeck MA, et al. National
140 Healthcare Safety Network (NHSN) Report, data summary for 2006, issued June 2007. *Am J Infect*
141 *Control* 2007;35(5):290-301.
142 54 Leimann BC, Koifman RJ. Official information systems for cryptococcal meningitis, state of Rio de
143 Janeiro, Southeastern Brazil. *Rev Saude Publica* 2009;43(4):717-20.
144 55 Yoshida M, Matsui T, Ohkusa Y, Kobayashi J, Ohyama T, Sugawara T, et al. Seasonal influenza
145 surveillance using prescription data for anti-influenza medications. *Jpn J Infect Dis* 2009;62(3):233-5.

146 56 Apisarnthanarak A, Buppunharun W, Tiengrim S, Sawanpanyalert P, Aswapokee N. An overview of
147 antimicrobial susceptibility patterns for gram-negative bacteria from the National Antimicrobial
148 Resistance Surveillance Thailand (NARST) program from 2000 to 2005. *J Med Assoc Thai* 2009;92
149 Suppl 4:S91-S94.
150 57 U.S.Department of Agriculture. Food Emergency Response Network.[cited 2014 Oct 16]. Available
151 from: URL: http://www.usda.gov/oig/webdocs/24601-6-AT.pdf
152 58 Castillo-Salgado C. Trends and directions of global public health surveillance. *Epidemiol Rev*
153 2010;32(1):93-109.
154 59 World Health Organization. Weekly epidemiological record / Relevé épidémiologique hebdomadaire.
155 [cited 2014 Oct 16];7:61-64. Available from: URL: http://www.who.int/wer/2005/en/wer8007.pdf
156 60 McBryde ES, Brett J, Russo PL, Worth LJ, Bull AL, Richards MJ. Validation of statewide surveillance
157 system data on central line-associated bloodstream infection in intensive care units in Australia. *Infect*
158 *Control Hosp Epidemiol* 2009;30(11):1045-9.
159 61 Choi JY, Cheong HJ, Chun BC, Park HK, Lee HS, Lee H, et al. Hospital-based surveillance of
160 infection-related mortality in South Korea. *Public Health Rep* 2009;124(6):883-8.
161 62 Zielinski A. Evidence for excessive incidence of infectious diseases at mass gatherings with special
162 reference to sporting events. *Przegl Epidemiol* 2009;63(3):343-51.
163 63 Epidemiological Consultation Team. Surveillance system in place for the 2006 Winter Olympic Games,
164 Torino, Italy, 2006. *Euro Surveill* 2006;11(2):E060209.
165 64 Coulombier D. Surveillance for the World Cup, France, 1998. *Euro Surveill* 1998;2(24).
166 65 Schenkel K, Williams C, Eckmanns T, Poggensee G, Benzler J, Josephsen J, et al. Enhanced
167 surveillance of infectious diseases : the 2006 FIFA World Cup experience, Germany. *Euro Surveill*
168 2006;11(12):234-8.
169 66 Ronveaux O. Infectious disease surveillance in Belgium during the Euro 2000 football tournament.
170 *Euro Surveill* 2000;4(25).
171 67 Goncalves G, Castro L, Correia AM, Queiros L. Infectious diseases surveillance activities in the north
172 of Portugal, during the EURO 2004 football tournament. *Euro Surveill* 2005;10(4):86-9.
173 68 Roberts JA, Grant KA, Yoon YK, Polychronopoulos S, Ibrahim A, Thorley BR. Annual report of the
174 Australian National Poliovirus Reference Laboratory, 2008. *Commun Dis Intell Q Rep* 2009;33(3):291-
175 7.

176 69 Scotch M, Mattocks K, Rabinowitz P, Brandt C. A qualitative study of state-level zoonotic disease
177 surveillance in new England. *Zoonoses Public Health* 2011;58(2):131-9.
178 70 Mostashari F, Kulldorff M, Hartman JJ, Miller JR, Kulasekera V. Dead bird clusters as an early
179 warning system for West Nile virus activity. *Emerg Infect Dis* 2003;9(6):641-6.
180 71 Eidson M, Komar N, Sorhage F, Nelson R, Talbot T, Mostashari F, et al. Crow deaths as a sentinel
181 surveillance system for West Nile virus in the northeastern United States, 1999. *Emerg Infect Dis*
182 2001;7(4):615-20.
183 72 Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal
184 infections among organ transplant recipients: results of the Transplant-Associated Infection
185 Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;50(8):1101-11.
186 73 Leekha S, Sampathkumar P, Berry DJ, Thompson RL. Should national standards for reporting surgical
187 site infections distinguish between primary and revision orthopedic surgeries? *Infect Control Hosp*
188 *Epidemiol* 2010;31(5):503-8.
189 74 Lindsey NP, Staples JE, Lehman JA, Fischer M. Surveillance for human West Nile virus disease -
190 United States, 1999-2008. *MMWR Surveill Summ* 2010;59(2):1-17.
191 75 Ziegler U, Seidowski D, Globig A, Fereidouni SR, Ulrich RG, Groschup MH. Sentinel birds in wild-
192 bird resting sites as potential indicators for West Nile virus infections in Germany. *Arch Virol*
193 2010;155(6):965-9.
194 76 Jespersen S, Sogaard OS, Schonheyder HC, Fine MJ, Ostergaard L. Clinical features and predictors of
195 mortality in admitted patients with community- and hospital-acquired legionellosis: a Danish historical
196 cohort study. *BMC Infect Dis* 2010;10:124.
197 77 Poggensee G, Gilsdorf A, Buda S, Eckmanns T, Claus H, Altmann D, et al. The first wave of pandemic
198 influenza (H1N1) 2009 in Germany: from initiation to acceleration. *BMC Infect Dis* 2010;10:155.
199 78 Johnson AS, Hu X, Dean HD. Epidemiologic differences between native-born and foreign-born black
200 people diagnosed with HIV infection in 33 U.S. states, 2001-2007. *Public Health Rep* 2010;125 Suppl
201 4:61-9.
202 79 Hokit G, Alvey S, Geiger JM, Johnson GD, Rolston MG, Kinsey DT, et al. Using undergraduate
203 researchers to build vector and West Nile virus surveillance capacity. *Int J Environ Res Public Health*
204 2013;10(8):3192-202.

205 80 Grills NJ, Rowe SL, Gregory JE, Lester RA, Fielding JE. Evaluation of *Campylobacter* infection
206 surveillance in Victoria. *Commun Dis Intell Q Rep* 2010;34(2):110-5.
207 81 Woeltje KF, McMullen KM. Developing information technology for infection prevention surveillance.
208 *Crit Care Med* 2010;38(8 Suppl):S399-S404.
209 82 Kahn MG, Steib SA, Dunagan WC, Fraser VJ. Monitoring expert system performance using continuous
210 user feedback. *J Am Med Inform Assoc* 1996;3(3):216-23.
211 83 Bellini C, Petignat C, Francioli P, Wenger A, Bille J, Klopotov A, et al. Comparison of automated
212 strategies for surveillance of nosocomial bacteremia. *Infect Control Hosp Epidemiol* 2007;28(9):1030-
213 5.
214 84 Azar FE, Masoori N, Meidani Z, Paul L. Proposal for a modernized Iranian notifiable infectious
215 diseases surveillance system: comparison with USA and Australia. *East Mediterr Health J*
216 2010;16(7):771-7.
217 85 Vinnard C, Winston CA, Wileyto EP, Macgregor RR, Bisson GP. Isoniazid resistance and death in
218 patients with tuberculous meningitis: retrospective cohort study. *BMJ* 2010;341:c4451.
219 86 Kim WL, Anneducharme C, Bucher BJ. Development and Implementation of a Surveillance Network
220 System for Emerging Infectious Diseases in the Caribbean (ARICABA). *Online J Public Health Inform*
221 2011;3(2).
222 87 Fukuda MM, Klein TA, Kochel T, Quandelacy TM, Smith BL, Villinski J, et al. Malaria and other
223 vector-borne infection surveillance in the U.S. Department of Defense Armed Forces Health
224 Surveillance Center-Global Emerging Infections Surveillance program: review of 2009
225 accomplishments. *BMC Public Health* 2011;11 Suppl 2:S9.
226 88 van WK, Rodenburg GD, Veenhoven RH, Spanjaard L, van der Ende A, Sanders EA. Nontypeable
227 *Haemophilus influenzae* invasive disease in The Netherlands: a retrospective surveillance study 2001-
228 2008. *Clin Infect Dis* 2011;53(1):e1-e7.
229 89 Chen TC, Lin WR, Lu PL, Lin CY, Lin SH, Lin CJ, et al. Computer laboratory notification system via
230 short message service to reduce health care delays in management of tuberculosis in Taiwan. *Am J*
231 *Infect Control* 2011;39(5):426-30.
232 90 Balter S, Gupta LS, Lim S, Fu J, Perlman SE. Pandemic (H1N1) 2009 surveillance for severe illness
233 and response, New York, New York, USA, April-July 2009. *Emerg Infect Dis* 2010;16(8):1259-64.

234 91 Wadl M, Rieck T, Nachtnebel M, Greutelaers B, an der HM, Altmann D, et al. Enhanced surveillance
235 during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga
236 toxin/verotoxin-producing *Escherichia coli* in Germany, May to June 2011. *Euro Surveill* 2011;16(24).
237 92 Filia A, Tavilla A, Bella A, Magurano F, Ansaldi F, Chironna M, et al. Measles in Italy, July 2009 to
238 September 2010. *Euro Surveill* 2011;16(29).
239 93 Xiao YH, Giske CG, Wei ZQ, Shen P, Heddini A, Li LJ. Epidemiology and characteristics of
240 antimicrobial resistance in China. *Drug Resist Updat* 2011;14(4-5):236-50.
241 94 Hlavsa MC, Roberts VA, Anderson AR, Hill VR, Kahler AM, Orr M, et al. Surveillance for waterborne
242 disease outbreaks and other health events associated with recreational water --- United States, 2007--
243 2008. *MMWR Surveill Summ* 2011;60(12):1-32.
244 95 Inaida S, Yasui Y, Tada Y, Taniguchi K, Okabe N. Geographic trends and spread of the pandemic
245 (H1N1) 2009 in the metropolitan areas of Japan studied from the national sentinel data. *Jpn J Infect Dis*
246 2011;64(6):473-81.
247 96 Goutard FL, Paul M, Tavornpanich S, Houisse I, Chanachai K, Thanapongtharm W, et al. Optimizing
248 early detection of avian influenza H5N1 in backyard and free-range poultry production systems in
249 Thailand. *Prev Vet Med* 2012;105(3):223-34.
250 97 Mughini-Gras L, Graziani C, Biorci F, Pavan A, Magliola R, Ricci A, et al. Surveillance of acute
251 infectious gastroenteritis (1992-2009) and food-borne disease outbreaks (1996-2009) in Italy, with a
252 focus on the Piedmont and Lombardy regions. *Euro Surveill* 2012;17(8).
253 98 Carrieri MP, Salmaso S, Bella A, D'Ancona F, Demicheli V, Marongiu C, et al. Evaluation of the SIMI
254 system, an experimental computerised network for the surveillance of communicable diseases in Italy.
255 *Eur J Epidemiol* 2000;16(10):941-7.
256 99 Chu TP, Li CC, Wang L, Hsu LW, Eng HL, You HL, et al. A surveillance system to reduce
257 transmission of pandemic H1N1 (2009) influenza in a 2600-bed medical center. *PLoS One*
258 2012;7(3):e32731.
259 100 Krauss S, Pryor SP, Raven G, Danner A, Kayali G, Webby RJ, et al. Respiratory tract versus cloacal
260 sampling of migratory ducks for influenza A viruses: are both ends relevant? *Influenza Other Respir*
261 *Viruses* 2013;7(1):93-6.

262 101 Gray DJ, Thrift AP, Williams GM, Zheng F, Li YS, Guo J, et al. Five-year longitudinal assessment of
263 the downstream impact on schistosomiasis transmission following closure of the Three Gorges Dam.
264 *PLoS Negl Trop Dis* 2012;6(4):e1588.
265 102 Takla A, Velasco E, Benzler J. The FIFA Women's World Cup in Germany 2011--a practical example
266 for tailoring an event-specific enhanced infectious disease surveillance system. *BMC Public Health*
267 2012;12:576.
268 103 Severi E, Heinsbroek E, Watson C, Catchpole M. Infectious disease surveillance for the London 2012
269 Olympic and Paralympic Games. *Euro Surveill* 2012;17(31).
270 104 Xu XH, Ye Y, Hu LF, Jin YH, Jiang QQ, Li JB. Emergence of serogroup C meningococcal disease
271 associated with a high mortality rate in Hefei, China. *BMC Infect Dis* 2012;12:205.
272 105 Meijer A, Jonges M, van BP, Swaan CM, Osterhaus AD, Daniels RS, et al. Oseltamivir-resistant
273 influenza A(H1N1)pdm09 virus in Dutch travellers returning from Spain, August 2012. *Euro Surveill*
274 2012;17(36):20266.
275 106 Almirante B, Limon E, Freixas N, Gudiol F. Laboratory-based surveillance of hospital-acquired
276 catheter-related bloodstream infections in Catalonia. Results of the VINCat Program (2007-2010).
277 *Enferm Infecc Microbiol Clin* 2012;30 Suppl 3:13-9.
278 107 Bergamasco MD, Garnica M, Colombo AL, Nucci M. Epidemiology of candidemia in patients with
279 hematologic malignancies and solid tumours in Brazil. *Mycoses* 2013;56(3):256-63.
280 108 Kronenberg A, Hilty M, Endimiani A, Muhlemann K. Temporal trends of extended-spectrum
281 cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in in- and outpatients in
282 Switzerland, 2004 to 2011. *Euro Surveill* 2013;18(21).
283 109 Gould LH, Walsh KA, Vieira AR, Herman K, Williams IT, Hall AJ, et al. Surveillance for foodborne
284 disease outbreaks - United States, 1998-2008. *MMWR Surveill Summ* 2013;62(2):1-34.
285 110 Paterson BJ, Durrheim DN. The remarkable adaptability of syndromic surveillance to meet public
286 health needs. *J Epidemiol Glob Health* 2013;3(1):41-7.
287 111 John TJ, Samuel R, Balraj V, John R. Disease surveillance at district level: a model for developing
288 countries. *Lancet* 1998;352(9121):58-61.
289 112 Heffernan R, Mostashari F, Das D, Besculides M, Rodriguez C, Greenko J, et al. New York City
290 syndromic surveillance systems. *MMWR Morb Mortal Wkly Rep* 2004;53 Suppl:23-7.

291 113 Public Health Agency of Canada. Canadian Nosocomial Infection Surveillance / Surveillance for
292 Methicillin-resistant *Staphylococcus aureus* (MRSA) In CNISP health care facilities / Program 2010 -
293 MRSA Surveillance Protocol. [cited 2014 Oct 16];Available from: URL:
294 http://publications.gc.ca/collections/collection_2012/aspc-phac/HP40-61-2011-eng.pdf
295 114 Simor AE, Gilbert NL, Gravel D, Mulvey MR, Bryce E, Loeb M, et al. Methicillin-resistant
296 *Staphylococcus aureus* colonization or infection in Canada: National Surveillance and Changing
297 Epidemiology, 1995-2007. *Infect Control Hosp Epidemiol* 2010;31(4):348-56.
298 115 Meyer E, Schwab F, Schroeren-Boersch B, Gastmeier P. Dramatic increase of third-generation
299 cephalosporin-resistant *E. coli* in German intensive care units: secular trends in antibiotic drug use and
300 bacterial resistance, 2001 to 2008. *Crit Care* 2010;14(3):R113.
301 116 Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance
302 --- United States, 2006-2008. *MMWR Morb Mortal Wkly Rep* 2010;59(48):1577-80.
303 117 Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA. Enterovirus surveillance--United States,
304 1970-2005. *MMWR Surveill Summ* 2006;55(8):1-20.
305 118 Schanzer DL, Langley JM, Dummer T, Aziz S. The geographic synchrony of seasonal influenza: a
306 waves across Canada and the United States. *PLoS One* 2011;6(6):e21471.
307 119 Passerini R, Biffi R, Riggio D, Pozzi S, Sandri M. Laboratory-based management of microbiological
308 alerts: effects of an automated system on the surveillance and treatment of nosocomial infections in an
309 oncology hospital. *Ecancermedicalscience* 2009;3:137.
310 120 Sar B, Keo C, Leng C, Saman M, Min DC, Chan S, et al. Anti-tuberculosis drug resistance and HIV co-
311 infection in Phnom Penh, Cambodia. *Southeast Asian J Trop Med Public Health* 2009;40(1):104-7.
312 121 Florez C, Martin-Mazuelos E, Ruiz M, Cisneros JM, Herrero M, Garcia MV, et al. In vitro
313 susceptibilities of bloodstream isolates of *Candida* spp.: results from a multicenter active surveillance
314 program in Andalusia. *Enferm Infecc Microbiol Clin* 2009;27(9):518-22.
315 122 Mongkolrattanothai K, Aldag JC, Mankin P, Gray BM. Epidemiology of community-onset
316 *Staphylococcus aureus* infections in pediatric patients: an experience at a Children's Hospital in central
317 Illinois. *BMC Infect Dis* 2009;9:112.
318 123 Imohl M, Reinert RR, Ocklenburg C, van der Linden M. Epidemiology of invasive *Streptococcus*
319 *pyogenes* disease in Germany during 2003-2007. *FEMS Immunol Med Microbiol* 2010;58(3):389-96.

320 124 Imohl M, Reinert RR, van der Linden M. Adult invasive pneumococcal disease between 2003 and 2006
321 in North-Rhine Westphalia, Germany: serotype distribution before recommendation for general
322 pneumococcal conjugate vaccination for children <2 years of age. *Clin Microbiol Infect*
323 2009;15(11):1008-12.
324 125 Cohen D, Gargouri N, Ramlawi A, Abdeen Z, Belbesi A, Al HB, et al. A Middle East subregional
325 laboratory-based surveillance network on foodborne diseases established by Jordan, Israel, and the
326 Palestinian Authority. *Epidemiol Infect* 2010;138(10):1443-8.
327 126 Playford EG, Nimmo GR, Tilse M, Sorrell TC. Increasing incidence of candidaemia: long-term
328 epidemiological trends, Queensland, Australia, 1999-2008. *J Hosp Infect* 2010;76(1):46-51.
329 127 Lee KS, Lai YL, Lo S, Barkham T, Aw P, Ooi PL, et al. Dengue virus surveillance for early warning,
330 Singapore. *Emerg Infect Dis* 2010;16(5):847-9.
331 128 Karki R, Bhatta DR, Malla S, Dumre SP. Cholera incidence among patients with diarrhea visiting
332 National Public Health Laboratory, Nepal. *Jpn J Infect Dis* 2010;63(3):185-7.
333 129 Villena I, Ancelle T, Delmas C, Garcia P, Brezin AP, Thulliez P, et al. Congenital toxoplasmosis in
334 France in 2007: first results from a national surveillance system. *Euro Surveill* 2010;15(25).
335 130 Melo-Cristino J, Santos L, Silva-Costa C, Friaes A, Pinho MD, Ramirez M. The Viriato study: update
336 on antimicrobial resistance of microbial pathogens responsible for community-acquired respiratory tract
337 infections in Portugal. *Paediatr Drugs* 2010;12 Suppl 1:11-7.
338 131 ANOFEL Cryptosporidium National Network. Laboratory-based surveillance for *Cryptosporidium* in
339 France, 2006-2009. *Euro Surveill* 2010;15(33):19642.
340 132 Cisterna R, Ezpeleta G, Telleria O. Nationwide sentinel surveillance of bloodstream *Candida* infections
341 in 40 tertiary care hospitals in Spain. *J Clin Microbiol* 2010;48(11):4200-6.
342 133 Dong BQ, Yang J, Wang XY, Gong J, von SL, Wang ML, et al. Trends and disease burden of enteric
343 fever in Guangxi province, China, 1994-2004. *Bull World Health Organ* 2010;88(9):689-96.
344 134 Singh RI, Xess I, Mathur P, Behera B, Gupta B, Misra MC. Epidemiology of candidaemia in critically
345 ill trauma patients: experiences of a level I trauma centre in North India. *J Med Microbiol* 2011;60(Pt
346 3):342-8.
347 135 Picazo J, Ruiz-Contreras J, Hernandez B, Sanz F, Gutierrez A, Cercenado E, et al. Clonal and clinical
348 profile of *Streptococcus pneumoniae* serotype 19A causing pediatric invasive infections: a 2-year
349 (2007-2009) laboratory-based surveillance in Madrid. *Vaccine* 2011;29(9):1770-6.

350 136 Cordoba S, Vivot W, Bosco-Borgeat ME, Taverna C, Szusz W, Murisengo O, et al. Species distribution
351 and susceptibility profile of yeasts isolated from blood cultures: results of a multicenter active
352 laboratory-based surveillance study in Argentina. *Rev Argent Microbiol* 2011;43(3):176-85.
353 137 Steindl G, Liu YL, Schmid D, Orendi U, Kormann-Klement A, Heuberger S. Epidemiology of invasive
354 meningococcal disease in Austria 2010. *Wien Klin Wochenschr* 2011;123 Suppl 1:10-4.
355 138 Hanquet G, Ducoffre G, Vergison A, Neels P, Sabbe M, Van DP, et al. Impact of rotavirus vaccination
356 on laboratory confirmed cases in Belgium. *Vaccine* 2011;29(29-30):4698-703.
357 139 Gomes T, Vinhas SA, Reis-Santos B, Palaci M, Peres RL, Aguiar PP, et al. Extrapulmonary
358 tuberculosis: *Mycobacterium tuberculosis* strains and host risk factors in a large urban setting in Brazil.
359 *PLoS One* 2013;8(10):e74517.
360 140 Deng Y, Wang Y, Wang J, Jing H, Yu C, Wang H, et al. Laboratory-based surveillance of extensively
361 drug-resistant tuberculosis, China. *Emerg Infect Dis* 2011;17(3):495-7.
362 141 Lambertsen LM, Ingels H, Schonheyder HC, Hoffmann S. Nationwide laboratory-based surveillance of
363 invasive beta-haemolytic streptococci in Denmark from 2005 to 2011. *Clin Microbiol Infect*
364 2014;20(4):O216-O223.
365 142 Polkowska A, Harjunpaa A, Toikkanen S, Lappalainen M, Vuento R, Vuorinen T, et al. Increased
366 incidence of *Mycoplasma pneumoniae* infection in Finland, 2010-2011. *Euro Surveill* 2012;17(5).
367 143 Heraud-Bousquet V, Lot F, Esvan M, Cazein F, Laurent C, Warszawski J, et al. A three-source capture-
368 recapture estimate of the number of new HIV diagnoses in children in France from 2003-2006 with
369 multiple imputation of a variable of heterogeneous catchability. *BMC Infect Dis* 2012;12:251.
370 144 Schweickert B, Noll I, Feig M, Claus H, Krause G, Velasco E, et al. MRSA-surveillance in Germany:
371 data from the Antibiotic Resistance Surveillance System (ARS) and the mandatory surveillance of
372 MRSA in blood. *Eur J Clin Microbiol Infect Dis* 2012;31(8):1855-65.
373 145 Imohl M, van der Linden M, Reinert RR, Ritter K. Invasive group A streptococcal disease and
374 association with varicella in Germany, 1996-2009. *FEMS Immunol Med Microbiol* 2011;62(1):101-9.
375 146 Ansaldi F, Canepa P, Bassetti M, Zancolli M, Molinari MP, Talamini A, et al. Sequential outbreaks of
376 multidrug-resistant *Acinetobacter baumannii* in intensive care units of a tertiary referral hospital in
377 Italy: combined molecular approach for epidemiological investigation. *J Hosp Infect* 2011;79(2):134-
378 40.

379 147 Mammina C, Parisi A, Guaita A, Aleo A, Bonura C, Nastasi A, et al. Enhanced surveillance of invasive
380 listeriosis in the Lombardy region, Italy, in the years 2006-2010 reveals major clones and an increase in
381 serotype 1/2a. *BMC Infect Dis* 2013;13:152.
382 148 Meiring ST, Quan VC, Cohen C, Dawood H, Karstaedt AS, McCarthy KM, et al. A comparison of
383 cases of paediatric-onset and adult-onset cryptococcosis detected through population-based
384 surveillance, 2005-2007. *AIDS* 2012;26(18):2307-14.
385 149 Madzivhandila M, Adrian PV, Cutland CL, Kuwanda L, Schrag SJ, Madhi SA. Serotype distribution
386 and invasive potential of group B streptococcus isolates causing disease in infants and colonizing
387 maternal-newborn dyads. *PLoS One* 2011;6(3):e17861.
388 150 Wiseman CA, Schaaf HS, Cotton MF, Gie RP, Jennings T, Whitelaw A, et al. Bacteriologically
389 confirmed tuberculosis in HIV-infected infants: disease spectrum and survival. *Int J Tuberc Lung Dis*
390 2011;15(6):770-5.
391 151 Mathieu E, Dorkenoo A, Otogbe FK, Budge PJ, Sodahlon YK. A laboratory-based surveillance system
392 for *Wuchereria bancrofti* in Togo: a practical model for resource-poor settings. *Am J Trop Med Hyg*
393 2011;84(6):988-93.
394 152 Mukhi SN, May-Hadford J, Plitt S, Preiksaitis J, Lee B. DIAL: A Platform for real-time Laboratory
395 Surveillance. *Online J Public Health Inform* 2010;2(3).
396 153 Kirk CJ, Shult PA. Developing laboratory networks: a practical guide and application. *Public Health*
397 *Rep* 2010;125 Suppl 2:102-9.
398 154 Kelly TA, O'Lorcain P, Moran J, Garvey P, McKeown P, Connell J, et al. Underreporting of viral
399 encephalitis and viral meningitis, Ireland, 2005-2008. *Emerg Infect Dis* 2013;19(9):1428-36.
400 155 Helferty M, Rotondo JL, Martin I, Desai S. The epidemiology of invasive pneumococcal disease in the
401 Canadian North from 1999 to 2010. *Int J Circumpolar Health* 2013;72.
402 156 Seo YB, Song JY, Cheong HJ, Cho YD, Wie SH, Jeong HW, et al. Hospital-based influenza morbidity
403 and mortality surveillance system for influenza-like illnesses: a comparison with national influenza
404 surveillance systems. *Influenza Other Respir Viruses* 2014;8(1):17-20.
405 157 Seo YB, Song JY, Cheong HJ, Cho YD, Wie SH, Jeong HW, et al. Hospital-based influenza morbidity
406 and mortality surveillance system for influenza-like illnesses: a comparison with national influenza
407 surveillance systems. *Influenza Other Respir Viruses* 2014;8(1):17-20.

408 158 Chen E, Chen Y, Fu L, Chen Z, Gong Z, Mao H, et al. Human infection with avian influenza A(H7N9)
409 virus re-emerges in China in winter 2013. *Euro Surveill* 2013;18(43).
410 159 Biggs HM, Hertz JT, Munishi OM, Galloway RL, Marks F, Saganda W, et al. Estimating leptospirosis
411 incidence using hospital-based surveillance and a population-based health care utilization survey in
412 Tanzania. *PLoS Negl Trop Dis* 2013;7(12):e2589.
413 160 Silva JC, Shah SC, Rumoro DP, Bayram JD, Hallock MM, Gibbs GS, et al. Comparing the accuracy of
414 syndrome surveillance systems in detecting influenza-like illness: GUARDIAN vs. RODS vs. electronic
415 medical record reports. *Artif Intell Med* 2013;59(3):169-74.
416 161 Li Y, Yin Z, Shao Z, Li M, Liang X, Sandhu HS, et al. Population-based surveillance for bacterial
417 meningitis in China, September 2006-December 2009. *Emerg Infect Dis* 2014;20(1):61-9.
418 162 Chaintoutis SC, Dovas CI, Papanastassopoulou M, Gewehr S, Danis K, Beck C, et al. Evaluation of a
419 West Nile virus surveillance and early warning system in Greece, based on domestic pigeons. *Comp*
420 *Immunol Microbiol Infect Dis* 2014;37(2):131-41.
421 163 Yukich JO, Butts J, Miles M, Berhane Y, Nahusenay H, Malone JL, et al. A description of malaria
422 sentinel surveillance: a case study in Oromia Regional State, Ethiopia. *Malar J* 2014;13:88.
423 164 Navarro TA, Dias JG, Quinten C, Hruba F, Busana MC, Lopalco PL, et al. European enhanced
424 surveillance of invasive pneumococcal disease in 2010: data from 26 European countries in the post-
425 heptavalent conjugate vaccine era. *Vaccine* 2014;32(29):3644-50.
426 165 Coman A, Maftei DN, Chereches RM, Zavrotchi E, Bria P, Dragnea C, et al. Avian influenza
427 surveillance in the danube delta using sentinel geese and ducks. *Influenza Res Treat* 2014;2014:965749.
428 166 Leal J, Gregson DB, Ross T, Flemons WW, Church DL, Laupland KB. Development of a novel
429 electronic surveillance system for monitoring of bloodstream infections. *Infect Control Hosp Epidemiol*
430 2010;31(7):740-7.
431 167 Billington EO, Phang SH, Gregson DB, Pitout JD, Ross T, Church DL, et al. Incidence, Risk Factors,
432 and Outcomes for Enterococcus spp. Blood Stream Infections: A Population-Based Study. *Int J Infect*
433 *Dis* 2014;26:76-82.
434 168 Patel M, Romero-Steiner S, Broderick MP, Thomas CG, Plikaytis BD, Schmidt DS, et al. Persistence of
435 serogroup C antibody responses following quadrivalent meningococcal conjugate vaccination in United
436 States military personnel. *Vaccine* 2014;32(30):3805-9.

437 169 Rubertone MV, Brundage JF. The Defense Medical Surveillance System and the Department of
438 Defense serum repository: glimpses of the future of public health surveillance. *Am J Public Health*
439 2002;92(12):1900-4.
440 170 Skoczynska A, Wasko I, Kuch A, Kadlubowski M, Golebiewska A, Forys M, et al. A decade of
441 invasive meningococcal disease surveillance in Poland. *PLoS One* 2013;8(8):e71943.
442 171 El MA, Pozo F, Ben Hadj Kacem MA, Ledesma J, Cuevas MT, Casas I, et al. Virological Surveillance
443 of Influenza Viruses during the 2008-09, 2009-10 and 2010-11 Seasons in Tunisia. *PLoS One*
444 2013;8(9):e74064.
445 172 Zhao H, Green H, Lackenby A, Donati M, Ellis J, Thompson C, et al. A new laboratory-based
446 surveillance system (Respiratory DataMart System) for influenza and other respiratory viruses in
447 England: results and experience from 2009 to 2012. *Euro Surveill* 2014;19(3).
448 173 Hsieh YH, Kuo MJ, Hsieh TC, Lee HC. Underreporting and underestimation of gonorrhea cases in the
449 Taiwan National Gonorrhea Notifiable Disease System in the Tainan region: evaluation by a pilot
450 physician-based sentinel surveillance on Neisseria gonorrhoeae infection. *Int J Infect Dis*
451 2009;13(6):e413-e419.
452 174 Meerhoff TJ, Mosnier A, Schellevis F, Paget WJ. Progress in the surveillance of respiratory syncytial
453 virus (RSV) in Europe: 2001-2008. *Euro Surveill* 2009;14(40).
454 175 Mardy S, Ly S, Heng S, Vong S, Huch C, Nora C, et al. Influenza activity in Cambodia during 2006-
455 2008. *BMC Infect Dis* 2009;9:168.
456 176 Savulescu C, Valenciano M, de MS, Larrauri A. Estimating the influenza vaccine effectiveness in
457 elderly on a yearly basis using the Spanish influenza surveillance network--pilot case-control studies
458 using different control groups, 2008-2009 season, Spain. *Vaccine* 2010;28(16):2903-7.
459 177 Larrauri A, de MS. Characterisation of swabbing for virological analysis in the Spanish Influenza
460 Sentinel Surveillance System during four influenza seasons in the period 2002-2006. *Euro Surveill*
461 2007;12(5):E5-E6.
462 178 Athanasiou M, Lytras T, Spala G, Triantafyllou E, Gkolfinopoulou K, Theocharopoulos G, et al. Fatal
463 cases associated with pandemic influenza A (H1N1) reported in Greece. *PLoS Curr* 2010;2:RRN1194.
464 179 Guy RJ, Kong F, Goller J, Franklin N, Bergeri I, Dimech W, et al. A new national *Chlamydia* Sentinel
465 Surveillance System in Australia: evaluation of the first stage of implementation. *Commun Dis Intell Q*
466 *Rep* 2010;34(3):319-28.

467 180 Ma E, Lam T, Chan KC, Wong C, Chuang SK. Changing epidemiology of hand, foot, and mouth
468 disease in Hong Kong, 2001-2009. *Jpn J Infect Dis* 2010;63(6):422-6.
469 181 Skowronski DM, Janjua NZ, De SG, Hottes TS, Dickinson JA, Crowcroft N, et al. Effectiveness of
470 AS03 adjuvanted pandemic H1N1 vaccine: case-control evaluation based on sentinel surveillance
471 system in Canada, autumn 2009. *BMJ* 2011;342:c7297.
472 182 Khuwaja S, Mgbere O, Awosika-Olumo A, Momin F, Ngo K. Using sentinel surveillance system to
473 monitor seasonal and novel H1N1 influenza infection in Houston, Texas: outcome analysis of 2008-
474 2009 flu season. *J Community Health* 2011;36(5):857-63.
475 183 Lee VJ, Yap J, Cook AR, Tan CH, Loh JP, Koh WH, et al. A clinical diagnostic model for predicting
476 influenza among young adult military personnel with febrile respiratory illness in Singapore. *PLoS One*
477 2011;6(3):e17468.
478 184 Centers for Disease Control and Prevention. Establishment of a viral hepatitis surveillance system--
479 Pakistan, 2009-2011. *MMWR Morb Mortal Wkly Rep* 2011;60(40):1385-90.
480 185 Mirand A, Henquell C, Archimbaud C, Ughetto S, Antona D, Bailly JL, et al. Outbreak of hand, foot
481 and mouth disease/herpangina associated with coxsackievirus A6 and A10 infections in 2010, France: a
482 large citywide, prospective observational study. *Clin Microbiol Infect* 2012;18(5):E110-E118.
483 186 Gamage CD, Amarasekera J, Palihawadana P, Samaraweera S, Mendis D, Janakan N, et al. Analysis of
484 hospital-based sentinel surveillance data on leptospirosis in Sri Lanka, 2005-2008. *Jpn J Infect Dis*
485 2012;65(2):157-61.
486 187 Liao M, Nie X, Pan R, Wang C, Ruan S, Zhang C, et al. Consistently low prevalence of syphilis among
487 female sex workers in Jinan, China: findings from two consecutive respondent driven sampling surveys.
488 *PLoS One* 2012;7(4):e34085.
489 188 Yang ZF, Zhan YQ, Chen RC, Zhou R, Wang YT, Luo Y, et al. A prospective comparison of the
490 epidemiological and clinical characteristics of pandemic (H1N1) 2009 influenza A virus and seasonal
491 influenza A viruses in Guangzhou, South China in 2009. *Jpn J Infect Dis* 2012;65(3):208-14.
492 189 Lin W, Chen S, Seguy N, Chen Z, Sabin K, Calleja JG, et al. Is the HIV sentinel surveillance system
493 adequate in China? Findings from an evaluation of the national HIV sentinel surveillance system.
494 *Western Pac Surveill Response J* 2012;3(4):76-85.

495 190 Wang L, Wang L, Norris JL, Li DM, Guo W, Ding ZW, et al. HIV prevalence and influencing factors
496 analysis of sentinel surveillance among men who have sex with men in China, 2. *Chin Med J (Engl)*
497 2012;125(11):1857-61.
498 191 Theo A, Liwewe M, Ndumba I, Mupila Z, Tambatamba B, Mutemba C, et al. Influenza surveillance in
499 Zambia, 2008-2009. *J Infect Dis* 2012;206 Suppl 1:S173-S177.
500 192 Petrovic V, Reguljev Z, Nedeljkovic J, Ristic M. Seroprevalence study in Vojvodina (Serbia) following
501 2009 pandemic influenza A(H1N1)V. *Srp Arh Celok Lek* 2012;140(11-12):751-5.
502 193 Zhou L, Situ S, Huang T, Hu S, Wang X, Zhu X, et al. Direct medical cost of influenza-related
503 hospitalizations among severe acute respiratory infections cases in three provinces in China. *PLoS One*
504 2013;8(5):e63788.
505 194 Kebede S, Conteh IN, Steffen CA, Vandemaele K, Wurie I, Alemu W, et al. Establishing a national
506 influenza sentinel surveillance system in a limited resource setting, experience of Sierra Leone. *Health*
507 *Res Policy Syst* 2013;11:22.
508 195 de LF, Dia A, Plumet S, Decam C, Leparc G, I, Deparis X. Dengue surveillance in the French armed
509 forces: a dengue sentinel surveillance system in countries without efficient local epidemiological
510 surveillance. *J Travel Med* 2013;20(4):259-61.
511 196 Choe YJ, Kim JW, Park YJ, Jung C, Bae GR. Burden of pertussis is underestimated in South Korea: a
512 result from an active sentinel surveillance system. *Jpn J Infect Dis* 2014;67(3):230-2.
513 197 Katz MA, Muthoka P, Emukule GO, Kalani R, Njuguna H, Waiboci LW, et al. Results from the first six
514 years of national sentinel surveillance for influenza in Kenya, July 2007-June 2013. *PLoS One*
515 2014;9(6):e98615.
516 198 Mullner P, Spencer SE, Wilson DJ, Jones G, Noble AD, Midwinter AC, et al. Assigning the source of
517 human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. *Infect*
518 *Genet Evol* 2009;9(6):1311-9.
519 199 Angelini P, Tamba M, Finarelli AC, Bellini R, Albieri A, Bonilauri P, et al. West Nile virus circulation
520 in Emilia-Romagna, Italy: the integrated surveillance system 2009. *Euro Surveill* 2010;15(16).
521 200 Gallegos D, Olea A, Sotomayor V, Gonzalez C, Munoz JC, Ramos M, et al. Rubella outbreaks
522 following virus importations: the experience of Chile. *J Infect Dis* 2011;204 Suppl 2:S669-S674.
523 201 Sitaula S, Awasthi GR, Thapa JB, Joshi KP, Ramaiya A. Measles outbreak among unvaccinated
524 children in Bajura. *JNMA J Nepal Med Assoc* 2010;50(180):273-6.

525 202 David JM, Sanders P, Bemrah N, Granier SA, Denis M, Weill FX, et al. Attribution of the French
526 human Salmonellosis cases to the main food-sources according to the type of surveillance data. *Prev*
527 *Vet Med* 2013;110(1):12-27.
528 203 Hyde TB, Andrus JK, Dietz VJ, Andrus JK, Hyde TB, Lee CE, et al. Critical issues in implementing a
529 national integrated all-vaccine preventable disease surveillance system. *Vaccine* 2013;31 Suppl 3:C94-
530 C98.
531 204 Centers for Disease Control and Prevention. Pediatric bacterial meningitis surveillance - African region,
532 2002--2008. *MMWR Morb Mortal Wkly Rep* 2009;58(18):493-7.
533 205 Hernandez-Avila JE, Rodriguez MH, Santos-Luna R, Sanchez-Castaneda V, Roman-Perez S, Rios-
534 Salgado VH, et al. Nation-wide, web-based, geographic information system for the integrated
535 surveillance and control of dengue fever in Mexico. *PLoS One* 2013;8(8):e70231.
536 206 Rizzo C, Salcuni P, Nicoletti L, Ciufolini MG, Russo F, Masala R, et al. Epidemiological surveillance
537 of West Nile neuroinvasive diseases in Italy, 2008 to 2011. *Euro Surveill* 2012;17(20).
538 207 Barzon L, Pacenti M, Franchin E, Pagni S, Lavezzo E, Squarzon L, et al. Large human outbreak of
539 West Nile virus infection in north-eastern Italy in 2012. *Viruses* 2013;5(11):2825-39.
540 208 Marka A, Diamantidis A, Papa A, Valiakos G, Chaintoutis SC, Doukas D, et al. West Nile virus state of
541 the art report of MALWEST Project. *Int J Environ Res Public Health* 2013;10(12):6534-610.
542 209 Johnson RP, Holtslander B, Mazzocco A, Roche S, Thomas JL, Pollari F, et al. Detection and
543 prevalence of verotoxin-producing *Escherichia coli* O157 and non-O157 serotypes in a Canadian
544 watershed. *Appl Environ Microbiol* 2014;80(7):2166-75.
545 210 Acar JF, Moulin G. Integrating animal health surveillance and food safety: the issue of antimicrobial
546 resistance. *Rev Sci Tech* 2013;32(2):383-92.
547 211 Silley P, Simjee S, Schwarz S. Surveillance and monitoring of antimicrobial resistance and antibiotic
548 consumption in humans and animals. *Rev Sci Tech* 2012;31(1):105-20.
549 212 Flory JH, Joffe M, Fishman NO, Edelstein PH, Metlay JP. Socioeconomic risk factors for bacteraemic
550 pneumococcal pneumonia in adults. *Epidemiol Infect* 2009;137(5):717-26.
551 213 Baggett HC, Chittaganpitch M, Thamthitiwat S, Prapasiri P, Naorat S, Sawatwong P, et al. Incidence
552 and epidemiology of hospitalized influenza cases in rural Thailand during the influenza A
553 (H1N1)pdm09 pandemic, 2009-2010. *PLoS One* 2012;7(11):e48609.

554 214 Jordan HT, Prapasiri P, Areerat P, Anand S, Clague B, Sutthirattana S, et al. A comparison of
555 population-based pneumonia surveillance and health-seeking behavior in two provinces in rural
556 Thailand. *Int J Infect Dis* 2009;13(3):355-61.
557 215 Santos MS, Ribeiro GS, Oliveira TQ, Santos RC, Gouveia E, Salgado K, et al. Burden of group A
558 streptococcal meningitis in Salvador, Brazil: report of 11 years of population-based surveillance. *Int J*
559 *Infect Dis* 2009;13(4):456-61.
560 216 Manning SD, Springman AC, Lehotzky E, Lewis MA, Whittam TS, Davies HD. Multilocus sequence
561 types associated with neonatal group B streptococcal sepsis and meningitis in Canada. *J Clin Microbiol*
562 2009;47(4):1143-8.
563 217 Arifeen SE, Saha SK, Rahman S, Rahman KM, Rahman SM, Bari S, et al. Invasive pneumococcal
564 disease among children in rural Bangladesh: results from a population-based surveillance. *Clin Infect*
565 *Dis* 2009;48 Suppl 2:S103-S113.
566 218 Rantala S, Vuopio-Varkila J, Vuento R, Huhtala H, Syrjanen J. Predictors of mortality in beta-
567 hemolytic streptococcal bacteremia: a population-based study. *J Infect* 2009;58(4):266-72.
568 219 Heath CH, Slavin MA, Sorrell TC, Handke R, Harun A, Phillips M, et al. Population-based surveillance
569 for scedosporiosis in Australia: epidemiology, disease manifestations and emergence of *Scedosporium*
570 *aurantiacum* infection. *Clin Microbiol Infect* 2009;15(7):689-93.
571 220 Torian LV, Eavey JJ, Punsalang AP, Pirillo RE, Forgione LA, Kent SA, et al. HIV type 2 in New York
572 City, 2000-2008. *Clin Infect Dis* 2010;51(11):1334-42.
573 221 Harrison KM, Song R, Zhang X. Life expectancy after HIV diagnosis based on national HIV
574 surveillance data from 25 states, United States. *J Acquir Immune Defic Syndr* 2010;53(1):124-30.
575 222 Lambert PJ, Dyck M, Thompson LH, Hammond GW. Population-based surveillance of *Clostridium*
576 *difficile* infection in Manitoba, Canada, by using interim surveillance definitions. *Infect Control Hosp*
577 *Epidemiol* 2009;30(10):945-51.
578 223 Wang XY, Riewpaiboon A, von SL, Chen XB, Kilgore PE, Ma JC, et al. Potential cost-effectiveness of
579 a rotavirus immunization program in rural China. *Clin Infect Dis* 2009;49(8):1202-10.
580 224 Plevneshi A, Svoboda T, Armstrong I, Tyrrell GJ, Miranda A, Green K, et al. Population-based
581 surveillance for invasive pneumococcal disease in homeless adults in Toronto. *PLoS One*
582 2009;4(9):e7255.

583 225 Klevens RM, Miller J, Vonderwahl C, Speers S, Alelis K, Sweet K, et al. Population-based surveillance
584 for hepatitis C virus, United States, 2006-2007. *Emerg Infect Dis* 2009;15(9):1499-502.
585 226 Scudeller L, Badano L, Crapis M, Pagotto A, Viale P. Population-based surveillance of infectious
586 endocarditis in an Italian region. *Arch Intern Med* 2009;169(18):1720-3.
587 227 Qazi R, Sultana S, Sundar S, Warraich H, un-Nisa T, Rais A, et al. Population-based surveillance for
588 severe rotavirus gastroenteritis in children in Karachi, Pakistan. *Vaccine* 2009;27 Suppl 5:F25-F30.
589 228 Simmerman JM, Chittaganpitch M, Levy J, Chantra S, Maloney S, Uyeki T, et al. Incidence,
590 seasonality and mortality associated with influenza pneumonia in Thailand: 2005-2008. *PLoS One*
591 2009;4(11):e7776.
592 229 Shah AS, Nisarga R, Ravi Kumar KL, Hubler R, Herrera G, Kilgore PE. Establishment of population-
593 based surveillance for invasive pneumococcal disease in Bangalore, India. *Indian J Med Sci*
594 2009;63(11):498-507.
595 230 Lott TJ, Frade JP, Lockhart SR. Multilocus sequence type analysis reveals both clonality and
596 recombination in populations of *Candida glabrata* bloodstream isolates from U.S. surveillance studies.
597 *Eukaryot Cell* 2010;9(4):619-25.
598 231 Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, et al. Incidence of
599 bloodstream infections due to Candida species and in vitro susceptibilities of isolates collected from
600 1998 to 2000 in a population-based active surveillance program. *J Clin Microbiol* 2004;42(4):1519-27.
601 232 Kao AS, Brandt ME, Pruitt WR, Conn LA, Perkins BA, Stephens DS, et al. The epidemiology of
602 candidemia in two United States cities: results of a population-based active surveillance. *Clin Infect Dis*
603 1999;29(5):1164-70.
604 233 Brooks WA, Goswami D, Rahman M, Nahar K, Fry AM, Balish A, et al. Influenza is a major
605 contributor to childhood pneumonia in a tropical developing country. *Pediatr Infect Dis J*
606 2010;29(3):216-21.
607 234 Rodriguez D, Almirante B, Cuenca-Estrella M, Rodriguez-Tudela JL, Mensa J, Ayats J, et al. Predictors
608 of candidaemia caused by non-albicans *Candida* species: results of a population-based surveillance in
609 Barcelona, Spain. *Clin Microbiol Infect* 2010;16(11):1676-82.
610 235 Weatherholtz R, Millar EV, Moulton LH, Reid R, Rudolph K, Santosham M, et al. Invasive
611 pneumococcal disease a decade after pneumococcal conjugate vaccine use in an American Indian
612 population at high risk for disease. *Clin Infect Dis* 2010;50(9):1238-46.

613 236 Reyes L, Arvelo W, Estevez A, Gray J, Moir JC, Gordillo B, et al. Population-based surveillance for
614 2009 pandemic influenza A (H1N1) virus in Guatemala, 2009. *Influenza Other Respir Viruses*
615 2010;4(3):129-40.
616 237 Andrade AL, Franco CM, Lamaro-Cardoso J, Andre MC, Oliveira LL, Kipnis A, et al. Non-typeable
617 *Streptococcus pneumoniae* carriage isolates genetically similar to invasive and carriage isolates
618 expressing capsular type 14 in Brazilian infants. *J Infect* 2010;61(4):314-22.
619 238 Rimoin AW, Mulembakani PM, Johnston SC, Lloyd Smith JO, Kisalu NK, Kinkela TL, et al. Major
620 increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the
621 Democratic Republic of Congo. *Proc Natl Acad Sci U S A* 2010;107(37):16262-7.
622 239 Wang SM, Ma JC, Hao ZY, Zhang ZY, Mason C, Sethabutr O, et al. Surveillance of shigellosis by real-
623 time PCR suggests underestimation of shigellosis prevalence by culture-based methods in a population
624 of rural China. *J Infect* 2010;61(6):471-5.
625 240 Klevens RM, Miller JT, Iqbal K, Thomas A, Rizzo EM, Hanson H, et al. The evolving epidemiology of
626 hepatitis a in the United States: incidence and molecular epidemiology from population-based
627 surveillance, 2005-2007. *Arch Intern Med* 2010;170(20):1811-8.
628 241 Laupland KB, Schonheyder HC, Ostergaard C, Knudsen JD, Valiquette L, Galbraith J, et al.
629 Epidemiology of *Haemophilus influenzae* bacteremia: a multi-national population-based assessment. *J*
630 *Infect* 2011;62(2):142-8.
631 242 Williams IT, Bell BP, Kuhnert W, Alter MJ. Incidence and transmission patterns of acute hepatitis C in
632 the United States, 1982-2006. *Arch Intern Med* 2011;171(3):242-8.
633 243 Makinen J, Marjamaki M, Haanpera-Heikkinen M, Marttila H, Endourova LB, Presnova SE, et al.
634 Extremely high prevalence of multidrug resistant tuberculosis in Murmansk, Russia: a population-based
635 study. *Eur J Clin Microbiol Infect Dis* 2011;30(9):1119-26.
636 244 Govender NP, Patel J, van WM, Chiller TM, Lockhart SR. Trends in antifungal drug susceptibility of
637 *Cryptococcus neoformans* isolates obtained through population-based surveillance in South Africa in
638 2002-2003 and 2007-2008. *Antimicrob Agents Chemother* 2011;55(6):2606-11.
639 245 Vila-Corcoles A, Ochoa-Gondar O, Gomez-Bertomeu F, Raga-Luria X. Invasive pneumococcal disease
640 in Catalonian elderly people, 2002-2009: serotype coverage for different anti-pneumococcal vaccine
641 formulations at the beginning of the new conjugate vaccines era. *Vaccine* 2011;29(43):7430-4.

642 246 Handel S, Klingler EJ, Washburn K, Blank S, Schillinger JA. Population-based surveillance for
643 neonatal herpes in New York City, April 2006-September 2010. *Sex Transm Dis* 2011;38(8):705-11.
644 247 Doshi S, Kamimoto L, Finelli L, Perez A, Reingold A, Gershman K, et al. Description of antiviral
645 treatment among adults hospitalized with influenza before and during the 2009 pandemic: United
646 States, 2005-2009. *J Infect Dis* 2011;204(12):1848-56.
647 248 Kim SH, Song JH, Chung DR, Thamlikitkul V, Yang Y, Wang H, et al. Changing trends in
648 antimicrobial resistance and serotypes of *Streptococcus pneumoniae* isolates in Asian countries: an
649 Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Antimicrob Agents*
650 *Chemother* 2012;56(3):1418-26.
651 249 Song JH, Lee NY, Ichiyama S, Yoshida R, Hirakata Y, Fu W, et al. Spread of drug-resistant
652 *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens
653 (ANSORP) Study. *Clin Infect Dis* 1999;28(6):1206-11.
654 250 Mackenzie GA, Plumb ID, Sambou S, Saha D, Uchendu U, Akinsola B, et al. Monitoring the
655 introduction of pneumococcal conjugate vaccines into West Africa: design and implementation of a
656 population-based surveillance system. *PLoS Med* 2012;9(1):e1001161.
657 251 Arguedas A, Abdelnour A, Soley C, Jimenez E, Jimenez AL, Ramcharran D, et al. Prospective
658 epidemiologic surveillance of invasive pneumococcal disease and pneumonia in children in San Jose,
659 Costa Rica. *Vaccine* 2012;30(13):2342-8.
660 252 Jules A, Grijalva CG, Zhu Y, Talbot KH, Williams JV, Dupont WD, et al. Estimating age-specific
661 influenza-related hospitalization rates during the pandemic (H1N1) 2009 in Davidson Co, TN. *Influenza*
662 *Other Respir Viruses* 2012;6(3):e63-e71.
663 253 Benavides JA, Ovalle OO, Salvador GR, Gray S, Isaacman D, Rodgers GL. Population-based
664 surveillance for invasive pneumococcal disease and pneumonia in infants and young children in
665 Bogota, Colombia. *Vaccine* 2012;30(40):5886-92.
666 254 Hortal M, Estevan M, Laurani H, Iraola I, Meny M. Hospitalized children with pneumonia in Uruguay:
667 pre and post introduction of 7 and 13-valent pneumococcal conjugated vaccines into the National
668 Immunization Program. *Vaccine* 2012;30(33):4934-8.
669 255 Stoto MA. The effectiveness of U.S. public health surveillance systems for situational awareness during
670 the 2009 H1N1 pandemic: a retrospective analysis. *PLoS One* 2012;7(8):e40984.

671 256 Kim SA, Kim DW, Dong BQ, Kim JS, Anh DD, Kilgore PE. An expanded age range for
672 meningococcal meningitis: molecular diagnostic evidence from population-based surveillance in Asia.
673 *BMC Infect Dis* 2012;12:310.
674 257 Spada E, Mele A, Mariano A, Zuccaro O, Tosti ME. Risk factors for and incidence of acute hepatitis C
675 after the achievement of blood supply safety in Italy: results from the national surveillance system. *J*
676 *Med Virol* 2013;85(3):433-40.
677 258 Mele A, Rosmini F, Zampieri A, Gill ON. Integrated epidemiological system for acute viral hepatitis in
678 Italy (SEIEVA): description and preliminary results. *Eur J Epidemiol* 1986;2(4):300-4.
679 259 Jhung MA, D'Mello T, Perez A, Aragon D, Bennett NM, Cooper T, et al. Hospital-onset influenza
680 hospitalizations--United States, 2010-2011. *Am J Infect Control* 2014;42(1):7-11.
681 260 Fleming-Dutra KE, Taylor T, Link-Gelles R, Garg S, Jhung MA, Finelli L, et al. Effect of the 2009
682 influenza A(H1N1) pandemic on invasive pneumococcal pneumonia. *J Infect Dis* 2013;207(7):1135-43.
683 261 Scott S, Altanseseg D, Sodbayer D, Nymadawa P, Bulgan D, Mendsaikhan J, et al. Impact of
684 *Haemophilus influenzae* Type b conjugate vaccine in Mongolia: prospective population-based
685 surveillance, 2002-2010. *J Pediatr* 2013;163(1 Suppl):S8-S11.
686 262 Mendsaikhan J, Watt JP, Mansoor O, Suvdmaa N, Edmond K, Litt DJ, et al. Childhood bacterial
687 meningitis in Ulaanbaatar, Mongolia, 2002-2004. *Clin Infect Dis* 2009;48 Suppl 2:S141-S146.
688 263 Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza R, et al.
689 Epidemiology and predictive factors for early and late mortality in *Candida* bloodstream infections: a
690 population-based surveillance in Spain. *Clin Microbiol Infect* 2014;20(4):O245-O254.
691 264 Ben-Shimol S, Sagi O, Greenberg D. Differences in prevalence of parasites in stool samples between
692 three distinct ethnic pediatric populations in southern Israel, 2007-2011. *Parasitol Int* 2014;63(2):456-
693 62.
694 265 Broor S, Dawood FS, Pandey BG, Saha S, Gupta V, Krishnan A, et al. Rates of respiratory virus-
695 associated hospitalization in children aged <5 years in rural northern India. *J Infect* 2014;68(3):281-9.
696 266 Rowlinson E, Dueger E, Taylor T, Mansour A, Van BC, Abukela M, et al. Incidence and clinical
697 features of respiratory syncytial virus infections in a population-based surveillance site in the Nile Delta
698 Region. *J Infect Dis* 2013;208 Suppl 3:S189-S196.

699 267 Liu SJ, Iqbal K, Shallow S, Speers S, Rizzo E, Gerard K, et al. Characterization of Chronic Hepatitis B
700 Cases Among Foreign-Born Persons in Six Population-Based Surveillance Sites, United States 2001-
701 2010. *J Immigr Minor Health* 2014;12:7-12.
702 268 Gwynn RC, Garg RK, Kerker BD, Frieden TR, Thorpe LE. Contributions of a local health examination
703 survey to the surveillance of chronic and infectious diseases in New York City. *Am J Public Health*
704 2009;99(1):152-9.

Conclusions et perspectives de la Partie I

Ce premier travail de revue de la littérature nous a permis d’identifier que le champ de la surveillance épidémiologique est complexe et varié, comme en témoigne la variété de stratégies et de catégories de données utilisables pour la réaliser. Nous avons également pu observer la place centrale des laboratoires de microbiologie clinique dans la surveillance des maladies infectieuses de part la quantité de données produites directement disponibles pour les systèmes de surveillance épidémiologique dans le monde. Ceci a par ailleurs été confirmé par la revue de la littérature disponible dans PubMed entre Janvier 2009 et mi-Juin 2014 sur le sujet (Figure 2, extraite de l’article 1 «Traditional and Syndromic Surveillance of Infectious Diseases and Pathogens»).

Figure 2. Les systèmes de surveillance des maladies infectieuses développés et bien décris dans le monde entre Janvier 2009 et mi-Juin 2014.

Nous avons également observé que la plupart des systèmes de surveillance décrits dans ce laps de temps étaient des systèmes de surveillance nationaux ou internationaux spécifiques orientés majoritairement vers la surveillance des pathogènes viraux et bactériens (Figure 3, extraite de l'article 1 «Traditional and Syndromic Surveillance of Infectious Diseases and Pathogens»).

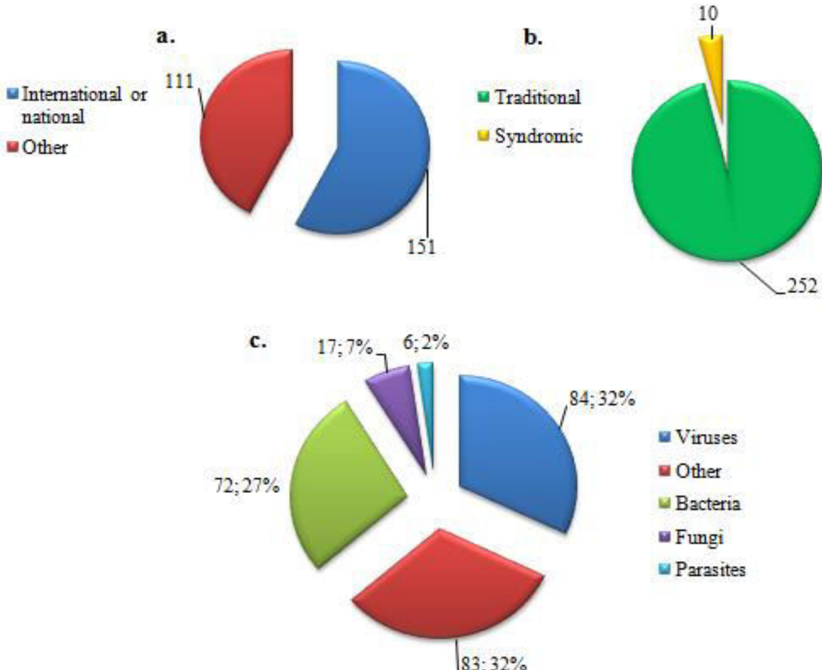


Figure 3. Caractéristiques des 262 systèmes de surveillance étudiés entre Janvier 2009 et le 13 Juin 2014. Le panel A présente le nombre de systèmes de surveillance nationaux et internationaux, ou autre. Le panel B présente le nombre de systèmes de

surveillance spécifiques (ou traditionnels) ou syndromiques. Le panel C présente une classification des systèmes de surveillance selon ce qu'ils surveillent.

En conclusion, ce travail nous a permis d'observer que la surveillance des maladies infectieuses et des pathogènes est un champ évoluant rapidement dans lequel de plus en plus de ressources et de pays sont engagés. L'avènement des big data, notamment par le biais d'internet, ouvre la voie à des changements plus profonds encore dans ce domaine. Ainsi, bien que la surveillance spécifique ait par le passé montré son efficacité avec l'éradication totale de la variole en 1978 et de la peste bovine en 2011, les progrès technologiques constants dans le domaine de la surveillance épidémiologique, notamment avec le développement de système de surveillance syndromique basé sur les données acquises par les internautes dans internet, laissent à penser que le futur de la surveillance des maladies infectieuses réside dans un système mondial de surveillance le plus exhaustif possible associant une surveillance spécifique et une surveillance syndromique en temps réel.

Partie II: Développement de nouveaux outils informatiques pour la surveillance en temps réel de phénomènes anormaux basés sur les données de microbiologie clinique du laboratoire de la Timone.

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<u>Article 2:</u> EPIMIC: a simple homemade computer program for real-time EPIdemiological surveillance and alert based on MICrobiological data. **Under review in PlosOne** **(IF: 3.234)**.

<u>Article 3:</u> Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry. **Published in JCM** **(IF: 3.993)**.

<u>Article 4:</u> A real-time microbiology laboratory surveillance system implemented for the detection of abnormal events and emerging infections, Marseille, France. **Published in Emerg Infect Dis** **(IF: 6.751)**.

<u>Article 5:</u> Description of a human infection due to *Sporolactobacillus laevolacticus*, Marseille, France. **Accepted in Emerg Infect Dis** **(IF: 6.751)**.

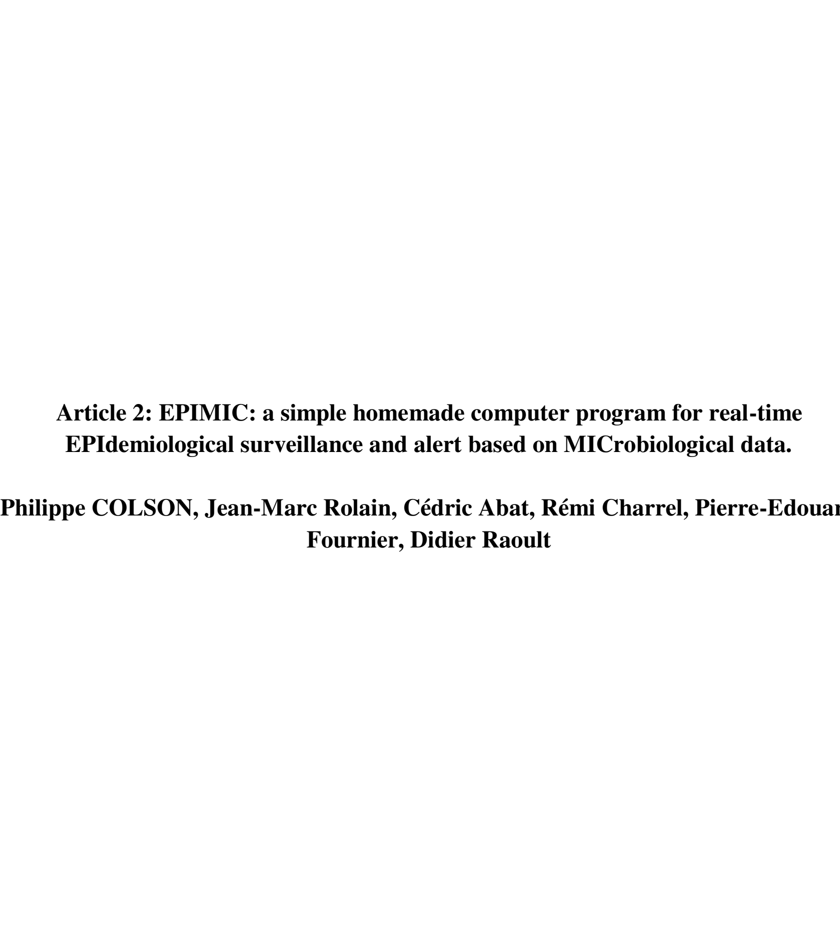
<u>Article 6:</u> Report of the first *Vagococcus lutrae* human infection, Marseille, France. **Under review in Emerg Infect Dis** **(IF: 6.751)**.

Avant propos

Sur la base du système de surveillance syndromique EPIMIC développé et mis en activité dès 2002 au sein de notre laboratoire de microbiologie clinique (article 2), nous avons décidé de mettre en place deux nouveaux outils informatiques pour la surveillance en temps réel des phénomènes épidémiques anormaux des maladies infectieuses sur la base de données produites par notre

laboratoire. Pour ce faire, nous avons dans un premier temps créé deux bases de données historiques (dont l'une est en partie publiée dans l'article 3) sur la base des identifications bactériennes et des résultats d'antibiogrammes bactériens réalisés en routine par notre laboratoire. Une fois constituées, ces dernières nous ont permis de développer deux nouveaux systèmes de surveillance, le premier nommé BALYSES (the BActerial real-time LaboratorY-based SurveillancE System) permettant la surveillance des 672 espèces bactériennes isolées au moins une fois dans notre laboratoire depuis 2002, et le second appelé MARSS (the Marseille Antibiotic Resistance Surveillance System) permettant de surveiller pour 15 espèces bactériennes d'intérêt clinique majeur les 54 phénotypes de résistance au β-lactamines plus 5 phénotypes "alarmes" correspondant à des résistances critiques sur le plan clinique.

La première base de données constituée nous a également permis d'identifier, après mise à jour avec les données du laboratoire de la Timone jusqu'en Mai 2015 et nettoyage, d'observer qu'une part importante des espèces bactériennes isolées en routine au moins une fois dans notre laboratoire n'avait jamais donné lieu à de "case report" publié. Nous avons donc entrepris, en nous basant sur les dossiers cliniques des patients infectés, de publier un certain nombre de cas d'infection par des espèces bactériennes rares (articles 5 et 6).



Article 2: EPIMIC: a simple homemade computer program for real-time EPIdemiological surveillance and alert based on MICrobiological data.

Philippe COLSON, Jean-Marc Rolain, Cédric Abat, Rémi Charrel, Pierre-Edouard Fournier, Didier Raoult

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ABSTRACT

Background & Aims: Infectious diseases (IDs) are major causes of morbidity and mortality and their surveillance is critical. In 2002, we implemented a simple and versatile homemade tool, named EPIMIC, for the real-time systematic automated surveillance of IDs at Marseille university hospitals, based on the data from our clinical microbiology laboratory, including clinical samples, tests and diagnoses.

Methods: This tool was specifically designed to detect abnormal events as IDs are rarely predicted and modeled. EPIMIC operates using Microsof Excel software and require no particular computer skills or resources. An abnormal event corresponds to an increase above, or a decrease below threshold values calculated based on the mean of historical data plus or minus 2 standard deviations, respectively.

Results: Between November 2002 and October 2013 (11 years), 293 items were surveyed weekly, including 38 clinical samples, 86 pathogens, 79 diagnosis tests, and 39 antibacterial resistance patterns. The mean duration of surveillance was 7.6 years (range, 1 month-10.9 years). A total of 108,427 Microsoft Excel file cells were filled with counts of clinical samples, and 110,017 cells were filled with counts of diagnoses. A total of 1,390,689 samples were analyzed. Among them, 172,180 were found to be positive for a pathogen. EPIMIC generated a mean number of 0.5 alerts/week on abnormal events.

Conclusions: EPIMIC proved to be efficient for real-time automated laboratory-

47 based surveillance and alerting at our university hospital clinical microbiology

48 laboratory-scale. It is freely downloadable from the following URL:

49 http://www.mediterranee-infection.com/article.php?larub=157&titre=bulletin-

50 epidemiologique.

51

TEXT

INTRODUCTION

Infectious diseases (IDs) are major causes of morbidity and mortality worldwide [1–4]. Their surveillance is therefore critical to improve their diagnosis, prevention, clinical management and treatment [5–7]. Many surveillance systems target a limited number of IDs, and not throughout the whole year, but rather only for periods during which, classically, they are known to occur. These are important drawbacks that considerably limit the capability to detect “abnormal” events, including infections with unusual/unexpected features, and emerging/re-emerging diseases. Indeed, IDs are rarely predicted or modeled, as emphasized during recent epidemics [8–10]. In addition, the majority of ID surveillance tools do not lead to real-time detection and alert, preventing the rapid prioritization of public health threats and impairing the timely implementation of control strategies [7].

One of the surveillance approaches for IDs is syndromic surveillance that is based on non-specific markers available before confirmed diagnosis and that can be early and powerful surrogate indicators [11,12]. Several examples during past decades have highlighted that syndromic surveillance and warning systems could reveal major infections and outbreaks. These included the detection in 1976 of an unexplained mortality rise in Philadelphia, USA, which led to the discovery of *Legionella pneumophila* as a causative agent of pneumonia in humans [13]; or the

73 warning concerning a few "abnormal" prescriptions of pentamidine in San
74 Francisco in 1981, which attracted attention on the first cases of acquired
75 immunodeficiency syndromes [14].
76 Clinical microbiology laboratories represent a wealth of information, including
77 data usable for syndromic surveillance consisting of numbers and types of clinical
78 samples collected and of tests prescribed by clinicians, in addition to diagnoses
79 [15,16]. In 2002, back from a stay in the USA for a mission on bioterrorism [17],
80 one of the authors (DR) decided to implement a simple and versatile tool for the
81 real-time systematic surveillance of IDs at Marseille university hospitals, based on
82 data from our clinical microbiology laboratory. This homemade system surveys
83 clinical samples, tests and diagnoses. We describe here its principle, skills and
84 limits.
85
86 **MATERIALS AND METHODS**
87 **Laboratory setting**
88 Between November 2002 and October 2013, we prospectively monitored
89 the weekly numbers of clinical samples received, tests performed and positive and
90 negative diagnoses obtained at the clinical microbiology laboratory of university
91 hospitals of Marseille. Marseille, the second largest French city, encompasses
92 ≈850,000 inhabitants (http://www.insee.fr; 2010). Its university hospitals comprise
93 4,000 beds and cumulate yearly ≈800,000 consultations and 790,000 days of

94 hospitalisation [18]. Our clinical microbiology laboratory performs annually
95 approximately 145,000 serological tests, 200,000 PCR and 220,000 cultures.

96 **Computer program operation**

97 Our homemade computer tool, named EPIMIC (for EPIdemiological
98 surveillance and alert based on MICrobiological data) was implemented using the
99 Microsof Excel software. Data were split into several files accessible via a shared
100 drive to any PC computer in the laboratory (Online appendix: S1 Table). Each of
101 these files encompasses a dozen parameters, fitting the capability of our standard
102 PC computers to open and run them; parameters from a given file are related to a
103 given clinical syndrome or technological platform. These files can be accessed
104 through hyperlinks from a Microsoft PowerPoint slide that presents our entire
105 surveillance activity, which is split into various infectious syndromes or
106 technological platforms (Figure 1). Laboratory data are collected weekly, either
107 manually or automatically from our laboratory computer system (LCS), then
108 entered manually into the different Microsoft excel files by a medical biology
109 resident. All entered data are anonymous. Basically, triplets of numbers are
110 entered, corresponding to weekly counts of clinical samples handled, tests
111 performed and positive diagnoses; proportions of positive diagnoses are
112 automatically calculated. Each of the newly-entered weekly counts grows the set of
113 historical data. Mean, standard deviation (SD) and mean±2 SD are automatically
114 calculated for these historical data, and counts from the week are automatically

115 compared to values corresponding to mean±2 SD. For instance, Figure 2A shows
116 the numbers of respiratory samples tested and found positive for viral pathogens,
117 and Figure 3A shows the numbers of stool samples tested and found positive for
118 rotavirus. Finally, all counts are automatically plotted on graphs showing weekly,
119 monthly and yearly numbers of events as shown in Figures 2B and 3B-D.

120 **Detection of abnormal events**

121 An abnormal event corresponds to an increase above, or a decrease below
122 threshold values calculated based on the mean of historical data plus or minus 2
123 SD, respectively. While entering weekly data, conditional formatting from the
124 Excel software automatically changes the font to red if numbers are above the
125 mean+2 SD and to blue if they are below the mean-2 SD. These automatically
126 calculated thresholds can be replaced by others chosen by the user. Computed data
127 are presented at least once a week during medical meetings, and interpreted by
128 microbiologists. Confirmed alerts are reported to clinicians, and, depending on
129 their nature, to a committee for the control of nosocomial infections, to the health
130 regional agency, or to other French sanitary surveillance institutions.

131 **Statistical analysis of antibiotic-resistance surveillance data**

132 Statistical analyses were performed for the surveillance of antibiotic-
133 resistance patterns using linear models and the LOESS regression (locally
134 weighted polynomial regression) curve to determine whether the proportion of
135 isolated bacterial strains presenting a particular resistance profile monitored by

136 EPIMIC significantly increased or decreased throughout the surveillance period.

137 The tests were two-sided, p-values < 0.05 being considered as statistically

138 significant, and were performed using the R program (Auckland, New-Zealand).

139 **Search for other laboratory-based surveillance systems for IDs**

140 In order to compare EPIMIC to other laboratory-based surveillance

141 systems, we identified these other systems through a PubMed (URL:

142 http://www.ncbi.nlm.nih.gov/pubmed) search over the last 5 years using

143 "laboratory-based surveillance" as keyword.

144 **Availability of the computer tool**

145 A ready-to-use EPIMIC file can be freely downloaded from the University

146 Hospital Institute (IHU) “Méditerranée Infection” foundation website (URL:

147 http://www.mediterranee-infection.com/article.php?larub=157&titre=bulletin-

148 epidemiologique).

149

150 **RESULTS**

151 **EPIMIC datasets**

152 Between November 2002 and October 2013 (11 years), 293 items were

153 surveyed weekly, including 38 clinical samples, 86 pathogens, 79 diagnosis tests,

154 and 39 antibacterial resistance patterns. The mean duration of surveillance was 7.6

155 years (range, 1 month-10.9 years). A total of 108,427 Microsoft Excel file cells

156 were filled with counts of clinical samples, and 110,017 cells were filled with

157 counts of diagnoses. EPIMIC was used at our laboratory by 15 senior biologists
158 and ≈30 residents in medical biology per year; the training period for each new
159 person was approximately 10 min.Table 1 summarizes numbers of samples and
160 diagnoses during the study period for the seven major types of samples surveyed
161 by EPIMIC and the major pathogens diagnosed. A total of 1,390,689 samples were
162 analyzed. Among them, 172,180 were found to be positive for a pathogen.
163 Pathogens that were the most frequently isolated from respiratory samples, urine,
164 stools, blood cultures and cerebrospinal fluids were respiratory syncytial virus
165 (4,939 positive diagnoses), *E. coli* (42,874 strains), rotavirus (2,464 positive
166 diagnoses), coagulase-negative *Staphylococcus* (7,006 strains) and enteroviruses
167 (922 positive diagnoses), respectively. At a one-year scale, in 2011, the most
168 numerous clinical samples received at our laboratory were urine samples (57,088),
169 followed by blood cultures (50,948 samples) and respiratory samples (24,338)
170 (Figure 4). *Escherichia coli* (5,137 strains) and coagulase-negative *Staphylococcus*
171 (1,130 strains) were the bacterial species most frequently isolated from urine and
172 blood, respectively. Regarding respiratory samples, influenza virus, respiratory
173 syncytial virus and metapneumovirus were the most frequently diagnosed viruses,
174 representing 960, 927 and 340 cases, respectively, *Pseudomonas aeruginosa* was
175 the most frequently isolated bacterium, representing 69 cases. In addition, the
176 pathogens by far the most frequently diagnosed from cerebrospinal fluids were
177 enteroviruses (in 110 cases).

178 **Examples of EPIMIC skills and use**

179 EPIMIC was efficient at detecting abnormal events for various IDs. The
180 surveillance of clinical samples was found to be more precocious in some cases
181 than that of diagnoses to detect a rise in some IDs, as the number of clinical
182 samples exceeded the warning threshold before the number of diagnoses. This was
183 the case for respiratory samples during fall 2009 and 2010, for cerebrospinal fluids
184 during summer 2007, or for stool samples during fall 2007, summer 2011 and
185 winters 2013 and 2014. EPIMIC allowed known seasonalities to be visualized, for
186 instance for influenza virus, respiratory syncytial virus or rotavirus infections
187 (Figure 2). Nonetheless, the period and intensity of these infections were found to
188 substantially vary according to the year, and unexpected features were observed,
189 including a dramatically low incidence of influenza virus infections in 2010,
190 following the 2009 H1N1 pandemic [8,9]. Moreover, EPIMIC revealed the
191 seasonality of invasive bacteremia caused by *Klebsiella pneumoniae* during the
192 summer months, which was previously unknown [19]. Another example of
193 abnormal event detected by EPIMIC was an increase in autochthonous hepatitis E
194 diagnosed during early 2011 [20]. This rise was associated with consumption of
195 raw pig liver sausage (traditionally eaten around Christmas and New Year eve in
196 Corsica) in 55% of cases, and the emergence in our geographical area of genotype
197 4 HEV infections, formerly found mainly in China, not in Europe. At about the
198 same time, early 2011, EPIMIC detected an abnormal increase in Group A

199 *Streptococcus* (GAS) infections [21]. The ensuing investigations revealed that
200 these infections mostly affected children, and as a study in UK concurrently
201 described cases of infections with influenza B and invasive GAS [22], we further
202 noted that 23 of 74 samples (31%) testing positive for GAS infection also tested
203 positive for influenza virus. Between December 2010 and April 2011, EPIMIC
204 also identified an abnormal increase in the number of *Acinetobacter baumannii*
205 strains exhibiting a carbapenem-resistant profile at Marseille university hospitals
206 [23]. Moreover, EPIMIC allowed the first report of a rise in 2012 of sexually
207 transmitted diseases, including gonorrhea, syphilis and primary HIV infection [24],
208 and the same year, a 71% incidence increase was observed compared to the
209 average yearly incidence reported during the ten previous years (2002–2011) [25].
210 Finally, EPIMIC allowed the rapid detection of hypervirulent and highly
211 transmissible *Clostridium difficile* clone 027 in our geographical area [26]. Overall,
212 between June 2013 and October 2014 (17 months), 12 abnormal events were
213 detected, corresponding to 0.46 such alerts per week. Moreover, EPIMIC allowed
214 us to survey specific antibiotic-resistance profiles for various bacterial species
215 defined as critical pathogens. This allowed , for instance, to observe that the
216 weekly percentage of samples positive for *S. aureus* strains resistant to methicillin
217 decreased significantly by 0.0099% on average throughout the study period (from
218 33.4% for the first week of December 2003 to 13.5% for the last week of
219 December 2013, $p < 10^{-5}$) (Figure 5). This finding is consistent with those recently

220 reported in France and worldwide, and recently described in our institution for
221 invasive methicillin-resistant *S. aureus* infections [27]. EPIMIC was also
222 contributive in the retrospective analysis of intrinsic colistin-resistant bacteria in
223 Marseille university hospitals in the context of an increasing burden of urinary tract
224 infections [28].

225 Finally, EPIMIC was an educational tool as it showed the infectious
226 syndromes and pathogens most frequently encountered at university hospitals of
227 Marseille and in our geographical area to ≈200 students who stayed each year in
228 our clinical microbiology laboratory for periods ranging from several days to
229 several semesters.

230 **Comparison with other laboratory-based surveillance tools**

231 A total of 76 other laboratory-based surveillance systems were identified
232 through a PubMed search over the last 5 years (Online appendix: S2 Table), in
233 Europe (n=31; 41%) America (19), Asia (11), Africa (7), the Middle East (2) and
234 the Pacific region (1); 5 systems (7%) were implemented for the purpose of global
235 surveillance. Amongst these 76 systems, 34 (45%) surveyed bacteria, 14 surveyed
236 viruses, 9 surveyed yeasts and 2 surveyed parasites; for 17 (22%), targeted
237 pathogens were not identified. Almost half (n=36) of these 76 surveillance systems
238 only surveyed one pathogen or topic (e.g., nosocomial infection, antimicrobial
239 resistance, or invasive diseases). Nine systems (12%) surveyed between 2 and 13
240 pathogens or topics. Finally, 31 systems (41%) surveyed an undefined number of

241 pathogens or topics. In contrast, during the study period EPIMIC surveyed 293
242 pathogens or topics. The mean (±standard deviation) duration of surveillance of the
243 76 surveillance systems was 10±10 years (range, 1-60 years), whereas mean
244 duration of surveillance with EPIMIC was 11 years. Finally, only one third (25) of
245 the 76 laboratory-based surveillance systems surveyed pathogens in real-time, and
246 in a large majority of cases they focused on a single pathogen. By contrast,
247 EPIMIC allowed the real-time surveillance of our entire clinical microbiology
248 laboratory dataset.
249
250 **DISCUSSION**
251 EPIMIC was implemented in our clinical microbiology laboratory to
252 allow the automatic and in real-time detection of any abnormal events related to
253 IDs, assuming that they are rarely predictable and modeled [8]. Over an 11-year
254 period, EPIMIC appeared as a simple, versatile and scalable tool that could be
255 applied to any infectious syndrome and pathogen, and that was capable of
256 managing a considerable amount of data at our clinical microbiology laboratory-
257 scale. Moreover, our tool was efficient for automated real-time monitoring of IDs
258 through both syndromic and traditional surveillance [6,7]. Thus, EPIMIC, in
259 addition to detecting known seasonalities or expected events related to IDs, also
260 identified abnormal events, including unexpected outbreaks and unknown seasonal
261 phenomena [19,21,26]. These findings allowed us to report to clinicians from our

262 institution, but also to regional and national institutions, and several of these
263 findings were worthy of publication. EPIMIC was also an interesting educational
264 tool for students, through objective assessment of the actual incidence and
265 prevalence of IDs and pathogens.

266 Other automated laboratory-based surveillance systems were described as
267 fruitful to identify rises in IDs. For instance, among these systems is the one
268 implemented at the country-scale by the Health Protection Agency in England and
269 Wales since the early 1990s, which counts infectious pathogens detected by
270 hospital and specialist laboratories, and allowed trends for invasive group B
271 streptococcal disease to be described in England and Wales, 1991-2010, and
272 various other pathogens over long periods [16;29-30]. Nevertheless, compared to
273 these other laboratory-based systems, EPIMIC continuously surveys and alerts on a
274 more comprehensive dataset, including clinical samples and tests, and not only
275 pathogens. Moreover, it does not focus on specific infectious threats during
276 specific periods but rather performs surveillance without *a priori*, which is a
277 prerequisite to detect unexpected events. In addition, historical data in EPIMIC are
278 available over more than a decade, which is a longer duration than for most of the
279 other systems. Importantly, EPIMIC generates automatic weekly alerts that are also
280 managed in real-time. Finally, our surveillance tool is user-friendly and can be
281 used by any microbiologist as it operates using Microsoft Excel and, therefore,
282 requires no specific computer skills. Over the study period, EPIMIC was used by

283 ≈300 residents and biologists trained within minutes. Furthermore, it can be
284 implemented in any setting including unsophisticated ones because it can operate
285 using basic PC computers with no specific cost. Thus, EPIMIC can be shared
286 easily; a ready-to-use EPIMIC file is freely-available from our institution website.
287 Some limits of our surveillance computer tool are, notwithstanding,
288 related to its absence of sophistication. Thus, some data are collected manually and
289 all data are entered manually. Such human interventions can generate errors and
290 false alerts, lowering the specificity of the surveillance system. Also, the statistical
291 method used to set alert thresholds (based on the mean ±SD) is the same for all
292 surveyed data, regardless of their amounts and variations during the year, and we
293 are aware that such a global approach may not be the most appropriate in all cases
294 [16,31-32]. Finally, the capabilities of EPIMIC in terms of performance and
295 scalability are now limited in view of growing data and needs in our laboratory. As
296 the development of epidemiological surveillance of IDs is one of the objectives of
297 Méditerranée Infection foundation, the introduction of new tools is on-going in
298 collaboration with epidemiologists and computer scientists. Computer resources
299 are expanding considerably; detection methods and alert thresholds will be
300 optimized and adapted according to the data, and alert statements will be displayed
301 continuously, available remotely, and transferred automatically to referents.
302 However, EPIMIC might be useful for other laboratories in various settings,
303 including in cases of limited computer resources.

304

305 **Acknowledgements**

306 We would like to thank all medical biology residents who participated in

307 the surveillance at our clinical microbiology laboratory since November 2002.

308 **REFERENCE LIST**

309

310 1. Wang H, Dwyer-Lindgren L, Lofgren KT, Rajaratnam JK, Marcus JR,
311 Levin-Rector A, et al. Age-specific and sex-specific mortality in 187
312 countries, 1970-2010: a systematic analysis for the Global Burden of
313 Disease Study 2010. Lancet. 2012; 380: 2071-2094.

314 2. Fauci AS, Touchette NA, Folkers GK. Emerging infectious diseases: a 10-
315 year perspective from the National Institute of Allergy and Infectious
316 Diseases. Emerg Infect Dis. 2005; 11: 519-525.

317 3. WHO. The top 10 causes of death. 2014. Available;
318 www.who.int/mediacentre/factsheets/fs310/en/.

319 4. WHO. World Health Statistics 2013. 2013; Available:
320 http://www.who.int/gho/publications/world_health_statistics/2013/en/.

321 5. Thacker SB, Berkelman RL, Stroup DF. The science of public health
322 surveillance. J Public Health Policy. 1989; 10: 187-203.

323 6. Texier G, Buisson Y. From outbreak detection to anticipation. Rev
324 Epidemiol Sante Publique. 2010; 58: 425-433.

325 7. Meynard JB, Chaudet H, Texier G, Queyriaux B, Deparis X, Boutin JP.
326 Real time epidemiological surveillance within the armed forces: concepts,
327 realities and prospects in France. Rev Epidemiol Sante Publique. 2008; 56:
328 11-20.

329 8. Raoult D. Molecular, epidemiological, and clinical complexities of
330 predicting patterns of infectious diseases. Front Microbiol. 2011; 2:25.

331 9. Nougairede A, Charrel RN, Raoult D. Models cannot predict future
332 outbreaks: A/H1N1 virus, the paradigm. Eur J Epidemiol. 2011; 26: 183-
333 186.

334 10. Raoult D. Microbe interactions undermine predictions. Science. 2011; 331:
335 144-145.

336 11. Sala Soler M, Fouillet A, Viso AC, Josseran L, Smith GE, Elliot AJ, et al.
337 Assessment of syndromic surveillance in Europe. Lancet. 2011; 378: 1833-
338 1834.

339 12. Henning KJ. Overview of Syndromic Surveillance What is Syndromic
340 Surveillance? Morb Mortal Wkly Rep. 2004; 53: 5-11.

341 13. Sharrar RG. Respiratory infection - Pennsylvania. Morb Mortal Wkly Rep.
342 1976; 25: 244.

343 14. Gottlieb MS, Shanker HM, Fan PT, Saxon A, Weisman JD, Pozalski I.
344 Pneumocystis Pneumonia - Los Angeles. Morb Mortal Wkly Rep. 1981; 30:
345 1-3.

346 15. Kman NE, Bachmann DJ. Biosurveillance: a review and update. Adv Prev
347 Med. 2012; 2012: 301408.

348 16. Enki DG, Noufaily A, Garthwaite PH, Andrews NJ, Charlett A, Lane C, et
349 al. Automated biosurveillance data from England and Wales, 1991-2011.
350 Emerg Infect Dis. 2013; 19: 35-42.

351 17. Raoult D. Rapport de mission. 2003. Available: http://ifr48.timone.univ-
352 mrs.fr/files/Documents-Raoult/bioterrorisme2003.pdf.

353 18. Colson P, Gouriet F, Badiaga S, Tamalet C, Stein A, Raoult D. Real-time
354 laboratory surveillance of sexually-transmissible infections in Marseille
355 University hospitals reveals rise of gonorrhoea, syphilis and human
356 immunodeficiency virus seroconversions in 2012. Euro Surveill. 2013; 18:
357 4.

358 19. Anderson DJ, Richet H, Chen LF, Spelman DW, Hung YJ, Huang AT, et al.
359 Seasonal variation in Klebsiella pneumoniae bloodstream infection on 4
360 continents. J Infect Dis. 2008; 197: 752-756.

361 20. Colson P, Romanet P, Moal V, Borentain P, Purgus R, Benezech A, et al.
362 Autochthonous infections with hepatitis E virus genotype 4, France. Emerg
363 Infect Dis. 2012; 18: 1361-1364.

364 21. Parola P, Colson P, Dubourg G, Million M, Charrel R, Minodier P, et al.
365 Letter to the editor. Group A streptococcal infections during the seasonal
366 influenza outbreak 2010/11 in South East England. Euro Surveill. 2011; 16:
367 19816.

368 22. Scaber J, Saeed S, Ihekweazu C, Efstratiou A, McCarthy N, O'Moore E.
369 Group A streptococcal infections during the seasonal influenza outbreak
370 2010/11 in South East England. Euro Surveill. 2011; 16: 19780.

371 23. Kempf M, Rolain JM, Azza S, Diene S, Joly-Guillou ML, Dubourg G, et al.
372 Investigation of Acinetobacter baumannii resistance to carbapenems in
373 Marseille hospitals, south of France: a transition from an epidemic to an
374 endemic situation. APMIS. 2013; 121: 64-71.

375 24. Colson P, Gouriet F, Badiaga S, Tamalet C, Stein A, Raoult D. Real-time
376 laboratory surveillance of sexually-transmissible infections in Marseille
377 University hospitals reveals rise of gonorrhoea, syphilis and human
378 immunodeficiency virus seroconversions in 2012. Euro Surveill. 2013; 18:
379 4.

380 25. Aherfi S, Ninove L, Zandotti C, Crepey P, Richet H, Nougairede A, et al.
381 Increased incidence of acute parvovirus B19 infections in Marseille, France,
382 in 2012 compared with the 2002-2011 period. Clin Microbiol Infect. 2014;
383 20(3):O176-81.

384 26. Lagier JC, Dubourg G, Cassir N, Fournier PE, Colson P, Richet H, et al.
385 Clostridium difficile 027 emerging outbreak in Marseille, France. Infect
386 Control Hosp Epidemiol. 2013; 34: 1339-1341.

387 27. Rolain JM, Abat C, Brouqui P, Raoult D. Worldwide decrease in
388 methicillin-resistant *Staphylococcus aureus*: do we understand something?
389 Clin Microbiol Infect. 2015; pii: S1198-743X(15)00428-0.

390 28. Abat C, Desboves G, Olaitan AO, Chaudet H, Roattino N, Fournier PE, et
391 al. Increasing burden of urinary tract infections due to intrinsic colistin-
392 resistant bacteria in hospitals in Marseille, France. Int J Antimicrob Agents.
393 2015; 45: 144-150.

394 29. Lamagni TL, et al. Emerging trends in the epidemiology of invasive group
395 B streptococcal disease in England and Wales, 1991-2010. Clin Infect Dis.
396 2013; 57(5):682-8.

397 30. Okike IO, Ribeiro S, Ramsay ME, Heath PT, Sharland M, Ladhani SN.
398 Trends in bacterial, mycobacterial, and fungal meningitis in England and
399 Wales 2004-11: an observational study. Lancet Infect Dis. 2014; 14(4):301-
400 7.

401 31. Dab W, Festy B. In search for the threshold: variations on the decision-
402 making role of epidemiologic surveillance. Rev Epidemiol Sante Publique.
403 1998; 46: 249-252.

404 32. Hartfield M, Alizon S. Introducing the outbreak threshold in epidemiology.
405 PLoS Pathog. 2013; 9: e1003277.

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Table 1. Summary of the main types of clinical samples surveyed by EPIMIC and, by sample type, of main pathogens surveyed

Sample type (surveillance period)	Total number of samples		Mean number of samples per week		Standard deviation of the number of samples per week		Main pathogens isolated from the samples	Total number of positive samples	Mean number of positive samples	Standard deviation of the weekly number of positive samples
	Tested	Positive	Tested	Positive	Tested	Positive				
Respiratory samples (from 11/11/2002 to 30/10/2013)	169 147	29 597	320	53	142	44	Respiratory syncytial virus	4,939	9	18
							Pseudomonas aeruginosa	584	1	1
							Staphylococcus aureus	531	1	1
							Influenza virus	2,976	15	39
Urine samples (from 04/11/2002 to 30/10/2013)	560 955	84 174	972	146	165	52	*E. coli*	42,874	74	24
							P. aeruginosa	4,007	7	3
Bone samples (from 04/11/2002 to 30/10/2013)	8 801	2 142	20	5	10	3	N.a.	N.a.	N.a.	N.a.
Ocular samples (from 04/11/2002 to 30/10/2013)	3 211	299	6	0,5	4	1	N.a.	N.a.	N.a.	N.a.
Stool samples (from 04/11/2002 to 30/10/2013)	94 045	5 118	163	9	60	7	*Salmonella* sp.	384	1	1
							Clostridium difficile	633	2	3
							Rotavirus	2,464	4	6
							Calicivirus	661	3	4
Blood cultures (from 03/11/2003 to 30/10/2013)	496 891	33 619	937	63	158	26	*Streptococcus* sp.	2,175	4	2
							S. aureus	2,369	4	3
							Coagulase-negative *staphylococcus*	7,006	13	8
Cerebrospinal fluid (from 04/11/2002 to 30/12/2013)	-	17 231	-	3	-	4	*Neisseria meningitidis*	48	<1	<1
							Streptococcus pneumoniae	78	<1	<1
							Enterovirus	922	2	3

N.a., not available

FIGURE LEGENDS

Figure 1: EPIMIC organization chart.

Groups of items currently monitored by EPIMIC, classified according to infectious syndromes or platforms based on specific technologies or dedicated to specific pathogens.

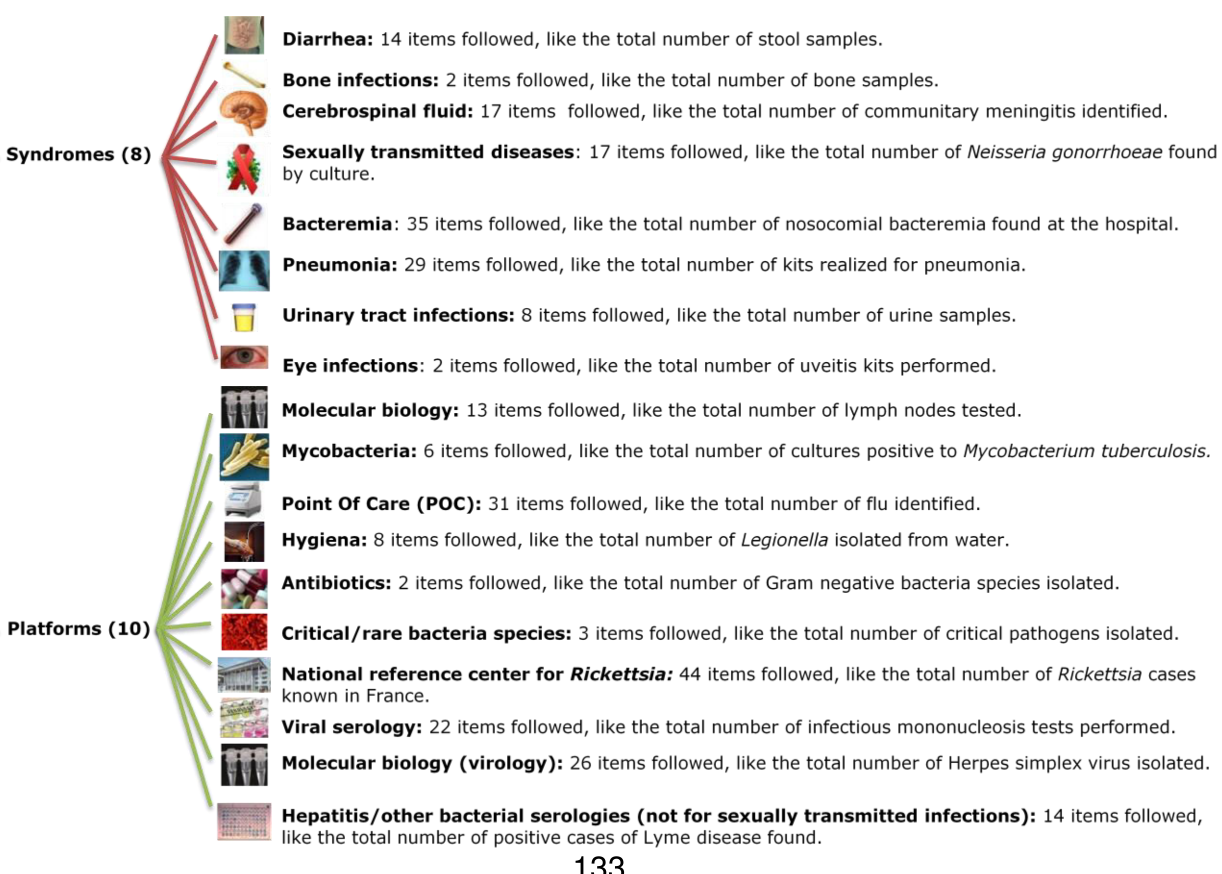
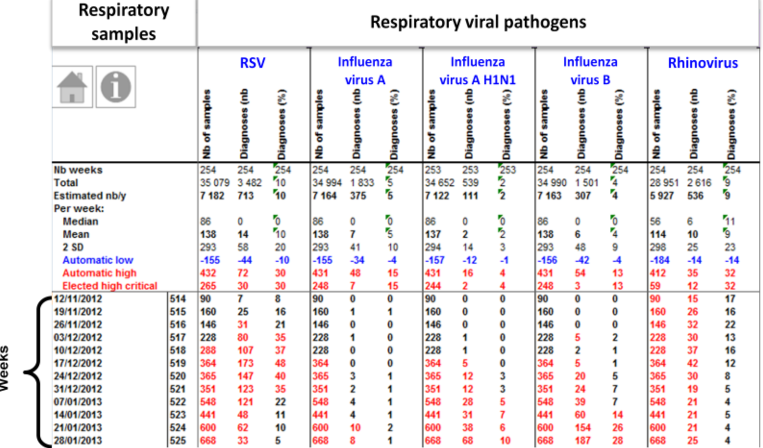

Syndromes (8)
Platforms (10)
Diarrhea: 14 items followed, like the total number of stool samples.
Bone infections: 2 items followed, like the total number of bone samples.
Cerebrospinal fluid: 17 items followed, like the total number of communitary meningitis identified.
Sexually transmitted diseases: 17 items followed, like the total number of *Neisseria gonorrhoeae* found by culture.
Bacteremia: 35 items followed, like the total number of nosocomial bacteremia found at the hospital.
Pneumonia: 29 items followed, like the total number of kits realized for pneumonia.
Urinary tract infections: 8 items followed, like the total number of urine samples.
Eye infections: 2 items followed, like the total number of uveitis kits performed.
Molecular biology: 13 items followed, like the total number of lymph nodes tested.
Mycobacteria: 6 items followed, like the total number of cultures positive to *Mycobacterium tuberculosis.*
Point Of Care (POC): 31 items followed, like the total number of flu identified.
Hygiena: 8 items followed, like the total number of *Legionella* isolated from water.
Antibiotics: 2 items followed, like the total number of Gram negative bacteria species isolated.
Critical/rare bacteria species: 3 items followed, like the total number of critical pathogens isolated.
National reference center for *Rickettsia:* 44 items followed, like the total number of *Rickettsia* cases known in France.
Viral serology: 22 items followed, like the total number of infectious mononucleosis tests performed.
Molecular biology (virology): 26 items followed, like the total number of Herpes simplex virus isolated.
Hepatitis/other bacterial serologies (not for sexually transmitted infections): 14 items followed, like the total number of positive cases of Lyme disease found.

Figure 2: Examples of EPIMIC respiratory infection surveillance tables and plots.

Table (top; A) shows counts of respiratory samples and viral diagnoses entered each week in an EPIMIC Microsoft Excel file; numbers in red font are those above the alert threshold corresponding to the mean plus 2 standard deviations calculated for historical data and shown in the top rows of the table. Plot (bottom; B) shows trends of weekly numbers of samples positive for respiratory viruses.

Nb, number; RSV, respiratory syncytial virus

A.



Respiratory samples		RSV			Influenza virus A			Influenza virus A H1N1		
		Nb of samples	Diagnoses (nb	Diagnoses (%)	Nb of samples	Diagnoses (nb	Diagnoses (%)	Nb of samples	Diagnoses (nb	Diagnoses (%)
Nb weeks		254	254	254	254	254	254	253	253	253
Total		35 079	3 482	10	34 994	1 833	5	34 652	539	2
Estimated nb/y		7 182	713	10	7 164	375	5	7 122	111	2
Per week:										
Median		86	0	0	86	0	0	86	0	0
Mean		138	14	10	138	7	5	137	2	2
2 SD		293	58	20	293	41	10	294	14	3
Automatic low		-155	-44	-10	-155	-34	-4	-157	-12	-1
Automatic high		432	72	30	431	48	15	431	16	4
Elected high critical		265	30	30	248	7	15	244	2	4
12/11/2012	514	90	7	8	90	0	0	90	0	0
19/11/2012	515	160	25	16	160	1	1	160	0	0
26/11/2012	516	146	31	21	146	0	0	146	0	0
03/12/2012	517	228	80	35	228	1	0	228	1	0
10/12/2012	518	288	107	37	228	0	0	228	1	0
17/12/2012	519	364	173	48	364	0	0	364	5	0
24/12/2012	520	365	147	40	365	3	1	365	12	3
31/12/2012	521	351	123	35	351	2	1	351	12	3
07/01/2013	522	548	121	22	548	4	1	548	28	5
14/01/2013	523	441	48	11	441	4	1	441	31	7
21/01/2013	524	600	62	10	600	10	2	600	38	6
28/01/2013	525	668	33	5	668	8	1	668	68	10

Respiratory samples		Influenza virus B			Rhinovirus		
		Nb of samples	Diagnoses (nb	Diagnoses (%)	Nb of samples	Diagnoses (nb	Diagnoses (%)
Nb weeks		254	254	254	254	254	254
Total		34 990	1 501	4	28 951	2 616	9
Estimated nb/y		7 163	307	4	5 927	536	9
Per week:							
Median		86	0	0	56	6	11
Mean		138	6	4	114	10	9
2 SD		293	48	9	298	25	23
Automatic low		-156	-42	-4	-184	-14	-14
Automatic high		431	54	13	412	35	32
Elected high critical		248	3	13	59	12	32
12/11/2012	514	90	0	0	90	15	17
19/11/2012	515	160	0	0	160	26	16
26/11/2012	516	146	0	0	146	32	22
03/12/2012	517	228	5	2	228	30	13
10/12/2012	518	228	2	1	228	37	16
17/12/2012	519	364	5	1	364	42	12
24/12/2012	520	365	20	5	365	30	8
31/12/2012	521	351	24	7	351	19	5
07/01/2013	522	548	39	7	548	21	4
14/01/2013	523	441	60	14	441	21	5
21/01/2013	524	600	154	26	600	21	4
28/01/2013	525	668	187	28	668	25	4

Respiratory viral pathogens

Weeks

B.

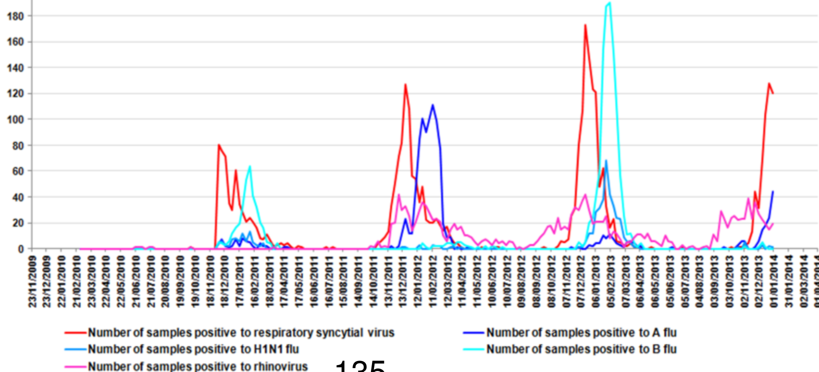


Figure 3: Examples of EPIMIC stool sample and rotavirus diagnosis surveillance tables and plots.

Table (A) shows counts of stool samples and positive diagnoses of rotavirus entered each week into an EPIMIC Microsoft Excel file; numbers in red font are those above the alert threshold corresponding to the mean plus 2 standard deviations calculated for historical data and shown in the top rows of the table. Plots B, C and D show cumulated weekly numbers of stool samples received at our laboratory, of positive rotavirus diagnosis, along with the proportions of positive samples per season (B), year (C) and month (D).

A.

Stool samples		Rotavirus		
		Nb of samples	Diagnoses (nb)	Diagnoses (%)
Nb weeks		563	563	563
Total		24 473	2 462	10
Estimated nb/y		2 260	227	10
Per week:				
Median		42	2	5
Mean		43	4	10
2 SD		41	11	17
Automatic low critical threshold (CT)		2	-7	-7
Automatic high critical threshold		85	15	27
Elected high critical threshold		85	15	27
20/12/2010	425	73	7	10
27/12/2010	426	78	8	10
03/01/2011	427	60	14	23
10/01/2011	428	84	19	23
17/01/2011	429	82	19	23
24/01/2011	430	82	23	28
31/01/2011	431	92	29	32
07/02/2011	432	125	34	27
14/02/2011	433	88	14	16
21/02/2011	434	97	29	30
28/02/2011	435	94	28	30
07/03/2011	436	81	29	36
14/03/2011	437	82	30	37
21/03/2011	438	69	15	22
28/03/2011	439	73	13	18
04/04/2011	440	85	16	19
11/04/2011	441	55	8	15
18/04/2011	442	79	9	11
25/04/2011	443	68	9	13

Weeks

B.

9 000 8 000 7 000 6 000 5 000 4 000 3 000 2 000 1 000 0
Nb
7 675
6 442
4 932
5 301
1 086
998
132
238
Winter Spring Summer Autumn
18 16 14 12 10 8 6 4 2 0
%
Nb of samples
Diagnoses (nb)
Diagnoses (%)

C.

4 000 3 500 3 000 2 500 2 000 1 500 1 000 500 0
Nb
1475 1401 1345 2158 1868 2808 2980 3442 3237 2377
181 97 128 138 159 214 405 367 429 231 86
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
16 14 12 10 8 6 4 2 0
%
Nb of samples
Diagnoses (nb)
Diagnoses (%)

D.

3 500 3 000 2 500 2 000 1 500 1 000 500 0
Nb
3165 3177 2867 2422 2121 1916 1934 1998 2008 2047 2244 2710
458 573 586 386 175 60 49 47 67 94 140 268
January February March April May June July August September October November December
25 20 15 10 5 0
%
Nb of samples
Diagnoses (nb)
Diagnoses (%)

Figure 4: Examples of number of samples handled and positive diagnoses performed in 2011 at our laboratory.

Respiratory samples	
Samples	24338
Bronchoalveolar fluids	263
Pleural liquid	45
Diagnoses	5742
Bacteria	3558
Viruses	2229
Influenza virus	960
Respiratory syncytial virus	927
Metapneumovirus	340
Streptococcus pneumoniae in urines *	146
Pseudomonas aeruginosa	69
Mycoplasma pneumoniae	65
Staphylococcus aureus	47
Legionella pneumophila in urines *	18
Klebsiella pneumoniae	10
Bordetella pertussis	10
Parainfluenzavirus 1,2 3	10
Adenovirus	4
Chlamydia pneumoniae	1
Chlamydia psittaci	0

Mycobacteria	
Samples	10749
Diagnoses	
Culture	120
PCR	108
M. tuberculosis	64

* Rapid test

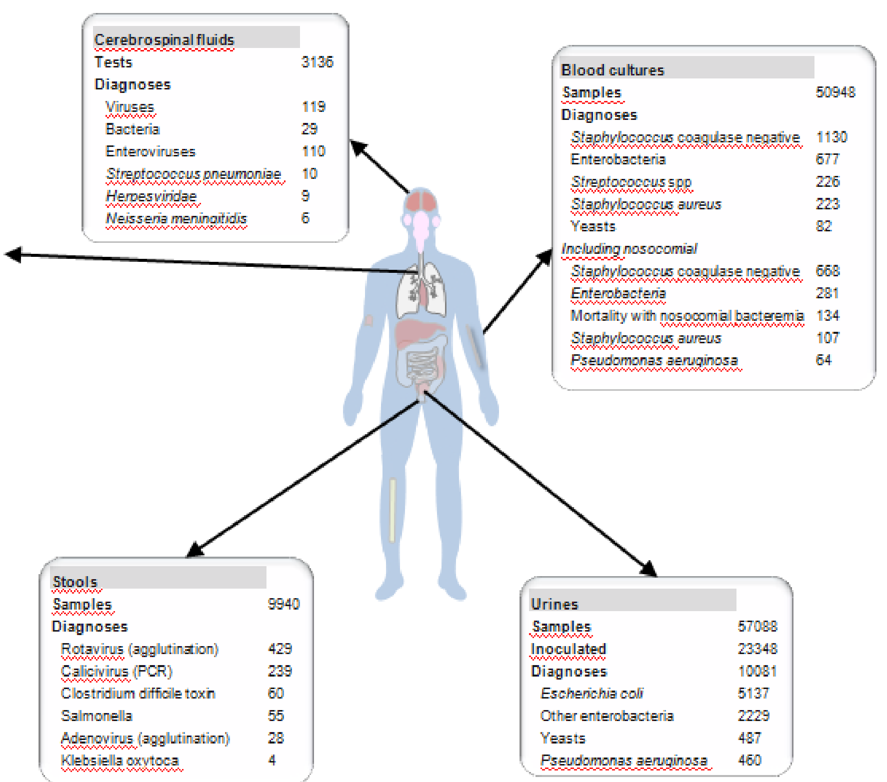
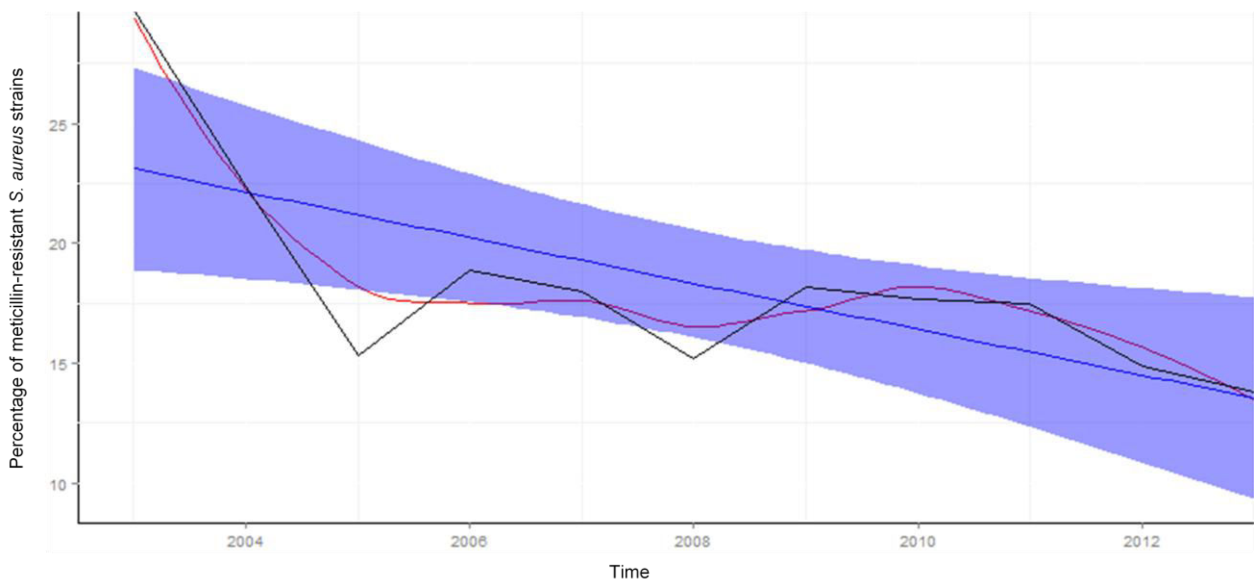


Figure 5: EPIMIC methicillin-resistant *Staphylococcus aureus* profile surveillance plot.



SUPPORTING INFORMATION

S1 Table. A ready-to-use EPIMIC file as freely-available from our institution website (http://www.mediterranee-infection.com/article.php?larub=157&titre=bulletin-epidemiologique).

S2 Table. Main features of laboratory-based surveillance systems identified through a PubMed search over the last 5 years.

S1 Table

Infectious syndrome	Infectious agent X								
	Nb of samples			Diagnoses (nb)			Diagnoses (%)		
Nb weeks	20			20			20		
Total	951			308			32		
Estimated nb/y	2 473			801			32		
Per week:									
Median	43			12			27		
Mean	48			15			32		
2 SD	35			25					
Automatic low critical threshold (CT)	13			-10					
Automatic high critical threshold	82			41					
Elected high critical threshold	82			41					
% of weeks with increased values	0,1			0,1			0,0		
		Mean/wk			Mean/wk				
Seasons		High CT			High CT			High CT	
Winter	687	53		220	17		32		
(Dec-Jan-Feb)		84			45			85	
Spring	111	37		19	6		17		
(Mar-Apr-May)		37			13			34	
Summer	0			0					
(Jun-Jul-Aug)									
Autumn	0			0					
(Sep-Oct-Nov)									
Weeks / No. Sem.		Vs. global CT	Vs. monthly CT		Vs. global CT	Vs. monthly CT		Vs. global CT	Vs. monthly CT
Years									
2015	449	41		80	7		18		
2016	0			0					
2017	0			0					
2018	0			0					
2019	0			0					
2020	0			0					
2021	0			0					
			Vs. global CT			Vs. global CT			Vs. global CT
Months									
January	237	47		75	15		32	-	
		67			36			77	
February	200	40		19	4		10	-	
		45			10			26	
March	111	37		19	6		17	-	
		37			13			34	
April	0			0				-	
May	0			0				-	
June	0			0				-	
July	0			0				-	
August	0			0				-	
September	0			0				-	
October	0			0				-	
November	0			0				-	
December	349	70		159	32		46	-	
		89			47			68	

CT= critical threshold; H= higher than high critical threshold; L= lower than low critical threshold
The elected high critical threshold is the one that is automatically, except when a more relevant threshold has been elected by the laboratory team

Infectious syndrome		**Infectious agent X**		
		Nb of samples	**Diagnoses (nb)**	**Diagnoses (%)**
Nb weeks		20	20	20
Total		951	308	32
Estimated nb/y		**2 473**	**801**	**32**
Per week:				
Median		43	12	27
Mean		**48**	**15**	**32**
2 SD		35	25	
Automatic low critical threshold (CT)		**13**	**-10**	
Automatic high critical threshold		**82**	**41**	
Elected high critical threshold		**82**	**41**	
% of weeks with increased values		0,1	0,1	0,0
01/01/2015	**1**	12	5	42
08/01/2015	**2**	25	14	56
15/01/2015	**3**	49	18	37
22/01/2015	**4**	67	32	48
29/01/2015	**5**	71	37	52
05/02/2015	**6**	85	42	49
12/02/2015	**7**	67	28	42
19/02/2015	**8**	66	22	33
26/02/2015	**9**	60	30	50
05/03/2015	**10**	51	21	41
12/03/2015	**11**	36	13	36
19/03/2015	**12**	51	8	16
26/03/2015	**13**	39	3	8
02/04/2015	**14**	40	9	23
09/04/2015	**15**	42	0	0
16/04/2015	**16**	43	4	9
23/04/2015	**17**	36	3	8
30/04/2015	**18**	37	5	14
07/05/2015	**19**	37	10	27
14/05/2015	**20**	37	4	11

CT= critical threshold; H= higher than high critical threshold; L= lower than low critical threshold
The elected high critical threshold is the one that is automatically, except when a more relevant threshold has been elected by the laboratory team

S2 Table

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
	S. pneumoniae , *H. influenzae* and *N. meningitidis*	19 Latin American countries	1993	Retrospective	Castañeda E. et al. Laboratory-based surveillance of Streptococcus pneumoniae invasive disease in children in 10 Latin American countries: a SIREVA II project, 2000-2005. Pediatr Infect Dis J. (2009), 28(9):e265-70.
	N. meningitidis	Austria	1995	Not precised	Steindl G. et al. Epidemiology of invasive meningococcal disease in Austria 2010. Wien Klin Wochenschr. (2011), 123 Suppl 1:10-4.
	M. tuberculosis	Brazil	From 1998 to 2007	Not precised	Gomes T. et al. Extrapulmonary tuberculosis: Mycobacterium tuberculosis strains and host risk factors in a large urban setting in Brazil. PLoS One. (2013), 8(10):e74517.
	M. tuberculosis	Cambodia	From March 2003 to February 2005	Real-time	Sar B. et al. Anti-tuberculosis drug resistance and HIV co-infection in Phnom Penh, Cambodia. Southeast Asian J Trop Med Public Health. (2009), 40(1):104-7.
	Salmonella spp.	China	Not precised	Real-time	Dong BQ. et al. Trends and disease burden of enteric fever in Guangxi province, China, 1994-2004. Bull World Health Organ. (2010), 88(9):689-96.
	M. tuberculosis	China	From November 2004 to April 2007	Real-time	Deng Y. et al. Laboratory-based surveillance of extensively drug-resistant tuberculosis, China. Emerg Infect Dis. (2011), 17(3):495-7.
	Invasive meningococcal disease	China	From 2000 to 2010	Real-time	Xu XH. Et al. Emergence of serogroup C meningococcal disease associated with a high mortality rate in Hefei, China. BMC Infect Dis. (2012), 12:205.
	E.coli O157:H7, *V. cholerae* , *Salmonella* typhi and paratyphi, *Shigella* , *Y. enterocolitica* , *C. jejuni* , *L. monocytogenes,* and other bacteria pathogens including *N. meningitidis* , *Y. pestis* , *L. interrogans* , and *S. suis* .	China	2004	Real-time	Li W. et al. PulseNet China, a model for future laboratory-based bacterial infectious disease surveillance in China. Front Med. (2012), 6(4):366-75.
	Beta-haemolytic streptococci	Denmark	From 2005 to 2011	Real-time	Lambertsen LM. Et al. Nationwide laboratory based surveillance of invasive beta-haemolytic streptococci in Denmark from 2005 to 2011. Clin Microbiol Infect. (2013).
	N. gonorrhoeae	Europe	2004	Retrospective	Cole MJ. et al. The European gonococcal antimicrobial surveillance programme, 2009. Euro Surveill. (2011), 16(42).

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
Bacteria	*C. difficile*	Finland	January 2008	Retrospective	Kotila SM. et al. Incidence, case fatality and genotypes causing Clostridium difficile infections, Finland, 2008. Clin Microbiol Infect. (2011), 17(6):888-93.
	M. pneumoniae	Finland	1995	Retrospective	Polkowska A. et al. Increased incidence of Mycoplasma pneumoniae infection in Finland, 2010-2011. Euro Surveill. (2012), 17(5).
	Invasive pneumococcal disease	Germany	From 2003 to 2007	Retrospective	Imöhl M. et al. Adult invasive pneumococcal disease between 2003 and 2006 in North-Rhine Westphalia, Germany: serotype distribution before recommendation for general pneumococcal conjugate vaccination for children <2 years of age. Clin Microbiol Infect. (2009), 15(11):1008-12.
	Antimicrobial resistance for 13 bacterial species	Germany	February 2000	Retrospective	Meyer E. et al. Dramatic increase of third-generation cephalosporin-resistant E. coli in German intensive care units: secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008. Crit Care. (2010), 14(3):R113.
	Shiga toxin/verotoxin-producing *Escherichia coli*	Germany	May 25 2011	Retrospective	Wadl M. et al. Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing Escherichia coli in Germany, May to June 2011. Euro Surveill. (2011), 16(24).
	K. pneumoniae (others not precised)	Italy	2008	Retrospective	Sisto A. et al. Carbapenem non-susceptible Klebsiella pneumoniae from Micronet network hospitals, Italy, 2009 to 2012. Euro Surveill. (2012), 17(33).
	Invasive listeriosis	Italy	2005	Retrospective	Mammina C. et al. Enhanced surveillance of invasive listeriosis in the Lombardy region, Italy, in the years 2006-2010 reveals major clones and an increase in serotype 1/2a. BMC Infect Dis. (2013), 13:152.
	V. cholerae	Nepal	From June 2008 to January 2009	Not precised	Karki R. et al. Cholera incidence among patients with diarrhea visiting National Public Health Laboratory, Nepal. Jpn J Infect Dis. (2010), 63(3):185-7.
	H. influenza , *N. meningitidis* and *S. pneumoniae*	Netherlands	1975	Not precised	van Wessel K. et al. Nontypeable Haemophilus influenzae invasive disease in The Netherlands: a retrospective surveillance study 2001-2008. Clin Infect Dis. (2011), 53(1):e1-7.

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
	S. pneumoniae , *H. influenzae, M. catarrhalis* and *S. pyogenes*	Portugal	1999	Not precised	Melo-Cristino J. et al. The Viriato study: update on antimicrobial resistance of microbial pathogens responsible for community-acquired respiratory tract infections in Portugal. Paediatr Drugs. (2010), 12 Suppl 1:11-7.
	S. agalactiae	South Africa	From January 2004 to December 2008	Prospective	Madzivhandila M. et al. Serotype distribution and invasive potential of group B streptococcus isolates causing disease in infants and colonizing maternal-newborn dyads. PLoS One. (2011), 6(3):e17861.
	M. tuberculosis	South Africa	From the January 2004 to December 2006	Prospective	Wiseman CA. et al. Bacteriologically confirmed tuberculosis in HIV-infected infants: disease spectrum and survival. Int J Tuberc Lung Dis. (2011), 15(6):770-5.
	Invasive pneumococcal disease	Spain	From May 2007 to April 2008	Retrospective	Picazo J. et al. Clonal and clinical profile of Streptococcus pneumoniae serotype 19A causing pediatric invasive infections: a 2-year (2007-2009) laboratory-based surveillance in Madrid. Vaccine. (2011), 29(9):1770-6.
	S. pneumoniae	Spain	From January 1997 to June 2009	Retrospective	Fenoll A. et al. Increase in serotype 19A prevalence and amoxicillin non-susceptibility among paediatric Streptococcus pneumoniae isolates from middle ear fluid in a passive laboratory-based surveillance in Spain, 1997-2009. BMC Infect Dis. (2011), 11:239.
	Bacterial meningitidis	Sudan	From 2004 to 2005	Retrospective	Afifi S. et al. Laboratory-based surveillance for patients with acute meningitis in Sudan, 2004-2005. Eur J Clin Microbiol Infect Dis. (2009), 28(5):429-35.
	M. tuberculosis	Taiwan	June 14 2005	Real-time	Chen TC. et al. Computer laboratory notification system via short message service to reduce health care delays in management of tuberculosis in Taiwan. Am J Infect Control. (2011), 39(5):426-30.
	W. bancrofti	Togo	2006	Not precised	Mathieu E. et al. A laboratory-based surveillance system for Wuchereria bancrofti in Togo: a practical model for resource-poor settings. Am J Trop Med Hyg. (2011), 84(6):988-93.
	S. aureus	USA	From January 2005 to June 2008	Not precised	Mongkolrattanothai K. et al. Epidemiology of community-onset Staphylococcus aureus infections in pediatric patients: an experience at a Children's Hospital in central Illinois. BMC Infect Dis. (2009), 9:112.
	Antimicrobial resistance for enteric bacteria	USA	1999	Not precised	Lynch MF. et al. Typhoid fever in the United States, 1999-2006. JAMA. (2009), 302(8):859-65.
	S. pneumoniae , group A and group B *Streptococcus* , *N. meningitidis* , and *H. influenzae*	USA	1995	Not precised	Rosen JB. et al. Geographic variation in invasive pneumococcal disease following pneumococcal conjugate vaccine introduction in the United States. Clin Infect Dis. (2011), 53(2):137-43.

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
	Lyme disease	USA	1996	Not precised	Ertel SH. et al. Effect of surveillance method on reported characteristics of Lyme disease, Connecticut, 1996-2007. Emerg Infect Dis. (2012), 18(2):242-7.
	Gram-positive rods	USA	2003	Not precised	Begier EM. et al. Gram-positive rod surveillance for early anthrax detection. Emerg Infect Dis. (2005), 11(9):1483-6.
	Mycobacterium tuberculosis	USA	1953	Not precised	Vinnard C. et al. Isoniazid resistance and death in patients with tuberculous meningitis: retrospective cohort study. BMJ. (2010), 341:c4451.
	Non-Typhi *Salmonella* isolate, *Salmonella* Typhi, *Shigella* isolate, and *E. coli* O157 and *Campylobacter*	USA	1996	Not precised	Crump JA. et al. Antimicrobial resistance among invasive nontyphoidal Salmonella enterica isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. Antimicrob Agents Chemother. (2011), 55(3):1148-54.
	Various infectious diseases	Belgium	1983	Retrospective	Hanquet G. et al. Impact of rotavirus vaccination on laboratory confirmed cases in Belgium. Vaccine. (2011), 29(29-30):4698-703.
	Nosocomial infections	Canada	1995	Retrospective	Simor AE. et al. Methicillin-resistant Staphylococcus aureus colonization or infection in Canada: National Surveillance and Changing Epidemiology, 1995-2007. Infect Control Hosp Epidemiol. (2010), 31(4):348-56.
	Various infectious diseases	England	Not precised	Real-time surveillance	Severi E. et al. Infectious disease surveillance for the London 2012 Olympic and Paralympic Games. Euro Surveill. (2012), 17(31).
	All pathogens	England and Wales	From July 1996 to June 2006	Real-time surveillance	Trotter CL. et al. Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era: England and Wales, 1996-2006. J Infect. (2010), 60(3):200-8.
	Both bacteria and fungus species isolated from blood	Finland	Not precised	Retrospective	Poikonen E. et al. Secular trend in candidemia and the use of fluconazole in Finland, 2004-2007. BMC Infect Dis. (2010), 10:312.
	Antimicrobial resistance for all clinical pathogens and sample types	Germany	From 2008 to 2010	Retrospective	Schweickert B. et al. MRSA-surveillance in Germany: data from the Antibiotic Resistance Surveillance System (ARS) and the mandatory surveillance of MRSA in blood. Eur J Clin Microbiol Infect Dis. (2012), 31(8):1855-65.
	Varicella-associated invasive group A streptococcus infections	Germany	January 1996	Not precised	Imöhl M. et al. Invasive group A streptococcal disease and association with varicella in Germany, 1996-2009. FEMS Immunol Med Microbiol. (2011), 62(1):101-9.

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
Undefined	23 alerts defined	Italy	From May 2006 to Septembre 2008	Retrospective	Passerini R. et al. Laboratory-based management of microbiological alerts: effects of an automated system on the surveillance and treatment of nosocomial infections in an oncology hospital. Ecancermedicalscience. (2009), 3:137.
	Healthcare-associated infections	Italy	From January 2007 to May 2010	Retrospective	Ansaldi F. et al. Sequential outbreaks of multidrug-resistant Acinetobacter baumannii in intensive care units of a tertiary referral hospital in Italy: combined molecular approach for epidemiological investigation. J Hosp Infect. (2011), 79(2):134-40.
	Foodborne diseases (mainly *Salmonella* spp.)	Jordan, the Palestinian authority and Israel	July 2005	Not precised	Cohen D. et al. A Middle East subregional laboratory-based surveillance network on foodborne diseases established by Jordan, Israel, and the Palestinian Authority. Epidemiol Infect. (2010), 138(10):1443-8.
	Haemophilus influenzae type b, meningococcal and pneumococcal diseases, and HIV	South Africa	July 1999	Prospective	Wolter N. et al. Molecular characterization of emerging non-levofloxacin-susceptible pneumococci isolated from children in South Africa. J Clin Microbiol. (2009), 47(5):1319-24.
	Nosocomial infections	Spain	2006	Retrospective	Almirante B. et al. Laboratory-based surveillance of hospital-acquired catheter-related bloodstream infections in Catalonia. Results of the VINCat Program (2007-2010). Enferm Infecc Microbiol Clin. (2012), 30 Suppl 3:13-9.
	Antimicrobial resistance and consumption	Switzerland	Not precised	Not precised	Kronenberg A. et al. Temporal trends of extended-spectrum cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae isolates in in- and outpatients in Switzerland, 2004 to 2011. Euro Surveill. (2013), 18(21).
	Nosocomial infections	Turkey	From January 2008 to December 2011	Retrospective	Tekin R. et al. A 4-year surveillance of device-associated nosocomial infections in a neonatal intensive care unit. Pediatr Neonatol. (2013), 54(5):303-8.
	Various infectious diseases	USA	2004	Not precised	Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev. (2010), 32(1):93-109.

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
	Respiratory infections, Febrile and vector-borne infections, Gastrointestinal infections, Antimicrobial resistant organisms and Sexually-transmitted infections	Worldwide	1997	Real-time	Fukuda MM. et al. Malaria and other vector-borne infection surveillance in the U.S. Department of Defense Armed Forces Health Surveillance Center-Global Emerging Infections Surveillance program: review of 2009 accomplishments. BMC Public Health. (2011), 11 Suppl 2:S9.
	Any targeted disease tested	Canada	2009	Retrospective	Mukhi SN. et al. DIAL: A Platform for real-time Laboratory Surveillance. Online J Public Health Inform. (2010), 2(3).
Parasite	Congenital toxoplasmosis	France	June 2007	Real-time	Villena I. Congenital toxoplasmosis in France in 2007: first results from a national surveillance system. Euro Surveill. (2010), 15(25).
Parasite	Cryptosporidiosis	France	2004	Real-time	ANOFEL Cryptosporidium National Network. Laboratory-based surveillance for Cryptosporidium in France, 2006-2009. Euro Surveill. (2010), 15(33):19642.
Virus	HIV	France	2001	Real-time	Héraud-Bousquet V. et al. A three-source capture-recapture estimate of the number of new HIV diagnoses in children in France from 2003-2006 with multiple imputation of a variable of heterogeneous catchability. BMC Infect Dis. (2012), 12:251.
Virus	Influenza viruses	Italy	Not precised	Retrospective	Surveillance Group for New Influenza A(H1N1) Virus Investigation in Italy. Virological surveillance of human cases of influenza A(H1N1)v virus in Italy: preliminary results. Euro Surveill. (2009), 14(24).
Virus	Dengue virus	Mayotte	2007	Not precised	Lernout T. et al. Emergence of dengue virus serotype 3 on Mayotte Island, Indian Ocean. East Afr J Public Health. (2011), 8(2):155-6.
Virus	Dengue virus	Puerto Rico	More than 30 years	Real-time	Muñoz-Jordán JL. et al. Highly sensitive detection of dengue virus nucleic acid in samples from clinically ill patients. J Clin Microbiol. (2009), 47(4):927-31.
Virus	Dengue virus	Singapore	2005	Not precised	Lee KS. et al. Dengue virus surveillance for early warning, Singapore. Emerg Infect Dis. (2010), 16(5):847-9.
Virus	Measles viruse	South Korea	2006	Not precised	Choe YJ. et al. Current status of measles in the Republic of Korea: an overview of case-based and seroepidemiological surveillance scheme. Korean J Pediatr. (2012), 55(12):455-61.
Virus	Enterovirus	USA	From 2006 to 2008	Not precised	Centers for Disease Control and Prevention (CDC). Nonpolio enterovirus and human parechovirus surveillance --- United States, 2006-2008. MMWR Morb Mortal Wkly Rep. (2010), 59(48):1577-80.
Virus	HEV	USA	From 2005 to 2012	Not precised	Drobeniuc J. et al. Laboratory-based surveillance for hepatitis E virus infection, United States, 2005-2012. Emerg Infect Dis. (2013), 19(2):218-22.

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
	H1N1 influenza virus	USA	Not precised	Not precised	Balter S. et al. Pandemic (H1N1) 2009 surveillance for severe illness and response, New York, New York, USA, April-July 2009. Emerg Infect Dis. (2010), 16(8):1259-64.
	Influenza viruses	Worldwide	1995	Real-time	Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev. (2010), 32(1):93-109.
	Dengue	Worldwide	1995	Real-time	Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev. (2010), 32(1):93-109.
	Rabies	Worldwide	End of 1990s	Real-time	Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev. (2010), 32(1):93-109.
	Avian influenza viruses	Worldwide	2006	Real-time	Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev. (2010), 32(1):93-109.
	Influenza viruses	Canada	1998	Retrospective	Schanzer DL. et al. The geographic synchrony of seasonal influenza: a waves across Canada and the United States. PLoS One. (2011), 6(6):e21471.
Yeast	Yeasts species	Argentina	From June 2007 to June 2008	Prospective	Córdoba S. et al. Species distribution and susceptibility profile of yeasts isolated from blood cultures: results of a multicenter active laboratory-based surveillance study in Argentina. Rev Argent Microbiol. (2011), 43(3):176-85.
	Candidemia	Brazil	From March 2003 to December 2007	Not precised	Bergamasco MD. et al. Epidemiology of candidemia in patients with hematologic malignancies and solid tumours in Brazil. Mycoses. (2013), 56(3):256-63.
	Yeasts species	China	From August 2009 to July 2010	Real-time	Wang H. et al. In vitro susceptibilities of yeast species to fluconazole and voriconazole as determined by the 2010 National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study. J Clin Microbiol. (2012), 50(12):3952-9.
	Candida spp.	India	From April 2008 to December 2009	Real-time surveillance	Singh RI. et al. Epidemiology of candidaemia in critically ill trauma patients: experiences of a level I trauma centre in North India. J Med Microbiol. (2011), 60(Pt 3):342-8.
	Candida spp.	Italy	From January to December 2009	Retrospective	Tortorano AM. et al. A 1-year prospective survey of candidemia in Italy and changing epidemiology over one decade. Infection. (2013), 41(3):655-62.

Type of pathogen	Pathogens	Country	Date of implementation or duration	Type of surveillance	Related articles
	Cryptococcal disease	South Africa	From January 2005 to 31 December 2007	Prospective	Meiring ST. et al. A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. AIDS. (2012), 26(18):2307-14.
	Candida spp.	Spain	From Octobre 2005 to Septembre 2006	Retrospective	Flórez C. et al. In vitro susceptibilities of bloodstream isolates of Candida spp.: results from a multicenter active surveillance program in Andalusia. Enferm Infecc Microbiol Clin. (2009), 27(9):518-22.
	Candida spp.	Spain	From June 2008 to June 2009	Retrospective	Cisterna R. et al. Nationwide sentinel surveillance of bloodstream Candida infections in 40 tertiary care hospitals in Spain. J Clin Microbiol. (2010), 48(11):4200-6.
	Candida spp.	Australia	1999	Retrospective	Playford EG. et al. Increasing incidence of candidaemia: long-term epidemiological trends, Queensland, Australia, 1999-2008. J Hosp Infect. (2010), 76(1):46-51.

Article 3: Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry.

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Identification of Rare Pathogenic Bacteria in a Clinical Microbiology Laboratory: Impact of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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During the past 5 years, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has become a powerful tool for routine identification in many clinical laboratories. We analyzed our 11-year experience in routine identification of clinical isolates (40 months using MALDI-TOF MS and 91 months using conventional phenotypic identification [CPI]). Among the 286,842 clonal isolates, 284,899 isolates of 459 species were identified. The remaining 1,951 isolates were misidentified and required confirmation using a second phenotypic identification for 670 isolates and using a molecular technique for 1,273 isolates of 339 species. MALDI-TOF MS annually identified 112 species, i.e., 36 species/10,000 isolates, compared to 44 species, i.e., 19 species/10,000 isolates, for CPI. Only 50 isolates required second phenotypic identifications during the MALDI-TOF MS period (i.e., 4.5 reidentifications/10,000 isolates) compared with 620 isolates during the CPI period (i.e., 35.2/10,000 isolates). We identified 128 bacterial species rarely reported as human pathogens, including 48 using phenotypic techniques (22 using CPI and 37 using MALDI-TOF MS). Another 75 rare species were identified using molecular methods. MALDI-TOF MS reduced the time required for identification by 55-fold and 169-fold and the cost by 5-fold and 96-fold compared with CPI and gene sequencing, respectively. MALDI-TOF MS was a powerful tool not only for routine bacterial identification but also for identification of rare bacterial species implicated in human infectious diseases. The ability to rapidly identify bacterial species rarely described as pathogens in specific clinical specimens will help us to study the clinical burden resulting from the emergence of these species as human pathogens, and MALDI-TOF MS may be considered an alternative to molecular methods in clinical laboratories.

Early and accurate microbial identification is a critical requisite for early, adequate antibiotic treatment. The number of newly described bacteria has risen impressively during the past few decades (1, 2). Notably, the identification of new pathogens in clinical microbiology has been spectacularly improved during previous decades by the use of molecular identification, especially 16S rRNA gene sequencing (3–8). Molecular identification is one of the most useful techniques but remains expensive and requires a workload that is not adapted for routine use. Moreover, clinical definitions of some species do not match those used for 16S rRNA identification, such as the mismatched definitions used for streptococci (9–11).

Bacterial identification directly from colonies and samples using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has been described as a revolutionary tool perfectly adapted to the clinical microbiology laboratory (12, 13). MALDI-TOF MS has been used to identify bacterial species and subspecies (14, 15), and in some outbreaks, MALDI-TOF MS has been reported to be able to identify the lineages of strains (16–18). Recently, MALDI-TOF MS has also been used to detect clinical pathogens previously misidentified or ambiguously identified (19–24). Detection of antimicrobial resistance using MALDI-TOF MS has been reported for *Staphylococcus aureus* (25–32), *Acinetobacter baumannii* (26), *Escherichia coli*, and other members of the family *Enterobacteriaceae* (33–35). Several new bacterial species emerging as human pathogens have been identified using MALDI-TOF MS (36–45).

In the present study, we examined data from a large collection of clinical isolates routinely identified during the last 11 years in our laboratory to evaluate the performance of MALDI-TOF MS for routine bacterial identification compared with conventional phenotypic identification (CPI). Particularly, we evaluated the capacity of MALDI-TOF MS to identify bacterial species that were rarely reported as human pathogens compared with conventional phenotypic and molecular identifications.

MATERIALS AND METHODS

Specimen collection. Clinical isolates were recovered from blood samples, cerebrospinal fluid samples, wounds, exudate samples, abscesses, respiratory tract samples, genitourinary samples, bone-joint infection samples, digestive samples, stools, and other clinical samples from 1 January 2002 through 31 December 2012, excluding December 2002 (data not available). In September 2008, an anaerobic laboratory with anaerobic chamber, preincubation of agar plates in strictly anaerobic condition, and a team of dedicated technicians was created with the opening of another laboratory at the North University Hospital, Marseille, France (600 beds) in our 4,000-bed university hospital.

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TABLE 1 Summary of 11 years of bacterial identification in our laboratory[a]

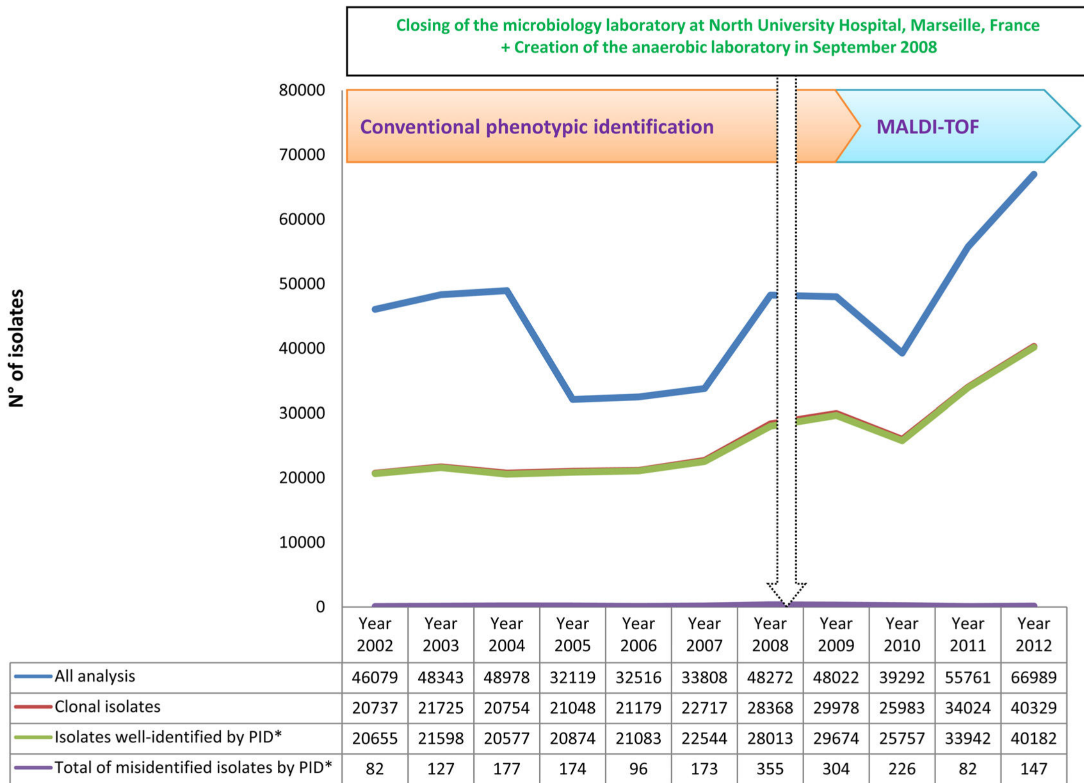
Identification technique (study period [day-mo-yr])	Study period (no. of months)	Total no. of analyses	No. of clonal isolates	No. of isolates identified by 1st PID	No. of species identified by 1st PID	No. of bacterial species identified/year	No. of isolates confirmed by 2nd PID	No. of isolates identified by molecular identification	No. of isolates misidentified by 1st PID	% misidentified
CPI period (1-Jan-02 to 30-Aug-09)	91	322,291	175,999	174,636	336	44	620	743	1,363	0.77
MALDI-TOF MS period (1-Sep-09 to 30-Dec-12)	40	177,888	110,843	110,263	382	112	50	530	580	0.52
AutoFlex II (1-Sep-09 to 30-Nov-10)	15	52,695	34,839	34,497	264	211	32	310	342	0.98
MicroFlex (1-Dec-10 to 31-Dec-12)	25	125,193	76,004	75,766	340	163	18	220	238	0.31
Total	131	500,179	286,842	284,899	459	42	670	1,273	1,951	0.68

[a] We identified 459 bacterial species among 284,899 clinical isolates during nearly 11 years. We identified 112 species per year using MALDI-TOF MS compared with 44 identified using conventional phenotypic identification (CPI) (Gram staining, API, Vitek 2 system identification). PID, phenotypic identification.

Bacterial identification. All isolates were identified after aerobic, microaerophilic, and anaerobic incubation of clinical specimens on 5% sheep blood, chocolate, Mueller-Hinton, Trypticase soy, and MacConkey agar plates (bioMérieux).

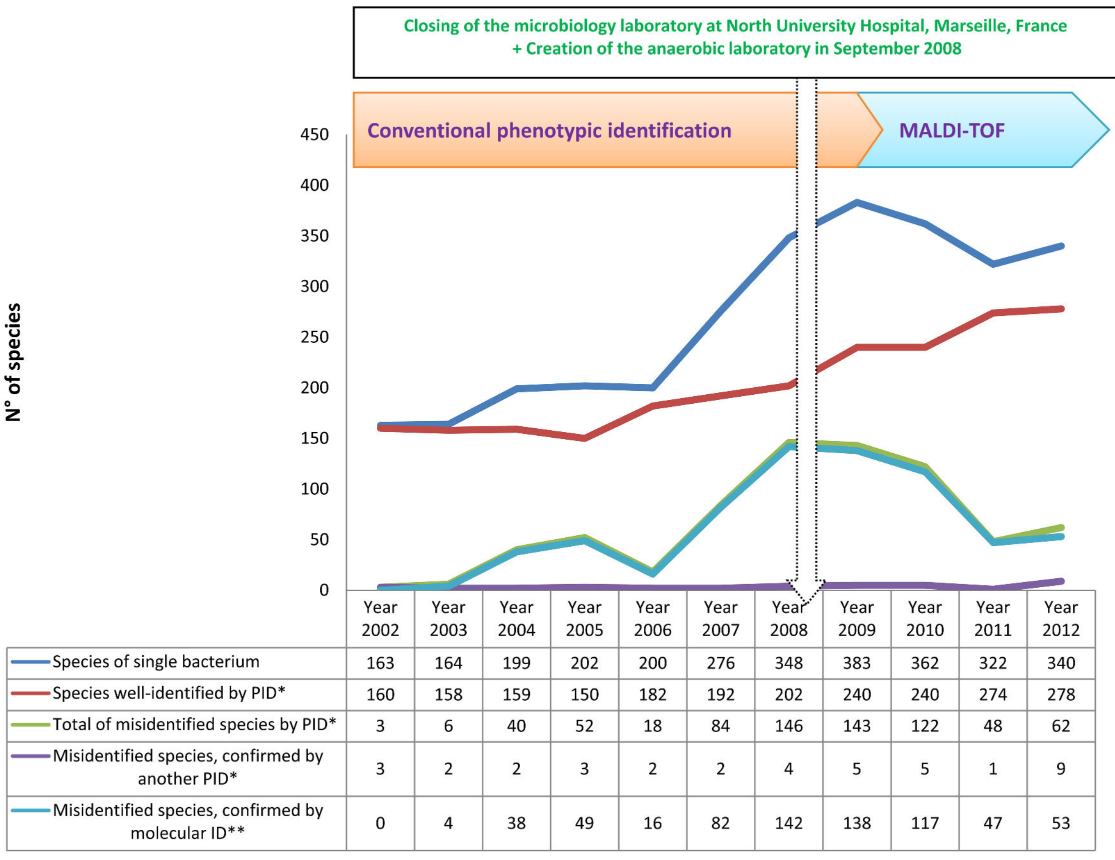
(i) Conventional phenotypic identification period. In CPI, we used semiautomated Gram staining (Aerospray Wiescor; Elitech), determined catalase and oxidase activities, and used the Vitek 2 system (bioMérieux), with 330 microorganism strains as references or the API 20A identification strip for anaerobes (bioMérieux) to identify bacterial species from 1 January 2002 to 30 August 2009. Correct identification of an isolate using the Vitek 2 system was confirmed when the T index was ≥0.25; identification using the API system was confirmed when the percentage of identification was ≥90%, and the T index was ≥ 0.25 (46). We reidentified organisms by Gram staining rather than by using the Vitek 2 system. API identification strips included API 20A, API Coryne, API Campy, API 20E, API 20NE, API Strep, API Staph, API NH, and API Listeria strips (bioMérieux) as the second phenotypic identification in the CPI period to identify uncertainly identified isolates at the species level.

(ii) MALDI-TOF MS identification period. ***(a) MALDI-TOF MS analysis.*** We used MALDI-TOF MS as a routine bacterial identification tool to categorize bacterial species from direct colonies, and the procedure was performed as previously described (12). We used a MALDI-TOF MS AutoFlex II system (Brüker Daltonik) for the first part of the MALDI-TOF MS identification period, from 1 September 2009 to 30 November 2010 and a MicroFlex LT mass spectrometer (Brüker Daltonik) for the second part of the MALDI-TOF MS identification period, from 1 December 2010 to 31 December 2012.



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
All analysis	46079	48343	48978	32119	32516	33808	48272	48022	39292	55761	66989
Clonal isolates	20737	21725	20754	21048	21179	22717	28368	29978	25983	34024	40329
Isolates well-identified by PID*	20655	21598	20577	20874	21083	22544	28013	29674	25757	33942	40182
Total of misidentified isolates by PID*	82	127	177	174	96	173	355	304	226	82	147

FIG 1 Time course of the total numbers of isolates analyzed, clonal isolates analyzed, and clonal isolates identified and misidentified using phenotypic identification (PID*) during 11 years of routine identification in our clinical laboratory.



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Species of single bacterium	163	164	199	202	200	276	348	383	362	322	340
Species well-identified by PID*	160	158	159	150	182	192	202	240	240	274	278
Total of misidentified species by PID*	3	6	40	52	18	84	146	143	122	48	62
Misidentified species, confirmed by another PID*	3	2	2	3	2	2	4	5	5	1	9
Misidentified species, confirmed by molecular ID**	0	4	38	49	16	82	142	138	117	47	53

FIG 2 Time course of the numbers of species of clonal bacteria identified, species identified using an initial phenotypic identification (PID*), total species misidentified, species confirmed by another PID*, and species confirmed by molecular identification (molecular ID**) over 11 years of routine identification in our clinical laboratory.

(b) MALDI-TOF mass spectrum database. The Brüker database updated with a laboratory collection of spectra from clinical isolates identified by 16S rRNA gene sequencing was used from 1 September 2009 to 31 December 2012. For each organism updated, a consensus spectrum was obtained by using the Biotyper MSP (mean spectrum projection) creation standard method from a total of 12 spots made for each isolate, and the manipulation was repeated in two independent runs. The Fisher exact test was used to evaluate the reproducibility. We determined the sensitivity of MALDI-TOF MS by identification of 10 colonies of the same bacterial species in another independent run. Our MALDI-TOF mass spectrum database has 6,213 reference microorganism strain spectra, and we updated the primary Brüker database containing 3,993 microorganism spectra (3,670 of bacteria, 7 of *Archaea*, and 316 of *Eukaryota*) with laboratory bacterial spectra including spectra from well-typed bacterial strains and other human-pathogenic bacteria identified by using a molecular technique.

(c) MALDI-TOF MS identification. Bacterial species were directly identified from one bacterial colony; each colony was covered with 2 ml of matrix solution (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) without other supplements and extracted as previously described (12). We used MALDI Biotyper 3.0 software to compare the first 100 peaks of each spectrum to our MALDI-TOF mass spectrum database previously updated as described below. An isolate was considered correctly identified at the species level by using MALDI-TOF MS if 2 spectra had scores of ≥1.9. Uncertainly identified isolates at the species level (scores of <1.9) were identified with certainty by MALDI-TOF MS analysis of 2 additional spectra. A second run of MALDI-TOF MS identification with 4 spectra was done for unsatisfied species identification in the MALDI-TOF MS period.

(iii) Molecular identification. Isolates misidentified by the second CPI or MALDI-TOF MS analyses were identified with certainty using molecular identification using 16S rRNA or *rpoB* gene sequencing as described elsewhere (4, 12, 47, 48). An isolate was correctly identified when (i) its 16S rRNA gene sequence yielded ≥98.7% identity with the sequence of the most closely related bacterial species in GenBank (49) or (ii) when its *rpoB* gene sequence yielded ≥97% identity with the sequence of the most closely related bacterial species in GenBank or a local database (12, 48).

Database analysis. Our database included bacterial identification results and their associated clinical information; 500,174 identifications of clinical isolates were performed during the study period. All results were extracted into Microsoft Excel files for further analysis. Duplicate analyses were eliminated by retaining only a single bacterial identification per sample. We also excluded all samples for which there were phenotypic or molecular identifications of fungi, environmental isolates, *Mycobacterium*, and other intra- and extralaboratory strains that were not of human origin.

Meaning of rare species. Rare species were defined as bacterial species with ≤10 reports designating them as human pathogens retrieved from the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/). The possibility of inaccurate classifications as rare species due to taxonomy changes was checked using the National Center for Biotechnology Information (NCBI) taxonomy database (http://www.ncbi.nlm.nih.gov/guide/taxonomy/).

Time, cost, and training requirement evaluation of a MALDI-TOF MS identification technique. We evaluated the time required for the MALDI-TOF mass spectrometry identification as the period between the deposit of a bacterial colony on the MALDI-TOF MS plate by a technician

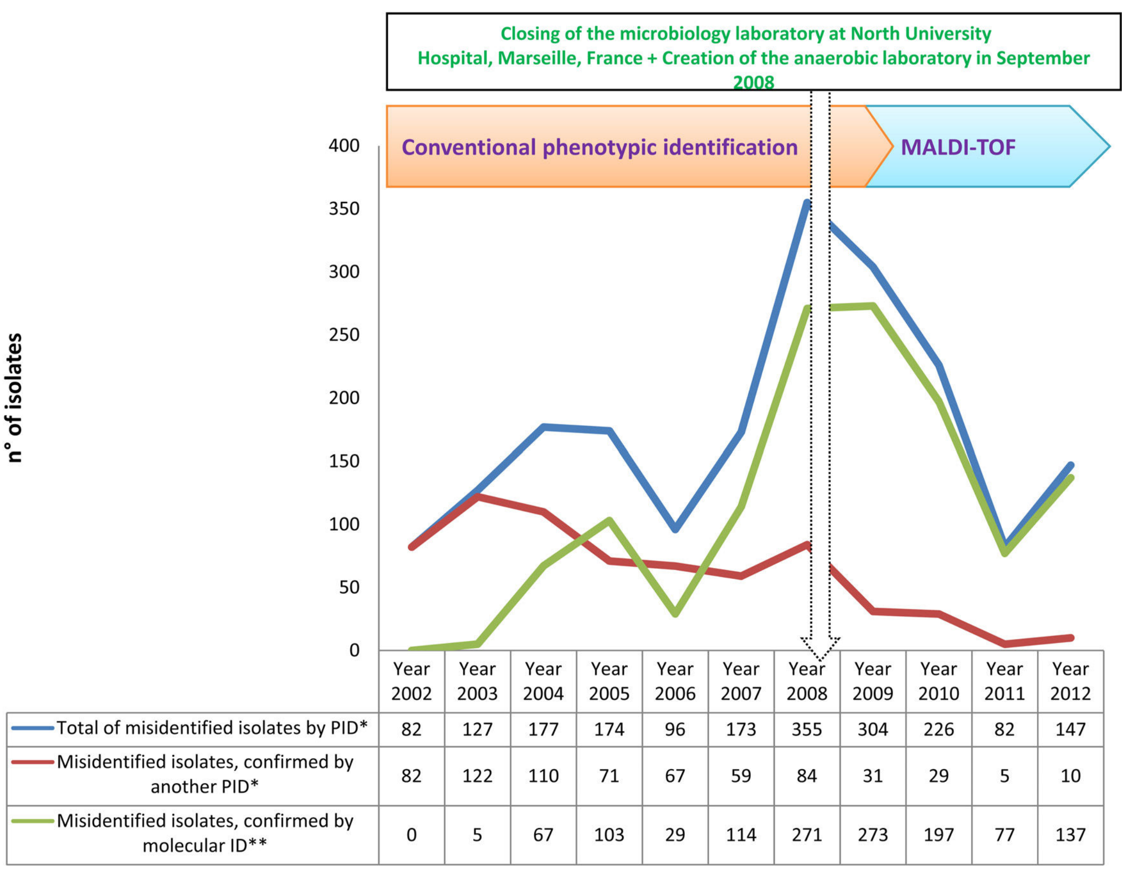


FIG 3 Time course of the numbers of total isolates misidentified using phenotypic identification (PID*), isolates confirmed by a second PID* and isolates confirmed by molecular identification (ID**) over 11 years of routine identification in our clinical laboratory.

and the completion of the informatics interpretation of the resulting spectra (i.e., identification ready to be transmitted to a clinician). The costs of identification were evaluated by adding the costs of matrix reagents, plates, positive controls, and technician salary, with provisions for 5-year depreciations of the apparatuses used (Gram staining apparatus, microscope, identification apparatus, and mass spectrometer) on the basis of ≈67,000 isolates analyzed per year (the number of samples analyzed in 2012 in our laboratory).

Statistical analysis. Data analyses were performed using IBM SPSS Statistics software version 20.0. Proportions were compared using the chi-squared or Fisher's exact two-tailed tests. A P value of <0.05 was considered statistically significant.

RESULTS

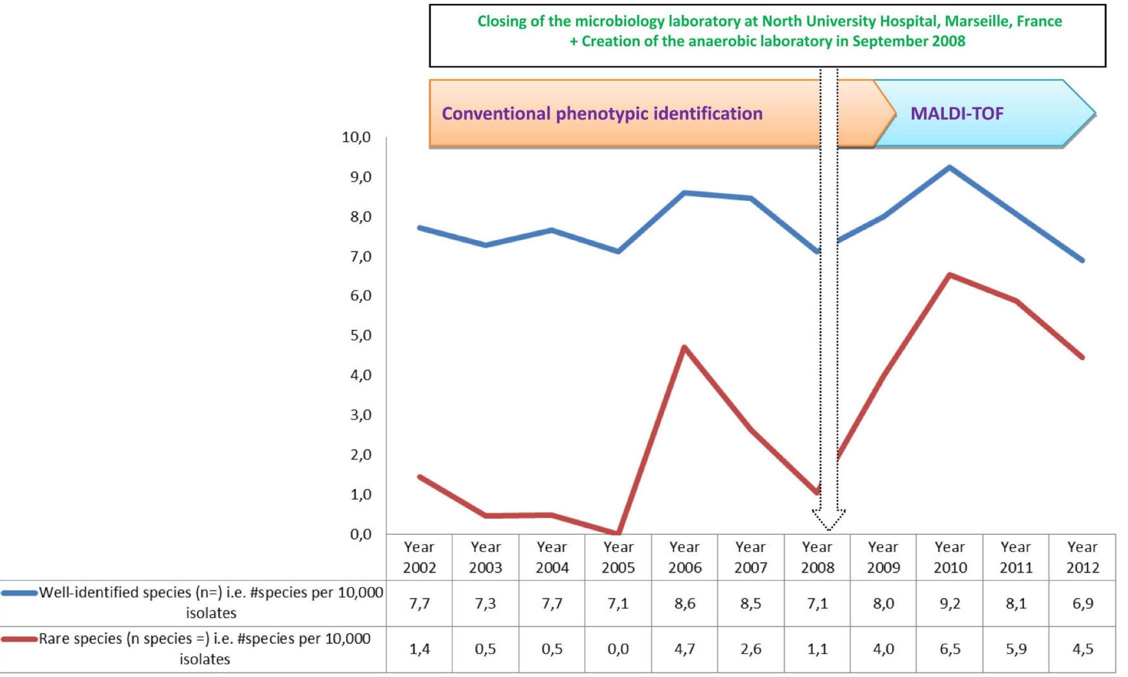
Over 11 years, we performed 500,179 bacterial identifications in our laboratory (Table 1). We grew our capacity for identification between 2002 and 2012, increasing the number of analyses from 46,079 per year to 66,989 per year, by creating an anaerobic laboratory and joining with another microbiology laboratory located at North University Hospital, Marseille, France, in September 2008 (Fig. 1). The implementation of a new tool for identification (MALDI-TOF MS) has spectacularly improved our capacity to identify more clinical isolates and more human-pathogenic bacteria. We identified 160 bacterial species during 2002 and 278 species during 2012 (Fig. 2).

Among 286,842 clonal isolates identified, phenotypic identification methods (CPI or MALDI-TOF MS) correctly identified 284,899 isolates including 459 species of 134 genera and 6 phyla. Another 1,951 isolates were misidentified and required identification by another phenotypic or molecular method (Table 1 and Fig. 3).

CPI identified 174,636 isolates, including 336 species of 120 genera and 6 phyla, over the 91 months from 1 January 2002 through 30 August 2009, whereas MALDI-TOF MS identified 110,263 isolates classified in 382 species of 114 genera and 6 phyla over the 40 months from 1 September 2009 through 31 December 2012. Thus, MALDI-TOF MS yearly identified 32,430 isolates of 112 species, i.e., 36 species/10,000 isolates, compared with 22,692 isolates of 44 species, i.e., 19 species per 10,000 isolates, for CIP ($P < 0.0001$) (Table 1 and Fig. 4).

Among the 459 bacterial species identified during 2002 to 2012, 76 species (17%) were identified using only CPI over a 91-month period, 124 species (27%) were identified using only MALDI-TOF MS during a 40-month period (see Table S1 and Table S2 in the supplemental material), and 258 species (56%) were identified using both methods.

In the group of bacterial species identified only by CPI, 15 (20%) of the 76 isolates were absent from our MALDI-TOF mass spectrum database. In the phylum *Actinobacteria*, 16 species of 11 genera were identified using only CPI, and 3 species were absent from our MALDI-TOF MS database. In the phylum *Bacteroidetes*, 5 species of 3 genera were identified using CPI exclusively, and 1 species was absent from the MALDI-TOF MS database. In the



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Well-identified species (n=) i.e. #species per 10,000 isolates	7,7	7,3	7,7	7,1	8,6	8,5	7,1	8,0	9,2	8,1	6,9
Rare species (n species =) i.e. #species per 10,000 isolates	1,4	0,5	0,5	0,0	4,7	2,6	1,1	4,0	6,5	5,9	4,5

FIG 4 Biodiversity of rare species identified in the routine identification of all clinical isolates tested (identified plus misidentified) during the last 11 years.

phylum *Firmicutes*, 19 species of 10 genera were identified using only CPI, and 3 were missing from the MALDI-TOF MS database. In the phylum *Fusobacteria*, 3 species of 2 genera were identified using only CPI, and 1 was missing from the MALDI-TOF MS database. In the phylum *Proteobacteria*, 33 species of 22 genera were identified using CPI exclusively, and 7 were missing from the MALDI-TOF MS database (see Table S1 in the supplemental material).

In the group of bacterial species identified only by MALDI-TOF MS, 21 (17%) of the 124 isolates were present in the Vitek 2 database, whereas 103 (83%) were not (see Table S2 in the supplemental material). In the phylum *Actinobacteria*, 21 species of 12 genera were identified using only MALDI-TOF MS and were lacking in the Vitek 2 database. In the phylum *Bacteroidetes*, 10 species of 7 genera were identified by using MALDI-TOF MS exclusively, and 9 species were absent from the Vitek 2 database. In the phylum *Firmicutes*, 54 species of 18 genera were identified using only MALDI-TOF MS, and 41 were missing from the Vitek 2 database. In the phylum *Fusobacteria*, *Fusobacterium periodonticum* was identified using only MALDI-TOF MS and was missing from the Vitek 2 database. In the phylum *Proteobacteria*, 38 species of 20 genera were identified using MALDI-TOF MS exclusively, and 31 were missing from the Vitek 2 database. No species in the phylum *Tenericutes* was identified by using MALDI-TOF MS exclusively (see Table S2 in the supplemental material).

During the study period, 1,951 isolates were misidentified and required confirmation by another round of phenotypic identification for 670 isolates of 21 species (see Table S3 in the supplemental material) and by molecular identification for 1,273 isolates of 339 species (see Table S4 in the supplemental material). Among 339 species that required confirmation by molecular identification, 63 species were absent from the initial Brüker database, which contained 3,993 bacterial spectra, and only 24 were missing from our updated MALDI-TOF mass spectrum database (6,213 bacterial spectra). Among 24 bacterial species of 46 isolates missed from our MALDI-TOF MS database, 16 species of 32 isolates were identified by a molecular method in the CPI period, and 11 species of 14 isolates were identified by a molecular method in the MALDI-TOF MS period. Despite their presence in our MALDI-TOF database, 315 other species had to be examined by molecular identification; this included 228 species of 711 isolates and 196 species of 516 isolates in the CPI period and the MALDI-TOF MS period, respectively.

We identified 40 species of 1,506 anaerobic organisms before MALDI-TOF MS by using the API 20A system (bioMérieux), and we identified 103 species of 1,564 anaerobic organisms at the species level using MALDI-TOF MS identification.

During the CPI period, 1,363 isolates (0.77%) were misidentified; the 1,363 isolates included 620 isolates reidentified using a second CPI as described below (i.e., 35.2 per 10,000 isolates) and 743 confirmed using a molecular technique (i.e., 42 per 10,000 isolates). During the MALDI-TOF MS period, 580 isolates (0.52%) were misidentified; the 580 isolates included 50 isolates reidentified using a second run of identification by MALDI-TOF MS, i.e., 4.5 species per 10,000 isolates, and 530 isolates confirmed using a molecular technique, i.e., 47 species per 10,000 isolates (Table 1 and Fig. 3).

The molecular identification requirements were similar during the CPI and MALDI-TOF MS periods at 42 and 47 molecular identifications/10,000 isolates, respectively. However, a decreasing trend was observed during the final 2 years, with 47 and 53 during 2011 and 2012, respectively, compared with 142 molecular identifications in 2008 (Fig. 2 and Fig. 3).

During 11 years of routine identification, we identified 123 rare species of bacteria that were reported to be human pathogens fewer than or equal to 10 times in the literature (PubMed data-

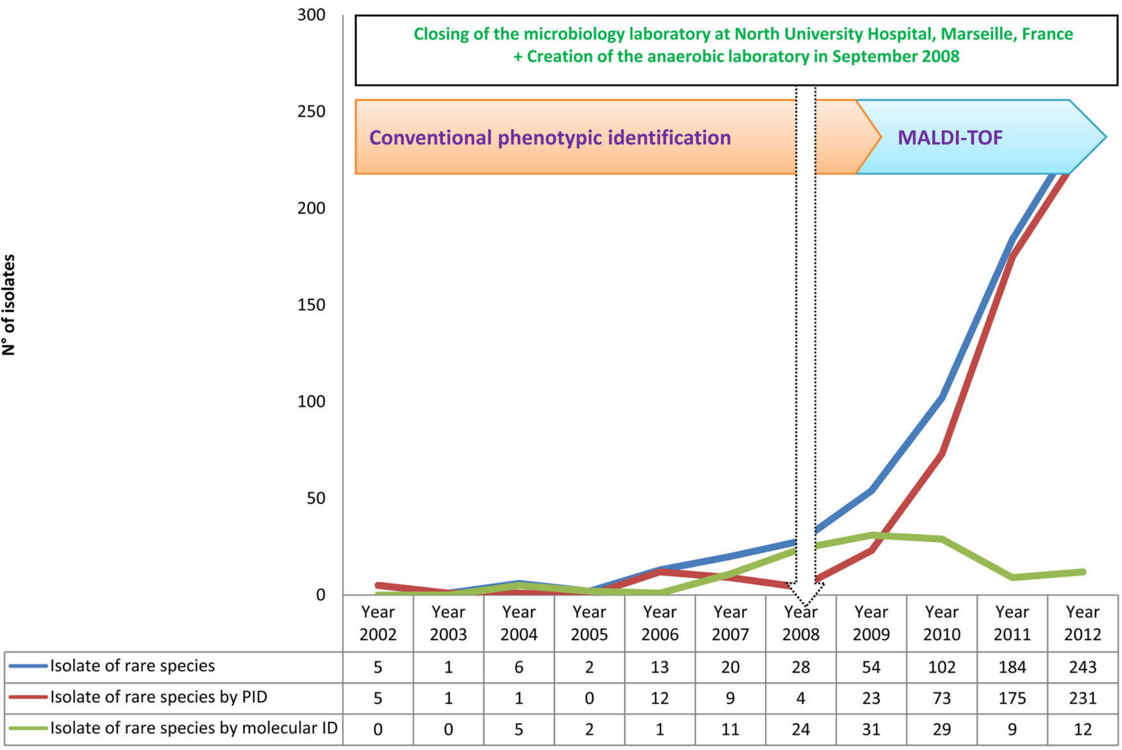


FIG 5 Time course of the numbers of isolates of 128 rare species, 48 of which were identified using phenotypic identification (PID), and 75 of which were identified using molecular identification (ID).

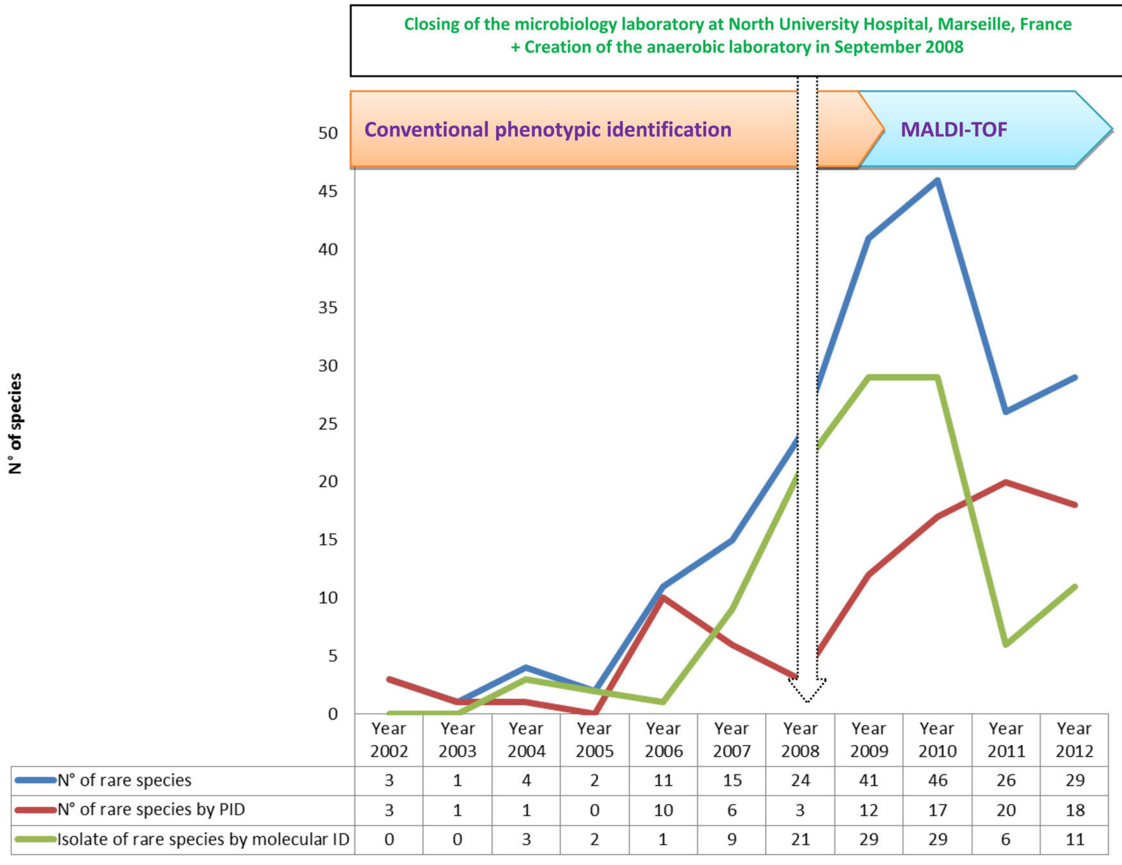
base). Among these species, 48 were identified by phenotypic identification. Another 75 species were confirmed by molecular identification. In addition, CPI identified only 22 rare species during 91 months, and MALDI-TOF MS identified 37 such rare species during 40 months (Fig. 5, Fig. 6, and Fig. 7). Among 196 species of 516 isolates that were not satisfactorily identified in the MALDI-TOF MS period, 365 (71%) isolates represented 10 genera, including *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Acinetobacter*, *Actinomyces*, *Staphylococcus*, *Bacillus*, *Enterobacter*, *Enterococcus*, and *Nocardia*, that frequently required molecular identification (Fig. 8).

Identification of 11 of the 48 rare species identified using phenotypic methods was performed using only CPI, and 26 other rare species were identified using only MALDI-TOF MS (Table 2). In the phylum *Actinobacteria*, 18 rare species were identified, including 9 exclusively identified using MALDI-TOF MS, 5 using CPI, and 4 species using both techniques. In the phylum *Bacteroidetes*, 6 rare species were identified; the 6 species included 2 exclusively identified using MALDI-TOF MS, 1 using CPI, and 3 using both techniques. In the phylum *Firmicutes*, 12 rare species were identified, including 7 exclusively identified using MALDI-TOF MS, 2 using CPI, and 3 using both techniques. In the phylum *Fusobacteria*, 2 rare species were totally identified using CPI. In the phylum *Proteobacteria*, 10 rare species were identified, including 8 exclusively identified using MALDI-TOF MS, 1 using CPI, and 1 using both techniques (Table 2).

Looking in detail at the group of 48 rare species identified using phenotypic methods, 4 of these were identified more than 10 times in our laboratory during the last 11 years, including 12 isolates of *Actinomyces europaeus*, 20 isolates of *Actinomyces radingae*, 31 isolates of *Pandoraea pulmonicola*, 95 isolates of *Peptoniphilus harei*, and 272 isolates of *Enterobacter kobei* (Table 2).

The rare species identified using phenotypic methods were mostly recovered from bloodstream and urinary traction infections (see Table S5 in the supplemental material). *Enterobacter kobei* was the most frequently identified among the 48 rare species (see Table S5 in the supplemental material). In the following analysis, using MALDI-TOF MS, we identified two bacterial species, *Brevibacterium ravenspurgense* and *Corynebacterium fastidiosum*, that had never been reported as human pathogens in PubMed (Table 2).

Moreover, molecular techniques identified 75 rare species among 124 isolates including 23 that were identified as rare species using phenotypic identification methods (Table 3). In all, 57 of the 75 rare species identified using molecular techniques were absent from the Brüker database and 18 were absent from our MALDI-TOF database. Among 57 bacterial rare species identified by molecular methods which spectrum present in our MALDI-TOF database, 39 species were recently created during the study. Fourteen of 18 rare species exclusively identified in the CPI period were recently created. Twenty-five of 39 rare species identified in the MALDI-TOF MS period were recently created in our database. Other 14 rare species that were present in the database but that needed molecular identification in the MALDI-TOF MS period were *Actinomyces europaeus* (2 isolates), *Corynebacterium argentoratense* (2 isolates), *Corynebacterium confusum* (1), *Corynebacterium coyleae* (4 isolates), *Corynebacterium imitans* (1 isolate), *Corynebacterium kroppenstedtii* (1 isolate), *Corynebacterium mucifaciens* (3 isolates), *Corynebacterium riegelii* (1 isolate), *Corynebacterium ureicelerivorans* (1 isolate), *Microbacterium aurum* (1



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
N° of rare species	3	1	4	2	11	15	24	41	46	26	29
N° of rare species by PID	3	1	1	0	10	6	3	12	17	20	18
Isolate of rare species by molecular ID	0	0	3	2	1	9	21	29	29	6	11

FIG 6 Time course for the numbers of species identified among 128 rare species, 48 of which were identified using phenotypic identification (PID) and 75 of which were identified using molecular identification (ID).

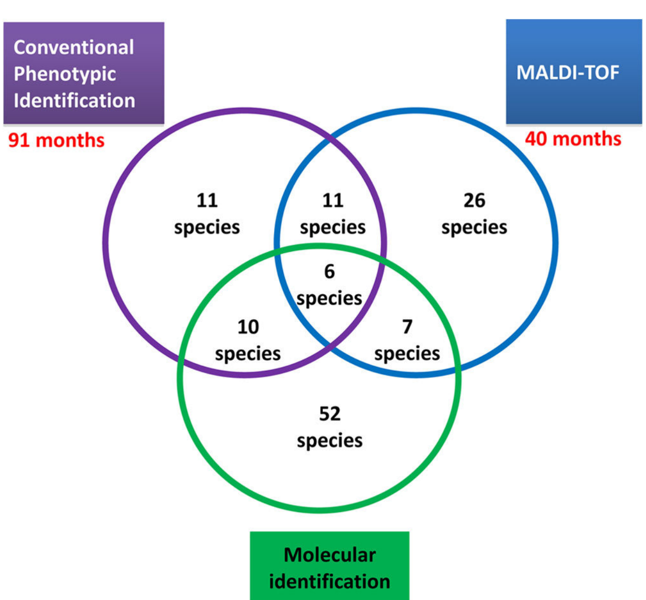


FIG 7 Of 48 rare species identified using phenotypic techniques, MALDI-TOF MS identified 37 rare species and conventional phenotypic identification identified 22 rare species in 40 and 91 months of study, respectively. Seventy-five rare species were identified using molecular techniques.

isolate), *Streptococcus criceti* (3 isolates), *Streptococcus peroris* (1 isolate), *Enterobacter kobei* (3 isolates), and *Pandoraea pulmonicola* (3 isolates).

The time required for identification of one clinical isolate using MALDI-TOF MS was 6 to 8 min 30 s for the AutoFlex II system (Brüker Daltonik) and 1 min 46 s for the MicroFlex LT mass spectrometer (Brüker Daltonik). The cost of identification of one clinical isolate using MALDI-TOF MS was 1.43 euros for the AutoFlex II system (Brüker Daltonik) and 1.35 euros for the MicroFlex LT mass spectrometer (Brüker Daltonik) (Table 4). In comparison, the time required for identification for one clinical isolate using 16S rRNA or *rpoB* sequencing was 24 h. In addition, the cost of bacterial isolate identification using gene sequencing was 137.70 euros.

DISCUSSION

During the last 11 years, our clinical laboratory has seen an increased ability to analyze bacteriological samples due to several reasons: first, the establishment of another laboratory at the North University Hospital, Marseille, France, and second, the creation of an anaerobic laboratory in September 2008. By optimizing the new tool of MALDI-TOF mass spectrometry for routine identification, we were able to increase our yearly analysis capacity from 46,079 analyses in 2002 to 66,989 in 2012.

In 2008, we evaluated the performance of MALDI-TOF MS to identify 1,660 clinical isolates in a 16-week period by comparing it

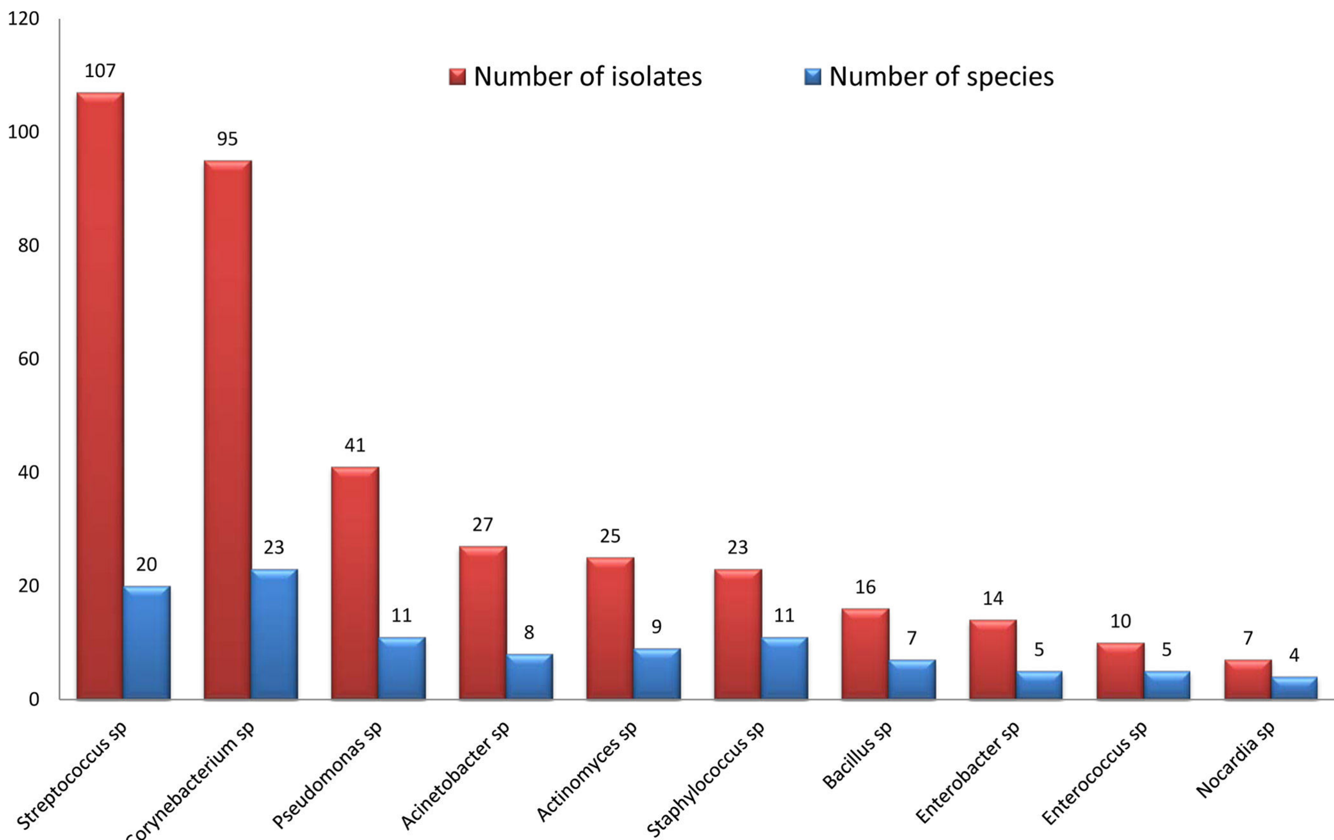


FIG 8 Ten genera of 365 (71%) isolates that frequently required molecular identification among 196 species of 516 isolates identified unsatisfactorily in the MALDI-TOF period.

with routine phenotypic identification methods, such as semiautomated Gram staining (Aerospray Wescor; Elitech), catalase and oxidase assays and automated identifications using the Vitek 2 and API 20A systems (bioMérieux). Since then, more than 300 scientific publications have confirmed that MALDI-TOF MS can be adapted to achieve performances similar to the routine identification methods used in clinical laboratories (14, 50–53). Many clinical laboratories have, like us, adopted bacterial identification using MALDI-TOF MS for biotyping microbes to replace all of the traditional phenotypic methods used for routine diagnoses directly from colony or clinical samples (13, 45, 54–58).

Recently, MALDI-TOF MS was used in culturomics studies to identify 32 new bacterial species and another 177 bacterial species that had never been reported to occur in the human gut microbiota that may explain the involvement of microorganisms in human diseases such as obesity (59, 60). MALDI-TOF MS has been used to identify 233 of 349 bacterial species from 4 stool samples by direct identification from 36,500 colonies. MALDI-TOF MS has also identified 116 unknown bacterial species with the score $<$ 1.9 that was needed to identify by 16S rRNA gene sequencing. Seventy-one of 116 (61%) bacterial species were previously absent in our MALDI-TOF database. Among 45 (39%) species present in our MALDI-TOF database, 24 (20%) have only 1 reference spectrum, and only one serovar of 18 serovars of *Acinetobacter pittii* has more than 10 spectra in the database (59–61). We used an incremental database with each spectra identified by 16S rRNA gene sequencing from the first three stool samples that allowed us to use the culturomics study of Dubourg et al. (61) for the fourth stool sample; in the study of Dubourg et al., only 4 of 4,000 bacterial colonies needed molecular identification (61).

The capacity of MALDI-TOF MS to identify an unknown bacterial species before molecular identification has been previously observed by Bizzini et al. (62) and confirmed after updating the MALDI-TOF database. Among 410 bacterial strains that were not satisfactorily identified by the Vitek 2 and API systems (bioMérieux), 62% of them were concordantly identified by MALDI-TOF MS and 16S rRNA gene sequencing. Failure to identify 85 other bacterial species was due to the absence of spectra of 78 species in the MALDI-TOF database (62).

The 196 species (516 isolates) that were not identified included 57 rare bacterial species present in the MALDI-TOF database that needed molecular identification in the MALDI-TOF period can be attributed to two causes. The first cause is the absence of reference spectrum. The second cause was the presence of a low number of spectra in the database that does not allow MALDI-TOF to identify the bacteria in the groups with biodiversity within species. As an example, 10 genera that frequently needed molecular identification in the MALDI-TOF MS period in spite of the presence of some reference spectra were *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Acinetobacter*, *Actinomyces*, *Staphylococcus*, *Bacillus*, *Enterobacter*, *Enterococcus*, and *Nocardia*.

In addition to the capacity to analyze more isolates as shown in the present study, MALDI-TOF MS has annually identified 2.5 times more species than CPI, identifying 112 species (i.e., 36 spe-

TABLE 2 Species of clinical isolates that were identified by phenotypic identification as species that had been rarely reported as human pathogens[a]

Phylum	Genus	Bacterial rare species identified by PID	No. of isolates	Identification method(s)	No. of isolates identified by CPI	No. of isolates identified by MALDI-TOF MS	No. of reports in PubMed
Actinobacteria	*Actinobaculum*	*Actinobaculum massiliense*	1	MALDI-TOF MS	0	1	4
	Actinomadura	*Actinomadura cremea*	1	CPI	1	0	6
	Actinomyces	*Actinomyces europaeus*	12	CPI and MALDI-TOF MS	3	9	9
		Actinomyces radicidentis	3	MALDI-TOF MS	0	3	4
		Actinomyces radingae	20	CPI and MALDI-TOF MS	5	15	10
	Arthrobacter	*Arthrobacter cumminsii*	5	CPI and MALDI-TOF MS	3	2	4
	Brevibacterium	*Brevibacterium luteolum*	1	CPI	1	0	4
		Brevibacterium massiliense	1	MALDI-TOF MS	0	1	2
		Brevibacterium paucivorans	1	MALDI-TOF MS	0	1	3
		Brevibacterium ravenspurgense	1	MALDI-TOF MS	0	1	0
	Corynebacterium	*Corynebacterium auriscanis*	3	CPI	3	0	5
		Corynebacterium coyleae	7	CPI and MALDI-TOF MS	2	5	7
		Corynebacterium fastidiosum	2	MALDI-TOF MS	0	2	0
		Corynebacterium imitans	2	MALDI-TOF MS	0	2	2
		Corynebacterium mucifaciens	5	MALDI-TOF MS	0	5	6
	Microbacterium	*Microbacterium schleiferi*	1	MALDI-TOF MS	0	1	6
	Pseudoclavibacter	*Pseudoclavibacter bifida*	1	CPI	1	0	1
	Varibaculum	*Varibaculum cambriense*	2	CPI	3	9	2
Bacteroidetes	*Alistipes*	*Alistipes finegoldii*	3	CPI and MALDI-TOF MS	0	3	4
	Bacteroides	*Bacteroides cellulosilyticus*	4	MALDI-TOF MS	5	15	2
	Butyricimonas	*Butyricimonas virosa*	1	MALDI-TOF MS	3	2	1
	Porphyromonas	*Porphyromonas somerae*	9	CPI and MALDI-TOF MS	1	0	1
	Prevotella	"*Candidatus* Prevotella conceptionensis"	3	CPI and MALDI-TOF MS	0	1	1
		Prevotella massiliensis	1	CPI	0	1	2
Firmicutes	*Acidaminococcus*	*Acidaminococcus intestini*	2	CPI and MALDI-TOF MS	0	1	2
	Anaerococcus	*Anaerococcus lactolyticus*	3	MALDI-TOF MS	3	40	9
		Anaerococcus octavius	7	MALDI-TOF MS	2	5	3
	Eubacterium	*Eubacterium tenue*	2	MALDI-TOF MS	0	2	6
		Eubacterium yurii	1	MALDI-TOF MS	0	2	10
	Facklamia	*Facklamia languida*	1	CPI	0	5	2
	Peptoniphilus	*Peptoniphilus harei*	95	CPI and MALDI-TOF MS	0	1	7
	Robinsoniella	*Robinsoniella peoriensis*	3	MALDI-TOF MS	1	0	8
	Sporosarcina	*Sporosarcina ginsengisoli*	1	CPI	2	0	1
	Streptococcus	*Streptococcus massiliensis*	4	MALDI-TOF MS	1	2	1
	Turicibacter	*Turicibacter sanguinis*	3	CPI and MALDI-TOF MS	0	4	3
	Veillonella	*Veillonella montpellierensis*	1	MALDI-TOF MS	0	1	3
Fusobacteria	*Leptotrichia*	*Leptotrichia goodfellowii*	1	CPI	1	8	5
		Leptotrichia trevisanii	3	CPI	1	2	3
Proteobacteria	*Acinetobacter*	*Acinetobacter parvus*	2	MALDI-TOF MS	1	0	8
	Comamonas	*Comamonas kerstersii*	2	MALDI-TOF MS	1	1	3
	Enterobacter	*Enterobacter cowanii*	3	MALDI-TOF MS	0	3	9
		Enterobacter kobei	272	MALDI-TOF MS	0	7	10
	Ochrobactrum	*Ochrobactrum grignonense*	1	MALDI-TOF MS	0	2	8
	Pandoraea	*Pandoraea pulmonicola*	31	MALDI-TOF MS	0	1	7
	Paracoccus	*Paracoccus yeeii*	2	CPI and MALDI-TOF MS	1	0	1
	Pseudomonas	*Pseudomonas hibiscicola*	2	MALDI-TOF MS	11	84	4
	Roseomonas	*Roseomonas ludipueritiae*	1	CPI	0	3	4
	Serratia	*Serratia ureilytica*	1	MALDI-TOF MS	1	0	6

[a] List of 48 species of 534 clinical isolates that were identified by phenotypic identification as species that had been rarely reported as human pathogens, with ≤10 reports in PubMed. PID, phenotypic identification; CPI, conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).

cies/10,000 isolates) compared with 44 species (i.e., 19 species/10,000 isolates), respectively. This performance of MALDI-TOF MS in annually identifying more species per isolate tested can be explained first by the increasing numbers of colonies analyzed from each clinical sample and a tendency to identify systematically all isolates from a polymicrobial clinical specimen. Second, the MALDI-TOF database is now 10 times larger than the Vitek 2 database (bioMérieux, Durham, NC), with 6,213 reference strains compared with 330 reference strains, respectively.

Another benefit of MALDI-TOF MS in routine identification revealed in this study is the reduced need for secondary phenotypic identification, which significantly decreased the cost and time required to provide results to clinicians. Only 50 secondary phenotypic identifications of 110,263 clonal-bacterial isolates tested (i.e., 4.5 reidentifications/10,000 isolates) were required during the MALDI-TOF MS period compared with 620 of 175,999 isolates during the CPI period (i.e., 35.2 reidentifications/10,000 isolates).

TABLE 3 Rare bacterial species identified using molecular identification[a]

Phylum	Genus	Bacterial species confirmed by molecular identification	No. of isolates	No. of isolates identified in the CPI period	No. of isolates identified in the MALDI-TOF MS period	No. of reports in PubMed	48 rare species by PID	Presence/absence of species in our MALDI-TOF MS database	Presence/absence of species in MALDI-TOF MS database (Brüker)
Actinobacteria	*Actinomyces*	*Actinomyces europaeus*	3	1	2	9	Yes	Present	Present
		Actinomyces lingnae	1	0	1	1	No	Absent	Absent
		Actinomyces radingae	5	3	2	10	Yes	Present	Absent
		Actinomyces urogenitalis	2	0	2	4	No	Present	Absent
	Arthrobacter	*Arthrobacter cumminsii*	5	4	1	4	Yes	Present	Absent
	Bifidobacterium	*Bifidobacterium scardovii*	1	1	0	5	No	Present	Absent
	Brachybacterium	*Brachybacterium muris*	1	0	1	3	No	Present	Absent
		Brachybacterium sacelli	1	0	1	3	No	Absent	Absent
	Brevibacterium	*Brevibacterium massiliense*	1	1	0	2	Yes	Absent	Absent
		Brevibacterium otitidis	1	1	0	9	No	Absent	Absent
		Brevibacterium paucivorans	2	1	1	3	Yes	Present	Absent
		Brevibacterium ravenspurgense	1	1	0	0	Yes	Present	Absent
		Brevibacterium sanguinis	1	1	0	2	No	Present	Absent
		Brevibacterium stationis	1	0	1	10	No	Present	Absent
	Corynebacterium	*Corynebacterium argentoratense*	2	0	2	3	No	Present	Present
		Corynebacterium auriscanis	3	3	0	5	Yes	Present	Present
		Corynebacterium confusum	1	0	1	2	No	Present	Present
		Corynebacterium coyleae	4	0	4	7	Yes	Present	Present
		Corynebacterium durum	1	1	0	3	No	Present	Absent
		Corynebacterium fastidiosum	1	0	1	0	Yes	Absent	Absent
		Corynebacterium imitans	1	0	1	2	Yes	Present	Present
		Corynebacterium kroppenstedtii	1	0	1	9	No	Present	Present
		Corynebacterium mucifaciens	3	0	3	6	Yes	Present	Present
		Corynebacterium riegelii	1	0	1	6	No	Present	Present
		Corynebacterium ureicelerivorans	1	0	1	3	No	Present	Present
	Dietzia	*Dietzia cinnamea*	1	1	0	10	No	Present	Absent
	Janibacter	*Janibacter hoylei*	1	0	1	2	No	Present	Absent
	Microbacterium	*Microbacterium aurum*	2	1	1	5	No	Present	Present
		Microbacterium chocolatum	1	1	0	1	No	Absent	Absent
		Microbacterium flavum	1	0	1	5	No	Present	Absent
	Nesterenkonia	*Nesterenkonia lacusekhoensis*	1	0	1	4	No	Present	Absent
	Propionimicrobium	*Propionimicrobium lymphophilum*	2	1	1	3	No	Present	Absent
	Trueperella	*Trueperella abortisuis*	1	1	0	5	No	Present	Absent
	Zimmermannella	*Zimmermannella bifida*	1	1	0	1	Yes	Absent	Absent
Bacteroidetes	*Alistipes*	*Alistipes finegoldii*	1	1	0	4	Yes	Present	Absent
	Bacteroides	*Bacteroides dorei*	1	1	0	8	No	Absent	Absent
	Butyricimonas	*Butyricimonas virosa*	2	0	2	1	Yes	Present	Absent
	Chryseobacterium	*Chryseobacterium hominis*	1	0	1	4	No	Present	Absent
		Chryseobacterium vrystaatense	1	0	1	3	No	Absent	Absent
	Peptoniphilus	*Candidatus Peptoniphilus massiliensis*	1	0	1	0	No	Absent	Absent
	Porphyromonas	*Porphyromonas uenonis*	4	4	0	2	No	Present	Absent
	Prevotella	"*Candidatus* Prevotella conceptionensis"	1	1	0	1	Yes	Present	Absent
	Wautersiella	*Wautersiella falsenii*	2	1	1	4	No	Present	Absent
Firmicutes	*Aerosphaera*	*Aerosphaera taetra*	1	1	0	0	No	Present	Absent
	Anaerococcus	*Anaerococcus octavius*	2	2	0	3	Yes	Present	Absent
	Anaerotruncus	*Anaerotruncus colihominis*	2	1	1	2	No	Present	Absent
	Lysinibacillus	*Lysinibacillus massiliensis*	1	0	1	8	No	Absent	Absent
	Catabacter	*Catabacter hongkongensis*	1	1	0	6	No	Absent	Absent
	Clostridium	*Clostridium aldenense*	1	0	1	3	No	Present	Absent
	Dialister	*Dialister micraerophilus*	1	0	1	3	No	Present	Absent
	Granulicatella	*Granulicatella para-adiacens*	1	0	1	2	No	Present	Absent
	Peptoniphilus	*Peptoniphilus harei*	3	2	1	7	Yes	Present	Absent
	Streptococcus	*Streptococcus criceti*	3	0	3	10	No	Present	Present

(Continued on following page)

TABLE 3 (Continued)

Phylum	Genus	Bacterial species confirmed by molecular identification	No. of isolates	No. of isolates identified in the CPI period	No. of isolates identified in the MALDI-TOF MS period	No. of reports in PubMed	48 rare species by PID	Presence/absence of species in our MALDI-TOF MS database[b]	Presence/absence of species in MALDI-TOF MS database (Brüker)[b]
		Streptococcus massiliensis	2	2	0	1	Yes	Present	Present
		Streptococcus peroris	1	0	1	6	No	Present	Present
	Turicibacter	*Turicibacter sanguinis*	1	1	0	3	Yes	Present	Absent
Fusobacteria	*Leptotrichia*	*Leptotrichia trevisanii*	5	4	1	3	Yes	Present	Absent
Proteobacteria	*Acetobacter*	*Acetobacter indonesiensis*	2	2	0	9	No	Absent	Absent
	Acinetobacter	*Acinetobacter parvus*	1	1	0	8	Yes	Present	Present
		Acinetobacter septicus	5	4	1	3	No	Present	Absent
	Aurantimonas	*Aurantimonas altamirensis*	1	0	1	9	No	Present	Absent
	Blastomonas	*Blastomonas ursincola*	1	1	0	5	No	Present	Present
	Desulfovibrio	*Desulfovibrio intestinalis*	1	1	0	5	No	Absent	Absent
	Enterobacter	*Enterobacter kobei*	3	0	3	10	Yes	Present	Present
	Hematobacter	*Hematobacter massiliensis*	3	1	2	2	No	Absent	Absent
	Pandoraea	*Pandoraea pulmonicola*	3	0	3	7	Yes	Present	Present
	Pantoea	*Pantoea brenneri*	1	0	1	1	No	Absent	Absent
		Pantoea eucrina	1	0	1	2	No	Present	Absent
	Pseudochrobactrum	*Pseudochrobactrum asaccharolyticum*	1	0	1	2	No	Present	Absent
	Pseudomonas	*Pseudomonas lurida*	1	0	1	3	No	Present	Absent
	Ralstonia	*Ralstonia insidiosa*	1	0	1	5	No	Present	Absent
	Roseomonas	*Roseomonas genomospecies 5*	1	1	0	6	No	Absent	Absent
	Rothia	*Rothia aeria*	1	1	0	8	No	Present	Absent
	Serratia	*Serratia nematodiphila*	1	0	1	3	No	Absent	Absent
	Sphingomonas	*Sphingomonas mucosissima*	1	1	0	2	No	Present	Absent

[a] List of 75 rare bacterial species identified using molecular identification; 18 of these species were absent from our MALDI-TOF database, and 57 species from the Brüker database. PID, phenotypic identification; CPI, conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).

Over 3 years of experience in routine identification using MALDI-TOF MS, we observed a rise in the numbers of isolates and species that were identified using MALDI-TOF MS. The ability to expand the database by incorporation of laboratory spectra for bacteria that had been identified previously by molecular techniques has improved the performance of MALDI-TOF MS in identifying human-pathogenic bacteria.

TABLE 4 Comparison of time, cost, and level of training required for routine identification of one isolate using the different techniques in our clinical laboratory

Identification technique	Time required for identification of one isolate	Cost (euros)	Level of training
Gram staining	6 min	0.6	Medium to high
API system identification (bioMérieux)	18–48 h	4.6–6	Medium
Vitek 2 system identification (bioMérieux)	5–8 h	5.9–8.23	Medium
Molecular identification by 16S rRNA or *rpoB* sequencing	24 h	137.7	Medium to high
MALDI-TOF MS by AutoFlex II system (Brüker Daltonik)	6–8 min 30 s	1.43	Low to medium
MALDI-TOF MS by MicroFlex LT mass spectrometer (Brüker Daltonik)	1 min 46 s	1.35	Low to medium

Interestingly, MALDI-TOF MS identified more bacterial species that had been rarely reported as human pathogens than CPI did. A total of 37 of 48 rare species (77%) identified by phenotypic techniques were identified using MALDI-TOF MS. A systematic identification of all colonies derived from clinical samples will increase the capacity to identify more rare species in the future.

We also evaluated the time and cost-effectiveness of MALDI-TOF MS, which reduced by 55-fold and 169-fold the time required for identification and reduced by 5- and 96-fold the cost compared with CPI and gene sequencing, respectively (12). The time required for identification has been newly improved to 1 min 46 s using the MicroFlex LT mass spectrometer (Brüker Daltonik) compared with the AutoFlex II system, which took 6 to 8 min 30 s for identification of one isolate. The cost was evaluated at 1.35 euros for the MicroFlex LT mass spectrometer and 1.43 euros for the AutoFlex II system.

Conclusion. We have shown the effectiveness and performance of MALDI-TOF MS in the identification of clinical isolates and bacterial species in routine bacterial identification in a clinical laboratory over 11 years of study.

The ability of MALDI-TOF MS to identify a large number of bacterial species well is leading many clinical laboratories to abandon traditional phenotypic identification. We have shown that MALDI-TOF MS is not only a powerful tool for routine bacterial identification in the clinical laboratory but also a powerful tool to identify rare bacterial species implicated in human infectious diseases.

This capacity to identify rare species as human pathogens using MALDI-TOF MS could be an alternative to molecular methods in the clinical laboratory. The rapid identification of bacterial species that were rarely or never previously described as pathogens in specific clinical specimens will help us to study the clinical burden due to the emergence of these species as human pathogens and to implement their real-time surveillance.

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REFERENCES

1. **Woo PC, Lau SK, Teng JL, Tse H, Yuen KY.** 2008. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Infect. **14:**908–934.
2. **Drancourt M, Raoult D.** 2005. Sequence-based identification of new bacteria: a proposition for creation of an orphan bacterium repository. J. Clin. Microbiol. **43:**4311–4315.
3. **Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D.** 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clin. Microbiol. **38:**3623–3630.
4. **Drancourt M, Berger P, Raoult D.** 2004. Systematic 16S rRNA gene sequencing of atypical clinical isolates identified 27 new bacterial species associated with humans. J. Clin. Microbiol. **42:**2197–2202.
5. **Fenollar F, Roux V, Stein A, Drancourt M, Raoult D.** 2006. Analysis of 525 samples to determine the usefulness of PCR amplification and sequencing of the 16S rRNA gene for diagnosis of bone and joint infections. J. Clin. Microbiol. **44:**1018–1028.
6. **Janda JM, Abbott SL.** 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J. Clin. Microbiol. **45:**2761–2764.
7. **Al Masalma M, Armougom F, Scheld WM, Dufour H, Roche PH, Drancourt M, Raoult D.** 2009. The expansion of the microbiological spectrum of brain abscesses with use of multiple 16S ribosomal DNA sequencing. Clin. Infect. Dis. **48:**1169–1178.
8. **Schlaberg R, Simmon KE, Fisher MA.** 2012. A systematic approach for discovering novel, clinically relevant bacteria. Emerg. Infect. Dis. **18:**422–430.
9. **Teles C, Smith A, Ramage G, Lang S.** 2011. Identification of clinically relevant viridans group streptococci by phenotypic and genotypic analysis. Eur. J. Clin. Microbiol. Infect. Dis. **30:**243–250.
10. **Ikryannikova LN, Lapin KN, Malakhova MV, Filimonova AV, Ilina EN, Dubovickaya VA, Sidorenko SV, Govorun VM.** 2011. Misidentification of alpha-hemolytic streptococci by routine tests in clinical practice. Infect. Genet. Evol. **11:**1709–1715.
11. **Maeda Y, Goldsmith CE, Coulter WA, Mason C, Dooley JS, Lowery CJ, Millar BC, Moore JE.** 2011. Comparison of five gene loci (rnpB, 16S rRNA, 16S-23S rRNA, sodA and dnaJ) to aid the molecular identification of viridans-group streptococci and pneumococci. Br. J. Biomed. Sci. **68:**190–196.
12. **Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D.** 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis. **49:**543–551.
13. **La Scola B, Raoult D.** 2009. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One **4:**e8041. doi:10.1371/journal.pone.0008041.
14. **Seng P, Rolain JM, Fournier PE, La Scola B, Drancourt M, Raoult D.** 2010. MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol. **5:**1733–1754.
15. **Drancourt M.** 2010. Detection of microorganisms in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review. Clin. Microbiol. Infect. **16:**1620–1625.
16. **Bittar F, Cassagne C, Bosdure E, Stremler N, Dubus JC, Sarles J, Reynaud-Gaubert M, Raoult D, Rolain JM.** 2010. Outbreak of Corynebacterium pseudodiphtheriticum infection in cystic fibrosis patients, France. Emerg. Infect. Dis. **16:**1231–1236.
17. **Williamson YM, Moura H, Woolfitt AR, Pirkle JL, Barr JR, Carvalho MDAG, Ades EP, Carlone GM, Sampson JS.** 2008. Differentiation of *Streptococcus pneumoniae* conjunctivitis outbreak isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl. Environ. Microbiol. **74:**5891–5897.
18. **Griffin PM, Price GR, Schooneveldt JM, Schlebusch S, Tilse MH, Urbanski T, Hamilton B, Venter D.** 2012. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry to identify vancomycin-resistant enterococci and investigate the epidemiology of an outbreak. J. Clin. Microbiol. **50:**2918–2931.
19. **Werno AM, Christner M, Anderson TP, Murdoch DR.** 2012. Differentiation of *Streptococcus pneumoniae* from nonpneumococcal streptococci of the *Streptococcus mitis* group by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **50:**2863–2867.
20. **Hinic V, Lang C, Weisser M, Straub C, Frei R, Goldenberger D.** 2012. *Corynebacterium tuberculostearicum*: a potentially misidentified and multiresistant *Corynebacterium* species isolated from clinical specimens. J. Clin. Microbiol. **50:**2561–2567.
21. **Djelouadji Z, Roux V, Raoult D, Kodjo A, Drancourt M.** 2012. Rapid MALDI-TOF mass spectrometry identification of Leptospira organisms. Vet. Microbiol. **158:**142–146.
22. **Alvarez-Buylla A, Culebras E, Picazo JJ.** 2012. Identification of Acinetobacter species: is Bruker biotyper MALDI-TOF mass spectrometry a good alternative to molecular techniques? Infect. Genet. Evol. **12:**345–349.
23. **Lista F, Reubsaet FA, De Santis R, Parchen RR, de Jong AL, Kieboom J, van der Laaken AL, Voskamp-Visser IA, Fillo S, Jansen HJ, Van der Plas J, Paauw A.** 2011. Reliable identification at the species level of Brucella isolates with MALDI-TOF-MS. BMC Microbiol. **11:**267. doi:10.1186/1471-2180-11-267.
24. **Fournier PE, Couderc C, Buffet S, Flaudrops C, Raoult D.** 2009. Rapid and cost-effective identification of Bartonella species using mass spectrometry. J. Med. Microbiol. **58:**1154–1159.
25. **Hrabak J, Walkova R, Studentova V, Chudackova E, Bergerova T.** 2011. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **49:**3222–3227.
26. **Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunel JM, Drissi M, Mesli E, Touati A, Rolain JM.** 2012. Rapid detection of carbapenem resistance in Acinetobacter baumannii using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One **7:**e31676. doi:10.1371/journal.pone.0031676.
27. **Edwards-Jones V, Claydon MA, Evason DJ, Walker J, Fox AJ, Gordon DB.** 2000. Rapid discrimination between methicillin-sensitive and methicillin-resistant Staphylococcus aureus by intact cell mass spectrometry. J. Med. Microbiol. **49:**295–300.
28. **Walker J, Fox AJ, Edwards-Jones V, Gordon DB.** 2002. Intact cell mass spectrometry (ICMS) used to type methicillin-resistant Staphylococcus aureus: media effects and inter-laboratory reproducibility. J. Microbiol. Methods **48:**117–126.
29. **Jackson KA, Edwards-Jones V, Sutton CW, Fox AJ.** 2005. Optimisation of intact cell MALDI method for fingerprinting of methicillin-resistant Staphylococcus aureus. J. Microbiol. Methods **62:**273–284.
30. **Du Z, Yang R, Guo Z, Song Y, Wang J.** 2002. Identification of Staphylococcus aureus and determination of its methicillin resistance by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Chem. **74:**5487–5491.
31. **Rajakaruna L, Hallas G, Molenaar L, Dare D, Sutton H, Encheva V, Culak R, Innes I, Ball G, Sefton AM, Eydmann M, Kearns AM, Shah HN.** 2009. High throughput identification of clinical isolates of Staphylococcus aureus using MALDI-TOF-MS of intact cells. Infect. Genet. Evol. **9:**507–513.
32. **Majcherczyk PA, McKenna T, Moreillon P, Vaudaux P.** 2006. The discriminatory power of MALDI-TOF mass spectrometry to differentiate between isogenic teicoplanin-susceptible and teicoplanin-resistant strains of methicillin-resistant Staphylococcus aureus. FEMS Microbiol. Lett. **255:**233–239.
33. **Camara JE, Hays FA.** 2007. Discrimination between wild-type and ampicillin-resistant Escherichia coli by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Bioanal. Chem. **389:**1633–1638.
34. **Russell SC, Edwards N, Fenselau C.** 2007. Detection of plasmid insertion

in Escherichia coli by MALDI-TOF mass spectrometry. Anal. Chem. **79:** 5399–5406.
35. **Sparbier K, Schubert S, Weller U, Boogen C, Kostrzewa M.** 2012. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against beta-lactam antibiotics. J. Clin. Microbiol. **50:**927–937.
36. **Zbinden A, Mueller NJ, Tarr PE, Eich G, Schulthess B, Bahlmann AS, Keller PM, Bloemberg GV.** 2012. *Streptococcus tigurinus*, a novel member of the *Streptococcus mitis* group, causes invasive infections. J. Clin. Microbiol. **50:**2969–2973.
37. **Tani A, Sahin N, Matsuyama Y, Enomoto T, Nishimura N, Yokota A, Kimbara K.** 2012. High-throughput identification and screening of novel Methylobacterium species using whole-cell MALDI-TOF/MS analysis. PLoS One **7:**e40784. doi:10.1371/journal.pone.0040784.
38. **Chan JF, Lau SK, Curreem SO, To KK, Leung SS, Cheng VC, Yuen KY, Woo PC.** 2012. First report of spontaneous intrapartum *Atopobium vaginae* bacteremia. J. Clin. Microbiol. **50:**2525–2528.
39. **Gouriet F, Million M, Henri M, Fournier PE, Raoult D.** 2012. Lactobacillus rhamnosus bacteremia: an emerging clinical entity. Eur. J. Clin. Microbiol. Infect. Dis. **31:**2469–2480.
40. **Angelakis E, Million M, Henry M, Raoult D.** 2011. Rapid and accurate bacterial identification in probiotics and yoghurts by MALDI-TOF mass spectrometry. J. Food Sci. **76:**M568–M572.
41. **Dridi B, Raoult D, Drancourt M.** 2012. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of Archaea: towards the universal identification of living organisms. APMIS **120:**85–91.
42. **Fernandez-Olmos A, Morosini MI, Lamas A, Garcia-Castillo M, Garcia-Garcia L, Canton R, Maiz L.** 2012. Clinical and microbiological features of a cystic fibrosis patient chronically colonized with *Pandoraea* sputorum identified by combining 16S rRNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **50:**1096–1098.
43. **Ng LS, Sim JH, Eng LC, Menon S, Tan TY.** 2012. Comparison of phenotypic methods and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry for the identification of aero-tolerant Actinomyces spp. isolated from soft-tissue infections. Eur. J. Clin. Microbiol. Infect. Dis. **31:**1749–1752.
44. **Huber H, Ziegler D, Pfluger V, Vogel G, Zweifel C, Stephan R.** 2011. Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet. Res. **7:**6. doi:10.1186/1746-6148-7-6.
45. **La Scola B, Fournier PE, Raoult D.** 2011. Burden of emerging anaerobes in the MALDI-TOF and 16S rRNA gene sequencing era. Anaerobe **17:** 106–112.
46. **Carroll KC, Weinstein MP.** 2007. Manual and automated systems for detection and identification of microorganisms, p 192–217. *In* Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (ed), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, DC.
47. **Grosse-Herrenthey A, Maier T, Gessler F, Schaumann R, Bohnel H, Kostrzewa M, Kruger M.** 2008. Challenging the problem of clostridial identification with matrix-assisted laser desorption and ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Anaerobe **14:**242–249.
48. **Khamis A, Raoult D, La Scola B.** 2005. Comparison between *rpoB* and 16S rRNA gene sequencing for molecular identification of 168 clinical isolates of *Corynebacterium.* J. Clin. Microbiol. **43:**1934–1936.
49. **Adekambi T, Drancourt M, Raoult D.** 2009. The rpoB gene as a tool for clinical microbiologists. Trends Microbiol. **17:**37–45.
50. **van Veen SQ, Claas EC, Kuijper EJ.** 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J. Clin. Microbiol. **48:**900–907.
51. **Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G.** 2010. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J. Clin. Microbiol. **48:**1549–1554.
52. **Cherkaoui A, Hibbs J, Emonet S, Tangomo M, Girard M, Francois P, Schrenzel J.** 2010. Comparison of two matrix-assisted laser desorption ionization–time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J. Clin. Microbiol. **48:**1169–1175.
53. **Eigner U, Holfelder M, Oberdorfer K, Betz-Wild U, Bertsch D, Fahr AM.** 2009. Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. Clin. Lab. **55:**289–296.
54. **Stevenson LG, Drake SK, Murray PR.** 2010. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **48:** 444–447.
55. **Christner M, Rohde H, Wolters M, Sobottka I, Wegscheider K, Aepfelbacher M.** 2010. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption-ionization time of flight mass spectrometry fingerprinting. J. Clin. Microbiol. **48:**1584–1591.
56. **Ferreira L, Sanchez-Juanes F, Gonzalez-Avila M, Cembrero-Fucinos D, Herrero-Hernandez A, Gonzalez-Buitrago JM, Munoz-Bellido JL.** 2010. Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **48:**2110–2115.
57. **Christensen JJ, Dargis R, Hammer M, Justesen US, Nielsen XC, Kemp M, Danish MALDI-TOF MS Study Group.** 2012. Matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of Gram-positive, catalase-negative cocci not belonging to the *Streptococcus* or *Enterococcus* genus and benefits of database extension. J. Clin. Microbiol. **50:**1787–1791.
58. **Yan Y, Meng S, Bian D, Quinn C, Li H, Stratton CW, Tang YW.** 2011. Comparative evaluation of Bruker Biotyper and BD Phoenix systems for identification of bacterial pathogens associated with urinary tract infections. J. Clin. Microbiol. **49:**3936–3939.
59. **Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, Trape JF, Koonin EV, La Scola B, Raoult D.** 2012. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin. Microbiol. Infect. **18:**1185–1193.
60. **Lagier JC, Million M, Hugon P, Armougom F, Raoult D.** 2012. Human gut microbiota: repertoire and variations. Front. Cell. Infect. Microbiol. **2:**136. doi:10.3389/fcimb.2012.00136.
61. **Dubourg G, Lagier JC, Armougom F, Robert C, Hamad I, Brouqui P, Raoult D.** 2013. The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics. Eur. J. Clin. Microbiol. Infect. Dis. **32:**637–645.
62. **Bizzini A, Jaton K, Romo D, Bille J, Prod'hom G, Greub G.** 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-to-identify bacterial strains. J. Clin. Microbiol. **49:**693–696.

Article 4: A real-time microbiology laboratory surveillance system implemented for the detection of abnormal events and emerging infections, Marseille, France.

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Real-Time Microbiology Laboratory Surveillance System to Detect Abnormal Events and Emerging Infections, Marseille, France

Cédric Abat, Hervé Chaudet, Philippe Colson, Jean-Marc Rolain, Didier Raoult

Infectious diseases are a major threat to humanity, and accurate surveillance is essential. We describe how to implement a laboratory data–based surveillance system in a clinical microbiology laboratory. Two historical Microsoft Excel databases were implemented. The data were then sorted and used to execute the following 2 surveillance systems in Excel: the Bacterial real-time Laboratory-based Surveillance System (BALYSES) for monitoring the number of patients infected with bacterial species isolated at least once in our laboratory during the study periodl and the Marseille Antibiotic Resistance Surveillance System (MARSS), which surveys the primary β-lactam resistance phenotypes for 15 selected bacterial species. The first historical database contained 174,853 identifications of bacteria, and the second contained 12,062 results of antibiotic susceptibility testing. From May 21, 2013, through June 4, 2014, BALYSES and MARSS enabled the detection of 52 abnormal events for 24 bacterial species, leading to 19 official reports. This system is currently being refined and improved.

Although infectious diseases were declared under control and considered to be a past public health problem during the second half of the 20th century (*1*), these diseases, including those that are well-known, emerging, and reemerging, remain a major threat to humanity. Indeed, infectious pathogens possess an amazing common capacity to emerge and spread in unpredictable ways before they are detected by public health institutions (*2*). Infectious diseases have a substantial effect on both global human demographics (they are the second leading cause of death in humans worldwide, accounting for ≈15 million deaths) (*3*) and the economy (*4*), which has led the public health community to reconsider them as a real threat. This alarming observation has led public health authorities to try to improve infectious disease surveillance.

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One of these strategies, known as traditional public health surveillance of infectious diseases, has been to use clinical case reports from sentinel laboratories or laboratory networks and direct reports of positive results from clinical laboratories to survey the presence of microbial agents known to be dangers to health in a precise population (*5*). Some examples of surveillance systems implemented by using this strategy are the National Tuberculosis Surveillance System in the United States (*6*), the surveillance system of the Netherlands Reference Laboratory for Bacterial Meningitis (*7*) and the European Gonococcal Antimicrobial Surveillance Programme (*8*).

Another strategy, known as syndromic surveillance, consists of developing real-time surveillance systems capable of detecting abnormal epidemiologic events, not on the basis of infectious disease diagnosis data, but rather on the basis of nonspecific health indicators, such as absenteeism, chief complaints, and prescription drug sales (*5,9*). Such surveillance systems can be implemented nationally, such as the Emergency Department Syndromic Surveillance System in England (*10*) or the National Retail Data Monitor in the United States (*11*), and regionally, such as the Emergency Department Syndromic Surveillance in Canada (*12*) or the European Antimicrobial Resistance Surveillance Network in Europe (*13*), or the systems can be administered by laboratories with large quantities of data and the financial and human resources to apply the information.

On the basis of our experience at the Assistance Publique–Hôpitaux de Marseille (AP-HM), we describe all the steps necessary for implementing a laboratory data–based syndromic surveillance system in a laboratory. Because of its simplicity, we believe that it can be rapidly applied and used as a first surveillance tool in well-established laboratories. We also show the advantages and limits of this surveillance system.

Materials and Methods

Study Setting

Marseille is the second-most populous French city (estimated population 850,726 persons in 2010). All data

analyzed in this article came from the 4 university hospitals of Marseille (North, South, Conception, and Timone hospitals). Cumulatively, these hospitals represent ≈3,700 beds, including ≈1,500 beds for the Timone Hospital, ≈600 beds for the North Hospital, ≈700 beds for the Conception Hospital, and ≈900 beds for the South Hospital. The AP-HM clinical microbiology laboratory is located at Timone Hospital; the laboratory performed ≈145,000 serologic tests and ≈200,000 PCRs and cultures of microorganisms from 220,000 samples in 2012 (*14*). This amount of data allowed us to implement our own laboratory-data–based syndromic surveillance system.

Organization of Surveillance Activity on Tools of AP-HM

The AP-HM laboratory–based surveillance consists of 3 following syndromic surveillance tools founded on Excel software (Mircosoft Corp., Redmond, WA, USA): 1 previously described system called EPIMIC (EPIdemiological biosurveillance and alert based on MICrobiologic data) (*15,16*), 1 surveillance system implemented for the surveillance of bacterial antibiotic resistance (MARSS, Marseille Antibiotic Resistance Surveillance System), and BALYSES (BActerial real-time LaboratorY-based SurveillancE System), which was developed for the surveillance of the number of patients infected by each bacteria species identified at least once in our laboratory. Our surveillance systems are defined as syndromic surveillance systems because no surveillance data are specifically collected for their use. The flow of information needed for each of the 3 surveillance systems is summarized in Figure 1. However, only BALYSES and MARSS are further described.

All of the data routinely used for the 2 surveillance systems are manually collected from the Timone Hospital laboratory information management systems and processed by using Microsoft Excel software (2007 version). Data are then entered in the 2 surveillance systems according to their nature. The 2 systems automatically compare the entered data with their specific thresholds. Alarms are emitted by the systems if the entered values exceed thresholds. The emitted alarms are analyzed weekly during a specific thematic epidemiology meeting with laboratory staff. If alarms are validated, further investigations are immediately conducted by biologists, clinicians, and medical residents. After the alarm is signaled, our institution's team in charge of nosocomial infections, called the Centre de Coordination de la Lutte contre les Infections Nosocomiales, initiates an investigation. Finally, if these investigations reveal that the alarm events were real epidemiologic events (thereafter called true alarms), official reports can be sent to an official regional public health institution, the Agence Régionale de la Santé (ARS).

Laboratory Data–Based Syndromic Surveillance System

BALYSES

The BALYSES surveillance system was implemented and has been routinely used since January 2013. The first version of BALYSES was implemented to automatically compare the weekly number of samples positive for each bacterial species identified at least once at our institution with the mean historical weekly values ± 2 SDs (Table 1, http://wwwnc.cdc.gov/EID/article/21/8/14-1419-T1htm). In October 2013, BALYSES was improved to survey the weekly number of patients infected by each bacterial species (Figure 2; Table 1). Then, if alarms are emitted that indicate an abnormal increase in the number of isolations of a specific bacterial species, an additional Microsoft Excel interface is used to show more details, including the hospitals and units in which the patients received care, the types of samples from which the bacterial species were isolated, and the patients' identification numbers. BALYSES also automatically classifies the bacterial species from most to least abundant, according to the weekly number of infected patients, and calculates their weekly rank. It finally calculates the maximum number of patients infected by each of the bacterial species monitored, indicates the date of first isolation of the bacterial species at AP-HM, and identifies the historical rank (on the basis of the historical number of patients infected) among the other bacterial species.

MARSS

The MARSS surveillance program has been used since April 2013. Fifteen bacterial species are monitored by MARSS, including *Escherichia coli, Klebsiella pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, *Enterobacter cloacae*, *Enterobacter aerogenes, Morganella morganii*, *Serratia marcescens, Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Streptococcus agalactiae*, *Enterococcus faecalis, Enterococcus faecium*, *Staphylococcus aureus,* and *S. epidermidis*. MARSS automatically compares the weekly number of isolates exhibiting a given β-lactam resistance phenotype to the mean value ± 2 SDs for the historical number of strains harboring this phenotype (Figure 3). Alarms are emitted when this threshold is exceeded. In parallel, MARSS emits alarms for key phenotypes to allow for their rapid identification and verification (Tables 2, 3).

Historical Databases

The detection of abnormal events necessitates the calculation of expected references, previously called historical thresholds. To define the expected references, 2 historical databases were built by using data extracted from the laboratory information management systems of the 4 university hospitals of Marseille. The first historical database consisted

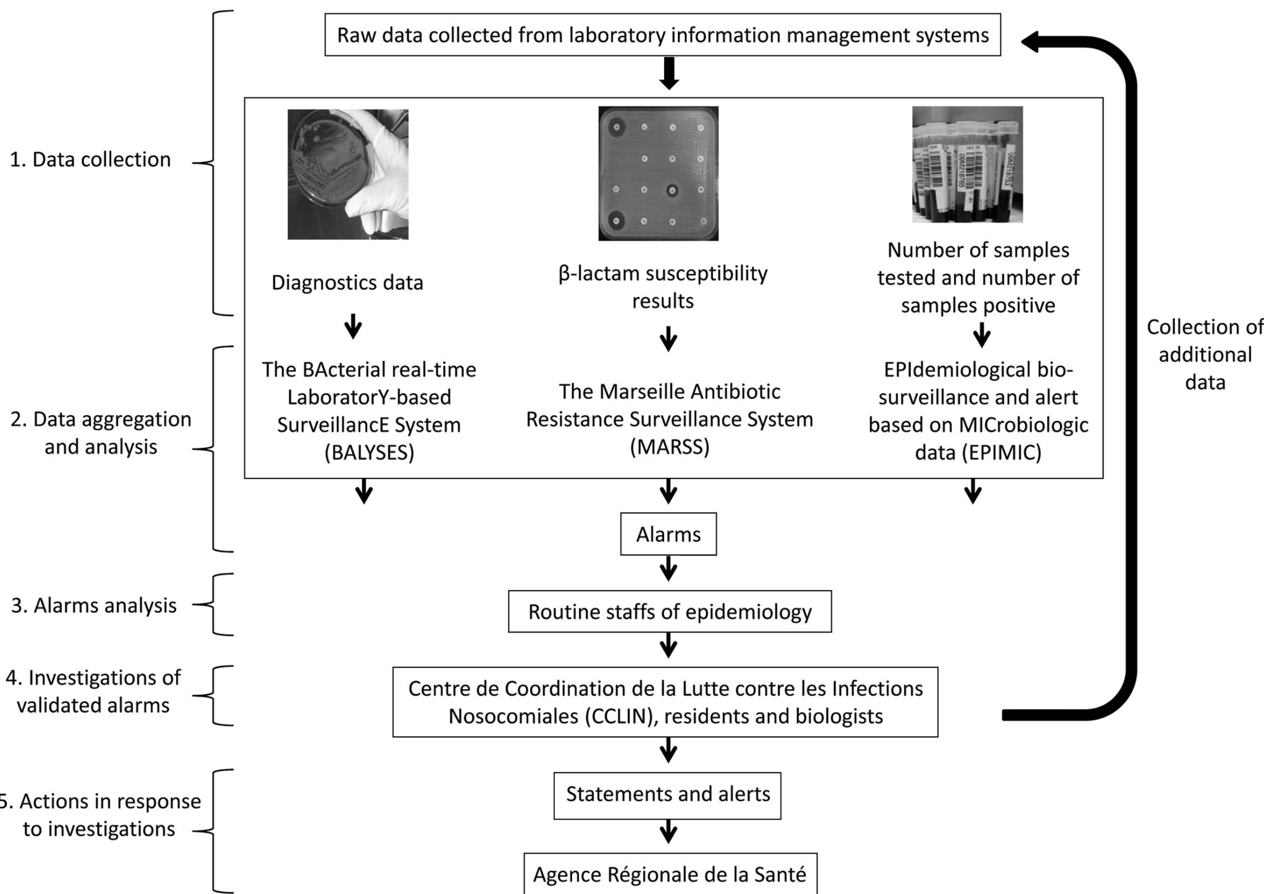


Figure 1. Workflow of real-time surveillance systems used by Institut Hospitalo–Universitaire Méditérranée Infection, Assistance Publique-Hôpitaux de Marseille, Marseille, France.

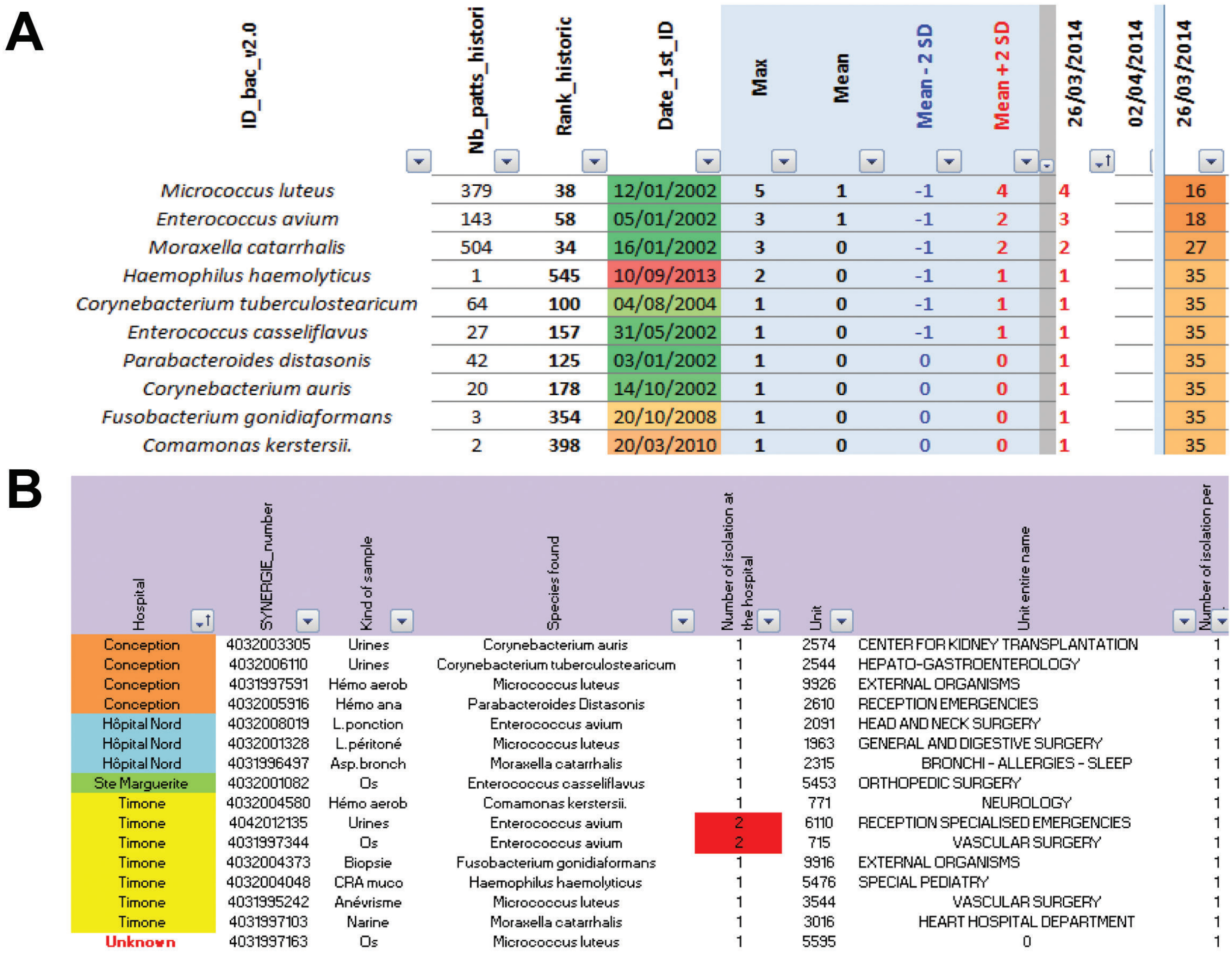
of all of the bacterial identifications obtained from January 2002 to December 2013 (excluding December 2002, data unavailable), including those described in a previous work (*17*), and a second database consisted of most antimicrobial resistance profiles obtained from October 2012 through March 2013. These data were then processed with Microsoft Excel software (2007 version) and sorted. The first database was then sorted, and only samples from which bacterial species were properly identified were conserved. Then, the duplicates for patient and bacterial species were removed. The second database was sorted into different Microsoft Excel spreadsheets for the most frequently isolated bacterial species. Duplicates occurring within the same week were then removed on the basis of the same methods.

Results

Databases and Surveillance Systems

The first version of the 11-year historical BALYSES database contained 161,374 bacterial identifications corresponding to 568 different bacterial species. The 10 most numerous bacterial species were *E. coli* (37,560 patients), *S. aureus* (23,562 patients), *S. epidermidis* (11,091 patients), *P. aeruginosa* (9,113 patients), *K. pneumoniae* (7,576 patients), *E. faecalis* (7,403 patients), *S. agalactiae* (4,473 patients), *E. cloacae* (4,453 patients), *P. mirabilis* (4,415 patients), and *Haemophilus influenzae* (2,424 patients). The 2013 updates increased the number of bacterial identifications to 174,853 and the number of monitored bacterial species to 611 (43 new bacterial species were added). Among them, 384 bacterial species, defined here as rare bacterial species, were identified <11 times in the 12-year period.

The historical MARSS database included 12,062 antibiograms from October 2012 to March 2013. Here, the 10 most frequently isolated bacterial species were *E. coli* (3,293 strains), *S. aureus* (1,613 strains), *Achromobacter xylosoxidans* (1,478 strains), *S. epidermidis* (822 strains), *E. faecalis* (749 strains), *K. pneumoniae* (729 strains), *P. mirabilis* (455 strains), *S. agalactiae* (322 strains), *E. cloacae* (278 strains), and *Staphylococcus hominis* (153 strains).



A

ID_bac_v2.0	Nb_patts_histori	Rank_historic	Date_1st_ID	Max	Mean	Mean - 2 SD	Mean + 2 SD	26/03/2014	02/04/2014	26/03/2014
Micrococcus luteus	379	38	12/01/2002	5	1	-1	4	4		16
Enterococcus avium	143	58	05/01/2002	3	1	-1	2	3		18
Moraxella catarrhalis	504	34	16/01/2002	3	0	-1	2	2		27
Haemophilus haemolyticus	1	545	10/09/2013	2	0	-1	1	1		35
Corynebacterium tuberculostearicum	64	100	04/08/2004	1	0	-1	1	1		35
Enterococcus casseliflavus	27	157	31/05/2002	1	0	-1	1	1		35
Parabacteroides distasonis	42	125	03/01/2002	1	0	0	0	1		35
Corynebacterium auris	20	178	14/10/2002	1	0	0	0	1		35
Fusobacterium gonidiaformans	3	354	20/10/2008	1	0	0	0	1		35
Comamonas kerstersii.	2	398	20/03/2010	1	0	0	0	1		35

B

Hospital	SYNERGIE_number	Kind of sample	Species found	Number of isolation at the hospital	Unit	Unit entire name	Number of isolation per
Conception	4032003305	Urines	Corynebacterium auris	1	2574	CENTER FOR KIDNEY TRANSPLANTATION	1
Conception	4032006110	Urines	Corynebacterium tuberculostearicum	1	2544	HEPATO-GASTROENTEROLOGY	1
Conception	4031997591	Hémo aerob	Micrococcus luteus	1	9926	EXTERNAL ORGANISMS	1
Conception	4032005916	Hémo ana	Parabacteroides Distasonis	1	2610	RECEPTION EMERGENCIES	1
Hôpital Nord	4032008019	L.ponction	Enterococcus avium	1	2091	HEAD AND NECK SURGERY	1
Hôpital Nord	4032001328	L.péritoné	Micrococcus luteus	1	1963	GENERAL AND DIGESTIVE SURGERY	1
Hôpital Nord	4031996497	Asp.bronch	Moraxella catarrhalis	1	2315	BRONCHI - ALLERGIES - SLEEP	1
Ste Marguerite	4032001082	Os	Enterococcus casseliflavus	1	5453	ORTHOPEDIC SURGERY	1
Timone	4032004580	Hémo aerob	Comamonas kerstersii.	1	771	NEUROLOGY	1
Timone	4042012135	Urines	Enterococcus avium	2	6110	RECEPTION SPECIALISED EMERGENCIES	1
Timone	4031997344	Os	Enterococcus avium	2	715	VASCULAR SURGERY	1
Timone	4032004373	Biopsie	Fusobacterium gonidiaformans	1	9916	EXTERNAL ORGANISMS	1
Timone	4032004048	CRA muco	Haemophilus haemolyticus	1	5476	SPECIAL PEDIATRY	1
Timone	4031995242	Anévrisme	Micrococcus luteus	1	3544	VASCULAR SURGERY	1
Timone	4031997103	Narine	Moraxella catarrhalis	1	3016	HEART HOSPITAL DEPARTMENT	1
Unknown	4031997163	Os	Micrococcus luteus	1	5595	0	1

Figure 2. Screen shots from the Bacterial Real-Time Laboratory-based Surveillance System. A) List of the 652 bacterial species followed by the Bacterial Real-time Surveillance System and all of the contained information. B) Interface summarizing information from the alarms. ID_bac_v2.0, all the bacterial species followed by the surveillance system; Nb_patts_histori, the historical number of patients infected by the bacterium; Rank_historic, the historical rank of a precise bacterium under surveillance; Date_1st_ID, the date of first identification of the bacterium

Alarms Validated and Investigated, May 21, 2013– June 4, 2014

From May 21, 2013, through June 4, 2014 (55 weeks), BALYSES detected 21 alarms (6 confirmed events and 15 unconfirmed events), corresponding to ≈0.4 alarms per week. These alarms led to 5 official reports to the ARS of the Provence-Alpes-Côte d'Azur (PACA) region, France (Table 1; Figure 4). The positive predictive value for the study period was 0.28. Sixteen bacterial species triggered alarms in this surveillance system. The bacterial species that triggered alarms were *E. aerogenes* (3 alarms), *Aeromonas hydrophila* (2 alarms), *E. cloacae* (2 alarms), *K. oxytoca* (2 alarms), *M. morganii* (2 alarms), *E. coli* (1 alarm), *E. faecium* (1 alarm), *Gardnerella vaginalis* (1 alarm), *Haemophilus parahaemolyticus* (1 alarm), *Moraxella catarrhalis* (1 alarm), *Raoultella ornithinolytica* (1 alarm), *Staphylococcus capitis* (1 alarm), *Staphylococcus gallolyticus* (1 alarm), *Staphylococcus hominis* (1 alarm), and *Staphylococcus saprophyticus* (1 alarm). As an example of the system's usefulness, BALYSES allowed us to detect a real nosocomial transmission of *R. ornithinolytica* between 2 patients in the intensive care unit at the Timone Hospital on June 4, 2013 (Table 1).

In parallel, MARSS detected 31 alarms (16 confirmed events and 15 unconfirmed events, ≈0.6 alarms/week), which led to 15 official reports to the ARS of the PACA region, France (Table 4, http://wwwnc.cdc.gov/EID/article/21/8/14-1419-T4htm; Figure 4). The positive predictive value for the study period was 0.52. Thirteen bacterial species triggered alarms in MARSS. Here, the bacterial species, in order according to the number of alarms triggered, were *K. pneumoniae* (13 alarms), *E. cloacae* (3

A

Sum_week

Resistance phenotypes	AMX	TIC	AMC	TCC	CRO	FEP	IPM	Maximum for one specific week	Mean number of isolation	Standard deviation	Mean + 2 standard deviation	Mean − 2 standard deviation	03/10/2014
													130
Wild	S	S	S	S	S	S	S	72	57	10	77	36	63
Low level penicillinase	I/R	I/R	S	S	S	S	S	33	17	6	30	5	23
Inhibitors resistant penicillinase	I/R	I/R	R	R	S	S	S	36	17	6	30	5	14
High level penicillinase	I/R	I/R	I/R	I/R	S	S	S	41	22	7	35	9	23
Extended-spectrum β-lactamase	R	R	S/I/R	S/I/R	I/R	I/R	S	17	9	3	16	3	5
High level cephalosporinase	R	R	R	R	R	S	S	5	1	1	4	−1	2

B

Figure 3. Marseille Antibiotic Resistance Surveillance System (MARSS) interface for *Escherichia coli*. A) Screen shot showing list of most of the β-lactam antibiotic resistance profiles coded for *E. coli* in MARSS. B) Example of graph created by using MARSS showing the evolution of the antibiotic resistance of *E. coli*.

alarms), *P. mirabilis* (3 alarms), *E. coli* (2 alarms), *E. aerogenes* (2 alarms), *Salmonella* spp. (2 alarms), *P. aeruginosa* (1 alarms), *Citrobacter koseri* (1 alarm), *M. morganii* (1 alarm), *S. marcescens* (1 alarm), *S. epidermidis* (1 alarm), and *S. agalactiae* (1 alarm). As an example of the system's usefulness, MARSS allowed us to detect a local outbreak of oxicillinase-48 carbapenemase–producing *K. pneumoniae* from July 2013 to October 2013 (11 patients infected) (unpub. data; Table 4, http://wwwnc.cdc.gov/EID/article/21/8/14-1419-T4.htm).

For clarification, not all of the true alarms led to official reports because we did not identify the reasons why these abnormal increases occurred (Tables 1, 4). Nevertheless, investigations are ongoing to try to elucidate these phenomena.

Discussion

Analysis of 2 Real-Time Laboratory-Based Surveillance Systems

Implementing surveillance systems on the basis of data that were not specifically collected for surveillance is one of the advantage of our systems. Indeed, these types of systems, syndromic surveillance systems, are well suited in places and situations in which surveillance tools are urgently needed (*18*). In our situation, this approach allowed us to rapidly implement the system and quickly detect abnormal events related to bacterial infections occurring in our institution (19 official reports) (Tables 1, 4; Figure 4).

The fact that all of the emitted alarms are systematically validated during epidemiologic meetings with microbiologists (Figure 1) is also a strength of this laboratory surveillance system. Thus, the system enables rapid verification and filtering of false alarms to ensure that the official reports sent to the regional health authorities (ARS) are correct. This facilitates a rapid public health response to counter possible epidemics. As an example, EPIMIC, our third surveillance system not described here (Figure 1) (*15,16*), allowed us to detect a nosocomial outbreak of the hypervirulent *Clostridium difficile* ribotype O27 that started in March 2013 (*19*). As we continue to fight this major public health problem, a list of recommended containment measures, such as systematic isolation of infected patients in special care units or systematic screening of patients at risk, is being published and transmitted to our institutional and regional health care providers.

Our 2 surveillance systems have been implemented by using Microsoft Excel software. This strategy makes the

Table 2. Summary of the normal phenotypes registered in MARSS*

		β-lactam antibiotics										
Bacterial species	Resistance phenotypes	AMX	TIC	AMC	TCC	TZP	FOX	OXA	CRO	FEP	CAZ	IPM
Escherichia coli												
	Wild-type	S	S	S	S				S	S		S
	Low-level penicillinase	I/R	I/R	S	S				S	S		S
	Inhibitor-resistant penicillinase	I/R	I/R	R	R				S	S		S
	High-level penicillinase	I/R	I/R	I/R	I/R				S	S		S
	ESBL	R	R	S/I/R	S/I/R				I/R	I/R		S
	High-level cephalosporinase	R	R	R	R				R	S		S
Klebsiella pneumoniae	Wild-type			S		S			S	S		S
	ESBL			I/R		I/R			I/R	I/R		S
	High-level cephalosporinase			I/R		I/R			I/R	S		S
	ESBL-TZP-sensible			I/R		S			I/R	I/R		S
Proteus mirabilis	Wild	S	S	S	S				S	S		S
	Low-level penicillinase	I/R	I/R	S	S				S	S		S
	Inhibitor-resistant penicillinase	R	R	R	R				S	S		S
	High-level penicillinase	R	R	I/R	I/R				S	S		S
	ESBL	R	R	I/R	I/R				I/R	I/R		S
	High-level cephalosporinase	R	R	R	R				R	S		S
Klebsiella oxytoca	Wild-type			S		S				S		S
	ESBL			I/R		I/R				I/R		S
	High-level penicillinase			I/R		S/I/R				S		S
	Low-level penicillinase			S		R				S		S
	ESBL–TZP-sensible			I/R		S				I/R		S
Enterobacter aerogenes	Wild-type				S	S			S	S		S
	Inhibitors-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Morganella morganii	Wild-type				S	S			S	S		S
	Inhibitor-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Serratia marcescens	Wild-type				S	S			S	S		S
	Inhibitor-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Enterobacter cloacae	Wild-type				S	S			S	S		S
	Inhibitor-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Pseudomonas aeruginosa	Wild-type		S		S	S				S	S	S
	Penicillinase		R		R/I/S	I/S				S	S	S
	High-level penicillinase		I/R		I/R	I/R				S	S	S
	ESBL		I/R		I/R	I/R				I/R	I/R	S
	Selective permeability to imipenem		S		S	S				S	S	R
	Penicillinase, loss of D2 porine		R		R	S				S	S	R
Acinetobacter baumannii	Wild-type		S		S						S	S
	Penicillinase		R		R/I/S						S	S
	ESBL		I/R		I/R						I/R	S
Streptococcus agalactiae	Wild							S	S			
	Oxacillin-resistant							I/R	S			
Enterococcus faecalis	Wild-type	S										
E. faecium	Wild-type	I/R										
Staphylococcus aureus	Wild-type						S					
	Methicillin-resistant						I/R					
S. epidermidis	Wild-type						S					
	Methicillin-resistant						I/R					

*MARSS, Marseille Antibiotic Resistance Surveillance System; AMC, amoxicillin; TIC, ticarcillin; AMC, amoxicillin-clavulanic acid; TIC, ticarcillin-clavulanic acid; TZP, piperacillin-tazobactam; FOX, cefoxitin; OXA, oxacillin; CRO, ceftriaxone; FEP, cefepime; CAZ, ceftazidime; IMP, imipenem; S, susceptible ; I, intermediate ; R, resistant; ESBL, extended-spectrum β-lactamase.

systems easy to handle and allows rapid modifications and improvements without the need for in-depth computer skills. These advantages may not be the case for fully designed website surveillance systems such as the Swiss Antibiotic Resistance Surveillance database (*20*) or the Real-Time Outbreak and Disease Surveillance (RODS) (*21*). These aspects

Table 3. Summary of the alarm phenotypes defined in MARSS*

Bacteria species	Alarm triggering key phenotypes
Escherichia coli, Proteus mirabilis	Carbapenem resistance
Klebsiella pneumoniae	Carbapenem resistance
Klebsiella oxytoca	Carbapenem resistance
Enterobacter aerogenes, *Morganella morganii*, *Serratia marcescens, Enterobacter cloacae*	Carbapenem resistance
Pseudomonas aeruginosa	Carbapenem resistance
Acinetobacter spp.	Carbapenem and colistin resistance
Streptococcus agalactiae	Ceftriaxone resistance
Enterococcus faecalis	Amoxicillin resistance
Enterococcus faecium	Amoxicillin susceptible
Staphylococcus aureus	Vancomycin resistance

*MARSS, Marseille Antibiotic Resistance Surveillance System.

are key factors for the optimal long-term use at the hospital level because surveillance systems can be considered complex socio-technical systems with the objective of assisting users during abnormal epidemic events (*22*).

The implementation of our 2 surveillance systems required 1 full-time PhD student for 4 months and a computer with standard configuration equipped with Microsoft Office version 2003 or 2007. In France, the national research agency requires that the minimum salary of a PhD student is 33,000€ per year. Considering that the average price for a basic computer equipped with Microsoft Office is ≈500€ and that the PhD student's salary for the 4 months was 11,000€, plus the administrative and management costs, the total consolidated cost of these surveillance systems was ≈13,800€ (US $17,000).

The use of our own microbiology laboratory data ensures the availability and the completeness of the data. These problems are frequently mentioned when surveillance systems collect data from various health care institutions. For example, the designers of the German Surveillance System of Antibiotic Use and Bacterial Resistance encountered problems comparing antibiogram data between participating intensive care units. Indeed, in Germany, laboratories did not apply 1 standard to determine antibiotic-resistance profiles of the bacterial species (*23*). Moreover, the increasing number of intensive care units joining the surveillance system may effect the comparability of collected data because recently added intensive care

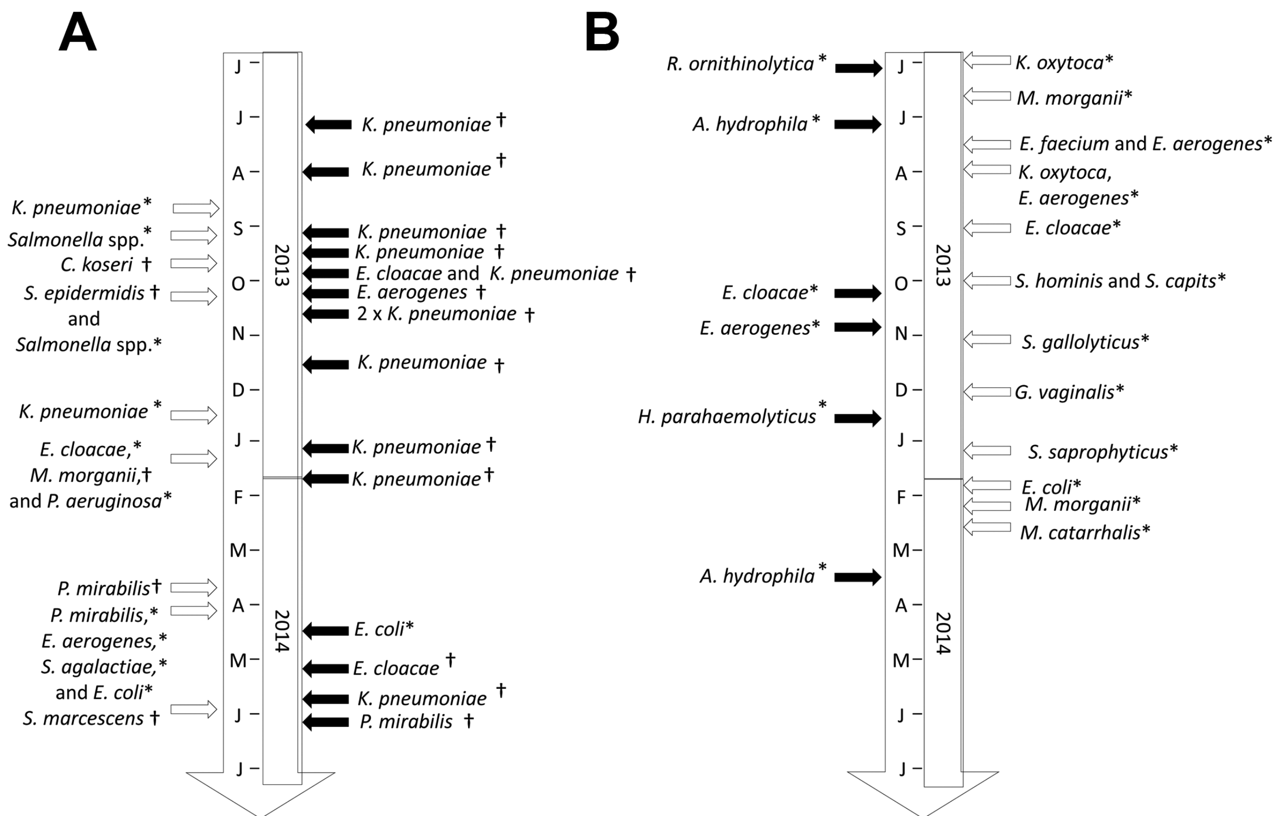


Figure 4. Time chart of the confirmed and unconfirmed events identified by the Marseille Antibiotic Resistance Surveillance System (MARSS) and the Bacterial real-time Laboratory-based Surveillance System (BALYSES). A) List of all the abnormal events (confirmed or not) detected by MARSS. B) List of all the abnormal events (confirmed or not) detected by BALYSES. Open arrows, unconfirmed events; solid arrows, confirmed events; asterisk (*), alarm due to abnormal increases or abnormal isolations; dagger (†), alarm due to strain with abnormal antibiotic susceptibility results.

units may use different antibiotic drugs, thus leading to different antimicrobial resistance profiles (*24*). Poor quality data were also observed in the emergency department syndromic surveillance system in New York, primarily because of the lack of human resources (*25*).

However, our surveillance systems have 2 main limitations. The first limitation is the statistical analysis used for the detection of abnormal events. As described before, our surveillance systems compared entered data with the historical means ± 2 SDs. For our purposes, this tool was simple to develop and was used effectively to detect abnormal events. However, these statistics do not consider seasonal variations in pathogen isolation, especially for rare bacterial species. To address this problem, Enki et al. improved the detection algorithms according to the frequency of isolation of the 3,303 pathogens included in the 20-year LabBase surveillance database recovered from the UK Health Protection Agency (*26*). They discovered that although all of these organisms varied greatly in their isolation frequency, most of them could be surveyed by using quasi-Poisson or negative binomial models for which the variance is proportional to the mean. In MARSS, the use of moving averages in our kinetic graphs or of cumulative sum control charts, as has been done in RODS (http://openrods.sourceforge.net/), could also be effective improvements for the detection of abnormal events.

The second limitation was that all of the data in our system were manually collected and entered into the surveillance system. This aspect can introduce bias into our data analysis. For example, we have already observed false alarms after shifts in data collection because of national holidays or because of the lack of human resources, which is a problem also observed in other surveillance systems, such as the emergency department syndromic surveillance system in New York (*25*). To address these issues, simple solutions can be developed, such as implementing and using informatic tools for automatic collection and processing of the collected data. This solution was implemented by the designers of ASTER, the French military decision-supported surveillance system (*22*).

With knowledge of the previously mentioned weaknesses, we are currently working to improve our 2 surveillance systems. Thus, a surveillance platform that will merge all of the surveillance activities and will contain stronger statistical tools for the surveillance of abnormal events is under development. This platform will help us survey abnormal events by using all of the clinical microbiology data available in the laboratory. Moreover, our monitoring activity is expanding to other laboratories in the PACA region. We are implementing a regional laboratory surveillance system that will allow us, on the basis of the clinical microbiology data that are collected every week, to gain a better understanding of the local dissemination of pathogens at the regional level and to survey weekly isolation frequencies. Finally, another surveillance system based on matrix-assisted laser desorption/ionization–time of flight spectra of bacteria is currently under development in our laboratory. A prototype is used weekly in our laboratory to try to detect epidemics, including the possible nosocomial transmission of bacterial clones.

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Mr. Abat is a PhD student at the Institut Hospitalo-Universitaire Méditerranée Infection, Aix-Marseille Université. His research interest is the implementation of computer tools for real-time epidemiologic surveillance of abnormal events based on clinical microbiology laboratory data.

References

1. Raoult D. Les causes de l'émergence des agents infectieux. Responsabilité et Environnement. 2008;51:21–5. http://dx.doi.org/10.3917/re.051.0021
2. Raoult D. Molecular, epidemiological, and clinical complexities of predicting patterns of infectious diseases. Front Microbiol. 2011;2:25. http://dx.doi.org/10.3389/fmicb.2011.00025 PMID: 21687417
3. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature. 2004;430:242–9. http://dx.doi.org/10.1038/nature02759
4. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451:990–3. http://dx.doi.org/10.1038/nature06536
5. Zeng D, Chen H, Castillo-Chavez C, Thurmond M. Clinical laboratory data for biosurveillance. In: Zeng D., Chen H., Castillo-Chavez C., Lober W.B., Thurmond M., editors. Infectious disease informatics and biosurveillance. New York: Springer; 2011. p. 67–87.
6. Vinnard C, Winston CA, Wileyto EP, Macgregor RR, Bisson GP. Isoniazid resistance and death in patients with tuberculous meningitis: retrospective cohort study. BMJ. 2010;341:c4451. http://dx.doi.org/10.1136/bmj.c4451
7. van Wessel K. Rodenburg GD, Veenhoven RH, Spanjaard L, van der Ende A, Sanders EA. Nontypeable *Haemophilus influenzae* invasive disease in The Netherlands: a retrospective surveillance study 2001–2008. Clin Infect Dis. 2011;53:e1–7.
8. Cole MJ, Unemo M, Hoffmann S, Chisholm SA, Ison CA, van de Laar MJ. The European gonococcal antimicrobial surveillance programme, 2009. Euro Surveill. 2011;16: 19995.
9. Sala Soler M, Fouillet A, Viso AC, Josseran L, Smith GE, Elliot AJ, et al. Assessment of syndromic surveillance in Europe. Lancet. 2011;378:1833–4. http://dx.doi.org/10.1016/S0140-6736(11)60834-9
10. Severi E, Heinsbroek E, Watson C, Catchpole M. Infectious disease surveillance for the London 2012 Olympic and Paralympic Games. Euro Surveill. 2012;17: 20232.
11. Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev. 2010;32:93–109. http://dx.doi.org/10.1093/epirev/mxq008

12. van Dijk A, Aramini J, Edge G, Moore KM. Real-time surveillance for respiratory disease outbreaks, Ontario, Canada. Emerg Infect Dis. 2009;15:799–801. http://dx.doi.org/10.3201/eid1505.081174
13. Magiorakos AP, Suetens C, Monnet DL, Gagliotti C, Heuer OE. The rise of carbapenem resistance in Europe: just the tip of the iceberg? Antimicrob Resist Infect Control. 2013;2:6. http://dx.doi.org/10.1186/2047-2994-2-6
14. Colson P, Gouriet F, Badiaga S, Tamalet C, Stein A, Raoult D. Real-time laboratory surveillance of sexually-transmissible infections in Marseille University hospitals reveals rise of gonorrhoea, syphilis and human immunodeficiency virus seroconversions in 2012. Euro Surveill. 2013;18:4.
15. Parola P, Colson P, Dubourg G, Million M, Charrel R, Minodier P, et al. Group A streptococcal infections during the seasonal influenza outbreak 2010/11 in South East England. Euro Surveill. 2011;16:19815.
16. Kempf M, Rolain JM, Azza S, Diene S, Joly-Guillou ML, Dubourg G, et al. Investigation of *Acinetobacter baumannii* resistance to carbapenems in Marseille hospitals, south of France: a transition from an epidemic to an endemic situation. APMIS. 2013;121:64–71. http://dx.doi.org/10.1111/j.1600-0463.2012.02935.x
17. Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al. Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol. 2013;51:2182–94. http://dx.doi.org/10.1128/JCM.00492-13
18. Calain P. From the field side of the binoculars: a different view on global public health surveillance. Health Policy Plan. 2007;22:13–20. http://dx.doi.org/10.1093/heapol/czl035
19. Lagier JC, Dubourg G, Cassir N, Fournier PE, Colson P, Richet H, et al. *Clostridium difficile* 027 emerging outbreak in Marseille, France. Infect Control Hosp Epidemiol. 2013;34:1339–41. http://dx.doi.org/10.1086/673995
20. Kronenberg A, Hilty M, Endimiani A, Muhlemann K. Temporal trends of extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in in- and outpatients in Switzerland, 2004 to 2011. Euro Surveill. 2013;18: 20284.
21. Silva JC, Shah SC, Rumoro DP, Bayram JD, Hallock MM, Gibbs GS, et al. Comparing the accuracy of syndrome surveillance systems in detecting influenza-like illness: GUARDIAN vs. RODS vs. electronic medical record reports. Artif Intell Med. 2013;59:169–74. http://dx.doi.org/10.1016/j.artmed.2013.09.001
22. Chaudet H, Anceaux F, Beuscart MC, Pelayo S, Pellegrin L. Facteurs humains et ergonomie en informatique médicale. In: Venot A, Burgun A, Quantin C, editors. Informatique médicale, e-santé—fondements et applications. New York: Springer; 2013. p. 495–520.
23. Meyer E, Jonas D, Schwab F, Rueden H, Gastmeier P, Daschner FD. Design of a surveillance system of antibiotic use and bacterial resistance in German intensive care units (SARI). Infection. 2003;31:208–15.
24. Meyer E, Schwab F, Schroeren-Boersch B, Gastmeier P. Dramatic increase of third-generation cephalosporin-resistant *E. coli* in German intensive care units: secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008. Crit Care. 2010;14:R113. http://dx.doi.org/10.1186/cc9062
25. Das D, Weiss D, Mostashari F, Treadwell T, McQuiston J, Hutwagner L, et al. Enhanced drop-in syndromic surveillance in New York City following September 11, 2001. J Urban Health. 2003;80(Suppl 1):i76–88.
26. Enki DG, Noufaily A, Garthwaite PH, Andrews NJ, Charlett A, Lane C, et al. Automated biosurveillance data from England and Wales, 1991–2011. Emerg Infect Dis. 2013;19:35–42. http://dx.doi.org/10.3201/eid1901.120493

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etymologia

Escherichia coli [esh"ə-rik'e-ə co'lī]

A gram-negative, facultatively anaerobic rod, *Escherichia coli* was named for Theodor Escherich, a German-Austrian pediatrician. Escherich isolated a variety of bacteria from infant fecal samples by using his own anaerobic culture methods and Hans Christian Gram's new staining technique. Escherich originally named the common colon bacillus *Bacterium coli commune*. Castellani and Chalmers proposed the name *E. coli* in 1919, but it was not officially recognized until 1958.

"Escherich, Theodor" by Unknown, retouched by Lichtspiel. Licensed under Public Domain via Wikimedia Commons - https://commons.wikimedia.org/wiki/File:Escherich,_Theodor.jpg#/media/File:Escherich,_Theodor.jpg

Sources

1. Oberbauer BA. Theodor Escherich—Leben und Werk. Munich: Futuramed-Verlag; 1992.
2. Shulman ST, Friedmann HC, Sims RH. Theodor Escherich: the first pediatric infectious diseases physician? Clin Infect Dis. 2007;45:1025–9. http://dx.doi.org/10.1086/521946

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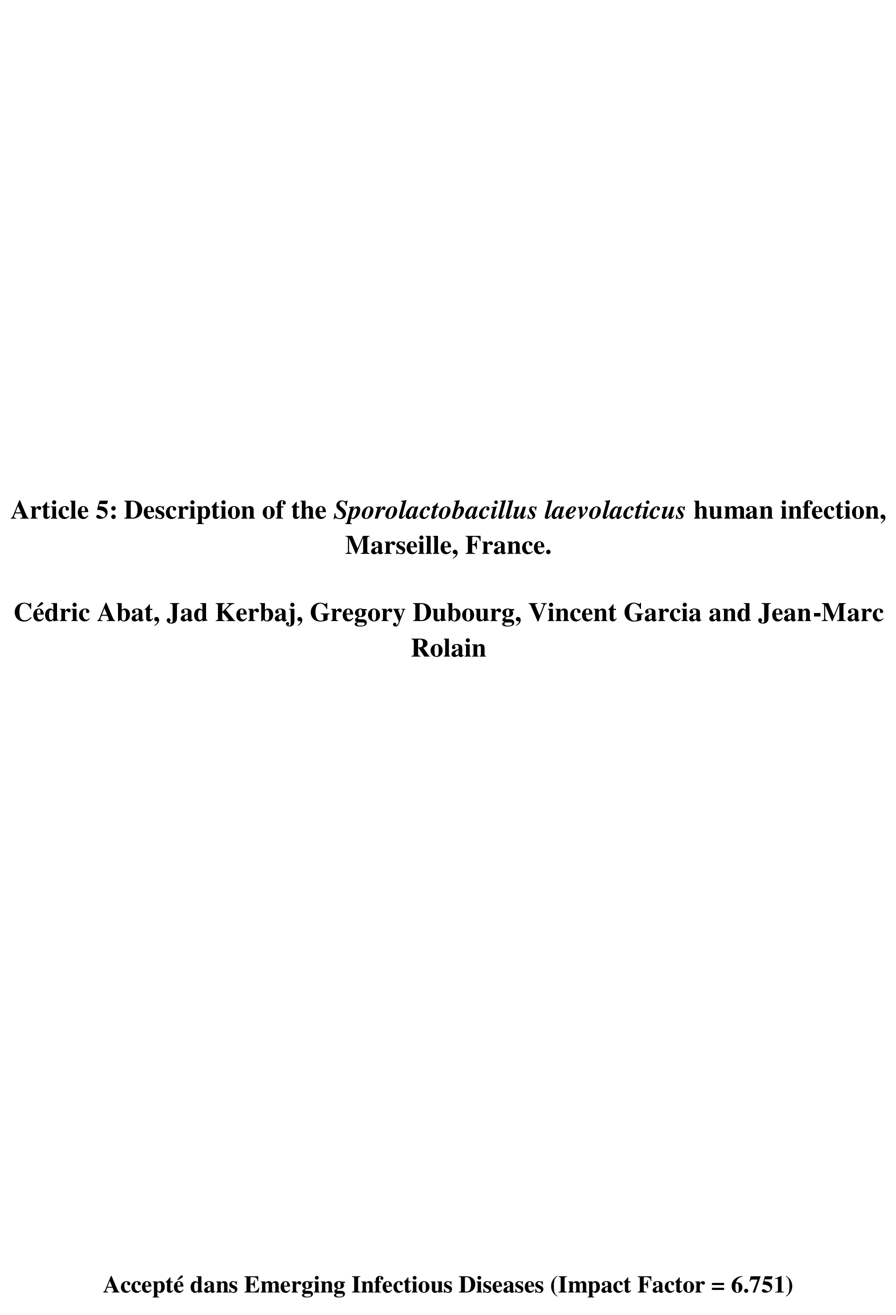
DOI: http://dx.doi.org/10.3201/eid2108.ET2108



Article 5: Description of the *Sporolactobacillus laevolacticus* human infection, Marseille, France.

Cédric Abat, Jad Kerbaj, Gregory Dubourg, Vincent Garcia and Jean-Marc Rolain

Accepté dans Emerging Infectious Diseases (Impact Factor = 6.751)

1 TITLE PAGE

2

3 **Full-length title:** Description of a human infection due to *Sporolactobacillus*

4 *laevolacticus*, Marseille, France

5 **Short title (for the running head):** *Sporolactobacillus laevolacticus* human

6 infection

7 **Author list:** Cédric Abat [1], Jad Kerbaj [1], Gregory Dubourg, Vincent Garcia and

8 Jean-Marc Rolain [*]

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16 **Footnote:** [1] These first authors contributed equally to this article.

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23

24 **Key words:** blood, cellulitis, infection, bacteria

25

26 **To the Editor**: *Sporolactobacillus laevolacticus,* formerly called *Bacillus*
27 *laevolacticus*, is a Gram positive acid-tolerant, catalase-positive, facultatively
28 anaerobic and mesophilic bacteria isolated from the rhizosphere of wild plants
29 (1,2). However, since then, it has never been isolated from humans. In this paper,
30 we report *S. laevolacticus* wound infection and cellulitis in a patient hospitalised in
31 our facility in Marseille, France.
32 In March 2015, a 47-year old man without any underlying disease was admitted to
33 the emergency unit of the North Hospital in Marseille, France. He presented an
34 infected wound on his right foot following a barefoot jogging during a vacation in
35 the Comoros. The patient did not know what wounded him. The patient did not
36 take any anti-inflammatory drugs. The foot became swollen, red, hot and painful.
37 He so visited a doctor during his travel who prescribed him anti-inflammatory
38 drugs and antibiotics including second generation cephalosporin and ofloxacin.
39 The patient came back to Marseille but the infection was not cured. On admission,
40 the patient was apyretic but with high C-Reactive Protein (85.7 mg/L (814.15
41 nmol.L)) and fibrinogen (8.35 g/L), reflecting an inflammation. The white blood
42 cell count was normal (9.29 G/L) but procalcitonine (0.19µg/L) was increased,
43 suggesting that the infection of the foot had not been cured. A cellulitis abscess
44 was suspected and the patient was hospitalised for surgical cleaning and drainage
45 (Figure 1A and B). Samples were collected during surgery and probabilistic
46 antibiotherapy, including tazocillin, clindamycin and vancomycin was initiated in

47 order to treat the patient. Puncture liquid collected during surgery was sterile when

48 incubated directly on Columbia and Polyvitex agar plate (Biomérieux, Craponne,

49 France). However, surgical sample pre-incubated into blood culture bottle grew

50 after 4 days and gram staining yielded gram-positive bacilli. Subcultures colonies

51 were identified using MALDI-TOF MS (Leipzig, Germany) as *Sporolactobacillus*

52 *laevolaticus* with a score of 1.88. Identification was confirmed by amplification of

53 the 16S RNA gene (3). A 944-bp sequence yielded 99.5% similarity with

54 *Sporolactobacillus laevolaticus* (Genbank AB362648) using NCBI BLAST

55 (http://www.ncbi.nlm.nih.gov). The *S. laevolaticus* strain was susceptible to

56 amoxicillin, amoxicillin/clavulanate, imipenem, metronidazole, clindamycin and

57 vancomycin. Antibiotic regimen was changed for administering clindamycine and

58 trimethoprime-sulfamethoxazole with an excellent clinical outcome. The patient

59 was considered clinically cured 7 weeks later (Figure 1C and D).

60 *S. laevolacticus* has been studied for its capacity to survive in extreme conditions

61 and its fermentation process (4–8). However, it has never before been isolated in

62 humans. This may be due to the fact that this bacteria was isolated from the plant

63 rhizosphere in Japan only (2). It can also be explained by the fact that conventional

64 identification methods such as the VITEK 2 system or API system cannot identify

65 *S. laevolacticus*. Thus, since September 2009, we have used MALDI-TOF

66 technology for the routine identification of bacterial species isolated from clinical

67 samples (9). This strategy increases our capacity to detect rare bacterial species,

68 including emerging pathogens (10). In the present report, the bacterial species was
69 accurately identified by MALDI-TOF and was then confirmed by 16S RNA
70 polymerase chain reaction. Figure 1D summarises the characteristics of the patient
71 infected by *S. laevolacticus*. Because the bacteria was isolated from the plant
72 rhizosphere and the patient was admitted to our hospital with an open wound in the
73 foot, we can speculate that the infection was the direct result of close extended
74 contact between the wound and soil infected with the bacteria. This case confirms
75 that *S. laevolaticus* can be responsible for human infections and leads us to suggest
76 that this bacterial species could be an emerging opportunistic pathogen responsible
77 for human infections.
78

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81 contributing to this journal do not necessarily reflect the opinions of the Centers for

82 Disease Control and Prevention or the institutions with which the authors are

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86

87 **REFERENCES**

88 1. Andersch I, Pianka S, Fritze D, Claus D. Description of *Bacillus*
89 *laevolacticus* (ex Nakayama and Yanoshi 1967) sp. nov., nom. rev. Int J
90 Syst Bacteriol. 1996;46(3):839–839.

91 2. Yanagida, F., K.I. Suzuki, T. Kaneko, M. Kozaki and KK. Spore-bearing
92 lactic acid bacteria isolated from rhizosphere I. Taxonomic studies on
93 *Bacillus laevolacticus* nov. sp. and *Bacillus racemilacticus* nov. sp. J Gen
94 Appl Microbiol. 1967;33:139–53.

95 3. Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, et
96 al. Complementarity between targeted real-time specific PCR and
97 conventional broad-range 16S rDNA PCR in the syndrome-driven
98 diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis. 2015
99 Mar;34(3):561–70.

100 4. Mowlick S, Inoue T, Takehara T, Kaku N, Ueki K, Ueki A. Changes and
101 recovery of soil bacterial communities influenced by biological soil
102 disinfestation as compared with chloropicrin-treatment. AMB Express.
103 Springer; 2013 Aug 17;3(1):46.

104 5. Sawai H, Na K, Sasaki N, Mimitsuka T, Minegishi S, Henmi M, et al.
105 Membrane-Integrated Fermentation System for Improving the Optical
106 Purity of D-Lactic Acid Produced during Continuous Fermentation. Biosci
107 Biotechnol Biochem. 2011;75(12):2326–32.

108 6. Li Y, Wang L, Ju J, Yu B, Ma Y. Efficient production of polymer-grade d-
109 lactate by *Sporolactobacillus laevolacticus* DSM442 with agricultural
110 waste cottonseed as the sole nitrogen source. Bioresour Technol. 2013
111 Aug;142:186–91.
112 7. Gulati HK, Chadha BS, Saini HS. Production and characterization of
113 thermostable alkaline phytase from Bacillus laevolacticus isolated from
114 rhizosphere soil. J Ind Microbiol Biotechnol. 2007;34(1):91–8.
115 8. Wang H, Wang L, Ju J, Yu B, Ma Y. Genome Sequence of
116 *Sporolactobacillus laevolacticus* DSM442, an Efficient Polymer-Grade D-
117 Lactate Producer from Agricultural Waste Cottonseed as a Nitrogen
118 Source. Genome Announc. American Society for Microbiology; 2013 Dec
119 26;1(6):e01100–13 – e01100–13.
120 9. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et
121 al. Ongoing revolution in bacteriology: routine identification of bacteria by
122 matrix-assisted laser desorption ionization time-of-flight mass
123 spectrometry. Clin Infect Dis. 2009 Aug 15;49(4):543–51.
124 10. Seng P, Abat C, Rolain JM, Colson P, Lagier J-C, Gouriet F, et al.
125 Identification of rare pathogenic bacteria in a clinical microbiology
126 laboratory: impact of matrix-assisted laser desorption ionization-time of
127 flight mass spectrometry. J Clin Microbiol. 2013 Jul;51(7):2182–94.
128

129 **FIGURE LEGENDS**

130 **Figure 1.** Picture of the foot of the patient and general information on infection due to *Sporolactobacillus laevolaticus*. A) and B) show the drainage of the cellulitis abcess on

131 the right foot . C) shows the extent to which the wound on the arch of the foot had healed six weeks after surgery and antibiotherapy. D) summarizes the information on foot

132 infection caused by *Sporolactobacillus laevolaticus.*

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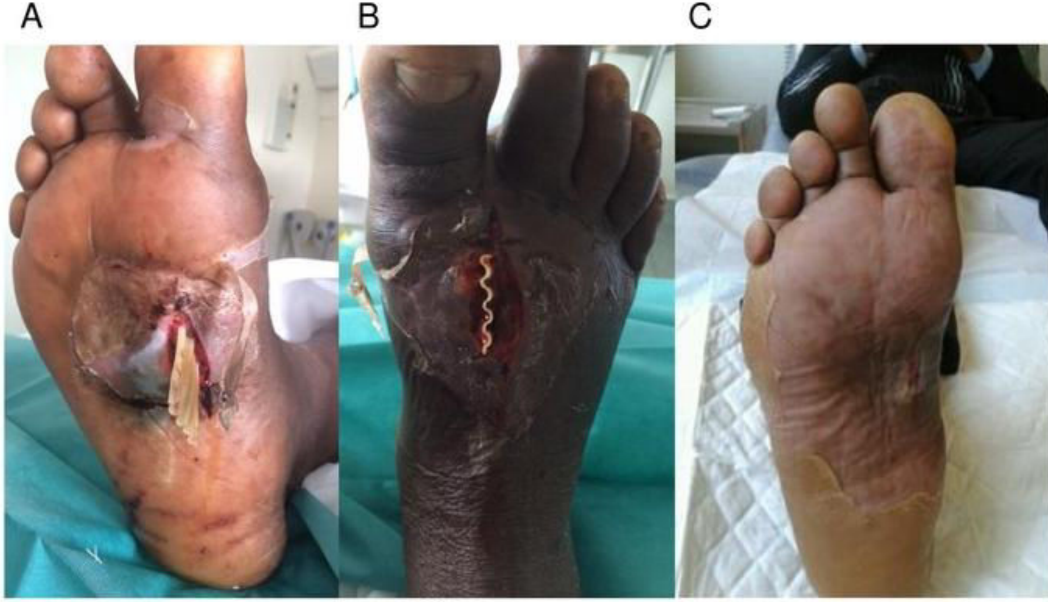

D

Characteristics of the patient and of the infection	Data
City, Country	Marseille, France
Sex/age (years)	Male/47
Sample date	10/03/2015
Context	Trip to the Comoros
Underlying disease	No
Clinical symptoms	Inflammation, abscess, ache
Type of sample	Surgical sample
Type of infection	Foot cellulitis abscess, bacteraemia
Diagnostic method	16 S RNA standard PCR
Antibiotherapy	Clindamycin and Cotrimoxazole
Outcome	Cured

134

Article 6: Report of the first *Vagococcus lutrae* human infection, Marseille, France.

Vincent Garcia, Cédric Abat, Jean-Marc Rolain,

Soumis dans Emerging Infectious Diseases (Impact Factor = 6.751)

TITLE PAGE

Article Summary Line: Here we report the first human infection due to *Vagococcus lutrae*

Running title: ***Vagococcus lutrae*: an unexpected cutaneous infection**

Key words: skin infection, *Vagococcus lutrae*, bacteria, MALDI-TOF

Title: Report of the first *Vagococcus lutrae* human infection, Marseille, France

Author list: Vincent GARCIA [1], Cédric ABAT [1] and Jean-Marc ROLAIN [1*]

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Words = 619 / Abstract = 42

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26 **ABSTRACT**

27 *Vagococcus lutrae* is a Gram-positive coccus initially isolated from the common otter (*Lutra*
28 *lutra*) but that has never been reported as a human pathogen. In this paper we describe the
29 first case of human infection due to *Vagococcus lutrae* in Marseille, France.

30

31

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32 **INTRODUCTION**

33 *Vagococcus lutrae* is a Gram positive catalase-negative, facultatively anaerobic, motile
34 coccus initially isolated and identified in 1999 from blood, liver, lungs and spleen samples
35 from a common otter (*Lutra lutra*) killed by a road traffic accident on the Isle of Mull in the
36 United Kingdom [1]. Since that, the bacterium has been isolated from the intestine of a
37 largemouth bass (*Micropterus salmoides*) caught in the wilds of Maine, USA [2]. However,
38 this bacterial species has never been isolated from humans. We herein report the first human
39 case of *V. lutrae* infection ever reported in the world from a patient hospitalized in our
40 settings, Marseille, France.

41 **CASE REPORT**

42 A 58-year old man was admitted to the intensive care unit of the Conception
43 hospital, Marseille, on January 7, 2015 for extensive skin lesions following four months bed
44 rest. The patient was morbidly obese with a body mass index of 41 (1m80, 135kg) and had
45 experienced chronic depressive syndrome for five years. Upon admission, the patient
46 presented with skin lesions located on the right side of his abdomen and on the right upper
47 and lower limbs. Skin lesions were erythematous, superficial and covered 50% of his body
48 surface (Figure 1A). Skin lesions were probably maceration lesions following prolonged bed
49 rest and carlessness with issuance of feces and urine directly in the bed. Upon admission, the
50 patient was dehydrated. Biochemical analysis showed hyponatremia (132 mmol/L),
51 hyperkaliemia (4.82 mmol/L). White blood cells were high (19.62 G/L). Blood cultures and
52 skin biopsies were collected during cleaning of the lesions. After growing, *Vagococcus* spp.
53 was identified from the skin biopsies by MALDI-TOF. 16S RNA standard polymerase chain
54 reaction (99.9% sequence homology) indicated that the strains belonged to the species *V.*
55 *lutrae*. After antibiotic-susceptibility testing, the strains were determined to be susceptible to
56 amoxicillin, ceftriaxone, gentamicin, erythromycin, rifampicin, clindamycin, doxycycline and

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57 vancomycin. Kaliemia and dehydratation were treated with insulin drip and glucose solution.
58 Skins lesions were cleaned and treated with dressings containing sulfadiazine (Figure 1B).
59 *V. lutrae* infection was treated by amoxicillin. After 15 days, the patient was considered
60 cured.

61 **DISCUSSION**

62 Globally, *V. lutrae* is rarely isolated worldwide, which can be the result of the
63 ineffectiveness of conventional identification methods such as the VITEK 2 system or API
64 system to properly identify *V. lutrae*. In our settings, we routinely use MALDI-TOF
65 technology for the identification of bacterial species isolated from clinical samples [3]. As
66 previously published [4], this strategy allows us to considerably increase our capacity to
67 detect rare bacterial species, including emerging pathogens. Herein, the genus *Vagococcus*
68 was accurately identified by MALDI-TOF and the species was identified by 16S RNA
69 polymerase chain reaction.

70 *V. lutrae* is generally only isolated from marine animals, suggesting that the bacterial
71 species is a member of fish and marine animal microbiome. This is supported by the fact that
72 the post-mortem examination of the otter concluded that the animal was in good bodily
73 conditions and did not suffer from the *V. lutrae* colonization, suggesting that the bacterial
74 species was not a pathogen of the otter [1]. Table 1 summarizes the characteristics of the
75 patient experiencing *V. lutrae* skin infection. In our case, we can speculate that the infection
76 originated from a food-mediated acquisition of the pathogen, particularly through fish and
77 seafood-based food. Then, due to the patient's poor hygiene, the bacterium was excreted *via*
78 the feces released directly onto his bed, leading to the patient's skin infections, facilitated by
79 the maceration lesions due to his prolonged bed rest.

80 All together, our observations allowed us to identify that under certain conditions,
81 such as poor hygiene, a marine animal commensal bacterial species like *V. lutrae* can be

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82 responsible for human infection, suggesting that this bacterial species can be an emerging
83 opportunistic human pathogen.

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86 **Acknowledgments:** We thank Trad Online for English correction of this article

87 **Conflict of interest:** Vincent GARCIA, Cédric ABAT and Jean-Marc ROLAIN, no
88 conflict.

89 **Disclaimers**: The opinions expressed by authors contributing to this journal do not
90 necessarily reflect the opinions of the Centers for Disease Control and Prevention or the
91 institutions with which the authors are affiliated.

92 **Biographical Sketch:**

93 Mr. Vincent GARCIA is a student in medical biology at the Institut Hospitalo-
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95 the identification and genomic analysis of pathogenic bacteria of interest including emerging
96 pathogens and bacterial clones responsible for outbreak infections.
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99 **REFERENCES**

100 1. Lawson PA, Foster G, Falsen E, Ohlén M, Collins MD. *Vagococcus lutrae* sp. nov.,
101 isolated from the common otter (*Lutra lutra*). Int J Syst Bacteriol. 1999 Jul;49 Pt 3:1251–
102 4.

103 2. Lebreton F, Valentino MD, Duncan LB, Zeng Q, Manson McGuire A, Earl AM, Gilmore
104 MS. High-Quality Draft Genome Sequence of *Vagococcus lutrae* Strain LBD1, Isolated
105 from the Largemouth Bass *Micropterus salmoides*. Genome Announc. 2013;1(6).

106 3. Seng P, Rolain J-M, Fournier PE, La Scola B, Drancourt M, Raoult D. MALDI-TOF-
107 mass spectrometry applications in clinical microbiology. Future Microbiol. 2010
108 Nov;5(11):1733–54.

109 4. Seng P, Abat C, Rolain JM, Colson P, Lagier J-C, Gouriet F, Fournier PE, Drancourt M,
110 La Scola B, Raoult D. Identification of rare pathogenic bacteria in a clinical microbiology
111 laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass
112 spectrometry. J Clin Microbiol. 2013 Jul;51(7):2182–94.

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114 **TABLE**

115 **Table 1**. General information concerning the patient and type of infection caused by

116 *Vagococcus lutrae*.

Characteristics of skin infection	Data
City, Country	Marseille, France
Sex/age (years) of patient	Male/58
Underlying disease	Morbidly obese, chronic depressive syndrome
Sample date	January 7, 2015
Cause	Prolonged bed rest, carlessness
Sample type	Skin biopsy
Type of infection	Cutaneous infection
Diagnostic method	MALDI- TOF and 16 S RNA standard PCR
Antibiotherapy/Time	Amoxicillin/15 days
Outcome	Cured

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FIGURE LEGENDS

Figure 1. Infected skin lesions due to *Vagococcus lutrae*.

Panel A shows erythematous and infected skin lesions caused by *Vagococcus lutrae*. Panel B shows skin lesions healing after 10 days of treatment.

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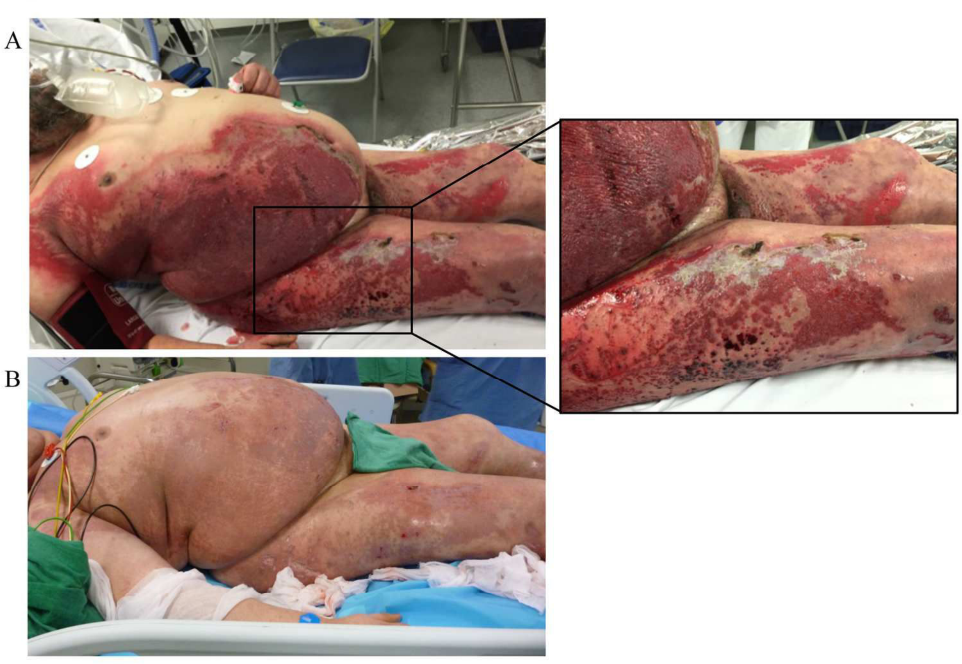
Figure 1.



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Conclusions et perspectives de la Partie II

Le développement des deux bases de données Microsoft Excel a permis, par la suite, de développer dans Microsoft Excel deux nouveaux systèmes de surveillance BALYSES et MARSS. La première base de données développée, partiellement publiée dans l'article 3, contient actuellement, après mise à jour avec les données du laboratoire de la Timone jusqu'en Mai 2015 et nettoyage, plus de 200 000 lignes de données pour plus de 120 000 patients. Elle regroupe 672 espèces bactériennes différentes dont 187 ont été identifiées une seule fois dans notre laboratoire depuis 2002. La Figure 4 présente les 50 espèces bactériennes les plus isolées à l'APHM sur cet intervalle de temps.

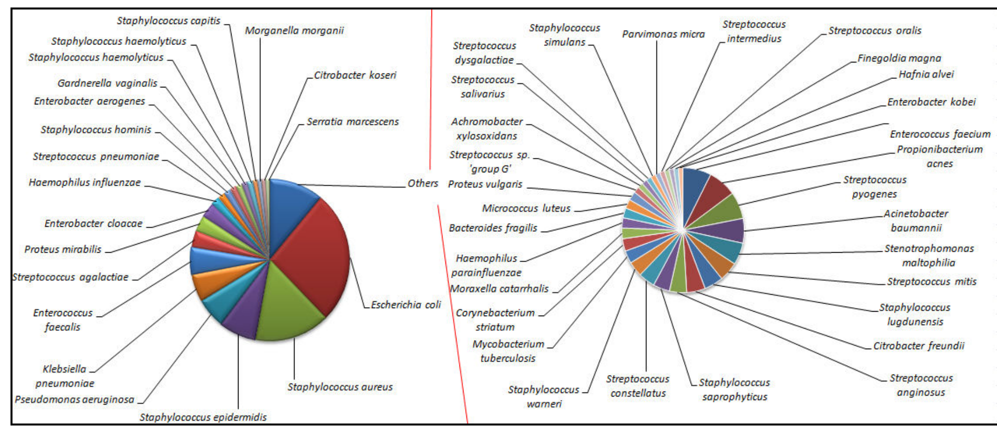

Figure 4. Liste des 50 espèces bactériennes les plus isolées en routine au laboratoire entre Janvier 2002 et Mai 2015. La taille des quartiers des diagrammes circulaires est proportionnelle au nombre dédoublonné de patients infectés par chacune des espèces bactériennes présentées sur la période d'étude.

La seconde base de données contient, quant à elle, après mise à jour avec les données récentes, plus de 50 000 lignes de données pour plus de 30 000 patients. Les données relatives aux 15 espèces bactériennes d'intérêt clinique suivies par MARSS sont présentées Figure 5.

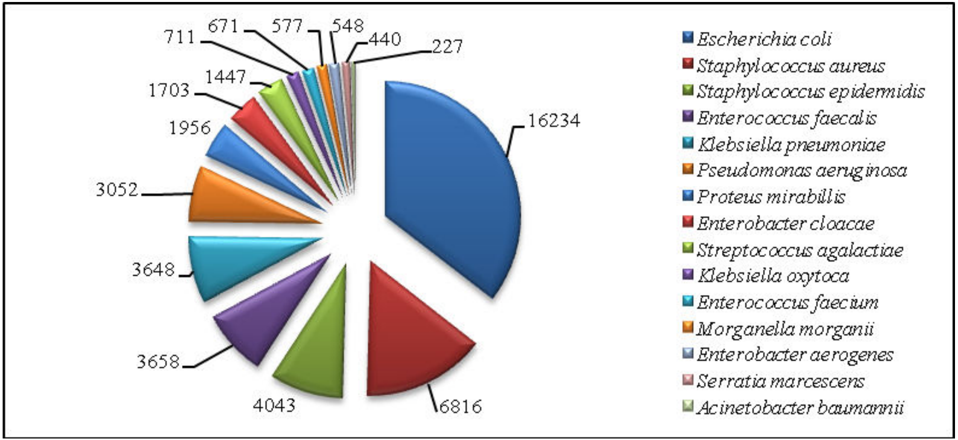

Figure 5. Liste des 15 espèces bactériennes d'intérêt clinique suivies par MARSS. La taille des quartiers des diagrammes circulaires est proportionnelle au nombre dédoublonné de patients infectés par chacune des espèces bactériennes présentées entre Janvier 2013 et Juillet 2015.

Les deux systèmes de surveillance BALYSES et MARSS ont émis, en deux ans de surveillance, 111 alarmes qui ont été par la suite investiguées (56 se sont finalement avérées fausses et 55 vraies). Parmi ces alarmes, 33 alarmes impliquant 10 espèces bactériennes (*Aeromonas hydrophila* (1 déclaration), *Enterococcus faecalis* (1), *Proteus mirabilis* (1), *Raoultella ornithinolytica* (1), *Streptococcus pyogenes* (1), *Enterobacter aerogenes* (2), *Escherichia coli* (2),

Enterobacter cloacae (3), *Acinetobacter baumannii* (4) et *Klebsiella pneumoniae* (17)) ont par la suite donné lieu à des déclarations officielles à l'ARS.

Bien qu'efficace, ces outils ne permettent actuellement pas une surveillance épidémiologique fine dans nos hôpitaux. C'est pourquoi nous travaillons actuellement au développement d'un nouvel outil de surveillance basé sur les spectres MALDI-TOF (Matrix Assisted Laser Desorption Ionisation) produits lors de l'identification des espèces bactériennes en routine au laboratoire de la Timone (Figure 6).

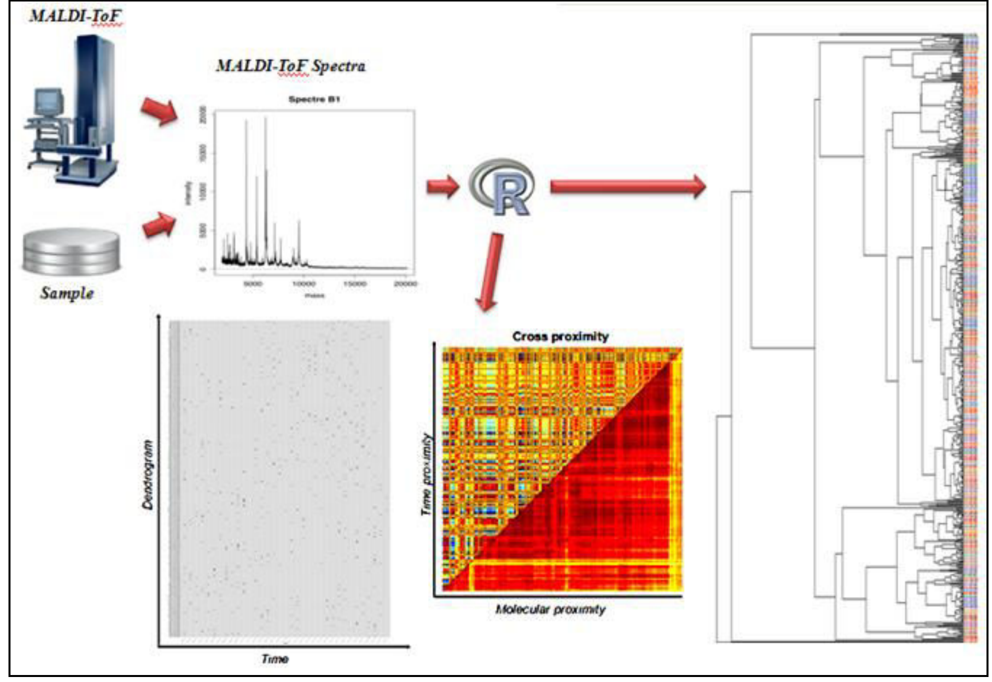

Figure 6. Schéma de fonctionnement du nouvel outil de surveillance des clones bactériens basé sur les spectres produits en routine lors de l'identification des espèces

bactériennes en utilisant la technologie MALDI-TOF (Matrix Assisted Laser Desorption Ionisation).

Brièvement, cet outil nous permet actuellement, sur la base de fichier Microsoft Excel regroupant les informations d'identification bactérienne sur une période donnée, de collecter automatiquement les spectres associés aux données du fichier Microsoft Excel et de les analyser en utilisant un scripte développé sous R. Une fois analysés, R produits automatiquement un dendrogramme "taggant" les spectres avec des couleurs plus ou moins chaudes selon leur date d'inclusion dans le dendrogramme, mais également un chronogramme permettant de visualiser, par l'intermédiaire de points représentant les spectres analysés dans un espace à deux dimensions, la proximité de spectres collectés tant sur le plan moléculaire que temporel, permettant ainsi d'observer de possibles émergences de clones bactériens nosocomiaux ou communautaires.

Partie III: Description d'événements épidémiologiques identifiés par les différents systèmes de surveillance développés au sein de l'Institut Hospitalo-Universitaire Méditerranée Infection.

Liste des articles

Article 7: Increasing burden of urinary tract infections due to intrinsic colistin-resistant bacteria in hospitals in Marseille, France. **Published in IJAA (IF: 4.296)**.

Article 8: Increasing trend of invasive group B streptococcal infections, Marseille, France. **Published in CID (IF: 8.886)**.

Article 9: Dramatic decrease of *Streptococcus pneumoniae* in Marseille, 2009-2014. **Published in EJCMID (IF: 2.668)**.

Article 10: *Enterococcus cecorum* human infection, France. **Published in NMNI (IF: NA)**.

Article 11: *Citrobacter amalonaticus* urinary-tract human infections, Marseille, France. **To be submitted in IJID (IF: 2.33)**.

Article 12: Worldwide decrease in methicillin-resistant *Staphylococcus aureus*: do we understand something? **Published in CMI (IF: 5.768)**.

Article 13: Low level of resistance in Enterococci strains isolated in four French hospitals, Marseille, France. **Published in MDR (IF: NA)**.

Avant propos

Une fois émises par les différents systèmes de surveillance épidémiologique développés dans le cadre de l'IHU, toutes les alarmes sont présentées lors d'un staff épidémiologique hebdomadaire pour être validées ou infirmées (article 4). Les alarmes validées donnent par la suite lieu à des

investigations visant à confirmer leur véracité. En cas de confirmation, une alarme peut donner lieu à une déclaration à l'ARS et être valorisée par la publication d'articles scientifiques.
La partie suivante fait l'inventaire de tous les événements épidémiques anormaux confirmés et publiés au cours de mon travail de thèse en les classant par système de surveillance.

La surveillance hebdomadaire des données classées par syndromes et plateformes technologiques dans EPIMIC a permis de détecter, d'investiguer et de publier un certain nombre d'événements anormaux, dont l'**article 7**.
L'**article 7** est une étude épidémiologique rétrospective présentant les résultats de l'investigation épidémiologique réalisée après identification d'une augmentation de fréquence d'émission d'alarmes par EPIMIC pour les espèces bactériennes intrinsèquement résistantes à la colistine, synonyme d'une augmentation de leur prévalence à l'échelle communautaire et/ou hospitalière. En reprenant les données historiques de nos hôpitaux relatives à la bactérie sur la période Janvier 2009-Décembre 2013, cette investigation nous a permis de confirmer cette tendance à l'échelle communautaire et hospitalière, mais surtout d'identifier dans les services d'hospitalisation longue durée et de réanimation une corrélation franche entre l'augmentation de la consommation de colistine sous sa forme aérosol et l'augmentation de la prévalence de ces espèces, démontrant ainsi la nécessité

d'utiliser cet antibiotique avec parcimonie et avec pleine conscience du danger que représente le fait de l'utiliser massivement pour le traitement des malades.

La surveillance hebdomadaire des espèces bactériennes isolées au moins une fois à l'AP-HM depuis 2002 par BALYSES a permis de rédiger 4 articles.
L'**article 8** est une lettre présentant le résultat d'une investigation épidémiologique réalisée après avoir identifié par le biais de BALYSES que *Streptococcus agalactiae* faisait anormalement partie des 10 espèces les plus isolées en routine au laboratoire sur plusieurs semaines consécutives. Après avoir récupéré les données historiques de nos hôpitaux relatives à l'espèce bactérienne entre Juillet 2008 et Septembre 2013, cette investigation nous a permis d'observer une augmentation annuelle de la prévalence du nombre d'infections invasives causées par la bactérie dans nos hôpitaux, ce qui avait été précédemment observé dans une étude Anglaise et Galloise (18).
La rédaction de l'**article 9** a été initiée après avoir identifié dans BALYSES une diminution progressive de l'incidence hebdomadaire de *Streptococcus pneumoniae* dans nos hôpitaux, tous sites infectieux confondus. En analysant les données rétrospectives de nos hôpitaux sur la période Janvier 2003-Décembre 2014, nous avons pu observer une diminution globale de prévalence sur la période d'étude, mais aussi une corrélation significative entre la diminution de prévalence observée chez les patients âgés de moins de 21 ans et ceux âgés de plus de 21 ans,

démontrant l'impact des programmes de vaccination nationaux à l'échelle de notre région.

Enfin, les **articles 10 et 11** sont des rapports de cas décrivant des infections par des espèces bactériennes rarement identifiées comme pathogène chez l'homme (*Enterococcus cecorum* et *Citrobacter amalonaticus*) chez des patients hospitalisés à l'AP-HM identifiées sur la base d'alarmes émises par BALYSES.

La surveillance hebdomadaire des niveaux de résistance aux β-lactamines des 15 espèces bactériennes d'intérêt clinique surveillées par le système de surveillance MARSS à permis de rédiger une lettre à Clinical Microbiology and Infection (**article 12**) présentant le faible niveau de résistance à la méthicilline des souches de *Staphylococcus aureus* responsables d'infections invasives chez des patients hospitalisés à l'AP-HM. Cet article a été rédigé après avoir observé une constante diminution de ce niveau de résistance chez toutes les souches isolées en routine au laboratoire depuis le début de la surveillance en Janvier 2013.

Enfin, cette surveillance nous a également permis d'identifier que les souches d'entérocoques isolées et testées par antibiogramme en routine au laboratoire de la Timone présentaient un faible niveau de résistance aux antibiotiques, et que ce niveau de résistance ainsi que le ratio du nombre de souches d'*E. faecium /E. faecalis* isolées à partir de prélèvements invasifs était plus faible dans nos hôpitaux que celui de la majorité des pays Européens participant au rapport annuel de

l’EARS-Net en 2012 (**article 13**).

EVENEMENT ANORMAL IDENTIFIE SUR LA BASE D’ALARMES EMISES PAR EPIMIC

Article 7: Increasing burden of urinary tract infections due to intrinsic colistin-resistant bacteria in hospitals in Marseille, France.

Cédric Abat, Guillaume Desboves, Abiola Olumuyiwa Olaitan, Hervé Chaudet, Nicole Roattino, Pierre-Edouard Fournier, Philippe Colson, Didier Raoult, Jean-Marc Rolain

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Increasing burden of urinary tract infections due to intrinsic colistin-resistant bacteria in hospitals in Marseille, France

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ABSTRACT

The emergence of multidrug-resistant (MDR) Gram-negative bacteria has become a major public health problem, eliciting renewed interest in colistin, an old antibiotic that is now routinely used to treat MDR bacterial infections. Here we investigated whether colistin use has affected the prevalence of infections due to intrinsic colistin-resistant bacteria (CRB) in university hospitals in Marseille (France) over a 5-year period. All data from patients infected by intrinsic CRB were compiled from January 2009 to December 2013. *Escherichia coli* infections were used for comparison. Colistin consumption data were also collected from pharmacy records from 2008 to 2013. A total of 4847 intrinsic CRB infections, including 3150 *Proteus* spp., 847 *Morganella* spp., 704 *Serratia* spp. and 146 *Providencia* spp., were collected between 2009 and 2013. During this period, the annual incidence rate of hospital-acquired CRB infections increased from 220 per 1000 patients to 230 per 1000 patients and that of community-acquired CRB infections increased from 100 per 1000 patients to 140 per 1000 patients. In parallel, colistin consumption increased 2.2-fold from 2008 to 2013, mainly because of an increase in the use of colistin aerosol forms (from 50 unitary doses to 2926 unitary doses; $P < 10^{-5}$) that was significantly correlated with an increase in the number of patients positive for CRB admitted to ICUs and units of long-term care between 2009 and 2013 ($r = 0.91$; $P = 0.03$). The global rise in infections due to intrinsic CRB is worrying and surveillance is warranted to better characterise this intriguing epidemiological change.
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1. Introduction

Antimicrobial resistance represents a major public health concern worldwide. Following the appearance in the 1980s of extended-spectrum β-lactamase-producing Gram-negative bacteria, which threaten both hospital settings and the community [1], carbapenems have been considered as the last-resource drugs and have been widely used in healthcare units [2]. However, since the early 2000s, various acquired carbapenemases, primarily *Klebsiella pneumoniae* carbapenemase (KPC) type [2] or, more recently, the New Delhi metallo-β-lactamase (NDM) [3], have emerged and spread worldwide [4], further limiting therapeutic options. These limits have forced clinicians and researchers to develop new treatment strategies and practices, including the use of alternative treatment options. The polymyxins are cationic cyclic polypeptide antibiotics composed of five chemical compounds (polymyxins A–E) [5,6]. Polymyxins are bactericidal antibiotics effective against most Gram-negative bacteria except bacteria of the genera *Proteus*, *Providencia*, *Serratia*, *Morganella* and *Burkholderia* that are intrinsically resistant [5]. Colistin (polymyxin E) was extensively used between the 1960s and 1980s to treat patients infected by Gram-negative bacteria but was gradually abandoned in the 1980s owing to nephrotoxicity and neurotoxicity [5,6]. In this context, colistin has recently been reconsidered as a treatment of last resort to treat patients with ventilator-associated pneumonia and bacteraemia due to carbapenemase-producing bacteria, mainly *K. pneumoniae*, *Acinetobacter* spp. and *Pseudomonas* spp. [5–7]. Unfortunately, the increased use of colistin as a 'last-line' therapeutic drug for the treatment of patients infected with these multidrug-resistant (MDR) Gram-negative bacteria has led to the recent emergence

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of colistin-resistant bacteria (CRB) among these bacterial species [5,8–12].

This increasing public health concern led us to investigate whether the use of colistin currently affects the biodiversity of bacterial pathogens isolated from hospitals towards an increase of intrinsic CRB. A 5-year (January 2009 to December 2013) retrospective analysis of data on intrinsic CRB from the four university hospitals of Marseille was performed, using *Escherichia coli* infections as a control, and these data were correlated with colistin consumption in the four hospitals during the same period.

2. Materials and methods

2.1. Study setting

The Assistance Publique–Hôpitaux de Marseille (AP-HM) comprises the four university hospitals (North, South, Conception and Timone Hospitals) of Marseille, which is the second largest city in France (2010 estimated city population, 850 726). Cumulatively, these hospitals include 4000 beds (ca. 1500 beds in Timone Hospital, 900 in the North Hospital, 700 in Conception Hospital and 600 in the South Hospital [13]).

2.2. Retrospective analysis of intrinsic colistin-resistant bacteria in the database

To perform this study, a 5-year retrospective Microsoft Excel database (January 2009 to December 2013) that only included samples from which intrinsic CRB were isolated was implemented. In this study, intrinsic CRB included *Morganella* spp., *Serratia* spp., *Proteus* spp. and *Providencia* spp. All of the data were collected from an 11-year historical bacterial database that included previously published data [14]. Duplicates were deleted, and only one bacterial identification per sample and patient was considered. To ensure that the observed trends were not due to a rise in the number of samples that were processed in the laboratory during the study period, the annual number of patients who were infected by *E. coli* was used as a control to calculate annual ratios. EPIMIC, which is a simple Microsoft Excel tool that is based on clinical microbiology data from the four university hospitals [15], was also used to retrospectively plot the weekly number of patients who were infected by intrinsic CRB and to set up a threshold to detect an increase in CRB, i.e. a number above the mean weekly number plus 2 standard deviations. Data from 2002 to 2008 were not included in this study because some gaps in these data were observed. Indeed, before 2009, two laboratories performed clinical microbiology analysis (one in Timone Hospital and the other in the North Hospital). Since 2009, only one laboratory performs all the analysis.

2.3. AP-HM colistin consumption

Annual AP-HM colistin consumption data were retrospectively collected from pharmacy records of all medical units from 2008 to 2013, according to prescriptive unitary doses and form (spray or fluid injection).

2.4. Hospital-acquired infections, community-acquired infections and others

The data analysed in the present study did not contain the date of hospitalisation. Therefore, based on the category of each healthcare unit where CRB were isolated, hospital-acquired CRB infections were defined as CRB infections observed in patients admitted to intensive care units (ICUs) or hospitalised in units of long-term healthcare. Community-acquired CRB infections were defined as infections observed in patients admitted to emergency units or units of short-term healthcare. Finally, other units were included in another group not included in the analysis of colistin consumption evolution.

2.5. Statistical analysis

Statistical analyses were performed using Pearson's χ^2 test and Pearson's coefficient correlation test. Finally, linear models were defined to analyse historical trends (the annual trend in the mean number of patients infected by CRB strains or the annual trend in the mean number of colistin units used). All of the tests were two-sided, and *P*-values of <0.05 were considered statistically significant. Data were analysed using the Epi Info v.3.01 (http://www.openepi.com/Menu/OE_Menu.htm) and R software (The R Project, Auckland, New Zealand).

3. Results

3.1. Total number of patients infected by intrinsic colistin-resistant strains, genus distribution and global trends

During this 5-year study, 4847 patients in the different units of the university hospitals of Marseille were identified to be infected by at least one CRB. Among the genera of interest, *Proteus* spp. were the most common pathogens (3150 isolates), followed by *Morganella* spp. (847 isolates), *Serratia* spp. (704 isolates) and *Providencia* spp. (146 isolates) (Table 1). During the same period, 23 436 patients were infected by *E. coli* (Table 1). The increase in the number of patients infected by CRB strains was predominantly due to *Proteus* spp., with a 1.7-fold increase between 2009 and 2013, and more precisely to an increase in hospital- and community-acquired urinary tract infections due to *Proteus* spp. (the annual trends in the number of patients infected by CRB were 33.8 patients and 38.9 patients, respectively) (Table 1). The number of CRB strains isolated from hospital-acquired infections increased 1.4-fold (584 vs. 799) throughout the study period, leading to an increase in the annual incidence rate of hospital-acquired infections caused by CRB strains from 220 per 1000 patients to 230 per 1000 patients (Table 2). Comparison between the annual number of patients infected by *E. coli* and CRB isolated from hospital-acquired infections revealed that this increase was not significant between 2009 and 2013 ($P = 0.19$) (Table 2) but was significant between 2010 and 2013 ($P = 0.01$) and between 2011 and 2013 ($P = 0.001$). Community-acquired CRB infections also increased over the study period (2.4-fold, from 198 to 476 between 2009 and 2013; annual incidence rate increase from 100 per 1000 patients to 140 per 1000 patients). Comparison between the annual number of patients infected by *E. coli* and CRB isolated from community-acquired infections revealed that this increase was significant over the study period ($P < 10^{-3}$) (Table 2). The ratio of the annual rate of CRB hospital-acquired infections to community-acquired CRB infections decreased over time from 2.1 to 1.7 (Table 2). Evolution of the number of CRB strains isolated per hospital, sample and year is presented in Table 3. CRB infections significantly increased for three of the four studied hospitals, with annual trends in the number of patients infected by CRB equal to 55.3 patients for Conception Hospital ($P = 0.03$), 63.8 patients for the North Hospital ($P = 0.005$) and 63.6 patients for Timone Hospital ($P = 0.04$) (Table 3). For these three hospitals, this increase was mainly due to rises in the number of urine samples positive for CRB (the annual trends in the number of patients infected were 34.8, 40.9 and 38.5 patients for Conception Hospital, the North Hospital and Timone Hospital, respectively), which were significant ($P = 0.06$, 0.01 and 0.048, respectively) (Table 3). Finally, retrospective analysis of the data per week (using EPIMIC) is presented in Fig. 1. The automated comparison between the weekly numbers of

Table 1
Number of isolations and historical trends of colistin-resistant *Morganella* spp., *Proteus* spp., *Providencia* spp. and *Serratia* spp. strains included in this study, classified by kind of sample and unit where they were isolated, January 2009 to December 2013.

Year	Sample	*Proteus* spp.			*Morganella* spp.			*Serratia* spp.			*Providencia* spp.			*Escherichia coli*
		HAI	CAI	O	HAI	CAI	O	HAI	CAI	O	HAI	CAI	O	
2009	Urine	221	119	17	48	16	7	25	6	0	9	1	0	
	Respiratory samples	26	3	4	7	0	1	42	2	3	2	0	0	
	Mucosal and cutaneous samples and biopsies	78	23	3	28	8	2	29	5	5	6	1	0	
	Blood cultures	14	2	2	9	3	1	14	4	2	0	0	0	
	Others	16	5	0	5	0	0	4	1	3	1	0	0	
2010	Urine	160	76	10	35	15	1	17	6	2	7	4	0	
	Respiratory samples	14	1	2	2	0	2	20	3	3	1	0	0	
	Mucosal and cutaneous samples and biopsies	69	25	3	23	6	0	23	6	3	2	0	0	
	Blood cultures	17	11	3	4	1	0	10	4	0	0	0	0	
	Others	10	4	0	7	1	1	8	0	0	0	0	0	
2011	Urine	191	128	18	61	28	3	16	8	2	9	6	0	
	Respiratory samples	18	3	6	5	0	0	31	5	6	2	0	1	
	Mucosal and cutaneous samples and biopsies	73	33	11	36	11	2	21	8	2	5	1	0	
	Blood cultures	16	19	2	7	2	1	13	7	1	0	0	0	
	Others	7	5	3	7	3	0	5	3	1	1	1	0	
2012	Urine	292	213	19	81	34	12	21	9	2	12	7	0	
	Respiratory samples	35	1	3	12	2	3	28	6	14	4	0	0	
	Mucosal and cutaneous samples and biopsies	103	46	10	35	14	6	24	14	3	7	1	2	
	Blood cultures	17	10	1	7	0	2	9	2	1	0	0	0	
	Others	25	6	2	8	4	1	8	3	0	4	1	0	
2013	Urine	324	245	23	75	49	6	35	12	3	11	23	1	
	Respiratory samples	43	4	6	10	0	3	44	9	6	1	0	0	
	Mucosal and cutaneous samples and biopsies	101	69	15	45	15	1	31	12	0	4	1	2	
	Blood cultures	17	11	1	3	3	2	11	6	0	1	1	1	
	Others	26	7	4	11	4	0	5	4	3	1	1	0	
Historical trends per bacterial species and samples [a]	Urine	33.8	38.9	2.1	10.0	8.5	0.9	2.4	1.5	0.6	0.9	4.7	0.2	
	Respiratory samples	5.5	0.2	0.5	1.6	0.2	0.5	1.2	1.7	1.7	0.1	0	$<10^{-5}$	
	Mucosal and cutaneous samples and biopsies	8.0	11.3	3.1	4.6	2.2	0.4	0.5	2.2	−1.0	0.1	0.1	0.6	
	Blood cultures	0.6	1.7	−0.4	−0.9	−0.1	0.4	−0.7	0.2	−0.3	0.2	0.2	0.2	
	Others	3.5	0.6	1.0	1.3	1.1	$<10^{-5}$	0.2	0.9	$<10^{-5}$	0.4	0.3	0	
Annual number of strains isolated per bacterial species	2009		533			135			145			20		
	2010		405			98			105			14		
	2011		533			166			129			26		
	2012		783			221			144			38		
	2013		896			227			181			48		
Total number of strains isolated for each bacterial genus		3150			847			704			146			23 436

HAI, hospital-acquired infections; CAI, community-acquired infections; O, infections observed in units not classified.
[a] Historical trends calculated using linear models, each value giving the annual trend of the mean number of patients infected by colistin-resistant bacteria strains.

Table 2
Number of patients with hospital- or community-acquired colistin-resistant bacteria (CRB) infections, January 2009 to December 2013.

Year	Hospital-acquired infections				Community-acquired infections				Ratio of the AIR of hospital-acquired/ community-acquired CRB infections
	CRB	*Escherichia coli*	*P*-value[a]	AIR	CRB	*E. coli*	*P*-value[a]	AIR	
2009	584	2113		0.22	198	1759		0.10	2.1
2010	429	1697		0.20	163	1581		0.09	2.2
2011	525	2158	0.19	0.20	271	2180	$<10^{-3}$	0.11	1.8
2012	732	2428		0.23	373	2593		0.13	1.8
2013	799	2670		0.23	476	3033		0.14	1.7
Total	3069	11 066			1481	11 146			

AIR, annual incidence rate.
[a] Analyses performed using a two-sided Pearson χ^2 test; *P*-values of <0.05 were considered statistically significant.

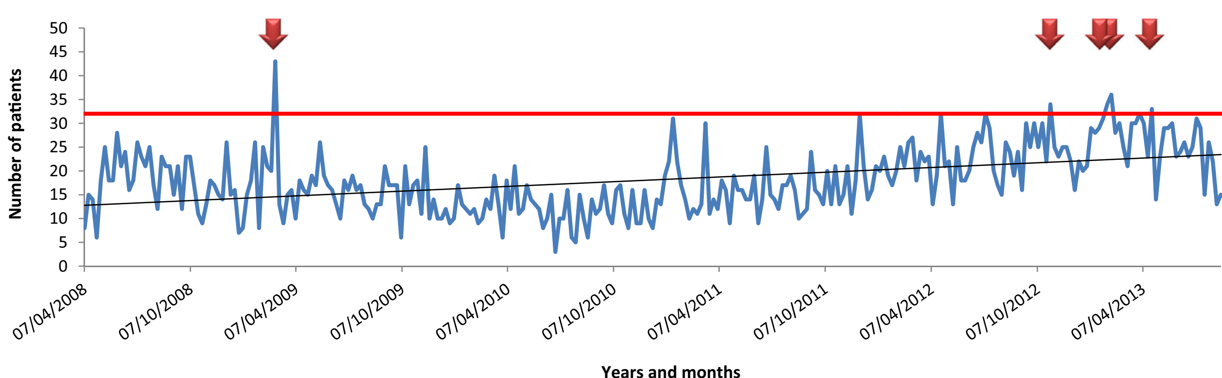

Fig. 1. Number of patients infected by intrinsic colistin-resistant bacteria (CRB) from 7th April 2008 to December 2013. The red line indicates an automatic threshold to detect an increase in CRB, defined as the mean value of historical data plus 2 standard deviations. Arrows indicate an abnormal increase in the number of patients infected by CRB.

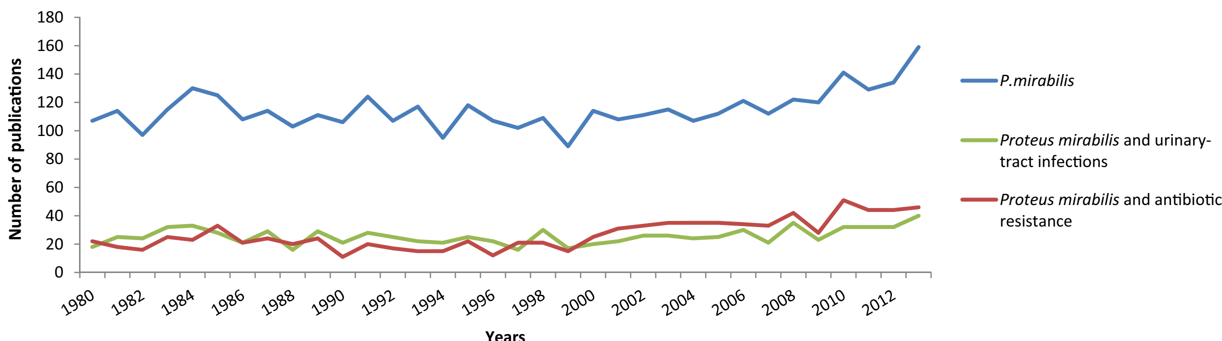

Fig. 2. Global evolution of the number of publications dealing with '*P. mirabilis*', '*Proteus mirabilis* AND urinary-tract infections' and '*Proteus mirabilis* AND antibiotic resistance'.

patients infected by intrinsic CRB and the calculated mean threshold (value = 33) allowed us to detect five abnormal increases, the first one occurring during the week of 2 March 2009.

3.2. *Colistin consumption trends and origin of patients infected by colistin-resistant bacteria*

Colistin consumption data allowed us to detect a 2.2-fold increase in the number of prescriptions in the four hospitals, primarily due to a significant increase in the use of colistin aerosol forms in ICUs and units of long-term care (from 50 units to 2926 units between 2008 and 2013; $P < 10^{-5}$) (Table 4). Over the same period, the number of colistin units prescribed in emergency units and units of short-term healthcare decreased (from 347 units to 250 units). A significant correlation was found between the increase in the number of patients positive for CRB admitted to ICUs and units of long-term care and the increase in the use of colistin aerosol forms in these units between 2009 and 2013 ($r = 0.91$; $P = 0.03$) (Table 4).

4. Discussion

To the best of our knowledge, here we present the largest series of human infections due to intrinsic CRB that has been published worldwide. This study allowed us to identify interesting epidemiological changes of intrinsic CRB isolated from the university hospitals of Marseille, with an increasing number of hospital- and community-acquired CRB infections over the study period.

4.1. *Hospital-acquired infections*

Based on these results, an increase was observed in the number of patients with hospital-acquired CRB infections linked to an increasing consumption of colistin in ICUs and units of long-term

Table 3
Kinetics of the number of colistin-resistant bacteria (CRB) isolated per hospital, 2009–2013.

Sample	Hospital and year																							
	Conception						North hospital						South hospital						Timone					
	2009	2010	2011	2012	2013	T	2009	2010	2011	2012	2013	T	2009	2010	2011	2012	2013	T	2009	2010	2011	2012	2013	T
Urine	146	100	151	218	261	34.8	107	97	156	206	257	40.9	94	33	32	38	43	−9.7	116	95	124	232	240	38.5
Blood cultures	14	17	20	19	21	1.6	10	19	16	11	18	0.08	11	7	3	0	0	−2.9	16	7	29	19	17	1.4
Cutaneous and mucosal samples and biopsies	49	48	85	88	111	16.4	46	49	45	72	71	7.3	30	8	9	8	13	−3.4	61	55	63	89	98	10.8
Respiratory samples	5	9	8	2	8	−0.1	16	14	27	45	59	11.7	40	10	0	0	4	−8.2	28	15	41	61	54	9.8
Others	13	8	4	20	20	2.6	4	10	16	15	17	3.1	4	1	0	1	3	−0.2	14	12	16	25	23	3.1
Total	227	182	268	347	421	55.3	183	189	260	349	422	63.8	179	59	44	47	63	−24.4	235	184	273	426	432	63.6

T, historical trend calculated using linear models, each value giving the annual trend of the mean number of patients infected by CRB strains.

healthcare (Tables 2 and 4). Colistin is currently widely used in hospitals to face increasing levels of resistance to carbapenems, mainly in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *K. pneumoniae* [16], as resistance to carbapenem compounds is now endemic in several countries worldwide and has led to an increased use of colistin. However, the fact that a significant correlation between the increasing use of colistin aerosol forms in ICUs and units of long-term healthcare and a rise in the number of hospital-acquired CRB infections between 2009 and 2013 (Table 4) was observed is interesting. This result can be explained by the fact that colistin has been extensively used as a treatment of last resort for patients with ventilator-associated pneumonia due to carbapenemase-producing bacteria, mainly *K. pneumoniae*, *Acinetobacter* spp. and *Pseudomonas* spp. [5–7], even in our hospitals [15]. Similar observations were reported in a few studies. In Argentina, clinicians from a hospital in Lanús, a province of Buenos Aires, reported a MDR *Serratia marcescens* outbreak from November 2007 to April 2008 associated with 40% mortality following colistin use in adult patients presenting with carbapenem-resistant *A. baumannii* postsurgical meningitis [17]. In a retrospective analysis, these authors discovered that the use of colistin, starting in 2005, had been followed by a significant increase in the frequency of infections due to *S. marcescens* but also to *Proteus mirabilis* in 2006, showing that the routine use of colistin impacted the frequency of isolation of intrinsic CRB at the hospital scale [17]. Similarly, in 2012, Hayakawa et al. detected a 5-year increase in the number of *Providencia stuartii* strains that were isolated from the Detroit Medical Center (Detroit, MI) [18]. Here, increased colistin use was a response to the increased frequency of isolation of *A. baumannii* and carbapenem-resistant Enterobacteriaceae in the hospital. After 5 years of use, the authors clearly observed that the number of *P. stuartii* strains isolated from their medical centre increased dramatically. They also demonstrated a significant correlation between colistin consumption and numbers of infections due to *P. stuartii* [18]. Finally, in Crete (Greece), Samonis et al. recently observed a 5-year increase in the number of patients infected by the same bacterial species (*Proteus* spp., *Serratia* spp., *Morganella morganii* and *Providencia* spp.) as studied here [19]. In their study, no correlation was observed between the number of intrinsically resistant bacterial species of interest and the use of colistin, but an association between colistin consumption and the number of hospital-acquired isolates belonging to these species was observed, especially in ICUs [19]. Table 5 summarises the increases that were observed for the four intrinsic CRB that were analysed in the current study and those published by the three previous studies.

4.2. Community-acquired infections

The fact that a significant increase in the number of community-acquired CRB infections was observed from 2009 to 2013 (Table 2), mainly due to increasing numbers of patients admitted for urinary tract infections, is an intriguing result. To the best of our knowledge, nobody has studied such a phenomenon in intrinsic CRB. Some hypotheses can be so proposed. First, it is possible that intrinsic CRB infections are mediated by a common source, such as food or the environment [20]. Second, we can suppose that this phenomenon can be due to a *Proteus* spp. clone that emerged and persisted in the Marseillan population (which can be supported by the abrupt increase in the number of publications dealing with *P. mirabilis*; Fig. 2), or at a larger scale. Such events has been previously described in some bacterial species, such as in *E. coli* with the pandemic *E. coli* clone sequence type 131 (ST131) [21], the O104:H4 clone [22] or the O15:K52:H1 clone [23]. To verify these hypotheses, systematic genotyping using the multilocus sequence

Table 4
Comparison between colistin consumption and the number of patients infected by intrinsic colistin-resistant bacteria (CRB), depending on the type of infection (hospital-acquired infections versus community-acquired infections) from 2009 to 2013.

Year	Colistin consumption (number of doses)						Number of intrinsic CRB (number of patients infected)	
	ICUs or units of long-term healthcare			Emergency units or units of short-term healthcare			ICUs or units of long-term health care	Emergency units or units of short-term health care
	Fluid injection	Aerosol	Total	Fluid injection	Aerosol	Total		
2008	4936	50	4986	347	0	347	N/A	N/A
2009	8664	1399	10 063	29	1	30	584	198
2010	7597	1131	8728	275	0	275	429	163
2011	9960	1576	11 536	491	67	558	525	271
2012	10 843	2002	12 845	277	0	277	732	373
2013	7938	2926	10 864	250	0	250	799	476

ICU, intensive care unit; N/A, data not available for 2008 for CRB strains.

typing (MLST) approach should be done on CRB isolates causing community-acquired infections.

4.3. Limitations

This study has some limitations. The definitions of hospital- and community-acquired CRB infections are not based on the duration of hospitalisation. Moreover, some strains were classified in another group. Therefore, the classifications are objectionables. However, these definitions allowed us to classify 93.9% (4550) of the 4847 CRB strains and 94.8% (22 212) of the 23 436 *E. coli* strains in hospital- and community-acquired infection groups (Tables 1 and 2). Moreover, we were not able to collect data on colistin consumption prior to 2008, which may have prevented us seeing an offset in time between the beginning of use of colistin and the increase of nosocomial CRB infections in our institution as has been observed elsewhere [17–19].

Table 5
Comparison between the increases observed for the four genera in this study with those observed and published after the routine use of colistin in other hospitals.

Species, date and number of isolates		Observed increase	Reference
Proteus spp.		1.7-fold	This study
2009	2013		
533	896		
Morganella spp.		1.7-fold	
2009	2013		
135	227		
Serratia spp.		1.2-fold	
2009	2013		
145	181		
Providencia spp.		2.4-fold	
2009	2013		
20	48		
Serratia marcescens[a]		2.5-fold	[17]
2002	2011		
16	40		
Proteus mirabilis[a]		1.3-fold	
2002	2011		
44	57		
Providencia stuartii		1.7-fold	[18]
2005	2009		
168	288		
Proteus spp.		1.4-fold	[19]
2006	2010		
167	232		
Morganella spp.		2.9-fold	
2006	2010		
11	32		
Serratia spp.		2-fold	
2006	2010		
32	64		
Providencia spp.		1.4-fold	
2006	2010		
8	11		

[a] Approximate values.

5. Conclusion

We argue that the extensive use of colistin may lead to the selection of intrinsic CRB and facilitate their spread as nosocomial agents in hospitals. This phenomenon is well known in the context of cystic fibrosis where colistin use by aerosols occasionally has led to the selection of intrinsic CRB, including *Inquilinus limosus*, *Brevundimonas diminuta*, *Ochrobactrum anthropi*, *Pandoraea* spp., *Chryseobacterium indologenes* and *Burkholderia* spp. [5,24,25]. However, other factors could be responsible for the increase in CRB infections especially in the community. In parallel, the increase in the number of community-acquired CRB infections challenges us about the possibility for CRB clones to disseminate in the global population using animal-based food or in other unsuspected ways. This should be further investigated in the future. We therefore believe that colistin use should be restricted to treat patients with sepsis and severe infections due to carbapenemase-producing bacteria and should be avoided for selective digestive decontamination [26]. Clinicians must remain vigilant when they use colistin and should use it in combination with at least another antibiotic such as doripenem, ceftazidime, tigecycline or rifampicin to avoid the selection and spread of intrinsic CRB [5,17–19]. Indeed, most of the countries with a carbapenem-resistant Enterobacteriaceae burden have also reported the emergence of CRB, including Greece [10], Italy [27], Argentina [11], the USA [28] and South Korea [29,30]. Finally, surveillance of the emergence of resistance to colistin and colistin consumption is warranted to limit the emergence and spread of such bacteria worldwide.

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References

[1] Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14:933–51.
[2] Livermore DM, Woodford N. The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends Microbiol 2006;14:413–20.

[3] Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010;10:597–602.
[4] Rolain JM, Parola P, Cornaglia G. New Delhi metallo-β-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect 2010;16: 1699–701.
[5] Biswas S, Brunel JM, Dubus JC, Reynaud-Gaubert M, Rolain JM. Colistin: an update on the antibiotic of the 21st century. Expert Rev Anti Infect Ther 2012;10:917–34.
[6] Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant Gram-negative bacterial infections. Clin Infect Dis 2005;40:1333–41.
[7] Stein A, Raoult D. Colistin: an antimicrobial for the 21st century? Clin Infect Dis 2002;35:901–2.
[8] Mohanty S, Maurya V, Gaind R, Deb M. Phenotypic characterization and colistin susceptibilities of carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. J Infect Dev Ctries 2013;7:880–7.
[9] Lesho E, Yoon EJ, McGann P, Snesrud E, Kwak Y, Milillo M, et al. Emergence of colistin-resistance in extremely drug-resistant *Acinetobacter baumannii* containing a novel *pmrCAB* operon during colistin therapy of wound infections. J Infect Dis 2013;208:1142–51.
[10] Kontopoulou K, Protonotariou E, Vasilakos K, Kriti M, Koteli A, Antoniadou E, et al. Hospital outbreak caused by *Klebsiella pneumoniae* producing KPC-2 β-lactamase resistant to colistin. J Hosp Infect 2010;76:70–3.
[11] Arduino SM, Quiroga MP, Ramírez MS, Merkier AK, Errecalde L, Di Martino A, et al. Transposons and integrons in colistin-resistant clones of *Klebsiella pneumoniae* and *Acinetobacter baumannii* with epidemic or sporadic behaviour. J Med Microbiol 2012;61:1417–20.
[12] Rolain JM, Diene SM, Kempf M, Gimenez G, Robert C, Raoult D. Real-time sequencing to decipher the molecular mechanism of resistance of a clinical pan-drug-resistant *Acinetobacter baumannii* isolate from Marseille, France. Antimicrob Agents Chemother 2013;57:592–6.
[13] Colson P, Gouriet F, Badiaga S, Tamalet C, Stein A, Raoult D. Real-time laboratory surveillance of sexually-transmissible infections in Marseille university hospitals reveals rise of gonorrhoea, syphilis and human immunodeficiency virus seroconversions in 2012. Euro Surveill 2013;18:4.
[14] Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al. Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 2013;51:2182–94.
[15] Kempf M, Rolain JM, Azza S, Diene S, Joly-Guillou ML, Dubourg G, et al. Investigation of *Acinetobacter baumannii* resistance to carbapenems in Marseille hospitals, south of France: a transition from an epidemic to an endemic situation. APMIS 2013;121:64–71.
[16] Michalopoulos A, Fotakis D, Virtzili S, Vletsas C, Raftopoulou S, Mastora Z, et al. Aerosolized colistin as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant Gram-negative bacteria: a prospective study. Respir Med 2008;102:407–12.
[17] Merkier AK, Rodríguez MC, Togneri A, Brengi S, Osuna C, Pichel M, et al. Outbreak of a cluster with epidemic behavior due to *Serratia marcescens* after colistin administration in a hospital setting. J Clin Microbiol 2013;51:2295–302.
[18] Hayakawa K, Marchaim D, Divine GW, Pogue JM, Kumar S, Lephart P, et al. Growing prevalence of *Providencia stuartii* associated with the increased usage of colistin at a tertiary health care center. Int J Infect Dis 2012;16:e646–8.
[19] Samonis G, Korbila IP, Maraki S, Michailidou I, Vardakas KZ, Kofteridis D, et al. Trends of isolation of intrinsically resistant to colistin Enterobacteriaceae and association with colistin use in a tertiary hospital. Eur J Clin Microbiol Infect Dis 2014;33:1505–10.
[20] Morales AS, Fragoso de Araújo J, de Moura Gomes VT, Reis Costa AT, dos Prazeres Rodrigues D, Porfida Ferreira TS, et al. Colistin resistance in *Escherichia coli* and *Salmonella enterica* strains isolated from swine in Brazil. ScientificWorldJournal 2012;2012:109795.
[21] Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, et al. Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci U S A 2014;111:5694–9.
[22] Jandhyala DM, Vanguri V, Boll EJ, Lai Y, McCormick BA, Leong JM. Shiga toxin-producing *Escherichia coli* O104:H4: an emerging pathogen with enhanced virulence. Infect Dis Clin North Am 2013;27:631–49.
[23] Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob Agents Chemother 2009;53:2733–9.
[24] Menuet M, Bittar F, Stremler N, Dubus JC, Sarles J, Raoult D, et al. First isolation of two colistin-resistant emerging pathogens, *Brevundimonas diminuta* and *Ochrobactrum anthropi*, in a woman with cystic fibrosis: a case report. J Med Case Rep 2008;2:373.
[25] Chen FL, Wang GC, Teng SO, Ou TY, Yu FL, Lee WS. Clinical and epidemiological features of *Chryseobacterium indologenes* infections: analysis of 215 cases. J Microbiol Immunol Infect 2013;46:425–32.
[26] Strenger V, Gschliesser T, Grisold A, Zarfel G, Feierl G, Masoud L, et al. Orally administered colistin leads to colistin-resistant intestinal flora and fails to prevent faecal colonisation with extended-spectrum β-lactamase-producing enterobacteria in hospitalised newborns. Int J Antimicrob Agents 2011;37:67–9.
[27] Mammina C, Bonura C, Di Bernado F, Aleo A, Fasciana T, Sodano C, et al. Ongoing spread of colistin-resistant *Klebsiella pneumoniae* in different wards of an acute general hospital, Italy, June to December 2011. Euro Surveill 2012;17:pii: 20248.
[28] Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM, Rudin S, et al. Outbreak of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in metropolitan Detroit, Michigan. Antimicrob Agents Chemother 2011;55:593–9.
[29] Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother 2007;60:1163–7.
[30] Suh JY, Son JS, Chung DR, Peck KR, Ko KS, Song JH. Nonclonal emergence of colistin-resistant *Klebsiella pneumoniae* isolates from blood samples in South Korea. Antimicrob Agents Chemother 2010;54:560–2.

EVENEMENTS ANORMAUX IDENTIFIES SUR LA BASE D'ALARMES EMISES PAR BALYSES

Article 8: Increasing trend of invasive group B streptococcal infections, Marseille, France.

Cédric Abat, Hervé Chaudet, Didier Raoult, Philippe Colson

Publié dans Clinical Infectious Diseases (Impact Factor = 8.886)

Increasing Trend of Invasive Group B Streptococcal Infections, Marseille, France

To the Editor—We read with interest the article by Lamagni et al that describes a steady rise from 700–800 to 1652 per annum during the 1991–2010 period of invasive group B streptococcus (GBS) infections in England and Wales, most pronounced among adults [1]. This was identified based on routine microbiology laboratory reports undertaken across these countries through an automated biosurveillance system [1, 2], and was triggered by the description of an increase of invasive GBS disease in nonpregnant adults in the United States [3]. Since 2002, a weekly surveillance system of infections based on clinical microbiology data was implemented in our center, which is similar to that described in England and Wales and aims at detecting abnormal events [4]. In spring 2013, we extended our surveillance panel to all bacterial species found from 2002 through 2012 in our laboratory, including 459 different species identified from approximately 500 000 bacterial isolates [5]. Unexpectedly, we detected that GBS was, from weeks 16–25 of 2013, the ninth most frequently identified bacteria. These data and Lamagni et al's findings prompted us to analyze the incidence since mid-2008 (no earlier comprehensive data being available) of invasive GBS infections in our institution that gathers university hospitals of Marseille, the second-largest French city.

A total of 334 invasive GBS infections were diagnosed over the July 2008–

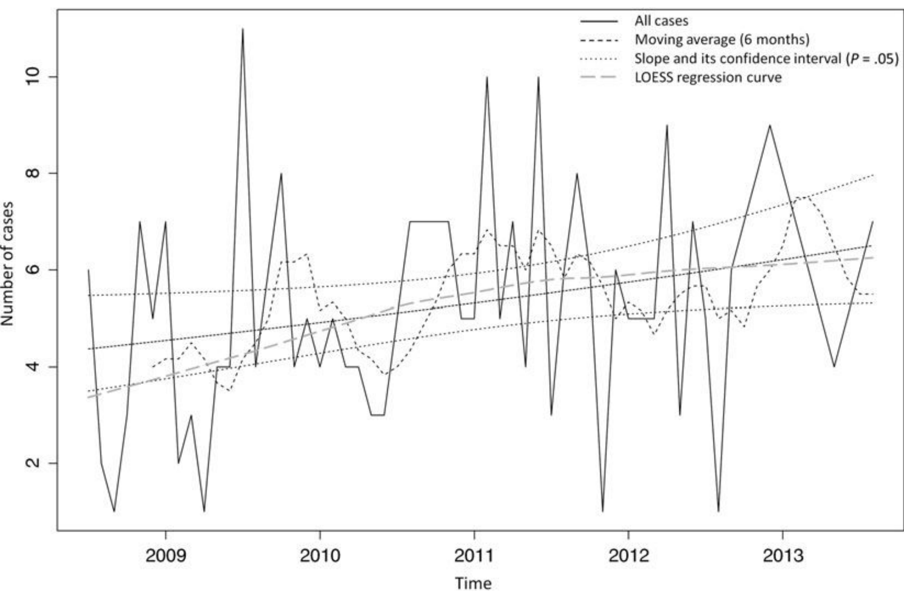

Figure 1. Evolution of numbers of invasive group B streptococcal infections diagnosed in Marseille university hospitals, 2008–2013. The black solid line indicates the monthly number of diagnoses, and the black dashed line indicates the corresponding moving average (6 months). The black dotted lines show the slope and its confidence interval for an error of .005 (Poisson distribution), and the gray dashed line shows the locally weighted polynomial regression (LOESS) curve.

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September 2013 period, based on culture from blood in 42% of cases, or from cerebrospinal fluids (2%), joints or bones (12%), and other normally sterile sites. We found an increasing trend of the number of invasive GBS infections (slope = 0.0065, $P = .0345$, Poisson regression) with a 1.4-fold increase, from 49 to 71, of the number of cases diagnosed between the first and last 12-month periods of follow-up (Figure 1); a 1.5-fold rise (from 44 to 67) was observed among patients older than 15 years. A structural change analysis suggested a change point of the regression coefficient on August 2011. This knee-point may be observed on the LOESS regression (locally weighted polynomial regression) curve, with a strong progression until the change point (slope = 0.0145, $P = .0346$, Poisson regression), followed by a plateau since this date (slope = 0.0065, $P = .0475$, Poisson regression).

Although we diagnosed in our single center far less invasive GBS infections than in nationwide studies conducted in England and Wales over 20 years (21 386) [1] or in the United States (in 10 states) over 6–17 years (14 573 and 19 512 in 2 studies [3, 6]), we observed an increasing trend of invasive GBS infections over the past 5 years. Reasons for such increases are unresolved [1, 3]. These data demonstrate the relevance of systematic surveillance of infections at various scales and in different geographical areas, which enable awareness of epidemiological changes and of their geographical spread.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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References

1. Lamagni TL, Keshishian C, Efstratiou A, et al. Emerging trends in the epidemiology of invasive group B streptococcal disease in England and Wales, 1991–2010. Clin Infect Dis **2013**; 57:682–8.
2. Enki DG, Noufaily A, Garthwaite PH, et al. Automated biosurveillance data from England and Wales, 1991–2011. Emerg Infect Dis **2013**; 19:35–42.
3. Skoff TH, Farley MM, Petit S, et al. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. Clin Infect Dis **2009**; 49:85–92.
4. Parola P, Colson P, Dubourg G, et al. Letter to the editor. Group A streptococcal infections during the seasonal influenza outbreak 2010/11 in south east England. Euro Surveill **2011**; 16:19816.
5. Seng P, Abat C, Rolain JM, et al. Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol **2013**; 51: 2182–94.
6. Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA **2008**; 299:2056–65.

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Article 9: Dramatic decrease of *Streptococcus pneumoniae* in Marseille, 2009-2014.

Cédric Abat, Didier Raoult, Jean-Marc Rolain

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ORIGINAL ARTICLE

Dramatic decrease of *Streptococcus pneumoniae* infections in Marseille, 2003–2014

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Abstract We studied the evolution of the prevalence of pneumococcal infections in university hospitals in Marseille, France, from January 2003 to December 2014, and compared our observations and results to available international data. We collected data referring to patients hospitalised for *Streptococcus pneumoniae* infections in the four university hospitals of Marseille from January 2003 to December 2014. We then calculated percentages of positiveness to pneumococcal strains by dividing the annual number of patients infected by pneumococcal strains by the annual number of patients found to be infected by at least one bacterial species in the settings of interest throughout the study period. Overall, 2442 non-redundant patients were infected by *S. pneumoniae* strains throughout the study period. We observed that the annual percentage of patients infected by *S. pneumoniae* significantly decreased throughout the study period (from 1.99 % in 2003 to 0.77 % in 2014, p-value < 10^{-4}). A significant correlation was obtained comparing the annual evolution of the percentage of patients positive to pneumococcal strains aged under 21 years to that of patients aged over 21 years ($r = 0.93$, p-value < 10^{-5}). Our results allowed us to prove that national immunisation programmes effectively impact on the pneumococcal infection prevalence in young and elderly populations, even on the regional scale.

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Introduction

Streptococcus pneumoniae is a Gram-positive bacterium present in the nasopharynx of humans, especially in young humans, in which the carriage prevalence varies from 27 % in developed countries to 85 % in developing countries [1].

S. pneumoniae is known to be a major life-threatening bacterial species, mainly for old people and children. It is classified as the first worldwide cause of bacterial meningitidis, with a 30 % associated mortality rate [2]. In children, it has been estimated to be a main cause of sepsis, meningitis and bacterial pneumonia, with 700,000 to 1 million annual deaths among children, while in the elderly, the fatality rate of pneumococcal bacteraemia ranges from 30 to 40 % of the cases [1, 3].

Vaccination programmes have been developed worldwide to massively vaccinate children against *S. pneumoniae* to prevent meningitis [4]. This led to a dramatic decrease in the frequency of all invasive pneumococcal diseases (IPDs) due to serotypes present in the vaccine. Recently, a study identified that the proportion of the worldwide population of children younger than 5 years old dying because of pneumococcal pneumonia decreased from 30.1 % in 1990 (around 652,400 deaths) to 29.2 % in 2013 (264,000 deaths), mainly in high-incomes countries thanks to conjugate vaccines [5]. It was also observed that the worldwide proportion of deaths due to pneumococcal strains fell from 26.9 to 22.4 % [5].

In France, two pneumococcal vaccines have been successively introduced in the French immunisation schedule since 2000. In 2002, the 7-valent pneumococcal conjugate vaccine (PCV-7), which targets the seven serotypes 4, 6B, 9V, 14, 18C, 19F and 23F [6], was introduced and recommended for at-risk children under 2 years of age before being recommended for all children under 2 years old in June 2006 [7]. Then, the 13-valent pneumococcal conjugate vaccine (PCV-13), which targets all the serotypes covered by the PCV-7 plus

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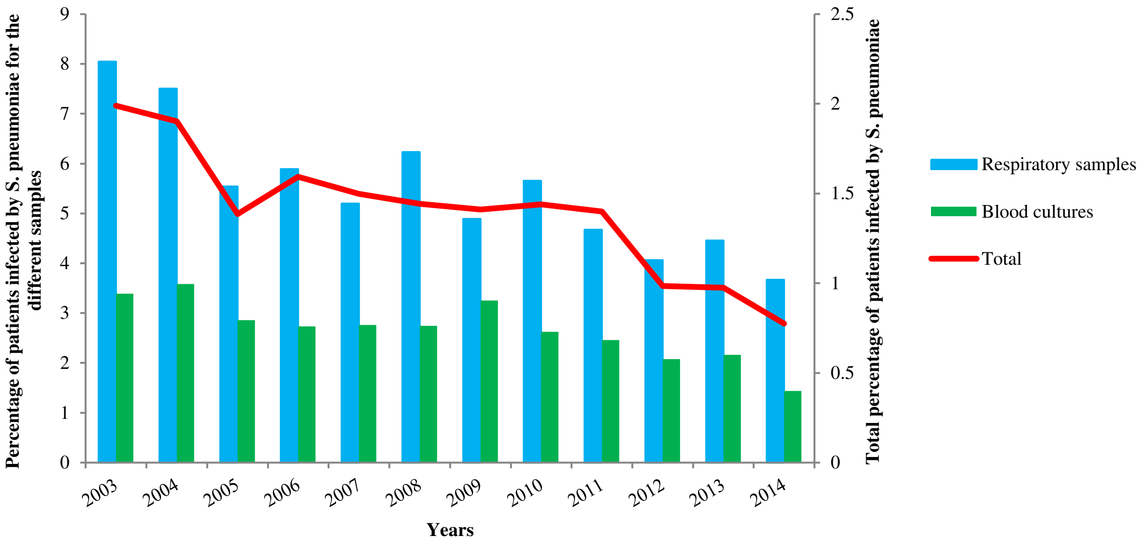

Fig. 1 Evolution of the percentage of patients infected by *Streptococcus pneumoniae* in the four university hospitals of Marseille from January 2003 to December 2014 (globally and per the main kinds of samples, 2442 infected patients)

Table 1 Evolution of the age class distribution of the 2442 patients infected by pneumococcal strains, of the 186,507 patients infected by at least one bacterial species and of the overall percentage of patients infected by *Streptococcus pneumoniae* in the four university hospitals of Marseille, January 2003 to December 2014

Years	Number of patients infected by *S. pneumoniae* classified by age class										Total
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	≥91	
2003	61	7	4	27	33	30	24	25	15	6	232
2004	59	6	7	20	22	28	20	19	20	1	202
2005	35	4	6	15	22	21	18	18	9	3	151
2006	40	5	2	21	25	15	24	26	11	3	172
2007	45	5	10	14	20	31	19	13	14	2	173
2008	65	5	17	19	21	28	23	27	13	0	218
2009	60	7	10	22	26	44	21	22	16	1	229
2010	53	3	10	15	25	21	24	27	12	3	193
2011	76	12	13	16	23	39	37	17	13	6	252
2012	59	5	10	14	27	32	23	21	13	4	208
2013	59	8	15	13	25	37	34	27	7	5	230
2014	46	3	7	11	27	31	32	17	3	5	182
Total	658	70	111	207	296	357	299	259	146	39	2442
	Number of patients infected by at least one bacterial species in the four university hospitals classified by age class[a]										
2003	1138	519	965	1115	1176	1579	1645	2018	1245	263	11,663
2004	1201	507	741	948	1111	1492	1507	1805	1068	242	10,622
2005	1238	528	800	908	1111	1547	1638	1809	1096	226	10,901
2006	1241	559	835	965	1073	1456	1468	1792	1124	281	10,794
2007	1306	565	936	1037	1135	1593	1614	1809	1258	291	11,544
2008	1841	677	1324	1238	1413	1981	2147	2439	1682	371	15,113
2009	2161	830	1394	1357	1617	2113	2198	2435	1834	297	16,236
2010	1828	612	1201	1048	1302	1656	1965	1993	1532	269	13,406
2011	2695	903	1553	1356	1587	2317	2537	2618	2029	402	17,997
2012	3182	995	1944	1692	1995	2365	3066	2896	2441	568	21,144
2013	3615	1130	2397	2056	1960	2679	3333	2994	2739	680	23,583
2014	3532	1052	2513	2012	2022	2713	3235	2987	2718	720	23,504

Table 1 (continued)

Years	Number of patients infected by *S. pneumoniae* classified by age class										Total
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	≥91	
Total	24,978	8877	16,603	15,732	17,502	23,491	26,353	27,595	20,766	4610	186,507
	Percentage of patients infected by *S. pneumoniae* classified by age class[b]										
2003	5.4	1.3	0.4	2.4	2.8	1.9	1.5	1.2	1.2	2.3	
2004	4.9	1.2	0.9	2.1	2.0	1.9	1.3	1.1	1.9	0.4	
2005	2.8	0.8	0.8	1.7	2.0	1.4	1.1	1.0	0.8	1.3	
2006	3.2	0.9	0.2	2.2	2.3	1.0	1.6	1.5	1.0	1.1	
2007	3.4	0.9	1.1	1.4	1.8	1.9	1.2	0.7	1.1	0.7	
2008	3.5	0.7	1.3	1.5	1.5	1.4	1.1	1.1	0.8	0.0	
2009	2.8	0.8	0.7	1.6	1.6	2.1	1.0	0.9	0.9	0.3	
2010	2.9	0.5	0.8	1.4	1.9	1.3	1.2	1.4	0.8	1.1	
2011	2.8	1.3	0.8	1.2	1.4	1.7	1.5	0.6	0.6	1.5	
2012	1.9	0.5	0.5	0.8	1.4	1.4	0.8	0.7	0.5	0.7	
2013	1.6	0.7	0.6	0.6	1.3	1.4	1.0	0.9	0.3	0.7	
2014	1.3	0.3	0.3	0.5	1.3	1.1	1.0	0.6	0.1	0.7	
Historical trends by age classes (*p*-value)[c]	−0.3 (10^{-4})	−0.05 (0.02)	−0.01 (0.6)	−0.2 (10^{-5})	−0.1 (10^{-3})	−0.04 (0.2)	−0.04 (0.06)	−0.05 (0.06)	−0.1 (10^{-3})	−0.05 (0.3)	

[a] Data from the historical database of the four university hospitals of Marseille, January 2003 to December 2014. Duplicates were removed by patients and bacterial species

[b] Percentages calculated dividing the annual number of patients infected by *S. pneumoniae* included in each range of ages by the total annual number of patients infected by at least one bacterial species in the same range of ages over the January 2003 to December 2014 period

[c] Historical trends calculated using linear models, each value giving the annual trend of the percentage of patients infected by *S. pneumoniae* strains included in the different age classes in the four university hospitals of Marseille. A *p*-value < 0.05 means that changes in historical trends are statistically significant over the study period for the age class of interest

serotypes 1, 3, 5, 6A, 7F and 19A [6], was introduced to the French market in 2010 to replace the PCV-7 [8].

Here, we study and discuss the evolution of the prevalence of *S. pneumoniae* infections in Marseille, France, from January 2003 to December 2014.

Methods

We retrospectively retrieved data on patients hospitalised for *S. pneumoniae* infections in the four university hospitals of Marseille from a 13-year historical database that has been partially published [9]. Only data from January 2003 to December 2014 were analysed herein. Duplicates were deleted per patients and bacterial species. We then calculated yearly percentages of positiveness to pneumococcal strains by dividing the annual number of patients infected by pneumococcal strains by the annual number of patients found to be infected by at least one bacterial species in the four university hospitals of Marseille. The classification of the infections was done according to the sample from which each *S. pneumoniae* strain was isolated. Moreover, for respiratory infections, a patient was considered infected by *S. pneumoniae* if we isolated at least 10^7 *S. pneumoniae* colony-forming units (CFU)/mL plus polynuclear neutrophils from sputum, 10^5 *S. pneumoniae* CFU/mL plus polynuclear neutrophils from bronchial aspiration or 10^4 *S. pneumoniae* CFU/mL plus polynuclear neutrophils from bronchoalveolar fluid.

Statistical analysis was performed with R (Auckland, New-Zealand) using a two-sided Pearson's Chi-square test or Fisher's exact test, as appropriate, and Pearson's coefficient correlation test. Linear models were used to define historical trends of the percentages of patients infected by *S. pneumoniae* strains throughout the study, meaning the annual mean percentage of patients infected by the bacterium. All of the tests performed were two-sided, and *p*-values < 0.05 were considered statistically significant.

Results

2442 non-redundant patients were infected by *S. pneumoniae* strains throughout the study period. The annual percentage of patients infected by *S. pneumoniae* significantly decreased during the study period (from 1.99 % in 2003 to 0.77 % in 2014, *p*-value < 10^{-4}) (Fig. 1). Most of the infections occured

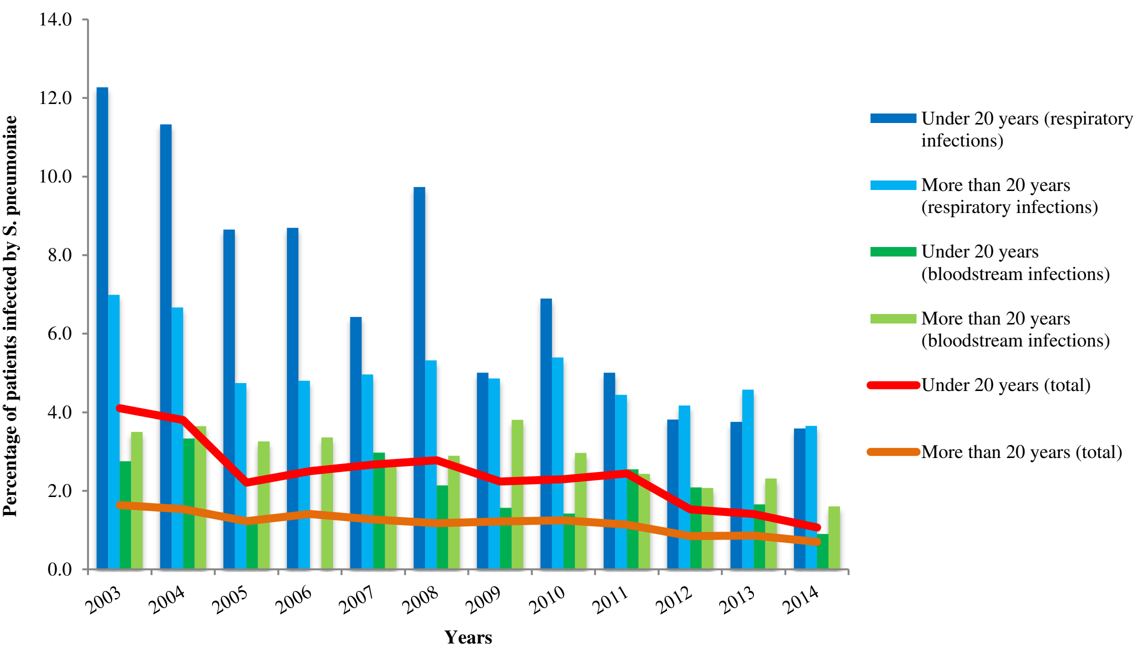

Fig. 2 Evolution of the annual percentage of patients infected by *S. pneumoniae* per age class in the four university hospitals of Marseille from January 2003 to December 2014 (2442 infected patients, birth dates were not available for 21 and 22 patients experiencing non-pneumococcal respiratory bacterial infections and non-pneumococcal bacteraemia, respectively)

in patients under 10 years old (26.9 %) and in patients between 41 and 70 years old (38.9 %) (Table 1). Moreover, the annual percentage of patients infected by *S. pneumoniae* decreased for all age classes over the years (Fig. 2 and Table 1) and was statistically significant for the 0–10, 11–20, 31–40, 41–50 and 81–90 years age classes (Table 1). Significant correlations were found between the decrease in the percentage of patients included in the 0–10 years age class and that of the percentage of patients included in the 31–40 years age class ($r = 0.88$, p-value $< 10^{-3}$), the 61–70 years age class ($r = 0.62$, p-value $= 0.03$) and the 81–90 years age class ($r = 0.88$, p-value $< 10^{-3}$) (Table 1). We also observed a significant correlation between the global percentage of patients positive to pneumococcal strains aged under 21 years and that of patients aged over 21

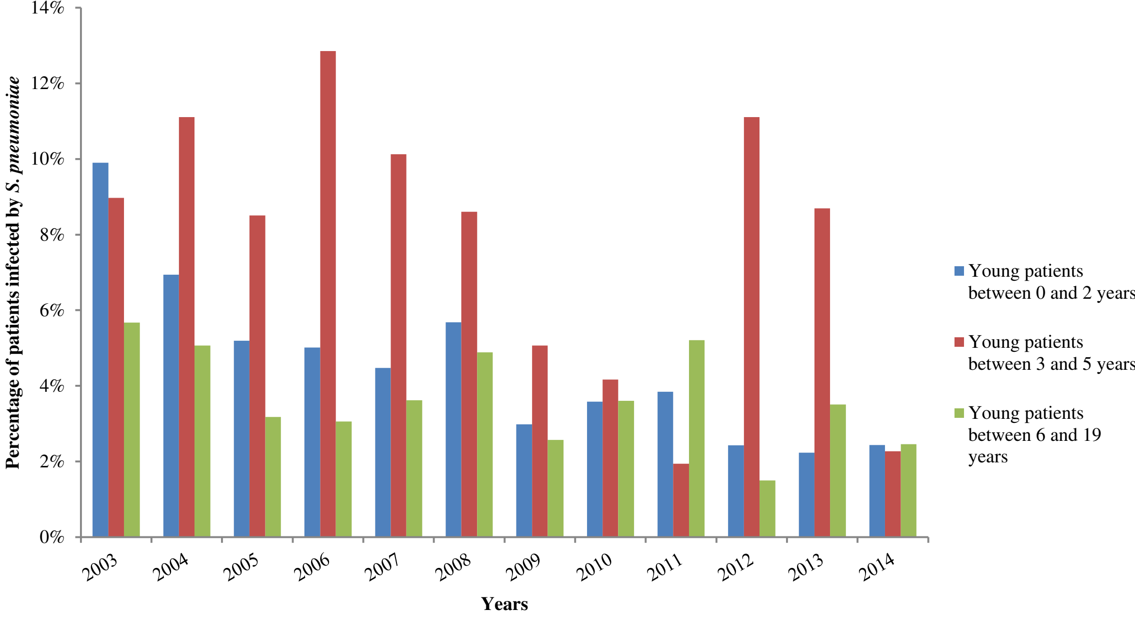

Fig. 3 Evolution of the annual percentage of young patients with respiratory and bloodstream infections due to *S. pneumoniae* per age class (0–2 years, 3–5 years and 6–19 years) in the four university hospitals of Marseille from January 2003 to December 2014 (459 infected patients)

Table 2 Evolution of the number of patients with pneumococcal strains (1866 patients), of the number of patients infected by at least one bacterial species (48,702 patients) and of the overall percentage of patients infected by *S. pneumoniae* strains isolated from the main kinds of samples in the four university hospitals of Marseille, January 2003 to December 2014

Year	Main kinds of samples	Number of patients infected by pneumococcal strains (percentage of patients infected by pneumococcal strains for this biological site)	Number of patients infected by at least by one bacterial species[a] (percentage of patients infected by bacterial strains for this biological site)	Annual percentage of *S. pneumoniae* strains isolated per kind of sample[b]	Annual proportion of the sample in pneumococcal infections
2003	Respiratory samples	132 (56.9)	1641 (14)	8.0	56.9
	Blood cultures	61 (26.3)	1800 (15.3)	3.4	26.3
	Cerebrospinal fluid	2 (0.9)	15 (0.1)	13.3	0.9
2004	Respiratory samples	96 (47.5)	1279 (12)	7.5	47.5
	Blood cultures	61 (30.2)	1702 (15.9)	3.6	30.2
	Cerebrospinal fluid	2 (1)	32 (0.3)	6.3	1.0
2005	Respiratory samples	78 (51.6)	1408 (12.9)	5.5	51.7
	Blood cultures	47 (31.1)	1645 (15.1)	2.9	31.1
	Cerebrospinal fluid	1 (0.7)	18 (0.2)	5.6	0.7
2006	Respiratory samples	98 (57)	1664 (15.4)	5.9	57.0
	Blood cultures	48 (27.9)	1758 (16.2)	2.7	27.9
	Cerebrospinal fluid	0 (0)	35 (0.3)	0.0	0.0
2007	Respiratory samples	90 (52)	1732 (15)	5.2	52.0
	Blood cultures	43 (24.8)	1557 (13.5)	2.8	24.9
	Cerebrospinal fluid	1 (0.6)	40 (0.3)	2.5	0.6
2008	Respiratory samples	109 (50)	1750 (11.6)	6.2	50.0
	Blood cultures	58 (26.6)	2113 (14)	2.7	26.6
	Cerebrospinal fluid	4 (1.8)	37 (0.2)	10.8	1.8
2009	Respiratory samples	89 (38.8)	1819 (11.2)	4.9	38.9
	Blood cultures	76 (33.2)	2336 (14.4)	3.3	33.2
	Cerebrospinal fluid	1 (0.4)	40 (0.2)	2.5	0.4
2010	Respiratory samples	79 (40.9)	1397 (10.4)	5.7	40.9
	Blood cultures	59 (30.6)	2250 (16.8)	2.6	30.6
	Cerebrospinal fluid	5 (2.6)	52 (0.4)	9.6	2.6
2011	Respiratory samples	94 (37.3)	1990 (11.1)	4.7	36.9
	Blood cultures	68 (27)	2767 (15.4)	2.5	27.0
	Cerebrospinal fluid	1 (0.4)	61 (0.3)	1.6	0.4
2012	Respiratory samples	96 (46.1)	2364 (11.2)	4.1	46.2
	Blood cultures	55 (26.4)	2649 (12.5)	2.1	26.4
	Cerebrospinal fluid	4 (1.9)	61 (0.3)	6.6	1.9
2013	Respiratory samples	109 (47.4)	2445 (10.4)	4.5	47.4
	Blood cultures	57 (24.8)	2635 (11.2)	2.2	24.8
	Cerebrospinal fluid	1 (0.4)	72 (0.3)	1.4	0.4
2014	Respiratory samples	98 (53.8)	2669 (11.4)	3.7	53.8
	Blood cultures	41 (22.5)	2846 (12.1)	1.4	22.5
	Cerebrospinal fluid	2 (1.1)	23 (0.1)	8.7	1.1
Total number of strains per samples	Respiratory samples	1168 (47.8)	22,158 (11.9)	0.6	47.8
	Blood cultures	674 (27.6)	26,058 (13.9)	0.4	27.6
	Cerebrospinal fluid	24 (23.6)	486 (0.3)	0.0	1.0
Historical trends per samples (*p*-value)[c]	Respiratory samples	−0.3 (10^{-3})			
	Blood cultures	−0.1 (10^{-3})			
	Cerebrospinal fluid	−0.2 (0.5)			

[a] Data from the historical database of the four university hospitals of Marseille, January 2003 to December 2014. Duplicates were removed by patients and bacterial species

[b] Percentages calculated dividing the number of patients infected by *S. pneumoniae* in a precise kind of sample from January 2003 to December 2014 by the total number of patients found to be infected by at least one bacterial species in the same sample over the same period

[c] Historical trends calculated using linear models, each value giving the annual trend of the percentage of patients infected by *S. pneumoniae* strains in the different biological sites of interest in the four university hospitals of Marseille. A *p*-value < 0.05 means that changes in historical trends are statistically significant over the study period for the biological site of interest

years ($r = 0.93$, $p\text{-value} < 10^{-5}$) (Fig. 2). Most of the pneumococcal infections were respiratory infections (1168 patients infected), followed by blood infections (674) and meningitis (24) (Table 2). Respiratory samples mostly included bronchial

Table 3 Comparison between the evolution of the number of pneumococcal infections in our study with those observed and published in other hospitals in recent years

Country	Parameter under surveillance	Years and observed decrease		Overall decrease	Reference of the study
France (Marseille)	Number of patients infected by pneumococcal strains causing IPDs	2009 229 patients	2014 182 patients	0.8-fold	Our study
France	Number of children infected by pneumococcal strains causing acute otitis media infections	2001 1694 patients	2011 560 patients	0.3-fold	[8]
France	Incidence rates of IPDs	2001 9.3/100,000 people	2011 9.1/100,000 people	0.9-fold	[10]
USA	Incidence rates of pneumococcal meningitidis	1997 0.81/100,000 people	2010 0.3/100,000 people	0.4-fold	[2]
South Africa	Incidence rates of IPDs	From 2005 to 2008 9.4/100,000 people	2012 5.7/100,000 people	0.6-fold	[14]
England	Children hospital admission episodes due to pneumococcal meningitidis and septicaemia	From 1968 to 1985 Between 1.13 and 2.29 admission episodes/ 100,000 children	2011 2.03 admission episodes/ 100,000 children[a]	Between 1.8 and 0.9-fold	[11]
Spain (Madrid)	Incidence rates of IPDs in people aged 60 years and over	2008 19.99/100,000 people	2011 15.21/100,000 people	0.8-fold	[1]
Scotland	Incidence rates of IPDs	Prior PCV-7 use 4550 patients[a]	From 2006 to 2010 2380 patients	0.5-fold	[18]
Portugal	Incidence rates of IPDs in children under 18 years of age	From 2008 to 2009 8.19/100,000 people	From 2011 to 2012 4.52/100,000 people	0.5-fold	[12]

IPD Invasive pneumococcal disease; *PCV-7* 7-valent pneumococcal conjugate vaccine

[a] Approximate values

aspirates (58 % of the 1168 respiratory samples), sputum (30.1 %) and bronchoalveolar fluids (5.1 %). We observed that the annual trend of the percentage of patients diagnosed to have a respiratory, blood or cerebrospinal fluid infections due to *S. pneumoniae* significantly decreased over the years (Table 2). A significant correlation was also found when comparing the annual evolution of the percentage of patients experiencing pneumococcal respiratory infections aged under 21 years to that of patients aged over 21 years (Fig. 2). The classification of children into three classes of age (0 to 2 years, 3 to 5 years and 6 to 19 years) merging together respiratory or blood infections (Fig. 3) allowed us to identify that the number of young patients experiencing respiratory and blood infections due to the bacterium globally decreased over the years (from 8.3 % in 2003 to 2.4 % in 2014, p-value < 10^{-4}), especially those included in the 0 to 2 years age class (from 9.9 % in 2003 to 2.4 % in 2014, p-value < 10^{-4}).

Discussion

At the beginning of the 21st century, pneumococcal strains were identified to be the first cause of bacterial invasive diseases in France [10]. This led to the successive national introduction of the PCV-7 and the PCV-13 between 2002 and 2010 [8], which can explain our results. That is supported by the fact that other worldwide studies observed similar phenomena after the introduction of new vaccines (Table 3).

Our results also allowed to observe that the decrease in the percentage of pneumococcal infections in the young population aged under 21 years is correlated with that observed in older patients, especially for respiratory infections (Figs. 2 and 3). Similar observations were noted in France [10], but equally in the USA [13], in South Africa [14] and in Kenya [15]. These results clearly show the positive effect of childhood vaccination on adult pneumococcal infections prevalence [16, 17].

The major limitation of our study was the fact that we did not serotype the strains to assess *S. pneumoniae* serotype changes across our region over the years. This must be done in a future study.

In conclusion, our results allowed to conclude that national immunisation programmes effectively impact on the global pneumococcal infection prevalence in young and elderly populations [5], even on the regional scale. However, this may be balanced by the possible serotype replacement in IPDs in the global population [8, 6, 13, 18], confirming the need for vaccines to be developed covering more pneumococcal serotypes in the future.

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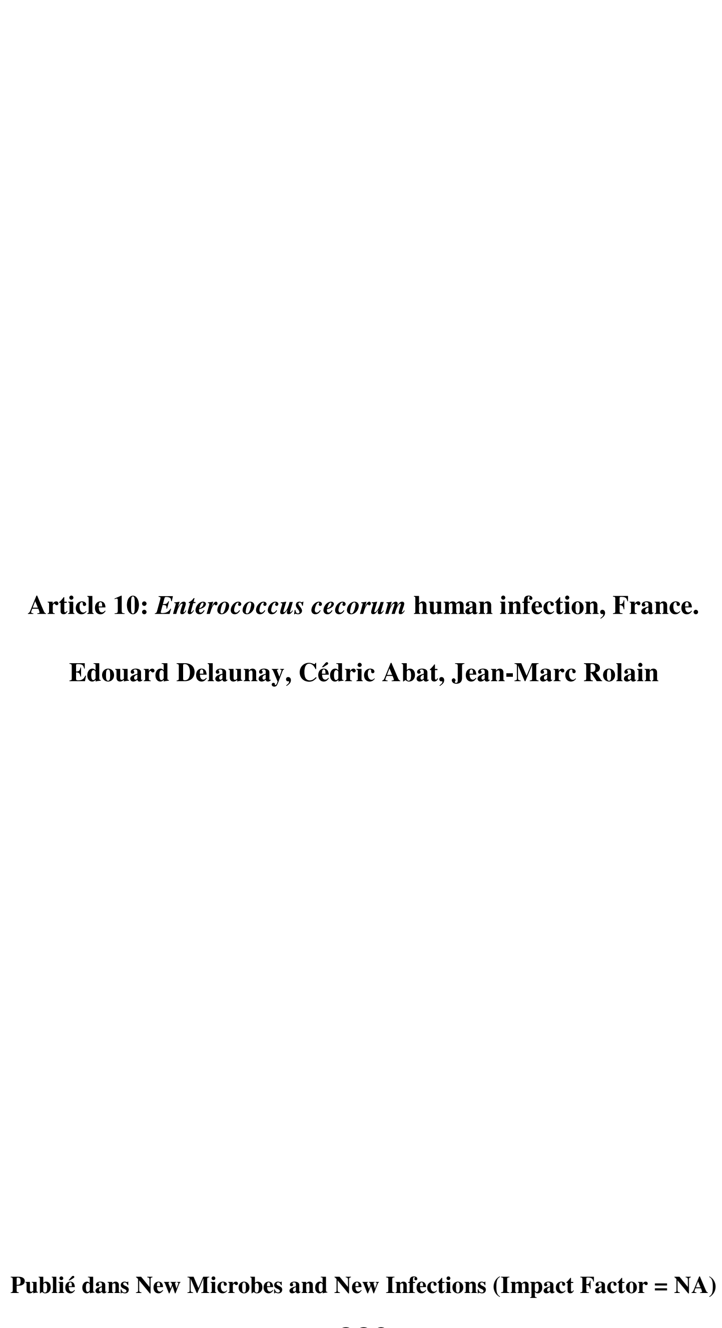
Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gutierrez Rodriguez MA, Ordobas Gavin MA, Garcia-Comas L, Sanz Moreno JC, Cordoba Deorador E, Lasheras Carbajo MD, Taveira Jimenez JA, Martin Martinez F, Iniesta Fornies D, Arce Arnaez A (2014) Effectiveness of 23-valent pneumococcal polysaccharide vaccine in adults aged 60 years and over in the Region of Madrid, Spain, 2008–2011. Euro Surveill 19:20922
2. Castelblanco RL, Lee M, Hasbun R (2014) Epidemiology of bacterial meningitis in the USA from 1997 to 2010: a population-based observational study. Lancet Infect Dis 14:813–819
3. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, Mccall N, Lee E, Mulholland K, Levine OS, Cherian T; Hib and Pneumococcal Global Burden of Disease Study Team (2009) Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet 374:893–902
4. Tin Tin Htar M, Madhava H, Balmer P, Christopoulou D, Menegas D, Bonnet E (2013) A review of the impact of pneumococcal polysaccharide conjugate vaccine (7-valent) on pneumococcal meningitis. Adv Ther 30:748–762
5. GBD 2013 Mortality and Causes of Death Collaborators (2015) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385:117–171
6. Weinberger DM, Malley R, Lipsitch M (2011) Serotype replacement in disease after pneumococcal vaccination. Lancet 378:1962–1973
7. Lepoutre A, Varon E, Georges S, Gutmann L, Lévy-Bruhl D (2008) Impact of infant pneumococcal vaccination on invasive pneumococcal diseases in France, 2001–2006. Euro Surveill 13. pii: 18962
8. Kempf M, Varon E, Lepoutre A, Gravet A, Baraduc R, Brun M, Chardon H, Cremniter J, Croizé J, Dalmay F, Demachy MC, Fosse T, Grelaud C, Hadou T, Hamdad F, Koeck JL, Luce S, Mermond S, Patry I, Péchinot A, Raymond J, Ros A, Segonds C, Soullié B, Tandé D, Vergnaud M, Vernet-Garnier V, Wallet F, Gutmann L, Ploy MC, Lanotte P (2015) Decline in antibiotic resistance and changes in the serotype distribution of *Streptococcus pneumoniae* isolates from children with acute otitis media; a 2001–2011 survey by the French Pneumococcal Network. Clin Microbiol Infect 21:35–42
9. Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, Fournier PE, Drancourt M, La Scola B, Raoult D (2013) Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 51:2182–2194
10. Lepoutre A, Varon E, Georges S, Dorléans F, Janoir C, Gutmann L, Lévy-Bruhl D; Microbiologists of Epibac; ORP Networks (2015) Impact of the pneumococcal conjugate vaccines on invasive pneumococcal disease in France, 2001–2012. Vaccine 33:359–366
11. Martin NG, Sadarangani M, Pollard AJ, Goldacre MJ (2014) Hospital admission rates for meningitis and septicaemia caused by Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae in children in England over five decades: a population-based observational study. Lancet Infect Dis 14:397–405
12. Aguiar SI, Brito MJ, Horacio AN, Lopes JP, Ramirez M, Melo-Cristino J (2014) Decreasing incidence and changes in serotype distribution of invasive pneumococcal disease in persons aged under 18 years since introduction of 10-valent and 13-valent conjugate vaccines in Portugal, July 2008 to June 2012. Euro Surveill 19:20750
13. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG, Moore MR; Active Bacterial Core Surveillance/Emerging Infections Program Network (2010) Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 201:32–41
14. von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, Madhi SA, Zell ER, Verani JR, O'Brien KL, Whitney CG, Klugman KP, Cohen C; GERMS-SA Investigators (2014) Effects of vaccination on invasive pneumococcal disease in South Africa. N Engl J Med 371:1889–1899
15. Hammitt LL, Akech DO, Morpeth SC, Karani A, Kihuha N, Nyongesa S, Bwanaali T, Mumbo E, Kamau T, Sharif SK, Scott JA (2014) Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* in Kilifi, Kenya: findings from cross-sectional carriage studies. Lancet Glob Health 2:e397–e405
16. Klugman KP (2014) Herd protection induced by pneumococcal conjugate vaccine. Lancet Glob Health 2:e365–e366
17. Walter ND, Taylor TH Jr, Dowell SF, Mathis S, Moore MR; Active Bacterial Core Surveillance System Team (2009) Holiday spikes in pneumococcal disease among older adults. N Engl J Med 361:2584–2585
18. Lamb KE, Flasche S, Diggle M, Inverarity D, Greenhalgh D, Jefferies JM, Smith A, Edwards GF, Denham B, McMenamin J, McDonald E, Mitchell TJ, Clarke SC, Robertson C (2014) Trends in serotypes and sequence types among cases of invasive pneumococcal disease in Scotland, 1999–2010. Vaccine 32:4356–4363



Article 10: ***Enterococcus cecorum*** **human infection, France.**

Edouard Delaunay, Cédric Abat, Jean-Marc Rolain

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FIRST CLINICAL CASE REPORT

Enterococcus cecorum human infection, France

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Abstract

Enterococcus cecorum is a bacterium of the intestinal tract of many domestic animals that is rarely reported as human pathogen. Here we report the first case of incisional hernia plate infection and the first case of urinary tract colonization due to *E. cecorum* from patients in Marseille, France.
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Keywords: *Enterococcus cecorum*, food-mediated acquisition, immunosuppressors, MALDI-TOF, urinary-tract infection
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Enterococcus cecorum is a species that was first isolated from the intestines of poultry but also occurs in pigs, calves, ducks, cats and dogs [1]. It is an uncommon human pathogen, with only five reported clinical cases in the literature: one septicemia, two peritonitis, one thoracic empyema and one endocarditis [2–6]. Here we report a case of incisional hernia plate infection and a case of urinary tract colonization due to *E. cecorum* from patients in Marseille, France.

The first case comprised a 56-year-old man with Crohn diseases who was referred to our digestive surgery department in February 2012 for surgical management of an infectious syndrome with persistence of a purulent discharge from the parietal abdomen. At admission, the patient was afebrile; he had a pain in the right iliac fossa. The white blood cell count was normal (7.5×10^9/L), hemoglobin was 1270 g/L and C-reactive protein was elevated (1160 nmol/L). The patient underwent surgery with resection of a loop of the fistulized small intestine and ablation of the incisional hernia plate. The surgical samples of the incisional hernia plate cultures were positive for *Enterococcus cecorum,* which was identified by MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight mass spectrometry). The isolate was susceptible to amoxicillin, gentamicin 500, vancomycin, rifampicin and erythromycin. Antibiotic treatment with amoxicillin was initiated for 30 days. The patient was discharged 10 days after his surgery and was considered cured.

The second case comprised a 39-year-old woman who consulted with our nephrology department in December 2013 for her termly checkup after kidney transplantation in September 2012. At admission, the patient was afebrile, without any sign of infection. White blood cell count was normal (5.7×10^9/L); hemoglobin was 1550 g/L. A urine sample was collected; leukocyturia was 5 elements/mm^3, and bacteriuria was 10^4/mm^3 with positive culture for *Enterococcus cecorum,* which was identified by MALDI-TOF. The isolate was susceptible to amoxicillin, gentamicin 500, vancomycin, teicoplanin, linezolid and nitrofurantoin. No antibiotic treatment was initiated for this asymptomatic urinary colonization.

Enterococcus cecorum is a bacterium rarely involved in human infections. The rarity of these infections can be explained by the fact that *E. cecorum* is difficult to identify correctly and has probably been underestimated by the past. In fact, conventional methods such as the VITEK 2 or API systems are less efficient than MALDI-TOF [7] and 16S RNA for identification of non-*faecalis* and non-*faecium Enterococcus* species [8]. The characteristics of patients with *E. cecorum* infections are outlined in Table 1. Close contact with animals was previously assumed to be a major risk factor for *E. cecorum* human infection [2,6]. No available data on our patients helped us learn whether they had exposure to domestic animals. Nevertheless, because food

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TABLE 1. Characteristics of patients with *Enterococcus cecorum* infection

Patient no.	Age (years)/ Sex	Infection type	Underlying disease or condition	Bacteriology source for *E. cecorum*	Identification method	Antimicrobial therapy	Outcome	Study
1	44/F	Septicemia	Morbid obesity, malnutrition, skin lesions	Blood culture (2)	SDS-PAGE	Imipenem	Cure	Greub [2]
2	44/M	Peritonitis	Decompensated liver cirrhosis (alcohol related) with ascites, and hepatorenal syndrome, peritoneal dialysis	Dialysate	16S RNA	Cefazolin + gentamicin	Cure	De Baere [3]
3	60/M	Peritonitis	Decompensated liver cirrhosis (hepatitis B virus related) with ascites and hepatic encephalopathy	Blood culture (1), ascites fluid	16S RNA	Cefoxitin	Died	Hsueh [4]
4	44/M	Empyema thoracis	Decompensated liver cirrhosis (Wilson disease related) with ascites	Pleural fluid	16S RNA	Cefotaxime	Cure	Woo [5]
5	58/M	Infectious endocarditis	No anterior valvulopathy, teeth extraction 5 weeks before admission	Blood culture (1), aortic valve	16S RNA	Amoxicillin + gentamicin	Cure	Ahmed [6]
6	56/M	Incisional hernia plate infection	Crohn disease, stenosing and fistulizing	Incisional hernia plate	MALDI-TOF	Amoxicillin	Cure	This study
7	39/F	Urinary tract colonization	Kidney transplantation	Urine culture	MALDI-TOF	None	Cure	This study

MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight analysis; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis mass spectrometry.

animals can be a reservoir of *E. cecorum* [1], we hypothesize that the infections originated from a food-mediated acquisition of the pathogen, probably facilitated by the immunosuppressive drug intake of the two patients. *E. cecorum* was susceptible to all the antibiotics tested, including amoxicillin and glycopeptides (vancomycin, teicoplanin), with a low level of resistance to gentamicin. These two cases confirm that *E. cecorum* can be responsible for human infections.

Conflict of Interest

None declared.

References

[1] Devriese LA, Hommez J, Wijfels R, Haesebrouck F. Composition of the enterococcal and streptococcal intestinal flora of poultry. J Appl Bacteriol 1991;71:46–50.

[2] Greub G, Devriese LA, Pot B, Dominguez J, Bille J. *Enterococcus cecorum* septicemia in a malnourished adult patient. Eur J Clin Microbiol Infect Dis 1997;16:594–8.

[3] De Baere T, Claeys G, Verschraegen G, Devriese LA, Baele M, Van Vlem B, et al. Continuous ambulatory peritoneal dialysis peritonitis due to *Enterococcus cecorum*. J Clin Microbiol 2000;38:3511–2.

[4] Hsueh PR, Teng LJ, Chen YC, Yang PC, Ho SW, Luh KT. Recurrent bacteremic peritonitis caused by *Enterococcus cecorum* in a patient with liver cirrhosis. J Clin Microbiol 2000;38:2450–2.

[5] Woo PC, Tam DM, Lau SK, Fung AM, Yuen KY. *Enterococcus cecorum* empyema thoracis successfully treated with cefotaxime. J Clin Microbiol 2004;42:919–22.

[6] Ahmed FZ, Baig MW, Gascoyne-Binzi D, Sandoe JA. *Enterococcus cecorum* aortic valve endocarditis. Diagn Microbiol Infect Dis 2011;70:525–7.

[7] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. On-going revolution in bacteriology: routine identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.

[8] Fang H, Ohlsson AK, Ullberg M, Ozenci V. Evaluation of species-specific PCR, Bruker MS, VITEK MS and the VITEK 2 system for the identification of clinical *Enterococcus* isolates. Eur J Clin Microbiol Infect Dis 2012;31:3073–7.

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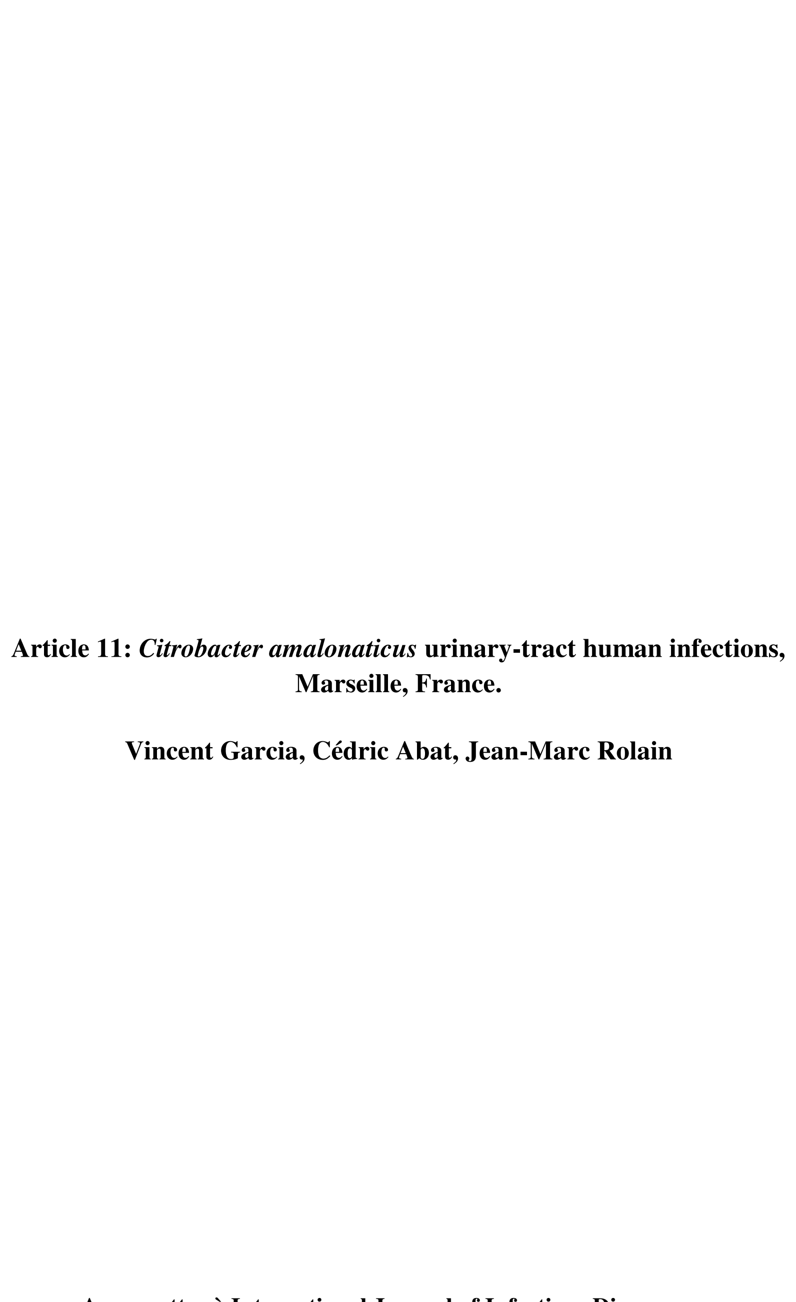

Article 11: ***Citrobacter amalonaticus*** **urinary-tract human infections, Marseille, France.**

Vincent Garcia, Cédric Abat, Jean-Marc Rolain

A soumettre à International Journal of Infections Diseases

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1 **TITLE PAGE**

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22 **1** Table and **2** Figures

23 **Abstract**

24 *Citrobacter amalonaticus* is a bacterium that has been rarely reported as
25 human pathogen in the past. Here we report 4 cases of *C. amalonaticus* infections
26 occurring in patients hospitalized in Marseille, France and reviewed all cases
27 published in the literature.

28

Introduction

Citrobacter amalonaticus, formerly called *Levinea amalonatica*, was firstly studied and described in 1971 after being isolated from various human samples coming from hospitalized patients, especially feces (1). Since that, this bacterial species have been isolated from the environment (2;3), but equally sporadically isolated from human, mainly from fecal samples, urine, wounds, and respiratory samples (1;4-9). Recently, our syndromic clinical laboratory-based surveillance system called BALYSES (the BActerial real-time LaboratorY-based SurveillancE System) detected two consecutive *C. amalonaticus* kidney infections in 2 renal transplant patients hospitalized in the same nephrology unit in Conception hospital of Marseille, France. Additionally 2 cases of *C. amalonaticus* infections were then detected the following weeks in the others university hospitals of Marseille, France. We report here all cases from the literature to date.

Case reports

Case 1. A 75-year old woman with chronic renal failure due to membranoproliferative glomerulonephritis (MPGN), transplanted since December 2010, was admitted in Nephrology unit in Conception Hospital for regular check of her renal transplant. Since her transplantation, the patient developed MPGN recidive on the kidney transplant, urinary tract infections (UTIs) and diabete due to immunosuppressive therapy. On admission, in December 2014, urine sample showed leucocyturia (29,8 elements /mm^3) and bacteriuria (10^4 /mL *Citrobacter amalonaticus* identified by MALDI TOF MS (Bruker Daltonics, Germany)).

51 Antimicrobial susceptibility testing showed that the isolate was resistant to
52 amoxicillin and susceptible to third of cephalosporins, carbapenem, cotrimoxazole,
53 ciprofloxacin and amoxicillin/clavulanate. The patient was successively treated
54 with amoxicillin/clavulanate during seven days and considered cured.
55 **Case 2.** A 61-year old man, renal transplanted since November 2014,
56 consultated in Nephrology unit in Conception hospital for his weekly check up in
57 December 2014. On urine samples collected, leucocyturia was 25,2 elements/mm^3,
58 bacteriuria was 10^4/mL with *Citrobacter amalonaticus* which was identified by
59 MALDI TOF-MS from December 2014. In this case, *Citrobacter amalonaticus*
60 was resistant to amoxicillin, ticarcillin and cotrimoxazole and susceptible to
61 amoxicillin/clavulanate, ticarcilline/clavulanate, third generation of cephalosporins,
62 carbapenems, nitrofurantoïn, gentamicin and ciprofloxacin. The patient was treated
63 a first time in December with ciprofloxacin during seven days and then antibiotic
64 was recommended only during periods of urological surgery. It was treated a
65 second time from 11/03/15 to 03/04/15.
66 **Case 3**. 4 years child with Leigh syndrome, which is a mitochondrial
67 cytopathy due to heterozygote mutation on SURF1 gene was admitted in intensive
68 care unit in Timone Hospital for cardiogenic shock due to Epstein Barr Virus
69 (EBV) infection on 16/02/2015. On 06/04/15, an urine sample was collected and
70 showed a leucocyturia was 35 elements/mm^3, a hematuria was 8 elements/mm3
71 and bacteriuria at 10^4/mL. The bacterium *Citrobacter amalonaticus* identified by
72 MALDI TOF was resistant to amoxicillin, amoxicillin/clavulanate, ticarcillin,

73 ticarcillin/clavulanate and sensible to carbapenems, third generation of
74 cephalosporins, carbapenems, nitrofurantoïn, ciprofloxacin and cotrimoxazole.
75 Patient was treated with cotrimoxazole and cured after seven days of traitement.

76 **Case 4.** A 27 days newborn female consulted in pediatric unit in North
77 Hospital on 04/03/2015 for rhinitis and fever (38.3°C). Urine sample was collected
78 that showed a leucocyturia at 13,2 elements/mm^3 and a bacteriuria at 10^7/mL
79 *Citrobacter amalonaticus*. The strain was susceptible to amoxicillin/clavulanate,
80 ticarcilline/clavulanate, ciprofloxacin, cotrimoxazole, nitrofurantoïn, carbapenems
81 and third generation of cephalosporins but resistant to amoxicillin and ticarcillin.
82 The patient was symptomatically treated with paracetamol for fever and no
83 antibiotic treatment was started for asymptomatic urinary colonization.

84 **Epidemiological features**

85 Because the two initial cases were reported in nephrology unit in renal
86 transplant patients, we look at our updated 13 years historical database (10) and
87 retrospectively found that 36 patients experienced *C. amalonaticus* infections in
88 our settings before 2015 (Figure 1). Most of the infections occurred in males (21
89 males and 15 females), were hospital-acquired infections (22 infections, 62%), and
90 were urinary-tract infections (29 infections, i.e 80% of all the 36 infections). We
91 also identified a peak in the number of infected patients in 2012 (12 patients
92 infected). Comparing the global number of patients experiencing *C. amalonaticus*
93 UTI before 2012 and since 2012 to the number of patients experiencing *E. coli* UTI
94 over the two same periods based on our updated 13 years historical database (10),

95 we observed that there were statistically more UTI infections due to *C.*
96 *amalonaticus* after the 2012 peak than before (11 on 25,789 patient-bacteria pairs
97 from 2002 to 2011 *vs* 18 on 13,502 patient-bacteria pairs from 2012 to 2014,
98 p=0.003). Moreover, we observed that the majority of the strains were collected
99 from young children (11 patients, 31% of all the infected patients). Comparing the
100 proportion of patients experiencing *C. amalonaticus* and aged under 11 years to
101 that of *E. coli* based on our historical database (10), we identified that the
102 proportion of patients under 11 years infected by *C. amalonaticus* was statistically
103 higher than that of *E. coli* (11 on 6,361 patient-bacteria pairs *vs* 25 on 43,169
104 patient-bacteria pairs, p=0.003). Furthermore, we statistically identified more
105 patients infected by *C. amalonaticus* after the introduction of the MALDI-TOF
106 technology for the routine bacterial identifications in our laboratory (10) in 2009
107 than before (9 on 82,436 patient-bacteria pairs from 2002 to 2008 *vs* 27 on 115,922
108 patient-bacteria pairs from 2009 to 2014, p=0.04) (Figure 1).

109 **Discussion**

110 *C. amalonaticus* has been rarely isolated in humans (Table). In 2009, a
111 chinese woman with kidney transplantation contracted a peritonitis due to *C.*
112 *amalonaticus.* In the other study, a 63 years old woman with bone marrow
113 transplant developed wound infection caused by *C. amalonaticus*. An other case
114 concerned a 75 years old man with pancreas cancer who contracted biliary tract
115 infection and bacteraemia due to *C. amalonaticus*. In Italy, it recovered from urine
116 sample of one patient with renal graft since ten months. In the USA, between 1972

117 and 1978, at the Seattle Veterans Administration Medical Center, *Citrobacter*
118 *amalonaticus* were identified in urine and fluids samples in 5 patients. Among
119 these patients, 2 patients had UTIs. Then in Thaïland, a man contracted enteric
120 fever and *C. amalonaticus* was incriminated. In our study, we identified the last
121 four *C. amalonaticus* using MALDI TOF. The four spectra were then included in a
122 dendrogram built with Bruker MALDI Biotyper software 3.0 (Bruker Daltonics,
123 Bremen, Germany) (Figure 2). Among all cases reported, including our studies, we
124 observed 5 patients with kidney transplantation or urinary tract abnormality (case
125 6). Also, we observed that patients were mainly immunocompromised, including
126 patients with renal graft, newborn (case report 4) and patients with leukaemia and
127 pancreas cancer (case 2 and 3). All together, our observations, linked to the
128 previously published reports (Table), lead us to think that this bacterium is an
129 opportunistic pathogen, especially in patients suffering from urinary-tract failures.
130 The fact that *C. amalonaticus* infections was rarely reported in the past (Table)
131 may be explained by the fact that this bacterium is difficult to identify. Thus,
132 conventional methods such as API system and RapID onE may underestimate their
133 prevalence by misidentifying the strains (7;11-14). The fact we statistically
134 identified more *C. amalonaticus* infections after the routine use of the MALDI-
135 TOF in our settings may result in an improvement in the identification of this
136 bacterial species. However, we found a statistically higher prevalence of the
137 number of UTIs due to this bacterium even after routine use of MALDI TOF

138 (afeter 2012) likely suggesting that this bacteria could be an emerging pathogen

139 responsible for UTIs in immunocompromised patients.

140

141 **REFERENCES**

142 (1) Young VM, Kenton DM, Hobbs BJ, Moody MR. Levinea, a New
143 Genus of the Family Enterobacteriaceae. International Journal of
144 Systematic Bacteriology 1971;21(1):58-63.

145 (2) Nawaz M, Khan AA, Khan S, Sung K, Steele R. Isolation and
146 characterization of tetracycline-resistant *Citrobacter* spp. from
147 catfish. Food Microbiol 2008 Feb;25(1):85-91.

148 (3) Urbanova E, Pacova Z. [Identification of *Citrobacter* species and their
149 occurrence in raw products and foods]. Vet Med (Praha) 1997
150 Mar;42(3):87-91.

151 (4) Aschbacher R, Pagani L, Doumith M, Pike R, Woodford N,
152 Spoladore G, et al. Metallo-beta-lactamases among
153 Enterobacteriaceae from routine samples in an Italian tertiary-care
154 hospital and long-term care facilities during 2008. Clin Microbiol
155 Infect 2011 Feb;17(2):181-9.

156 (5) Farmer JJ, III, Davis BR, Hickman-Brenner FW, McWhorter A,
157 Huntley-Carter GP, Asbury MA, et al. Biochemical identification of
158 new species and biogroups of Enterobacteriaceae isolated from
159 clinical specimens. J Clin Microbiol 1985 Jan;21(1):46-76.

160 **(6) Lai CC, Tan CK, Lin SH, Liu WL, Liao CH, Huang YT, et al.**
161 **Bacteraemia caused by non-freundii, non-koseri *Citrobacter* species in**
162 **Taiwan. J Hosp Infect 2010 Dec;76(4):332-5.**

163 **(7) Lipsky BA, Hook EW, III, Smith AA, Plorde JJ. *Citrobacter***
164 **infections in humans: experience at the Seattle Veterans**
165 **Administration Medical Center and a review of the literature. Rev**
166 **Infect Dis 1980 Sep;2(5):746-60.**

167 **(8) Suwansrinon K, Wilde H, Sitprija V, Hanvesakul R. Enteric fever-**
168 **like illness caused by infection with *Citrobacter amalonaticus*. J Med**
169 **Assoc Thai 2005 Jun;88(6):837-40.**

170 **(9) Wong MY, Lau SK, Tang SC, Curreem SO, Woo PC, Yuen KY. First**
171 **report of peritoneal dialysis-related peritonitis caused by *Citrobacter***
172 ***amalonaticus*. Perit Dial Int 2012 Mar;32(2):224-5.**

173 **(10) Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al.**
174 **Identification of rare pathogenic bacteria in a clinical microbiology**
175 **laboratory: impact of matrix-assisted laser desorption ionization-time**
176 **of flight mass spectrometry. J Clin Microbiol 2013 Jul;51(7):2182-94.**

177 **(11) Jeffery J, Sulaiman S, Oothuman P, Vellayan S, Zainol-Ariffin P,**
178 **Paramaswaran S, et al. Domiciliary cockroaches found in restaurants**

179 in five zones of Kuala Lumpur Federal Territory, peninsular
180 Malaysia. Trop Biomed 2012 Mar;29(1):180-6.

181 (12) Kitch TT, Jacobs MR, Appelbaum PC. Evaluation of RapID onE
182 system for identification of 379 strains in the family
183 Enterobacteriaceae and oxidase-negative, gram-negative
184 nonfermenters. J Clin Microbiol 1994 Apr;32(4):931-4.

185 (13) Morgan MG, Stuart C, Leanord AT, Enright M, Cole GF. *Citrobacter*
186 *diversus* brain abscess: case reports and molecular epidemiology. J
187 Med Microbiol 1992 Apr;36(4):273-8.

188 (14) York MK, Brooks GF, Fiss EH. Evaluation of the autoSCAN-W/A
189 rapid system for identification and susceptibility testing of gram-
190 negative fermentative bacilli. J Clin Microbiol 1992 Nov;30(11):2903-
191 10.

192 (15) Mugnaioli C, Luzzaro F, De LF, Brigante G, Amicosante G, Rossolini
193 GM. Dissemination of CTX-M-type extended-spectrum beta-
194 lactamase genes to unusual hosts. J Clin Microbiol 2005
195 Aug;43(8):4183-5.

TABLE

Table. Characteristics of the patients with *Citrobacter amalonaticus* infections reported in our studies and elsewhere. Only studies reporting fully described infections with well identified *C. amalonaticus* were included.

Case and number of patient (country)	Age[1] /Sex	Infection type	Underlying condition	Sampling date	Identification method	Antibiotic therapy	Issue	Reference
1, 1 (China)	47/F	Peritonitis	IgA nephropathy, renal graft, intermittent peritoneal dialysis	NA	Biochemical tests and16S rRNA	CAZ AK	Cure	-9
2, 1 (Italy)	63/F	Wound infection	ABMT, AML, intracranial haemorrhages	NA	Vitek 2 system	TG	Cure	-4
3, 1 (Taïwan)	75/M	Bacteraemia	Amoulla vater cancer	NA	Phoenix automated system	CMZ	Cure	-6
4, 1 (Italy)	NA	NA	Renal allograft 10 months earlier	NA	API 20E	NA	NA	-15
5, 1 (Thaïland)	53/M	Enteric fever	Fever, water diarrhea, headache, Travelers in Asia	NA	Biochemical tests	CRO SXT	Cure	-8
6, 5 (USA)	2 patients	NA	Urinary tract abnormally, Diabetes, Malignancy	NA	Biochemical tests	NA	NA	-7
	3 patients	NA	Diabetes mellitus	NA		NA	NA	
Case 1, 1 (France)	77/F	Urinary tract infection	Renal graft, Chronical nephropathy, diabetes	02/03/15	MALDI-TOF	AMC	Cure	Our study
Case 2, 1 (France)	61/M	Urinary tract infection	Renal graft, Lymphatic cyst of graft, Ureter stenosis, diabetes	10/12/14 04/03/15	MALDI-TOF	CIP	Cure	Our study
Case 3, 1 (France)	4/M	Urinary tract infection	Leigh syndrom	04/02/15	MALDI-TOF	SXT	Cure	Our study
Case 4, 1 (France)	0/F	Asymptomatic urinary colonization	Fever, Rhinitis	04/03/15	MALDI-TOF	No	Cure	Our study

[1]: years, CMZ = cefmetazole, CAZ = ceftazidime, SXT = cotrimoxazole, CIP = ciprofloxacin, AMC = amoxicillin/clavulanate, CRO = ceftriaxone, and TG = tigécycline.

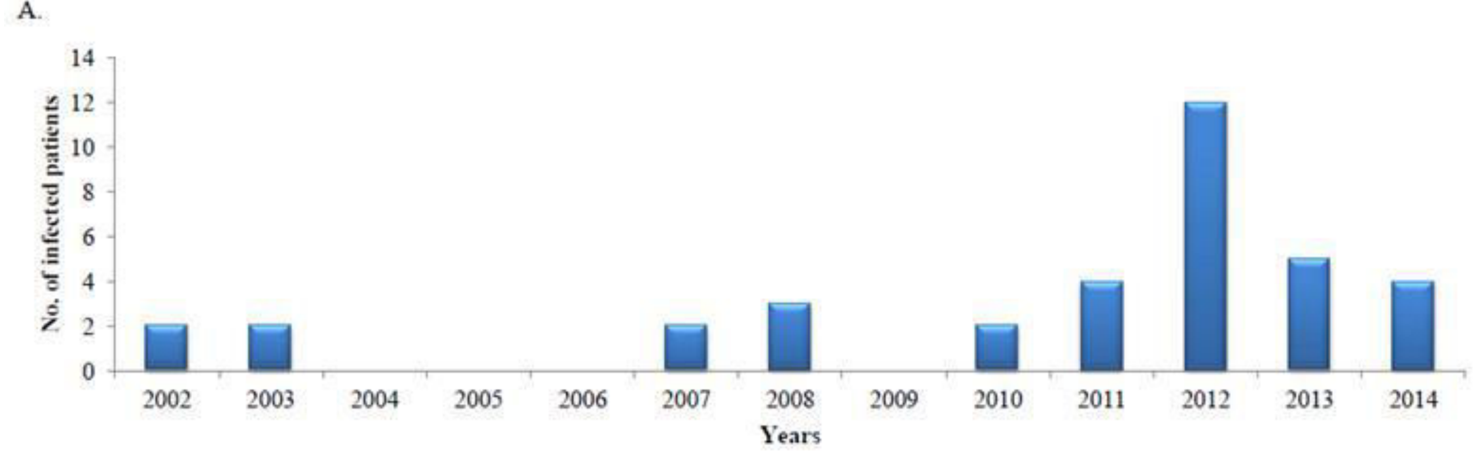
NA: Non-available data, M: male, F: female, IgA: immunoglobulin A, ABMT: allogenic bone marrow transplantation, AMT: acute myelogenous leukaemia

FIGURE LEGEND

Figure 1. Main features of the 36 patients who experienced *C. amalonaticus* infections in our settings from January 2002 to December 2014. Panel A presents the annual evolution of the number of patients experiencing *C. amalonaticus* infections. Panel B presents the age distribution of the patients infected by *C. amalonaticus*. Panel C shows the different kinds of samples from which the bacterium was isolated.

Figure 2. Main-spectrum dendrogram of 4 ours *C. amalonaticus* isolates built from protein spectra

Figure 1.



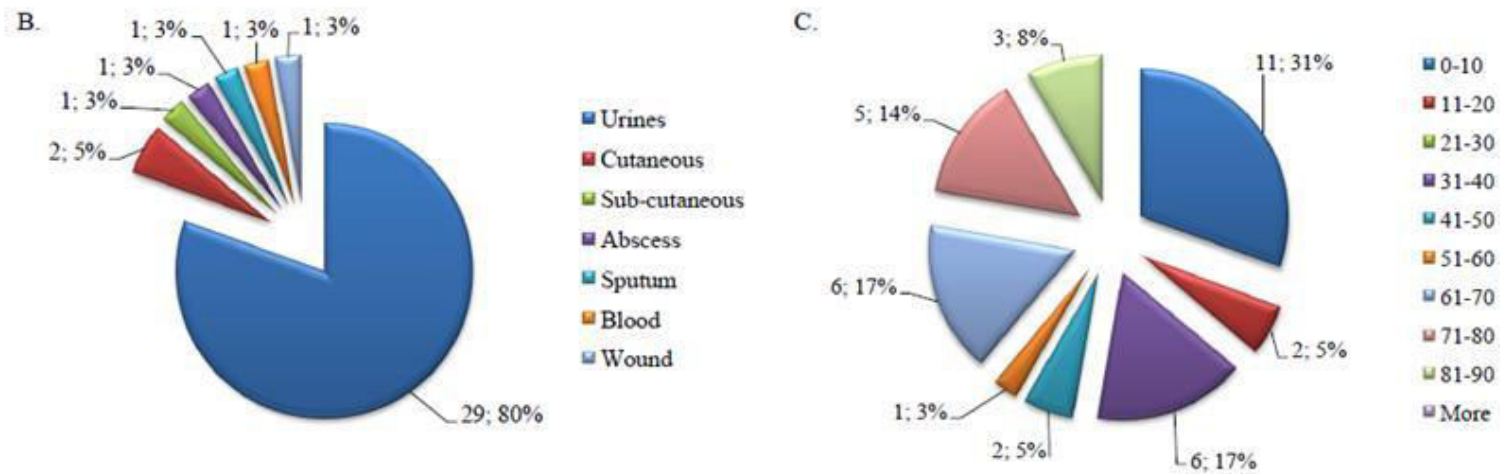
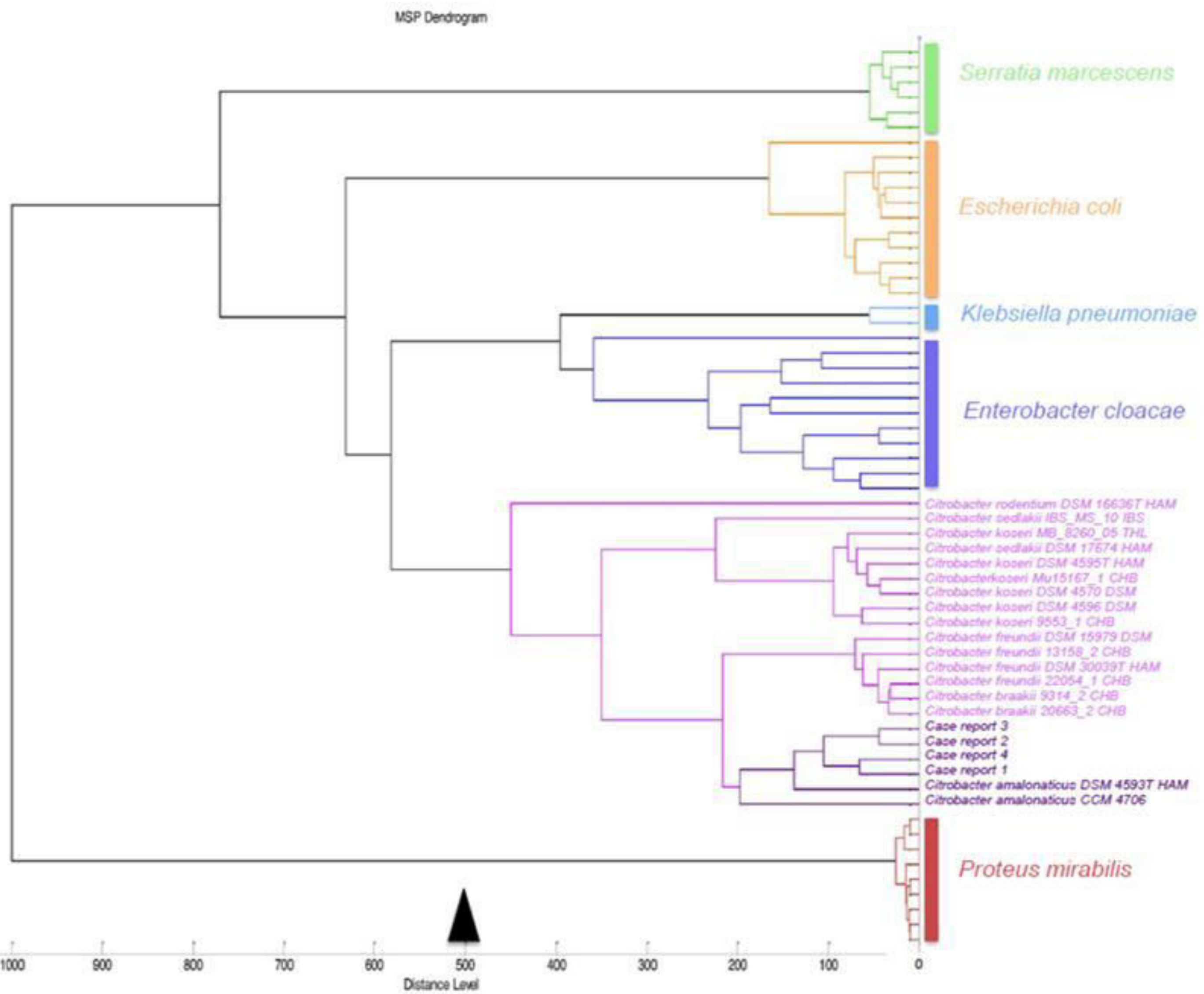


Figure 2.



EVENEMENTS ANORMAUX IDENTIFIES SUR LA BASE D’ALARMES EMISES PAR MARSS

Article 12: Worldwide decrease in methicillin-resistant *Staphylococcus aureus*: do we understand something?

Jean-Marc Rolain, Cédric Abat, Didier Raoult

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Worldwide decrease in methicillin-resistant *Staphylococcus aureus*: do we understand something?

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Sir, the 'effectiveness' of screening and isolation strategies to control methicillin-resistant *Staphylococcus aureus* (MRSA) has recently been brought into question [1]. A significant amount of evidence has emerged worldwide of a decrease in the prevalence of MRSA. Many reasons have been proposed for this but no definitive explanation has been given except that we understand nothing on the current epidemiology of MRSA. The progressive introduction and use of antimicrobial agents was associated with an increase in MRSA in the 1980s, mainly in hospitals (hospital-acquired MRSA). No convincing explanation for the recent replacement of hospital-acquired MRSA by community-acquired MRSA has yet been given. Indeed, our knowledge of the epidemiology of MRSA is poor and the processes of transmission are not yet understood. Although hospital-acquired MRSA is due to only five clonal complexes, the methicillin-susceptible *S. aureus* (MSSA) population is highly diverse, with many different clones in circulation, rendering it impossible to understand the epidemiology of the disease. The rate of invasive MRSA in the EU was 18.0% in 2013, which had been decreasing since 2001, when the level of resistance was >30%. This decrease was also observed in France for invasive MRSA, with a decrease from 33.4% in 2001 to 17.1% in 2013 (Fig. 1a). Although this decrease has been attributed to infection control strategies [2], the same trend was also observed in our institution in Marseille in the south of France, for invasive strains (31.0% in 2001 versus 27.4% in 2010 and 12.8% in 2015), where no screening and/or isolation procedures are performed (Fig. 1b, c). The prevalence of MRSA in non-invasive strains was even lower, with only 11.2% of MRSA in 2015 (Fig. 1c). Hence, all strategies for MRSA infection control that have so far been proposed cannot explain this trend, because the decrease is observed without any infection control policies being in place. One possible explanation for this decrease in MRSA is the cyclical success of some MSSA clones, which tend to replace dominant MRSA clones.

What are the reservoirs of MRSA?

It is now well established that pigs are a major source of MRSA; they can act as zoonotic agents and spread the disease to humans [3]. In addition, a recent study comparing 458 USA300 MRSA strains from different US cities reported that households are a long-term reservoir for this epidemic MRSA clone, in which the same MRSA strain freely circulates between members of the same family [4]. Finally, other sources of MRSA have been identified: persistent carriage of MRSA in healthy people, including healthcare workers and family members, can reintroduce MRSA into the hospital through intrafamilial spread from and to healthcare workers [5]. Indeed, the prevalence of MRSA transmission among household contacts within a family in the community is very high [6], and so isolation procedures at hospital and taking precautions to limit contact between patients and healthcare workers may be ineffective, because the reintroduction of MRSA could be the result of contact with family members during visits. Moreover, isolating patients has negative psychological effects upon them, which also render such a strategy ineffective. Furthermore, and above all, a recent study on possible patient-to-patient intra-hospital transmission of both MSSA and MRSA [7] did not clearly identify closely related *S. aureus* isolates between patients with invasive infections. Screening and topical MRSA decolonization therapy is not as effective as expected, as exemplified by the intrafamilial transmission of MRSA from a healthy nurse who was decolonized because of Panton–Valentine leukocidin-positive MRSA carriage as per the institutional protocol for healthcare workers in Australia. The same Panton–Valentine leukocidin-positive MRSA clone was transmitted by the nurse 6 months later to her husband, who died from severe necrotizing pneumonia [8]. Another problem linked to the decolonization

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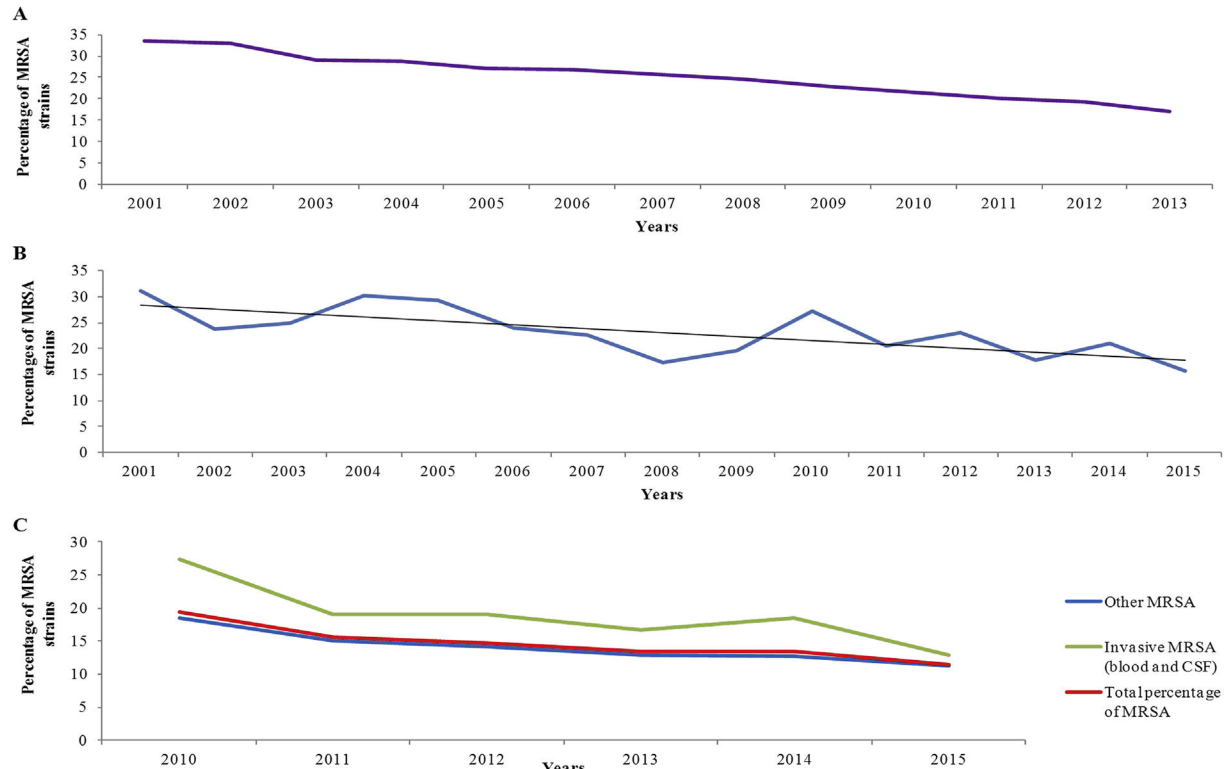

FIG. 1. Current trends of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in France and in Marseille hospitals according to different data sources from 2001 to 2015*. (a) 2001–2013 data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) invasive MRSA database (http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx), France only. Duplicates have been removed from this database. (b) 2001–2015 data from the information management system of the four university hospitals of Marseille. These data only included data on bacteraemia due to MRSA strains. These data contained redundant data. (c) 1 January 2010 to 8 April 2015 data from the information management system of the four university hospitals of Marseille. These data only included data on bacteraemia and CSF infections due to MRSA strains. Duplicates have been removed from this database. CSF, cerebrospinal fluid; *data from 1 January 2015 to 8 April 2015.

strategy is the emergence of MRSA strains that are resistant to either antiseptics (chlorhexidine-resistant MRSA) [9] or to mupirocin [10]. This clearly shows that the MRSA search-and-destroy policy has not really been adapted to the rapidly changing epidemiology of MRSA. Moreover, because multiple MRSA strains may circulate within communities, even under low antibiotic pressure and in healthy people, it is impossible to predict the success of any hospital control policies. Hence, because of our lack of knowledge on MRSA epidemiology, particularly on the success of some epidemic clones, despite the expense of current infection control policies employed in hospitals, their effectiveness remains to be demonstrated.

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References

[1] Fätkenheuer G, Hirschel B, Harbarth S. Screening and isolation to control meticillin-resistant *Staphylococcus aureus*: sense, nonsense, and evidence. Lancet 2014;385:1146–9.

[2] Vos MC, Behrendt MD, Melles DC, Mollema FPN, de Groot W, Parlevliet G, et al. 5 years of experience implementing a methicillin-resistant *Staphylococcus aureus* search and destroy policy at the largest University Medical Center in the Netherlands. Infect Control Hosp Epidemiol 2009;30:977–84.

[3] Johnson AP. Methicillin-resistant *Staphylococcus aureus*: the European landscape. J Antimicrob Chemother 2011;66(Suppl. 4):iv43–8.

[4] Alam MT, Read TD, Petit RA, Boyle-Vavra S, Miller LG, Eells SJ, et al. Transmission and microevolution of USA300 MRSA in U.S. households: evidence from whole-genome sequencing. MBio 2015;6:e00054–15.

[5] Wagenvoort JHT, De Brauwer EIGB, Sijstermans MLH, Toenbreker HMJ. Risk of re-introduction of methicillin-resistant

Clinical Microbiology and Infection © 2015 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved,

Staphylococcus aureus into the hospital by intrafamilial spread from and to healthcare workers. J Hosp Infect 2005;59:67–8.

[6] Johansson PJH, Gustafsson EB, Ringberg H. High prevalence of MRSA in household contacts. Scand J Infect Dis 2007;39:764–8.

[7] Long SW, Beres SB, Olsen RJ, Musser JM. Absence of patient-to-patient intrahospital transmission of *Staphylococcus aureus* as determined by whole-genome sequencing. MBio 2014;5:e01692–14.

[8] Pozzi Langhi SA, Robinson JO, Pearson JC, Christiansen KJ, Coombs GW, Murray RJ. Intrafamilial transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 2009;15:1687–9.

[9] Edgeworth JD. Has decolonization played a central role in the decline in UK methicillin-resistant *Staphylococcus aureus* transmission? A focus on evidence from intensive care. J Antimicrob Chemother 2010;66: ii41–7.

[10] Antonov NK, Garzon MC, Morel KD, Whittier S, Planet PJ, Lauren CT. High prevalence of mupirocin resistance in *Staphylococcus aureus* isolates from a pediatric population. Antimicrob Agents Chemother 2015 [epub ahead of print].

Article 13: Low level of resistance in enterococci strains isolated in four French hospitals, Marseille, France.

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1 Low level of resistance in enterococci isolated in four hospitals, Marseille, France.

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15 **Key words:** resistant enterococci; antimicrobial resistance; automated

16 surveillance.

17 **ABSTRACT**

18 Enterococci are Gram-positive cocci responsible for various infections worldwide,
19 and their prevalence of antibiotic resistance greatly varies worldwide. This study
20 investigates the prevalence of resistance to antibiotics in enterococci from patients
21 admitted in the 4 university hospitals of Marseille between January 2013 to
22 September 2014. 2,976 patients-bacteria couples were identified (2,507 *E. faecalis*
23 and 469 *E. faecium*) in the 4 university hospitals of Marseille. 1.3%, 8.9%, 1.4%
24 and 0% of *E. faecalis* strains were resistant to amoxicillin, gentamicin, teicoplanin
25 and vancomycin, respectively, and 83.9%, 49.2%, 1.3% and 0.2% of *E. faecium*
26 strains were resistant to amoxicillin, gentamicin, teicoplanin and vancomycin,
27 respectively. Resistance to aminoglycosides and vancomycin in strains isolated
28 from blood cultures was significantly lower than that of most European countries
29 included in the 2012 European Antimicrobial Resistance Surveillance Network
30 report. Our low percentage of antibiotic resistance in enteroccocci is likely due to a
31 low level of *E. faecium* infections, underlining the need to implement surveillance
32 systems, especially to monitor the *E. faecalis* / *E. faecium* ratio evolution in blood
33 cultures and others.

34

35

36

37

38 **INTRODUCTION**

39 Enterococci are Gram-positive cocci responsible for various infections
40 worldwide including urinary-tract infections (UTIs), blood-stream infections,
41 endocarditis [2], but also respiratory tract infections and cellulitis [15]. Emergence of
42 vancomycin-resistant enterococci (VRE), which was first reported in England in an
43 outbreak of vancomycin-resistant *E. faecium* infections in the last 1980s [4,25], has
44 spread worldwide and is becoming challenging because of limited therapeutic
45 options [2].

46 The prevalence of antibiotic resistance in enterococci varies worldwide. In the
47 United States, before the 1990s, *E. faecalis* accounted for 90-95% of the strains
48 collected from patients at the hospital level [2]. Since that, the increasing use of
49 vancomycin and broad-spectrum antimicrobials lead to an increase of the
50 prevalence of hospital-acquired infections due to *E. faecium* which became almost
51 as prevalent as *E. faecalis* in hospitals settings, resulting in a dramatic increase in
52 the number of VRE infections in US hospitals between 2000 and 2006 [2]. In
53 Europe, the prevalence of VRE collected from invasive infections ranges from 0%
54 in Sweden and the Netherlands to 44% in Ireland according to the 2012 European
55 Antimicrobial Resistance Surveillance Network (EARS-Net) report [8]. At the
56 national level, low outbreaks of VRE hospital-acquired infections occured in the
57 North of France between 2004 and 2008 [7,12–14,23]. Nevertheless, no outbreak has
58 ever been reported in the South of France to date.

59 In this context, we herein investigate the prevalence of resistance to antibiotics in
60 enterococci from patients admitted in Marseille university hospitals between
61 January 2013 to September 2014, and compare our results to available data.

62 MATERIAL AND METHODS

63 All the data we analyzed herein came from the four University hospitals
64 of Marseille (North, South, Conception and Timone hospitals). These hospitals
65 include 3,700 beds with approximately 1,500 beds for the Timone hospital, 600
66 beds for the North hospital, 700 beds for the Conception hospital and 900 beds for
67 the South Hospital. We retrospectively collected raw data of antibiotic
68 susceptibility testing results for amoxicillin, gentamicin, vancomycin, and
69 teicoplanin for *E. faecalis* and *E. faecium* strains isolated from January 2013 to
70 September 2014 from the information management system of the four Marseille
71 University hospitals. The enterococci strains analyzed herein were identified using
72 Matrix Assisted Laser Desorption Ionisation –Time Of Flight (MALDI-TOF) mass
73 spectrometers [22] which ensure good bacterial identification even for enterococci
74 strains [9]. In our laboratory, we decide to follow the EUCAST recommendations.
75 According to these recommendations, all the antibiotic susceptibility testing results
76 were obtained after using the disk diffusion method. Moreover, E-test for
77 vancomycin must be performed to validate or invalidate possible VRE resistance
78 phenotype. Data were then sorted in a Microsoft Excel database from which
79 duplicates were removed to finally conserve only one bacterial identification per

80 patient. The classification of the infections was done according the sample from
81 which each enterococci strain was isolated.
82 Data on the percentage of resistance to gentamicin and vancomycin for *E. faecalis*
83 and *E. faecium* strains studied in our study were compared to those available from
84 different European countries included in the EARS-Net report [8]. In this report, only
85 one record per infected patient was conserved, and only data referring to invasive
86 enterococcal bacteremia or meningitis (both community and hospital acquired
87 infections) were published.
88 Statistical analyses were done using Pearson's Chi Square test or Fisher exact test
89 as appropriate using the Epi-Info 3.03 software
90 (http://www.openepi.com/Menu/OE_Menu.htm). All were two-sided, and p-values
91 < 0.05 were considered as statistically significant.

92 **RESULTS**

93 2,976 patients-bacteria couples were identified throughout the study
94 period including 2,507 *E. faecalis* (84.2%) and 469 *E. faecium* (15.8%) with UTIs
95 being the most common type of clinical isolates (69% and 62% for *E. faecalis* and
96 *E. faecium*, respectively) (Figure 1). 1.3%, 8.9%, 1.4% and 0% of *E. faecalis*
97 strains were resistant to amoxicillin, gentamicin, teicoplanin and vancomycin,
98 respectively. Conversely 83.9%, 49.2%, 1.3% and 0.2% of *E. faecium* strains were
99 resistant to amoxicillin, gentamicin, teicoplanin and vancomycin, respectively. To
100 be able to compare our resistance level to that of the other European countries
101 included in the EARS-Net report [8], we divided resistance data from enterococci

102 strains isolated from blood cultures (invasive infections) to that from strains
103 isolated from other infection sites, including urine. The percentage of resistance to
104 all the antibiotics tested in our laboratory of the *E. faecalis* and *E. faecium* strains
105 isolated from blood cultures are presented Table 1. Gentamicin resistance was
106 significantly more prevalent in *E. faecium* strains isolated from blood cultures than
107 in *E. faecalis* strains isolated from blood cultures ($p < 10^{-5}$). In these enterococci
108 strains, we also observed that the percentage of resistance to aminoglycosides and
109 vancomycin was significantly lower than the percentage of resistance reported in
110 25 European countries for aminoglycosides and 7 countries for vancomycin (Table
111 1).

112 **DISCUSSION**

113 It is well known that *E. faecium* strains have an extraordinary genome
114 plasticity allowing them to be more frequently resistant than *E. faecalis* strains [2,5].
115 In the US, the increase in the number of *E. faecium* strains isolated from patients
116 led to an increase of isolation of VRE from 0% in the 1980s to 80% in 2007 and
117 currently the prevalence of nosocomial infections due to *E. faecium* species is
118 almost the same as *E. faecalis* [2]. Similar observations were made in France where
119 the ratio *E. faecium/E. faecalis* infections increased from 10%/90% at the end of
120 the 1990s to 27%/73% in 2010, leading to an increase of resistance of enterococci
121 strains isolated in hospitalized patients [5]. The French national reference center for
122 antibiotic resistance observed that more than 95% of the VRE received nationwide
123 were *E. faecium* strains from 2006 to 2013 (http://www cnr-resistance-

124 antibiotiques fr/bilans-dactivites html) and mainly those included in the clonal
125 complex 17 (CC17), a clade of strains that is pandemic and associated with
126 hospital-acquired *E. faecium* infections [10]. The common capability of the CC17
127 strains to colonize and harbour resistance genes was directly involved in the
128 increase of isolation of *E. faecium* strains in hospitalized patients in US and
129 European hospitals [2,5,8].
130 In Europe, avoparcin, a glycopeptide antibiotic used to promote food-producing
131 animals growth in agriculture [5,11], was suspected to select for VRE gut carriage [3]
132 and was banned for animal husbandry in 1996 [2,5]. Several years after, studies
133 demonstrated that the number of VRE declined in food-producing animals,
134 including chicken and pigs in Denmark and France [5], likely explaining the lower
135 prevalence of VRE in Europe as compared to the US. Nevertheless, numerous
136 European studies identified *E. faecium* VRE colonization in non wild animals
137 including buzzards [19], migratory wild birds [24] and mullets fish [1], supporting the
138 possible role of wild animals as VRE reservoir.
139 Our results also allowed us to observe that the percentage of resistance to
140 gentamycin in *E. faecium* strains isolated from blood cultures was significantly
141 higher as compared to *E. faecalis* strains (Table 1). This was also observed in a
142 2006-2009 Danish population-based cohort study [17], in Australia in 2010 [6] and in
143 Greece [18]. High level gentamicin resistance gene *aac(6')-Ie-aph(2") (aacA-aphD)*,
144 is usually located on Tn*4001*-type transposons, that can be carried by highly

145 transferable plasmids [20,21] among *E. faecium* strains reported in Greece and
146 Australia [6,18].
147 Interestingly, whole genome sequencing of the major clones of *E. faecium* causing
148 outbreaks has shown that the presence of Clustered Regularly Interspaced Short
149 Palindromic Repeats (CRISPRs) elements, small DNA elements that protect
150 bacteria against acquisition of foreign DNA, were inversely correlated with the
151 presence of resistant genes in most multidrug-resistant enterococci clonal isolates,
152 especially in the hospital-adapted CC17 *E. faecium* strains [16]. This finding shows
153 that acquired resistance is not established and that bacteria may eliminate foreign
154 DNA when it did not confer a useful phenotype. It also shows that resistance level
155 may not be cumulative but results from epidemic clones favored by
156 environemental factors.
157 Our study suffered from a major limitation, the fact we did not perform any
158 molecular analyses to check if our strains, especially the *E. faecium* strains,
159 belonged to special clonal complex like the CC17. We believe that such molecular
160 analyses should be performed in our region in a future molecular epidemiological
161 study based on human and animal samples.
162 In conclusion, our low level of antibiotic resistance in enteroccocci is likely due to
163 a low level of *E. faecium* infections, maybe due to a regional low animal carriage
164 of this bacterial species.

165 These observations underlined the need to implement integrated surveillance

166 systems to quickly identify resistant clones outbreaks, survey the CC17 incidence

167 and to monitor the *E. faecalis* / *E. faecium* ratio evolution.

168

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177

178 **REFERENCES**

179 1. Araújo, C., C. Torres, A. Gonçalves, C. Carneiro, M. López, H. Radhouani, *et*

180 *al.* 2011. Genetic detection and multilocus sequence typing of vanA-containing

181 Enterococcus strains from mullets fish (Liza ramada). Microb. Drug Resist.

182 17:357–361.

183 2. Arias, C.A., and B.E. Murray. 2012. The rise of the Enterococcus: beyond

184 vancomycin resistance. Nat. Rev. Microbiol. 10:266–278.

185 3. Van der Auwera, P., N. Pensart, V. Korten, B.E. Murray, and R. Leclercq. 1996.

186 Influence of oral glycopeptides on the fecal flora of human volunteers: selection of

187 highly glycopeptide-resistant enterococci. J. Infect. Dis. 173:1129–1136.

188 4. Bonten, M.J., R. Willems, and R.A. Weinstein. 2001. Vancomycin-resistant

189 enterococci: why are they here, and where do they come from? Lancet. Infect. Dis.

190 1:314–325.

191 5. Cattoir, V., and R. Leclercq. 2012. Twenty-five years of shared life with

192 vancomycin-resistant enterococci: is it time to divorce? J. Antimicrob. Chemother.

193 68:731–742.

194 6. Coombs, G.W., J.C. Pearson, K. Christiansen, T. Gottlieb, J.M. Bell, N. George,

195 *et al.* 2013. Australian Group on Antimicrobial Resistance Enterococcus

196 Surveillance Programme annual report, 2010. Commun. Dis. Intell. Q. Rep.

197 37:E199–E209.

198 7. Escaut, L., S. Bouam, M. Frank-Soltysiak, E. Rudant, F. Saliba, N. Kassis, *et al.*
199 2013. Eradication of an outbreak of vancomycin-resistant Enterococcus (VRE): the
200 cost of a failure in the systematic screening. Antimicrob. Resist. Infect. Control
201 2:18.

202 8. European Centre for Disease Prevention and Control. 2013. Antimicrobial
203 resistance surveillance in Europe 2012. Available at
204 http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-
205 surveillance
206 europe-2012.pdf. (Online.)

207 9. Fang, H., A.-K. Ohlsson, M. Ullberg, and V. Ozenci. 2012. Evaluation of
208 species-specific PCR, Bruker MS, VITEK MS and the VITEK 2 system for the
209 identification of clinical Enterococcus isolates. Eur. J. Clin. Microbiol. Infect. Dis.
210 31:3073–3077.

211 10. Galloway-Peña, J., J.H. Roh, M. Latorre, X. Qin, and B.E. Murray. 2012.
212 Genomic and SNP analyses demonstrate a distant separation of the hospital and
213 community-associated clades of *Enterococcus faecium*. PLoS One 7:e30187.

214 11. Hammerum, A.M., C.H. Lester, and O.E. Heuer. 2010. Antimicrobial-resistant
215 enterococci in animals and meat: a human health hazard? Foodborne Pathog. Dis.
216 7:1137–1146.

217 12. Henard, S., T. Cao-Huu, C. Loos-Ayav, P. Chanet, M. Kessler, and C. Rabaud.
218 2009. [Interventions implemented to control an outbreak of vancomycin-resistant

219 enterococci in a university hospital]. Néphrologie & Thérapeutique 5 Suppl
220 4:S265–S271.

221 13. Lucet, J.-C., L. Armand-Lefevre, J.-J. Laurichesse, A. Macrez, E. Papy, R.
222 Ruimy, *et al.* 2007. Rapid control of an outbreak of vancomycin-resistant
223 enterococci in a French university hospital. J. Hosp. Infect. 67:42–48.

224 14. Marcadé, G., J.-B. Micol, H. Jacquier, L. Raskine, J.-L. Donay, S. Nicolas-
225 Viaud, *et al.* 2014. Outbreak in a haematology unit involving an unusual strain of
226 glycopeptide-resistant *Enterococcus faecium* carrying both vanA and vanB genes.
227 J. Antimicrob. Chemother. 69:500–505.

228 15. Murray, B.E. Diversity among multidrug-resistant enterococci. Emerg. Infect.
229 Dis. 4:37–47.

230 16. Palmer, K.L., and M.S. Gilmore. 2010. Multidrug-resistant enterococci lack
231 CRISPR-cas. MBio 1.

232 17. Pinholt, M., C. Østergaard, M. Arpi, N.E. Bruun, H.C. Schønheyder, K.O.
233 Gradel, *et al.* 2014. Incidence, clinical characteristics and 30-day mortality of
234 enterococcal bacteraemia in Denmark 2006–2009: a population-based cohort
235 study. Clin. Microbiol. Infect. 20:145–151.

236 18. Protonotariou, E., E. Dimitroulia, S. Pournaras, V. Pitiriga, D. Sofianou, and A.
237 Tsakris. 2010. Trends in antimicrobial resistance of clinical isolates of
238 *Enterococcus faecalis* and *Enterococcus faecium* in Greece between 2002 and
239 2007. J. Hosp. Infect. 75:225–227.

240 19. Radhouani, H., L. Pinto, C. Coelho, R. Sargo, C. Araújo, M. López, *et al.* 2010.
241 MLST and a genetic study of antibiotic resistance and virulence factors in vanA-
242 containing Enterococcus from buzzards (Buteo buteo). Lett. Appl. Microbiol.
243 50:537–541.

244 20. Rosvoll, T.C.S., B.L. Lindstad, T.M. Lunde, K. Hegstad, B. Aasnaes, A.M.
245 Hammerum, *et al.* 2012. Increased high-level gentamicin resistance in invasive
246 *Enterococcus faecium* is associated with aac(6')Ie-aph(2″)Ia-encoding transferable
247 megaplasmids hosted by major hospital-adapted lineages. FEMS Immunol. Med.
248 Microbiol. 66:166–176.

249 21. Sadowy, E., A. Sieńko, I. Gawryszewska, A. Bojarska, K. Malinowska, and W.
250 Hryniewicz. 2013. High abundance and diversity of antimicrobial resistance
251 determinants among early vancomycin-resistant *Enterococcus faecium* in Poland.
252 Eur. J. Clin. Microbiol. Infect. Dis. 32:1193–1203.

253 22. Seng, P., M. Drancourt, F. Gouriet, B. La Scola, P.-E. Fournier, J.M. Rolain, *et*
254 *al.* 2009. Ongoing revolution in bacteriology: routine identification of bacteria by
255 matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin.
256 Infect. Dis. 49:543–551.

257 23. Servais, A., L. Mercadal, F. Brossier, M. Venditto, B. Issad, C. Isnard-Bagnis,
258 *et al.* 2009. Rapid curbing of a vancomycin-resistant *Enterococcus faecium*
259 outbreak in a nephrology department. Clin. J. Am. Soc. Nephrol. 4:1559–1564.

260 24. Silva, N., G. Igrejas, A. Felgar, A. Gonçalves, R. Pacheco, and P. Poeta. 2012.
261 Molecular characterization of vanA-containing Enterococcus from migratory birds:
262 song thrush (Turdus philomelos). Braz. J. Microbiol. 43:1026–1029.

263 25. Uttley, A.H., C.H. Collins, J. Naidoo, and R.C. George. Vancomycin-resistant
264 enterococci. Lancet 1:57–58.

265

FIGURE LEGENDS

Figure 1. Summary of the 10 main types of sample *E. faecalis* (panel A) and *E. faecium* (panel B) isolates have been isolated.

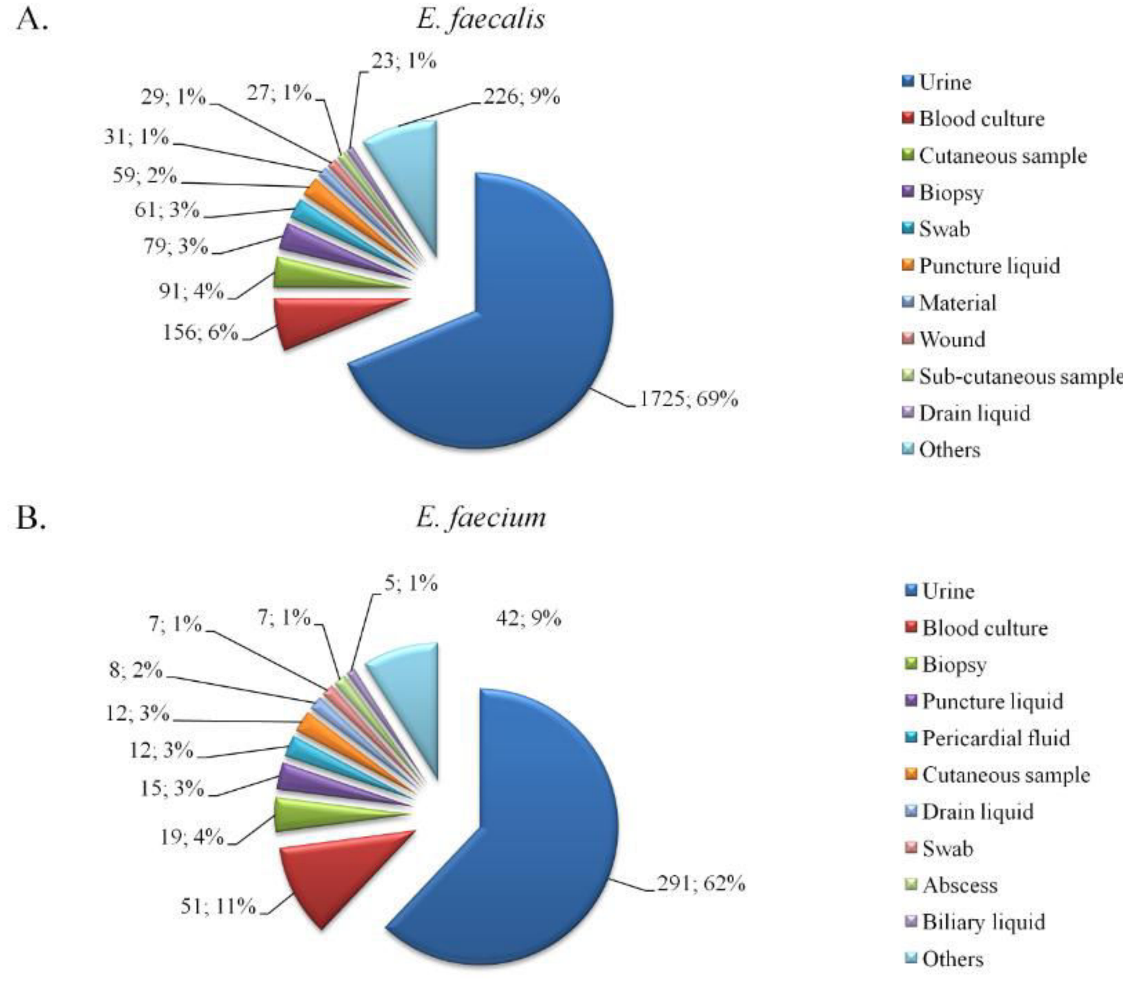

Table 1. Summary of the percentage of resistance of our *E. faecalis* and *E. faecium* strains isolated from blood cultures (invasive infections) to the antibiotics routinely tested in our laboratory for enterococci, and comparison of the percentage of resistance of our *E. faecalis* and *E. faecium* strains isolated from blood cultures (invasive infections) to gentamicin and vancomycin, respectively, to those published in the European Antimicrobial Resistance Surveillance Network report classified by country (data from the 2012 European Antimicrobial Resistance Surveillance Network report, http://www.ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2012.pdf).

Study or country	**Bacterial species**		**Antibiotic tested and percentages of resistance (number of strains)**								**P-value** [a]	
			E. faecalis				*E. faecium*					
	E. faecalis	*E. faecium*	AMX	GM	VA	TEC	AMX	GM	VA	TEC	*E. faecalis*	*E. faecium*
Our study	156	51	1.3% (2)	7.2% (11)	0% (0)	0% (0)	82.4% (42)	54% (27)	0% (0)	3.9% (2)		
Austria	425	376		29.2% (124)					3.2% (12)		$p < 10^{-3}$	p = 0.4
Belgium	396	212		24.5% (97)					1.4% (3)		$p < 10^{-3}$	p = 1
Bulgaria	78	42		38.5% (30)					0% (0)		$p < 10^{-3}$	p = 1
Croatia	160	61		38.8% (62)					0% (0)		$p < 10^{-3}$	p = 1
Cyprus	77	29		10.4% (8)					10.3% (3)		p = 0.4	p = 0.1
Czech Republic	581	262		41.7% (242)					11.5% (30)		$p < 10^{-3}$	p = 0.01
Denmark	112	593		27.7% (31)					2% (12)		$p < 10^{-3}$	p = 0.7
Estonia	19	40		42.1% (8)					0% (0)		p = 0.003	p = 1
Finland	0	274		0% (0)					0.7% (2)		NA	p = 1
France	1,528	614		16.7% (255)					0.8% (5)		$p < 10^{-3}$	p = 1
Germany	680	647		35.6% (242)					16.2% (105)		$p < 10^{-3}$	p = 0.004

Greece	667	418	28.3% (189)	17.2% (72)	$p < 10^{-3}$	p = 0.003
Hungary	452	142	56.2% (254)	3.5% (5)	$p < 10^{-3}$	p = 0.4
Iceland	20	14	11.8% (2)	0% (0)	p = 1	p = 1
Ireland	279	386	32.6% (91)	44% (170)	$p < 10^{-3}$	$p < 10^{-3}$
Italy	300	435	51% (153)	6% (26)	$p < 10^{-3}$	p = 0.1
Latvia	55	18	29.1% (16)	5.6% (1)	$p < 10^{-3}$	p = 0.5
Lithuania	59	36	50.8% (30)	5.6% (2)	$p < 10^{-3}$	p = 0.4
Luxembourg	45	20	22.2% (10)	0% (0)	p = 0.01	p = 1
Malta	0	6	0% (0)	NA	NA	NA
Netherlands	287	484	30.7% (88)	0% (0)	$p < 10^{-3}$	p = 1
Norway	123	168	30.1% (37)	0.6% (1)	$p < 10^{-3}$	p = 1
Poland	105	157	39% (41)	8.3% (13)	$p < 10^{-3}$	p = 0.06
Portugal	347	257	42.9% (149)	23.3% (60)	$p < 10^{-3}$	$p < 10^{-3}$
Slovakia	179	82	50.3% (90)	4.9% (4)	$p < 10^{-3}$	p = 0.3
Slovenia	129	95	34.9% (45)	0% (0)	$p < 10^{-3}$	p = 1
Spain	878	537	38.3% (336)	1.5% (8)	$p < 10^{-3}$	p = 1
Sweden	792	404	14.8% (117)	0% (0)	p = 0.02	p = 1
United Kingdom	377	362	29.4% (111)	13.3% (48)	$p < 10^{-3}$	p = 0.01

[a]: Analyses performed using two-sided Pearson Chi Square or Fisher exact tests as appropriate, p-value < 0.05, comparing the number of *E. faecalis* or *E. faecium* strains resistant to gentamicin and vancomycin in our study to that of the different countries presented in the European Antimicrobial Resistance Surveillance Network report.

NA indicates that the countries did not provide data for the antibiotic and the bacterial species of interest.

Amoxicillin (AMX), teicoplanin (TEC), vancomycin (VA) and gentamicin (GM).

Conclusions et perspectives de la Partie III

Le développement des deux nouveaux systèmes de surveillance a permis, couplé au système de surveillance EPIMIC, de détecter de vrais événements épidémiologiques qui ont donnés lieu à 14 publications scientifiques dont 9 sont cités dans cette thèse (articles 5 à 13). Néanmoins, ces trois systèmes de surveillance nécessitent d'être améliorées. En effet, les données sont actuellement collectées à la main ce qui ne permet pas une surveillance en temps réel des événements anormaux des maladies infectieuses par nos systèmes. Par ailleurs, nos outils statistiques ne sont pas optimums pour la surveillance des événements anormaux des maladies infectieuses, notamment car ils ne prennent actuellement pas en compte la saisonnalité de certains pathogènes suivis. Afin de les améliorer, nous sommes actuellement en train de mettre en place une plateforme informatique appelé MIDaS (Méditerranée Infection Datawarehousing and Surveillance) qui va regrouper toutes les activités de surveillance développées ou à venir dans le cadre de l'IHU en améliorant les algorithmes statistiques utilisés pour la surveillance des événements anormaux des maladies infectieuses et en permettant une collecte automatique des données utilisées pour la surveillance (Figure 7).

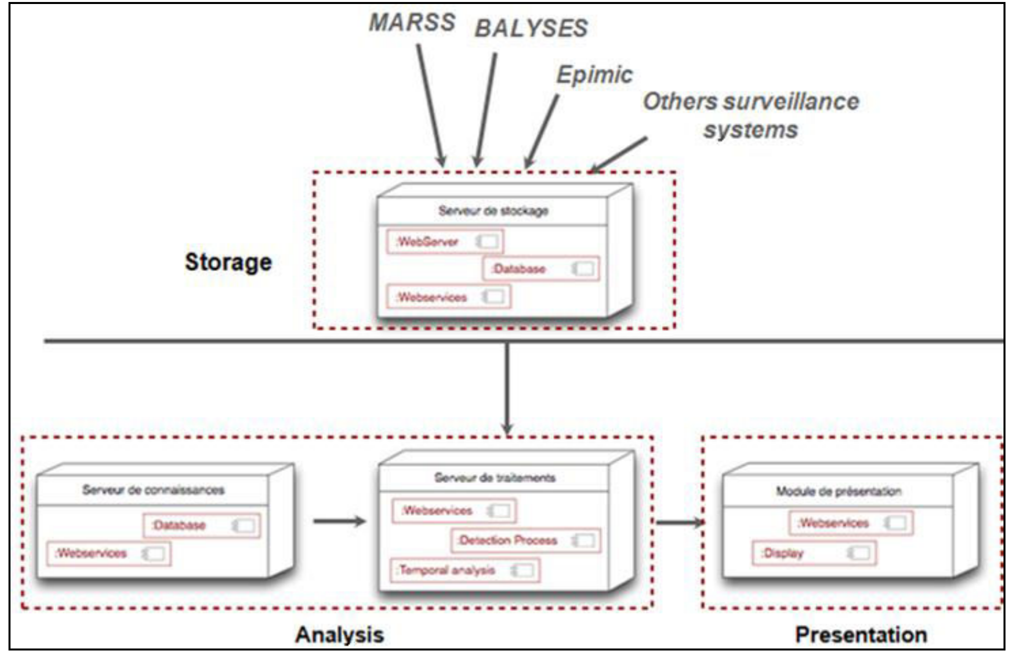

Figure 7. Organisation globale de MIDaS (Méditerranée Infection Datawarehousing and Surveillance).

Conclusions et perspectives

Mon travail de thèse a finalement abouti au développement rapide et à faible coût de deux bases de données historiques, de deux nouveaux outils informatiques de surveillance épidémiologiques uniques de par leur capacité de détection d'événements anormaux en temps réel ainsi que par leur exhaustivité et à l'optimisation d'une stratégie d'investigation et de validation d'événements épidémiologiques détectés (Figure 8, extraite de l'article 4 « A real-time microbiology laboratory surveillance system implemented for the detection of abnormal events and emerging infections, Marseille, France »).

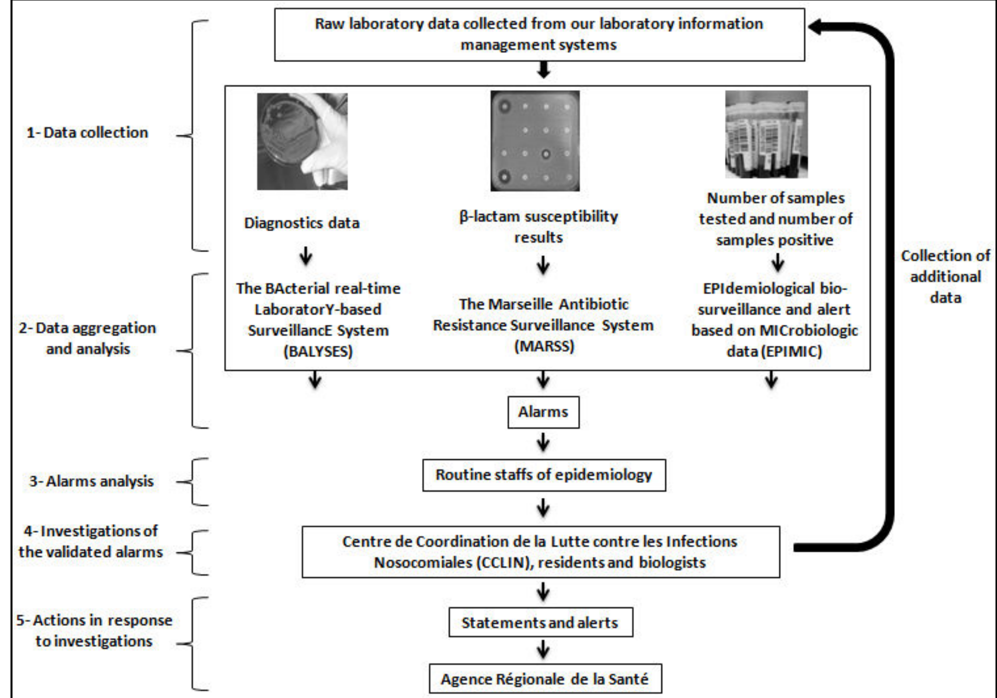

Figure 8. Le système de surveillance en temps réel de l'Institut Hospitalo-Universitaire Méditerranée Infection, Assistance Publique-Hôpitaux de Marseille.

En deux ans, ces systèmes ont permis d'identifier 111 événements épidémiologiques anormaux dont 55 ont été validés et ont donné lieu à des investigations (1 alarme validée émise en moyenne par semaine) et à 33 déclarations officielles à l'ARS. Ces systèmes de surveillance ont également permis de contribuer à la mise en place et la mise à jour d'une "souchothèque" dont l'objectif est d'inventorier toutes les espèces bactériennes isolées au moins une fois dans le laboratoire à partir d'un prélèvement clinique issu d'un patient. Ils ont enfin permis de réaliser 14 publications scientifiques décrivant des événements épidémiologiques confirmés, incluant 9 articles cités dans ce manuscrit de thèse.

Ces systèmes sont en cours d'automatisation complète au sein d'une interface web nommée MIDaS qui réalisera la surveillance épidémiologique au sein de l'IHU à l'aide d'outils mathématiques de détection plus perfectionnés que ceux existant (Figure 7).

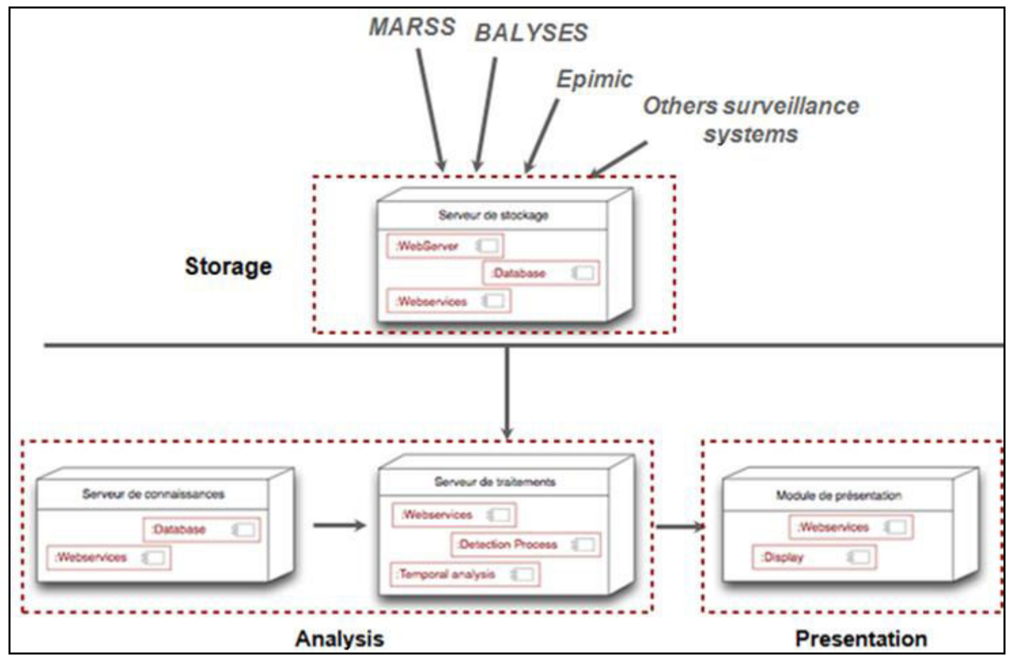

Figure 7. Organisation globale de MIDaS (Méditerranée Infection Datawarehousing and Surveillance).

Cette interface contient déjà une version améliorée d'EPIMIC, le tout premier système de surveillance syndromique développé dans le cadre de l'IHU, accessible *via* internet à l'adresse ci-jointe: https://139.124.153.17/MIDaS/epimic/surveillance/POC.php (Figure 9).

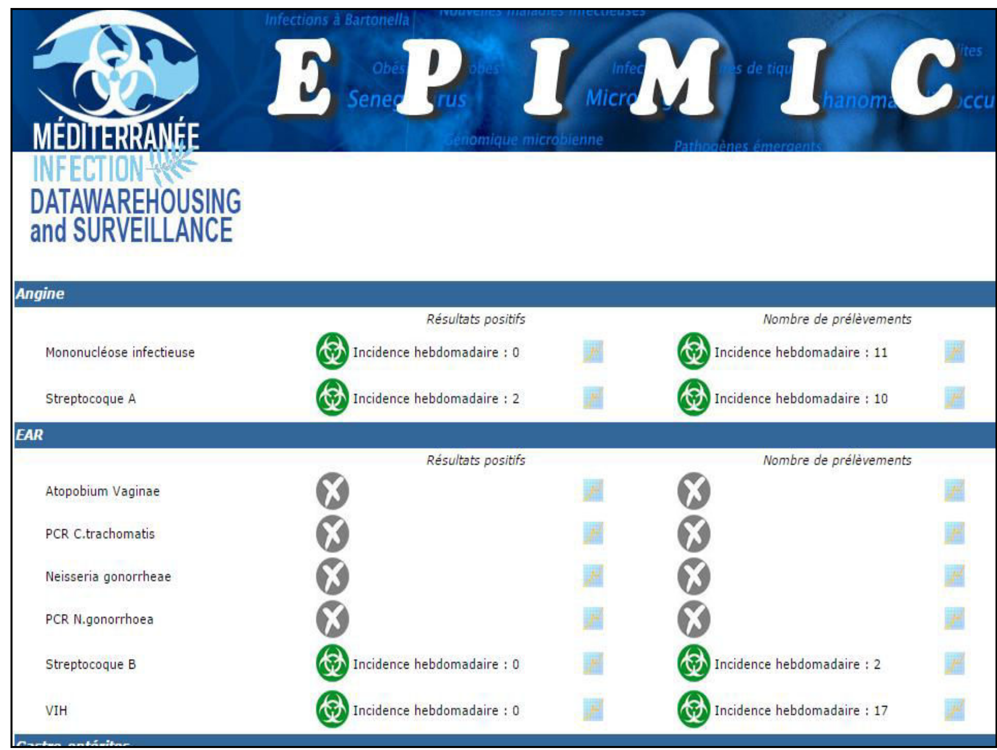

Figure 9. La nouvelle interface d'EPIMIC développée sous MIDaS.

Néanmoins, force est de constater que la surveillance épidémiologique comme nous la concevons actuellement a des limites et doit, de par ce fait, se moderniser pour assumer l'évolution rapide du monde qui nous entoure. Dans cette optique, l'interface développée dans le cadre de l'IHU intégrera un concentrateur de données médicales sur lequel viendront se connecter MIDaS ainsi que d'autres outils de surveillance épidémiologiques en cours de développement ou à venir. Ainsi, face aux risques de santé publique que représentent certains clones bactériens tels que le clone usa300 chez *S. aureus* (19) ou le clone *Escherichia coli*

O104:H4 (20;21), nous développons actuellement un outil de surveillance des clones bactériens fondé sur les spectres MALDI-TOF produits par l'activité de routine d'identification bactérienne de notre laboratoire. Nous sommes également entrain de déployer la surveillance hebdomadaire des espèces bactériennes à la région PACA en récupérant et analysant les données d'activité de laboratoires de ville ou d'hôpitaux de la région. Cette interface permettra donc, *in fine*, d'assurer la surveillance la plus exhaustive possible des événements épidémiologiques à l'échelle de l'IHU et des hôpitaux publics de Marseille, mais également de la région PACA (Figure 10).

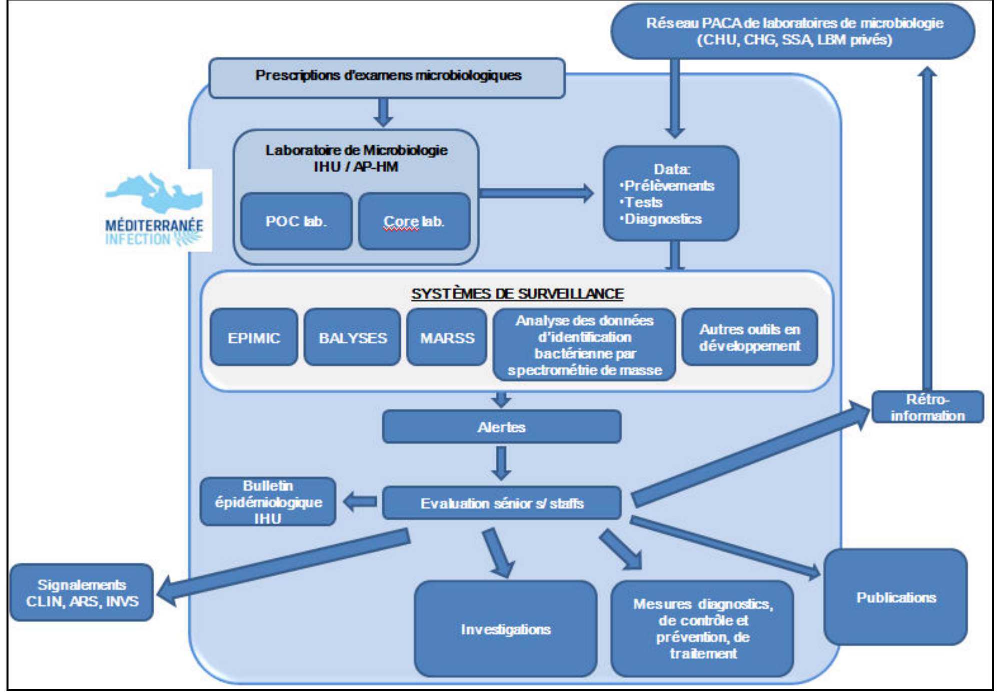

Figure 10. Structure globale de la surveillance des événements anormaux des maladies infectieuses de l'Institut Hospitalo-Universitaire Méditerranée Infection.

Enfin, nous projetons, dans le cadre des projets de collaboration internationale GIRAFE (Groupement International de Recherche en AFrique sur l'Emergence) et REMEDIER (REcherche MEDiterranéenne dans les Infections Emergentes et Réemergentes) entre l'IHU et ses partenaires africains, d'étendre la surveillance épidémiologique développée au sein de l'IHU à l'Afrique Sub-Saharienne (Sénégal, Mali, Guinée, Côte d'ivoire, Bénin, Burkina Faso, Tchad et Niger) et au Maghreb (Mauritanie, Maroc, Tunisie, Libye et Algérie) (Figures 11 et 12).

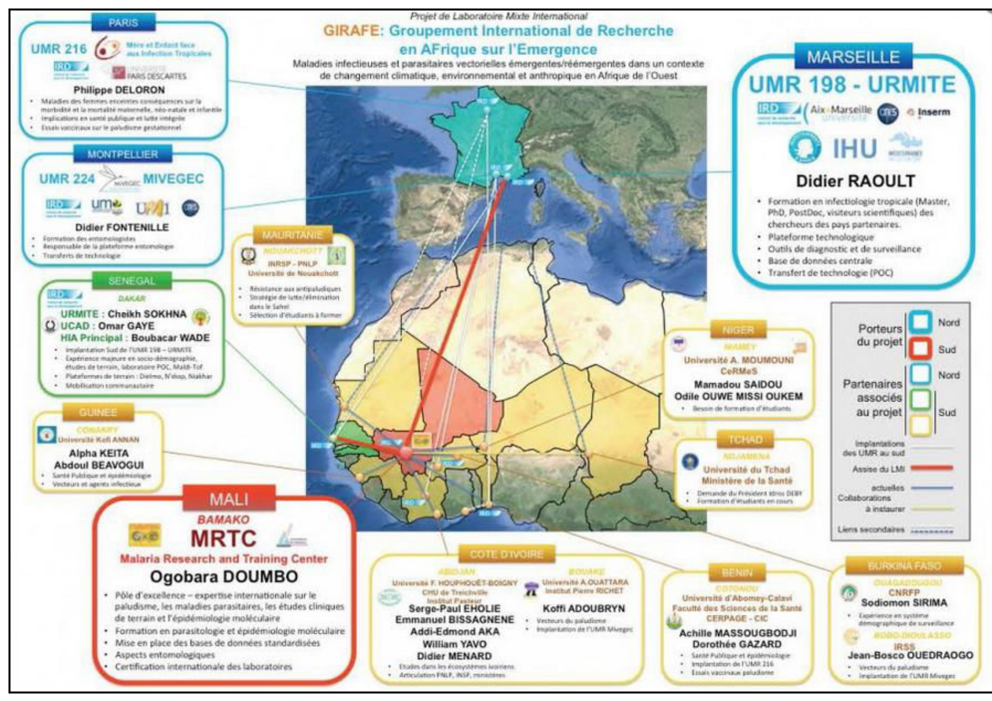

Figure 11. Structure du projet de collaboration de laboratoire mixte international GIRAFE (Groupement International de Recherche en AFrique sur l'Emergence).

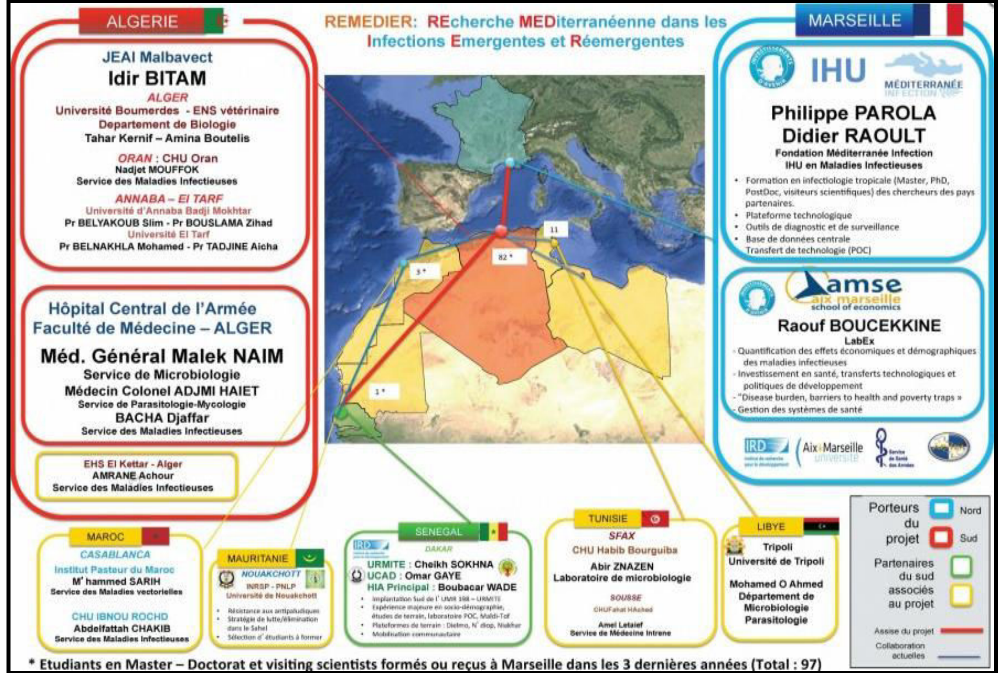

Figure 12. Structure du projet de collaboration de laboratoire mixte international REMEDIER (REcherche MEDiterranéenne dans les Infections Emergentes et Réemergentes).

Liste des articles

Article 14: Correlation between sputum and bronchoalveolar lavage fluid cultures. **Published in JCM (IF: 3.993)**.

Article 15: Emergence of clusters of CRF02_AG and B human immunodeficiency viral strains among men having sex with men exhibiting HIV primary infection in southeastern France. **Published in J Med Virol (IF: 2.347)**.

Article 16: Molecular epidemiology and distribution of serotypes, genotypes, and antibiotic resistance genes of *Streptococcus agalactiae* clinical isolates from Guelma, Algeria, and Marseille, France. **In press in EJCMID (IF: 2.668)**.

Annexes

Le premier travail présenté dans ces annexes (**article 14**) est une analyse comparative de la valeur prédictive positive des crachats et celle des liquides broncho-alvéolaires récupérés après lavage broncho-alvéolaire pour l'identification des espèces bactériennes responsables d'infections respiratoires chez des patients hospitalisés à l'AP-HM. Cette comparaison nous a permis d'identifier que la culture sur crachats avait une valeur prédictive positive comparable à celle des liquides broncho-alvéolaires pour l'identification des espèces bactériennes responsables d'infections respiratoires.

Les deux travaux suivants (**articles 15 et 16**) sont les résultats de collaborations transversales. L'**article 15** a permis d'identifier que l'augmentation des primo-

infections au VIH dans nos hôpitaux était due à l'émergence de variant CRF02_AG et B chez des patients homosexuels au niveau local. L'**article 16** est une étude épidémiologique comparative sur la base de souches de *S. agalactiae* isolées de prélèvements cliniques provenant de Guelma en Algérie et de souches provenant de notre laboratoire à Marseille.

Article 14: Correlation between sputum and bronchoalveolar lavage fluid cultures.

Grégory Dubourg, Cédric Abat, Jean-Marc Rolain, Didier Raoult

Publié dans Journal of Clinical Microbiology (Impact Factor = 3.993)

JCM Accepts published online ahead of print

Correlation between sputum and BAL cultures

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28 **ABSTRACT**

29 A correlation study of the cultured bacteria from paired sputum and bronchoalveolar lavage
30 was performed. The rates of concordant, culture-positive paired specimens that were isolated
31 within one or seven days were 93.7% and 96.5%, respectively, suggesting that culture of
32 readily collectable sputum specimens may result in useful microbiologic diagnosis.

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52 The usefulness of sputum culture has been widely debated. For example, one study found a
53 lack of sensitivity of *Streptococcus pneumoniae* detection from expectorated sputum when
54 compared with a more invasive specimen-collection method (1). However Gram staining and
55 sputum sample culture were recommended for the diagnosis of community-acquired
56 pneumonia (CAP) in immunocompetent patients (2) as studies have shown the usefulness of
57 these methods in immunocompromised subjects (3). Complementarity between the two
58 sampling methods was also reported in immunocompromised patients (4). When sputum
59 induction is not possible, such as in ventilator-associated pneumonia (VAP), the value of
60 bronchoalveolar lavage (BAL) culture is significant, as the survival rate is dependent on the
61 empirical therapy (5-7). Finally, sputum provides sufficient information when compared to
62 bronchoscopy in cystic fibrosis patients (8), particularly in those infected with *Pseudomonas*
63 *aeruginosa* (9). Herein, we compare the results of standard culture techniques from patients
64 who underwent both bronchoalveolar lavage (BAL) and sputum specimen collection in a
65 retrospective study to determine the sputum culture positive predictive value for BAL
66 positivity.

67 All samples were recovered from clinical units from the Assistance Publique Hôpitaux de
68 Marseille (APHM) and were analyzed in a microbiology laboratory from January 2002 to
69 June 2014 according to the Société Française de Microbiologie (SFM) recommendations and
70 EUCAST 2014 (10). Briefly, the specimens were screened for initial quality regarding the
71 presence of white blood cells and epithelial cells and were then inoculated onto Chocolate
72 agar, Colistin – nalidixic acid agar and MacConkey agar plates (Biomerieux, Marcy l'Etoile,
73 France) and incubated aerobically with 5% CO_2 for 48 hours. Each colony that grew from the
74 BAL and sputum specimens with a bacterial load $\geq 10^4$ UFC/ml and $\geq 10^7$ UFC/ml,
75 respectively, was identified. Colony identification was performed until September 2009 using
76 the VITEK 2 apparatus (BioMérieux); thereafter, colony identification was performed using

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77 MALDI-TOF mass spectrometry (11). A total of 25,926 positive samples that were recovered
78 from 6,918 patients between January 2002 and June 2014 were analyzed in this study.
79 Overall, 169,608 sputum samples and 19,536 BAL samples were received, of which 21,760
80 (12.8%) and 4,166 (21.3%) were positive for microorganisms, respectively. After removing
81 duplicates, 3,159 positive BAL and 8,470 positive sputum samples were included in the
82 analysis. Of these, 511 of the culture-positive, paired specimens were identified and
83 characterized using microbiological analyses of BAL and sputum samples on the same day
84 and again within seven days. Pairs were more significantly obtained from patients who were
85 hospitalized in long-term healthcare units (436 of 511) compared to short-term units (75 of
86 511) ($p < 10^{-5}$). When sampling of pairs was not performed within 24 hours, sputa were
87 significantly obtained before BAL (234 VS 111, $p > 10^{-5}$). For samples for which several
88 microorganisms were found, if one organism was missing from one specimen, the pair was
89 considered “mismatched”.

90 In total, 146 different bacterial species were isolated from the sputum samples, and 84 were
91 isolated from the BAL samples. Eight of the 10 most common bacterial species for each
92 sample type were concordant between the types. Regarding the discrepancies, *Moraxella*
93 *catarrhalis* and *Serratia marcescens* were commonly identified in the sputum samples,
94 whereas *Staphylococcus epidermidis* and *Enterobacter aerogenes* were commonly identified
95 in the BAL samples (Table 1). Using the formula $H' = -\Sigma\ pi^* \ln(pi)$ (12), the Shannon
96 diversity index values were estimated to be 2.73 and 2.75 for the BAL and sputum samples,
97 respectively.

98 Of the cultures performed within 7 days, 511 sputum-BAL pairs were identified, and the same
99 microorganism was found in 479 cases (93.7%). Finally, the concordance of methicillin
100 susceptibility/resistance for the available *S. aureus* pairs (66 of 117 pairs) was 100%. Of the
101 cultures performed on the same day, 285 sputum-BAL pairs were identified, and the same

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102 microorganism was found in 275 of the cases (96.5%). These results yielded a positive
103 predictive value (PPV) of 96.5% for sputum culture, considering BAL culture as the gold
104 standard. The correlations were not affected by the identification method, and the sputum
105 PPV when the specimens were collected within one day was 97.1% when identification was
106 performed using a Vitek-2 apparatus and 96.3% when performed using MS MALDI-TOF
107 (e.g. before and after September 2009, respectively). We then analyzed the discrepancies
108 between the results of the two sampling methods. Finally, we assessed the misdetections of
109 bacteria that were considered strict pathogens for each specimen type, including
110 *Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Burkholderia*
111 *cepacia,* and *Haemophilus influenzae.* Of these 20 discrepancies, fourteen were considered
112 major errors, and a specific pathogen was not detected in BAL in 6 cases and in sputum in 8
113 cases. Cases when two different microorganisms where identified from each sample where
114 not stated (Table 2).

115 This 12-year retrospective study shows good agreement between BAL and sputum sample
116 microbiological analyses, which were performed in the same laboratory using the same
117 methods. Indeed, when the analyses were performed within seven days, the PPV of sputum
118 culture was 93.7%. The interval of seven days may have been too long to evaluate the PPV of
119 sputum for BAL positivity, leading us to consider a shorter interval of 24 hours, for which the
120 PPV was 96.5%. The similar Shannon index values obtained in this study (2.73 VS 2.75), as a
121 high correlation of 8 of the 10 most represented bacteria in each group, could reflect the
122 ecology of the medical center due to biased recruitment of patients who were hospitalized in
123 long term health-care units. Because of respiratory tract flora contamination in sputum
124 samples, BALs have been considered the best biological sample to identify a bacterial agent
125 for years, but these samples may also be contaminated. The major bias of this retrospective
126 study is the inclusion of strictly positive samples. These data show that if microbiological

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127 examination of BAL may be valuable for the management of VAP (5-7) sputum analysis is
128 more cost effective and has a similar efficiency compared to invasive sampling methods. This
129 study will be further used prospectively with clinicians to de-escalate antibiotics, if started,
130 and/or to change antibiotic therapy according to sputum sample culture results.

131 **TABLES AND FIGURES**

132 **TABLE 1.** Characteristics of the BAL and sputum groups and the 10 most commonly
133 identified bacteria for each specimen type. Discrepancies are in bold.

134 **TABLE 2**. Characteristics of the 20 discrepancies observed in this series.

135 **Declarations**

136 **Funding:** This work was funded by the IHU Mediterranée Infection.

137 **Competing Interests:** None of the authors have a conflict of interest.

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139 Reference List

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141

142 (1) Bartlett JG. 1994. Invasive diagnostic techniques in pulmonary infections. Respiratory
143 Infections: Diagnosis and Management, 3rd Ed Pennington J (Editor) Raven Press Ltd,
144 NY 73-99.

145 (2) Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC,
146 Dowell SF, File TM, Musher DM, Niederman MS, Torres A, Whitney CG. 2007.
147 Infectious Diseases Society of America/American Thoracic Society consensus
148 guidelines on the management of community-acquired pneumonia in adults. Clin.
149 Infect. Dis. 44(Supplement 2):S27-S72.

150 (3) Cordero E, Pachón J, Rivero A, Girón -Gonzàlez J, Gomez-Mateos J, Merino M,
151 Torres-Tortosa M, González-Serrano M, Aliaga L, Collado A, Hernández-Quero J,
152 Barrera A, Nuño E. 2002. Usefulness of sputum culture for diagnosis of bacterial
153 pneumonia in HIV-infected patients. Eur. J. Clin. Microbiol. Infect. Dis. 21(5):362-7.

154 (4) Rano A, Agusti C, Jimenez P, Angrill J, Benito N, Danes C, González J, RoviraM,
155 Pumarola T, Moreno A, Torres A. 2001. Pulmonary infiltrates in non-HIV
156 immunocompromised patients: a diagnostic approach using non-invasive and
157 bronchoscopic procedures. Thorax 56(5):379-87.

158 (5) Luna CM, Vujacich P, Niederman MS, Vay C, Gherardi C, Matera J, EC Jolly. 1997.
159 Impact of BAL data on the therapy and outcome of ventilator-associated pneumonia.
160 CHEST 111(3):676-85.

161 (6) Michel F, Franceschini B, Berger P, Arnal JM, Gainnier M, Sainty JM, Papazian L.
162 2005. Early Antibiotic Treatment for BAL-Confirmed Ventilator-Associated
163 PneumoniaA Role for Routine Endotracheal Aspirate Cultures. CHEST 127(2):589-
164 97.

165 (7) Timsit JF, Cheval C, Gachot B, Bruneel F, Wolff M, Carlet J, Regnier B. 2001.
166 Usefulness of a strategy based on bronchoscopy with direct examination of
167 bronchoalveolar lavage fluid in the initial antibiotic therapy of suspected ventilator-
168 associated pneumonia. Intensive Care Med. 27(4):640-7.

169 (8) Henig NR, Tonelli MR, Pier MV, Burns JL, Aitken ML. 2001. Sputum induction as a
170 research tool for sampling the airways of subjects with cystic fibrosis. Thorax
171 56(4):306-11.

172 (9) Aaron SD, Kottachchi D, Ferris WJ, Vandemheen KL, Denis MS, Plouffe A, Doucette
173 SP, Saginur R, Chan FT, Ramotar K. 2004. Sputum versus bronchoscopy for
174 diagnosis of *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Eur. Respir. J.
175 24(4):631-7.

176 (10) Leclercq R, Cantón R, Brown DF, Giske CG, Heisig P, MacGowan AP, Mouton JW,
177 Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG

JCM Accepts published online ahead of print

178 Kahlmeter G. 2013. EUCAST expert rules in antimicrobial susceptibility testing. Clin.
179 Microbiol. Infect. 19(2):141-60.

180 (11) Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. 2009.
181 Ongoing revolution in bacteriology: routine identification of bacteria by matrix-
182 assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis.
183 49(4):543-51.

184 (12) Hutcheson K. 1970. A test for comparing diversities based on the Shannon formula. J.
185 Theor. Biol. 29(1):151-4.

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Sputum group N=8470		BAL group N=3159	
Shannon diversity index: 2.73		Shannon diversity index: 2.75	
Microorganism	N (%)	Microorganism	N (%)
Staphylococcus aureus	1883 (22.2)	*Staphylococcus aureus*	626 (19.8)
Pseudomonas aeruginosa	1827 (21.6)	*Pseudomonas aeruginosa*	622 (19.7)
Haemophilus influenzae	841 (9.9)	*Haemophilus influenzae*	361 (11.4)
Escherichia coli	543 (6.4)	*Escherichia coli*	225 (7.1)
Streptococcus pneumoniae	454 (5.4)	*Streptococcus pneumoniae*	180 (5.7)
Klebsiella pneumoniae	399 (4.7)	*Klebsiella pneumoniae*	178 (5.6)
Stenotrophomonas maltophilia	315 (3.7)	***Staphylococcus epidermidis***	101 (3.2)
Enterobacter cloacae	261 (3.1)	*Stenotrophomonas maltophilia*	98 (3.1)
Serratia marcescens	168 (2)	*Enterobacter cloacae*	97 (3.1)
Moraxella catarrhalis	154 (1.8)	***Enterobacter aerogenes***	88 (2.8)

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Organism in sputum	Delay between sputum and BAL sampling	Organism in BAL	Error Type
Staphylococcus aureus	7 days earlier	*Enterobacter aerogenes*	Major
Staphylococcus aureus	5 days earlier	***Klebsiella pneumoniae***	Not Stated
Staphylococcus aureus	4 days earlier	*Escherichia coli*	Major
Staphylococcus aureus	2 days earlier	*Pseudomonas aeruginosa*	Major
Staphylococcus aureus	1 days earlier	*Serratia marcescens*	Major
Staphylococcus aureus	same day	***Streptococcus pneumoniae***	Not Stated
Staphylococcus aureus	1 day after	***Haemophilus influenzae***	Not Stated
Burkholderia cepacia	2 days earlier	***Staphylococcus aureus***	Not Stated
Enterobacter aerogenes	2 days after	***Staphylococcus aureus***	Major
Escherichia coli	2 days after	***Staphylococcus aureus***	Major
Proteus mirabilis	1 day earlier	***Staphylococcus aureus***	Major
Staphylococcus haemolyticus	4 days after	***Staphylococcus aureus***	Major
Streptococcus anginosus	5 days earlier	***Staphylococcus aureus***	Major
Streptococcus pneumoniae	4 days after	***Staphylococcus aureus***	Not Stated
Citrobacter freundii	same day	***Haemophilus influenzae***	Major
Streptococcus pneumoniae	6 days after	***Haemophilus influenzae***	Not Stated
Escherichia coli	7 days earlier	***Klebsiella pneumoniae***	Major
Haemophilus influenzae	2 days earlier	*Proteus mirabilis*	Major
Klebsiella pneumoniae	1 day earlier	*Pseudomonas aeruginosa*	Major
Pseudomonas aeruginosa	1 day earlier	***Streptococcus pneumoniae***	Major

Article 15: Emergence of clusters of CRF02_AG and B human immunodeficiency viral strains among men having sex with men exhibiting HIV primary infection in southeastern France.

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Emergence of Clusters of CRF02_AG and B Human Immunodeficiency Viral Strains Among Men Having Sex With Men Exhibiting HIV Primary Infection in Southeastern France

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The number of new HIV diagnoses is increasing in the western world and transmission clusters have been recently identified among men having sex with men despite Highly Active Antiretroviral Therapy efficacy. The objective of this study was to assess temporal trends, epidemiological, clinical and virological characteristics of primary HIV infections. A retrospective analysis of 79 patients presenting primary HIV infections from 2005 to 2012 was performed in Marseille University Hospitals, southeastern France. Clinical, epidemiological and immunovirological data including phylogeny based on the polymerase gene were collected. 65 males and 14 females were enrolled. The main transmission route was homosexual contact (60.8%). Patients were mostly infected with subtype B (73.4%) and CRF02_AG (21.5%) HIV-1 strains. An increase in the annual number of HIV seroconversions among new HIV diagnoses from 5% in 2005 to 11.2% in 2012 ($P = 0.06$) and of the proportion of CRF02_AG HIV strains among primary HIV infections in 2011–2012 as compared to 2005–2010 ($P = 0.055$) was observed. Phylogenetic analysis revealed four transmission clusters including three transmission clusters among men having sex with men: two large clusters of nine CRF02_AG, six B HIV strains; and one small cluster of three B HIV strains. Clusters involved more frequently men ($P = 0.01$) belonging to caucasian ethicity ($P = 0.05$), with a higher HIV RNA load at inclusion ($P = 0.03$). These data highlight the importance of improving epidemiological surveillance and of implementing suitable prevention strategies to control the spread of HIV transmission among men having sex with men. ***J. Med. Virol. 87:1327–1333, 2015.***
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KEY WORDS: primary HIV infection; MSM; transmission clusters; CRF02_AG; B subtype; France

INTRODUCTION

Primary infections with human immunodefiency virus are an important source of transmission [Koopman et al., 1997; Hayes et al., 2006], which is primarily due to the substantial plasma viral load reached during the first week following contamination [Quinn et al., 2000]. The number of these primary HIV infections represented an estimated 10% of all new human immunodeficiency virus (HIV) diagnoses in France in 2011 [Cazein et al., 2011].

Of note, an outbreak of HIV epidemics among men who have sex with men was observed worldwide [Beyrer et al., 2012] and in many European countries

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[Bezemer et al., 2010; Fisher et al., 2010; Brenner et al., 2011]. Use of phylogenetic analysis allowed defining different patterns of clusters involving men having sex with men [Kouyos et al., 2010; Brenner et al., 2011; Leigh Brown et al., 2011; Frange et al., 2012]. It was shown that clustering in men having sex with men cohorts was most often associated with early-stage infection [Brenner et al., 2007, 2011; Lewis et al., 2008].

An increase of primary HIV infections diagnosed in the clinical microbiology laboratory at Marseille University Hospitals was recently identified [Colson et al., 2013a]. This retrospective analysis aimed at studying primary HIV infections in Marseille University Hospitals.

PATIENTS AND METHODS

Study Population

Between 2000 and 2012, 133,842 sera were tested for HIV diagnosis, 1,192 HIV infections were newly diagnosed (0.08%), and 79 patients presented primary HIV infection (6.6% of new HIV diagnoses).

Enrolment criteria for this retrospective study were a Western blot profile compatible with ongoing seroconversion (incomplete Western blot with absence of antibodies to pol proteins), or a detectable plasma HIV RNA with a negative or weakly reactive (serum to threshold ratio ELISA comprised between 0.9 and 2.0) ELISA, or a documented negative screening test followed by a positive test within a 3-month period. Clinical and epidemiological data are reported.

Results of serological screening tests for syphilis [Architect Syphilis TA assay (Abbott Diagnostics) and Venereal Disease Research Laboratory test (VDRL), hepatitis B and C viruses (Architect chemo-luminescent microparticle immunoassay, Abbott Diagnostics] are reported. Primary HIV infection was defined as symptomatic according to French guidelines [Morlat, 2013] if at least one symptom related to the HIV acute viral syndrome was present: fever, pharyngitis, rash, poly-adenopathy, myalgia, asthenia, arthralgia, diarrhea, nausea, mouth or genital ulcers, neurological symptoms (meningitis, encephalitis, facial palsy), and/or biological abnormalities: thrombocytopenia, neutropenia, or lymphocytosis in the setting of a mononucleosis syndrome, cytolysis.

Virological Analysis

HIV viral load. HIV-1 RNA was quantified with the Cobas Taqman HIV-1 V1.0 or V1.5 assay (Roche Diagnostics, Meylan, France) or the Abbott Real Time HIV-1 PCR assay (Abbott Diagnostics) as recommended by the manufacturers.

RNA extraction, amplification and sequencing. A 1,200 base pair (bp) fragment of the polymerase gene including full length protease and partial reverse transcriptase was amplified and sequenced from viral RNA extracted from plasma obtained at first diagnosis as described elsewhere [Yahi et al., 2005].

Resistance to nucleos(t)ide reverse transcriptase inhibitors (NRTI), non-nucleoside RT inhibitors (NNRTI), and protease inhibitors (PI) was defined according to the 2012 ANRS HIV-1 genotype resistance interpretation algorithm (www.hivfrenchresistance.org). Seventy-nine HIV-1 sequences were retrieved using a 3130XL Genetic Analyzer (Applied Biosystems Branchburg, NJ) then analyzed using Seqscape v2.5 (Applied Biosystems).

Phylogenetic analysis. HIV RNA sequences obtained here were aligned using the ClustalX v2.0 software with those from group M subtypes and circulating recombinant forms available at the NCBI GenBank nucleotide sequence database, and with their best match obtained through BLAST searches against our local laboratory nucleotide sequence database, named after "Marseille database" (composed of 15,100 RT sequences) [Tamalet et al., 2003; Colson et al., 2013b]. Pairwise nucleotide similarities were generated using BioEdit (http://www.mbio.ncsu.edu/bioedit/page2.html). The phylogenetic tree was built using the 672 bp alignment of 146 HIV RNA sequences including the 79 patients' sequences each with their best Blast hit from the Marseille sequence database.

The phylogenetic tree was built using MEGA v5.1 software [Tamura et al., 2011] with the Maximum-Likelihood method based on the most appropriate model: distances were calculated using the General Time Reversible (GTR) model and a discrete three categories Gamma distribution was used to model evolutionary rate distances among sites, allowing a proportion of invariant sites (G + I). Branch supports were assessed by performing 1,000 bootstrap replicates.

Definition of clusters was based on the criteria of high bootstrap values (>98%), short genetic distances (<0.045) according to Hue et al. [2004], [Brenner and Wainberg, 2013] and similarity in polymorphisms and mutation motifs.

Primary HIV infections were stratified into three transmission patterns: unique transmission (1 primary HIV infection), small cluster (2–4 primary HIV infections per cluster), and large cluster (≥5 primary HIV infections per cluster) according to Brenner et al.'s [2011] description.

Statistical Analysis

Statistical analysis was performed using SPSS® v17.02 software for Windows (SPSS Inc., Chicago, IL) and R (Oakland, New Zealand). The association of univariate predictors was calculated by use of the Chi-squared test or Fischer's exact test when appropriate. A logistic regression was also performed to evaluate the significancy of the increase in the number of HIV seroconverters over time. Comparison of means was done by use of ANOVA. A two-sided *P*

value of <0.05 was considered statistically significant. Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were calculated to estimate relative risks when appropriate. Comparisons between clustered and nonclustered primary HIV infections were made using the Chi-squared or the Fisher's exact test for categorical variables and the *t*-test or the Wilcoxon test for continuous variables. Multivariable logistic regression was performed to select the best significant predictive set of variables. All reverse transcriptase nucleotide sequences were submitted to GenBank (accession numbers: KC788426-KC788461; KJ396396-KJ396432).

RESULTS

Temporal changes in the number of primary HIV infection cases are summarized in Figure 1. The baseline epidemiological and virological characteristics of the 79 patients with primary HIV infection are reported in Table I. There were 65 males (82.3%) and 14 females (17.7%). Transmission route was homosexual (60.8%), heterosexual (35.6%), Injection drug use (IDU) (2.7%), and homobi-sexual (1.2%). Patients were mostly infected with B subtype HIV strains (n = 58, 73.4%), CRF02_AG strains (n = 16, 21.5%), F subtype (n = 3, 3.7%), C subtype (n = 1, 1.2%) and CRF22 01_AE (n = 1, 1.2%). Seven patients (7/54, 12.9%) had an active syphilis (both positive VDRL and TPIA tests), 5/68 (6.3%) had a positive HCV serology, no patient had a positive hepatitis B surface antigen (HBs Ag).

"There was a tendency to increase in the number of HIV seroconversions that varied from 5% of new diagnoses in 2005 (8/159) to 11.2% in 2012 (17/152) ($P = 0.06$) (17/152 cases) (Fig. 1). Patients with HIV seroconversion were mostly young men. All 17 cases diagnosed with HIV seroconversion in 2012 were men whose mean age was 38.6 ± 14.3 years (range: 21–72 years) and among whom 6 of 17 (35.3%) were younger than 30 years. Among persons who experienced HIV seroconversion, a significant rise of the male/female sex ratio from 2005 to 2010 (37 men among 50 cases) and the period 2011–2012 (28 men among 29 cases) ($P = 0.011$) was observed as well as a 1.9-fold rise of the annual number of men having sex with men who experienced HIV seroconversion in the period from 2005 to 2010 (31 cases) and the period 2011–2012 (24 cases). Ten (12.7%) of the 79 HIV seroconversions originated from foreign countries. A trend to increase in the proportion of CRF02_AG strains in 2011–2012 as compared to 2005–2010 was observed although not significantly (OR: 3.1, 95% CI 0.94–11.4, $P = 0.055$).

The phylogenetic analysis revealed that 20 primary HIV infections (25%) segregated into four transmission clusters (Fig. 2). The remaining 59 primary HIV infections were not clustered with each other. However, 38 of these 59 remaining primary HIV infections (64.4%) were clustered with one sequence of the

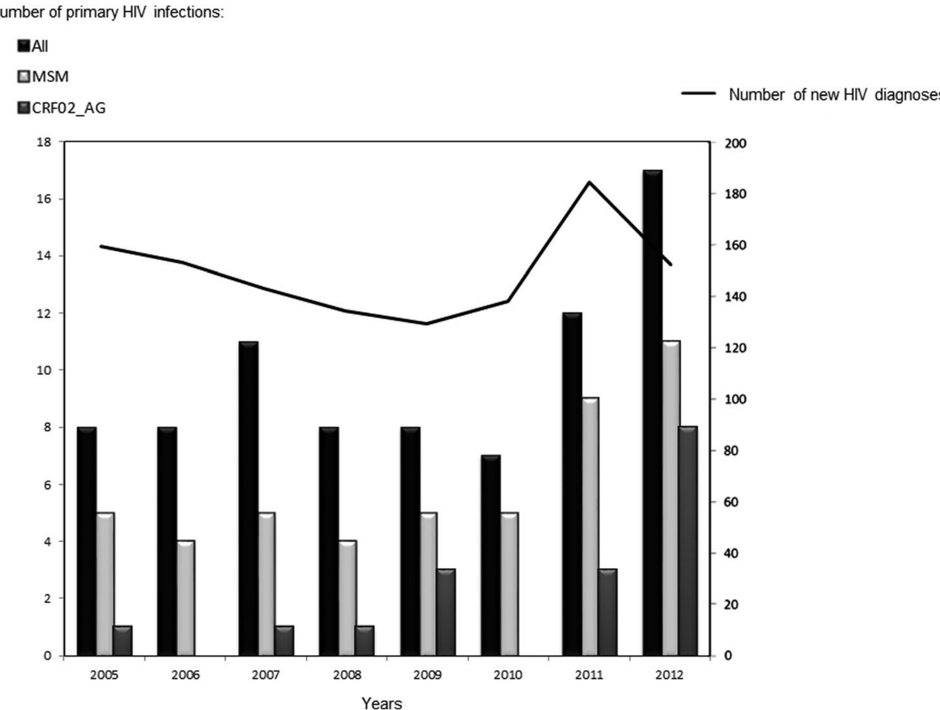

Fig. 1. Temporal trends of new HIV diagnoses, primary HIV infection in MSM, and in CRF02_AG infected patients.

TABLE I. Baseline Epidemiological and Virological Characteristics of the 79 Patients With HIV-1 Seroconversion (2005–2012)

N (%) of male patients	65 (82.3)
Median age (years) (IQR)	36.8 (29–47)
Foreign origin n (%)	10 (12.6)
Median CD4 cell count in cells/μl (IQR)	507 (364–671)
Median HIV-1 RNA in $\log_{10}$ copies/ml (IQR)	3.6 (7.3–8.1)
Transmission mode	
Homosexual	48 (60.8)
Heterosexual	26 (35.6)
IVDU	2 (2.7)
Active syphilis	7 (12.9)
Mean number of WB bands ± SD	4.3 ± 3
Estimation duration of infection (mean days ± SD)	39 ± 3.8
Mean ratio ELISA Architect ± SD	101 ± 208
R5-tropism	50/53 (94.3)
Median proviral DNA in CPMC	1,122
Resistance to NRTIs	3 (3.8)
Resistance to NNRTIs	5 (6.3)
Resistance to PIs	4 (5.1)

CPMC: copies per million cells; IVDU: intravenous drug use; WB: Western blot.

Marseille database found as the best hit. These best blast hits corresponded to patients of Caucasian ethnicity diagnosed in Marseille Hospitals and whose HIV strains were sequenced between years 2003 and 2012.

Among the 20 primary HIV infections that segregated into transmission clusters, two large clusters (≥5 primary HIV infections) were observed, including a large cluster of nine CRF02_AG strains (mean genetic distance = 0.01) from homosexual men all recently infected (seven in 2012, one in 2011, one in 2009), and another large cluster of six B strains (mean genetic distance = 0.025) from homosexual men including four recently infected (two in 2012, and two in 2011). In addition, two small clusters were observed, one with two HIV B strains from two homosexual men infected in 2009 and 2012, and one with three HIV-B strains from an homobisexual man infected in 2007 and two heterosexual men. Nucleotide identity was higher between CRF02_AG and B sequences in these large clusters (98% and 96%, respectively) than between these sequences and their best match in the Marseille laboratory database (n = 15,100 sequences; 93%) or the NCBI Genbank database (95%). In addition, these CRF02_AG and B sequences were similar to some recovered in West Africa [Tebit et al., 2009; Diop-Ndiaye et al., 2010; Kebe et al., 2013] and, interestingly, in recently diagnosed primary HIV infections in France (Genbank references: JQ292318; JQ2923287; JQ292308) [Frange et al., 2012].

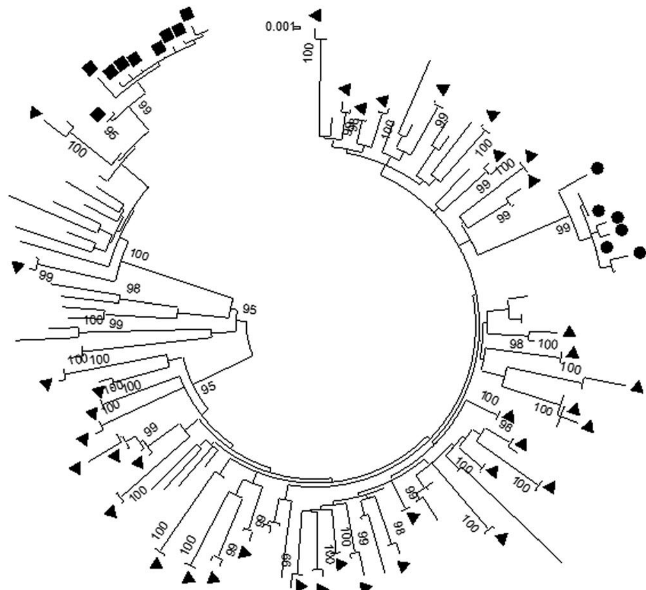

Fig. 2. Phylogenetic tree based on nucleotide sequences corresponding to the partial polymerase gene (1,200 nucleotides) from 79 primary HIV infection sequences recovered in the present study, and their best BLAST hits in the Marseille HIV sequence database. CRF2_AG and B sequence in large transmission cluster are denoted by black squares and black circles, respectively. B sequences in small transmission clusters and transmission pairs are denoted by black triangles.

Of note, a resistance mutation was found in only one patient included in the large cluster of CRF02_AG strains, infected by a CRF02_AG strain that harbored a T215N revertant, a resistance mutation inducing a possible resistance to zidovudine and stavudine according to ANRS algorithm, and a low level resistance to zidovudine and stavudine plus a possible low level resistance according to Stanford-HIV db algorithm [Rhee et al., 2003].

The characteristics of the patients involved into transmission clusters were analyzed and showed that these patients were more often of male gender ($P = 0.01$), of caucasian ethicity ($P = 0.05$) and had a higher HIV RNA load ($P = 0.03$) than patients not involved into transmission clusters (Table II). The multivariable analysis indicated that no factor was independently associated with clustered events.

TABLE II. Comparison of the Characteristics of Patients That Are Not Part of a Transmission Cluster

Patients (n = 79)	Clustered transmissions (n = 20)	Non-clustered transmissions (n = 59)	*P*-value	Multivariate analysis		
				ODDS ratio	95% CI	*P*-value
Sex (male)	20/20	45/59	0.01	NC	NC	0.99
Mean age ± SD	37.6 ± 14	39.4 ± 11	0.48			
Mean CD4 cell count ± SD at inclusion	515 ± 212	535 ± 240	0.74			
Symptomatic PHI	17/18	42/53	0.27			
Caucasian ethnicity	20/20	47/57	0.05	NC	NC	0.99
Risk group (MSM)	15/20	34/59	0.26			
Viral subtype B	11/20	47/59	0.06	0.38	0.10–1.46	0.16
Viral resistance to						
NRTIs	1/20	2/59	0.58			
NNRTIs	0/20	5/59	0.22			
PIs	0/20	4/59	0.42			
Positive HCV serology	0/17	5/51	0.32			
Serological syphilis testing (positive TPIA and VDRL)	4/20	3/34	0.40			
Serological syphilis testing isolated positive TPIA	8/20	10/34	0.25			
Mean HIV viral load at inclusion	7.2 ± 7.5	6.6 ± 7.1	0.03	1	1.0–1.0	0.20

Values are given as counts per number of patients of which data are available. PHI: primary HIV infection; MSM: men having sex with men; VDRL: Venereal Diseases Research Laboratory test; TPIA: Treponema pallidum Immunoassay. The *P*-value for the Hosmer–Lemeshow test for the multivariable model is 0.931. NC: Not calculable because a null value is in one of the cells. Bold characters indicate significant p values.

In contrast, no association was observed with age, transmission route, HIV subtype, symptomatic primary HIV infection, the presence of drug resistance mutations, mean CD4+ T cell count, HBV or HCV co-infection, recent or past syphilis.

When the characteristics of the patients involved into small (2–4 primary HIV infections) or large (≥5 primary HIV infections) clusters were compared, only subtype B HIV infection was significantly less frequent in large transmission clusters ($P = 0.03$). Patients in large clusters were of younger mean age (36.8 ± 14.5 vs. 40 ± 16 years) although not significantly.

DISCUSSION

In this report, four clusters of primary HIV infections were identified, including a main cluster of nine CRF02_AG sequences involving only men having sex with men. These sequences were similar to sequences from West Africa, which is not surprising since apart from B subtypes that are the major HIV strains circulating in France, CRF02_AG subtypes are increasing in number due to successive migratory flows from French-speaking African countries. The second large cluster was composed of six subtype B sequences. It should be noted that present analyses were performed using stringent conditions since HIV sequences recovered from patients with primary HIV infection were compared to one another and concurrently with their best BLAST hit from the Marseille sequence database and from Genbank, and they shared between each other nucleotide similarity levels which were equal to or above those found with the genetically closest sequences from these two large sequences databases. In addition, the high degree of similarity between the sequences within these clusters was above the threshold observed for HIV quasispecies.

The proportion of sequences from primary HIV infections that segregated into the four clusters (25%) is intermediary between the 50% rate of seroconverters found in transmission clusters in Quebec [Brenner et al., 2008] and the lower 12.7% rate found by Frange et al. [2012] at the national level in France, and close to the 30% rate found by Yerly et al. [2001] in Switzerland. The proportion of HIV sequences in large clusters (≥5 PHIs) was lower in the present study (19%) than in Brenner's study [2008] (28%) but higher than in Frange's study [2012] (1.8%). It is possible that in Marseille and its geographical area, men having sex with men whose HIV sequences compose the large clusters share the same partners and frequent the same gay-friendly places. In addition, it should be noted that subjects within the same CRF02_AG cluster co-segregated with subjects from Paris included in the National study [Frange et al., 2012], suggesting therefore the circulation of an epidemic clonal CRF02_AG variant among the population of HIV-infected men having sex with men who are infected with HIV in France.

Interestingly, no close relationship was found by phylogenetic reconstruction (data not shown) between HIV from the primary HIV infections diagnosed in Marseille and some old or new complex Circulating Recombinant Forms (CRF) identified in 8.3% of patients diagnosed at the time of primary HIV infection in a recent French Nationwide study [Galimand et al., 2010] nor with the new emerging CRF56_cpx recently diagnosed in men having sex with men in France [Leoz et al., 2013].These findings suggest that these old and new CRF are not yet

spreading among men who have sex with men in southeastern France.

The main characteristics of patients involved into clusters were the higher frequency of patients of male gender and of caucasian ethnicity in agreement with two recent studies [Chalmet et al., 2010; Frange et al., 2012]. Being part of a cluster was also significantly associated with a higher HIV-1 RNA load reflecting a potentially highly infectious status facilitating HIV transmission. In addition, contrasting with a recent nationwide French study [Frange et al., 2012] that found a vast majority of B subtypes in large clusters (72.2%) or small clusters (86.9%), one of the two large clusters observed in the present study was exclusively composed of CRF02_AG subtypes that infected men having sex with men. This finding is in line with a recent French study indicating that 20% of non-B HIV-1 in recent infections segregated into clusters, the largest one of which involved men having sex with men infected by a CRF02_AG variant [Brand et al., 2014]. Finally, in the present study, patients involved in clustered transmissions were not characterized by a younger age at a significant level, as was the case in Frange et al.'s [2012] study, nor did they harbour drug resistant viruses as in Yerly et al.'s [2009] study, suggesting that resistant variants such as the T215N revertant mutant strain, the only resistant strain found within a large cluster in this study, have not yet spread within transmission clusters probably due to the lower transmissibility of these resistant strains [Brenner et al., 2008]. The overall differences found between the present results and previous studies results could be related to the sample population size, the timing and duration of inclusion of patients, routes of HIV infection including various proportions of men having sex with men, prevalence of non-B subtypes, and definition of clusters, which largely varied according to the studies.

The present data point out the failure of control of HIV transmission in France among men who have sex with men. Indeed, the yearly number of primary HIV infections diagnosed in Marseille has never been so high, increasing in 2008 and 2012, and the number of these primary HIV infections affecting men having sex with men also rose. Of note, this increase was confirmed in 2013 wherein 22 primary HIV infections involving 18 men having sex with men were observed (data not shown) [Dubourg et al., 2014].

This increase in the incidence of HIV seroconversions among men having sex with men is observed at the national scale in France [Semaille et al., 2009; Le Vu et al., 2010], in Europe [Likatavicius et al., 2008] and worldwide [Beyrer et al., 2012]. This high and increasing incidence is maintained despite the effects of antiretroviral therapy to decrease transmission at the population level [Montaner et al., 2010], due to multifactorial reasons: a higher transmission risk related to anal receptive intercourse (approximately 18 times higher than by vaginal intercourse) [Baggaley et al., 2010], high rates of partner changes, increase in unprotected anal sex and number of sexual partners, increase in HIV transmission in case of another concurrent sexually transmitted infections [Bouyssou et al., 2011], and missed opportunities for HIV testing [Champenois et al., 2013]. Another finding was the increase in the non-B subtypes especially CRF02_AG subtypes in primary HIV infections diagnosed in Marseille, and more specifically, the emergence of a circulating epidemic clonal CRF02_AG variant among the population of HIV-infected men having sex with men. This latter finding was recently reinforced by a French study highlighting the spread of recent non-B HIV-1 infections including CRF02_AG variants clustering in men having sex with men [Brand et al., 2014].

CONCLUSION

In conclusion, these data highlight the importance of close epidemiological surveillance, including surveillance of HIV genotypes at the local scale to provide information on current epidemiological trends, and the need to implement appropriate prevention strategies to control the spread of B and non-B subtype infections in men having sex with men.

REFERENCES

Baggaley RF, White RG, Boily MC. 2010. HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. Int J Epidemiol 39:1048–1063.

Beyrer C, Baral SD, van Griensven F, Goodreau SM, Chariyalertsaak S, Wirtz AL, Brookmeyer R. 2012. Global epidemiology of HIV infection in men who have sex with men. Lancet 380:367–377.

Bezemer D, van Sighem A, Lukashov VV, van der Hoek L, Back N, Schuurman R, Boucher CA, Claas EC, Boerlijst MC, Coutinho RA, de Wolf F. ATHENA observational cohort. 2010. Transmission networks of HIV-1 among men having sex with men in the Netherlands AIDS 24:271–282.

Bouyssou A, Janier M, Dupin N, Alcaraz I, Vernay-Vaisse C, Basselier B, Spenatto N, Dhotte P, Castanos F, Semaille C, Gallay A. 2011. La syphilis en France: analyse des données de surveillance sur 10 ans, 2000–2009. Bull Epidemiol Hebd 26-27-28:295–300.

Brand D, Moreau A, Cazein F, Lot F, Pillonel J, Brunet S, Thierry D, Le Vu S, Plantier JC, Semaille C, Barin F. 2014. Characteristics of patients recently infected with HIV-1 non-B subtypes in France: a nested study within the mandatory notification of new HIV diagnoses. J Clin Microbiol 52:4010–4016.

Brenner BG, Wainberg MA. 2013. Future of phylogeny in HIV prevention. J Acquir Immune Defic Syndr 63:S248–254.

Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, Baril JG, Thomas R, Rouleau D, Bruneau J, Leblanc R, Legault M, Tremblay C, Charest H, Wainberg MA. Quebec Primary HIV Infection Study Group. 2007. High rates of forward transmission events after acute/early HIV-1 infection. J Infect Dis 195:951–959.

Brenner BG, Roger M, Moisi DD, Oliveira M, Hardy I, Turgel R, Charest H, Routy JP, Wainberg MA. Montreal PHI Cohort and HIV Prevention Study Groups. 2008. Transmission networks of drug resistance acquired in primary/early stage HIV infection. AIDS 22:2509–2515.

Brenner BG, Roger M, Stephens D, Moisi D, Hardy I, Weinberg J, Turgel R, Charest H, Koopman J, Wainberg MA. Montreal PHI

Cohort Study Group. 2011. Transmission clustering drives the onward spread of the HIV epidemic among men who have sex with men in Quebec. J Infect Dis 204:1115–1119.

Cazein F, Pinget R, Lot F, Pillonel J, Le Strat Y, Sommen C, Brunet S, Thierry D, Brand D, leclerc M, Benyelles L, Da Costa C, Barin F, Semaille C. 2011. New HIV and AIDS diagnoses. France, 2003–2011. Bull Epidemiol Hebd 28–29:333–340. Available from: http://invs.sante.fr

Chalmet K, Staelens D, Blot S, Dinakis S, Pelgrom J, Plum J, Vogelaers D, Vandekerckhove L, Verhofstede C. 2010. Epidemiological study of phylogenetic transmission clusters in a local HIV-1 epidemic reveals distinct differences between subtype B and non-B infections. BMC Infect Dis 10:262.

Champenois K, Cousien A, Cuzin L, Le Vu S, Deuffic-Burban S, Lanoy E, Lacombe K, Patey O, Béchu P, Calvez M, Semaille C, Yazdanpanah Y. 2013. Missed opportunities for HIV testing in newly-HIV-diagnosed patients, a cross sectional study. BMC Infect Dis 13:200.

Colson P, Gouriet F, Badiaga S, Tamalet C, Stein A, Raoult D. 2013a. Real-time laboratory surveillance of sexually-transmissible infections in Marseille university hospitals reveals rise of gonorrhea, syphilis and human immunodeficiency virus seroconversions in 2012. Eurosurveillance 18:4.

Colson P, Bregigeon S, Tourres C, Solas C, Poizot-Martin I, Tamalet C. 2013b. Relapse of hepatitis C virus after 14 months of sustained virological response following pegylated-interferon alpha plus ribavirin therapy in a human immunodeficiency virus type 1 infected patient. J Clin Virol 58:309–314.

Diop-Ndiaye H, Toure-Kane C, Leye N, Ngom-Gueye NF, Montavon C, Peeters M, Mboup S. 2010. Antiretroviral drug resistance mutations in antiretroviral-naive patients from Senegal. AIDS Res Hum Retroviruses 26:1133–1138.

Dubourg G, Colson P, Tamalet C, Fournier PE, Raoult D. 2014. Increase in sexually transmitted infections during Europride 2013 in Marseille, France. Lancet Infect Dis 14:667–678.

Fisher M, Pao D, Brown AE, Sudarshi D, Gill ON, Cane P, Buckton AJ, Parry JV, Johnson AM, Sabin C, Pillay D. 2010. Determinants of HIV-1 transmission in men who have sex with men: a combined clinical, epidemiological and phylogenetic approach. AIDS 24:1739–1747.

Frange P, Meyer L, Deveau C, Tran L, Goujard C, Ghosn J, Girard PM, Morlat P, Rouzioux C, Chaix ML. French ANRS CO6 PRIMO Cohort Study Group. 2012. Recent HIV-1 infection contributes to the viral diffusion over the French territory with a recent increasing frequency. PLoS ONE 7:e31695.

Galimand J, Frange P, Rouzioux C, Deveau C, Avettand-Fenoël V, Ghosn J, Lascoux C, Goujard C, Meyer L, Chaix ML. 2010. Evidence of HIV type 1 complex and second generation recombinant strains among patients infected in 1997–2007 in France: ANRS CO06 PRIMO Cohort. AIDS Res Hum Retroviruses 26:645–651.

Hayes RJ, White RJ. 2006. Amplified HIV transmission during early-stage infection J Infect Dis 193:604–605.

Hue S, Clewley JP, Cane PA, Pillay D. 2004. HIV-1 pol gene variation is sufficient for reconstruction of transmissions in the era of antiretroviral therapy. AIDS 18:719–728.

Kebe K, Thiam M, Diagne Gueye NR, Diop H, Dia A, Signate Sy H, Charpentier C, Belec L, Mboup S, Toure Kane C. 2013. High rate of antiretroviral drug resistance mutations in HIV type 1-infected Senegalese children in virological failure on first-line treatment according to the World Health Organization guidelines. AIDS Res Hum Retroviruses 29:242–249.

Koopman JS, Jacquez JA, Welch GW, Simon CP, Foxman B, Pollock SM, Barth-Jones D, Adams AL, Lange K. 1997. The role of early HIV infection in the spread of HIV through populations. J Acquir Immune Defic Syndr Hum Retrovirol 14:249–258.

Kouyos R, Von Wyl V, Yerly S, Boni J, Taffe P, Shah C, Klimkait T, Weber R, Hirschel B, Cavassini M, Furrer H, Battegay M, Vernazza P, Bernasconi E, Rickenbach M, Ledergerber B, Bonhoeffer S, Gunthard H. The Swiss HIV Cohort Study. 2010. Molecular epidemiology reveals long-term changes in HIV Type 1 subtype B transmission in Switzerland. J Infect Dis 201:1488–1497.

Le Vu S, Le Strat Y, Barin F, Pillonel J, Cazein F, Bousquet V, Brunet S, Thierry D, Semaille C, Meyer L, Desenclos JC. 2010. Population-based HIV-1 incidence in France, 2003–08: a modelling analysis. Lancet Infect Dis 10:682–687.

Leigh Brown AJ, Lycett SJ, Weinert L, Hughes GJ, Fearnhill E, Dunn DT. UK HIV Drug Resistance Collaboration. 2011. Transmission network parameters estimated from HIV sequences for a nationwide epidemic. J Infect Dis 204:1463–1469.

Leoz M, Feyertag F, Charpentier C, Delaugerre C, Wirden M, Lemee V, Plantier JC. 2013. Characterization of CRF56_cpx, a new circulating B/CRF02/G recombinant form identified in MSM in France. AIDS 27:2309–2312.

Lewis F, Hughes GJ, Rambaut A, Pozniak A, Leigh Brown AJ. 2008. Episodic sexual transmission of HIV revealed by molecular phylodynamics. PLoS Med 5:e50.

Likatavicius G, Klavs I, Devaux I, Alix J, Nardone A. 2008. An increase in newly diagnosed HIV cases reported among men who have sex with men in Europe. 2000–6: implications for a European public health strategy. Sex Transm Infect 84:499–505.

Montaner JS, Lima VD, Barrios R, Yip B, Wood E, Kerr T, Shannon K, Harrigan PR, Hogg RS, Daly P, Kendall P. 2010. Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. Lancet 376:532–539.

Morlat P. Prise en charge médicale des personnes vivant avec le VIH. In: Française LD, editor. Recommandations du groupe d'experts. Rapport Paris: La documentation Française 2013.

Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, Meehan MO, Lutalo T, Gray RH. 2000. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Sudy group. N Engl J Med 342:921–929.

Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. 2003. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nuclleic Acids Res 31:298–303.

Semaille C, Cazein F, Lot F, Pillonel J, Le Strat Y, Bousquet V, Velter A, Barin F. 2009. Recently acquired HIV infection in men who have sex with men (MSM) in France, 2003–2008. Euro Surveill 14: (pii:19425).

Tamalet C, Fantini J, Tourres C, Yahi N. 2003. Resistance of HIV-1 to multiple antiretroviral drugs in France: a 6-year survey (1997–2002) based on an analysis of over 7000 genotypes. AIDS 17:2383–22388.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEG A5: molecular evolutionary genetic analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol EVol 28:2731–2739.

Tebit DM, Sangaré L, Tiba F, Saydou Y, Makamtse A, Somlare H, Bado G, Kouldiaty BG, Zabsonre I, Yameogo SL, Sathiandee K, Drabo JY, Kräusslich HG. 2009. Analysis of the diversity of the HIV-1 pol gene and drug resistance associated changes among drug-naïve patients in Burkina Faso. J Med Virol 81:1691–1701.

Yahi N, Fantini J, Henry M, Tourrès C, Tamalet C. 2005. Structural analysis of reverse transcriptase mutations at codon 215 explains the predominance of T215Y over T215F in HIV-1 variants selected under antiretroviral therapy. Biomed Sci 12:701–710.

Yerly S, Vora S, Rizzardi P, Chave JP, Vernazza PL, Flepp M, Telenti A, Battegay M, Veuthey AL, Bru JP, Rickenbach M, Hirschel B, Perrin L. Swiss HIV Cohort Study. 2001. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. AIDS 15:2287–2292.

Yerly S, Junier T, Gayet-Ageron A, Amari EB, von Wyl V, Günthard HF, Hirschel B, Zdobnov E, Kaiser L. Swiss HIV Cohort Study. 2009. The impact of transmission clusters on primary drug resistance in newly diagnosed HIV-1 infection. AIDS 23:1415–1423.

Article 16: Molecular epidemiology and distribution of serotypes, genotypes, and antibiotic resistance genes of *Streptococcus agalactiae* clinical isolates from Guelma, Algeria, and Marseille, France.

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Molecular epidemiology and distribution of serotypes, genotypes, and antibiotic resistance genes of *Streptococcus agalactiae* clinical isolates from Guelma, Algeria and Marseille, France

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Abstract

This study describes, for the first time, the genetic and phenotypic diversity among 93 *Streptococcus agalactiae* (group B *Streptococcus*, GBS) isolates collected from Guelma, Algeria and Marseille, France. All strains were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The molecular support of antibiotic resistance and serotyping were investigated by polymerase chain reaction (PCR). The phylogenetic lineage of each GBS isolate was determined by multilocus sequence typing (MLST) and grouped into clonal complexes (CCs) using eBURST. The isolates represented 37 sequence types (STs), 16 of which were novel, grouped into five CCs, and belonging to seven serotypes. Serotype V was the most prevalent serotype in our collection (44.1 %). GBS isolates of each serotype were distributed among multiple CCs, including cps III/CC19, cps V/CC1, cps Ia/CC23, cps II/CC10, and cps III/CC17. All isolates presented susceptibility to penicillin, whereas resistance to erythromycin was detected in 40 % and tetracycline in 82.2 % of isolates. Of the 37 erythromycin-resistant isolates, 75.7 % showed the macrolide–lincosamide–streptogramin B (MLS_B)-resistant phenotype and 24.3 % exhibited the macrolide (M)-resistant phenotype. Constitutive MLS_B resistance (46 %) mediated by the *ermB* gene was significantly associated with the Guelma isolates, whereas the M resistance phenotype (24.3 %) mediated by the *mefA*/*E* gene dominated among the Marseille isolates and belonged to ST-23. Tetracycline resistance was predominantly due to *tetM*, which was detected alone (95.1 %) or associated with *tetO* (3.7 %). These results provide epidemiological data in these regions that establish a basis for monitoring increased resistance to erythromycin and also provide insight into correlations among clones, serotypes, and resistance genes.

Introduction

Streptococcus agalactiae (group B *Streptococcus*, GBS) is a Gram-positive species commensal of human gastrointestinal and genitourinary flora and is responsible for severe diseases in susceptible hosts [1]. Moreover, this species can cause life-threatening invasive diseases in pregnant women and

newborns [2]. The Centers for Disease Control and Prevention (CDC) recommends a strategy based on intrapartum chemoprophylaxis for pregnant women to decrease GBS infections in neonates [3]. However, in the last several decades, GBS strains have also been associated with invasive disease in non-pregnant adults, the elderly, and patients with underlying medical conditions, such as malignancy, diabetes, or liver disease [4–6]. To prevent *S. agalactiae* infections in newborns, β-lactams are recommended as a first-line antibiotic prophylaxis in parturient women, and macrolides–lincosamides remain the therapeutic alternative in cases of allergy to β-lactam [7]. However, the use of these antibiotics as alternative agents for prophylaxis is questioned because of increasing trends in the rates of resistance to erythromycin and clindamycin among *S. agalactiae* [8]. The first erythromycin resistance mechanism reported in GBS was the *erm* gene-encoded modification of their ribosomal targets via methylation, resulting in cross-resistance to macrolide–lincosamide–streptogramin B (MLS_B) antibiotics [9, 10]. Erythromycin resistance can also be due to efflux pumps, mediated by the *mefA*/*E* gene, which causes resistance to 14- and 15-membered macrolide compounds and produces the so-called M phenotype [11, 12]. The increasing resistance to macrolides observed worldwide emphasizes the need for more detailed studies on GBS macrolide-resistant populations [13, 14]. Similarly, the resistance to tetracycline of GBS is also common and frequently associated with the *tetM* gene [15], and tetracycline resistance genes are often found on the same mobile genetic elements that carry macrolide resistance genes [16]. Capsular serotyping is the classical method used in epidemiological studies, which defines ten GBS serotypes (Ia, Ib, II–IX) [17]. One of the most important factors involved in virulence is capsular polysaccharide (CPS) [18], and the multivalent CPS–protein conjugate vaccines have been developed in the last decade, raising the possibility of preventing perinatal GBS disease via maternal vaccination [19]. Among the methods applied for GBS typing, several molecular tools have been developed, especially multilocus typing sequence (MLST), which has a high discriminatory power [20]. Indeed, the MLST approach has contributed to a better characterization of GBS isolates and the classification of bacterial genogroups [21] and has been utilized in epidemiological studies of the population genetics of human pathogenic bacteria [20]. More recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful, cost-effective, and rapid tool for bacterial identification in clinical laboratories

and biotyping [22]. Other aspects of this technology are potentially interesting, such as its contribution to the field of new species characterization and the detection of certain mechanisms of resistance and simultaneous identification of strains belonging to the highly virulent ST-17 or emerging ST-1 clones [23, 24]. Only one report focused on GBS isolates from Algeria, which investigated risk factors associated with newborn infections by GBS and serotyping using the sero-agglutination method [25]. However, in this study, for the first time, we targeted molecular support of antibiotic resistance, molecular serotyping, and the clonality of a collection of GBS clinical isolates from Guelma, Algeria and compared them to those from Marseille, France.

Materials and methods

Bacterial strains

Ninety-three GBS isolates were collected using vaginal swabs in Guelma, Algeria ($n = 44$), between January 2011 and February 2012 and from different samples, including vaginal swabs ($n = 30$), urine ($n = 10$), and blood culture ($n = 9$), from the microbiology laboratory of Timone Hospital at Marseille, France, between October 2013 and January 2014.

Identification of GBS

All isolates were grown on the selective medium Todd–Hewitt broth (bioMérieux, France) and incubated at 37 °C for 24 h. After that, the isolates were cultivated on Columbia agar base enriched with 5 % sheep blood (bioMérieux, France) in a 10 % CO_2 atmosphere. The identification was carried out on the basis of the following criteria: hemolysis on blood agar, negative reaction with catalase reagent, a positive CAMP (Christie–Atkins–Munch–Petersen) test, and Lancefield grouping with type B antisera (Pastorex™ Strep B, Bio-Rad, France). The identification of isolates was confirmed using MALDI-TOF MS (Bruker Daltonics, Germany), and their obtained specific spectra were used to build a main spectrum profile (MSP) dendrogram using Biotyper 3.0 software (Bruker Daltonics, Leipzig, Germany), as described previously [26, 27]. We used MALDI-TOF MS to search for GBS biomarkers associated with ST-1 and ST-17 strains at the species identification stage [23].

Antimicrobial susceptibility

The antibiotic susceptibility of our isolates was assessed using the disk diffusion method on Muller–Hinton agar plates supplemented with 5 % blood (bioMérieux, France), according to the recommendation of the French Society for Microbiology standards (CA-SFM). The tested antibiotics were penicillin G, amoxicillin, gentamicin, tetracycline, erythromycin, clindamycin, pristinamycin, rifampicin, and vancomycin (Oxoid, France). The erythromycin (15 µg)–clindamycin (2 µg) double-disk test was used to determine constitutive MLS_B ($cMLS_B$) resistance, inducible MLS_B ($iMLS_B$) resistance, and the M resistance phenotype, as previously described [10]. Erythromycin and clindamycin minimum inhibitory concentrations (MICs) were determined using the Etest method (bioMérieux, France).

Molecular characterization of antibiotic resistance-encoding genes

DNA extraction of all isolates was performed using EZ1 Advanced XL extractor with EZ1® DNA Tissue Kit (Qiagen, Courtaboeuf, France) and DNA Bacteria Card (Qiagen), according to the manufacturer's instructions. Antibiotic resistance genes (*ermA*, *ermB*, *mefA*/*E*, *tetM*, *tetO*, *tetK*, and *tetL*) were searched by polymerase chain reaction (PCR), as previously described [28, 29].

Sequencing

All obtained PCR products were purified and sequenced using the BigDye Terminator® v1.1 Cycle Sequencing Kit (Applied Biosystems, Courtaboeuf, France). The sequencing products were then processed using an ABI PRISM 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA) [30]. The obtained sequences were aligned and compared with those in GenBank using the BLAST program against the NCBI and ARG-ANNOT databases [31].

Determination of capsular serotypes

Identification of the capsular type (Ia, Ib, II–IX) of all GBS isolates was performed by the multiplex PCR assay according to a previously published procedure [17]. Non-typeable isolates were designated as NT.

Multilocus sequence typing (MLST)

Seven housekeeping genes were used for GBS characterization using the MLST scheme, including *adhP* (alcohol dehydrogenase), *pheS* (phenylalanyl transfer RNA synthetase), *atr* (amino-acid transporter protein), *glnA* (glutamine synthetase), *sdhA* (L-serine dehydratase), *glcK* (glucose kinase), and *tkt* (transketolase), as described previously [20]. An online database (http://pubmlst.org/sagalactiae/) was used for assigning allele numbers and sequence types (STs). GBS 2603 (serotype V; ST-110) was used as a reference strain. The sequences of the seven loci obtained were concatenated and used to build a phylogenetic tree by the MEGA5 program with the neighbor-joining method [32]. The eBURST program was used to group isolates into clonal complexes (CCs), the members of which shared at least six of the seven MLST loci [33]; otherwise, an ST was considered a singleton.

Statistical analysis

Fisher's exact test was used to evaluate differences in the distributions of isolates using Epi Info software version 7, according to CDC recommendations (http://www.openepi.com/Menu/OE_Menu.htm). A p-value ≤ 0.05 was considered significant.

Results

Bacterial isolation and identification

The study collection consisted of 93 isolates of *S. agalactiae*, 44 from Guelma, Algeria and 49 from Marseille, France. The most prevalent source of isolates from Marseille was vaginal samples (30/49; 61.2 %), followed by urine (20.4 %) and blood cultures (18.4 %) (Figs. 1 and 2). Using MALDI-TOF MS, all GBS isolates were correctly identified at the species level, with score values >2.1 using Bruker Biotyper 3.0 software. The MSP dendrogram of our isolates revealed three clusters according to the geographical origin to an arbitrary cut-off at a distance level of 700, as shown in Fig. 1. The 49 isolates from Marseille contained 36 *S. agalactiae* grouped into two clusters as follows: cluster C1 (vaginal; $n = 8$ samples) and cluster C2 (vaginal; $n = 9$, blood; $n = 9$, and urine; $n = 10$ samples). Conversely, cluster C3 contained 42 isolates of *S. agalactiae* from Guelma (vaginal samples). Clusters C1 and C2 were significantly associated with Marseille isolates, whereas cluster C3 was associated with Algerian isolates ($p < 10^{-6}$) (Fig. 1). Additionally, MALDI-TOF MS identified a

6250-Da protein specific to sequence type ST-1 strains (and no mass peak at 6888 Da) ($n = 15$) and a 7625-Da protein specific to ST-17 strains ($n = 3$). However, these two peaks were also present in other STs, including a peak at 6250 in ST-460 ($n = 1$) and ST-693 ($n = 2$), and a peak at 7625 in ST-106 ($n = 1$), as shown in Fig. 2.

Fig. 1

Cluster analysis of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra of *Streptococcus agalactiae* isolates from Guelma and Marseille [main spectrum profile (MSP) dendrogram]

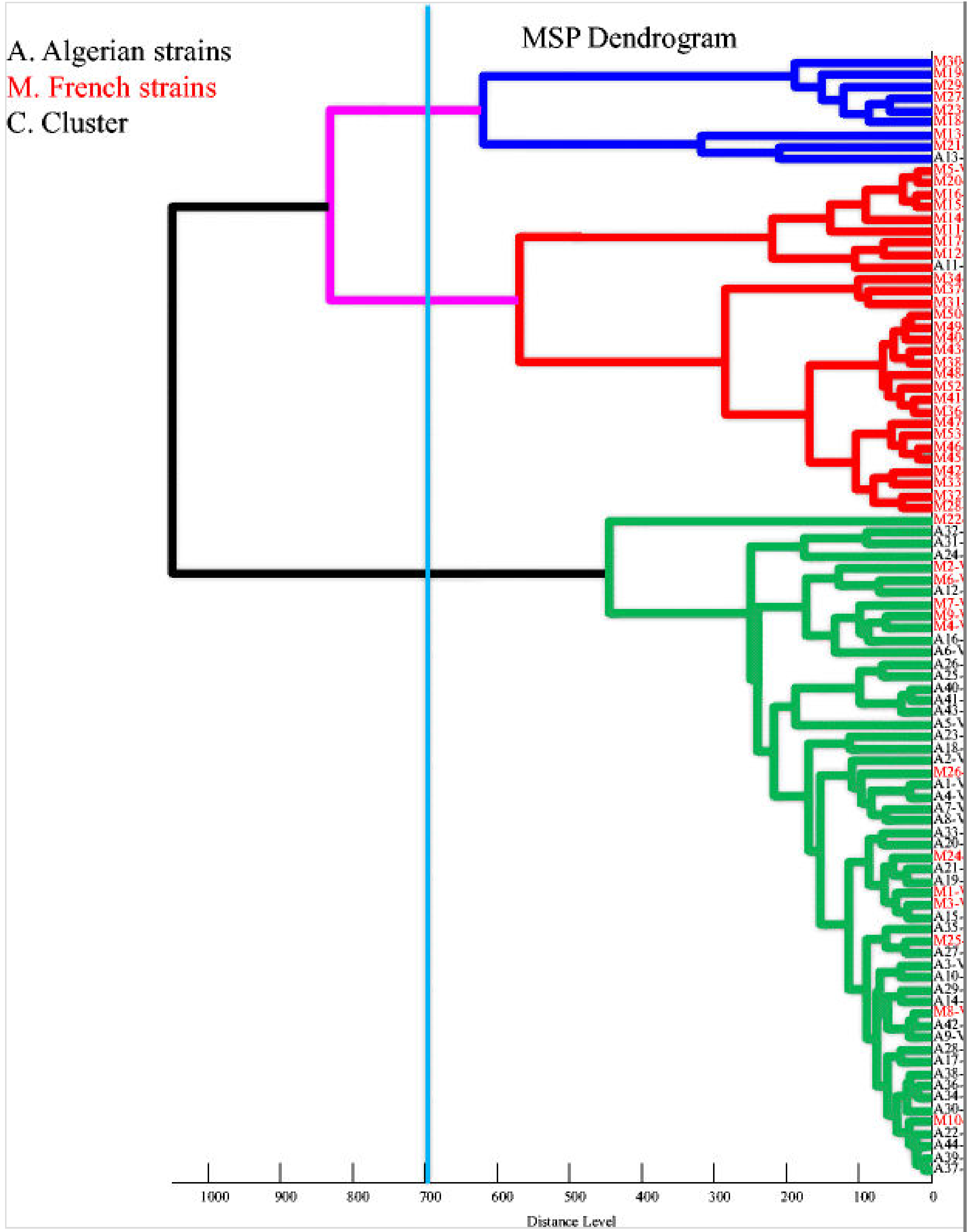
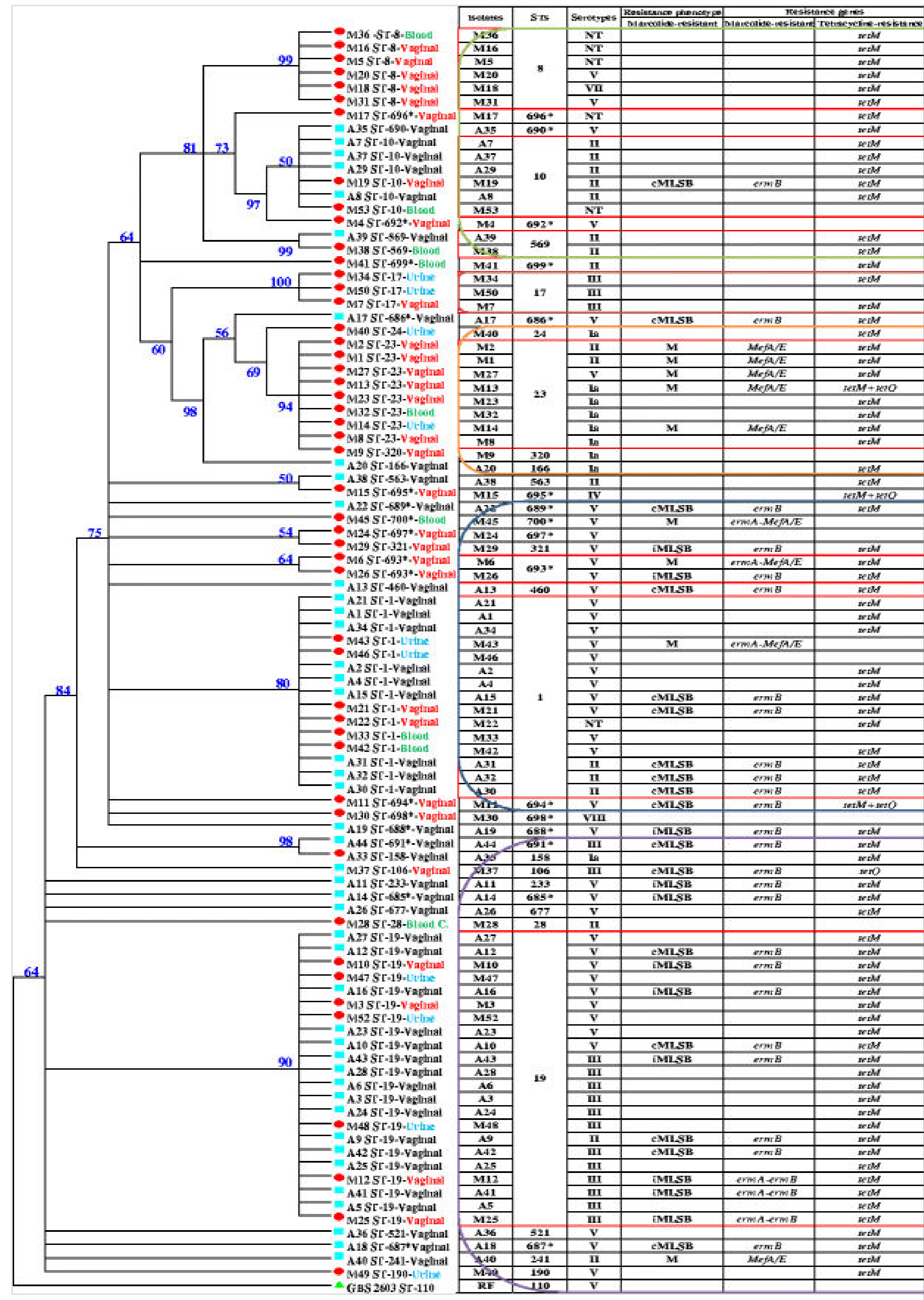


Fig. 2

Neighbor-joining tree of 37 STs constructed from multilocus sequence typing (MLST) analysis found using the MEGA5 program among 93 *S. agalactiae* isolates. The dendrogram shows genetic diversity and phenotypic characterization of macrolide and tetracycline *S. agalactiae* resistant isolates, genetic relationships among the different serotypes, resistance genes,

sequences type, and clonal complexes. *CC*, clonal complexes; *ST*, sequence type; *, new ST; *NT*, not typeable; *c/iMLSB*, constitutive/induced macrolides, lincosamides, and streptogramins B-type resistance; *ermA/B*, erythromycin ribosome methylase A/B; *M*, macrolides resistance phenotype; *Mef*, macrolide efflux; *RF*, reference strain



Isolates	STs	Serotypes	Resistance phenotype: Macrolide-resistant	Resistance genes: Macrolide-resistant	Resistance genes: Tetracycline-resistance
M36	8	NT			tetM
M16		NT			tetM
M5		NT			tetM
M20		V			tetM
M18		VII			tetM
M31		V			tetM
M17	696*	NT			tetM
A35	690*	V			tetM
A7	10	II			tetM
A37		II			tetM
A29		II			tetM
M19		II	cMLSB	ermB	tetM
A8		II			tetM
M53		NT			
M4	692*	V			
A39	569	II			tetM
M38		II			tetM
M41	699*	II			tetM
M34	17	III			tetM
M50		III			
M7		III			tetM
A17	686*	V	cMLSB	ermB	tetM
M40	24	Ia			tetM
M2	23	II	M	MefA/E	tetM
M1		II	M	MefA/E	tetM
M27		V	M	MefA/E	tetM
M13		Ia	M	MefA/E	tetM+tetO
M23		Ia			tetM
M32		Ia			tetM
M14		Ia	M	MefA/E	tetM
M8		Ia			tetM
M9	320	Ia			
A20	166	Ia			tetM
A38	563	II			tetM
M15	695*	IV			tetM+tetO
A22	689*	V	cMLSB	ermB	tetM
M45	700*	V	M	ermA-MefA/E	
M24	697*	V			
M29	321	V	iMLSB	ermB	tetM
M6	693*	V	M	ermA-MefA/E	tetM
M26		V	iMLSB	ermB	tetM
A13	460	V	cMLSB	ermB	tetM
A21	1	V			tetM
A1		V			tetM
A34		V			tetM
M43		V	M	ermA-MefA/E	
M46		V			
A2		V			tetM
A4		V			tetM
A15		V	cMLSB	ermB	tetM
M21		V	cMLSB	ermB	tetM
M22		NT			tetM
M33		V			
M42		V			tetM
A31		II	cMLSB	ermB	tetM
A32		II	cMLSB	ermB	tetM
A30		II	cMLSB	ermB	tetM
M11	694*	V	cMLSB	ermB	tetM+tetO
M30	698*	VIII			
A19	688*	V	iMLSB	ermB	tetM
A44	691*	III	cMLSB	ermB	tetM
A33	158	Ia			tetM
M37	106	III	cMLSB	ermB	tetO
A11	233	V	iMLSB	ermB	tetM
A14	685*	V	iMLSB	ermB	tetM
A26	677	V			tetM
M28	28	II			
A27	19	V			tetM
A12		V	cMLSB	ermB	tetM
M10		V	iMLSB	ermB	tetM
M47		V			tetM
A16		V	iMLSB	ermB	tetM
M3		V			tetM
M52		V			tetM
A23		V			tetM
A10		V	cMLSB	ermB	tetM
A43		III	iMLSB	ermB	tetM
A28		III			tetM
A6		III			tetM
A3		III			tetM
A24		III			tetM
M48		III			tetM
A9		II	cMLSB	ermB	tetM
A42		III	cMLSB	ermB	tetM
A25		III			tetM
M12		III	iMLSB	ermA-ermB	tetM
A41		III	iMLSB	ermA-ermB	tetM
A5		III			tetM
M25		III	iMLSB	ermA-ermB	tetM
A36	521	V			tetM
A18	687*	V	cMLSB	ermB	tetM
A40	241	II	M	MefA/E	tetM
M49	190	V			tetM
RF	110	V			

Antimicrobial susceptibility testing

Among the 93 clinical isolates, we found that they were all susceptible to

penicillin. However, we also found that 34 out of 74 (46 %) vaginal isolates (74/93) were resistant to erythromycin (20 out of 44 isolates in Guelma versus 14 out of 30 in Marseille, $p = 0.91$). Resistance to clindamycin was found in 37.8 %. All the resistance phenotypes detected are provided in Table 1 . For tetracycline, 100 % of the isolates from Guelma were resistant versus 86.6 % from Marseille (Table 1).

Table 1

Distribution of phenotypes and genotypes for erythromycin- and tetracycline-resistant isolates among Guelma, Algeria and Marseille, France

AQ1

Strains ($n = 93$)	**Phenotypes**		**Genotypes (n)**	
	Macrolide resistant (n)	**Tetracycline resistant (n)**	**Macrolide resistant (n)**	**Tetracycline resistant (n)**
Guelma ($n = 44$) Vaginal samples	$cMLS_B$ (13)	44	*ermB* (13)	*tetM* (44)
	$iMLS_B$ (6)		*ermA* + *ermB* (1)	
			ermB (5)	
	M (1)		*mefA/E* (1)	
Marseille ($n = 30$) Vaginal samples	$cMLS_B$ (4)	26	*ermB* (4)	*tetM* (22)
	$iMLS_B$ (5)		*ermA* + *ermB* (2)	*tetO* (1)
	M (5)		*ermB* (3)	*tetM* + *tetO* (3)
			mefA/E (4)	*ermA*+*mefA/E* (1)
Marseille ($n = 10$) Urine samples	M (2)	7	*mefA/E* (1)	*tetM* (7)
			ermA+*mefA/E* (1)	
Marseille ($n = 9$) Blood samples	M (1)	5	*ermA*+*mefA/E* (1)	*tetM* (5)
Total number	$n = 37$	$n = 82$	$n = 37$	$n = 82$

c/iMLS$_B$ constitutive/induced macrolides, lincosamides, and streptogramins B-type resistance, *ermA/B* erythromycin ribosome methylase A/B, *M* macrolides resistance phenotype, *mef* macrolide efflux, *tet* tetracycline, *n* total number

Macrolide resistance genotypes and tetracycline resistance determinants

Among 34 out of 74 isolates resistant to erythromycin, the most prevalent determinant of resistance was the *ermB* gene (73.5 %), and significantly more isolates carrying the *ermB* gene were isolated in Guelma ($n = 18$) compared to Marseille ($n = 7$; $p = 0.009$). Conversely, the *mefA*/*E* gene (14.7 %) was significantly more frequently detected in the isolates from Marseille ($n = 4$) compared to those from Guelma ($n = 1$; $p = 0.05$). An exception occurred for four strains that exhibited a combination of *ermA*/*ermB* genes (8.8 %), represented by one isolate in Guelma and two isolates in Marseille, and *ermA*/*mefA*/*E* genes (3 %), represented only by one isolate in Marseille. All *ermA*, *ermB*, and *mefA*/*E* gene-positive isolates expressed iMLS$_B$, cMLS$_B$, and the M resistance phenotypes, respectively, as shown in Table 1 . The *tetM* gene was detected in 100 % of the isolates from Guelma and 84.6 % of the isolates from Marseille; *tetO* was found in only 3.8 % of the isolates, and a co-occurrence of both *tetM* and *tetO* was only found in 11.6 % of the Marseille isolates. In contrast, *tetK* and *tetL* were not detected in our isolates (Tables 1 and 2).

Table 2

Correlation between serotypes, phenotypes, resistance genes profiles, ar desorption/ionization time-of-flight mass spectrometry (MLST) analysis (STs) of gro strains from Guelma, Algeria and Marseille, France

Serotypes (*n*)	Origin	Source and number of isolates	Phenotypes (*n*)	Resistance genes		
				Macrolide resistant (*n*)	Tetracycline resistant (*n*)	
Ia (9)	A	Vagina (2)			*tetM* (2)	ST-1:
	F	Vagina (4)	M (2)	*mefA*/*E* (2)	*tetM* (5)	5(ST-
		Urine (2)			*tetM* + *tetO* (1)	
		Blood (1)				
	A	Vagina (11)	cMLS$_B$ (4) M (1)	*ermB* (4) *mefA*/*E* (1)	*tetM* (11)	4(ST- ST-1! 569

II (17)	F	Vagina (3)	cMLS$_B$ (1)	*ermB* (1)	*tetM* (5)	2(ST- 569 ST-69
		Blood (3)	M (2)	*mefA*/*E* (2)		
III (17)	A	Vagina (10)	cMLS$_B$ (2) iMLS$_B$ (2)	*ermB* (2) *ermA* + *ermB* (1) *ermB* (1)	*tetM* (10)	9(ST-
	F	Vagina (4) Urine (3)	cMLS$_B$ (1) iMLS$_B$ (2)	*ermB* (1) *ermA* + *ermB* (2)	*tetM* (5) *tetO* (1)	3(ST-
IV (1)	F	Vagina (1)			*tetM* + *tetO* (1)	ST-69
V (41)	A	Vagina (21)	cMLS$_B$ (7) iMLS$_B$ (4)	*ermB* (7) *ermB* (4)	*tetM* (21)	6(ST- ST-2 677 (ST- 685,6
	F	Vagina (12) Urine (5) Blood (3)	cMLS$_B$ (2) iMLS$_B$ (3) M (4)	*ermB* (2) *ermB* (3) *mefA*/*E* (1) *ermA* + *mefA*/*E* (3)	*tetM* (13) *tetM* + *tetO* (1)	5(ST- ST-2 (2(ST 693),
VII (1)	F	Vagina (1)			*tetM* (1)	ST-8
VIII (1)	F	Vagina (1)				698[N
NT (6)	F	Vagina (4) Blood (2)			*tetM* (5)	3(ST- 696[N

c/iMLS$_B$ constitutive/induced macrolides, lincosamides, and streptogramins B-type erythromycin ribosome methylase A/B, *M* macrolides resistance phenotype, *mef* m tetracycline, *NT* non-typeable, *STs* sequences type, [NST] new sequence type, *A* Alge

Serotype identification

The isolates studied represented seven capsular types. The most represented serotypes isolated in Guelma and Marseille were type V (44.6 %), followed by serotypes II and III (19 % both) and serotype Ia (8 %). Moreover, serotypes IV, VII, and VIII (1.3 % each) were found only

in Marseille isolates. Finally, 5.4 % of the isolates from Marseille were non-typeable ($p = 0.005$) compared to Guelma, as shown in Table 2 and Fig. 2.

Multilocus sequence typing (MLST)

The MLST results of the *S. agalactiae* isolates were analyzed and presented in a phylogenetic tree; isolates demonstrated the existence of different genetic lineages (Fig. 2). A total of 37 individual STs were identified (Fig. 2). Moreover, 16 novel STs were detected: seven in Guelma and nine in Marseille. These new STs were entered in the *S. agalactiae* MLST database (STs 685 to 700). Thirty-one of the STs were clustered into five CCs, and six were singleton STs (Fig. 2). Among these, 93.5 % (87/93) of the isolates were found within five CCs; CC1, CC10, CC17, CC19, and CC23; 6.5 % (6/93) of the isolates identified were not part of a cluster. The most prevalent of these complexes was CC19 (including STs: 19, 28, 106, 158, 190, 241, 233, 521, 677, 685*, 687*, 691*; 35.5 %), followed by CC1 (including STs: 1, 321, 460, 689*, 693*, 694*, 697*, 700*; 24.7 %), which regrouped all vaginal isolates from Guelma and Marseille, then CC10 (18.3 %), CC23 (11.8 %), and CC17 (3.2 %), which were more common among the Marseille isolates (Fig. 2).

Correlation between phenotype, genotype, serotype, and MLST analysis

Our results showed that the $cMLS_B$ phenotype was significantly more common among the Guelma isolates as compared to the Marseille isolates ($p = 0.03$) and carried the *ermB* gene ($p = 0.009$), whereas the M phenotype was associated with the Marseille isolates ($p = 0.02$), expressed the *mefA*/*E* gene, and belonged to ST-23 ($p = 10^{-7}$) (Fig. 2). The MLST analysis showed that 82.6 % of serotype V GBS clustered into CC1 (ST-1/V, $p = 0.012$), 72.7 % of serotype Ia GBS clustered into CC23 (ST-23/Ia, $p = 10^{-7}$), 100 % of serotype III GBS clustered into CC17 (ST-17, $p = 10^{-4}$), and 70.6 % of serotype II and the non-typeable capsular serotype clustered into CC10 (ST-10/II, $p = 2.10^{-5}$ and ST-8/NT, $p = 7.10^{-6}$). In contrast, 45.4 and 42.4 %, respectively, of serotype V and serotype III isolates clustered into CC19 (ST-19/V, $p = 0.7312$ and ST-19/III, $p = 4.10^{-7}$) (Table 2 and Fig. 2).

Discussion

This report presents, for the first time, a comprehensive molecular analysis of GBS isolates circulating in Guelma, Algeria and Marseille, France. The MSP dendrogram clustering of isolates using MALDI-TOF MS [27] showed significant clusters according to the geographical source. Such grouping of isolates has recently been reported for *Klebsiella pneumoniae* isolates [22]. Moreover, Lartigue et al. report that MALDI-TOF MS analysis was also able to identify strains belonging to the highly virulent ST-17 clone or to the emerging ST-1 clone [23]. However, this was not true in our hands, as four isolates outside these two STs harbored these peaks.

One of the main objectives of this investigation was to determine the genetic basis of antibiotic resistance. In this study, all strains remained uniformly susceptible to penicillin [5, 15, 34]. However, the overall rate of erythromycin resistance among our isolates analyzed was 40 % (45.4 and 34.7 % in Guelma and Marseille, respectively). Such a level of resistance has been reported in Taiwan (44 %), Tunisia (40 %), Morocco (38.5 %), Switzerland (30 %), France (20.2–35.3 %), the USA (32–54 %) [12, 14, 16, 35–38], and extremely high in China (85.7 %) [39]. Due to this high level of resistance, the CDC guidelines no longer recommend erythromycin [40]. The increasing emergence of resistance to macrolides among GBS is a therapeutic problem among patients allergic to β-lactams. This observation emphasizes the need for the continuous monitoring of antimicrobial susceptibility profiles.

In our study, there was a predominance of the $cMLS_B$ phenotype in Guelma isolates mediated by the *ermB* gene, whereas the M phenotype was more common in Marseille isolates, which carried the *mefA*/*E* genes. A predominance of the MLS_B phenotype has been reported in Australia, Switzerland, and Tunisia [8, 14, 16], whereas in Brazil and Italy, the $cMLS_B$ and M phenotypes were detected with equal frequencies [13, 19]. In France (Paris), the $iMLS_B$ phenotype was more common in 2001, yet $cMLS_B$ was more dominant from 2007 to 2010 [38, 41]. Interestingly, we detected a coexistence of *ermA*/*ermB* and *ermA*/*mefA*/*E* genes in $iMLS_B$ and M phenotypes, respectively. The co-occurrence of both genes has been documented previously [10, 42].

We also report a high rate of tetracycline resistance in our study (82.2 %), as already described in Tunisia (97.3 %), France (94 %), Malaysia

(71.8 %), and Italy (69.9 %) [5, 7, 16, 43]. Moreover, according to our results, the *tetM* gene has spread throughout all strains. Thus, we also observed that the majority of isolates carrying the *ermB* gene also harbored the *tetM* gene (96.4 %). The contemporary presence of both genes was previously described by Gherardi et al. [15]. Seroprevalence studies are an important measure for determining the incidence and proportion of serotypes that are circulating in a given population [44].

Among the 93 GBS strains studied, all capsular serotypes except VI and IX were found. Our data show that serotype V was the predominant one among isolates (44.1 %), as also reported in Kuwait [45] and Japan [46]. However, other studies showed a predominance of other serotypes, such as serotype III in Morocco and France, serotype IV in the United Arab Emirates, serotype Ia in Brazil, and serotypes VI–VIII in Japan [2, 19, 34, 47]. Furthermore, global serotyping distribution studies have shown that the serotype distribution of GBS varies both geographically and over time [15, 44]. All serotypes (I, III, and V) are frequently associated worldwide with GBS infections [44, 48–50]. The proportion of NT strains showed higher percentages (5.4 %) among Marseille isolates, which could be the result of acquisition of an uncharacterized capsule gene cluster or mutations in capsule genes [44, 51]. Usually, erythromycin-resistant isolates were more frequently found in serotype V [2, 52]. However, in our study, we did not find this association [13], though an association between ST-23 and the M phenotype was established [47].

The population structures of GBS exhibit a remarkable clonal population, with large differences within groups of clones. This is the first report of MLST analysis in GBS strains circulating in Algeria. In this study, 37 STs were identified. The main STs identified in this study have also been observed as major STs for strains isolated among large collections during infectious diseases [19, 47, 53]. Despite this high genetic diversity, all STs found were grouped into five CCs, i.e., CC1, CC10, CC23, CC17, and CC19, which have been previously identified worldwide [54, 55]. The most prevalent of these CCs was CC19, followed by CC1. In addition, the main STs included in these two CCs, such as ST-19 and ST-1, were over-represented among carriage isolates of GBS [20]. Such diverse clonal populations have also been found in other countries, including Italy, Poland, France, the USA, and Senegal [15, 47, 54–56]. Therefore, GBS from these CCs have been shown to cause the majority of both neonatal

and adult GBS infections [54, 55]. The diversity of the genetic lineages between countries suggests that most diseases combined with GBS are caused by certain clonal lineages [6].

In conclusion, the data obtained in this study shed new light on the need for a more rigorous characterization and detection of correlations among serotypes, resistance genes, and clonal clusters of GBS isolates circulating in the study areas. Comparative genetic studies of *S. agalactiae* will be essential to perform epidemiological comparisons between countries and the evolution of isolates, as well as for vaccine development. Finally, as erythromycin resistance rates in GBS have increased, local antibiotic resistance surveillance is advisable in guiding empirical antibiotic therapy to prevent the development of such infections. Further epidemiological studies in other cities in France and in other Algerian cities are needed to support our findings.

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References

1. Wang NY, Patras KA, Seo HS, Cavaco CK, Rösler B, Neely MN, Sullam PM, Doran KS (2014) Group B streptococcal serine-rich repeat proteins promote interaction with fibrinogen and vaginal colonization. J Infect Dis 210(6):982–991

2. Otaguiri ES, Morguette AE, Tavares ER, dos Santos PM, Morey AT, Cardoso JD, Perugini MR, Yamauchi LM, Yamada-Ogatta SF (2013) Commensal *Streptococcus agalactiae* isolated from patients seen at University Hospital of Londrina, Paraná, Brazil: capsular types, genotyping, antimicrobial susceptibility and virulence determinants. BMC Microbiol 13:297

3. Mukhopadhyay S, Eichenwald EC, Puopolo KM (2013) Neonatal early-onset sepsis evaluations among well-appearing infants: projected impact of changes in CDC GBS guidelines. J Perinatol 33(3):198–205

4. Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, Harrison LH, Lynfield R, Mohle-Boetani J, Zansky S, Albanese BA, Stefonek K, Zell ER, Jackson D, Thompson T, Schrag SJ (2009) Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. Clin Infect Dis 49(1):85–92

5. Eskandarian N, Ismail Z, Neela V, van Belkum A, Desa MN, Amin Nordin S (2015) Antimicrobial susceptibility profiles, serotype distribution and virulence determinants among invasive, non-invasive and colonizing *Streptococcus agalactiae* (group B streptococcus) from Malaysian patients. Eur J Clin Microbiol Infect Dis 34(3):579–584

6. Tien N, Ho CM, Lin HJ, Shih MC, Ho MW, Lin HC, Lin HS, Chang CC, Lu JJ (2011) Multilocus sequence typing of invasive group B *Streptococcus* in central area of Taiwan. J Microbiol Immunol Infect 44(6):430–434

7. Poyart C, Jardy L, Quesne G, Berche P, Trieu-Cuot P (2003) Genetic basis of antibiotic resistance in *Streptococcus agalactiae* strains isolated in a French hospital. Antimicrob Agents Chemother 47(2):794–797

8. Garland SM, Cottrill E, Markowski L, Pearce C, Clifford V, Ndisang D, Kelly N, Daley AJ; Australasian Group for Antimicrobial Resistance-GBS Resistance Study Group (2011) Antimicrobial resistance in group B streptococcus: the Australian experience. J Med Microbiol 60(Pt 2):230–235

9. Buter CC, Mouton JW, Klaassen CH, Handgraaf CM, Sunnen S, Melchers WJ, Sturm PD (2010) Prevalence and molecular mechanism of macrolide resistance in beta-haemolytic streptococci in The Netherlands. Int J Antimicrob Agents 35(6):590–592

10. Culebras E, Rodriguez-Avial I, Betriu C, Redondo M, Picazo JJ (2002) Macrolide and tetracycline resistance and molecular relationships of clinical strains of *Streptococcus agalactiae*. Antimicrob

Agents Chemother 46(5):1574–1576

11. DiPersio LP, DiPersio JR, Beach JA, Loudon AM, Fuchs AM (2011) Identification and characterization of plasmid-borne *erm*(T) macrolide resistance in group B and group A *Streptococcus*. Diagn Microbiol Infect Dis 71(3):217–223

12. DiPersio LP, DiPersio JR (2006) High rates of erythromycin and clindamycin resistance among OBGYN isolates of group B *Streptococcus*. Diagn Microbiol Infect Dis 54(1):79–82

13. Pinto TC, Costa NS, Vianna Souza AR, Silva LG, Corrêa AB, Fernandes FG, Oliveira IC, Mattos MC, Rosado AS, Benchetrit LC (2013) Distribution of serotypes and evaluation of antimicrobial susceptibility among human and bovine *Streptococcus agalactiae* strains isolated in Brazil between 1980 and 2006. Braz J Infect Dis 17(2):131–136

14. Capanna F, Emonet SP, Cherkaoui A, Irion O, Schrenzel J, Martinez de Tejada B (2013) Antibiotic resistance patterns among group B *Streptococcus* isolates: implications for antibiotic prophylaxis for early-onset neonatal sepsis. Swiss Med Wkly 143:w13778

15. Gherardi G, Imperi M, Baldassarri L, Pataracchia M, Alfarone G, Recchia S, Orefici G, Dicuonzo G, Creti R (2007) Molecular epidemiology and distribution of serotypes, surface proteins, and antibiotic resistance among group B streptococci in Italy. J Clin Microbiol 45(9):2909–2916

16. Hraoui M, Boutiba-Ben Boubaker I, Rachdi M, Slim A, Ben Redjeb S (2012) Macrolide and tetracycline resistance in clinical strains of *Streptococcus agalactiae* isolated in Tunisia. J Med Microbiol 61(Pt 8):1109–1113

17. Imperi M, Pataracchia M, Alfarone G, Baldassarri L, Orefici G, Creti R (2010) A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*. J Microbiol Methods 80(2):212–214

18. Lemire P, Houde M, Lecours MP, Fittipaldi N, Segura M (2012) Role of capsular polysaccharide in Group B *Streptococccus* interactions with dendritic cells. Microbes Infect 14(12):1064–1076

19. De Francesco MA, Caracciolo S, Gargiulo F, Manca N (2012) Phenotypes, genotypes, serotypes and molecular epidemiology of erythromycin-resistant *Streptococcus agalactiae* in Italy. Eur J Clin Microbiol Infect Dis 31(8):1741–1747

20. Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F, Glaser P, Rusniok C, Crook DW, Harding RM, Bisharat N, Spratt BG (2003) Multilocus sequence typing system for group B streptococcus. J Clin Microbiol 41(6):2530–2536

21. Lanotte P, Perivier M, Haguenoer E, Mereghetti L, Burucoa C, Claverol S, Atanassov C (2013) Proteomic biomarkers associated with *Streptococcus agalactiae* invasive genogroups. PLoS One 8(1):e54393

22. Berrazeg M, Diene SM, Drissi M, Kempf M, Richet H, Landraud L, Rolain JM (2013) Biotyping of multidrug-resistant *Klebsiella pneumoniae* clinical isolates from France and Algeria using MALDI-TOF MS. PLoS One 8(4):e61428

23. Lartigue MF, Kostrzewa M, Salloum M, Haguenoer E, Héry-Arnaud G, Domelier AS, Stumpf S, Quentin R (2011) Rapid detection of “highly virulent” Group B *Streptococcus* ST-17 and emerging ST-1 clones by MALDI-TOF mass spectrometry. J Microbiol Methods 86(2):262–265

24. Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunel JM, Drissi M, Mesli E, Touati A, Rolain JM (2012) Rapid detection of carbapenem resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One 7(2):e31676

25. Ait AA, Hamidechi AM (2003) Incidence of group b streptococcus (GBS) in neonates born in Constantine (Algeria) and two of its suburbs usefullness of serotyping. Med Mal Infect 33(8):417–421

26. Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, Fournier PE, Drancourt M, La Scola B, Raoult D (2013) Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 51(7):2182–2194

27. Lartigue MF, Héry-Arnaud G, Haguenoer E, Domelier AS, Schmit PO, van der Mee-Marquet N, Lanotte P, Mereghetti L, Kostrzewa M, Quentin R (2009) Identification of *Streptococcus agalactiae* isolates from various phylogenetic lineages by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 47(7):2284–2287

28. Malhotra-Kumar S, Lammens C, Piessens J, Goossens H (2005) Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. Antimicrob Agents Chemother 49(11):4798–4800

29. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L (1996) Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother 40(11):2562–2566

30. Belbel Z, Chettibi H, Dekhil M, Ladjama A, Nedjai S, Rolain JM (2014) Outbreak of an *armA* methyltransferase-producing ST39 *Klebsiella pneumoniae* clone in a pediatric Algerian Hospital. Microb Drug Resist 20(4):310–315

31. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain JM (2014) ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58(1):212–220

32. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

33. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG (2004) eBURST: inferring patterns of evolutionary descent among clusters of

related bacterial genotypes from multilocus sequence typing data. J Bacteriol 186(5):1518–1530

34. Aitmhand R, Moustaoui N, Belabbes H, Elmdaghri N, Benbachir M (2000) Serotypes and antimicrobial susceptibility of group B streptococcus isolated from neonates in Casablanca. Scand J Infect Dis 32(3):339–340

35. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, Craig AS, Schaffner W, Zansky SM, Gershman K, Stefonek KR, Albanese BA, Zell ER, Schuchat A, Schrag SJ; Active Bacterial Core surveillance/Emerging Infections Program Network (2008) Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA 299(17):2056–2065

36. Sahnoun O, Ben Abdallah H, Noomen S, Ben Elhadj Khélifa A, Mastouri M (2007) Antimicrobial susceptibility of *Streptococcus agalactiae* strains in Monastir. Med Mal Infect 37(11):734–737

37. Janapatla RP, Ho YR, Yan JJ, Wu HM, Wu JJ (2008) The prevalence of erythromycin resistance in group B streptococcal isolates at a University Hospital in Taiwan. Microb Drug Resist 14(4):293–297

38. Tazi A, Morand PC, Réglier-Poupet H, Dmytruk N, Billoët A, Antona D, Trieu-Cuot P, Poyart C (2011) Invasive group B streptococcal infections in adults, France (2007–2010). Clin Microbiol Infect 17(10):1587–1589

39. Wang P, Tong JJ, Ma XH, Song FL, Fan L, Guo CM, Shi W, Yu SJ, Yao KH, Yang YH (2015) Serotypes, antibiotic susceptibilities, and multi-locus sequence type profiles of *Streptococcus agalactiae* isolates circulating in Beijing, China. PLoS One 10(3):e0120035

40. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC) (2010) Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recomm Rep 59(RR-10):1–36

41. Fitoussi F, Loukil C, Gros I, Clermont O, Mariani P, Bonacorsi S, Le Thomas I, Deforche D, Bingen E (2001) Mechanisms of macrolide resistance in clinical group B streptococci isolated in France. Antimicrob Agents Chemother 45(6):1889–1891

42. Betriu C, Culebras E, Gómez M, Rodríguez-Avial I, Sánchez BA, Agreda MC, Picazo JJ (2003) Erythromycin and clindamycin resistance and telithromycin susceptibility in *Streptococcus agalactiae*. Antimicrob Agents Chemother 47(3):1112–1114

43. Lambiase A, Agangi A, Del Pezzo M, Quaglia F, Testa A, Rossano F, Martinelli P, Catania MR (2012) In vitro resistance to macrolides and clindamycin by Group B *Streptococcus* isolated from pregnant and nonpregnant women. Infect Dis Obstet Gynecol 2012:913603

44. Ippolito DL, James WA, Tinnemore D, Huang RR, Dehart MJ, Williams J, Wingerd MA, Demons ST (2010) Group B streptococcus serotype prevalence in reproductive-age women at a tertiary care military medical center relative to global serotype distribution. BMC Infect Dis 10:336

45. Boswihi SS, Udo EE, Al-Sweih N (2012) Serotypes and antibiotic resistance in Group B streptococcus isolated from patients at the Maternity Hospital, Kuwait. J Med Microbiol 61(Pt 1):126–131

46. Ueno H, Yamamoto Y, Yamamichi A, Kikuchi K, Kobori S, Miyazaki M (2012) Characterization of group B streptococcus isolated from women in Saitama city, Japan. Jpn J Infect Dis 65(6):516–521

47. van der Mee-Marquet N, Fourny L, Arnault L, Domelier AS, Salloum M, Lartigue MF, Quentin R (2008) Molecular characterization of human-colonizing *Streptococcus agalactiae* strains isolated from throat, skin, anal margin, and genital body sites. J Clin Microbiol 46(9):2906–2911

48. Abat C, Chaudet H, Raoult D, Colson P (2014) Increasing trend of invasive group B streptococcal infections, Marseille, France. Clin Infect Dis 58(5):750–751

49. Martins ER, Andreu A, Correia P, Juncosa T, Bosch J, Ramirez M, Melo-Cristino J; Microbiologist Group for the Study of Vertical Transmission Infections from the Catalan Society for Clinical Microbiology and Infectious Diseases (2011) Group B streptococci causing neonatal infections in barcelona are a stable clonal population: 18-year surveillance. J Clin Microbiol 49(8):2911–2918

50. Martins ER, Melo-Cristino J, Ramirez M; Portuguese Group for the Study of Streptococcal Infections (2012) Dominance of serotype Ia among group B Streptococci causing invasive infections in nonpregnant adults in Portugal. J Clin Microbiol 50(4):1219–1227

51. Ramaswamy SV, Ferrieri P, Flores AE, Paoletti LC (2006) Molecular characterization of nontypeable group B streptococcus. J Clin Microbiol 44(7):2398–2403

52. von Both U, Ruess M, Mueller U, Fluegge K, Sander A, Berner R (2003) A serotype V clone is predominant among erythromycin-resistant *Streptococcus agalactiae* isolates in a southwestern region of Germany. J Clin Microbiol 41(5):2166–2169

53. Manning SD, Springman AC, Lehotzky E, Lewis MA, Whittam TS, Davies HD (2009) Multilocus sequence types associated with neonatal group B streptococcal sepsis and meningitis in Canada. J Clin Microbiol 47(4):1143–1148

54. Sadowy E, Matynia B, Hryniewicz W (2010) Population structure, virulence factors and resistance determinants of invasive, non-invasive and colonizing *Streptococcus agalactiae* in Poland. J Antimicrob Chemother 65(9):1907–1914

55. Bohnsack JF, Whiting A, Gottschalk M, Dunn DM, Weiss R, Azimi PH, Philips JB 3rd, Weisman LE, Rhoads GG, Lin FY (2008) Population structure of invasive and colonizing strains of *Streptococcus agalactiae* from neonates of six U.S. Academic Centers from 1995 to 1999. J Clin Microbiol 46(4):1285–1291

56. Brochet M, Couvé E, Bercion R, Sire JM, Glaser P (2009) Population structure of human isolates of *Streptococcus agalactiae*

from Dakar and Bangui. J Clin Microbiol 47(3):800–803

References

(1) Thacker SB, Berkelman RL. Public health surveillance in the United States. Epidemiol Rev 1988;10:164-90.

(2) Graunt J. Natural and Political Observations made upon the bills of mortality. London: Roycroft and Dica.1652.

(3) Wagner, M. M., Moore, A. W., and Aryel, R. M. Handbook of Biosurveillance. USA: Academic Press. 2006.

(4) Koski E. Clinical laboratory data for biosurveillance. In: Zeng D, Chen H, Castillo-Chavez C, and Thurmond M, editors. Clinical laboratory data for biosurveillance. Springer. 2011. pp. 67-87.

(5) Cazein F, Le Strat Y, Sarr A, Ramus C, Bouche N, Le Vu S, et al. Dépistage de l'infection par le VIH en France, 2003-2013. Bull Epidémiol Hebd 2014;32-33:534-40.

(6) Heraud-Bousquet V, Lot F, Esvan M, Cazein F, Laurent C, Warszawski J, et al. A three-source capture-recapture estimate of the number of new HIV diagnoses in children in France from 2003-2006 with multiple imputation of a variable of heterogeneous catchability. BMC Infect Dis 2012;12:251.

(7) Cole MJ, Unemo M, Hoffmann S, Chisholm SA, Ison CA, van de Laar MJ. The European gonococcal antimicrobial surveillance programme, 2009. Euro Surveill 2011;16(42).

(8) Sala Soler M, Fouillet A, Viso AC, Josseran L, Smith GE, Elliot AJ, et al. Assessment of syndromic surveillance in Europe. Lancet 2011 Nov 26;378(9806):1833-4.

(9) Chen H, Zeng D, and Yan P. Infectious Disease Informatics: Syndromic Surveillance for Public Health and Bio-Defense. New York: Springer; 2010.

(10) Babin S, Magruder S, Hakre S, et al. Part I: System Design and Implementation. In: Lombardo JS and Buckeridge DL, editors. Disease Surveillance: A Public Health Informatics Approach. Hoboken: John Wiley & Sons; 2006. pp. 43-91.

(11) M'ikanatha NO, Lynfield R, Van Beneden CA, et al. Infectious Disease Surveillance. 2nd ed. Wiley-Blackwell; 2013.

(12) Castillo-Salgado C. Trends and directions of global public health surveillance. Epidemiol Rev 2010 Apr;32(1):93-109.

(13) Wagner MM, Tsui FC, Espino J, Hogan W, Hutman J, Hersh J, et al. National Retail Data Monitor for public health surveillance. MMWR Morb Mortal Wkly Rep 2004 Sep 24;53 Suppl:40-2.

(14) Lombardo J, Burkom H, Elbert E, Magruder S, Lewis SH, Loschen W, et al. A systems overview of the Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE II). J Urban Health 2003 Jun;80(2 Suppl 1):i32-i42.

(15) Lewis SL, Feighner BH, Loschen WA, Wojcik RA, Skora JF, Coberly JS, et al. SAGES: a suite of freely-available software tools for electronic disease surveillance in resource-limited settings. PLoS One 2011;6(5):e19750.

(16) Lombardo JS, Burkom H, Pavlin J. ESSENCE II and the framework for evaluating syndromic surveillance systems. MMWR Morb Mortal Wkly Rep 2004 Sep 24;53 Suppl:159-65.

(17) Meynard JB, Chaudet H, Texier G, Queyriaux B, Deparis X, Boutin JP. [Real time epidemiological surveillance within the armed forces: concepts, realities and prospects in France]. Rev Epidemiol Sante Publique 2008 Feb;56(1):11-20.

(18) Lamagni TL, Keshishian C, Efstratiou A, Guy R, Henderson KL, Broughton K, et al. Emerging trends in the epidemiology of invasive group B streptococcal disease in England and Wales, 1991-2010. Clin Infect Dis 2013 Sep;57(5):682-8.

(19) Alam MT, Read TD, Petit RA, III, Boyle-Vavra S, Miller LG, Eells SJ, et al. Transmission and microevolution of USA300 MRSA in U.S. households: evidence from whole-genome sequencing. MBio 2015;6(2):e00054.

(20) Frank C, Werber D, Cramer JP, Askar M, Faber M, an der HM, et al. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. N Engl J Med 2011 Nov 10;365(19):1771-80.

(21) Scheutz F, Nielsen EM, Frimodt-Moller J, Boisen N, Morabito S, Tozzoli R, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill 2011;16(24).