AIX-MARSEILLE UNIVERSITE

FACULTE DE MEDECINE DE MARSEILLE

ECOLE DOCTORALE DES SCIENCES DE LA VIE ET DE LA SANTE

THESE DE DOCTORAT

Présentée par

Van Thuan HOANG

EPIDEMIOLOGIE DES INFECTIONS RESPIRATOIRES ET GASTROINTESTINALES CHEZ LES VOYAGEURS INTERNATIONAUX DANS LE CONTEXTE DE RASSEMBLEMENT DE MASSE

Soutenue le 01 Juillet 2021

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

Spécialité : Biologie de santé, spécialité Maladies infectieuses

Membres du Jury :

Mme. La Professeure Florence FENOLLAR	Présidente du Jury
M. Le Docteur Philippe GAUTRET	Directeur de thèse
M. Le Docteur Vincent POMMIER DE SANTI	Co-directeur de thèse
Mme. La Professeure Marie KEMPF	Rapporteure
M. Le Professeur Michel CARLES	Rapporteur
M. Le Docteur Paul-Henry CONSIGNY	Examinateur
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Laboratoire d'accueil :

Vecteurs-Infections Tropicales et Méditerranéennes (VITROME), Institut Hospitalo-Universitaire Méditerranée Infection, IRD, APHM, Aix-Marseille Université

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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.

Prof. Didier RAOULT

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A mes chers parents, mes frères et mes sœurs

A ma chérie Dao Loi

A mes petites

TABLE DES MATIERES

RESUME

Les voyages internationaux se sont beaucoup développés au cours des cinq dernières décennies. Les maladies tropicales bien connues telles que le paludisme, la dengue ou la fièvre typhoïde continuent d'être observées chez les voyageurs. Les maladies infectieuses émergentes et les épidémies augmentent encore plus les risques infectieux lors de voyages à l'étranger. Les voyageurs internationaux participant à des rassemblements de masse sont particulièrement exposés aux risques infectieux. Au cours des 10 dernières années, les inquiétudes se sont accrues en ce qui concerne les menaces sur la sécurité sanitaire mondiale posée par plusieurs maladies infectieuses émergentes et ré-émergentes, en lien avec les rassemblements de masse. Notre revue de littérature a montré que les maladies infectieuses liées aux rassemblements de masse étaient dominées par les infections des voies respiratoires et gastro-intestinales. Des épidémies de méningite bactériennes ont également été rapportées. La transmission interhumaine par voie aérogène des agents pathogènes respiratoires est favorisée par la concentration temporelle et spatiale des personnes. Dans ce travail, nous étudions l'épidémiologie des infections respiratoires et gastro-intestinales chez les pèlerins de Marseille participant au Hajj, ou pèlerinage à La Mecque en Arabie Saoudite et les pèlerins Sénégalais participant au Grand Magal de Touba.

Les participants de notre étude ont été invités à répondre aux deux questionnaires standardisés : (1) – un questionnaire avant leur départ, afin de collecter les données socio-démographiques et les comorbidités préexistantes et (2) – un questionnaire au retour du pèlerinage, afin de recueillir des indicateurs sur les mesures de prévention et les infections apparues pendant leur voyage. Des prélèvements par écouvillonnage nasal, pharyngé et rectal ont été systématiquement réalisés avant et après le voyage. Les principaux pathogènes respiratoires et gastro-intestinaux ont été recherchés par technique de biologie moléculaire (polymérase chain reaction (PCR) en temps réel). La présence de bactéries multi-résistantes aux antibiotiques et porteuses de gènes de résistance à la colistine ont été recherchés par culture et séquençage du génome. Les facteurs de risques associés aux infections respiratoires et gastro-intestinales chez les pèlerins ont été recherchés par l'analyse statistique.

Le Hajj, est l'un des plus grands rassemblements de masse religieux annuel du monde, avec plus de 2 millions de personnes venant de plus de 184 pays, dont environ 2000 de Marseille. Notre étude prospective chez les pèlerins du Hajj marseillais, de 2012 à 2018, a montré que l'âge moyen variait de 57,3 à 61,7 ans. De 81,2% à 93,4% des pèlerins ont présenté des symptômes respiratoires au cours de leur séjour. L'étude du portage de pathogènes respiratoires avant, pendant et après le

séjour montrait une acquisition significative en lien avec le pèlerinage. Les virus les plus fréquemment acquises étaient les rhinovirus (27,7%), coronavirus communs (8,3%) et influenza virus (3,7%). Les bactéries les plus fréquemment acquises étaient *Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae* et *Klebsiella pneumoniae*. Nous avons aussi observé une dynamique spécifique du portage de chaque pathogène au cours du séjour en Arabie Saoudite. En étudiant les interactions entre les agents pathogènes, nous avons observé que le portage du rhinovirus et le portage de *S. aureus* était statiquement associé. Une co-infection comparable a été aussi retrouvé avec *H. influenzae* et *Moraxella catarrhalis*. Les génotypes d'*H. influenzae* acquis différaient de ceux présents avant le Hajj, en faveur donc d'un remplacement de la flore au cours des séjours. Nous avons observé une grande biodiversité et une absence de clonalité d'*H. influenzae* parmi les pèlerins français. De plus, la prévalence du portage d'*H. influenzae* avant le Hajj montrait des variations importantes, de 0,9% à 52,8%, selon les années entre 2014 et 2018. Cependant, la prévalence du portage post-Hajj était constamment élevée, variant de 41,0 à 67,8%. Cela suggère que la circulation d'*H. influenzae* au Hajj était permanente au cours de la période d'étude et pose la question de l'intérêt éventuel de la vaccination. Enfin, nous avons observé des liens statiquement significatifs entre le portage de certains agents pathogènes, la présence de pathologies chroniques, des caractéristiques démographiques, de l'application de mesures préventives, y compris la vaccination contre la grippe et la symptomatologie respiratoire.

Pendant le Hajj, 18,6% des pèlerins présentaient au moins un symptôme gastro-intestinal, la diarrhée étant le symptôme le plus fréquent (13,8%). Une proportion de 36,4% des pèlerins ont acquis au moins un agent pathogène entérique pendant le pèlerinage, principalement bactérien. Le sexe féminin, l'obésité et l'acquisition d'*Escherichia coli* entérohémorragique étaient significativement associés avec les symptômes gastro-intestinaux.

Au moins un pathogène respiratoire était retrouvé sur près de 50% des prélèvements environnementaux (poignées de porte, tables, tentes, fontaines à eau, boutons d'ascenseur…). *K. pneumoniae* était la bactérie la plus fréquemment retrouvée (57,1%), suivie de *S. pneumoniae* (12,9%), *S. aureus* (10,0%) et *H. influenzae* (7,1%). De plus, 32,9% des échantillons ont été testés positifs pour le rhinovirus et 1,4% pour le coronavirus.

Nous avons observé une prévalence importante d'utilisation inappropriée d'antibiotiques chez les pèlerins, ce qui est un des facteurs de risque de sélection et d'acquisition de bactéries multirésistantes. De fait, nos études ont montré que 19,4% des pèlerins ont acquis au moins une bactérie multirésistante au cours du Hajj. Par ailleurs, la prévalence de bactéries porteuses de gènes de résistance à la colistine était de 8,6%, avec l'identification pour la première fois de la circulation du gène de résistance *mcr-4* au Hajj.

Le Grand Magal à Touba au Sénégal est à la fois une cérémonie religieuse et une fête. Il est le plus grand rassemblement religieux musulman en Afrique de l'Ouest. En 2017, nous avons conduit une étude prospective avec 110 participants. La durée du séjour à Touba a été courte (3 jours). L'âge médian était de 20 ans. Au cours de la période d'étude, 41,8% pèlerins ont signalé au moins un symptôme respiratoire. La prévalence des rhinovirus, des coronavirus et des adénovirus a augmenté après le Magal avec des taux d'acquisition de 13,0, 16,7 et 4,6% respectivement. L'acquisition de bactéries variait de 3,7% pour *S. pneumoniae* à 26,9% pour *H. influenzae*. Au total, 14,6% des pèlerins ont signalé des symptômes gastro-intestinaux. L'acquisition de virus et de parasites gastro-intestinaux était faible, tandis que l'acquisition bactérienne variait de 2,2% pour *Campylobacter jejuni* à 33,3% pour *E. coli* entéropathogène.

En décembre 2019, une nouvelle épidémie d'infection respiratoire aigüe, nommée COVID-19, causée par un nouveau coronavirus, le SARS-CoV-2, a émergé à Wuhan, en Chine. Cette épidémie s'est rapidement étendue dans le monde entier. L'Organisation Mondiale de la Santé a déclaré l'état de pandémie le 11 mars 2020. En conséquence, les rassemblements de masse ont été suspendus ou limités, au vu de la situation du COVID-19 dans les pays hôtes. Nous avons réalisé deux revues de littérature sur le risque de COVID-19 lié à ces événements. Afin de contrôler la propagation de l'épidémie de COVID-19, les Jeux olympiques de Tokyo 2020 ont été reportés à 2021. Compte tenu de la forte contagiosité de la maladie et de l'épidémiologie du COVID-19 au Japon, cette décision était appropriée et importante afin de protéger les athlètes et le public. Le risque de transmission de virus respiratoires, y compris du SARS-CoV-2, est particulièrement élevé en raison des conditions de surpeuplement pendant le Hajj et à l'Omra. Le profil des pèlerins du Hajj, dont beaucoup sont âgés et présentent de multiples comorbidités, correspond à celui des individus à risque de COVID-19 sévère. Afin d'éviter une épidémie de COVID-19 susceptible de se propager dans de nombreux pays par le retour des pèlerins, l'Arabie Saoudite a suspendu l'Omra et l'accès au Hajj 2020 était très limité. Une relation claire entre la suspension précoce des rassemblements religieux de masse et une fréquence moindre de transmission du COVID-19 dans les pays qui ont pris rapidement de telles mesures a été remarquée.

ABSTRACT

International travel has grown significantly over the past five decades. Several tropical diseases such as malaria, dengue fever, or enteric fever are still observed among travelers. Emerging infectious diseases and epidemics further increase the risk of infection related to traveling abroad. International travelers participating in mass gatherings are particularly exposed to infectious risks. Over the past 10 years, concerns have grown up about threats to global health security posed by several emerging and re-emerging infectious diseases, linked to mass gatherings. Our review of the literature showed that infectious diseases associated with mass gatherings were dominated by respiratory and gastrointestinal infections. Meningitis outbreaks have also been reported in some cases. The human-to-human transmission of airborne diseases is favored by the temporal and spatial concentration of participants. In this work, we are studying the epidemiology of respiratory and gastrointestinal infections in pilgrims from Marseille participating to the Hajj, Saudi Arabia and Senegalese pilgrims participating to the Grand Magal de Touba.

The Hajj or pilgrimage to Mecca, Saudi Arabia, is one of the largest annual religious mass gatherings in the world, with over 2 million people coming from over 184 countries, including around 2,000 from Marseille. Our prospective study among Hajj pilgrims from Marseille, from 2012 to 2018 showed that the average age of pilgrims from Marseille ranged from 57.3 to 61.7 years. From 81.2% to 93.4% of the pilgrims presented at least one respiratory symptoms during the stay. By studying the carriage before, during and after the stay by PCR, we observed a significant acquisition of respiratory pathogens. Rhinovirus (27.7%), common coronavirus (8.3%) and influenza virus (3.7%) were the most frequent viruses while *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae* were the respiratory bacteria most frequently acquired by pilgrims. We also observed a specific dynamic of the carriage of pathogens during the stay in Saudi Arabia. By studying the interactions between pathogens, we observed that the carriage of rhinovirus and *S. aureus* were positively associated. This positive interaction was also found between *H. influenzae* and *Moraxella catarrhalis*. The genotypes of *H. influenzae* acquired were completely different from those present before the Hajj. We observed high biodiversity and a lack of *H. influenzae* clonality among French pilgrims. In addition, the prevalence of *H. influenzae* before Hajj revealed significant variations, from 0.9% to 52.8%, depending on the year between 2014 and 2018. However, the post-Hajj prevalence was consistently high, varying from 41.0 to 67.8%. This suggests that the circulation of *H. influenzae* at the Hajj was permanent during the study period and raises the question of the possible benefit of vaccination. We also observed associations between the carriage of certain pathogens, the presence

of chronic diseases, the demographic characteristics of the pilgrims, the use of preventive measures, including vaccination against influenza and respiratory symptoms.

During the Hajj, 18.6% of pilgrims had at least one gastrointestinal symptom with diarrhea being the most common symptom (13.8%). 36.4% of the pilgrims acquired at least one enteric pathogen during the pilgrimage, mainly gastroenteric bacteria. Female gender, obesity, and acquisition of Enterohemorrhagic *Escherichia coli* were positively associated with gastrointestinal symptoms.

Almost 50% of environmental samples (door handles, tables, tents, faucet of water fountains...) were positive for at least one respiratory pathogen with *K. pneumoniae* the most frequent (57.1%), followed by *S. pneumoniae* (12.9%), *S. aureus* (10.0%) and *H. influenzae* (7.1%). In addition, 32.9% of positive samples tested positive for rhinovirus and 1.4% for coronavirus.

We observed a high prevalence of inappropriate antibiotic use among pilgrims during their pilgrimage which is one of the risk factors for the acquisition of multidrug-resistant bacteria. Indeed, our results showed that 19.4% of the pilgrims acquired at least one multidrug resistant bacteria at the Hajj. The prevalence of acquisition of colistin resistance genes in pilgrims from Marseille was 8.6%. In addition, we report here for the first time, the circulation of mcr-4 genes to the Hajj.

The Grand Magal de Touba, Senegal is both a religious ceremony and a celebration. It is the largest Muslim religious gathering in West Africa. In 2017, we conducted a prospective study with 110 participants. The length of stay in Touba was short (3 days). The median age was 20 years old. During the study period, 41.8% pilgrims reported at least one respiratory symptom. The prevalence of rhinoviruses, coronaviruses and adenoviruses increased after participating to the Magal with acquisition rates of 13.0, 16.7 and 4.6% respectively. Acquisition of bacteria ranged from 3.7% for *S. pneumoniae* to 26.9% for *H. influenzae*. A total of 14.6% pilgrims reported gastrointestinal symptoms. Acquisition of viruses and gastrointestinal parasites was low, while bacterial acquisition ranged from 2.2% for *C. jejuni* to 33.3% for enteropathogenic *Escherichia coli*.

In December 2019, a new outbreak of acute respiratory infection (named COVID-19), caused by a novel coronavirus (SARS-CoV-2) emerged in Wuhan, China. This epidemic quickly spread around the world. The World Health Organization declared a pandemic on March 11, 2020.

As a result, mass gatherings have been suspended or limited, in view of the COVID-19 situation in host countries. We carried out two reviews of the literature on the risk of COVID-19 linked to these events. In order to control the spread of the COVID-19 epidemic, the Tokyo 2020 Olympic Games have been postponed to 2021. Given the high contagiousness of the disease and the epidemiology of COVID-19 in Japan, this decision was appropriate and important to protect athletes and the public. The risk of transmission of respiratory viruses, including SARS-CoV-2, is particularly high due to overcrowded conditions during Hajj and Umrah. The profile of Hajj pilgrims, many of whom are elderly and have multiple co-morbidities, matches that of individuals at risk of severe COVID-19. In order to avoid a COVID-19 epidemic that could spread to many countries by returning pilgrims, Saudi Arabia suspended Umrah and access to Hajj 2020 was very limited. A clear relationship between the early suspension of mass religious gatherings and a lower frequency of transmission of COVID-19 in countries that have taken such measures quickly has been noted.

PREAMBULE

Les voyages internationaux se sont beaucoup développés au cours des cinq dernières décennies. Les arrivées de touristes internationaux dans le monde ont augmenté de 4% en 2019 pour atteindre 1,5 milliard, selon les données disponibles pour les destinations du monde entier [1]. Les arrivées devraient atteindre 1,8 milliard de personnes d'ici 2030 et les destinations touristiques continuent de se diversifier [1]. Ces tendances mettent davantage de voyageurs en contact avec des agents pathogènes infectieux, qui peuvent avoir de graves conséquences sur la santé individuelle et collective et peuvent contribuer à la globalisation de certaines maladies infectieuses [2].

À mesure que les voyages internationaux augmentent en fréquence, les voyageurs contractent de plus en plus de pathologies exotiques et peuvent servir de sentinelles pour repérer des épidémies dans des pays où les moyens diagnostiques font défaut. La prévalence des maladies liées aux voyages, comme par exemple la diarrhée du voyageur ou turista, peut culminer à 80%, selon les destinations [3,4]. Les voyages internationaux concernent aussi des voyageurs ayant des besoins spécifiques comme les personnes âgées, les enfants, les femmes enceintes, les personnes immunodéprimées et les migrants [4]. Alors que les maladies tropicales bien connues telles que le paludisme, la dengue ou encore les fièvres typhoïde et paratyphoïde continuent d'être observées chez les voyageurs, les itinéraires en dehors des destinations traditionnelles et les conditions parfois extrêmes de certains voyages offerts par certains prestataires exposent à une gamme croissante d'infections [5]. Les maladies infectieuses émergentes et les épidémies augmentent encore les risques infectieux des voyageurs internationaux [5]. Plusieurs épidémies récentes, comme le Zika, la maladie à virus Ebola et le syndrome respiratoire du Moyen-Orient (MERS), ont été caractérisées par leur propagation internationale rapide [6-8]. Les infections liées aux voyages peuvent avoir des conséquences graves et nécessiter un traitement urgent ou des mesures de contrôle sanitaires autour des cas d’importation. Les flambées nosocomiales du MERS en Corée du Sud et en Arabie Saoudite ont démontré que ces infections peuvent présenter un risque grave lorsqu’elles sont insoupçonnées dans les établissements de soins de santé [7,9]. Récemment, en décembre 2019, une épidémie de maladie infectieuse respiratoire (COVID-19) due à un nouveau coronavirus (à l'époque officiellement nommé SRAS-CoV-2) est apparue dans la ville de Wuhan, dans la province chinoise du Hubei [10]. Cette maladie s'est ensuite propagée dans le monde par le biais de voyageurs internationaux infectés. L'épidémie a été déclarée urgence de santé publique de portée internationale le 30 janvier 2020 et pandémie le 11 mars 2020 [10]. À minuit, le 11 avril 2021, cette pandémie avait touché 219 pays et territoires à travers le monde avec 136 734 084 cas confirmés et 2 951 407 décès [11]. Bien que la probabilité d’une infection grave liée au voyage soit relativement faible, les infections respiratoires et digestives et l'acquisition de pathogènes sont très fréquentes chez les voyageurs [12]. En outre, des co-infections peuvent se produire [13].

Les voyageurs internationaux participant à des rassemblements de masse (RM) sont exposés à des risques infectieux spécifiques. Nombre de ces risques peuvent être réduits en prenant des précautions avant, pendant et après un voyage.

L'Organisation Mondiale de la Santé (OMS) définit les RM comme une « concentration de personnes à un endroit précis à des fins spécifiques sur une période donnée qui a le potentiel de mettre à rude épreuve les ressources de planification et d'intervention du pays ou de la communauté » [14]. Les RM peuvent être spontanés ou planifiés et sporadiques ou récurrents [14]. Les RM planifiés peuvent inclure des événements culturels, religieux, sportifs, sociaux et politiques. Les exemples sont : Le Hajj en Arabie Saoudite, le Grand Magal de Touba au Sénégal, les festivals de musique ou d'autres activités culturelles, et les grands évènements sportifs comme les Jeux Olympiques [15]. Les RM spontanés, compte tenu de leur nature, sont plus difficiles à planifier et peuvent inclure des événements, tels que les funérailles de personnalités religieuses et politiques [14, 15]. Les RM peuvent également inclure les rassemblements de populations déplacées en raison de catastrophes naturelles, de conflits et de guerres [14]. Les RM peuvent présenter d'importants défis de santé publique liés à la santé des participants et de la population du pays hôte et des services de santé [14]. Divers risques pour la santé sont associés aux RM, y compris la transmission de maladies infectieuses, les maladies non transmissibles, les traumatismes et blessures, les maladies liées à l'utilisation de drogues et d'alcool ou à l'environnement et les actes délibérés, tels que les attaques terroristes [14, 16]. Les maladies infectieuses sont particulièrement préoccupantes dans les RM [12].

Le concept de médecine de RM comme spécialité a été proposé lors d'une conférence sur le Hajj en 2009, qui a eu lieu pendant la pandémie de grippe HIN1pdm09 en 2009 [17]. La première Conférence internationale sur la médecine des RM s'est tenue à Djeddah, en Arabie saoudite, en octobre 2010, où la série de « *The Lancet Infectious Diseases*» sur les RM a été lancée [18]. Cette conférence a conduit à la création d'un groupe d'experts de l'OMS pour guider l'élaboration et la mise à jour des directives optimales de santé publique et de prévention et de traitement médicaux lors d'événements de RM [19]. La médecine de RM a été soulignée comme nouvelle discipline à l'Assemblée mondiale de la Santé des ministres de la Santé à Genève en mai 2014. Au cours des 10 dernières années, les inquiétudes se sont accrues en ce qui concerne les menaces sur la sécurité sanitaire mondiale posée par plusieurs maladies infectieuses émergentes et ré-émergentes, en lien avec les RM.

Dans ce travail, nous étudions l'épidémiologie des infections respiratoires (IRs) et gastro-intestinales (IGIs) chez des voyageurs internationaux ou domestiques participant à des RM, sur deux populations cibles : les pèlerins de Marseille participant au Hajj, en Arabie Saoudite et les pèlerins Sénégalais participant au Grand Magal de Touba.

Le Hajj ou pèlerinage à La Mecque, en Arabie Saoudite, est l'un des plus grands RM religieux annuels du monde, avec plus de 2 millions de personnes venant de plus de 184 pays, dont environ 2 000 en provenance de la ville Marseille, France [19, 20]. Le Hajj est l'un des cinq piliers de l'Islam. Il est obligatoire, au moins une fois dans la vie, pour tous les musulmans adultes qui disposent des capacités physiques et financières pour l'accomplir. Le Hajj retrace les pas du prophète Mahomet sur plusieurs jours pendant lesquels les pèlerins doivent obligatoirement effectuer un ensemble de rituels, à différents lieux sacrés situés autour de la Mecque. Il se déroulent sur cinq jours pendant Dhul al-Hijjah - le dernier mois du calendrier islamique. Les pèlerins séjournent en Arabie Saoudite généralement de 3 à 4 semaines, pour visiter les Grandes Mosquées. Pendant le pèlerinage, les pèlerins séjournent dans des hôtels (à la Mecque et Médina) et des tentes (à Mina). La principale source de nourriture est représentée par les restaurants et les vendeurs ambulants soumis à des contrôles sanitaires stricts.

Le Grand Magal à Touba au Sénégal est à la fois une cérémonie religieuse et une fête. Environ 4 à 5 millions de mourides participent à cet événement chaque année, provenant de tout le Sénégal et des pays voisins, ainsi que de pays hors d'Afrique. Il est le plus grand rassemblement religieux musulman en Afrique de l'Ouest. Les deux événements centraux du Magal sont les visites à la Grande Mosquée de Touba, qui implique une circumambulation partielle de la mosquée, et au mausolée de Cheikh Ahmadou Bamba. Les pèlerins visitent également les mausolées de plusieurs autres dirigeants mourides importants qui étaient des descendants du Cheikh. De plus, les pèlerins visitent des lieux emblématiques à Touba qui sont associés à la vie sainte du Cheikh, y compris le "Puits de miséricorde", une source dont l'eau sacrée est réputée pour pouvoir guérir toutes sortes de maladies et de malheurs. La bibliothèque centrale de Touba qui contient les écrits du Cheikh et d'autres théologiens Mourides influents, et l'Université Mouride sont parmi les autres endroits visités par les pèlerins. Enfin, les pèlerins visitent leurs guides spirituels mourides personnels, ou marabouts qui reçoivent leurs disciples dans leur résidence personnelle dans la ville. De manière informelle, les pèlerins visitent également le grand marché temporaire de Touba, l'un des plus grands du pays où une large gamme de produits peut être trouvée avec des prix inférieurs à ceux de tout autre marché au Sénégal. Pendant cette période, les pèlerins sont hébergés dans des logements privés car il n'y a pas d'hôtels à Touba. Ces maisons peuvent être des résidences d'habitants locaux, où des membres de la famille vivant dans différentes régions du Sénégal ou qui ont émigré hors du pays, se réunissent pendant la période Magal, ou des maisons de Marabout d'une capacité pouvant aller jusqu'à des centaines d'individus. De nombreux pèlerins dorment sur des tapis, au sol, dans les maisons ou sur les terrasses extérieures. La nourriture est préparée collectivement par les membres de la famille ou par les adeptes du marabout. L'abattage des animaux se fait principalement dans les rues, devant les logements. Différentes associations communautaires de disciples mourides (dahiras) sont chargées de nourrir les pèlerins en leur fournissant gratuitement de la nourriture dans les rues et le long des routes menant à Touba [16].

INTRODUCTION

Les maladies infectieuses liées aux RMs sont dominées par les infections des voies respiratoires et gastro-intestinales. Des flambées de méningite bactérienne ont également été signalées dans certains cas. La transmission interhumaine par voie aérienne des agents pathogènes de maladies respiratoires est favorisée par la concentration temporelle et spatiale des participants.

Dans une étude conduite pendant le Hajj, les maladies infectieuses représentaient 53% des diagnostics ambulatoires. Les IRs supérieures et inférieures, les gastro-entérites aigues et les infections graves liées au diabète, la cellulite et la pneumonie étant les plus courantes [21]. Presque tous les pèlerins développent une infection des voies respiratoires pendant le Hajj, connue sous le nom de toux des pèlerins [22]. La prévalence de la diarrhée chez les pèlerins varie de 1,1% à 23,3%, selon les études. De nombreuses études menées auprès des pèlerins du Hajj ont aussi démontré la forte prévalence de l'acquisition de pathogènes, y compris des bactéries multi-résistantes aux antibiotiques [12, 23, 24].

Une enquête préliminaire, réalisée en 2015 pendant le pèlerinage du Grand Magal à Touba, a montré une prévalence élevée de maladies systémiques fébriles et de paludisme (4,9%), de maladies diarrhéiques (4,5%) et d'IRs (5,2%) parmi les pèlerins malades vus en consultation dans les structures de santé. Le taux global d'hospitalisation était de 3,4%, incluant les pathologies gynéco-obstétricales (16,2%) et les paludismes confirmés (14,5%) [16].

Étant donné que la distance sociale et la prévention des contacts lors des RMs sont des mesures difficiles à mettre en œuvre, des mesures préventives individuelles telles que l'hygiène, l'utilisation d'un masque facial et de mouchoirs jetables et l'hygiène des mains peuvent être recommandées. Néanmoins, l'efficacité de ces mesures a été peu étudiée dans le contexte des RMs (article 1).

<u>Article 1 :</u> REVIEW

Infectious Diseases and Mass gatherings

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TROPICAL, TRAVEL, AND EMERGING INFECTIONS (L CHEN AND A BOGGILD, SECTION EDITORS)

Infectious Diseases and Mass Gatherings

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Abstract

Purpose of Review Mass gatherings (MGs) are characterized by a high concentration of people at a specific time and location. Infectious diseases are of particular concern at MGs. The aim of this review was to summarize findings in the field of infectious diseases with a variety of pathogens associated with international MGs in the last 5 years.

Recent Findings In the context of Hajj, one of the largest religious MGs at Mecca, Saudi Arabia, respiratory tract infections are the leading cause of infectious diseases in pilgrims with a prevalence of 50–93%. The most commonly acquired respiratory viruses were human rhinovirus, followed by human coronaviruses and influenza A virus, in decreasing order. *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were the predominant bacteria. The prevalence of Hajj-related diarrhea ranged from 1.1 to 23.3% and etiologies included *Salmonella spp*., and *Escherichia coli*, with evidence of acquisition of antimicrobial-resistant bacteria. In other MGs such as Muslim, Christian, and Hindu religious events, sports events, and large-scale open-air festivals, outbreaks have been reported less frequently. The most common outbreaks at these events involved diseases preventable by vaccination, notably measles and influenza. Gastrointestinal infections caused by a variety of pathogens were also recorded.

Summary Because social distancing and contact avoidance are difficult measures to implement in the context of many MGs, individual preventive measures including vaccination, use of face mask, disposable handkerchief and hand hygiene may be recommended. Nevertheless, the effectiveness of these measures has been poorly investigated in the context of MGs.

Keywords Infectious diseases · Outbreaks · Mass gatherings · Hajj · Sport · Festival

Introduction

The WHO defines mass gatherings (MGs) as a "concentration of people at a specific location for a specific purpose over a set period of time which has the potential to strain the planning and response resources of the country or community" [1].

This article is part of the Topical Collection on *Tropical, Travel, and Emerging Infections*

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MGs can be either planned or spontaneous and recurrent or sporadic [1]. Planned MGs may include sporting, social, cultural, religious, and political events. Examples include music festivals, the Olympic Games, and the Hajj [2].

Spontaneous MGs, given their nature, are more difficult to plan for and may include events, such as funerals of religious and political figures [1, 2]. MGs may also include the gatherings of displaced populations due to natural disasters, conflicts, and wars [1]. Diverse health risks are associated with MGs, including transmission of infectious disease, non-communicable disease, trauma and injuries (occupational or otherwise), environmental effects (such as, heat-related illnesses, dehydration, hypothermia), illnesses related to the use of drugs and alcohol, and deliberate acts, such as terrorist attacks [1]. Infectious diseases are of particular concern at MGs [3]. In this review, we summarize recent findings in the field of infectious diseases associated with international MGs.

Results and Discussion

The Hajj (Table 1)

The Hajj, an annual Muslim pilgrimage to Mecca, Saudi Arabia, is one of the largest religious MGs in the world with about two million pilgrims from 185 countries [4]. As part of the Hajj rituals, pilgrims visit various sacred places around the city of Mecca. Most of them also travel to the city of Medina to visit the second holiest site of Islam, the Prophet's mosque containing the tomb of the Prophet Muhammad. The presence of a large number of pilgrims from different countries of the world and overcrowded condition considerably increases the risk of occurrence of infectious diseases, particularly respiratory and gastrointestinal diseases [5]. Furthermore, a vast majority of pilgrims are elderly people with a high prevalence of chronic diseases. In the past, Hajj-related cholera has been a public health problem and the main cause of morbidity and mortality among pilgrims, leading to major epidemics and international spread. Due to improved sanitary conditions in Saudi Arabia in general and at religious sites, large-scale cholera outbreaks have not been recorded during the last decades [6, 7]. Similarly, invasive meningococcal disease has been a Hajj-related public health concern with its last outbreaks (serogroup W-135) in the 2000s. However, with the strengthening of prevention through mandatory vaccination, no case of meningococcal disease has been reported in Mecca since 2006 [8, 9].

While gastrointestinal diseases and diarrhea have changed towards a lower prevalence, respiratory tract infections (RTIs) now account for the vast majority of health problems during the Hajj [4, 5]. The inevitable overcrowding conditions at the Grand Mosque in Mecca and the accommodation in tents in Mina with an average of 50 to 100 people per tent are likely responsible for the high rate of respiratory infections among Hajj pilgrims [5].

Table 1 Hajj and large-scale open-air festivals—key points

Hajj—key points
• Event: annual Muslim pilgrimage
• Place: Mecca, Saudia Arabia
• Population size: 2 million from 185 countries (high proportion of elderly pilgrims with co-morbidities)
• Prevalence of respiratory symptoms in overall population of pilgrims: 50–93%
• Pneumonia among the leading cause of admission in Saudi hospitals during the Hajj
• Acquisition of respiratory pathogens: 13.5–34.1% rhinovirus, 12.4–14.6% non-MERS coronavirus, 1.9–7.8% influenza A virus, 7.1–36.6% *Streptococcus pneumoniae*, 11.4% *Haemophilus influenzae*, 7.5% *Staphylococcus aureus*
• No case of MERS-CoV, *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* observed in pilgrims in recent years
• Antibiotic consumption in overall population of pilgrims: 45–61.8%
• Prevalence of diarrhea: 1.1–23.3%
• Hajj-associated diarrhea mostly due to bacteria including: *Salmonella spp.* and *Escherichia coli.*
• Evidence for acquisition of antimicrobial-resistant bacteria
Large-scale open-air festivals—key points
• Events: music festivals and other cultural festivals in various places in the world.
• Population size: variable but usually less than 400,000 participants (mostly young participants)
• Outbreaks of respiratory tract infections and gastrointestinal infections are regularly reported including notably measles and mumps, influenza, diarrhea due to bacterial and viral infections.

Respiratory Infections at the Hajj

Over the last 5 years, a significant number of publications from different countries based on both syndromic surveillance and PCR-based investigation of respiratory pathogen carriage were made available. Studies were conducted in out- and in-patients at health structures in Saudi Arabia or on return in pilgrim's country of origin and in cohorts of pilgrims regardless of symptoms [10–34] (Table 2).

RTIs are among the leading causes of admission to hospitals in Mina, Mecca, and Medina during the Hajj period (Table 2). Most cases are upper respiratory tract infections [10–16], but severe respiratory tract infections [17] and pneumonia are not uncommon among pilgrims [18, 19, 20•]. Respiratory diseases were the second cause of mortality in Indonesian pilgrims during the Hajj (following cardiovascular diseases) [35]. Among pathogens detected by PCR methods in ill pilgrims, the most common viruses were human rhinovirus (HRV), followed by human coronaviruses (HCoV) and influenza A virus (IAV). *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were the predominant bacteria isolated by culture [36, 37].

Cross-sectional and longitudinal cohort studies have recorded 53–93.4% prevalence of RTI symptoms among Hajj pilgrims [21–23, 26•, 28, 32, 34•]. The rate of ILI varied from 1.9 to 78.2% [21, 22, 26•, 27, 28, 30–33]. Cohort surveys allow evaluating the acquisition rate of respiratory pathogens regardless of symptoms. The most commonly acquired viruses were human rhinovirus (HRV) (13.5–34.1%), followed by human coronavirus E229 (HCoV-E229) (2.0–14.6%) and influenza virus (IAV) (1.9–20.0%) [21, 22, 27, 28, 30, 31•, 33]. The most commonly acquired bacteria were *S. pneumoniae* (7.1 to 36.6%) and *S. aureus* (7.5 to 22.8%) and *H. influenzae* (11.4%) [25, 28, 29, 31•, 34•]. *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* have not been detected in pilgrims in recent studies [13, 28, 31•]. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) that emerged in the Arabian Peninsula in 2012 is associated with severe acute respiratory infection with high

Table 2 Respiratory tract infections at the Hajj

Date of study	Clinical setting Place of study	Clinical findings	Number of pilgrims/cases	Pathogens isolated	Comments/highlights	References
2010	Retrospective survey of medical data registered by Iranian caravan physicians at Mecca, Medina, Arafat, and Mina, Saudi Arabia	RTIs 61.82%, cough 24%, rhinorrhea 19% *n*, sore throat 18%	107,074 ill Iranian pilgrims		RTIs were the most diagnosed diseases	[10]
2013–2015	Survey conducted among ill returning pilgrims presenting to hospitals in the Midlands South West and North England, UK		202 UK pilgrims with RTI	14.4% HRV, 13.9% IAV, 6.4% IBV, 5.0% PIV virus		[11]
2013–2016	In-patient of a survey admitted to the Kurmitola General Hospital isolation unit, Dhaka, Bangladesh		81 Bengali pilgrims hospitalized with RTIs	12.3% IAV, 10% IBV, 2.8% ADV, 2.8% MPV, 2.8% PIV		[12]
2014	Survey conducted among ill returning pilgrims consulting at 12 hospitals in Jordan	76% cough, 50% sore throat, 41% fever, 37% rhinorrhea, 16% dyspnea	125 Jordan pilgrims with RTIs	47% HRV/EV, 12.8% HCoV and 3% IAV		[13]
2014	Cross-sectional study conducted at primary health care services of the Al Noor Specialized Hospital in Medina, Saudi Arabia	66.8% acute rhinosinusitis	343 ill hospitalized pilgrims with RTI	93 (41.2%) bacterial rhinosinusitis, including 49.5% *S. aureus*, 15% *K. pneumoniae*, 8.6% *E. coli*, 4.3% *S. pneumoniae* and 3.2% *H. influenzae*		[14]
2014–2015	Cross-sectional survey conducted in returning Hajj and Umrah pilgrims with current respiratory symptoms or fever arriving from Mecca and landing at Srinagar Airport, India. Study based on face-to-face interview	89.7% cough, 86.3% rhinorrhea, 66.2% sore throat, 49.8% fever	300 Indian pilgrims	11% influenza virus		[15]
2016	Cross-sectional study conducted at an Indian medical mission in Mecca, Medina and Jeddah, Saudi Arabia	930 ill pilgrims needed secondary care, of whom 585 (62.9%) were hospitalized.	374,475 ill Indian pilgrims		Infectious diseases were most commonly due to overwhelming respiratory infections (49.4%)	[16]
2013–2016	Cross-sectional survey conducted in pilgrims admitted to Iranian hospitals on returning from the Hajj		3840 Iranian pilgrims hospitalized with severe acute respiratory infections	13% influenza virus		[17]
2004–2013	Retrospective cohort study conducted at Al-Ansar general hospital, Medina, Saudi Arabia	59.2% productive cough, 30.1% dry cough, 44.7% dyspnea, 26.8% sore throat. 31% admitted in ICU.	1059 hospitalized pilgrims with pneumonia (23% of total hospital admissions)	Organisms isolated from sputum cultures: 36.1% *S. aureus*, 29% *K. pneumoniae*, 24.3% *H. influenzae*, 6.5% community-acquired MRSA, 3.1% *P. aeruginosa*, 1% *S. pneumoniae* 14.9% multiple pathogen	The mortality rate was 2.4% in the ward and 21.45% in the ICU	[18]
2013	Cross-sectional study conducted at 15 healthcare facilities of Mecca and Medina, Saudi Arabia		38 pilgrims hospitalized with bilateral pneumonia	57.7% HRV, 23.1% IAV, 19.2% HCoV, 57.7% *H. influenzae*, 53.8% *S. pneumoniae*		[19]
2016	Prospective survey conducted in 13 hospitals in Mecca and Medina, Saudi Arabia	91% cough, 87.2% difficulty breathing, 32.3% chest	266 hospitalized pilgrims with confirmed	19% *S. pneumoniae* identified by culture		[20•]

Table 2 (continued)

Date of study	Clinical setting Place of study	Clinical findings	Number of pilgrims/cases	Pathogens isolated	Comments/highlights	References
		pain 83.1% fever, 79.4% tachypnea	community-acquired pneumonia			
2004–2009	Longitudinal survey conducted in pilgrims recruited at 1352 Hajj caravans. Study based on medical evaluation during travel	71.2% RTIs, 10.7% ILI, 0.5% pneumonia	254,823 Iranian pilgrims	357 samples tested were positive for respiratory bacteria with 15.8% *C. pneumoniae*, 6.6% *L. pneumophila*, 8.5% *streptococcus*, 9.1% *haemophilus* 105 pairs of pharyngeal samples tested were positive for respiratory virus with 36.2% ADV, 30% HRV, 20% IVB, 1.9% RSV		[21, 22]
2009	Longitudinal survey conducted in pilgrims recruited at primary healthcare centers in Riyadh, Saudi Arabia (for the mandatory pre-Hajj meningococcal vaccination). Study based on telephone interview on return	53% RTIs	1507 pilgrims		RTIs were leading cause of consultation (97% of ill pilgrims)	[23]
2010	Cross-sectional study conducted at Jeddah airport		1600 pilgrims	7.5% IAV		[24]
2012	Longitudinal survey conducted in pilgrims recruited at a specialized Hajj travel agency, Marseille, France. Study based on medical evaluation during travel		169 French pilgrims	The overall acquisition of nasal *S. aureus* carriage was 22.8%		[25]
2012–2014	Longitudinal survey conducted in pilgrims recruited at a specialized Hajj travel agency, Marseille, France. Study based on medical evaluation during travel	80.9% cough, 46.2% ILI, 91% sore throat, 78.7% rhinorrhea, 63.0% voice failure, 21.0% dyspnea	382 French pilgrims		The prevalence of cough was significantly higher among females than men. Pilgrims with chronic respiratory disease showed a slightly increased prevalence of cough.	[26•]
2012–2015	Cross-sectional study conducted at Cairo International Airport, Egypt	30.4% reported symptoms consistent with ILI 14.5% laboratory-confirmed influenza	3364 Egyptian pilgrims	14.5% influenza including, 3.5% H1N1, 5.6% H3N2, 5.4% IBV		[27]
2013	Longitudinal survey conducted in pilgrims recruited at a specialized Hajj travel agency, Marseille, France. Study based on medical evaluation during travel	Cough 86.8% Sore throat 82.9%, ILI 47.3%,	129 French pilgrims	HRV, HCoV-E229 and IAV prevalence was 13.5%, 12.4% and 7.8%, respectively. Of note, 36.6% of pilgrims acquired *S. pneumoniae* after the Hajj		[28]
2013	Prospective cohort study conducted among pilgrims recruited at King Abdul Aziz International airport Jeddah and Mina, Saudi Arabia		1175 pilgrims	The carriage rate of *S. pneumoniae* was 1.8% pre- and 7.1% post-Hajj		[29]

Table 2 (continued)

Date of study	Clinical setting Place of study	Clinical findings	Number of pilgrims/cases	Pathogens isolated	Comments/highlights	References
2013	Cross-sectional survey conducted in pilgrims recruited at Mina encampment. Study based on post-Hajj face-to-face interview	63.4% ILI, 46.3% cough, 34.7% sore throat, 23.8% rhinorrhea	164 Australian pilgrims	25% HRV, 4% IAV, 2% HCoV		[30]
2013	Cross-sectional survey (unpaired cohort) and longitudinal survey (paired cohort) conducted in pilgrims recruited on arrival at Jeddah airport. Study based on post-Hajj face-to-face interview conducted at Mina	62% ILI	1676 pilgrims	The acquisition rates of HRV, human coronavirus E229 (HCoV-E229) and IAV was 34.1%, 14.6%, and 1.9%, respectively and acquisition rates of *S. pneumoniae*, *H. influenzae* and *S. aureus* were 12%, 11.4%, and 7.5%, respectively		[31•]
2013	Cross-sectional survey conducted in pilgrims recruited at a Hajj course at Universiti Sains Malaysia (USM), Kelantan, Malaysia, at Hajj building complex, Malaysia and in Mecca, Saudi Arabia. Study based on post-Hajj self-questionnaires collected on return	93.4% respiratory symptoms 78.2% ILI 2.1% hospitalization	468 Malaysian pilgrims		Most of pilgrims acquired the infection intensely at Arafat (81.2%)	[32]
2013–2015	Longitudinal survey conducted in returning Hajj pilgrims arriving at Xinjiang and Gansu airports, China	ILI, 1.9%	847 Chinese pilgrims	4.0% IAV, 1.7% IBV, 0.5% MPV, 0.2% RSV		[33]
2016	Prospective multisite cohort study conducted in pilgrims recruited in 4 cities of India	76% pilgrims had at least one respiratory symptoms, cough 60.6%, sore throat 25.0%	807 Indian pilgrims	28% were positive for *S. pneumoniae* by culture methods (65% were symptomatic) and 65.9% were positive by qPCR (59% were symptomatic)	None of the participants reported receiving the pneumococcal vaccine	[34•]

RTIs respiratory tract infections, ILI influenza-like illness, ICU intensive care unit, *S. aureus Staphylococcus aureus*, *S. pneumoniae Streptococcus pneumoniae*, *K. pneumoniae Klebsiella pneumoniae*, *H. influenzae Haemophilus influenzae*, *P. aeruginosa: Pseudomonas aeruginosa*, *C. pneumoniae: Chlamydia pneumoniae*, *L. pneumophila: Legionella pneumophila*, *E. coli: Escherichia coli*, *MRSA* methicillin-resistant *staphylococcus aureus*, *HRV* human rhinovirus, *EV* enterovirus, *HCoV* human coronavirus, *IAV* influenza A virus, *IBV* influenza B virus, *PIV* parainfluenza virus, *RSV* respiratory syncytial virus, *ADV* adenovirus, *MPV* metapneumovirus mortality rates. Numerous studies were conducted in returning pilgrims with the aim of detecting MERS-CoV infections and all resulted negative [11–13, 15, 17, 24, 27, 28, 30, 33, 38–46].

Tuberculosis (TB) transmission is another concern at the Hajj, but there are no large-scale, specific studies to determine its prevalence among pilgrims [47]. A prospective cross-sectional study was conducted in Mecca, during the Hajj period in September 2015. One thousand one hundred sixty-four pilgrims with cough were selected from five countries in Africa and South Asia that are endemic for TB and 1.4% had active previously undiagnosed TB [48•]. During the Hajj in 2015, 44 cases of TB among pilgrims and non-pilgrims were diagnosed in Mecca hospitals [48•].

Gastrointestinal Diseases at the Hajj

A review on diarrhea at the Hajj published in 2015 showed a prevalence of diarrhea ranging from 1.1 to 23.3% in 14 cohort studies including 262,999 pilgrims from various countries between 2002 and 2013 [49•]. Five percent of pilgrims from Riyadh developed diarrheal symptoms during the 2009 Hajj [23]. Twenty-one percent of Iranian female pilgrims suffered from gastroenteritis during the 2011 Hajj [50]. In 2013, 23.3% pilgrims from Marseille, France, had diarrhea during the Hajj [51] while a 13.7% prevalence was recorded in 2016 [52]. In the latter study, *Escherichia coli* was the predominant pathogen isolated from pilgrims by PCR. Enteropathogenic *E. coli*, enteroaggregative *E. coli*, and Shiga-like toxin-producing *E. coli* were acquired by 29.9%, 10.2%, and 6.5% pilgrims, respectively [52]. Among persons infected during the 2011–2013 Hajj and hospitalized in Saudi hospitals, the pathogens responsible for enteric infection were mostly bacteria, with a prevalence of *Salmonella spp.* of 11.4%, while that of diarrhea associated *E. coli* ranged between 1.3 and 8.8% according to pathotypes [53]. Two cases of *Tropheryma whipplei* were recorded in a cohort of French pilgrims during the 2013 Hajj [51].

Antibiotic Consumption During the Hajj

The frequency of infectious diseases during the Hajj results in a significant demand for antibiotic use. The rate of antibiotic use among pilgrims varied according to their nationality and year with 61.8% in Malay pilgrims in 2013 [32], 53.8% in French pilgrims in 2012 [25], 45–48.3% in Indian pilgrims in 2016 [16, 34•], and 58.5% in Iranian pilgrims in 2012 [54]. A prospective study conducted among 218 pilgrims from Marseille, France, during the periods of Hajj in 2013–2014 showed that 54.8% of the population used antibiotics because of respiratory diseases and 5.4% because of diarrhea [55]. Although the dispensing of antibiotics without a prescription has been banned in Saudi Arabia for more than 30 years [56], 27 % of Australian pilgrims used antibiotics either delivered in Saudi Arabia without prescription or purchased in Australia before traveling [57].

Hajj and Antimicrobial-Resistant Bacteria

The predominance of bacterial pathogens in Hajj-related gastrointestinal infections poses a major risk to public health through the potential emergence and transmission of antimicrobial-resistant bacteria [53]. Methicillin-resistant *S. aureus* had been isolated in 28% of pilgrims with acute sinusitis in 2014 [14] and 63% of pilgrims with community-acquired infections hospitalized during the Hajj in 2015 [58•]. One study addressed the carriage of resistant *S. pneumoniae* in a multinational cohort of pilgrims and showed that 23% of isolates were resistant to multiple antibiotics (resistant to three or more classes of antibiotics) [29]. Extended spectrum beta-lactamase Enterobacteriaceae are also common among hospitalized pilgrims. During the 2014–2015 Hajj, 47% of pilgrims attending hospitals for urinary tract infections showed blaCTX-M genes in *E. coli* isolates [59].

During the 2013 and 2014 Hajj seasons, studies were conducted using rectal samples obtained before and after the Hajj in cohorts of French pilgrims to assess the carriage of the blaCTX-M gene. Acquisition rates of 31.0–34.8% were observed [55, 60]. There was also a significant increase in the number of pilgrims harboring *E. coli* resistant to ceftriaxone and ticarcillin-clavulanic acid [60].

The prevalence of C3G-resistance was observed in 90.6% *Acinetobacter baumannii* isolates in a cohort of French pilgrims in 2014 [61] and in 76.2% of isolates obtained from hospitalized pilgrims suffering from community-acquired infections in 2015 [58•]. Two French pilgrims carried *S. enterica*, resistant to ceftriaxone, gentamycin, and colistin after the 2013 Hajj [62]. Mrc-1 resistance gene screening from rectal swabs was conducted in French pilgrims in 2013–2014 and found an acquisition rate of 9.0% after Hajj [63].

Risk factors for the spread of antibiotic-resistant bacteria at the Hajj include international travel, misuse of antibiotics, and availability of over-the-counter antibiotics [64]. However, gastrointestinal diseases and diarrhea continue to occur in pilgrims, outbreaks of food poisoning are reported, and the acquisition of multi-resistant bacteria is emerging. The ongoing monitoring of these diseases is part of the public health response regarding the Hajj [49•, 55].

Meningococcal Carriage at the Hajj

Currently, meningococcal vaccination (A, C, Y, W-135) is mandatory for all pilgrims, national and international, as well as local residents of holy cities and workers in contact with pilgrims; however, polysaccharide vaccine which does not prevent meningococcal carriage is still in use in many countries. Mandatory oral ciprofloxacin prophylaxis is provided upon arrival to all the pilgrims coming from the "meningitis belt" of sub-Saharan Africa [8, 9, 65, 66]. A cross-sectional study among pilgrims arrived at King Abdul Aziz International Airport, in Jeddah for the Hajj in 2012 showed antibody titers under the level of protection against serogroups A, C, W, and Y of only 0.1%, 0.4%, 17.4%, and 9.4%, respectively. Most of them (98.2%) had received meningococcal vaccination in the three previous years [67]. In a prospective cohort study conducted in Turkish Hajj pilgrims during 2010, the carriage prevalence of *Neisseria meningitides*, assessed by culture method, was 13% before and 27.0% after the Hajj with the majority being serogroup W-135 [68]. In a prospective culture-based cohort study conducted among Iranian pilgrims in 2012, 1.4% acquired *N. meningitides* at the Hajj [54]. A prospective study conducted in 2014 among international pilgrims at King Abdul Aziz International Airport showed 3.0% *N. meningitides* carriage by culture method upon arrival and 0.9% upon departure, with the majority of typable isolates being serogroup B [69]. Outbreaks of the disease including those due to serogroups not included in the required vaccines, such as serogroups B and X, are therefore possible at the Hajj. Despite the wide use of polysaccharide vaccine, it does not prevent the carriage of serogroup W-135 and subsequent transmission to unvaccinated individuals by returning pilgrims.

Others Religious Meetings

The Grand Magal of Touba, the largest Muslim pilgrimage in Senegal, has specific features. Besides its setting in a tropical environment, its population is characterized by a large range of age groups since most pilgrims travel with their family, including young children. A preliminary survey in 2015 has showed a high rate of febrile systemic illnesses and malaria (4.9%), diarrheal diseases (4.5%), and RTIs (5.2%) among ill pilgrims consulting at health care structures during the pilgrimage. The overall hospitalization rate was 3.4% including gyneco-obstetric cases (16.2%) and confirmed malaria (14.5%) [70••].

The Kumbh Mela in India is the largest MG in the world with about 100 million visitors. It posed an exciting challenge to the provision of healthcare services. Increased population density, reduced sanitation, and exposure to environmental pollutants open the way for easy transmission of pathogens [71]. During Kumbh Mela in 2013, 412,703 patients consulted at hospitals. Respiratory infections accounted for 70% of illnesses and diarrheal diseases for 5%. In total, 4429 (1.1%) were hospitalized. Gastrointestinal disease risk, including cholera, is high because of potential contamination of water and food. In addition, vaccination against cholera is no longer considered adequate or even feasible in this context [72].

The Ashura MG at Karbala is an increasingly popular religious event in Iraq with about three to four million Muslims from within and outside Iraq. In 2010, a cross-sectional study conducted in three public hospitals at Karbala city showed that about 80% of the 18,415 consultations were at emergency rooms. Febrile illness was recorded seven times more frequently during this event compared to previous events, in relation to an eight-fold increase in the population in the area during the event [73].

Other notable events include the Moulay Abdellah Amghar Moussem, an 8-day annual gathering in Morocco, that documented an increase of gastrointestinal diseases from 11 to 14% between 2009 and 2010 [74]. During the 2010 anniversary of the death (Urs) of Baba Farid, an annual MG in Pakpattan, Pakistan, 58% of 5918 people seen at 15 healthcare facilities were affected by communicable diseases, including 26% gastrointestinal illnesses and 21% RTIs [75]. Also in 2010, a cross-sectional study of 700,000 attendees to the 5-day Eid Al Adha holiday, Aqaba (one of the largest Muslim MGs in Jordan), identified 23% and 33% increases in emergency department attendance and hospital admissions, respectively; however, no food poisoning outbreaks were reported [76]. Unlike the syndromic surveillance data mentioned above that lacked reliable identification of the responsible pathogen, *S. enterica* serotype typhimurium was determined to cause 64 cases of gastrointestinal illness among 9000 participants in a Christian religious festival in Hamilton County, Ohio; the outbreak was associated with the consumption of pulled pork prepared in a private house and sold at the festival [77].

Large-Scale Open-Air Festivals (Table 1)

Although numerous gastrointestinal and respiratory outbreaks have been documented at large-scale open-air festivals, particularly music festivals, with thousands of participants, these events are probably neglected, in terms of public health attention, as well as surveillance and prevention of infectious disease strategies, compared to other categories of MGs [78]. Since this review was published, several outbreaks were reported in the context of festivals.

Between July 10 and 24, 2013, during the annual independence celebrations in Kiribati, the Kiribati Syndromic Surveillance System reported an increase in children presenting with severe diarrhea due to Rotavirus. In total, 1118 cases of gastroenteritis were reported and 6 (0.5%) died among 103 (9.2%) hospitalized. Most of them (93.4%) were younger than 5 years of age [79].

An outbreak of measles with 44 cases identified at an international dog show occurred in November 2014 in Slovenia, where measles virus had not been circulating for many years. Twenty-three persons were infected there and 21 were presumable secondary and tertiary cases. Most cases (39) were adults. Five were unvaccinated children [80]. Also, a multistate measles outbreak that caught global attention occurred at the Disney theme parks in California, USA [81].

The 23rd World Scout Jamboree (WSJ) in Yamagushi, Japan, from July 28 to August 8, 2015, was a MG attended by more than 33,000 participants from 155 countries. The event is designed for scouts aged 14 to 17 years to live together, experience diverse cultures, and take part in recreational activities. In this event, six cases of invasive meningococcal disease related to the WSJ were reported, affecting 19.5 per 100,000 WSJ attendees, far exceeding the annual incidence rate in Japan in 2014 (0.03 per 100,000 population) [82•].

Finally, an outbreak of measles (52 cases) was reported at music and art festivals in England and Wales between June and October 2016. Almost half of the cases occurred in participants aged 15 to 19 years. Several people who contracted measles at a festival later attended another festival when they were contagious, resulting in multiple, interconnected outbreaks. Only one confirmed case was fully vaccinated. Forty-two were not vaccinated. Nine cases were not fully vaccinated, or their immunization status was unknown [83].

Sport Events (Table 3)

A review of outbreaks at large sport events including the Summer and Winter Olympics, FIFA world cup and the EURO football cup from 1984 through 2015 found little evidence for infectious outbreaks with the exception of 36 cases of influenza among participants to the Winter Olympiad in Salt Lake City in 2002 and a small outbreak of gastrointestinal disease affecting a single team during the pre-race sailing competition in Brazil in 2015, prior to the 2016 Olympics [84••].

An epidemic of measles occurred during the XXI Olympic Winter Games that were held in February 12–28, 2010, in Vancouver, Canada, with 82 cases [85]. Another epidemic of measles was noted during the 16th edition of the Italia Super Cup, international junior football tournament in Rimini, Italy, from June 2nd to 5th, 2011. Most ill individuals had not been vaccinated [86].

During the London 2012 Olympic and Paralympic Games, no major public health incidents occurred. Only a few outbreaks of gastrointestinal and respiratory infections were recorded during this period. No food-borne illness was directly linked to a Games venue, despite the tendency for those reporting them to label them as such [87]. During this event, 289 Olympic visitors were followed for sexually transmitted infections (STI), 47 new STI diagnoses were made including 15 non-specific genital infection, eight chlamydia, and eight genital warts (first episode) diagnoses. There were no new HIV or syphilis diagnoses [88].

Table 3 Sport events—key points

- Events: Summer and Winter Olympics, FIFA world cup, EURO football cup
- Population size: variable 1–6 million attendees at largest events
- Outbreaks of respiratory tract infections have been reported, notably measles and influenza
- Gastrointestinal infections and diarrhea due to *Salmonella spp*. and norovirus have been recorded.

During Euro 2012 European Football, according to national data from Ukraine, 1299 cases of acute gastroenteritis occurred in host cities, but daily notifications remained consistently below the epidemic threshold determined by Ukraine. Similarly, 109 measles cases were reported in the host cities during the tournament, only one of which occurred in a foreign visitor. This number represented about 10% of the new cases reported throughout Ukraine during the same period [89].

During the European Youth Olympic Festival in Utrecht, the Netherlands, in 2013, a prospective cohort study was conducted among 2272 participants from 49 countries. Forty-six cases of diseases were reported. Infection was the most commonly reported cause of illness (56.5% overall) with 43.5% patients reporting gastrointestinal symptoms and 26.1% respiratory symptoms [90].

Among the 2788 athletes in the Sochi 2014 Olympic Winter Games, a total of 249 illnesses were reported, resulting in an incidence of 8.9 illnesses per 100 athletes (95% CI 7.8 to 10.0). Most ill athletes suffered from respiratory symptoms (63.9%), followed by gastrointestinal symptoms (11%) with 58% caused by infections [91]. Only three cases of dengue fever were confirmed at the 2014 FIFA World Cup [92].

A recent multinational *Salmonella* outbreak was reported at an international youth ice hockey competition in Riga, Lativa in 2015 [93].

Among 11,274 athletes from 207 countries participating to the Rio de Janeiro 2016 Olympic Summer Games, 613 illnesses were reported, resulting in 5.4 illnesses per 100 athletes. Two hundred two individuals (47%) presented with respiratory symptoms and ($n = 131$; 21%) gastrointestinal symptoms with 56% ($n = 346$) due to infections [94]. Dengue case count was negligible and no case of Zika virus was detected [95, 96].

More recently, the Pyeong Ghang 2018 Winter Olympiad may have been hindered by a norovirus outbreak days before the event commenced. This outbreak affected mainly security staff for the games rather than athletes or visitors [97].

Conclusion

This review has some limitations. It was limited to articles written in English, which may have been a source of bias. There was a significant heterogeneity in the studies in relation to the populations studied, the clinical criteria for syndromic surveillance and the diagnostic methods applied.

Infectious diseases at MGs are dominated by respiratory tract and gastrointestinal infections. Meningitis outbreaks were also reported in some instances. Inter-human transmission of airborne diseases is favored by the temporal and spatial concentration of people. Because social distancing and contact avoidance are difficult measures to implement in the context of many MGs, individual preventive measures such as cough etiquette, the use of face mask and disposable handkerchiefs and hand hygiene may be recommended. Nevertheless, the effectiveness of these measures has been poorly investigated in the context of MGs. Most available data come from Hajj studies and results are contradictory [98].

Non-compliance with hygiene rules and inadequate sanitation are responsible for fecal–oral transmission of gastrointestinal infections. Public health measures aiming at provision of safe water and food supplies with rigorous quality control are likely the best way to limit the occurrence of gastrointestinal outbreaks at MGs. Planned organization by highly specialized teams of staff is a key element.

It should be noted that many MG-associated diseases are vaccine–preventable, including influenza, measles, mumps, meningococcal, and pneumococcal disease. Mandatory vaccination against meningitis has proven effective in the context of the Hajj. Measles and mumps and meningococcal vaccination status should certainly be verified and updated if needed in young people attending MGs. Influenza and pneumococcal vaccination should be recommended in at-risk individuals participating to MGs. This particularly applies to elderly people participating to religious MGs.

Finally, because of the evidence of circulation of resistant bacteria, at least in the Hajj context, rationalization of antibiotic consumption should be promoted.

Unfortunately, official recommendations for prevention at MGs are lacking, with the exception of the Hajj [99].

Compliance with Ethical Standards

Conflict of Interest Philippe Gautret and Van-Thuan Hoang declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Partie 1 :

Epidémiologie des infections respiratoires et gastrointestinales chez les pèlerins du Hajj

Préambule

Nous avons conduit une étude prospective afin d'évaluer l'épidémiologie des IRs et IGIs chez les pèlerins du Hajj de Marseille, de 2012 à 2018.

L'âge moyen des pèlerins variait de 57,3 à 61,7 ans. De 81,2% à 93,4% des pèlerins ont présenté des symptômes respiratoires au cours du séjour. En étudiant par biologie moléculaire (polymérase chain reaction (PCR) le portage d'agents pathogènes avant, pendant et après le séjour, nous avons observé une acquisition significative de virus respiratoires, surtout rhinovirus (27,7%), coronavirus communs (8,3%) et influenza virus (3,7%) (articles 2 et 3). Les virus étaient acquis par la majorité des pèlerins du Hajj peu après leur arrivée à La Mecque et la clairance virale est rapide (articles 2 et 3). Malgré certaines préoccupations concernant un risque d'épidémie de rougeole au Hajj, aucun cas n'a été identifié par le système de surveillance du Ministère Saoudien de la Santé. L'absence de circulation de la rougeole pendant le Hajj résulte très probablement de la structure d'âge des pèlerins ('âge moyen plus de 60 ans). Du fait de cet âge moyen élevé, une majorité de cette population est attendue être immunisée en raison d'une infection naturelle ou d'une vaccination antérieure (article 4). *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* et *Klebsiella pneumoniae* étaient les bactéries respiratoires les plus fréquemment acquises par les pèlerins (articles 3, 5 et 6). Nous avons observé une dynamique spécifique du portage de pathogènes au cours du séjour en Arabie Saoudite en réalisant des prélèvements séquentiels rapprochés (article 3). Une augmentation importante du portage du rhinovirus en parallèle avec *S. aureus* a été observée dès les 5ème et 6ème jours du pèlerinage, puis a diminué progressivement dans les échantillons suivants. Ce portage restait toujours plus élevé dans les échantillons post-Hajj que dans les échantillons pré-Hajj. En revanche, le portage d'*H. influenzae* a d'abord diminué les 5ème et 6ème ainsi que les 12ème et 13ème jours du pèlerinage, puis a augmenté dans les échantillons post-Hajj pour atteindre un taux de portage plus élevé que celui des échantillons pré-Hajj (article 3). En étudiant les interactions entre les agents pathogènes, nous avons observé que le portage du rhinovirus et le portage de *S. aureus* étaient positivement associés. Il en va de même pour *H. influenzae* et *Moraxella catarrhalis* (article 3). Les génotypes d'*H. influenzae* acquis différaient de ceux présents avant le Hajj. Nous avons observé une grande biodiversité et une absence de clonalité d'*H. influenzae* parmi les pèlerins français (article 7). La prévalence d*'H. influenzae* avant le Hajj montrait des variations importantes selon les années : 50,0% en 2014, 0,9% en 2015, 2,8% en 2016, 52,8% en 2017 et 35,5% en 2018. La prévalence post-Hajj restait constamment élevée, variant de 41,0 à 67,8%. Ces résultats suggèrent que la circulation d'*H. influenzae* au Hajj était permanente au cours de la période d'étude et pose la question de l'intérêt de la vaccination (article 8).

Nous avons observé des associations significatives entre le portage de certains pathogènes, la présence de pathologies chroniques, les caractéristiques démographiques des pèlerins, l'utilisation de mesures préventives et la symptomatologie respiratoire. Ainsi, la présence d'au moins un symptôme respiratoire était deux fois et cinq fois plus fréquente chez les porteurs de rhinovirus ou avec une co-infection par *H. influenzae* - *S. pneumonie*, respectivement. La toux était deux fois plus fréquente chez les pèlerins souffrant de maladies respiratoires chroniques et chez ceux porteurs de *H. influenzae* - *K. pneumoniae* associés. Les pèlerins qui toussaient étaient également plus susceptibles d'utiliser des mouchoirs jetables. Enfin, le syndrome pseudo-grippal était plus fréquent chez les femmes, chez les pèlerins atteints de maladies respiratoires chroniques et chez ceux qui étaient porteurs de *S. aureus* ou d'une association de virus et de bactéries. La vaccination antigrippale était associée à une diminution de la prévalence de syndrome pseudo-grippal (article 6). Aucune association significative n'a été observée entre les symptômes et la vaccination contre les maladies invasives à pneumocoque. Mais ce vaccin diminuait significativement le portage de *S. pneumoniae* (articles 5 et 6).

Concernant l'appareil digestif, 18,6% des pèlerins présentaient au moins un symptôme gastro-intestinal. La diarrhée était le symptôme le plus fréquent (13,8%) (article 9). Au total 36,4% des pèlerins ont acquis au moins un agent pathogène entérique pendant le pèlerinage. *Escherichia coli* entéropathogène (EPEC) (17,6%) et *E. coli* Enteroaggregative (EAEC) (14,4%) étaient les agents pathogènes les plus fréquemment acquis. Par ailleurs, une acquisition d'*E. coli* Entérohémorragique (EHEC), *Shigella* et *Salmonella*, était observée mais à des prévalences moins élevées. L'acquisition d'adénovirus et de rotavirus était rare alors que celle du norovirus était de 2,4%. Une proportion de 6,4% des pèlerins ont acquis plus d'un agent pathogène entérique (article 9). Aucun échantillon (avant ou après le Hajj) n'était positif pour l'astrovirus, *Entamoeba histolytica*, C*ryptosporidium spp*., *Campylobacter jejuni* (article 9) ou *Vibrio* spp (article 10).

Le sexe féminin était significativement associé avec la présence de symptômes gastro-intestinaux. L'acquisition d'EHEC était associée à un risque quatre fois plus élevé de symptômes gastro-intestinaux et de diarrhée. Un lavage des mains plus fréquent était associé à la présence de symptômes gastro-intestinaux et de diarrhée (article 9).

Article 2 :

Acquisition of respiratory viruses and presence of respiratory symptoms in French pilgrims during the 2016 Hajj: A prospective cohort study.

Hoang VT, Sow D, Dogue F, Edouard S, Drali T, Yezli S, Alotaibi B, Raoult D, Parola P, Pommier de Santi V, Gautret P.

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Acquisition of respiratory viruses and presence of respiratory symptoms in French pilgrims during the 2016 Hajj: A prospective cohort study

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ABSTRACT

Background: Viral respiratory tract infections are frequent among Hajj pilgrims. However, it is still not known whether viruses are responsible for the symptoms observed in sick pilgrims or whether they only colonize sick and asymptomatic pilgrims.
Patients and methods: A prospective cohort study was conducted among French Hajj pilgrims in 2016. Medical follow-up and systematic nasal swabbing were performed pre- and post-Hajj. Additional samples were obtained per-Hajj, at symptom onset in ill pilgrims. Viruses were identified using the BioFire FilmArray® Respiratory multiplex qualitative PCR panel.
Results: 109 pilgrims were included. 83.5% presented respiratory symptoms during Hajj and 39.5% were still symptomatic on return. 5.5% of pre-Hajj, 95.2% of per-Hajj (at symptom onset) and 46.5% of post-Hajj samples tested positive ($p < 0.0001$). Acquisition rates of rhinovirus/enterovirus, coronavirus 229E and influenza A virus were respectively 38.6%, 19.8% and 2.0%. Although rhinovirus/enterovirus, coronavirus 229E and influenza A clearance were respectively 70.6%, 71.4% and 100% on return, overall virus carriage proportion on return was 75.0% in pilgrims with influenza-like illness and 44.0% in those who have never experienced this symptoms or resolved it (OR = 4.05, 95% CI [1.02–16.02]).
Conclusions: Viruses likely play some role in the pathogenesis of the respiratory tract infections at the Hajj. Point of care-rapid multiplex PCR assays are valuable diagnosis tools in this context when used at respiratory symptom onset or soon after.

1. Introduction

Hajj pilgrimage has long been associated with enhanced transmission of infectious disease agents. Epidemics of cholera [1] and bacterial meningitis [2,3] are emblematic examples of the potential for international spread of life-threatening infections at the Hajj, given its international component with pilgrims originating from up to 180 countries and gathering in Mecca before returning to their home country [4]. More recently, respiratory tract infections at the Hajj have attracted the attention of the medical community because of the frequency of respiratory symptoms among pilgrims consulting at primary health care facilities or hospitalized in Saudi Arabia [5]. Cohort studies conducted in populations of pilgrims originating from different countries reported that a substantial proportion of pilgrims suffer from respiratory symptoms during their stay in Saudi Arabia [6]. On the other hand, numerous PCR-based studies have demonstrated the frequent acquisition of respiratory viruses following participation in Hajj [7,8]. Fortunately, SARS-CoV and MERS-Cov did not affect Hajj pilgrims so far [9,10]. However, rhinovirus, non-MERS coronaviruses and influenza viruses are commonly isolated from both asymptomatic returning pilgrims and pilgrims with acute respiratory symptoms [7,8]. Due to the lack of detailed clinical information in many Hajj studies and the high

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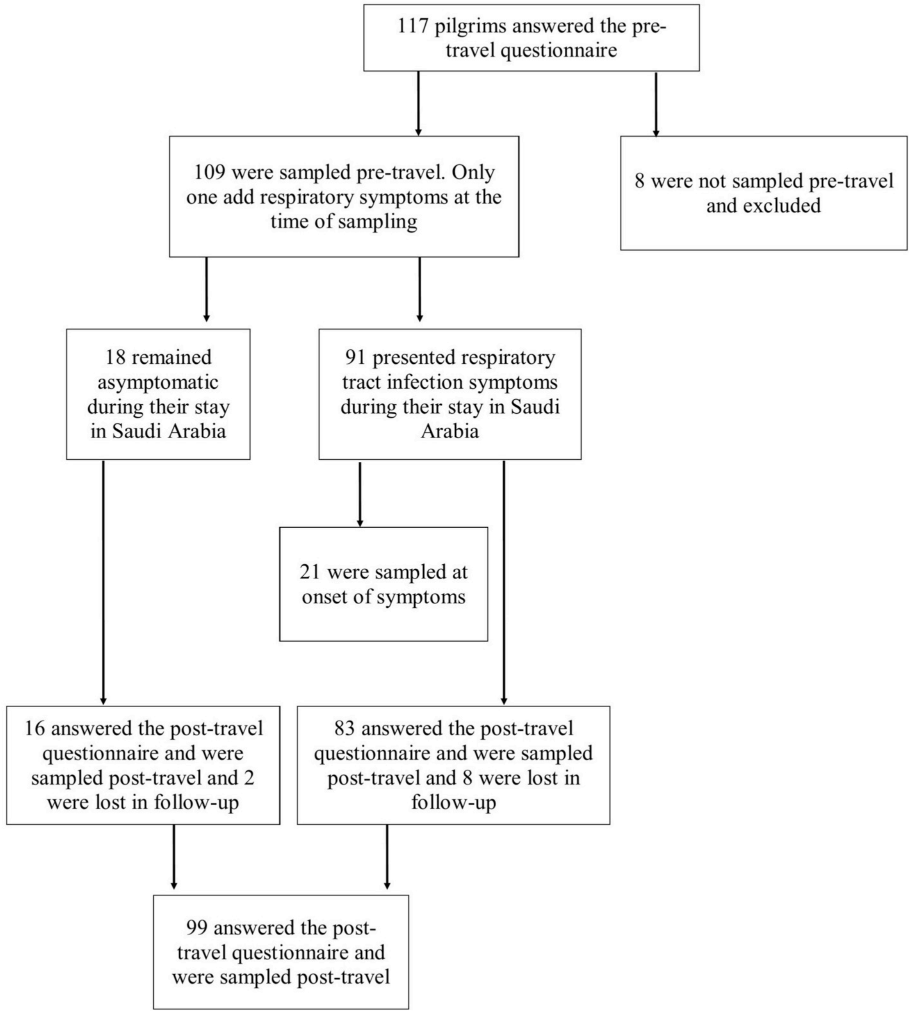

Fig. 1. Flow diagram of study participants.

sensitivity of PCR tools, the contribution of viruses to observed symptoms remains unknown.

The objective of the study was to evaluate the nasal carriage of respiratory viruses before and after traveling to the Hajj, and to investigate a possible relationship between viral carriage and respiratory symptoms with a careful clinical follow-up in a cohort of French Hajj pilgrims departing from Marseille.

2. Materials and methods

2.1. Study population

Fig. 1 details the procedure of this study. The study was conducted among French Hajj pilgrims traveling together to Mecca, from August 27th to September 20th, 2016, with one specialized travel agency in Marseille. Pilgrims older than 18 years were included on a voluntary basis, and participants were asked to sign a written consent form. Upon inclusion, the participants were questioned using a standardized pre-travel questionnaire that included demographic and chronic disease data and vaccination status. Health issues were recorded by a medical doctor who travelled with the group of pilgrims. We considered that participants suffered RTIs if they presented with cough and/or rhinitis and/or sore throat. Influenza like illness (ILI) was defined as the association of cough, sore throat and fever [11]. Each individual was classified in one of the three categories: i. asymptomatic (those who did not experience any respiratory symptoms during the entire stay in Saudi Arabia), ii. resolved respiratory tract infection (RTI) (those who experienced respiratory symptoms including cough and/or sore throat and/or rhinitis and/or voice failure during travel, but who recovered at the time of return to France and iii. ongoing RTI those with ongoing respiratory symptoms at the time of leaving Saudi Arabia).

The protocol was approved by our Institutional Review Board (July 23, 2013; reference No. 2013-A00961-44). It was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants gave a written informed consent.

2.2. Sample collection

The procedure included a systematic nasal swab 10 days before departing from France (pre-Hajj specimens) and just 1 day (on September 19th, 2016) before leaving the KSA (Kingdom of Saudi Arabia) (post-Hajj specimens) We previously showed that nasal swabs are more sensitive than pharyngeal swabs in detecting respiratory viruses in Hajj pilgrims, using real-time reverse transcriptase-polymerase chain reaction methods [12]. Nasal swabs were also performed among symptomatic pilgrims who spontaneously consulted the accompanying medical doctor at the time of onset (per-Hajj specimens). No per-Hajj sample was collected among asymptomatic pilgrims. Samples were collected using commercial rigid cotton-tipped swab applicators (Medical Wire & Equipment, Wiltshire, UK) which were inserted in the anterior nose and then placed in viral transport media

(Sigma Virocult®). This standardized procedure was previously explained to the pilgrims by the investigators. The swabs were stored at 20 °C before being transported to the Marseille laboratory for storage in a freezer at −80 °C within 48 h of collection for pre- and post-Hajj samples. Per-Hajj specimen were kept at 20 °C until the return to France.

2.3. Identification of respiratory pathogens

The analyses were carried out in Marseille, following return (thus results were not available during the stay in Saudi Arabia) with a validated multiplex qualitative PCR method [13]. The BioFire respiratory panel (BFRP, BioFire) includes the following virus targets: rhinovirus/enterovirus, adenovirus, human coronavirus (229E, HKU1, NL63 and OC43), human metapneumovirus, influenza virus A and B, parainfluenza virus (1, 2, 3 and 4) and respiratory syncytial virus. Three bacteria are targeted in the test including *Bordetella pertussis, Chlamydia pneumoniae and Mycoplasma pneumoniae.* Acquisition of pathogens was defined as the absence of a given pathogen in pre-Hajj samples and the presence of this pathogen in per- and or post-Hajj samples.

2.4. Statistical analysis

The Pearson's Chi-square test and Fisher's exact test, as appropriate, were applied to analyze the categorical variables. To evaluate the potential acquisition of respiratory viruses in Saudi Arabia, we used the McNemar's Test to compare their percentage before leaving France and in Saudi Arabia. Percentages and odds ratio (OR) with 95% confidence interval (CI) estimations and comparisons were carried out using the STATA 11.1 (Copyright 2009 StataCorp LP, http://www.stata.com). P values of 0.05 or less were considered significant.

3. Results

3.1. Demographics, and respiratory symptoms

A total of 109 pilgrims were included on departure from France. The median age was 63 years (interquartile: 55–69, range: 23–96), and 46.8% were males. 11 (10.1%) individuals reported a history of chronic respiratory disease and only one pilgrim had respiratory symptoms before leaving Marseille. At total, 26/109 (23.8%) pilgrims received influenza vaccination during the past 12 months, but only 2/109 (1.8%) reported having been vaccinated against pneumococcal (PCV-13) in the last 5 years. A total of 91 (83.5%) pilgrims presented at least one respiratory symptom during their stay in Saudi Arabia with cough, rhinitis and sore throat being most frequent (Table 1). The median time between arrival to Saudi Arabia and symptom onset was 9 days (range: 0–18 days). The majority of pilgrims had their onset of symptoms during their stay in Mecca and Mina (Fig. 2). Twenty-five (22.9%) pilgrims had influenza like illness. 30 (27.5%) pilgrims took antibiotics for the purpose of respiratory tract infection symptoms, based on the clinical judgment of the accompanying doctor and none was hospitalized. Forty-three (39.5%) were still symptomatic at the time of leaving Saudi Arabia.

Table 1
Clinical symptoms in 109 participants.

Symptoms	n (%)
At least one respiratory symptom	91 (83.5)
Cough	83 (76.2)
Expectoration	39 (35.8)
Dry cough	44 (40.4)
Rhinitis	62 (56.9)
Sore throat	61 (56.0)
Voice failure	39 (35.8)
Dyspnea	26 (23.9)
Fever	28 (25.7)
Myalgia	40 (36.7)
Conjunctivitis	3 (2.8)
Influenza like illness	25 (22.9)
Time between arrival in Kingdom of Saudi Arabia and onset of respiratory symptoms	
Median	9
Interquartile	7–12
Range	0–18

3.2. Acquisition of virus and bacteria carriage

Nasal swabs were obtained from 109 pilgrims (100%) before traveling to Saudi Arabia, from 21 patients at symptom onset in Saudi Arabia and from 99 (90.8%) pilgrims before leaving Saudi Arabia for France. Among these, 99 individuals (90.8%) underwent paired samples allowing the calculation of acquisition rates. The proportion of pilgrims positive for each potential pathogen detected is presented in Table 2. Of the pre-Hajj samples, 6 (5.5%) were positive, while a pathogen was found in 20 (95.2%) per-Hajj samples and in 46 (46.5%) post-Hajj samples ($p < 0.0001$). Overall, the acquisition rate of at least one pathogen during the stay in Saudi Arabia was 40.0%. The proportion of pilgrims positive for rhinovirus/enterovirus and coronavirus 229E were significantly higher in post-Hajj samples, compared to pre-Hajj samples, with acquisition rates of respectively 38.6% and 19.8%. Multiple infections were frequent accounting for 50.0% positive per-Hajj samples and 17.4% of positive post-Hajj samples with rhinovirus/enterovirus-coronavirus 229E mixed infection the most common (Table 3). No case was positive for the three bacteria tested.

3.3. Virus carriage according to respiratory symptoms

Among 108 asymptomatic pilgrims sampled in France before travel, 5/108 (4.6%) were positive for rhinovirus/enterovirus. The only pilgrim suffering respiratory symptoms before travel was positive for rhinovirus/enterovirus.

Among the 21 pilgrims sampled at symptom onset in Saudi Arabia, 20 tested positive for at least one virus with high proportion of rhinovirus/enterovirus (81.0%), coronavirus 229E (33.0%) and influenza A (23.8%) (Table 2). Seven of the 21 symptomatic pilgrims had ILI and their carriage rate did not significantly differ from that of other ill pilgrims (data not shown). Of the 21 pilgrims sampled at the time of symptom onset, 19 were resampled before leaving Saudi Arabia, allowing the proportion of individuals who allowed their viral transport back to the country to be calculated. The mean time between onset of symptoms and testing on leaving KSA was 15.4 days (ranging 8–19). Rhinovirus/enterovirus carriage was cleared in 70.6.1% cases, coronavirus 229E carriage in 71.4% cases and influenza virus A carriage in 100% cases, while the majority of cases were still symptomatic (71.4%) (Fig. 3).

Comparison of virus carriage at the time of return, according to the presence of respiratory symptoms at sampling time showed a highest proportion of virus positivity (at least one virus) in symptomatic patients (57.9%) compared to those who have never experienced cough or who resolved it (39.3%), but the difference was not significant (OR = 2.11, 95% CI [0.93–4.83]) (Table 4). However, carriage of at least one virus on return was significantly higher in patients with ongoing ILI symptoms (75.0%) compared to patients without ILI symptoms or with resolved ILI symptoms (42.5%), OR = 4.05, 95%CI [1.02–16.02] (Table 5). Breakdown by virus type; however, did not show statistically significant variations, although their percentage of positivity was higher in pilgrims presenting with at least one respiratory symptom or ILI symptoms at the time of sampling, in comparison with those who did not. Comparison of virus carriage in patients who experienced symptoms during the Hajj (ongoing and resolved) and in patients who were asymptomatic at any time did not show significant differences.

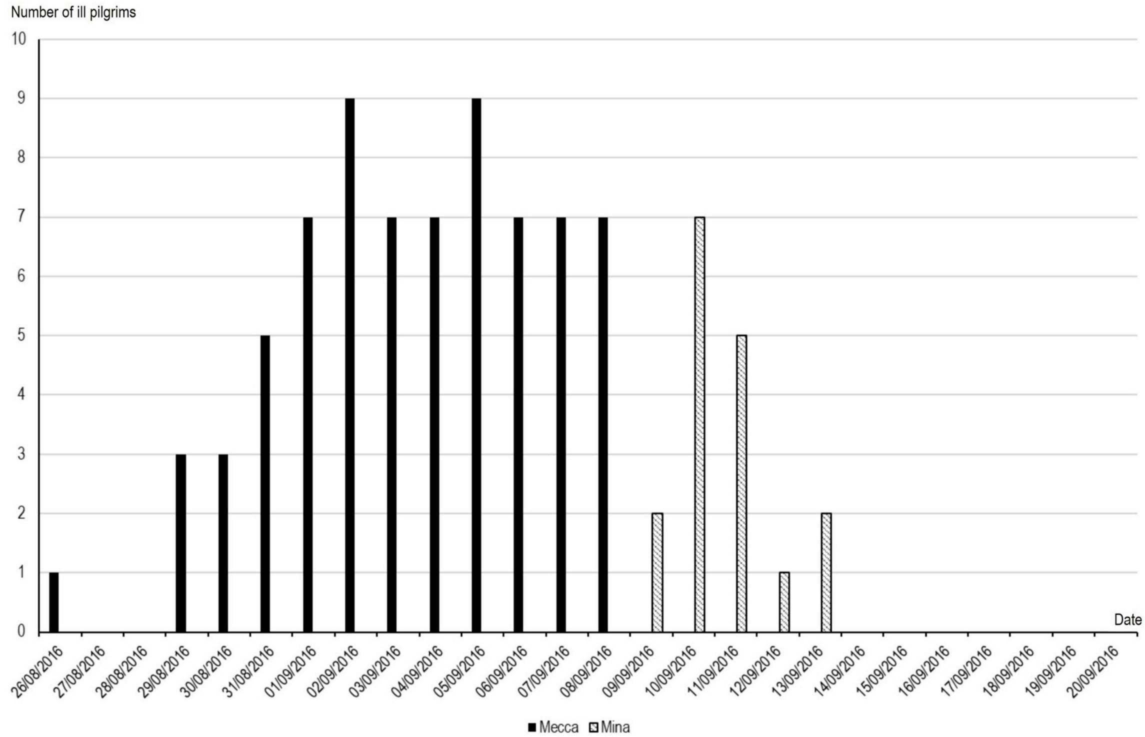

Fig. 2. Respiratory symptom onset date in 91 ill pilgrims.

Table 2
Frequency of pathogens detected in pre-, per- and post-Hajj samples.

	Pre-Hajj (N = 109) Number of individuals with positive sample (percentage)	Per-Hajj (N = 21) Number of individuals with positive sample (percentage)	Post-Hajj (N = 99) Number of individuals with positive sample (percentage)	Acquisition rate (pre- versus post-Hajj) (N = 99)	p value (pre- versus post-Hajj)*
Rhinovirus/enterovirus	6 (5.5)	17 (81.0)	32 (32.3)	28 (38.6)	< 0.0001
Adenovirus	0	0	3 (3.0)	3 (3.0)	0.08
Coronavirus 229E	0	7 (33.3)	15 (15.2)	15 (19.8)	< 0.0001
Coronavirus HKU1	0	1 (4.8)	1 (1.0)	1 (1.0)	0.32
Coronavirus NL63	0	1 (4.8)	1 (1.0)	1 (1.0)	0.32
Coronavirus OC43	0	3 (14.3)	2 (2.0)	2 (2.0)	0.15
Metapneumovirus	0	1 (4.8)	0	0 (0)	–
Influenza A	0	5 (23.8)	2 (2.0)	2 (2.0)	0.15
Influenza B	0	0	0	0	–
Parainfluenza virus type 1	0	0	0	0	–
Parainfluenza virus type 2	0	0	0	0	–
Parainfluenza virus type 3	0	0	0	0	–
Parainfluenza virus type 4	0	0	0	0	–
Respiratory syncitial virus	0	0	0	0	–
At least one virus	6 (5.5)	20 (95.2)	46 (46.5)	40 (40.0)	< 0.0001

* McNemar's Test.

4. Discussion

We observed both a high frequency of respiratory symptoms during the pilgrims' stay in KSA (76.2% cough and 22.9% ILI) and a significant acquisition of viral nasal carriage (40.0%), mostly due to rhinovirus/enterovirus and coronavirus 229E. This corroborates the results obtained from most recent studies conducted among French pilgrims by our team and among pilgrims from other nationalities [14–22]. The epidemic curves of onset of symptoms and virus carriage in samples obtained at onset of symptoms suggest an early acquisition of respiratory virus during the initial stay in Mecca, probably due to inter-human transmission as attested by the bi-modal pattern of the curves during this period of time. Crowded conditions at Al-Haram Mosque during the rituals, with up to 8 individuals per m^2 recorded close to the Kaaba [23], is likely to play a significant role in this process. Asymptomatic carriage of rhinovirus/enterovirus and coronaviruses was frequently observed when tested in returning pilgrims. Nevertheless, overall viral carriage in patients with ILI on return (75.0%) was significantly higher than in individuals without ILI (42.5%), which suggests that viruses play a role in the pathogenesis of the RTIs. Viruses were detected in almost all 21 pilgrims sampled at symptom onset, which reinforces this view. However, while most of these 21 pilgrims were still symptomatic at the post-Hajj sampling time; only a low proportion of those testing positive at onset remained positive in post-Hajj samples two weeks after first symptoms. These results, although based on small numbers of ill pilgrims clearly demonstrate that sampling at the time of leaving KSA results in underestimation of viral carriage in relation with symptoms during the stay because the majority of ill pilgrims already cleared their viral infection despite persisting symptoms, as observed in our survey. Since obtaining respiratory samples at onset of symptoms is challenging in the context of longitudinal cohort survey at the Hajj, most studies conducted so far were

Table 3
Frequency of carriage of multiple pathogen combinations.

	Pre-Hajj n (%) N = 6	Per-Hajj n (%) N = 20	Post-Hajj n (%) N = 46
Rhinovirus/enterovirus + adenovirus	–	–	3 (6.5)
Rhinovirus/enterovirus + coronavirus 229E	–	5 (25.0)	4 (8.7)
Rhinovirus/enterovirus + coronavirus HKU1	–	1 (5.0)	1 (2.2)
Rhinovirus/enterovirus + coronavirus NL63	–	1 (5.0)	–
Rhinovirus/enterovirus + coronavirus OC43	–	3 (6.0)	1 (2.2)
Rhinovirus/enterovirus + metapneumovirus	–	1 (2.0)	–
Rhinovirus/enterovirus + influenza A	–	2 (4.0)	1 (2.2)
Coronavirus 229E + Influenza A	–	3 (6.0)	–
Rhinovirus/enterovirus + adenovirus + coronavirus 229E	–	–	1 (2.2)
Rhinovirus/enterovirus + adenovirus + coronavirus OC43	–	–	1 (2.2)
Rhinovirus/enterovirus + coronavirus 229E + OC43	–	1 (2.0)	–
Rhinovirus/enterovirus + coronavirus 229E + OC43 + Influenza A	–	1 (2.0)	–
Total samples with multiple pathogens detected	–	10 (50.0)	8 (17.4)

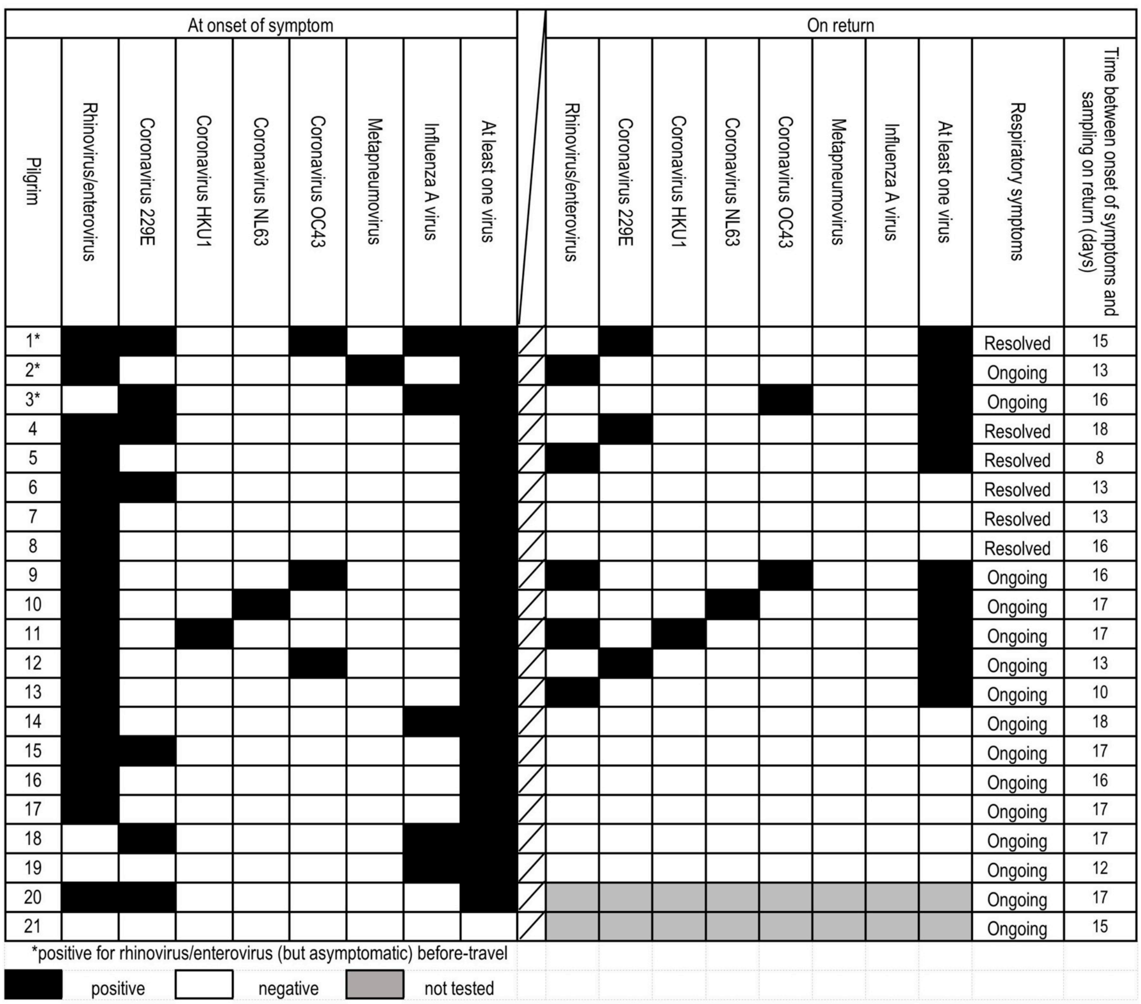

Fig. 3. Respiratory virus carriage among pilgrims sampled at symptom onset and on return.

based on systematic samples obtained at the time of return, days after the onset of symptoms occurred. Such a design evaluating viral carriage at return provides useful on the potential for respiratory viruses to spread upon return to the country of origin, assuming that some of the viruses detected by PCR are viable. It is however less appropriate for retrospectively investigating the responsibility of viruses in respiratory symptoms experienced during the stay in Saudi Arabia, given the rapid virus clearance. This was particularly obvious in our survey with a proportion of influenza A virus positivity of 2.0% in pilgrims screened on return, regardless of symptoms contrasting with 20% in pilgrims who actively consulted our medical investigator sampled at respiratory symptom onset. Studies conducted in different countries from 2009 through 2015 with the aim of screening influenza virus by PCR among returning pilgrims regardless of symptoms, reported a mean influenza carriage rate of 3.4%, ranging 0.4–7.8% [14,15,19,23–26], which is in line with the results reported here (1.9%). When similar screening studies were conducted, enrolling only pilgrims with respiratory symptoms on returning to their home country, a higher proportion of

Table 4
Frequency of pathogens in post-Hajj samples according to respiratory symptoms at the time of sampling.

		Asymptomatic (N = 16)	Resolved symptoms* (N = 45)	Ongoing symptoms* (N = 38)	OR [95%CI], P value (ongoing symptoms versus asymptomatic or resolved symptoms)	OR [95%CI], P value (ongoing or resolved symptoms versus asymptomatic)
Rhinovirus/ enterovirus	Yes	5 (31.3)	13 (28.9)	14 (36.8)	1.39 [0.59–3.29], P = 0.45	1.06 [0.34–3.36], P = 0.92
	No	11 (68.7)	32 (71.1)	24 (63.2)		
Adenovirus	Yes	0 (0)	1 (2.2)	2 (5.3)	3.33 [0.30–38.08], P = 0.33	–
	No	16 (100)	44 (97.8)	36 (94.7)		
Coronavirus 229E	Yes	0 (0)	8 (17.8)	7 (18.4)	1.50 [0.49–4.53], P = 0.48	–
	No	16 (100)	37 (82.2)	31 (81.6)		
Coronavirus HKU1	Yes	0 (0)	0 (0)	1 (2.6)	–	–
	No	16 (100)	45 (100)	37 (97.4)		
Coronavirus NL63	Yes	0 (0)	0 (0)	1 (2.6)	–	–
	No	16 (100)	45 (100)	37 (97.4)		
Coronavirus OC43	Yes	0 (0)	0 (0)	2 (5.3)	–	–
	No	16 (100)	45 (100)	36 (94.7)		
Influenza A	Yes	0 (0)	1 (2.2)	1 (2.6)	1.62 [0.10–26.72], P = 0.74	–
	No	16 (100)	44 (97.8)	37 (97.4)		
At least one virus	Yes	5 (31.3)	19 (42.2)	22 (57.9)	2.11 [0.93–4.83], P = 0.07	2.14 [0.69–6.72], P = 0.19
	No	11 (68.7)	26 (57.8)	16 (42.1)		

OR: odds ratio, CI: confidence interval.
* At least one respiratory symptom (cough, sore throat, rhinitis).

Table 5
Frequency of pathogens in post-Hajj samples according to influenza-like illness symptoms at the time of sampling.

		No ILI* (N = 75)	Resolved ILI* (N = 12)	Ongoing ILI* (N = 12)	OR [95%CI], P value (ongoing ILI versus no ILI or resolved ILI)	OR [95%CI], P value (ongoing or resolved ILI versus no ILI)
Rhinovirus/enterovirus	Yes	23 (30.7)	3 (25.0)	6 (50.0)	2.34 [0.70–7.96], P = 0.17	1.35 [0.52–3.55], P = 0.53
	No	52 (69.3)	9 (75.0)	6 (50.0)		
Adenovirus	Yes	3 (4.0)	0 (0)	0 (0)	–	–
	No	72 (96.0)	12 (100)	12 (100)		
Coronavirus 229E	Yes	10 (13.3)	1 (8.3)	4 (33.3)	3.45 [0.89–13.41], P = 0.07	1.71 [0.52–5.62], P = 0.38
	No	65 (86.7)	11 (91.7)	8 (66.7)		
Coronavirus HKU1	Yes	0 (0)	0 (0)	1 (8.3)	–	–
	No	75 (100)	12 (100)	11 (91.7)		
Coronavirus NL63	Yes	1 (1.3)	0 (0)	0 (0)	–	–
	No	74 (98.7)	12 (100)	12 (100)		
Coronavirus OC43	Yes	2 (2.7)	0 (0)	0 (0)	–	–
	No	73 (97.3)	12 (100)	12 (100)		
Influenza A	Yes	2 (2.7)	0 (0)	0 (0)	–	–
	No	73 (97.3)	12 (100)	12 (100)		
At least one virus	Yes	33 (44.0)	4 (33.3)	9 (75.0)	4.05 [1.02–16.02], P = 0.05	1.50 [0.59–3.79], P = 0.39
	No	42 (56.0)	8 (66.7)	3 (25.0)		

OR: Odds ratio, CI: confidence interval.
* ILI: influenza-like illness (cough, sore throat and fever).

10.6% influenza carriage was observed, ranging 3.0–14.5% when we found a slightly lower proportion of 2.1% in this study [27–31]. Finally, in studies conducted in sick pilgrims seen in Saudi or European hospitals for suspected MERS-Cov infection and therefore suffering from more severe respiratory symptoms, the percentage of confirmed influenza infection was 34.9%, ranging from 12.0 to 71.4% [21,32–35].

Our study has some limitations. First, it was conducted among a small number of pilgrims during one season of Hajj, and our results cannot be extrapolated to all pilgrims. Secondly, we were able to collect respiratory samples at symptom onset in only one fourth of patients who reported suffering respiratory symptoms during the study period. This low proportion is due to the nature of the recruitment since sampling at onset of symptoms was not systematic but performed in patients who spontaneously consulted the accompanying doctor at the early beginning of their illness. The sample type used is not FDA cleared or CE marked and the BFRP, BioFire performance characteristics using nasal swabs instead of nasopharyngeal swabs have not been established.

5. Conclusion

Viruses are acquired by the vast majority of Hajj pilgrims soon after their arrival in Mecca and likely responsible for respiratory symptoms, notably ILI. Viral clearance is rapid. Point of care-rapid multiplex PCR assays are valuable diagnosis tools for Hajj patients when used at respiratory symptom onset or soon after. In particular, they allow the detection of the influenza virus, which is particularly interesting because it has practical consequences for the early prescription of antivirals in people at risk. These tests are also useful for ruling-out MERC-CoV infection and deciding about isolation measure lifting.

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Article 3 :

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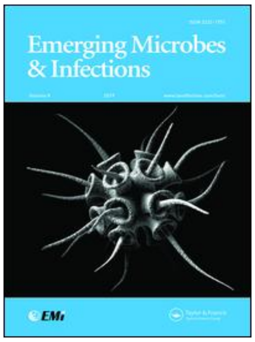

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The dynamics and interactions of respiratory pathogen carriage among French pilgrims during the 2018 Hajj

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ABSTRACT

We conducted this study to describe the dynamics of the acquisition of respiratory pathogens, their potential interactions and risk factors for possible lower respiratory tract infection symptoms (LRTI) among French pilgrims during the 2018 Hajj. Each participant underwent four successive systematic nasopharyngeal swabs before and during their stay in Saudi Arabia. Carriage of the main respiratory pathogens was assessed by PCR. 121 pilgrims were included and 93.4% reported respiratory symptoms during the study period. The acquisition of rhinovirus, coronaviruses and *Staphylococcus aureus* occurred soon after arrival in Saudi Arabia and rates decreased gradually after days 5 and 6. In contrast, *Streptococcus pneumoniae* and *Klebsiella pneumoniae* carriage increased progressively until the end of the stay in Saudi Arabia. *Haemophilus influenzae* and *Moraxella catarrhalis* carriage increased starting around days 12 and 13, following an initial clearance. Influenza viruses were rarely isolated. We observed an independent positive mutual association between *S. aureus* and rhinovirus carriage and between *H. influenzae* and *M. catarrhalis* carriage. Dual carriage of *H. influenzae* and *M. catarrhalis* was strongly associated with *S. pneumoniae* carriage (OR = 6.22). Finally, our model showed that *M. catarrhalis* carriage was negatively associated with *K. pneumoniae* carriage. Chronic respiratory disease was associated with symptoms of LRTI. *K. pneumoniae, M. catarrhalis-S. aureus* and *H. influenzae*-rhinovirus dual carriage was associated with LRTI symptoms. Our data suggest that RTIs at the Hajj are a result of complex interactions between a number of respiratory viruses and bacteria.

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KEYWORDS Hajj; pilgrims; respiratory tract infections; pathogen interaction

Introduction

Each year, an increasing number of people travel to the Kingdom of Saudi Arabia (KSA) for the Hajj and Umrah pilgrimages, which attract around 10 million pilgrims annually from more than 180 countries. More than two million pilgrims from outside Saudi Arabia participated in the Hajj pilgrimages in 2017 [1]. Each year, about 2,000 pilgrims from Marseille, France, participate in the Hajj [2]. The event presents major challenges for public health, including inter-human transmission of infectious diseases, notably respiratory tract infections (RTIs), due the crowded conditions experienced by pilgrims [1]. In a recent study on morbidity and mortality among Indian Hajj pilgrims, infectious diseases represented 53% of outpatient diagnoses, with RTIs and gastroenteritis being the most common [3]. Between 69.8% and 86.8% of French pilgrims presented RTI symptoms during the Hajj [4]. A recent literature review suggested that etiology of RTIs at the Hajj is complex; several studies showed a significant acquisition of respiratory pathogens by pilgrims following participation in the Hajj in both symptomatic and asymptomatic individuals [5]. In a systematic review of 31 studies, Al-Tawfiq et al. showed that human rhinovirus (HRV) and influenza viruses were the most common viral respiratory pathogens isolated from ill Hajj pilgrims [6]. In addition, human non-MERS coronaviruses (HCoV) were also a common cause of RTIs during the event [7]. On the other hand, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus* were shown to be the most commonly acquired respiratory bacteria at the Hajj [5].

RTIs are caused by the antagonistic and synergistic interactions between upper respiratory tract viruses

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and predominant bacterial pathogens [8]. Pathogens are usually studied individually, although in their natural environment they often compete or coexist with multiple microbial species. Similarly, the diagnosis of infections often proceeds via an approach which assumes a single agent etiology [9]. Nevertheless, complex interactions occur between the different infectious microorganisms living in the same ecological niche and mixed infections are frequent [10].

A better understanding of polymicrobial interactions in the nasopharynx among Hajj pilgrims is important for many reasons. Carriage of more than one pathogen is common among Hajj pilgrims, whether or not they present with respiratory symptoms [7]. Colonization is the initial step in the disease process [11]. Nasopharyngeal colonization is likely to be a reservoir for respiratory pathogens resulting in interhuman transmission between pilgrims during close contact experienced during the Hajj ritual. Furthermore, antibiotic use or vaccines, which target specific pathogen species, may alter polymicrobial interactions in the nasopharynx and have unanticipated consequences [12,13]. To our knowledge, the dynamics and interaction between the main respiratory pathogens acquired during the Hajj pilgrimage have not been specifically investigated, to date. Risk factors for possible lower respiratory tract infection (LRTI) symptoms at the Hajj have not been studied.

We conducted this study among French pilgrims during the 2018 Hajj, to describe the dynamics of the acquisition of respiratory pathogens and their potential interactions. In addition, we investigated risk factors for possible LRTI symptoms.

Materials and methods

Participants and study design (Figure 1)

Pilgrims travelling to Mecca, Saudi Arabia during the 2018 Hajj from Marseille, France, were recruited through a private specialized travel agency. Potential adult participants were invited to participate in the study. They were included and followed-up by two bilingual (Arabic and French) medical doctors who travelled with the group. All participants departed to KSA on the same date, were housed in the same accommodation during their stay and performed the rituals together. Upon inclusion, before departing from France, pilgrims were interviewed using a standardized pre-Hajj questionnaire that collected information about demographic characteristics, medical history and immunization status. Pilgrims were considered to have been immunized against influenza when they had been vaccinated within the past year and until before 10 days of the date of travel. Pilgrims were considered to be immune to invasive pneumococcal disease (IPD) when they had been vaccinated with the 13-valent conjugate pneumococcal vaccine (PCV-13) in the past five years [14,15]. A post-Hajj questionnaire was completed two days before the pilgrims' return to France. Clinical data, antibiotic intake and information on compliance with face masks use as well as hand washing, the use of hand gel disinfectant and disposable handkerchiefs was collected. To evaluate the dynamic and interaction of respiratory pathogens during the Hajj, all pilgrims underwent four successive systematic nasopharyngeal swabs at different times: pre-travel, five to six days post arrival, 12–13 days post arrival and just prior to leaving KSA (post-Hajj). The Hajj rituals took place from 19–24 August. Influenza-like illness (ILI) was defined as the presence of cough, sore throat and subjective fever [16]. Possible LRTI was defined by presence of productive cough without nasal or throat symptoms; febrile productive cough; dyspnea or febrile dyspnea [17]. Based on the WHO classification, "underweight" was defined as having a body mass index (BMI) below 18.5, "normal" corresponded to a BMI between 18.5 and 25, "overweight" corresponded to a BMI ≥25, and "obese" referred to those with a BMI ≥30 [18].

Respiratory specimen

Nasopharyngeal swabs were obtained from each pilgrim, transferred to Sigma-Virocult® medium and stored at −80°C until processing. The sampling was done by the doctors accompanying the group, in a standardized way (3 cm in the nostril, 5 turns; post wall of the pharynx, 5 streaks).

Identification of respiratory pathogens

The RNA and DNA were extracted from the samples using the EZ1 Advanced XL (Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendations. All quantitative real-time PCR were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA).

One-step simplex real-time quantitative RT–PCR amplifications were performed using Multiplex RNA Virus Master Kit (Roche Diagnostics, France) for influenza A, influenza B, HRV and internal controls MS2 phage [19]. HCoV was detected by one-step duplex quantitative RT–PCR amplifications of HCoV/ HPIV-R Gene Kit (REF: 71-045, BioMérieux, Marcy l'Etoile, France), according to the manufacturer's recommendations.

Real-time PCR amplifications were carried out using LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. The SHD gene of *H. influenzae*, phoE gene of *Klebsiella pneumoniae*, nucA gene of *S. aureus*, lytA CDC gene of *S. pneumoniae* and copB gene of

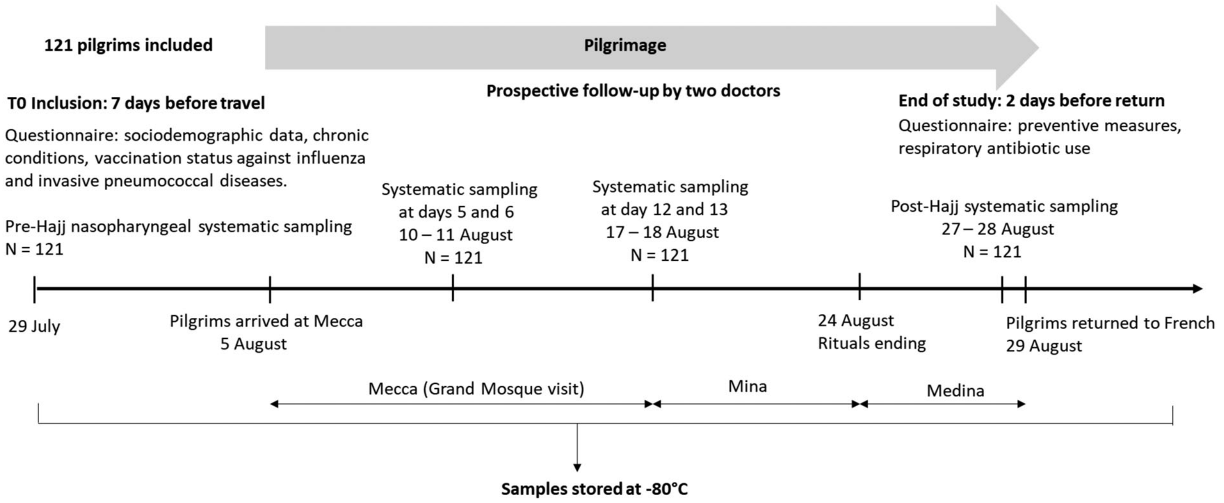

Figure 1. Study design of cohort survey among 121 French pilgrims in 2018 Hajj season.

Moraxella catarrhalis were amplified with internal DNA extraction controls TISS, as previously described [20,21]. Human adenovirus, human metapneumovirus, respiratory syncytial virus, *Bordetella pertussis* and *Mycoplasma pneumoniae* were not tested because a low proportion (<2%) of returning French pilgrims or international pilgrims were found positive for these pathogens in previous works [7,20,22,23].

Negative controls (PCR mix) and positive controls (DNA from bacterial strain or RNA from viral strain) were included in each run. Positive results of bacteria or virus amplification were defined as those with a cycle threshold (CT) value ≤35. A threshold value of 35 was used in each experimental run and we calculated the RFU Cut Off Value recommend by CFX Manager Software Version 3.1 (Bio-Rad) [24] in order to verify the positive cases. Results were considered positive when the cycle threshold value of real-time PCR was greater than the Cut Off Value.

Pilgrims were considered to be positive for respiratory pathogens during the Hajj if they were positive at the days 5 and 6 and/or days 12 and 13 sample.

Statistical analysis

STATA software version 14.2 (Copyright 1985–2015 StataCorp LLC, http://www.stata.com) was used for statistical analysis.

The main outcomes of interest were the relationships between respiratory pathogens among pilgrims during the Hajj. We evaluated the carriage of HRV, HCoV, *S. aureus, S. pneumoniae, H. influenzae, K. pneumoniae* and *M. catarrhalis* using logistic mixed models. Because each pilgrim provided four successive samples, we used a repeated measures design to take into account the variability of series samples from each pilgrim. To evaluate the effect of covariates on each respiratory pathogen carriage, we modelled carriage of HRV, HCoV, *S. aureus, S. pneumoniae, H. influenzae, K. pneumoniae* and *M. catarrhalis* separately. We did not separately model the outcome of carriage of influenza A and B viruses because of the low prevalence of these viruses. Only the variables with a prevalence ≥5.0% were considered for statistical analysis. Unadjusted associations between respiratory pathogen carriage with multiples factors: sociodemographic characteristics (gender, ≥60 years), chronic respiratory disease, BMI classification, smoking status; individual preventive measures (vaccination against influenza, vaccination against IPD, use of a face mask, hand washing, disinfectant gel and disposable handkerchiefs); antibiotic intake 10 days before each sample; respiratory virus or bacteria and dual carriage were analysed by univariable analysis. Variables with p values <0.2 in the univariable analysis were included in the multivariable analysis. A mixed model with the subject being random effect was used to estimate the relationships between respiratory pathogens and to take into account the repeated measures for pathogen carriage for each subject.

Regarding risk factors for LRTI, the outcome was possible LRTI symptoms reported during the Hajj. The independent factors were sociodemographic characteristics (gender, ≥60 years), chronic respiratory disease, smoking status, BMI classification; vaccination against influenza, vaccination against IPD, respiratory virus or bacteria and dual carriage during the Hajj. Unadjusted associations between multiple factors and possible LRTI symptoms were examined using univariable analysis. Variables with p values <0.2 in the univariable analysis were included in the multivariable analysis. A logistical regression model was used to estimate factors' adjusted odds ratios regarding possible LRTI.

The results were presented by odds ratio (OR) with a 95% confidence interval (95%CI). Results with a p value ≤0.05 was considered to be statistically significant.

Ethics Statement

The protocol was approved by the Aix-Marseille University institutional review board (23 July 2013; reference No. 2013-A00961-44).

The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments.

All participants provided their written informed consent.

Results

Characteristics of study participants

The study included 121 pilgrims. The sex ratio of the population was 1:1.3 and the median age was 61 years with 58.7% of pilgrims aged 60 years and over. Most pilgrims were born in North Africa (66.9%) and sub-Saharan Africa (26.5%). There was a high prevalence of overweight (46.3%), obesity (28.1%), diabetes mellitus (25.6%) and hypertension (25.6%) and 13.2% participants reported that they suffered from chronic respiratory disease. In line with French recommendation, 88/121 pilgrims (72.7%) had an indication for vaccination against IPD [14,15] (Table 1).

A total of 37/121 (30.6%) pilgrims reported that they had been vaccinated against influenza in the past year. Only 17/88 (19.3%) pilgrims with an indication for IPD had been vaccinated against pneumococcal disease (PCV-13) in the past five years. Regarding non-pharmaceutical preventive measures, 49/121 (40.5%) pilgrims reported using face masks during the pilgrimage. Also, 67/121 (55.4%) and 70/121 (57.8%) pilgrims reported washing their hands more often than usual and using hand gel, respectively during the pilgrimage. Finally, 106/121 (87.6%) reported using disposable handkerchiefs during the Hajj.

Table 1. Characteristics of participants.

Variables	$N = 121$	Prevalence (%)
Gender		
Male	52	43.0
Female	69	57.0
Age (years)		
Median	61	
Interquartile	56–66	
Min-Max	26–83	
Age ≥60	71	58.7
Place of birth		
France	8	6.6
North Africa	81	66.9
Sub-Saharan Africa	32	26.5
Comorbidities		
Diabetes mellitus	31	25.6
Hypertension	31	25.6
Chronic respiratory disease	16	13.2
Chronic heart disease	13	10.7
Chronic kidney disease	3	2.5
Immunodeficiency	4	3.3
Indication for vaccination against IPD[a]	88	72.7
Smoking status		
Yes, current	6	5.0
Yes, stopped	24	19.8
Never	91	75.2
BMI[b]		
Normal	31	25.6
Overweight	56	46.3
Obese	34	28.1

[a]Age over or equal to 60 years, diabetes mellitus, chronic respiratory disease, chronic heart disease, chronic kidney disease and immunodeficiency.
[b]Normal weight: BMI: 18.5–24.9, Overweight: BMI: 25.0–29.9, Obese: BMI ≥30.

Clinical symptoms

A total of 113/121 (93.4%) pilgrims presented at least one respiratory symptom during their stay in KSA. A cough and rhinitis were the most frequent symptoms affecting 86.8% and 69.4% of participants. Over half of the pilgrims (59.5%) reported expectoration and 27.3% reported a dry cough. Voice failure was reported by 37.2%, fever by 27.3% and ILI by 20.7% of participants. Antibiotic use for RTIs was reported by 58.7% pilgrims. Only one (0.8%) pilgrim was hospitalized in KSA. Regarding possible LRTI symptoms, 5/121 (4.1%) participants reported a productive cough without nasal or throat symptoms. In addition, 9/121 (7.4%), 16/121 (13.2%) and 25/121 (20.7%) pilgrims presented febrile dyspnoea, dyspnea and a febrile productive cough, respectively. At total of 51/113 (45.1%) pilgrims with respiratory symptoms were still symptomatic at return. The mean time between arrival in KSA and the onset of symptoms was 8.7 ± 4.6 days (range = 1-21 days) (data not shown). Most ill pilgrims presented the onset of respiratory symptoms when stationed at Mecca with a second minor wave in Mina (Figure 2).

Dynamics and interaction of respiratory pathogens carriage

Table 2 shows the prevalence of the carriage of respiratory pathogens according to sampling time and Figure 2 show the dynamics of most prevalent pathogens over the study period. Overall, 378/484 (78.1%) of all samples tested positive for at least one pathogen. *S. aureus* was the pathogen most frequently isolated with 33.3% of all samples testing positive. High positivity rates were also observed for *H. influenzae* (26.7%), *K. pneumoniae* (22.5%), HRV (21.1%) and *M. catarrhalis* (19.4%). Only 9.5% of the samples were positive for coronaviruses and 7.4% for *S. pneumoniae*. Very few samples tested positive for influenza viruses. No case was positive for HPIV. Of the positive samples, the proportion that was positive for more than one pathogen was 55.6% (210/378). A total of 138/378 (36.5%) samples were positive for two pathogens, 52/378 (13.8%) for three pathogens, 16/378 (4.2%) for four pathogens and 4/378 (1.1%) for five pathogens (data not shown).

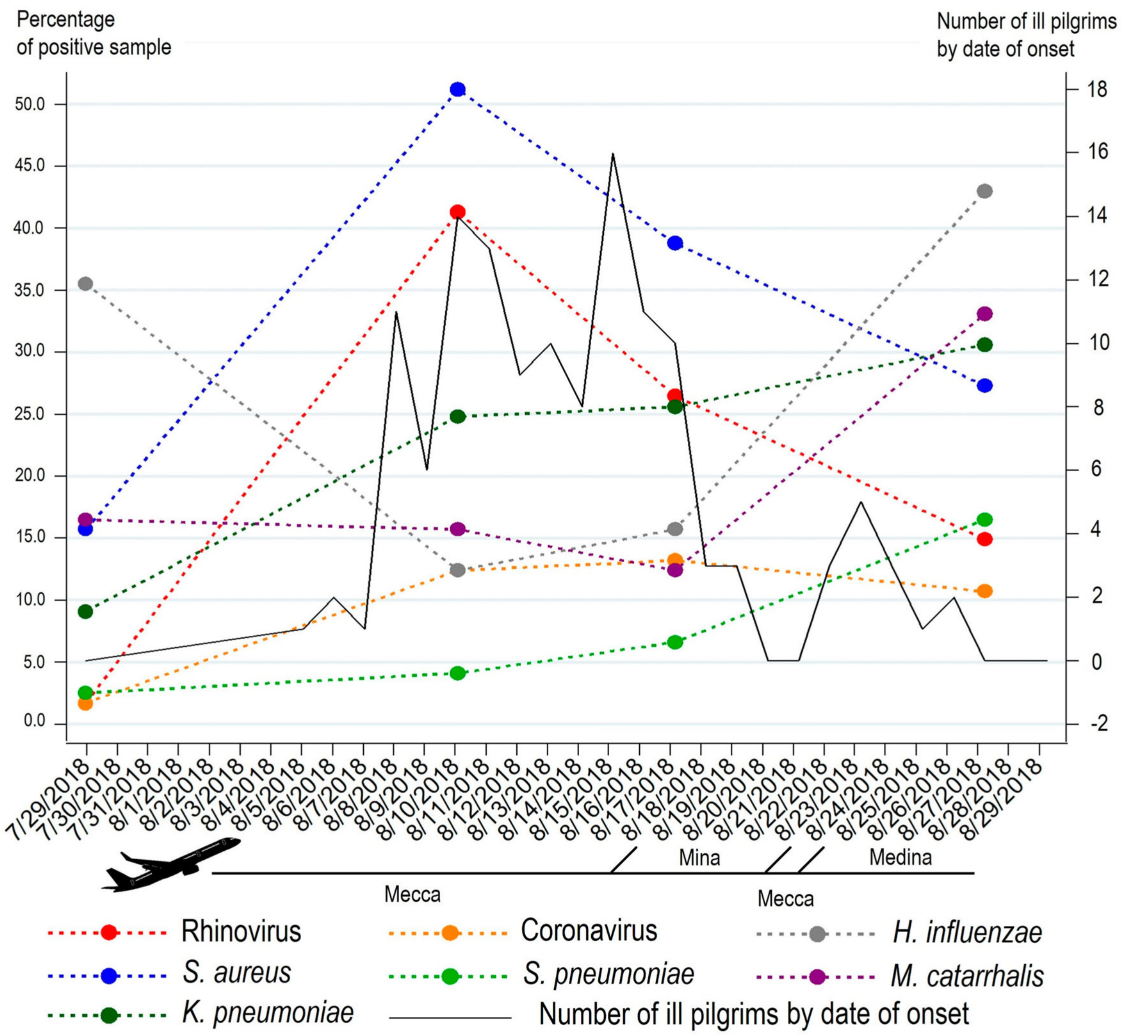

Figure 2. Dynamics of respiratory pathogen carriage during the 2018 Hajj and onset of respiratory symptoms among pilgrims.

In pre-travel samples, virus carriage was very low with only a few participants testing positive for HRV and HCoV (<2%). Bacterial carriage was higher, notably for *H. influenzae* (35.5%) *M. catarrhalis* (16.5%) and *S. aureus* (15.7%). *K. pneumoniae* and *S. pneumoniae* carriage were relatively low (9.1% and 2.5%, respectively).

A dramatic increase in HRV carriage was observed on days 5 and 6 of the pilgrimage with prevalence 24 times higher than that of pre-travel. HRV carriage decreased progressively in subsequent samples but was still eight times higher in post-Hajj samples compared to pre-Hajj. A seven-fold increase of HCoV carriage was observed on days 5 and 6 that persisted on into days 12 and 13 of the pilgrimage and tended to slightly decrease in post-Hajj samples. Regarding bacteria, carriage of *S. aureus* increased by a factor of three on days 5 and 6 and decreased progressively in subsequent samples but was still double in post-Hajj samples compared to pre-Hajj. Interestingly, the carriage curves of HRV and *S. aureus* were strictly parallel.

M. catarrhalis carriage was about 12-16% in pre-travel, days 5 and 6 and 12 and 13 samples and increased to 33% in post-Hajj samples. *K. pneumoniae* carriage increased three-fold between pre-Hajj and days 5 and 6 samples and slightly increased in subsequent samples. *S. pneumoniae* carriage increased constantly overtime with a seven-fold increase in post-Hajj samples compared to pre-Hajj. Finally, *H. influenzae* carriage first decreased on days 5 and 6 and 12 and 13 by a factor 2.5 and then increased in post-Hajj samples to a carriage rate which was higher than that of pre-Hajj samples.

Table 3 shows the factors that were independently associated with the carriage of respiratory pathogens on 484 swabs from 121 pilgrims. A positive association was observed between males and carriage of HRV and *S. pneumoniae*. Chronic respiratory disease was also associated with *S. pneumoniae* carriage. Finally, the use of disposable handkerchiefs was associated with a decreased carriage of *H. influenzae*. Antibiotic intake ten days before each sampling was positively associated with HCoV and *K. pneumoniae* carriage.

Regarding interactions between pathogens, we observed that HRV carriage and *S. aureus* carriage were mutually positively associated. The same applied to *H. influenzae* and *M. catarrhalis* carriage. Pilgrims carrying *S. pneumoniae* were more likely also to carry

Table 2. Prevalence of respiratory pathogens among pilgrims during the Hajj.

	Pre-travel *N* = 121		Days 5 and 6 *N* = 121		Days 12 and 13 *N* = 121		Post-Hajj *N* = 121		Total *N* = 484	
	n	%	n	%	n	%	n	%	n	%
Respiratory viruses										
Influenza A	0	0	0	0	0	0	3	2.5	3	0.6
Influenza B	0	0	2	1.7	1	0.8	1	0.8	4	0.8
Rhinovirus	2	1.7	50	41.3	32	26.5	18	14.9	102	21.1
Coronavirus	2	1.7	15	12.4	16	13.2	13	10.7	46	9.5
Respiratory bacteria										
S. aureus	19	15.7	62	51.2	47	38.8	33	27.3	161	33.3
S. pneumoniae	3	2.5	5	4.1	8	6.6	20	16.5	36	7.4
H. influenzae	43	35.5	15	12.4	19	15.7	52	43.0	129	26.7
K. pneumoniae	11	9.1	30	24.8	31	25.6	37	30.6	109	22.5
M. catarrhalis	20	16.5	19	15.7	15	12.4	40	33.1	94	19.4
Virus-bacteria combination										
Rhinovirus-coronavirus	0	0	5	4.1	1	0.8	2	1.7	8	1.7
S. aureus-rhinovirus	0	0	33	27.3	12	9.9	4	3.3	49	10.1
S. pneumoniae-rhinovirus	0	0	4	3.1	1	0.8	5	4.1	10	2.1
H. influenzae-rhinovirus	2	1.7	7	5.8	5	4.1	11	9.1	25	5.2
K. pneumoniae-rhinovirus	0	0	10	8.3	7	5.8	6	5.0	23	4.8
M. catarrhalis-rhinovirus	0	0	10	8.3	7	5.8	5	4.1	22	4.6
S. aureus-coronavirus	0	0	5	4.1	1	0.8	3	2.5	13	2.7
S. pneumoniae-coronavirus	0	0	1	0.8	2	1.7	2	1.7	4	0.8
H. influenzae-coronavirus	0	0	1	0.8	2	1.7	8	6.6	11	2.3
K. pneumoniae-coronavirus	0	0	5	4.1	3	2.5	2	1.7	10	2.1
M. catarrhalis-coronavirus	0	0	2	1.7	1	0.8	6	5.0	9	1.9
Bacteria combination										
S. aureus-S. pneumoniae	2	1.7	2	1.7	1	0.8	7	5.8	12	2.5
S. aureus-H. influenzae	9	7.4	8	6.6	13	10.7	19	15.7	49	10.1
S. aureus-K. pneumoniae	3	2.5	13	10.7	12	9.9	6	5.0	34	7.0
S. aureus-M. catarrhalis	2	1.6	11	9.1	4	3.3	14	11.6	31	6.4
S. pneumoniae-H. influenzae	0	0	0	0	2	1.7	12	9.9	14	2.9
S. pneumoniae-K. pneumoniae	1	0.8	3	2.5	2	1.7	6	5.0	12	2.5
S. pneumoniae-M. catarrhalis	0	0	1	0.8	0	0	11	9.1	12	2.5
H. influenzae-K. pneumoniae	2	1.7	2	1.7	6	5.0	13	10.7	23	4.8
H. influenzae-M. catarrhalis	8	6.6	1	0.8	3	2.5	22	18.2	34	7.0
K. pneumoniae-M. catarrhalis	3	2.5	4	3.3	3	2.5	7	5.8	17	3.5

M. catarrhalis. Patients with a dual carriage of *H. influenzae* and *S. pneumoniae* were six times more likely also to be carrying *S. pneumoniae*. By contrast, *M. catarrhalis* carriage was associated with a reduced carriage of *K. pneumoniae*.

Risk factors for possible lower respiratory tract infection among French pilgrims during the 2018 Hajj season

Table 4 shows the results of multivariable risk factor analysis for possible LRTI symptoms. Chronic

Table 3. Risk factors for the carriage of respiratory pathogens among pilgrims during the Hajj (484 swabs from 121 pilgrims) (multivariable analysis).

	OR [95% CI]						
	Rhinovirus	Coronavirus	*S. aureus*	*S. pneumoniae*	*H. influenzae*	*K. pneumoniae*	*M. catarrhalis*
Sociodemographic characteristics							
Gender = Male	1.99** [1.27–3.12]	–	–	4.14** [1.67–10.32]	–	–	–
Chronic respiratory disease	–	–	–	3.22* [1.09–9.50]	–	–	–
Disposable handkerchiefs	–	–	–	-	0.47** [0.29–0.78]	–	–
Antibiotic intake 10 days before sampling	–	1.91* [1.03–3.52]	–	–	–	3.62*** [1.81–7.22]	–
Respiratory carriage							
Rhinovirus	NA	–	3.88*** [2.01–7.49]	–	–	–	–
S. aureus	2.28*** [1.45–3.59]	–	NA	–	–	–	–
S. pneumoniae	–	–	–	NA	-	–	3.18* [1.22–8.31]
H. influenzae	–	–	–	–	NA	–	1.97* [1.07–3.60]
M. catarrhalis	–	–	–	–	2.11* [1.15–3.88]	0.42* [0.18–0.98]	NA
H. influenzae-M. catarrhalis	–	–	–	6.22*** [2.04–19.01]	NA	–	NA

*p-value ≤ 0.05.
**p-value ≤ 0.01.
***p-value ≤ 0.001.

Table 4. Risk factors for possible lower respiratory tract infection[1] during the 2018 Hajj (multivariable analysis).

	Febrile expectoration aOR [95%CI]	Febrile dyspnea aOR [95%CI]	Dyspnea aOR [95%CI]
Chronic respiratory disease	4.5** [1.40–14.43]	12.63* [1.61–99.14]	18.62*** [3.40–101.91]
Obesity			7.97** [1.74–36.49]
K. pneumoniae			4.50* [1.07–18.98]
M. catarrhalis-*S. aureus*		5.10* [1.10–23.57]	15.71** [2.41–102.37]
H. influenzae-rhinovirus			7.74* [1.04–57.43]

[1]Possible lower respiratory tract infection: productive cough with no nasal or throat symptoms; febrile productive cough; dyspnea or febrile dyspnea
Productive cough with no nasal or throat symptoms was not analysed due to the low number of subjects (5/121, 4.1%)
*p-value ≤ 0.05.
**p-value ≤ 0.01.
***p-value ≤ 0.001.

respiratory disease was associated with all possible LRTI symptoms. Obesity was associated with dyspnea. Carriage of *K. pneumoniae* or *M. catarrhalis*-*S. aureus* or *H. influenzae*-rhinovirus combination was associated with a four-fold, 16-fold and eight-fold increase of dyspnoea prevalence, respectively. Finally, *M. catarrhalis*-*S. aureus* dual carriage was associated with a five-fold increase in the prevalence of febrile dyspnea (Table 4).

Discussion

Despite the recommendation to take individual preventive measures to prevent RTIs [25,26], these infections remain common among Hajj pilgrims. Overcrowding during the event is thought to increase the risk of the transmission of infectious diseases, but interaction between respiratory pathogens is probably one factor contributing towards the development of RTIs. To our knowledge, no study on respiratory microbiota alteration among pilgrims during the Hajj has been conducted. Our results about the occurrence of RTI symptoms are in line with previous results obtained regarding French pilgrims [17] and others [1,20]. Notably, we observed that RTI symptoms occur soon after the pilgrims' arrival in Mecca, with most symptoms starting between 4and 13 days after arrival, corresponding to the period when pilgrims are stationed in Mecca hotels and are visiting the Grand Mosque daily, where highly crowded conditions are common [7]. We also confirmed that an overall increase in the carriage of respiratory viruses and bacteria can be seen when comparing pre-travel samples and post-Hajj samples, as previously documented [7,12,20,27–29]. Higher acquisition rates were observed for rhinovirus with a nine-fold increase when comparing pre-travel to post-Hajj carriage and for *S. pneumoniae* with a seven-fold increase, but an increase was observed for all pathogens tested in this study. The unique design of our study with sequential systematic sampling at regular intervals allows for a better understanding of the dynamics of pathogen carriage during the pilgrimage.

Carriage rates of bacteria and viruses in this study are in line with those observed during recent studies conducted on French pilgrims and in pilgrims of other nationalities, using the same methods of detection [7,20,27–29].

The acquisition of respiratory viruses and *S. aureus* occurred soon after arrival in Saudi Arabia and decreased gradually after days 5 and 6. By contrast, *S. pneumoniae* and *K. pneumoniae* carriage increased progressively until the end of the visit, *H. influenzae* and *M. catarrhalis* carriage increased later, after an initial clearance.

We hypothesize that the brutal acquisition of respiratory viruses upon arrival was the initial step that triggered subsequent changes in the relative abundance of resident bacteria [30] that were already present in the nasopharynx of pilgrims. The apparent simultaneity of viruses and *S. aureus* carriage increase and the initial wave of respiratory symptoms, suggests that this pathogen association was responsible for the RTIs that affected most pilgrims soon after arriving in Mecca. The subsequent increase in resident bacteria that occurred during the second half of pilgrims' stays in Saudi Arabia appears to be contemporaneous with a second wave of respiratory symptoms, suggesting that these RTIs were of bacterial origin.

Regarding interaction between respiratory pathogens, we observed a very clear pattern of positive association between the carriage of *S. aureus* and rhinovirus with acquisition curves which were strictly parallel. Furthermore, an independent positive mutual association between the carriage of the two pathogens was evidenced in our multivariate model. Several studies revealed a positive interaction between natural or experimental rhinovirus infection and *S. aureus* nasal carriers [31–35]. These studies also underlined that rhinovirus infection may facilitate the propagation of *S. aureus* from staphylococcal carriers to the environment and the transmission of the bacterium between humans. Among healthy persons who were experimentally infected by rhinovirus, the relative abundance of *S. aureus* first increased and then returned to its baseline level after the rhinovirus infection was cleared [36]. These results suggest that changes in the composition of the respiratory microbiota following rhinovirus infection may play a role in the development of bacterial superinfection. Morgene et al. proposed several potential mechanisms through which rhinovirus may increase bacterial infection [37]. Rhinovirus infection promotes pro-inflammatory cytokines and IFN production mainly through the activation of NFκB. In rhinovirus infected cells, the adherence of *S. aureus* was significantly higher compared to uninfected cells. The inflammation due to rhinovirus infection also increased cellular patterns that facilitate the adhesion and internalization of *S. aureus* within host cells [37].

We also observed a parallel increase of *H. influenzae* and *M. catarrhalis* carriage in days 12 and 13 and post-Hajj samples. An independent positive mutual association between the carriage of the two pathogens was evidenced in our multivariate model. Dual carriage of *H. influenzae* and *M. catarrhalis* strongly associated with *S. pneumoniae* carriage which in turns associated with *M catarrhalis* carriage. These results are consistent with those of several studies conducted among children with upper RTIs [38–40]. In these studies, the competitive interaction between *S. pneumoniae* and *H. influenzae* was dependent on neutrophils and complement. The additional carriage of *M. catarrhalis* might alter the competitive balance between *H. influenzae* and *S. pneumoniae* [39]. Co-colonization of *S. pneumoniae* or *H. influenzae* with *M. catarrhalis* associating with increased risk of otitis media has been documented [41]. Using in vivo models, mixed species biofilms play a role in increasing the persistence of ear disease [42]. Other proposed mechanisms for positive associations between bacterial species include interspecies quorum sensing and passive antimicrobial resistance, which have been observed in experimental models of otitis media [43].

Finally, our model showed that *M. catarrhalis* carriage was negatively associated with *K. pneumoniae* carriage which, to our knowledge, has not previously been published.

Additionally, we found that the male gender was independently associated with an increase in rhinovirus and *S. pneumoniae* carriage. We have no explanation for this unexpected observation. The carriage of *S. pneumoniae* was higher among pilgrims with chronic respiratory disease which support the current French recommendations that vaccination against IPD be proposed to at-risk pilgrims [44]. In one of our recent studies, pilgrims who were vaccinated against IPD were seven time less likely to harbour *S. pneumoniae* after the Hajj compared to unvaccinated pilgrims [27]. In this study, the use of disposable handkerchiefs was associated with a significant decrease in *H. influenzae* carriage. Non-pharmaceutical individual preventive measures such as cough etiquette, hand hygiene, use of a face mask, disinfectant gel and disposable handkerchiefs are recommended for Hajj pilgrims [26]. Nevertheless, the effectiveness of these measures has been poorly investigated and available results are contradictory [26]. The apparent association between antibiotic use and HCoV and *K. pneumoniae* carriage warrants further investigation to better explore this unexpected observation.

We also confirm that chronic respiratory disease is a risk factor for LRTI. We also evidenced the role of respiratory bacteria including *K. pneumoniae* and *M. catarrhalis-S. aureus* association and *H. influenzae*-rhinovirus association in the occurrence of possible LRTI symptoms. This reinforces the need for antibiotic use in case of LRTI symptoms [17].

Our study has some limitations. The study was conducted among French pilgrims only with a relatively small sample size and cannot be generalized to all pilgrims. qPCR used to detect respiratory pathogens does not distinguish between dead and living micro-organisms. Only a small number of respiratory pathogens were investigated. Respiratory bacteria serotypes were not investigated. Influenza viruses were not included in the model due to low carriage rates. In addition, we did not recruit a control group of individuals that did not participate to the Hajj. A study addressing interactions between respiratory pathogens in the general French adult population could be of interest. Nevertheless, our study is the first study on the dynamics of and interaction between the respiratory pathogens that are most frequently isolated among Hajj pilgrims. Our data suggest that RTIs at the Hajj are a result of complex interactions between a number of respiratory viruses and bacteria. Further studies aimed at studying the respiratory microbiota with tools allowing the identification of larger numbers of pathogens will be necessary to better elucidate these ecological changes and their potential role in the occurrence of respiratory symptoms.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Article 4 :

Measles outbreaks at mass gathering mostly occur at youth events.

Hoang VT and Gautret P.

Lancet Infect Dis. 2020 Jan;20(1):23. (IF = 24.446) of results from fresh versus biobanked samples was 93·4% (table). Positive percentage agreement was 85·2%, and nine samples turned negative after biobanking, suggesting that minor improvements in sensitivity might be observed when the FujiLAM assay is used on fresh samples. Negative percentage agreement was 97·5%; three (2·5%) of 121 samples that turned positive after biobanking are probably the result of differences caused by visual test interpretation (3·0% in our previous study[2]). Therefore, a higher proportion of false-positive results in fresh samples than in biobanked samples is unlikely to occur. Overall categorical agreement, positive percentage agreement, and negative percentage agreement were similar between HIV-negative and HIV-positive subgroups even in tubercolosis-positive, HIV-negative patients with lower LAM concentrations in urine.

In conclusion, the stability and availability of LAM, as detected by the FujiLAM assay, are high. The use of biobanked specimens delivers nearly equivalent results to the use of fresh specimens. A small reduction in positive percentage agreement suggests that marginal increases in diagnostic sensitivity in fresh samples are possible, but confirmation from an ongoing prospective study (NCT04089423) and after longer duration of storage is needed.

TB and CMD were previously employed by FIND. EM is currently employed by FIND. TB reports a patent application in the field of LAM detection. MM and ADK declare no competing interests. This work was supported by a Global Health Innovative Technology fund (number G2017-207) and the German Federal Ministry of Education and Research through KfW. The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The opinions, findings, and conclusions expressed in this manuscript reflect those of the authors alone.

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Measles outbreaks at mass gathering mostly occur at youth events

Despite some concerns regarding the risk of a measles outbreak at the 2019 Hajj, not a single case was identified by the Saudi Ministry of Health surveillance system, as noted by Saber Yezli and colleagues.[1] Although measles is potentially highly transmissible in crowded settings, few documented outbreaks have been reported in the context of mass-gathering events.[2]

An assessment of the age pattern of patients in publications with available age data (appendix) shows that in the vast majority of primary, secondary, and tertiary cases resulting from measles exposure at 23 different mass gatherings, patients were younger than 25 years. As exceptions, one outbreak at a sport event in Italy that led to further spread in Slovenia occurred in young adults aged 30–47 years, and another outbreak at a dog show in Slovenia that led to further spread in Italy occurred mostly in adults younger than 55 years. Most outbreaks occurred during youth sport competitions and youth religious meetings, at music festivals attended by young audiences, and at Disney theme parks. This pattern in age range mimics recent surveillance data from the USA for the period Jan 1 to Oct 1, 2019, in which 1249 new cases of measles were documented, with a median age of patients of 6 years, and no patients older than 49 years.[3]

Hajj pilgrims are characterised by their older age.[4] In a study in 2013 of 1206 Hajj pilgrims from 12 different nationalities, the mean age was 50 years (range 18–88 years). In another study, the mean age of French Marseille pilgrims participating in the 2018 Hajj was 61 years (range 26–83 years), with 71 (59%) of 121 pilgrims aged 60 years and older.[5] The absence of measles circulation during the 2019 Hajj was likely a result of the age pattern of the pilgrims, the vast majority of whom might be immune to measles because of natural infection or possibly previous vaccination. By contrast, the risk of measles outbreaks at youth mass-gathering events is real, and booster vaccinations of young people planning to participate in these events should be considered.

See **Online** for appendix

We declare no competing interests.

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Supplementary data

Measles outbreaks at mass gathering mostly occur at youth events

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Measles outbreaks at mass gathering events, with available information about age of patients

Place, year	Purpose of MG	Cumulated number of attendees	Total number of cases	Age of patients	International spread	Reference
Indiana, US, 1991	International gymnastic competition	64,400	3 primary cases and no secondary cases	15-16 years	No	1
Indiana, US, 2005	Church meeting	500	21 primary cases[1], 13 secondary cases, 1 with unknown status	88% under 20 years	No	2
Pennsylvania, US, 2007	International youth sporting event	265,000	2 primary cases[2], 1 secondary case[3], 2 tertiary cases.	12 years in primary cases, 40 years in the secondary case	No	3

				and 18 and 19 years in tertiary cases.		
Taizé, France, 2010	International youth religious meeting	3,500	13 primary cases; 17 secondary cases; 7 tertiary cases	Median age for primary cases: 17 years; for secondary cases: 15 years; for tertiary cases: 13 years	Germany	4
Granada, Spain, 2010	Large wedding	ND	25 primary cases; 58 secondary cases	97% of patients less than 16 years-old	ND	5

Rimini, Italy, 2011	International youth football tournament	ND	2 primary cases, 9 secondary cases4[3], 2 tertiary cases	16 and 18 years for primary cases, 11-27 years in secondary cases, 11 and 15 years in tertiary cases.	Germany	6
Rimini, Italy, 2011	International kickboxing tournament	ND	One primary case, 11 secondary cases, 1 tertiary case	34 years for the primary case, 30-47 years in secondary cases, 33 years in the tertiary case.	Slovenia	6
Vrtojba/Sempeter, Slovenia, 2015	International dog show	1,100	34 primary cases, 28 secondary and tertiary cases	5-55 years overall with the majority among adults (mean	Italy	7,8

				age 31 years in Italian cases)		
California, US, 2015	2 Disney theme parks	Annual attendance = 24 million	125 cases including at least 34 secondary cases.	Median age : 22 years (range 1-77 years)	Mexico and Canada	9,10
England and Whales, 2016	12 Art and Music festivals,	500-1000 to 144,000	52 cases	Median age: 19 years (range 1-52 years)	France, Germany, Spain	11

[1]index case was infected in Romania

[2]primary cases were in Japanese participant infected in Japan.

[3]Two additional secondary cases were documented in plane passenger travelling with the primary cases and in an airport employee.

[4]secondary transmission occurred during a music Festival, in Germany where one of the primary cases infected in Rimini participated a few days later.

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<u>Article 5 :</u>

Bacterial respiratory carriage in French Hajj pilgrims and the effect of pneumococcal vaccine and other individual preventive measures: A prospective cohort survey.

Hoang VT, Meftah M, Anh Ly TD, Drali T, Yezli S, Alotaibi B, Raoult D, Parola P, Pommier de Santi V, Gautret P.

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Bacterial respiratory carriage in French Hajj pilgrims and the effect of pneumococcal vaccine and other individual preventive measures: A prospective cohort survey

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Background: Viral respiratory tract infections are known to be common in Hajj pilgrims while the role of bacteria is less studied.

Methods: Clinical follow-up, adherence to preventive measures and PCR-based pharyngeal bacterial carriage pre- and post-Hajj, were assessed in a cohort of 119 French Hajj pilgrims.

Results: 55% had an indication for pneumococcal vaccination. Occurrence of respiratory symptoms was 76.5%, with cough (70.6%) and sore throat (44.5%) being the most frequent; fever was reported by 38.7% pilgrims and 42.0% took antibiotics. Respiratory symptoms, fever and antibiotic intake were significantly more frequent in pilgrims with indication for vaccination against pneumococcal infection. The prevalence of *S. pneumoniae* carriage (1.8% pre-, 9.8% post-Hajj), *H. influenzae* carriage (0.9%, 45.4%) and *K. pneumoniae* (2.8%, 9.8%) significantly increased post-Hajj. Pilgrims vaccinated with conjugate pneumococcal vaccine were seven time less likely to present *S. pneumoniae* carriage post-Hajj compared to those not vaccinated (3.2% vs. 18.0%, OR = 0.15; 95% CI [0.03–0.74], p = 0.02).

Conclusions: Pilgrims at risk for pneumococcal disease are more likely to suffer from febrile respiratory symptoms at the Hajj despite being immunized against pneumococcal disease and despite lowered *S. pneumoniae* carriage and should be targeted for reinforced prevention against respiratory infections.

1. Introduction

The Hajj is one of the largest annual religious mass gatherings in the world. Each year, Saudi Arabia attracts over 2 million pilgrims from more than 180 countries including about 2000 from Marseille, France [1]. The Hajj presents major challenges in public health and infection control and the crowding conditions favor the acquisition, dissemination and transmission of pathogenic microorganisms [2]. Respiratory diseases are particularly common during the pilgrimage, representing a significant cause of morbidity and a major cause of hospitalization with community-acquired pneumonia being a leading cause of serious illness among pilgrims [3]. The etiology of respiratory tract infection is multifactorial and complex. Whereas viruses are likely responsible for mild upper respiratory tract infections [4], some studies have identified *Streptococcus pneumoniae* as one of the main etiologic agents associated with severe respiratory infections in Hajj pilgrims [5]. Predisposing factors for pneumococcal infections at the Hajj include older age, chronic diseases, overcrowding conditions and environmental pollution [6]. The natural route of pneumococcal infection is initiated by nasopharyngeal colonization and may progress towards an invasive disease, especially if natural immunological barriers are crossed [7]. The reported rates of *S. pneumoniae* nasopharyngeal carriage depend on age, geographical area, genetic heritage and socio-economic conditions [7]. This nasopharyngeal carriage is considered as the principal source of person-to-person transmission of *S. pneumoniae* [7]. In addition to *S. pneumoniae* other bacterial pathogens such as *Haemophilus influenzae,*

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Klebsiella pneumoniae and *Staphylococcus aureus* were frequently isolated from Hajj pilgrims suffering from pneumonia [8–10]. However, available data about the carriage of *S. pneumoniae* and other respiratory bacteria in the overall population of Hajj pilgrims is limited [3,5].

The objective of this study was to investigate the prevalence of pharyngeal carriage of respiratory bacteria including *S. pneumoniae, H. influenzae, K. pneumoniae and S. aureus* in French pilgrims, before and after the Hajj in 2015.

2. Methods

2.1. Participants and study design

Pilgrims participating in the 2015 Hajj (from September 21st to 26th, 2015) were recruited in August 2015, at a private specialized travel agency in Marseille, France, which organizes trips to Mecca, Saudi Arabia. Potential adult participants were asked to participate in the study on a voluntary basis. They were included and followed up before departing from France and immediately before leaving Saudi Arabia by a medical bilingual (Arabic and French) doctor who traveled with the group. Upon inclusion (August 23rd to 30th, 2015), the participants were interviewed using a standardized pre-Hajj questionnaire that collected information on demographics, immunization status and medical history. Based on French recommendations [12,13], pilgrims with risk factors for invasive pneumococcal disease (IPD) such as age superior or equal to 60 years, diabetes mellitus, chronic respiratory disease, chronic heart disease, chronic kidney disease and immunodeficiency, were vaccinated with the 13-valent conjugate pneumococcal vaccine (PCV-13) unless they received the vaccine in the past 5 years. A post-Hajj questionnaire that collected clinical data and information on compliance with face masks use as well as hand washing, use of disinfectant hand gel and disposable handkerchiefs, was completed during a face-to-face interview, 2 days before the pilgrims' return to France. Pharyngeal swabs were collected upon inclusion on August 30th (pre-Hajj specimens) and 2 days prior to the return of the pilgrims on September 30th, 2015 (post-Hajj specimens). Influenza-like illness (ILI) was defined as the presence of cough, sore throat and subjective fever [14]. The protocol was approved by the Aix-Marseille University institutional review board (July 23rd, 2013; reference no. 2013-A00961-44). The study was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants provided written consent.

Respiratory specimen. Pharyngeal swabs were collected from each participant, transferred to Sigma-Virocult® medium and stored at −80 °C.

Identification of respiratory bacteria. The DNA extractions were concurrently performed using the EZ1 Advanced XL (Qiagen, German) with the DNA Kit v2.0 (Qiagen). Real-time PCR amplifications were carried out by using LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. All real-time PCR reactions were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, USA). Positive results of bacteria amplification were defined as those with a cycle threshold (CT) value ≤ 35. The SDD gene of *H. influenzae*, *phoE* gene of *K. pneumoniae*, *nucA* gene of *S. aureus* and *lytA* gene of *S. pneumoniae* were amplified with internal DNA extraction controls TISS, as previously described [15].

2.2. Statistical analysis

Differences in the proportions were tested by Pearson's chi-square or Fisher's exact tests when appropriate. To evaluate the potential acquisition of respiratory pathogens in Saudi Arabia, we used the McNemar's Test to compare their prevalence before leaving France and in Saudi Arabia (during and after the Hajj). Percentages and odds ratio (OR) with 95% confidence interval (CI) estimations and comparisons were carried out using STATA 11.1 (Copyright 2009 StataCorp LP, https://www.stata.com/). A p value of ≤0.05 was considered significant.

Table 1
Socio-demographic characteristics and comorbidities of 119 pilgrims according to vaccination status against invasive pneumococcal disease.

Socio-demographic characteristics and comorbidities	Total N = 119	Vaccinated pilgrims n = 62	Unvaccinated pilgrims n = 57
	n (%)	n (%)	n (%)
Male	62 (52.1)	30 (48.4)	32 (56.1)
Female	57 (47.9)	32 (51.6)	25 (43.9)
Median age [IQR][a] (years)	61 [52; 66]	65 [63; 69]	52.5 [34; 58]
Age ≥ 60 years[b]	59 (50.9)	57 (91.9)	2 (3.7)
previous Hajj	13 (10.9)	9 (14.5)	4 (7.0)
Diabetes mellitus	39 (32.8)	36 (58.1)	3 (5.3)
Chronic respiratory disease	12 (10.1)	12 (19.4)	0 (0)
Chronic heart disease	8 (6.7)	7 (11.3)	1 (1.8)
Chronic kidney disease	1 (0.8)	1 (1.6)	0 (0)
Immunodeficiency	0 (0)	0 (0)	0 (0)
Indication for vaccination against invasive pneumococcal disease[c]	65 (54.6)	62 (100)	3[d] (5.3)

[a] Interquartile range.
[b] Data based on 116 individuals, data from three participants were missing.
[c] Age of 60 years or over, diabetes mellitus, chronic respiratory disease, chronic heart disease, chronic kidney disease and immunodeficiency.
[d] Refused vaccination.

3. Results

3.1. Characteristics of study participants

A total of 119 persons of the 131 pilgrims who traveled with the travel agency (90.8%) agreed to participate and answered the pre- and post-travel questionnaires; 12 pilgrims were excluded because they joined the group in Saudi Arabia one week after the main group arrived. 62 pilgrims were male (52.1%) and 57 female (47.9%) with a gender ratio of 1:1. The median age was 61 years of age (ranging from 24 to 81 years). Fifty-nine (50.9%) of the 119 participants were over 60 years of age. Sixty-five pilgrims (54.6%) had an indication for vaccination against IPD according to French recommendation at the time of inclusion. Diabetes was the most common comorbidity (Table 1).

Regarding preventive measures, 4.2% (5/119) of pilgrims reported pneumococcal vaccination (PCV-13) in the past 5 years and 57 were vaccinated upon inclusion (three refused), so 62/119 (52.1%) of them were immunized. Ninety-eight pilgrims (82.4%) reported using face masks during the Hajj, including 68.1% (81/119) who reported using masks sometimes and 14.3% frequently (17/119). 47.1% (56/119) declared washing their hands during the Hajj more often than usual, 37.0% (44/119) used hand gel and 41.2% (49/119) used disposable handkerchiefs during their stay in Saudi Arabia.

3.2. Clinical features

During the three weeks of their stay in Saudi Arabia, 91 (76.5%) pilgrims reported at least one respiratory symptom with cough being the most frequent (70.6%, 84/119), followed by sore throat (44.5%, 53/119), rhinitis (37.0%, 44/119), voice failure (15.1%, 18/119) and dyspnea (6.7%, 8/119). 38.7% (46/119) reported fever and 20.2% (24/119) ILI. A proportion of 42.0% (50/119) received antibiotics for respiratory tract infection symptoms. The highest antibiotic consumption was β-lactams (42/50, 84.0%), followed by macrolides (5/50, 10.0%). None of the pilgrims suffered from pneumonia or other invasive pneumococcal diseases and none was hospitalized.

Overall, respiratory symptoms, ILI and antibiotic intake were more frequent in pilgrims vaccinated against IPD compared to those who were unvaccinated (Table 2). These differences were statistically

Table 2
Prevalence of symptoms and antibiotic intake during Hajj according to preventive measures.

Preventive measures		Cough n (%)	OR [CI $_{95\%}$] p	Dyspnea n (%)	OR [CI $_{95\%}$] p	Sore throat n (%)	OR [CI $_{95\%}$] p	Voice failure n (%)	OR [CI $_{95\%}$] p
Vaccination against IPD	Yes	50 (80.6)	2.82 [1.24–6.42]	7 (11.3)	7.12 [0.85–59.85]	33 (53.2)	2.1 [1.01–4.4]	11 (11.7)	1.54 [0.55–4.29]
	No	34 (59.7)	p = 0.01	1 (1.8)	p = 0.07	20 (35.1)	p = 0.05	7 (12.3)	p = 0.41
Mask	Yes	73 (74.5)	2.65 [1.01–7.00]	6 (6.1)	0.62 [0.12–3.31]	46 (46.9)	1.77 [0.66–4.76]	16 (16.3)	1.85 [0.39–8.75]
	No	11 (52.4)	p = 0.05	2 (9.5)	p = 0.58	7 (33.3)	p = 0.26	2 (9.5)	p = 0.44
Hand washing	More often than usual	38 (67.9)	0.78 [0.35–1.72] p = 0.54	5 (8.9)	1.96 [0.45–8.61] p = 0.37	23 (41.1)	0.77 [0.37–1.58] p = 0.47	11 (19.6)	1.96 [0.7–5.45] p = 0.2
	As usual	46 (73.0)		3 (4.8)		30 (47.6)		7 (11.1)	
Disinfectant gel	Yes	32 (72.7)	1.18 [0.52–2.69]	0 (0)	NA p = 0.02	22 (50.0)	1.42 [0.67–3]	7 (15.9)	1.1 [0.39–3.08]
	No	52 (69.3)	p = 0.7	8 (10.8)		31 (41.3)	p = 0.36	11 (14.7)	p = 0.86
Disposable handkerchiefs	Yes	36 (73.5)	1.27 [0.56–2.85]	2 (4.1)	0.45 [0.09–2.35]	21 (42.7)	0.89 [0.43–1.86]	7 (14.3)	0.89 [0.32–2.5]
	No	48 (68.6)	p = 0.56	6 (8.6)	p = 0.35	32 (45.7)	p = 0.76	11 (15.7)	p = 0.83

Preventive measures	Rhinitis n (%)	OR [CI $_{95\%}$] p	Fever n (%)	OR [CI $_{95\%}$] p	ILI n (%)	OR [CI $_{95\%}$] p	ATB n (%)	OR [CI $_{95\%}$] p
Vaccination against IPD	26 (41.9)	1.56 [0.74–3.32]	29 (46.8)	2.07 [0.97–4.4]	16 (13.4)	2.13 [0.83–5.45]	32 (51.6)	2.31 [1.09–4.88]
	18 (31.6)	p = 0.24	17 (29.8)	p = 0.06	8 (6.7)	p = 0.12	18 (31.6)	p = 0.03
Mask	38 (38.8)	1.58 [0.56–4.44]	42 (42.9)	3.19 [1.00–10.17]	23 (23.5)	6.13 [0.78–48.22]	46 (46.9)	3.76 [1.18–11.98]
	6 (28.6)	p = 0.38	4 (19.0)	p = 0.05	1 (0.8)	p = 0.08	4 (19.0)	p = 0.02
Hand washing	22 (34.9)	1.2 [0.57–2.54]	20 (35.7)	0.79 [0.38–1.66]	8 (6.7)	0.49 [0.19–1.25]	24 (42.9)	1.07 [0.51–2.21]
	22 (39.3)	p = 0.62	26 (41.3)	p = 0.54	16 (13.4)	p = 0.14	26 (41.3)	p = 0.86
Disinfectant gel	17 (38.6)	1.12 [0.52–2.41]	14 (31.8)	0.63 [0.29–1.37]	9 (7.6)	1.03 [0.41–2.6]	18 (40.9)	0.93 [0.44–1.98]
	27 (36.0)	p = 0.77	32 (42.7)	p = 0.24	15 (12.6)	p = 0.95	32 (42.7)	p = 0.85
Disposable handkerchiefs	20 (40.8)	1.32 [0.62–2.81]	23 (46.9)	1.81 [0.85–3.83]	10 (8.4)	1.02 [0.41–2.54]	22 (44.9)	1.22 [0.58–2.56]
	24 (34.3)	p = 0.47	23 (32.9)	p = 0.12	14 (11.8)	p = 0.96	28 (40.0)	p = 0.59

NA: not applicable.
IPD: invasive pneumococcal disease, OR: Odds ratio, CI $_{95\%}$: 95% confidence interval; ILI: influenza like illness, ATB: Antibiotic treatment.

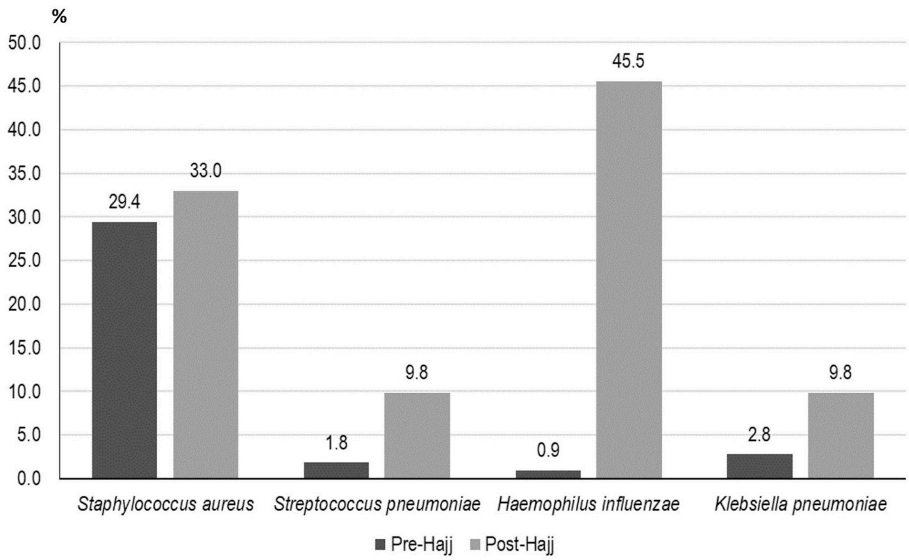

Fig. 1. Prevalence of bacterial carriage in pre- and post-Hajj pharyngeal samples.

significant for cough, sore throat and antibiotic intake. Prevalence of cough, fever and antibiotic intake was higher among those who reported using face masks.

3.3. Detection of respiratory bacteria

Pre- and post-Hajj specimens were collected from 109 (91.6%) and 112 (94.1%) participants, respectively. The prevalence of bacterial carriage significantly increased after participation in Hajj, compared to pre-Hajj status. *S. pneumoniae* carriage increased from 1.8% pre-Hajj to 9.8% post-Hajj ($p = 0.01$). The prevalence of *H. influenzae* carriage increased from 0.9% pre-Hajj to 45.4% post-Hajj ($p < 0.0001$). The prevalence of *K. pneumoniae* increased from 2.8% pre-Hajj to 9.8% post-Hajj ($p = 0.02$). *S. aureus* carriage increased from 29.4% in pre-Hajj samples to 33.0% in post-Hajj samples but the difference was not statistically significant with $p = 0.46$ (Fig. 1).

Pilgrims vaccinated against IPD were seven time less likely to harbor *S. pneumoniae* after the Hajj compared to unvaccinated pilgrims (3.2% vs. 18.0%, OR = 0.15; 95% CI [0.03–0.74], $p = 0.02$). Using disinfectant gel was significantly associated with an increased *S. aureus* carriage after the Hajj. Pilgrims using disposable handkerchiefs were 3 times less likely to be positive for *H. influenzae* in post-Hajj samples. Antibiotic intake was significantly associated with increased carriage of *K. pneumoniae* in post-Hajj samples (Table 3). We also compared the carriage rate of the four respiratory bacteria in pre-Hajj samples according to adherence to preventive measures during the Hajj for potential confounding factors and found no significant difference (data not shown). When analyzing the relationship between the carriage of the four respiratory bacteria in post-Hajj samples and the prevalence of clinical symptoms, no significant differences were observed.

4. Discussion

We confirm here that upper respiratory tract symptoms are very frequent in French Hajj pilgrims, leading to an excessive consumption of antibiotics [16]. On the other hand, our study showed a significantly

Table 3
Bacterial carriage in post-Hajj pharyngeal samples according to preventive measures and antibiotic intake.

Preventive measures		*Staphylococcus aureus*			*Streptococcus pneumoniae*			*Haemophilus influenzae*			*Klebsiella pneumoniae*		
		n (%)	OR CI 95%	p	n (%)	OR CI 95%	p	n (%)	OR CI 95%	p	n (%)	OR CI 95%	p
Vaccination against IPD	Yes	18 (29.0)	0.67	0.32	2 (3.2)	0.15	0.02	26 (41.9)	0.72	0.40	7 (11.3)	1.46	0.56
	No	19 (38.0)	[0.30–1.47]		9 (18)	[0.03–0.74]		25 (50.0)	[0.34–1.53]		4 (8.0)	[0.40–5.31]	
Mask	Yes	35 (36.1)	3.67	0.10	8 (8.2)	0.36	0.17	45 (46.4)	1.30	0.64	9 (9.3)	0.66	0.62
	No	2 (13.3)	[0.78–17.21]		3 (20.0)	[0.08–1.54]		6 (40.0)	[0.43–3.93]		2 (13.3)	[0.13–3.42]	
Hand washing	Much more	20 (35.7)	1.27 [0.58–2.81]	0.55	6 (10.7)	1.22 [0.35–4.27]	0.75	27 (48.2)	1.24 [0.59–2.61]	0.57	7 (12.5)	1.86 [0.51–6.74]	0.94
	As usual	17 (30.4)			5 (8.9)			24 (42.9)			4 (7.1)		
Disinfectant gel	Yes	19 (44.2)	2.24	0.05	7 (16.3)	3.16	0.08	17 (39.5)	0.67	0.32	6 (14.0)	2.08	0.25
	No	18 (26.1)	[1.01–5.03]		4 (5.8)	[0.87–11.53]		34 (49.3)	[0.31–1.46]		5 (7.2)	[0.59–7.27]	
Disposable handkerchiefs	Yes	12 (24.5)	0.49	0.09	3 (6.1)	0.45	0.26	16 (32.6)	0.39	0.02	6 (12.2)	1.62	0.45
	No	25 (39.7)	[0.22–1.12]		8 (12.7)	[0.11–1.79]		35 (55.6)	[0.18–0.84]		5 (7.9)	[0.46–5.65]	
Antibiotic treatment	Yes	18 (36.0)	1.27	0.55	4 (8.0)	0.68	0.56	22 (44.0)	0.89	0.77	9 (18.0)	6.58	0.02
	No	19 (30.6)	[0.58–2.81]		7 (11.3)	[0.19–2.48]		29 (46.8)	[0.42–1.89]		2 (3.2)	[1.35–32.06]	

IPD: invasive pneumococcal disease, OR: Odds ratio, CI $_{95\%}$: 95% confidence interval.

increased carriage of *S. pneumoniae, H. influenzae* and *K. pneumoniae* following participation in Hajj, confirming previous results obtained from cohort studies conducted among French pilgrims [17,18] and from a large cohort study enrolling pilgrims from 13 countries [15]. Such bacterial acquisition is worrying since these pathogens are frequently responsible for Hajj-associated pneumonia [8–10] or may be responsible for acute rhinosinusitis [11].

We demonstrate here that vaccination against IPD with a conjugate vaccine had a significant protective effect against pneumococcal carriage following participation in the Hajj. Additionally, none of the pilgrims in our cohort suffered from pneumonia. The impact of vaccination against IPD on the reduction of the acquisition of *S. pneumoniae* respiratory carriage and on the prevention of pneumococcal disease in adults is well-described in the literature, especially in at-risk patients, including those with bronchial asthma, chronic obstructive pulmonary disease, diabetes mellitus, chronic heart failure, splenectomy or immunodeficiency [19]. Consistent local or national official recommendations on the use of pneumococcal vaccine for Hajj pilgrims are lacking [6,20–22]. The recent recommendations of the Saudi Thoracic Society for pneumococcal vaccination before the Hajj season are to vaccinate all persons aged 50 years and over with combined pneumococcal conjugate vaccine 13 and pneumococcal polysaccharide vaccine 23 [23]. 54.6% of the participants in our study had chronic diseases, making them at risk for IPD, or were aged 60 years and over, which is the age cut-off for recommended pneumococcal vaccination in Hajj pilgrims according to French medical authorities [12]. If the Saudi recommendation were to be applied, this proportion would have been 73.3%. In our study, 32.8% of the participants were diabetic. A similar proportion was found in a study conducted in patients hospitalized for severe pneumonia during the 2013 Hajj with a 34.8% prevalence of diabetes mellitus [9]. Respiratory symptoms and antibiotic consumption were more frequent in pilgrims with indication for vaccination against IPD in our survey. This apparently paradoxical result is likely due to the fact that pilgrims with indication for vaccination against IPD were older, more likely to present with chronic respiratory disease, diabetes or other chronic conditions making them at higher risk for respiratory infections due to *S. pneumoniae* for which they were protected by vaccination or due other respiratory pathogens for which they were not protected. This finding indicates that criteria used to identify pilgrims at risk for IPD could indeed qualify for pre-screening of pilgrims at higher risk for any respiratory infection and help targeting them for reinforced measures against pulmonary communicable diseases including vaccination against influenza, reinforced hand hygiene practice and use of face masks.

When looking at non-pharmaceutical preventive measures against respiratory infections, our results showed that while a majority of pilgrims used face-masks at least sometimes during their stay in Saudi Arabia, only 14.3% used masks frequently and less than half reported compliance with frequent hand washing, use of disinfectant hand-gel and disposable handkerchiefs. Wearing masks especially when in crowded places, frequent hand washing with soap and water or disinfectant, especially after coughing and sneezing, and the use of disposable handkerchiefs are recommended by the Saudi Ministry of Health [24]. Only 8.4% of the Hajj pilgrims wore masks during the H1N1 outbreak in 2009 and less than 1% wore masks during the MERS-CoV epidemic in 2013 [5]. In our survey, cough, fever and antibiotic consumption were significantly more frequent in individuals using face masks, which likely accounts for the high willingness of pilgrims suffering from febrile cough to wear a face mask, with the aim of avoiding spreading diseases. We have no explanation for the positive association between the use of hand gel and the prevalence of *S. aureus* carriage in post-Hajj throat samples. The negative association between the use of disposable handkerchief and *H. influenzae* carriage in post-Hajj throat samples may possibly reflect an increased removal of organisms by cleaning of the nasopharynx. The 6-fold increase in *K. pneumoniae* carriage in post-Hajj samples in individuals who consumed antibiotics during their stay in Saudi Arabia compared to those who did not may possibly be indicative of the high capacity of *K. pneumoniae* to acquire antibiotic resistance genes under antibiotic selective pressure [25].

Our study had several limitations. The sample size was small and only limited numbers of patients infected with bacteria were recruited. The study was conducted in French pilgrims only and our results cannot be extrapolated to all pilgrims. Information regarding observance of prevention measures was self-reported and subjective. Also, qPCR does not distinguish between living and dead microorganisms and does not allow assessing antibiotic susceptibility. Finally, we did not investigate vaccine pneumococcal serotypes.

5. Conclusions

Hajj pilgrims acquire bacterial pathogens during Hajj, which is a risk for disease especially among the significant population of at-risk individuals among pilgrims. Vaccination with the pneumococcal conjugate vaccine appears to reduce the carriage rate of *S. pneumoniae* among the vaccinated. Further studies based on larger numbers of pilgrims are needed in order to confirm our preliminary findings.

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Article 6 :

Respiratory tract infections among French Hajj pilgrims from 2014 to 2017.

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Respiratory tract infections among French Hajj pilgrims from 2014 to 2017

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Respiratory tract infections (RTIs) are common among Hajj pilgrims, but risk factors for RTIs and respiratory pathogen acquisition during the Hajj are not clearly identified. Based on previous studies, most frequent pathogens acquired by Hajj pilgrims were investigated: rhinovirus, human coronaviruses, influenza viruses, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Haemophilus influenzae*. 485 pilgrims were included. 82.1% presented with RTIs. Respiratory chronic diseases were associated with cough, Influenza-like illness (ILI) and the acquisition of *H. influenzae*. Vaccination against invasive pneumococcal diseases (IPD) and influenza was associated with a decrease in the acquisition of *S. pneumoniae* and prevalence of ILI (aRR = 0.53, 95%CI [0.39–0.73] and aRR = 0.69, 95%CI [0.52–0.92] respectively). Individuals carrying rhinovirus and *H. influenzae-S. pneumoniae* together were respectively twice and five times more likely to have respiratory symptoms. Individual with *H. influenzae-K. pneumoniae* carriage were twice (p = 0.04) as likely to develop a cough. The use of disposable handkerchiefs was associated with a decrease in the acquisition of *S. aureus* (aRR = 0.75, 95%CI [0.57–0.97]). Results could be used to identify pilgrims at increased risk of RTIs and acquisition of respiratory pathogens. Results also confirm the effectiveness of influenza and IPD vaccinations in reducing ILI symptoms and acquisition of *S. pneumoniae* carriage respectively.

The Hajj is one of the largest annual religious mass gatherings in the world. Each year, Saudi Arabia attracts over 2 million pilgrims from over 180 countries, including about 2,000 from Marseille, France[1]. The Hajj presents major challenges in public health and infection control as the crowding conditions favor the acquisition, dissemination and transmission of pathogenic microorganisms[2]. Respiratory tract infections (RTIs) are particularly frequent during the pilgrimage and are responsible for most causes of hospitalization, with community-acquired pneumonia being a major cause of serious illness among pilgrims[3]. Many studies have been conducted among Hajj pilgrims over the last decade, demonstrating the high prevalence of respiratory symptoms and the frequent acquisition of respiratory pathogens[3–7]. The viruses most commonly acquired after the Hajj are human rhinovirus (HRV), human coronaviruses (HCoV) and influenza A virus (IAV). The most frequently acquired respiratory bacteria are *Streptococcus pneumoniae (S. pneumoniae)*, *Staphylococcus aureus (S. aureus)* and *Haemophilus influenzae (H. influenzae)*[3]. However, the etiology of RTIs at the Hajj is likely multifactorial and complex. The potential effects of vaccination against influenza and pneumococcus[8,9], of non-pharmaceutical preventive measures including face-mask use and hand hygiene practice[10–12] have been investigated, mostly based on clinical criteria, but results of studies are contradictory.

So far, to our knowledge, risk factors for pathogen acquisition during the Hajj are not clearly identified. Relationship between respiratory symptoms and carriage of respiratory pathogens at the Hajj also remain poorly understood making it difficult distinguishing between infection and colonization. We conducted this

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Variables		n	%
Pilgrimage year	2014	98	20.2
	2015	119	24.6
	2016	117	24.1
	2017	151	31.1
Gender	Male	212	43.7
	Female	273	56.3
Age*	Median	61.5	
	Interquartile	52–68	
	Min - max	21–96	
Age ≥ 60 years*		269	56.0
Place of birth	France	40	8.5
	North Africa	419	88.4
	Sub-Saharan Africa	13	2.7
	Others	2	0.4
Comorbidities*	Diabetes mellitus	136	28.6
	Hypertension	140	29.5
	Chronic respiratory disease	56	11.8
	Chronic heart disease	32	6.7
	Chronic kidney disease	5	1.1
	Immunodefiency	3	0.6
Indication for vaccination against IPD[1]		315	66.3
BMI[2]	Normal	130	26.8
	Underweight	3	0.6
	Overweight	220	45.4
	Obesity	132	27.2

Table 1. Characteristics of the study population, Hajj pilgrims 2014-2017 (N = 485). [1]Indication for vaccination against invasive pneumococcal diseases: Age superior or equal to 60 years, diabetes mellitus, chronic respiratory disease, chronic heart disease, chronic kidney disease and immunodeficiency, (n = 475, 10 missing data). [2]Body mass index. Normal weight: BMI: 18.5 – 24.9, Underweight: BMI <18.5, Overweight: BMI: 25.0 – 29.9, Obesity: BMI ≥30. [*]n = 480, 5 mission data.

study to identify risk factors for respiratory symptoms and respiratory pathogens carriage at the Hajj, including socio-demographics, vaccination against influenza and invasive pneumococcal diseases (IPD) and adherence to non-pharmaceutical preventive measures using data collected from 2014 to 2017. We also evaluated the relationship between pathogen carriage, including multiple carriage and respiratory symptoms.

Results

Characteristics of study participants. The study enrolled 485 pilgrims, 96.5% of whom filled both the pre- and post-travel questionnaires. The study population had a median age of 61.5 years (interquartile = (52 – 68 years), min = 21, max = 96 years) and a male:female ratio of 1:1.3 (Table 1). The majority (88.4%) of the pilgrims were from North Africa. 66.3% had an indication for vaccination against IPD according to the French recommendation at the time of inclusion[13–20]. Diabetes (28.6%) and hypertension (29.5%) were the most common comorbidities (Table 1).

With regard to preventive measures, 96 (20.2%) pilgrims reported having been vaccinated against pneumococcal (PCV-13) in the last 5 years, representing 30.5% of pilgrims with an indication for IPD. 26.7% (127/466) were vaccinated against influenza before their travel or in the past year. Two hundred sixty-one (56.0%) pilgrims reported using face masks during the Hajj. Also, 42.1% (196/466), 50.4% (235/466) and 73.6% (343/466) pilgrims declared washing their hands during the Hajj more often than usual, using hand gel, and using disposable handkerchiefs during their stay in Saudi Arabia, respectively.

Clinical features. Figure 1 shows the prevalence of respiratory symptoms among pilgrims during the Hajj. More than 80% of the pilgrims presented at least one respiratory symptoms, cough, sore throat and rhinitis being the most frequent. Influenza-like illness (ILI) was present in 16.9% of pilgrims. None suffered from pneumonia or other IPD, and only one was hospitalized during the 2017 Hajj season. Time between arrival in Saudi Arabia and onset of symptoms was 9.5 ± 4.8 days (min = 0, max = 22 days).

Detection of respiratory pathogens by qPCR. Pre- and post-Hajj specimens were collected from 456 (94.0%) and 451 (93.0%) participants, respectively. Furthermore, 32.6% (125/384) ill pilgrims were sampled at onset of respiratory symptoms. At total of 433 (89.3%) pilgrims had paired samples.

The prevalence of respiratory pathogens carriage increased after a participation in Hajj, compared to pre-Hajj status, and differences were significant for all bacterial pathogens and all viruses with the exception of

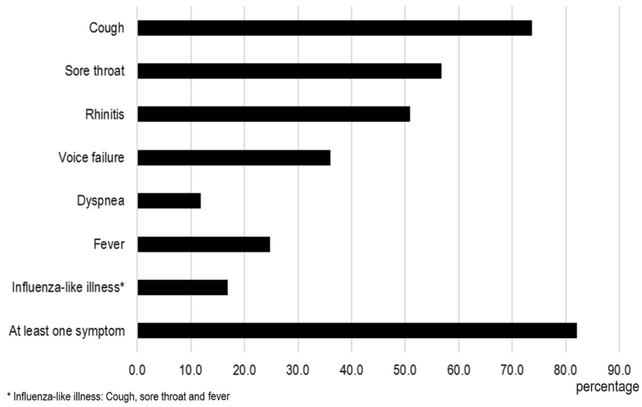

Figure 1. Prevalence of respiratory symptoms among pilgrims during the Hajj 2014–2017.

HCoV-HKU1 (Table 2). Overall, 33.0% of pilgrims acquired at least one respiratory virus, notably RHV (26.9%), HCoV (8.0%) and influenza viruses (3.7%). A similar proportion (35.3%) acquired at least one respiratory bacterium after the Hajj, mainly *H. influenzae* (31.7%), *Klebsiella pneumoniae (K. pneumoniae)* (20.9%), *S. pneumoniae* (17.9%) and *S. aureus* (14.0%). Overall, viral-bacterial acquisition proportion in returned Hajjes was 26.9%, the most common being *H. influenzae*-virus dual carriage (Table 2). Among the 125 pilgrims sampled at symptom onset, a high prevalence of HRV (47/125, 37.6%), *S. aureus* (35/125, 28.0%) and *H. influenzae* (49/125, 39.2%) carriage was observed.

To compare between pre- and per-Hajj specimens, 122 ill pilgrims have had paired samples. The acquisition proportion of HRV, *S. aureus* and *H. influenzae* was 39/122 (32.0%), 14/122 (11.5%) and 43/122 (35.3%) respectively (data not show).

Risk factors for respiratory symptoms. The Supplementary Table 1 and Table 3 show univariate and multivariate analyses results for factors associated with respiratory symptoms during the Hajj. Reporting of at least one respiratory symptom was twice as frequent and five times more frequent in rhinovirus carriers (adjusted relative risk (aRR): 1.98, 95%CI [1.03–3.78]) or *H. influenzae-S. pneumoniae* carriers, respectively (aRR: 4.75, 95%CI [1.17–19.35]) (Table 3). Coughing was twice as frequent in pilgrims suffering from chronic respiratory disease and among those carrying *H. influenzae-K. pneumoniae* together (aRR: 1.98, 95%CI [1.03–3.78]). Pilgrims who coughed were also more likely to use disposable handkerchiefs. Finally, ILI was more frequent in females, in pilgrims with chronic respiratory disease and among those carrying *S. aureus* or an association of virus and bacteria. In addition, pilgrims suffering ILI were more likely to use face mask. Influenza virus was not significantly associated with ILI. No significant association was observed between symptoms and vaccination against IPD. However, influenza vaccination was associated with a decrease in the prevalence of ILI (aRR: 0.69, 95%CI [0.52–0.92]).

We found no significant association between persistence of respiratory symptoms at return and pathogen carriage with the exception of *H. influenzae* carriage in association with viral carriage (aRR: 1.65, 95%CI [1.07–2.53], $p = 0.02$).

Risk factors for respiratory pathogens acquisition among pilgrims. The Supplementary Tables 2 and 3 show the univariate risk factors analysis for respiratory virus and bacteria acquisition, respectively. In multivariate analysis (Table 4), male gender was associated with decreased acquisition of HCoV and *K. pneumoniae* (aRR: 0.76, 95%CI [0.58–0.99]). Older age (≥60 years) was associated with an increased acquisition of influenza viruses, HCoV and *S. pneumoniae* (aRR: 1.39, 95%CI [1.01–1.93], aRR: 1.53, 95%CI [1.16–2.01] and aRR: 1.28, 95%CI [1.01–1.63] respectively). Chronic respiratory disease was associated with increased acquisition of *H. influenzae* (aRR: 1.69, 95%CI [1.14–2.50]). No effect of vaccination against influenza was found. By contrast, vaccination against IPD was associated with a decrease in the acquisition of *S. pneumoniae* (aRR: 0.53, 95%CI [0.39–0.73]). Of note, IPD vaccination did not significantly influenced *S. pneumoniae* carriage at baseline (in pre-Hajj samples) (RR: 0.47, 95%CI [0.11–1.99], $p = 0.29$). Acquisition of HRV was higher among pilgrims who reported using facemasks (aRR = 1.30, 95%CI [1.03–1.65]). The use of disposable handkerchiefs has been associated with a decrease in the acquisition of *S. aureus* (aRR: 0.75, 95%CI [0.57–0.97]). Hand hygiene and the use of a disinfectant gel do not have a significant effect on the acquisition of pathogens.

Discussion

Our results confirm that respiratory infections were very common among French Hajj pilgrims, with more than 80% of them reporting at least one symptom during the 2014–2017 Hajj seasons. Our result is in line with the previous results obtained from cohort studies conducted among French pilgrims[21] and from a large cohort study enrolling pilgrims from 13 different countries[22]. We also document the significant acquisition of almost all viral and bacterial pathogens included in our survey, following participation to the Hajj, as reported previously[3,23].

We found that male gender was independently associated with a decreased risk for ILI and for the acquisition of coronaviruses and *K. pneumoniae*. We have no explanation for this unexpected observation. Older age (≥60

Pathogens	Pre-Hajj		Per-Hajj		Post-Hajj		Acquisition		p*
	n = 456	%	n = 125	%	n = 451	%	n	%	
Viruses									
At least one virus	20	4.4	54	43.2	147	32.6	147	33.9	$<10^{-4}$
All influenza viruses	0	0	6	4.8	11	2.4	16	3.7	$<10^{-4}$
Influenza A	0	0	5	4.0	8	1.8	12	2.8	5.10^{-4}
Influenza B	0	0	1	0.8	3	0.7	4	0.9	0.05
Rhinovirus	18	4.0	47	37.6	117	25.9	120	27.7	$<10^{-4}$
All coronaviruses	2	0.4	17	13.6	29	6.4	36	8.3	$<10^{-4}$
Coronavirus 229E	2	0.4	11	8.8	20	4.4	27	6.2	$<10^{-4}$
Coronavirus HKU1	0	0	1	0.8	1	0.2	1	0.2	0.31
Coronavirus NL63	0	0	3	2.4	5	1.1	6	1.4	0.01
Coronavirus OC43	0	0	4	3.2	3	0.7	6	1.4	0.01
Bacteria									
At least one bacterium	217	47.6	78	62.4	344	76.3	159	36.7	$<10^{-4}$
S. aureus	68	14.9	35	28.0	97	21.5	63	14.5	3.10^{-3}
S. pneumoniae	18	4.0	5	4.0	89	19.7	80	18.5	$<10^{-4}$
H. influenzae	127	27.9	49	39.2	232	51.4	144	33.3	$<10^{-4}$
K. pneumoniae	58	12.7	15	12.0	115	25.5	94	21.7	$<10^{-4}$
Bacteria co-infections									
H. influenzae-S. pneumoniae	9	2.0	2	1.6	49	10.9	47	10.9	$<10^{-4}$
H. influenzae-K. pneumoniae	30	6.6	6	4.8	63	14.0	62	14.3	$<10^{-4}$
H. influenzae-S. aureus	8	1.8	14	11.2	47	10.4	52	12.0	$<10^{-4}$
S. pneumoniae-K. pneumoniae	4	0.9	1	0.8	23	5.1	24	5.5	2.10^{-4}
S. pneumoniae-S. aureus	1	0.2	2	1.6	21	4.7	22	5.1	$<10^{-4}$
K. pneumoniae-S. aureus	6	1.3	4	3.2	21	4.7	23	5.3	3.10^{-4}
Bacteria-virus co-infections									
At least one virus-bacteria combinaison	6	1.32	29	23.2	113	25.1	120	27.7	$<10^{-4}$
S. pneumoniae-virus	1	0.2	2	1.6	37	8.2	37	8.5	$<10^{-4}$
K. pneumoniae-virus	1	0.2	8	6.4	34	7.5	41	9.5	$<10^{-4}$
S. aureus-virus	2	0.4	13	10.4	36	8.0	39	9.0	$<10^{-4}$
H. influenzae-virus	4	0.9	16	12.8	76	16.9	83	19.3	$<10^{-4}$

Table 2. Acquisition of respiratory pathogens during the Hajj. *p value: pre-Hajj versus per-Hajj and/or post-Hajj, McNemar's Test.

years) was associated with the acquisition of influenza viruses, HCoV and *S. pneumoniae*. Although older age was not correlated with the increase in the proportion of respiratory symptoms in our study, our results support the current French recommendations that indicate influenza vaccination for all pilgrims and IPD vaccination for pilgrims aged ≥60 years old (or with chronic conditions)[24,25]. Furthermore, we showed that influenza vaccination was significantly associated with a lower prevalence of ILI in our survey. Similarly, in a meta-analysis, Alfelali *et al*. have shown that, influenza vaccine decreases the prevalence of ILI[26]. We also demonstrate here that vaccination against IPD with a decrease in the acquisition of *S. pneumoniae*, but had no effect on respiratory symptoms. A protective effect of pneumococcal vaccination against *S. pneumoniae* post-Hajj carriage was observed in only one out of 5 studies so far[9]. However, all studies showing no effect of pneumococcal vaccination were conducted on small groups of pilgrims (ranging 55 to 107 individuals), while in the two studies showing a protective effect, the number of pilgrims was much higher (1178 and 468) which may indicate that small surveys lacked statistical power. Finally, as expected, patients suffering from chronic respiratory diseases were more likely to suffer from cough and ILI but also to acquire *H. influenzae*. Our results confirm the need for influenza and IPD vaccination among the identified pilgrim populations at risk of RTIs and acquisition of respiratory pathogens. Vaccination rates in our cohort were clearly sub-optimal: 26.7% of pilgrims were vaccinated against influenza and 30.5% against IPD among those with an indication.

With regard to non-pharmaceutical preventive measures, the use of masks, especially in crowded areas, frequent hand washing with water and soap or disinfectant, especially after coughing and sneezing, and the use of disposable handkerchiefs are recommended by the Saudi Ministry of Health to Hajj pilgrims[27]. In a meta-analysis including 13 surveys of Hajj pilgrims, significant protection of face masks was found against RITs, but the end points varied considerably[12]. We found that there was a higher prevalence of ILI and rhinovirus acquisition among pilgrims who reported wearing face masks. Similarly, a higher prevalence of cough was observed among pilgrims who reported using disposable handkerchiefs. It is likely that such results indicate the higher willingness of symptomatic pilgrims to wear a face mask and use disposable handkerchiefs, with the aim of avoiding spreading diseases. In addition, the use of disposable handkerchiefs appears to be correlated with a decrease in the

Variables	Cough		ILI[†]		At least one symptom	
	aRR [95%CI]	p	aRR [95%CI]	p	aRR [95%CI]	p
Male gender	—		0.75 [0.58–0.96]	0.02	—	
Chronic respiratory disease	2.24 [1.04–4.82]	0.04	1.47 [1.01–2.16]	0.05	—	
Prevention measures						
Mask	—		1.42 [1.10–1.82]	0.007	—	
Disposable handkerchiefs	1.71[1.17–2.50]	0.006	—		—	
Vaccination against influenza	—		0.69 [0.52–0.92]	0.012	—	
Pathogens carriage during the Hajj						
Human rhinovirus	—		—		2.03 [1.14–3.61]	0.02
S. aureus	—		1.34 [1.01–1.82]	0.044	—	
H. influenzae – K. pneumoniae	1.98 [1.03–3.78]	0.04			—	
H. influenzae – S. pneumoniae	—		—		4.75 [1.17–19.35]	0.03
At least one virus-bacteria combinaison	—		1.33 [1.01–1.74]	0.04	—	

Table 3. Risk factor for respiratory symptoms during the Hajj (multivariate analysis). [†]ILI: influenza-like illness. *aRR: adjusted relative risk, CI: confidence interval, p: p value.*

Variables	Influenza viruses	Human rhinovirus	Human coronaviruses	*S. aureus*	*S. pneumoniae*	*H. influenzae*	*K. pneumoniae*
	aRR [95%CI] p	aRR [95%CI] p	aRR [95%CI] p	aRR [95%CI] p	aRR [95%CI] p	aRR [95%CI] p	aRR [95%CI] p
Socio-demographics characteristic							
Male gender			0.76 [0.58 – 0.99] 0.05				0.78 [0.62 – 0.98] 0.04
Age ≥60 years	1.39 [1.01 – 1.93] 0.04		1.53 [1.16 – 2.01] 0.003		1.28 [1.01 – 1.63] 0.05		
Chronic respiratory disease						1.69 [1.14 – 2.50] 0.01	
Preventive measures							
Vaccination against IPD					0.53 [0.39 – 0.73] < 0.0001		
Mask		1.30 [1.03 – 1.65] 0.03					
Handkerchief				0.75 [0.57 – 0.97] 0.03			

Table 4. Risk factor for acquisition of respiratory pathogens during the Hajj (multivariate analysis). *aRR: adjusted relative risk, CI: confidence interval, p: p value, IPD: invasive pneumococcal diseases.*

acquisition of *S. aureus* carriage, which may reflect a better elimination of organisms by cleaning the nasopharynx. We found that increased hand hygiene was not associated with reduced respiratory pathogens acquisition or a lower prevalence of respiratory symptoms. In a recent review paper, it was shown that while hand hygiene using non-alcoholic products was generally well accepted by Hajj pilgrims, there was no conclusive evidence of its effectiveness, which is consistent with our results[11].

Relationship between respiratory symptoms and carriage of respiratory pathogens at the Hajj are unclear. Because of the high frequency of respiratory symptoms, the distinction between infection and colonization is difficult to assess. Furthermore, asymptomatic carriage of potential pathogens is also observed among Hajj pilgrims[28]. Nevertheless, in the final model of multivariate analysis, the acquisition of *S. aureus* was associated with ILI and the acquisition of rhinovirus was associated with respiratory symptoms. Many studies showed that *S. aureus* and rhinovirus were among the predominant pathogens isolated from the Hajj pilgrims suffering RTIs[9,11,12,21,23–32]. Most cases of infections due to HRV are benign, self-limited cold-like illnesses. Nevertheless, HRV is also responsible for severe pneumonia in the elderly and immunocompromised patients, as well as exacerbations of chronic obstructive pulmonary disease and asthma[33]. HRV spreads mostly via direct contact or contact with a fomite, with inoculation to the eye or nose from fingertips[34]. The human-to-human transmission of rhinovirus among pilgrims may have been favored by the crowded conditions at pilgrim accommodations or during performing the Hajj rites.

S. aureus is also often part of the human microbiome in the anterior nares[35]. However, persistent *S. aureus* nasal carriage is associated with secondary staphylococcal respiratory infection, predisposing to invasive disease, especially in the case of detection of IAV[36–38]. To date, few studies addressed virus-bacteria carriage in relation with RTIs at the Hajj[4,28]. In our study, overall carrying virus-bacteria was associated with ILI. Dual *H. influenzae-K. pneumoniae* carriage was associated with twice the risk of coughing and *H. influenzae-S. pneumoniae* carriage with 5 times the risk for respiratory symptoms. Further works aiming at better understanding the role of overall carrying virus and bacteria in the pathogenesis of the RTIs at the Hajj are needed.

Our study had some limitations. The study was conducted among French pilgrims only and cannot be generalized to all pilgrims. Observance with individual preventive measures was self-reported and frequency of changing face mask and exact quantification of hand hygiene practice were not assessed. qPCR used to detect respiratory pathogens does not distinguish between dead and living micro-organisms. *S. pneumoniae* serotypes were not investigated. *Moraxella catarrhalis* that was recently shown to be relatively frequently acquired by Hajj pilgrims[39] was not included in our study. Finally, we did not to be any long-term follow-up to determine what infections might have had delayed presentation after return from the pilgrimage. Nevertheless, our study included a large samples size of pilgrims spanning four Hajj seasons giving it a stronger statistical power. A number of risk factors have been identified to recognize pilgrims at increased risk of RTIs and of acquisition of most common respiratory pathogens encountered in this setting. Also, the study confirmed the effectiveness of vaccination against influenza in reducing ILI symptoms and that of vaccination against IPD in reducing acquisition of *S. pneumoniae*. Given the limitations of the current study, the use of face mask and a reinforced hand hygiene should still be recommended for Hajj pilgrims until large-scale controlled studies are conducted to truly assess the effectiveness of these measures in the context of Hajj. The use of disposable handkerchief is, de facto, highly frequent among ill pilgrims and at least allows decreasing the acquisition of *S. aureus*.

Methods

Participants and study design. A convenience sample of pilgrims participating in the Hajj from 2014 to 2017 was surveyed. Potential adult participants were recruited at a private specialized travel agency from Marseille, France, organizing journeys to Mecca, Saudi Arabia and invited to participate in the study. They were included and followed-up by a medical bilingual (Arabic and French) doctor who traveled with the group. Upon inclusion before departing from France, the pilgrims were interviewed using a standardized pre-Hajj questionnaire that collected items about demographic characteristics (age, sex, place of birth), immunization status (vaccination against influenza and IPD) and medical history (chronic medical conditions, immunodeficiency, body mass index (BMI)). Pilgrims were considered to have been vaccinated immunized against influenza when they had been vaccinated within the last year, but before 10 days of the date of travel. Pilgrims were considered immune to IPD when they had been vaccinated with the 13-valent conjugate pneumococcal vaccine (PCV-13) in the past 5 years[13–20]. Clinical events occurring during the travel (type of respiratory symptoms, date of onset, duration of symptoms, antibiotic treatment provided) and persistence of respiratory symptoms at return were collected by a medical doctor using a post-Hajj questionnaire at 2 days before the pilgrims' return to France. The information on compliance with face masks use as well as hand washing, use of hand gel disinfectant and disposable handkerchiefs was documented. Ill pilgrims, who spontaneously consulted the accompanying medical doctor at the time of onset during the Hajj, underwent a complementary nasopharyngeal swab (per-Hajj specimens). ILI was defined as the presence of cough, sore throat and subjective fever[40]. Based on the WHO classification, underweight was define as a BMI below 18.5, normal with a BMI from 18.5 to less than 25, overweight with a BMI greater $\geq$25 and obesity with a BMI greater $\geq$30[41].

The protocol was approved by the Aix-Marseille University institutional review board (July 23rd, 2013; reference no. 2013-A00961-44). The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants provided an informed written consent.

Respiratory specimen. Nasopharyngeal swabs were obtained from each pilgrim, transferred to Sigma-Virocult® medium and stored at −80 °C until processing. Pre-Hajj and post-Hajj nasopharyngeal swabs were systematically collected at enrollment, prior traveling to Saudi Arabia and two days prior returning to France, respectively. In addition, nasopharyngeal swabs were collected at symptom onset when possible.

Respiratory specimens. DNA and RNA were extracted from the respiratory samples using the EZ1 Advanced XL (Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendations. Quantitative real-time PCRs were conducted using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA). Negative control (PCR mix) and positive control (DNA from bacterial strain or RNA from viral strain) were included in each run. Positive results of bacteria or virus amplification were defined as those with a cycle threshold (CT) value $\leq$35.

Identification of respiratory virus. One-step duplex quantitative RT-PCR amplifications of HCoV/HPIV-R Gene Kit (REF: 71–045, Biomérieux, Marcy l'Etoile, France) was used to detect HCoV and human para-influenza viruses according to the manufacturer's recommendations. One-step simplex real-time quantitative RT-PCR amplifications were performed using the Multiplex RNA Virus Master Kit (Roche Diagnostics, France) for HRV, IAV, influenza B and internal controls MS2 phage[42].

Identification of respiratory bacteria. Real-time PCR amplifications were carried out using the LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the recommendations of the manufacturer. The SDD gene of *H. influenzae*, *nucA* gene of *S. aureus*, *phoE* gene of *Klebsiella pneumoniae*, *lytA CDC* gene of *S. pneumoniae*, were amplified with internal DNA extraction controls TISS, as previously described[22].

The respiratory viruses and bacteria tested in this study were the most frequent microorganism detected among French pilgrims, according to previous studies[28,29]. All other pathogens (adenovirus, bocavirus, metapneumovirus, respiratory syncytial virus, parainfluenza viruses, *Bordetella pertussis, Mycoplasma pneumoniae, Neisseria meningitidis*) with prevalence <1% were not included in this study.

The acquisition of respiratory bacteria and viruses was defined as negative before travel and positive during the Hajj and/or when returning to France.

Statistical analysis. STATA software version 11.1 (Copyright 2009 StataCorp LP, http://www.stata.com) was used for statistical analysis. Differences in the proportions were tested by Pearson's chi-square or Fisher's exact tests when appropriate. In order to evaluate the potential acquisition of respiratory pathogens in Saudi Arabia, we used the McNemar's Test to compare their prevalence before leaving France and in Saudi Arabia (during and after the Hajj). Unadjusted associations between multiple factors and prevalence of respiratory pathogen acquisition and prevalence of respiratory symptom during the Hajj were examined by univariable analysis. The results were presented by percentages and risk ratio with 95% confidence interval (95%CI). Results with a p value ≤ 0.05 was considered statistically significant. Only the variables with a prevalence $\geq 5.0\%$ were considered for statistical analysis. Variables with p values < 0.2 in the univariable analysis were included in the multivariable analysis. Log-binomial regression was used to estimate factor's adjusted risk ratios regarding respiratory symptoms and respiratory pathogens acquisition[43].

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Author contributions
V.T.H., V.P.S. and P.G. contributed to experimental design, data analysis, statistics, interpretation and writing. V.T.H. and S.A.S. conducted the qPCR technique, K.B., M.M. and D.S. administered questionnaires, followed patients and collected samples, T.D.A.L., T.L.D. and L.N. provided technical assistance. S.Y., B.A., D.R. and P.P. contributed to critically reviewing the manuscript. P.G. coordinated the work.

Competing interests
The authors declare no competing interests.

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Supplementary data

Respiratory tract infections among French Hajj pilgrims from 2014 to 2017

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Table supplementary 1: Risk factor for respiratory symptoms during the Hajj (univariate analysis)

Variables		Cough		ILI		At least one symptom	
		n (%)	RR [95%CI] p	n (%)	RR [95%CI] p	n (%)	RR [95%CI] p
Socio-demographic characteristics							
Gender	Male	139 (68.5)	0.88 [0.79-0.99]	23 (11.3)	0.54 [0.34-.084]	159 (78.3)	0.92 [0.84-1.01]
	Female	206 (77.7)	0.02	56 (21.1)	0.005	225 (84.9)	0.07
Age	≥ 60 years	206 (77.4)	1.13 [1.01-1.27]	46 (17.3)	1.08 [0.72-1.63]	226 (85.0)	1.09 [0.99-1.19]
	<60 years	137 (68.5)	0.03	32 (16.0)	0.71	156 (78.0)	0.053
Country of birth	France	26 (65.0)	Ref	6 (15.0)	Ref	32 (80.0)	Ref
	North Africa	307 (74.5)	1.15 [0.91-1.45] 0.25	70 (17.0)	1.13 [0.53-2.44] 0.75	340 (82.5)	1.03 [0.88-1.21] 0.71
Comorbidities							
Diabetes mellitus	Yes	103 (78.0)	1.09 [0.97-1.22]	25 (18.9)	1.19 [0.78-1.84]	112 (84.8)	1.01 [0.96-1.15]
	No	240 (71.9)	0.17	53 (15.9)	0.42	270 (80.8)	0.31
Hypertension	Yes	102 (74.5)	1.02 [0.90-1.14]	23 (16.8)	1.01 [0.64-1.57]	117 (85.4)	1.06 [0.97-1.16]
	No	241 (73.3)	0.79	55 (16.7)	0.99	265 (80.5)	0.21
Chronic respiratory disease	Yes	48 (87.3)	1.22 [1.08-1.37]	15 (27.2)	1.78 [1.09-2.90]	51 (92.7)	1.15 [1.05-1.26]
	No	295 (71.8)	0.01	63 (15.3)	0.03	331 (80.5)	0.03
Chronic heart disease	Yes	26 (83.9)	1.15 [0.98-1.36]	7 (22.6)	1.38 [0.70-2.74]	29 (93.5)	1.15 [1.04-1.28]
	No	317 (72.9)	0.18	71 (16.3)	0.37	353 (81.1)	0.08
Indication for vaccination against IPD	Yes	239 (77.1)	1.16 [1.02-1.31]	58 (18.7)	1.46 [0.91-2.34]	262 (84.5)	1.10 [1.01-1.21]
	No	104 (66.7)	0.02	20 (12.8)	0.11	120 (76.9)	0.04
BMI[1]	Normal	90 (69.8)	Ref	23 (17.8)	Ref	104 (80.6)	Ref
	Overweight	158 (73.5)	1.05 [0.92-1.21] 0.46	31 (14.4)	0.81 [0.49-1.32] 0.40	175 (81.4)	1.01 [0.91-1.12] 0.86

	Obesity	94 (77.7)	1.11 [0.96-1.29] 0.16	24 (19.8)	1.11 [0.66-1.86] 0.69	102 (84.3)	1.04 [0.93-1.17] 0.44
Preventive measures							
Vaccination against influenza	Yes	92 (72.4)	0.98 [0.86-1.11]	16 (12.6)	0.69 [0.41-1.15]	100 (78.7)	0.95 [0.85-1.05]
	No	251 (74.0)	0.73	62 (18.3)	0.14	282 (83.2)	0.27
Vaccination against IPD	Yes	70 (72.9)	0.99 [0.86-1.13]	20 (20.8)	1.33 [0.84-2.10]	78 (81.3)	0.99 [0.89-1.10]
	No	273 (73.8)	0.86	58 (15.7)	0.23	304 (82.2)	0.84
Mask	Yes	197 (75.5)	1.01 [0.94-1.18]	56 (21.5)	1.91 [1.22-3.00]	218 (83.5)	1.04 [0.96-1.14]
	No	147 (71.7)	0.36	23 (11.2)	0.004	164 (80.0)	0.32
Hand washing	More often than usual	147 (75.0)	1.03 [0.92-1.15]	30 (15.3)	0.84 [0.56-1.28]	164 (83.7)	1.04 [0.95-1.13]
	As usual	197 (73.0)	0.62	49 (18.2)	0.42	218 (80.7)	0.42
Disinfectant gel	Yes	176 (74.9)	1.02 [0.92-1.15]	47 (20.0)	1.44 [0.96-2.18]	197 (83.8)	1.05 [0.96-1.14]
	No	168 (72.7)	0.59	32 (13.9)	0.08	185 (80.1)	0.29
Disposable handkerchiefs	Yes	267 (77.8)	1.24 [1.07-1.44]	61 (17.8)	1.22 [0.75-1.97]	289 (84.3)	1.11 [1.01-1.24]
	No	77 (62.6)	0.001	18 (14.6)	0.42	93 (75.6)	0.03
Respiratory pathogens carrying during the Hajj							
Respiratory virus							
At least one virus	Yes	131 (79.4)	1.12 [1.01-1.25]	35 (21.2)	1.41 [0.94-2.11]	147 (89.1)	1.13 [1.04-1.23]
	No	203 (71.0)	0.05	43 (15.0)	0.09	225 (78.7)	0.005
Human rhinovirus	Yes	108 (79.4)	1.11 [0.99-1.24]	28 (20.6)	1.30 [0.86-1.97]	122 (89.7)	1.13 [1.04-1.22]
	No	226 (71.7)	0.09	50 (15.9)	0.22	250 (79.4)	0.01
Human coronavirus	Yes	33 (86.8)	1.19 [1.04-1.37]	10 (26.3)	1.59 [0.90-2.83]	36 (94.7)	1.17 [1.07-1.27]
	No	300 (72.8)	0.06	68 (16.5)	0.13	335 (81.3)	0.03
Respiratory bacteria							
At least one bacteria	Yes	262 (74.4)	1.02 [0.89-1.17]	65 (18.5)	1.41 [0.81-2.44]	292 (83.0)	1.03 [0.92-1.14]

	No	72 (72.7)	0.73	13 (13.1)	0.22	80 (80.8)	0.62
S. aureus	Yes	78 (73.4)	0.99 [0.87-1.13]	26 (24.5)	1.61 [1.07-2.46]	85 (80.2)	0.97 [0.87-1.07]
	No	254 (74.1)	0.92	52 (15.2)	0.03	285 (83.1)	0.49
S. pneumoniae	Yes	70 (79.5)	1.10 [0.97-1.24]	12 (13.6)	0.76 [0.43-1.34]	77 (87.5)	1.08 [0.98-1.18]
	No	261 (72.5)	0.18	65 (18.1)	0.32	292 (81.1)	0.16
H. influenzae	Yes	182 (76.2)	1.02 [0.95-1.19]	41 (17.2)	1.01 [0.67-1.51]	202 (84.5)	1.06 [0.97-1.15]
	No	151 (71.6)	0.27	36 (17.1)	0.98	169 (80.1)	0.22
K. pneumoniae	Yes	93 (76.2)	1.03 [0.93-1.18]	23 (18.9)	1.14 [0.73-1.77]	104 (85.2)	1.05 [0.96-1.15]
	No	238 (73.0)	0.49	54 (16.6)	0.57	265 (81.3)	0.33
Bacteria co-infections							
H. influenzae – S.	Yes	42 (85.7)	1.18 [1.04-1.34]	7 (14.3)	0.81 [0.39-1.66]	47 (95.9)	1.19 [1.10-1.28]
pneumoniae	No	292 (72.6)	0.05	71 (17.7)	0.56	325 (80.8)	0.01
H. influenzae – K.	Yes	59 (84.3)	1.17 [1.04-1.32]	13 (18.6)	1.09 [0.64-1.86]	63 (90.0)	1.11 [1.01-1.22]
pneumoniae	No	275 (72.2)	0.03	65 (17.1)	0.76	309 (81.1)	0.07
H. influenzae – S.	Yes	40 (75.5)	1.02 [0.87-1.20]	12 (22.6)	1.37 [0.79-2.35]	43 (81.1)	0.98 [0.86-1.13]
aureus	No	294 (73.9)	0.80	66 (16.6)	0.27	329 (82.7)	0.78
S. pneumoniae – K.	Yes	21 (84.0)	1.14 [0.95-1.37]	2 (8.0)	0.45 [0.12-1.72]	23 (92.0)	1.12 [0.99-1.27]
pneumoniae	No	313 (73.5)	0.24	76 (17.8)	0.21	349 (81.9)	0.20
S. pneumoniae – S.	Yes	16 (72.7)	0.98 [0.76-1.27]	2 (9.1)	0.51 [0.13-1.95]	17 (77.3)	0.93 [0.74-1.18]
aureus	No	318 (74.1)	0.88	76 (17.7)	0.30	355 (82.8)	0.51
K. pneumoniae – S.	Yes	19 (76.0)	1.03 [0.82-1.29]	7 (28.0)	1.68 [0.87-3.26]	21 (84.0)	1.02 [0.85-1.22]
aureus	No	315 (73.9)	0.82	71 (16.7)	0.15	351 (82.4)	0.84
Virus-bacteria co-infections							
At least one virus-	Yes	104 (80.6)	1.13 [1.01-1.26]	32 (24.8)	1.73 [1.16-2.60]	115 (89.1)	1.12 [1.03-1.21]
bacteria combinaison	No	230 (71.4)	0.04	46 (14.3)	0.008	257 (79.8)	0.02

H. influenzae – virus	Yes	72 (82.8)	1.15 [1.02-1.29]	18 (20.7)	1.26 [0.78-2.01]	79 (90.8)	1.13 [1.04-1.23]
	No	262 (72.0)	0.04	60 (16.5)	0.35	293 (80.5)	0.02
S. pneumoniae - virus	Yes	31 (81.6)	1.11 [0.95-1.31]	8 (21.1)	1.24 [0.65-2.38]	34 (89.5)	1.09 [0.97-1.23]
	No	303 (73.4)	0.27	70 (17.0)	0.52	338 (81.8)	0.24
K. pneumoniae - virus	Yes	35 (83.3)	1.14 [0.98-1.32]	11 (26.2)	1.60 [0.92-2.78]	40 (95.2)	1.17 [1.08-1.27]
	No	299 (73.1)	0.15	67 (16.4)	0.11	332 (81.1)	0.02
S. aureus - virus	Yes	36 (81.8)	1.12 [0.96-1.30]	12 (27.3)	1.68 [0.99-2.86]	40 (90.9)	1.11 [1.00-1.24]
	No	298 (73.2)	0.21	66 (16.2)	0.07	332 (81.6)	0.12

IPD: Invasive pneumococcal disease, ILI: influenza-like illness

[1]Normal weight: BMI: 18.5 – 24.9, Overweight: BMI 25.0 – 29.9, Obesity: BMI ≥30

Table supplementary 2: Risk factor for acquisition of respiratory viruses during the Hajj (univariate analysis)

Variables		Influenza viruses		Human rhinovirus		Human coronaviruses	
		n (%)	RR [95%CI] p	n (%)	RR [95%CI] p	n (%)	RR [95%CI] p
Socio-demographic characteristics							
Gender	Male	4 (2.0)	0.43 [0.14-1.32]	51 (26.4)	0.97 [0.71-1.32]	10 (5.1)	0.50 [0.25-1.01]
	Female	12 (4.7)	0.13	69 (27.3)	0.84	26 (10.2)	0.05
Age	60 years	13 (5.1)	3.23 [0.93-11.19]	70 (27.6)	1.04 [0.76-1.42]	29 (11.3)	3.06 [1.37-6.84]
	60 years	3 (1.6)	0.05	50 (26.5)	0.80	7 (3.7)	0.004
Country of birth	France	2 (5.3)	Ref	8 (21.1)	Ref	1 (2.6)	Ref
	North Africa	13 (3.3)	0.63 [0.15-2.67] 0.75	110 (28.1)	1.33 [0.71-2.52] 0.38	35 (8.8)	3.36 [0.47-23.84] 0.23
Comorbidities							
Diabetes mellitus	Yes	4 (3.1)	0.84 [0.28-2.55]	35 (28.0)	1.04 [0.75-1.46]	11 (8.7)	1.04 [0.56-2.18]
	No	12 (3.8)	0.75	85 (26.8)	0.80	25 (7.8)	0.77
Hypertension	Yes	3 (2.3)	0.56 [0.16-1.94]	42 (32.8)	1.32 [0.97-1.81]	12 (9.2)	1.20 [0.62-2.33]
	No	13 (4.1)	0.35	78 (24.8)	0.09	24 (7.6)	0.59
Chronic respiratory disease	Yes	3 (5.6)	1.68 [0.49-5.69]	17 (32.1)	1.21 [0.79-1.85]	5 (9.3)	1.17 [0.48-2.88]
	No	13 (3.3)	0.41	103 (26.5)	0.39	31 (7.9)	0.73
Chronic heart disease	Yes	1 (3.2)	0.89 [0.12-6.54]	10 (33.3)	1.25 [0.73-2.12]	3 (9.7)	1.21 [0.40-3.75]
	No	15 (3.6)	0.91	110 (26.7)	0.43	33 (7.9)	0.73
Indication for vaccination against IPD	Yes	13 (4.4)	2.15 [0.62-7.44]	80 (27.2)	1.03 [0.73-1.39]	30 (10.0)	2.46 [1.05-5.77]
	No	3 (2.0)	0.21	40 (27.0)	0.97	6 (4.1)	0.03
BMI[1]	Normal	5 (4.1)	Ref	30 (24.6)	Ref	7 (5.7)	Ref
	Overweight	8 (3.9)	0.95 [0.32-2.83] 0.92	56 (26.7)	1.09 [0.74-1.59] 0.68	17 (8.2)	1.42 [0.61-3.34] 0.42

Obesity		3 (2.6)	0.64 [0.16-2.60] 0.53	35 (30.4)	1.24 [0.82-1.88] 0.32	12 (10.2)	1.77 [0.72-4.35] 0.21
Preventive measures							
Vaccination against influenza	Yes	5 (4.0)	1.18 [0.42-3.33]	37 (30.3)	1.17 [0.84-1.62]	10 (8.1)	0.99 [0.50-2.01]
	No	11 (3.4)	0.75	83 (25.9)	0.35	26 (8.1)	0.99
Vaccination against IPD	Yes	2 (2.1)	0.53 [0.12-2.31]	27 (28.7)	1.07 [0.75-1.54]	5 (5.3)	0.60 [0.24-1.51]
	No	14 (4.0)	0.39	93 (26.7)	0.70	31 (8.8)	0.27
Mask	Yes	9 (3.5)	0.98 [0.37-2.58]	79 (40.0)	1.42 [1.02-1.97]	21 (8.3)	1.06 [0.56-2.01]
	No	7 (3.6)	0.96	41 (21.8)	0.03	15 (7.8)	0.85
Hand washing	More often than usual	5 (2.6)	0.62 [0.22-1.76]	51 (27.3)	1.01 [0.74-1.38]	14 (7.4)	0.87 [0.46-1.65]
	As usual	11 (4.2)	0.36	69 (27.0)	0.94	22 (8.5)	0.67
Disinfectant gel	Yes	8 (3.5)	0.98 [0.37-2.56]	60 (26.8)	0.98 [0.72-1.33]	16 (7.1)	0.78 [0.42-1.47]
	No	8 (3.6)	0.96	60 (27.4)	0.88	20 (9.0)	0.44
Disposable handkerchiefs	Yes	13 (4.0)	1.55 [0.45-5.36]	89 (27.5)	1.05 [0.74-1.50]	27 (8.2)	1.08 [0.52-2.22]
	No	3 (2.5)	0.48	31 (26.1)	0.77	9 (7.6)	0.84

[1]BMI normal: 18.5 – 24.9, Overweight: BMI 25.0 – 29.9, Obesity: BMI ≥30

Table supplementary 3: Risk factor for acquisition of respiratory bacteria during the Hajj (univariate analysis)

Variables		***S. aureus***		***S. pneumoniae***		***H. influenzae***		***K. pneumoniae***	
		n (%)	**RR (95%CI) p**	**n (%)**	**RR (95%CI) p**	**n (%)**	**RR (95%CI) p**	**n (%)**	**RR (95%CI) p**
Socio-demographic characteristics									
Gender	Male	29 (14.8)	1.11 [0.70-1.75] 0.67	39 (20.0)	1.22 [0.82-1.82] 0.32	61 (31.6)	0.99 [0.76-1.31] 0.99	32 (16.3)	0.67 [0.46-0.98] 0.04
	Female	34 (13.4)		41 (16.3)		83 (31.7)		62 (24.4)	
Age	60 years	34 (13.2)	0.87 [0.55-1.37] 0.54	51 (20.2)	1.32 [0.87-2.00] 0.19	90 (35.0)	1.30 [0.97-1.72] 0.07	54 (21.2)	1.07 [0.74-1.56] 0.70
	60 years	29 (15.3)		29 (15.3)		53 (27.4)		38 (19.8)	
Country of birth	France	8 (21.1)	Ref	4 (10.8)	Ref	10 (25.6)	Ref	8 (20.5)	Ref
	North Africa	52 (13.1)	0.62 [0.62-1.21 0.17]	73 (18.6)	1.72 [0.67-4.45] 0.26	129 (32.3)	1.26 [0.72-2.19] 0.42	82 (20.9)	1.02 [0.53-1.94] 0.96
Comorbidities									
Diabetes mellitus	Yes	19 (14.8)	1.07 [0.65-1.76] 0.78	19 (15.1)	0.78 [0.49-1.25] 0.30	44 (33.8)	1.10 [0.83-1.48] 0.51	27 (21.6)	1.06 [0.71-1.58] 0.76
	No	44 (13.8)		61 (19.3)		99 (30.7)		65 (20.3)	
Hypertension	Yes	16 (12.3)	0.83 [0.49-1.40] 0.48	25 (19.4)	1.10 [0.72-1.69] 0.65	41 (30.8)	0.97 [0.72-1.31] 0.83	32 (24.8)	1.31 [0.90-1.90] 0.17
	No	47 (14.9)		55 (17.6)		102 (31.9)		60 (18.9)	
Chronic respiratory disease	Yes	8 (15.1)	1.08 [0.54-2.14] 0.83	10 (18.5)	1.03 [0.56-1.87] 0.93	27 (48.2)	1.65 [1.21-2.25] 0.004	11 (20.0)	0.96 [0.55-1.69] 0.90
	No	55 (14.0)		70 (18.0)		116 (29.2)		81 (20.8)	
Chronic heart disease	Yes	2 (0.44)	0.44 [0.11-1.71] 0.21	7 (22.6)	1.27 [0.64-2.52] 0.50	9 (29.0)	0.91 [0.52-1.61] 0.75	9 (29.0)	1.45 [0.81-2.59] 0.23
	No	61 (14.7)		73 (17.8)		134 (31.8)		83 (20.0)	
Indication for vaccination against IPD	Yes	40 (13.4)	0.86 [0.54-1.39] 0.55	54 (18.3)	1.04 [0.68-1.58] 0.87	98 (32.6)	1.10 [0.82-1.48] 0.52	61 (20.6)	0.99 [0.67-1.46] 0.96
	No	23 (15.5)		26 (17.7)		45 (29.6)		31 (20.8)	
BMI[1]	Normal	15 (12.3)	Ref	23 (18.9)	Ref	45 (36.6)	Ref	28 (22.8)	Ref
	Overweight	32 (15.3)	1.25 [0.70-2.20]	40 (19.2)	1.04 [0.64-1.62]	64 (30.2)	0.83 [0.61-1.12]	41 (19.7)	0.87 [0.57-1.33]

			0.45		0.93		0.22		0.51
Obesity		16 (13.8)	1.12 [0.58-2.16]	17 (15.0)	0.80 [0.45-1.41]	35 (29.9)	0.82 [0.57-1.17]	25 (21.6)	0.95 [0.59-1.52]
			0.73		0.44		0.28		0.82
Preventive measures									
Vaccination against influenza	Yes	19 (15.3)	1.12 [0.68-1.84]	24 (19.5)	1.11 [0.72-1.71]	36 (28.8)	0.88 [0.64-1.21]	24 (19.5)	0.92 [0.61-1.40]
	No	44 (13.7)	0.65	56 (17.6)	0.63	107 (32.6)	0.43	68 (21.1)	0.71
Vaccination against IPD	Yes	13 (13.8)	0.97 [0.55-1.72]	6 (6.4)	0.30 [0.13-0.67]	38 (40.0)	1.36 [1.02-1.83]	25 (26.6)	1.39 [0.93-2.08]
	No	50 (14.2)	0.93	74 (21.3)	0.001	105 (29.3)	0.05	67 (19.1)	0.11
Mask	Yes	39 (15.4)	1.23 [0.77-1.97]	39 (15.4)	0.71 [0.48-1.06]	88 (34.6)	1.19 [0.90-1.58]	48 (19.0)	0.81 [0.56-1.16]
	No	24 (12.5)	0.39	41 (21.6)	0.10	56 (29.0)	0.21	45 (23.4)	0.25
Hand washing	More often than usual	22 (11.6)	0.73 [0.45-1.18]	38 (20.1)	1.22 [0.82-1.81]	67 (35.6)	1.20 [0.92-1.57]	34 (18.1)	0.79 [0.54-1.15]
			0.20		0.33		0.19		0.21
	As usual	41 (16.0)		42 (16.5)		77 (29.7)		59 (23.0)	
Disinfectant gel	Yes	31 (13.7)	0.94 [0.60-1.49]	44 (19.8)	1.22 [0.82-1.81]	65 (29.1)	0.83 [0.63-1.08]	50 (22.3)	1.15 [0.80-1.65]
	No	32 (14.5)	0.80	36 (16.3)	0.33	79 (35.3)	0.17	43 (19.5)	0.46
Disposable handkerchiefs	Yes	39 (11.9)	0.58 [0.37-0.93]	67 (20.6)	1.85 [1.06-3.22]	100 (30.2)	0.80 [0.60-1.06]	72 (21.9)	1.21 [0.78-1.87]
	No	24 (20.3)	0.02	13 (11.1)	0.02	44 (37.9)	0.13	21 (18.1)	0.39
Antibiotic use	Yes	25 (14.0)	0.99 [0.62-1.57]	25 (14.1)	0.69 [0.44-1.06]	60 (33.5)	1.08 [0.82-1.41]	44 (24.7)	1.32 [0.93-1.90]
	No	38 (14.2)	0.95	55 (20.6)	0.08	84 (31.1)	0.59	50 (18.7)	0.12

[1]BMI normal: 18.5 – 24.9, Overweight: BMI 25.0 – 29.9, Obesity: BMI ≥30

Article 7 :

Dynamics and genetic diversity of *Haemophilus influenzae* carriage among French pilgrims during the 2018 Hajj: a prospective study

Hoang VT, Dao TL, Ly TDA, Belhouchat K, Larbi Chaht K, Yezli S, Alotaibi B, Raoult D, Parola P, Pommier de Santi V, Fournier PE, Gautret P

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Original article

Dynamics and genetic diversity of *Haemophilus influenzae* carriage among French pilgrims during the 2018 Hajj: A prospective cohort survey

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ABSTRACT

Background: To investigate the genetic diversity of *Haemophilus influenzae* positive samples among French pilgrims attending the 2018 Hajj pilgrimage.
Method: After screening by qPCR, multilocus sequence typing was performed for all *H. influenzae*-positive samples. The following housekeeping genes were amplified and assigned: *adk, atpG, frdB, fucK, mdh, pgi* and *recA*.
Results: 121 pilgrims were included. *H. influenzae* was positive in 35.5% pre-Hajj samples, 12.4% at day five post-arrival, 15.7% at day 12 post-arrival, and 43.0% post-Hajj. Of the 129 positive swabs for *H. influenzae*, only one sample at D12 was negative for all seven genes amplified by standard PCR. The *adk, atpG, frdB, mdh, pgi, recA* and *fucK* genes were positive in 123, 107, 122, 70, 127, 118 and 69 samples, respectively. One sequence of *atpG* and two of *recA* genes were not possible to assign. None of the sequences of *fucK* gene was successfully obtained. Consequently, a complete sequence type characterisation was not possible. Of the 128 obtained strains, 111 had distinct patterns of alleles.
Conclusion: H. influenzae genotypes acquired were completely different from those present at pre-Hajj. We observed a great biodiversity and a lack of clonality of *H. influenzae* among French pilgrims during the 2018 Hajj. Further studies aiming at studying the genome of Hajj-acquired *H. influenzae* isolates are needed to define the clinical burden of *H. influenzae* infection during Hajj and to evaluate the potential interest of vaccination in Hajj pilgrims.

1. Introduction

Every year, the Kingdom of Saudi Arabia (KSA) hosts more than two million people from about 180 countries during the Hajj pilgrimage. This number continues to increase, and the estimated number of annual attendees is expected to reach 4.5 million by 2030 [1]. Crowding and the extreme heat in Mecca, are known risk factors for the transmission of respiratory infections at the Hajj [2]. Infectious diseases accounted for 53% of diagnoses in outpatients consulting at the Indian Medical Mission in Mecca during the 2014–2016 Hajj, with respiratory tract infections (RTIs) and gastroenteritis being the most common [3]. The effectiveness of individual non-pharmaceutical preventive measures against infectious diseases is uncertain [3]. Despite several preventive measures [4], including hand hygiene, handkerchief use, face mask use, and vaccinations against influenza and pneumococcal infections in at-risk individuals being recommended, RTIs remain frequent among Hajj pilgrims. As an example, up to 90% of French pilgrims suffered an RTI during the 2012–2014 Hajj [5,6]. Besides the widespread acquisition of respiratory viruses, bacteria are also frequently isolated among ill and asymptomatic pilgrims with *Haemophilus influenzae* being the most common [7–10]. Most cohort surveys among pilgrims used real-time PCR to detect the carriage of *H. influenzae*. Based on a culture method,

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Nik Zuraina showed that *H. influenzae* was the most predominant bacterium isolated (60%) from Malaysian pilgrims with RTIs during the Hajj [9]. In a 2018 study conducted among French Hajj pilgrims the carriage of *Staphylococcus aureus, Moraxella catarrhalis, Streptococcus pneumoniae and Klebsiella pneumoniae* increased following participation in the Hajj [11]. In contrast, *H. influenzae* carriage first decreased on D5 and D12 post-arrival by a factor of 2.5 and then increased in post-Hajj samples to reach carriage rates higher than that of pre-Hajj samples [11]. The objective of this study was to investigate the genetic diversity of these *H. influenzae* positive samples among French pilgrims before, during and after the 2018 Hajj.

2. Methods

2.1. Participants and study design

This study included pilgrims travelling from Marseille, France, to Mecca, KSA during the 2018 Hajj. Participant were recruited at a single specialized travel agency and followed-up during the travel by two bilingual (French and Arabic) medical doctors. All pilgrims departed to KSA at the same date, were housed in the same accommodation and performed the rituals together during their stay. To evaluate the dynamic of *H. influenzae* during the Hajj, all pilgrims underwent four successive systematic nasopharyngeal swabs at different times: pre-travel, five to six days post arrival (D5 sample), 12–13 days post arrival (D12 sample) and just prior to leaving KSA (post-Hajj sample). The Hajj rituals took place from 19 to 24 August, corresponding to days 14–19 post-arrival (Fig. 1).

2.2. Respiratory specimen and screening for H. influenzae by real-time qPCR

The sampling was done by the doctors accompanying the group, in a standardized way (3 cm in the nostril, 5 turns; post wall of the pharynx, 5 streaks). Obtained swabs were transferred to Sigma-Virocult® medium and kept at room temperature (20 °C) during travel and then stored at −80 °C until processing.

The DNA were extracted from the samples using the EZ1 Advanced XL (Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendations. All quantitative real-time PCR were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA).

Real-time PCR amplifications were carried out using LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. The *SHD* gene of *H. influenzae* was amplified with internal DNA extraction controls TISS, as previously described [12]. Negative controls (PCR mix) and positive controls (DNA from *H. influenzae* strain) were included in each run. Positive results of DNA amplification were defined as those with a cycle threshold (CT) value ≤ 35.

2.3. Multilocus sequence typing (MLST) of H. influenzae

MLST was performed for all positively screened samples. The housekeeping genes *adk, atpG, frdB, fucK, mdh, pgi* and *recA* were amplified by standard PCR, as previously described [13], then sequenced. The sequences were compared to those existing in GenBank. Positive samples which presented at least one of seven genes using standard PCR were considered as being *H. influenzae* MLST positive samples. Purified PCR products were sequenced using specific primers and the BigDye Terminator® version 1.1 cycle sequencing ready reaction mix (Applied Biosystems, Foster City, CA, USA). Sequencing was performed using an Applied Biosystems 3130 platform (ABI PRISM, PE Applied Biosystems, USA). The sequences obtained were edited and assembled using Chromas Pro 1.77 (Technelysium Pty Ltd, Australia), aligned with *H. influenzae* strains from GenBank. Allele numbers and sequence types (ST) were assigned using the *H. influenzae* MLST website (http://haemophilus.mlst.net).

3. Results

3.1. Characteristics of study participants and clinical symptoms

The characteristics of study participants and clinical symptoms have been detailed elsewhere [11]. To summarise, 121 pilgrims were included, with a sex ratio of 1:1.3 and a median age of 61 years (interquartile = 56–66 years, range = 26–83 years). A total of 113/121 (93.4%) pilgrims presented at least one respiratory symptom during their stay in the KSA. Antibiotic use for RTIs was reported by 58.7% pilgrims. The mean time between arrival in the KSA and the onset of respiratory symptoms was 8.7 ± 4.6 days (range = 1–21 days).

3.2. Identification of H. influenzae among French pilgrims during the Hajj

3.2.1. H. influenzae screening by real-time PCR

Of the 484 swabs tested, 129 (26.7%) were positive [11]. Of the 121 pilgrims included, 75 (62.0%) at least one of their samples was positive for *H. influenzae* and all four samples of the remaining 46 were negative. The prevalence of *H. influenzae* carriage was 43/121 (35.5%) in samples obtained before leaving France, 15/121 (12.4%) at D5, 19/121 (15.7%) at D12 and 52/121 (43.0%) before leaving Saudi Arabia [11].

3.2.2. Diversity of encapsulated and non-capsulated H. influenzae using multi-locus sequence typing

Of the 129 swabs that were positive for *H. influenzae*, only one sample at D12 was negative for all seven genes amplified by standard PCR. Fig. 2 shows the distribution of the 128 MLST positive samples among the 75 pilgrims who presented at least one positive sample. A total of 40/121 (33.0%) pilgrims presented only one of the four samples that was positive for at least one of the seven genes (14 at pre-Hajj, 1 at D5, 3 at D12 and 12 at post-Hajj samples). In addition, 21/121 (17.4%), 10/121 (8.3%) and 4/121 (3.3%) pilgrims had two, three and four MLST positive samples, respectively.

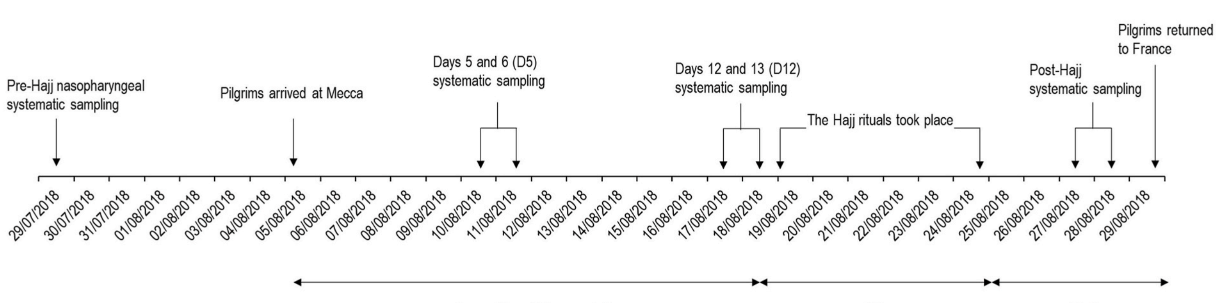

Fig. 1. Study design of cohort survey on the dynamic of *Haemophilus influenzae* carriage among 121 French pilgrims during the 2018 Hajj season.

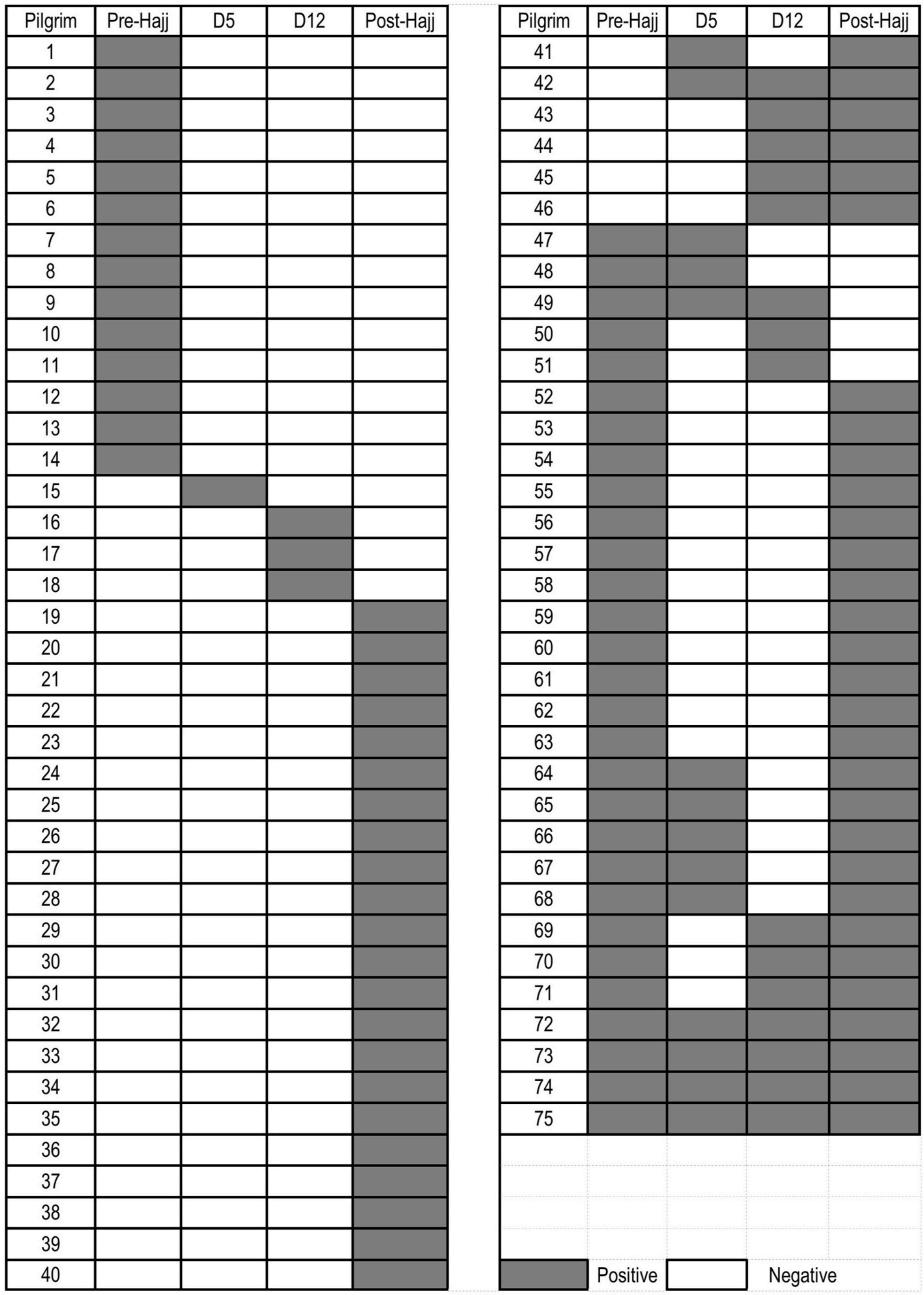

Fig. 2. Temporal distribution of 128 MLST positive samples among 75 pilgrims with at least one positive sample.

The *adk, atpG, frdB, mdh, pgi, recA* and *fucK* genes were positive in 123, 107, 122, 70, 127, 118 and 69 samples, respectively. All sequences of *adk, frdB, mdh* and *pgi* genes were obtained from GenBank or using the *H. influenzae* MLST website (GenBank accession numbers MN555328, MN607727–MN607848, MN617868–MN617990, MN617991–MN618060 and MN627489–MN627615). A total of 106/107 and 116/118 sequences of *atpG* and *recA* genes were assigned with success respectively (GenBank accession numbers MN607849 – MN607953 and MN627616 – MN627731). One sequence of *atpG* and two of *recA* genes were not possible to assign, using Chromas Pro 1.77. However, none of the sequences of the *fucK* gene was successfully obtained (Fig. 3). Consequently, complete sequence type characterisation was not possible. Nevertheless, our results enabled us to differentiate between strains. The supplementary data shows the diversity of the six genes obtained from pilgrim strains. Of the 128 obtained strains, 111 had distinct patterns of alleles. In post-Hajj samples, five distinct strains were each found in two pilgrims and one was found in three pilgrims. Samples with similar strains were also found in one pre-Hajj and one post-Hajj samples in two distinct individuals and another strain was found in one D12 and one post-Hajj samples in two other pilgrims.

4. Discussion

In this study, we evaluated the dynamics and genetic diversity of *H. influenzae* carriage among French pilgrims during the 2018 Hajj season. Our main results are as follows: 1 - *H. influenzae* carriage was

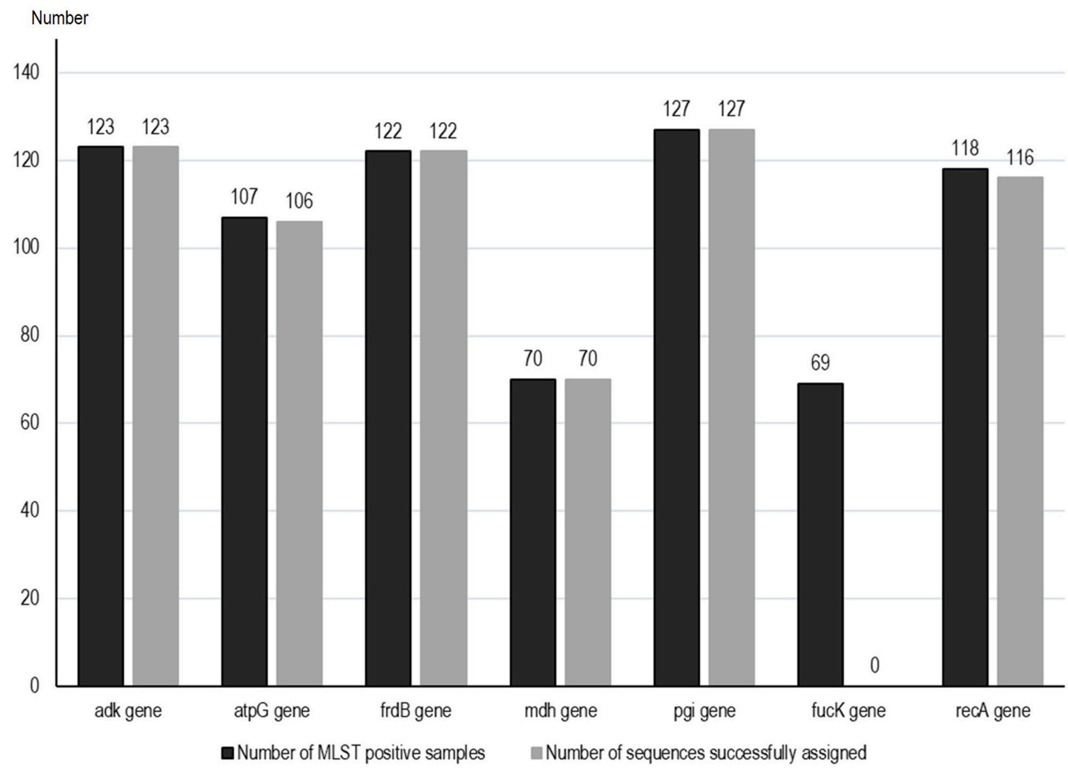

Fig. 3. Number of MLST positive samples and sequences successfully assigned of seven MLST genes.

frequent among pilgrims before leaving France and increased following participation in the Hajj, following a transient decrease; 2 - *H. influenzae* genotypes acquired in the KSA were completely different from those present before leaving France and 3 - we observed a great biodiversity and a lack of clonality of *H. influenzae* among pilgrims during the Hajj.

H. influenzae is a commensal organism of the human respiratory tract and is a common cause of upper and lower RTIs in adults [14]. Occasionally, it also causes life-threatening invasive diseases such as meningitis in adults [15]. This organism has been described as commensal in 75% of the healthy adult population [15] and typing of the *H. influenzae* isolates is essential in order to confirm its pathogenicity in pilgrims. Within the species, *H. influenzae* type b (Hib) is known to cause severe forms of infections [16]. Non-capsulated (non-typeable) *H. influenzae* is commonly carried in the pharynx and is one of the major causes of acute otitis media in children. It can cause diseases of the upper and lower respiratory tracts, including sinusitis and pneumonia [13]. However, previous studies conducted in some countries using the Hib conjugate vaccines reported that non-typeable and non-b serotypes caused most invasive diseases in adults over the age of 65 [17–19]. The World Health Organization recommends the epidemiological surveillance of *H. influenzae* to evaluate the current burden of its associated diseases and, consequently, to ascertain the practicality of Hib vaccine in the affected regions.

Pneumonia is a leading cause of hospitalisation during the annual Islamic pilgrimage. Memish conducted a study among pilgrims with severe community-acquired pneumonia who were admitted to 15 healthcare facilities in the cities of Makkah and Medina in Saudi Arabia [20]. Bacterial pathogens were detected in 84.6% of cases. *H. influenzae* and *S. pneumoniae* were the predominant bacteria, detected in 57.7% and 53.8% of patients, respectively. In a cohort study conducted among international pilgrims, Memish showed that the carriage of *H. influenzae* detected by qPCR was low. Only 2.3% of pilgrims were positive for this bacterium pre-Hajj and 11.7% post-Hajj [21]. Likewise, in a longitudinal survey conducted in 254,823 Iranian pilgrims recruited at 1352 Hajj caravans over a five-year period (2004–2009), *H. influenzae* was identified in only 9.1% of the 357 tested samples [22]. Among French pilgrims from Marseille, *H. influenzae* pre-Hajj prevalence varied according to the Hajj season: 50% in 2014, 0.9% in 2015, 2.8% in 2016 and 52.8% in 2017, while post-Hajj prevalence was consistently high with 67.8%, 45.5%, 41.0% and 53.5%, respectively [8, Gautret, unpublished data]. This suggests that *H. influenzae* carriage in Marseille may be subject to yearly variations. In contrast, a frequent acquisition of this bacterium at the Hajj is observed each year. In line with this result, Wilkinson conducted a prospective analysis in the United Kingdom, surveying 127 patients with chronic obstructive pulmonary disease (COPD) aged between 40 and 85 over a period of two years (from June 2011 to June 2012) for the occurrence of acute exacerbation and respiratory infections in COPD. Their results showed, for the first time, that changes in the yearly COPD exacerbation rate may be associated with variations in *H. influenzae* colonisation [23].

In our study, *H. influenzae* alleles varied according to the sampling time during Hajj. In pilgrims who were positive for *H. influenzae* in all four samples, *H. influenzae* genotypes were different in most samples, suggesting that pilgrims successively acquired different new *H. influenzae* genotypes during the pilgrimage. The reason for the initial clearance of *H. influenzae* carriage following arrival to the KSA may have resulted from the high rate of antibiotic consumption by pilgrims [24]. The large diversity of genotypes acquired during the stay in the KSA suggests that the source of infection was polyclonal and was likely to be external to the group of French pilgrims, with very little transmission between them.

In our study, none of the sequences of the *fucK* gene was successfully assigned. Other researchers have reported that certain *H. influenzae* isolates may lack the *fucK* gene, one of the seven genes used in the *H. influenzae* MLST scheme [13,25–27]. The absence of this gene has historically confounded attempts at *H. influenzae* speciation, such as the probable incorrect assignment of *fucK*-negative *H. influenzae* strains as *H. haemolyticus* [28]. These 'fuzzy' isolates should therefore be considered to be fucose-negative *H. influenzae*. It is unfortunate that the developers of the *H. influenza* MLST scheme inadvertently chose a variably present gene for genotyping purposes. Price confirmed the existence of fucose-negative *H. influenza* strains [29]. Redesign of the existing *H. influenzae* MLST scheme to incorporate a conserved seventh locus in *H. influenzae* and *H. haemolyticus* in place of the *fucK* gene may lead to greater uptake of this highly useful genotyping scheme around the world.

Our study has several limitations. First, we performed sequencing directly from samples, not on cultured isolates and we could not succeed in amplifying all seven genes in the MLST system. In addition, we could not eliminate the possibility that an individual sample could be positive for several genotypes of *H. influenzae*. The study was conducted among French pilgrims only and cannot be generalised to all pilgrims. Using qPCR to detect respiratory pathogens does not distinguish between dead and living micro-organisms. Nevertheless, our study is the first study on the dynamic and genetic diversity of *H. influenzae* in the Hajj context. Continuous monitoring of the molecular evolution in *H. influenzae* among Hajj pilgrims is necessary to explore how *H. influenzae* biofilm formation involves in the pathological process of infection and may help for clinical treatment. Moreover, constant vigilance and precaution such as a vaccine development is necessary to respond to new epidemiological trends of *H. influenzae* strains acquired by Hajj pilgrims. *H. influenzae* type b vaccination has been described by previous study that induces serum antibody production and reduces the nasopharyngeal carriage prevalence of this bacterium. Reduction in carriage also reduces transmission of *H. influenzae* between individuals [30]. Further studies aiming at studying the genome of Hajj-acquired *H. influenzae* isolates are needed to better elucidate their ecological changes, to define the clinical burden of *H. influenzae* infection during Hajj and to evaluate the potential interest of vaccination in Hajj pilgrims. Currently, vaccination against invasive *H. influenzae* disease is not recommended for Hajj pilgrims [4].

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CRediT authorship contribution statement

Van-Thuan Hoang: Data curation, Formal analysis, Writing - review & editing, contributed to experimental design, data analysis, interpretation and writing, conducted the laboratory technique. **Thi-Loi Dao:** conducted the laboratory technique. **Tran Duc Anh Ly:** conducted the laboratory technique. **Khadidja Belhouchat:** administered questionnaires, followed patients and collected samples. **Kamel Larbi Chaht:** administered questionnaires, followed patients and collected samples. **Saber Yezli:** Writing - review & editing, contributed to critically reviewing and editing the manuscript. **Badriah Alotaibi:** Writing - review & editing, contributed to critically reviewing and editing the manuscript. **Didier Raoult:** Writing - review & editing, contributed to critically reviewing and editing the manuscript. **Philippe Parola:** Writing - review & editing, contributed to critically reviewing and editing the manuscript. **Vincent Pommier de Santi:** Data curation, Formal analysis, Writing - review & editing, contributed to experimental design, data analysis, interpretation and writing. **Pierre-Edouard Fournier:** Data curation, Formal analysis, Writing - review & editing, contributed to experimental design, data analysis, interpretation and writing. **Philippe Gautret:** coordinated the work.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2020.101883.

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Supplementary data

Dynamics and genetic diversity of *Haemophilus influenzae* carriage among French pilgrims during the 2018 Hajj

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Running title: Dynamics and genetic diversity of *H. influenzae*

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Supplementary data: Distribution of the different alleles of the six MLST genes of *Haemophilus influenzae* in French pilgrims participating in the 2018 Hajj.

N° of sample	N° pilgrim	Time of sampling	*adk* gene	*atpG* gene	*frdB* gene	*mdh* gene	*pgi* gene	*recA* gene
1	1	Pre-Hajj	6	25	107	22	13	165
2	2	Pre-Hajj	55	12	107	77	13	10
3	3	Pre-Hajj **(1)**	1	25	1		41	5
4	4	Pre-Hajj	63	25	109		13	165
5	5	Pre-Hajj	55	12	109	202	41	11
6	6	Pre-Hajj	55	25	1	77	41	95
7	7	Pre-Hajj	6	15	107		39	10
8	8	Pre-Hajj	6	25	24	77	41	3
9	9	Pre-Hajj	11	25	16		56	10
10	10	Pre-Hajj	11	25	16		13	34
11	11	Pre-Hajj	11	12	5	77	13	8
12	12	Pre-Hajj	11	25	24	22		89
13	13	Pre-Hajj			28		19	3
14	14	Pre-Hajj	11	12	109	22	41	10
15	15	D5	6	25	16	77	13	99
16	16	D12		1	24		1	5
17	17	D12	22		5		53	5
18	18	D12	28	12			13	
19	19	Post-Hajj	1	54	1	15	1	5
20	20	Post-Hajj	33	15	16	17	2	29
21	21	Post-Hajj	25	1	16	23	8	5
22	22	Post-Hajj	33	1	16		2	29
23	23	Post-Hajj	1	1	1	15	1	5
24	24	Post-Hajj	25		16		2	29
25	25	Post-Hajj	33		5		1	5
26	26	Post-Hajj	1	8	16	17	2	29
27	27	Post-Hajj	11	15	16		2	5
28	28	Post-Hajj	1	1	17	102	1	5
29	29	Post-Hajj		15	15		1	3
30	30	Post-Hajj	18	14	5	1	24	1

31	31	Post-Hajj	21	8	16		24	34
32	32	Post-Hajj	1	1	5	15	1	5
33	33	Post-Hajj **(2)**	1	1	1		1	5
34	34	Post-Hajj **(2)**	1	1	1		1	5
35	35	Post-Hajj **(3)**	1	1	1	1	1	5
36	36	Post-Hajj **(3)**	1	1	1	1	1	5
37	37	Post-Hajj **(4)**	14	26	1	1	24	1
38	38	Post-Hajj **(4)**	14	26	1	1	24	1
39	39	Post-Hajj **(5)**	33	8	16	17	2	29
40	40	Post-Hajj **(6)**	14	26	1	1	267	1
41	41	D5			107		13	99
42	41	Post-Hajj	33	1	16	17	2	29
43	42	D5	55		28	77	41	165
44		D12	3	25	109		13	19
45		Post-Hajj	1		16		13	97
46	43	D12	14		107		13	
47		Post-Hajj **(7)**	1	15	17		1	5
48	44	D12	42	25	109		13	10
49		Post-Hajj	1	25	109		13	10
50	45	D12	28				13	1
51		Post-Hajj	14		16		24	5
52	46	D12		25	107		13	19
53		Post-Hajj	1		107		13	18
54	47	Pre-Hajj	55	12	107	22	2	97
55		D5	55	12	107	77	13	165
56	48	Pre-Hajj	11	25	5	77	13	
57		D5	63	11	16	23	267	5
58	49	Pre-Hajj	55	25	16		41	10
59		D5	42	25	28	22	41	
60		D12	6		107		41	97
61	50	Pre-Hajj	6	12	24	22	41	3
62		D12	22		5	22	41	95
63	51	Pre-Hajj	55	12	107		41	10
64		D12	42				41	
65	52	Pre-hajj	55		107	62	41	165
66		Post-Hajj	1	1	17		1	5
67	53	Pre-hajj	1	12	107	17	13	
68		Post-Hajj	5	25	7	17	41	29
69	54	Pre-hajj	6	25	107		13	5
70		Post-Hajj	11	12	109		13	5
71	55	Pre-hajj	33	12	16	22	13	10

72		Post-Hajj **(5)**	33	8	16	17	2	29
73	56	Pre-hajj	33	25	107		13	19
74		Post-Hajj	33	25	107		13	1
75	57	Pre-hajj	11	25	107	77	41	10
76		Post-Hajj	1	25	107		267	34
77	58	Pre-hajj	55	19	28	22	19	3
78		Post-Hajj	14	15	5	1	24	1
79	59	Pre-hajj	11	12	16	77	41	3
80		Post-Hajj **(7)**	1	15	17		1	5
81	60	Pre-hajj	11	25	16	22	41	34
82		Post-Hajj	33	14	16	1	24	1
83	61	Pre-hajj	6	25	16		41	8
84		Post-Hajj **(8)**	14	14	1	1	24	1
85	62	Pre-hajj	11	8	16	3	41	165
86		Post-Hajj	14	14		1	24	1
87	63	Pre-hajj	6	12	16		13	10
88		Post-Hajj	33	8	16		2	29
89	64	Pre-Hajj	6		24		13	10
90		D5	33	12	16		13	97
91		Post-Hajj	1	14	102	1	41	1
92	65	Pre-Hajj	11	12	16		13	1
93		D5	11		17	77	13	
94		Post-Hajj	18	15	5	1	41	10
95	66	Pre-Hajj	11	25	24	77	13	34
96		D5	6	12	107	77	13	165
97		Post-Hajj	55	14	107		3	3
98	67	Pre-Hajj	33	25		77	13	1
99		D5	42		107	62	41	
100		Post-Hajj	25	25	107		267	10
101	68	Pre-Hajj	11	25	16		56	95
102		D5	6		109	77	41	
103		Post-Hajj **(5)**	33	8	16	17	2	29
104	69	Pre-Hajj	63		107		41	29
105		D12	21	25	5		41	34
106		Post-Hajj	1	15	17	102	1	5
107	70	Pre-Hajj	6	12	28		13	10
108		D12	6	12	16		41	34
109		Post-Hajj	25	14	16	1	267	1
110	71	Pre-Hajj	11		16		13	95
111		D12 **(6)**	14	26	1	1	267	1
112		Post-Hajj **(8)**	14	14	1	1	24	1

113	72	Pre-Hajj	3	25	16	15	53	1
114		D5	55	25	28	22	41	165
115		D12	1		1		53	38
116		Post-Hajj **(1)**	1	25	1		41	5
117	73	Pre-Hajj	11	25	107	62	13	
118		D5	11	25	1		41	
119		D12	11	25	5	102	13	10
120		Post-Hajj	6	25	107		13	
121	74	Pre-Hajj	11	12	109	99	41	3
122		D5	6	4	5	77	13	10
123		D12	42	19	11	77	19	10
124		Post-Hajj	25	14	1	1	24	1
125	75	Pre-Hajj	55	25	16	74	56	165
126		D5	63		36	77	41	165
127		D12	14	12	109		41	10
128		Post-Hajj	14	1	109	77	41	10

(1), (2), (3), (4), (5), (6), (7), (8): pilgrims with same sequences of the six genes

Article 8 :

Annual variations of *Haemophilus influenzae* carriage among Hajj pilgrims

Hoang VT, Dao TL, Ly TDA, Pommier de Santi V, Gautret P

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Annual variations of *Haemophilus influenzae* carriage among Hajj pilgrims

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Dear Editor,

Every year, more than two million people from about 180 countries take part in the Hajj pilgrimage in the Kingdom of Saudi Arabia (KSA) [1], of whom approximately 20,000 are French (https://www.stats.gov.sa/en/28). The overcrowded conditions in Mecca are known risk factors for the transmission of infectious diseases during the Hajj [1]. During the 2014–2016 Hajj, infectious diseases accounted for 53% of diagnoses in outpatients consulting at the Indian Medical Mission, with respiratory tract infections (RTIs) being the most frequent [2]. Despite several preventive measures such as vaccinations against influenza and pneumococcal infections in at-risk individuals, and recommendations around the use of face masks, hand hygiene, and the disposable tissues, RTIs remain frequent among Hajj pilgrims [3]. Besides the widespread acquisition of respiratory viruses, bacteria are also frequently isolated among ill and asymptomatic pilgrims, with *Haemophilus influenzae* being the most common [4]. Co-carriage of *H. influenzae* - *Streptococcus pneumoniae* and *H. influenzae* – rhinovirus were associated with 5 times and 7 times increased risk for developing respiratory symptoms among French Hajj pilgrims, respectively [5,6]. Most cohort surveys among pilgrims used real-time PCR to detect the carriage of *H. influenzae* [4]. In this paper we report data about the annual variations of *H. influenzae* carriage among French pilgrims during the 2014–2018 Hajj seasons.

Potential adult participants departing from Marseille, France and travelling with a single travel agency were invited to participate in our study. Upon inclusion, pilgrims were interviewed using a standardised pre-Hajj questionnaire that collected information about demographic characteristics and medical history. Two days before the pilgrims' return to France, a post-Hajj questionnaire was completed to collect the information on clinical data, antibiotic intake and compliance with individual preventive measures (face masks use, hand washing, the use of hand gel disinfectant and disposable handkerchiefs). A total of 606 pilgrims were included. The sex ratio male:female was 1:1.3 and the median age was 61 years (interquartile = 53–67 years, min = 21, max = 96 years). In total, 84.9% of the pilgrims presented at least one respiratory symptom during their time in Saudi Arabia. Cough (76.4%) and sore throat (57.6%) were the most frequent symptoms, followed by rhinitis (54.7%), voice failure (36.3%), and dyspnoea (12.1%). A proportion of 17.7% pilgrims reported influenza-like illness (cough, sore throat, and subjective fever). Only two (0.3%) pilgrims were hospitalised in KSA for a respiratory infection.

Nasopharyngeal swabs were systematically obtained from each pilgrim prior to leaving France (pre-Hajj) and prior to leaving Saudi Arabia (post-Hajj). The *SHD* gene (Hinf_SHD_F: 5'-GCGGCGAGATATTGACCTGT-3', Hinf_SHD_R: 5'-GCAGTGGYGGTATGGCAAAA-3', Hinf_SHD_P: 6FAM-TGAATTTTTAAAGGCDRCCACAACGGC-TAMRA) was used to screen all isolates for *H. influenzae* using the quantitative real-time PCR method. The prevalence of *H. influenzae* pre-Hajj revealed significant variations according to year: 50.0% in 2014, 0.9% in 2015, 2.8% in 2016, 52.8% in 2017, and 35.5% in 2018, while post-Hajj prevalence was consistently high, ranging from 41.0 to 67.8% (Figure 1). This suggests that *H. influenzae* circulation at the Hajj was permanent over the study period. In a study that we conducted during the 2018 Hajj using a multilocus sequence typing method, *H. influenzae* genotypes acquired post-Hajj were completely different from those present at pre-Hajj. We also observed a lack of clonality and a great biodiversity of *H. influenzae* among French pilgrims, but we did not evaluate the carriage of *H. influenzae* type b (Hib) [7].

Individual preventive measures against infectious diseases are recommended during Hajj, but the effectiveness of individual non-pharmaceutical preventive measures against RTIs is uncertain [3]. The use of a face mask in the presence of respiratory symptoms and enhanced hand hygiene should always be recommended for Hajj pilgrims until large-scale controlled studies are conducted to truly assess effectiveness. Despite the high proportion of *H. influenzae* acquisition among Hajj pilgrims, no study on serotype of *H. influenzae* in this population was conducted to date. Further studies aiming at studying the genotype of *H. influenzae* isolates pre- and post-Hajj are needed to evaluate the respective proportion of Hib and of non-typable strains acquired at the Hajj and assess the potential value of Hib vaccination in pilgrims. Currently, vaccination against invasive *H. influenzae* disease is not recommended for Hajj pilgrims [8].

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Conflict of Interest

The authors declare that they have no conflict of interest.

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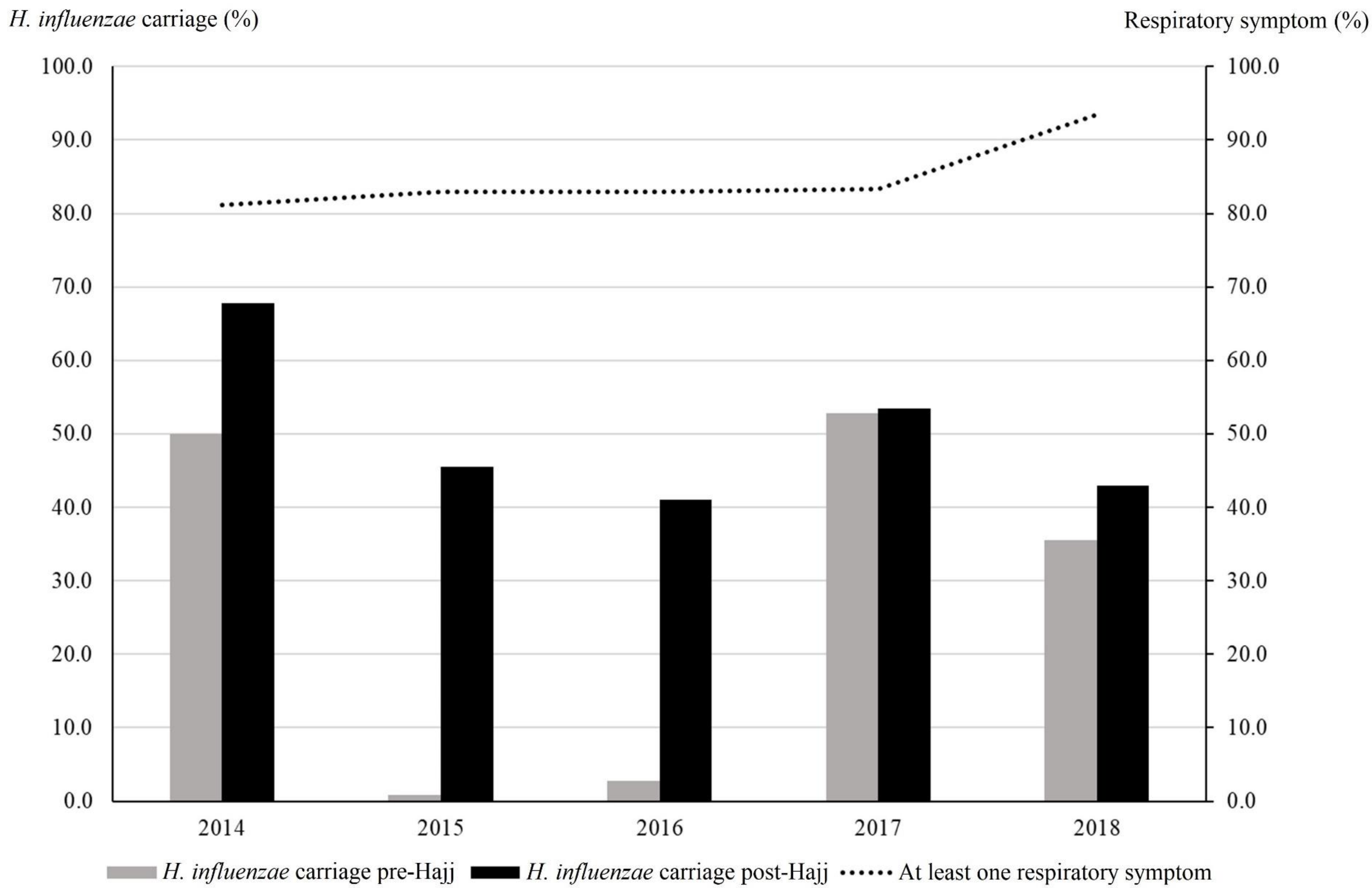
Figure 1: Seasonality of *Haemophilus influenzae* and respiratory tract infection symptoms among French Hajj pilgrims during the 2014 to 2018 Hajj



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Article 9 :

Gastrointestinal symptoms and the acquisition of enteric pathogens in Hajj pilgrims. A three-year prospective cohort study

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ORIGINAL ARTICLE

Gastrointestinal symptoms and the acquisition of enteric pathogens in Hajj pilgrims: a 3-year prospective cohort study

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Abstract
The acquisition of enteric pathogens and risk factors for Hajj-associated diarrhea in Hajj pilgrims is poorly documented. Pilgrims from Marseille participating in the Hajj in 2016–2018 underwent successive systematic rectal swabbing before and after their travel. Carriage of the main enteric pathogens was assessed by real-time PCR. Baseline demographics, adherence to individual preventive measures, gastrointestinal symptoms, and treatments were recorded. A total of 376 pilgrims were included. The median age was 62.0 years. During the Hajj, 18.6% presented at least one gastrointestinal symptom, 13.8% had diarrhea, and 36.4% had acquired at least one enteric pathogen. Enteropathogenic *Escherichia coli* (EPEC) and Enteroaggregative *E. coli* (EAEC) were the pathogens most frequently acquired by pilgrims (17.6% and 14.4%, respectively). Being female was associated with increased frequency of gastrointestinal symptoms during the pilgrimage (aOR = 2.38, $p = 0.004$). Enterohemorrhagic *Escherichia coli* (EHEC) acquisition was associated with a four-fold higher risk of reporting at least one gastrointestinal symptom and diarrhea (aOR = 3.68 and $p = 0.01$ and aOR = 3.96 and $p = 0.01$, respectively). Pilgrims who suffered from diarrhea were more likely to wash their hands more often (aOR = 2.07, $p = 0.03$) and to be either overweight (aOR = 2.71, $p = 0.03$) or obese (aOR = 2.51, $p = 0.05$). Enteric bacteria such as *E. coli* that are frequently associated with traveler's diarrhea due to the consumption of contaminated food and drink were frequently found in pilgrims. Respecting strict measures regarding food and water quality during the Hajj and adherence to preventive measures such as good personal hygiene and environmental management will help reduce the burden of gastrointestinal infections at the event.

Keywords Hajj · Pilgrims · Gastrointestinal · Risk factors · Acquisition · Diarrhea

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Introduction

The World Health Organization (WHO) defines mass gatherings (MGs) as a "concentration of people at a specific location for a specific purpose over a certain period of time which has the potential to strain the planning and response resources of the host country or community" [1]. Infectious diseases are of particular concern at MGs [2]. The Hajj in the Kingdom of Saudi Arabia (KSA) is one of the largest annual religious MGs in the world. During the 2019 Hajj season, around 2.5 million pilgrims from around the world participated in this event [3]. In the context of crowding, pilgrims are exposed to increased risk of infectious disease transmission, particularly respiratory tract, and gastrointestinal infections (GIs) [2, 4, 5]. In a recent review including 14 cohort studies with 262,999 Hajj pilgrims from several countries, the prevalence of diarrhea among pilgrims varied from 1.1 to 23.3% [6]. Despite the relative frequency of gastrointestinal diseases among pilgrims, the aetiologias of Hajj-associated diarrheal diseases remain largely unknown [6]. In a study conducted in 2012–2013 among Hajj pilgrims from various countries suffering diarrhea, *Salmonella* spp. was detected in 11.4% of ill pilgrims and *Escherichia coli* in 1.3–8.8%, according to pathotypes [7]. In another study, 43.5% of a cohort of French pilgrims acquired at least one gastrointestinal pathogen after participating in the 2016 Hajj, regardless of diarrheal symptoms [8]. Enteric bacteria were the agents most frequently detected, with 29.6% of samples testing positive for Enteropathogenic *Escherichia coli* (EPEC), followed by Enteroaggregative *E. coli* (EAEC) (10.2%). In addition, 10.2% of pilgrims acquired more than one pathogen (mixed infections) [8].

We conducted a study among French Hajj pilgrims in 2016–2018 with the aim of evaluating (1) the acquisition of gastrointestinal pathogens after participating in the Hajj and (2) the risk factors for Hajj-associated diarrhea and other gastrointestinal symptoms. Data from our survey conducted in 2016 [8] were included in the present study.

Materials and methods

Participants and study design

Through a private specialized travel agency, we recruited pilgrims traveling from Marseille, France, to KSA, during three successive Hajj seasons: 2016 (from August 27 to September 20), 2017 (from August 17 to September 10), and 2018 (from August 5 to August 29). All adult pilgrims were invited to participate in the study. Seven to ten days before departing from France, pilgrims were interviewed using a standardized pre-Hajj questionnaire that collected information about demographic characteristics and medical history. Pilgrims were followed-up during the Hajj by bilingual (French and Arabic) medical doctors who traveled with the group. Pilgrims stayed in the same hotels with breakfast included (in Mecca and Medina) and in the same tents (in Mina). Pilgrims were free to eat in restaurants of their choice for lunch and dinner while staying in Mecca and in Medina, while they ate at the same facilities during their stay in the holy sites of Mina and Arafat. Two days before the pilgrims' return to France, a post-Hajj questionnaire including clinical data and antibiotic intake was completed. Information on compliance with the use of hand gel disinfectant and hand washing was also collected. During the Hajj, as part of ablutions, pilgrims wash their hands before the five daily prayers. Hand washing more often than usual was defined by hand washing more than 5 times per day [9]. Diarrhea was defined as at least three loose or liquid stools per 24 h. Nausea, vomiting, and abdominal pain were also recorded. Based on the WHO classification, "obese," "overweight," "normal," and "underweight" were defined as having a body mass index (BMI) of ≥ 30, ≥ 25, 18.5–25, and below 18.5, respectively [10].

Gastrointestinal specimens and the identification of enteric pathogens

To identify enteric pathogens during the Hajj, all pilgrims underwent two rectal swabs: upon inclusion (pre-Hajj) and just prior to leaving KSA (post-Hajj). Rectal swabs were self-collected by each pilgrim, transferred to Sigma Virocult® medium and transported within 48 h of collection to our laboratory in Marseille for storage at −80 °C until processing. The standardized procedure was previously explained to the participants by the investigators.

Identification methods of enteric pathogens by PCR assay are detailed elsewhere [11, 12]. In summary, the RNAs were extracted from 200-μL clear supernatant using the EZ1 Advanced XL (Qiagen, Hilden, Germany) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendation. The semi-automated procedure was used for DNA extraction. A volume of 200 μL of rectal swabs was added to 350 μL of G2 lysis buffer (Qiagen) and glass powder in a tube. Then the tubes were disrupted in a FastPrep BIO 101 apparatus (Qbiogene, Strasbourg, France) at maximum power for 40 s. After incubation at 100 °C for 10 min, the tubes were centrifuged at 10,000 g for 1 min. Afterwards, 200 μL of supernatant was collected and 20 μL of proteinase K (20 mg/mL, Qiagen) was added for digestion. The samples were then incubated overnight at 56 °C. Finally, the automated procedure using the EZ1 Advanced XL (Qiagen, Hilden, German) with the DNA Tissue Kit (Qiagen) was used according to the manufacturer's recommendation.

The following pathogens were screened: norovirus, rotavirus, adenovirus, astrovirus, *Entamoeba histolytica* (18S gene), *Giardia lamblia* (18S gene), *Cryptosporidium* spp. (Hsp70 gene), *Salmonella* spp. (gene), *Shigella*/Enteroinvasive *E. coli* (EIEC)/Enterotoxigenic *E. coli* (ETEC) (ipaH gene), Enterohemorrhagic *Escherichia coli* (EHEC) (stx1 and stx2 genes), EPEC (EAF and eae genes), EAEC (pCVD432 gene), *Vibrio* spp. (toxR gene), and *Campylobacter jejuni* (mapA gene).

The Multiplex RNA Virus Master Kit (Roche Diagnosis, France) was used for detecting gastrointestinal virus and internal controls MS2 phage by one-step simplex real-time quantitative RT-PCR amplifications. Real-time PCR amplifications were carried out using the LightCycler® 480 Probes Master kit (Roche diagnostics, France) which was used to identify adenovirus, gastrointestinal bacteria, and parasites, according to the manufacturer's recommendations. All quantitative real-time PCR to detect respiratory and gastrointestinal pathogens were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA). Negative control (PCR mix) and positive control (DNA from bacterial strain or RNA from viral strain) were included in each run. A cycle threshold (CT) value of ≤ 35 was used to assess positive results of bacteria or virus amplification.

Statistical analysis

STATA software version 15.1 (copyright 1985–2017 StataCorp LLC, http://www.stata.com) was used for statistical analysis. Fisher's exact or Pearson's Chi-square tests were used for statistical analyses, when appropriate. The acquisition of gastrointestinal pathogens was defined as samples which were positive post-Hajj and negative pre-Hajj. The prevalence of enteric pathogens carriage before and after the Hajj was compared using the McNemar's test.

Unadjusted associations between multiple factors and the prevalence of gastrointestinal symptoms (at least one symptom and diarrhea) during the Hajj were examined by univariable analysis. The risk factors investigated were as follows: sociodemographic characteristics (Hajj seasons, sex, age, BMI), chronic conditions, preventive measures (hand hygiene and disinfectant gel use), and acquisition of gastrointestinal pathogens. Details on food and drink were not collected. The results were presented by odds ratio (OR) with 95% confidence interval (95%CI). The multivariable analysis model included only variables with p values < 0.2 in the univariable analysis. Logistical regression was used to estimate factors' adjusted OR regarding gastrointestinal symptoms. The results with a p value less than 0.05 were considered statistically significant.

Results

Characteristics of study participants

During the 2016–2018 Hajj seasons, the Marseille private travel agency recruited 389 pilgrims traveling to Saudi Arabia. Our study enrolled 376 pilgrims (96.6%) who completed both the pre- and post-travel questionnaires and provided paired rectal swabs. The study population had a median age of 62.0 years (interquartile = 53–67 years, min = 21, max = 96 years), and 43.4% (163/376) of the participants were male (Table 1). Hypertension (107/376, 27.7%) and diabetes (98/376, 26.1%) were the chronic comorbidities most frequently reported and (275/376, 73.1%) of the individuals were overweight or obese (Table 1).

Regarding preventive measures against gastrointestinal diseases, 58.5% (220/376) and 54.0% (203/376) of pilgrims reported washing their hands during the Hajj more often than usual and using hand gel during their stay in Saudi Arabia, respectively (Table 1).

Table 1 Characteristics of 376 French pilgrims participating in the Hajj between 2016 and 2018

Characteristics	*n*	%
Gender		
Male	163	43.4
Female	213	56.6
Age		
Median	62	
Interquartile	53–67	
Min-max	21–96	
≥ 60 years	226	60.1
Chronic conditions		
Diabetes mellitus	98	26.1
Hypertension	104	27.7
Chronic respiratory diseases	44	11.7
Chronic heart disease	26	6.9
Chronic kidney disease	6	1.6
Immunodeficiency	4	1.1
Body mass index		
Normal	101	26.9
Overweight	167	44.4
Obese	108	28.7
Individual preventive measures		
Hand washing more often than usual	220	58.5
Disinfectant gel use	203	54.0

Table 2 Gastrointestinal symptoms among 376 French Hajj pilgrims during the 2016 to 2018 Hajj seasons

Symptoms	*n*	%
At least one gastrointestinal symptom	70	18.6
Diarrhea	52	13.8
Nausea	17	4.5
Vomiting	10	2.7
Abdominal pain	28	7.5
Antibiotic intake for gastrointestinal symptoms*	9	12.8
Median time between departing from Marseille and onset of symptoms (days)**		
Median	14	
Interquartile	10–16	
Min-max	1–24	
Time between onset of symptoms and post-Hajj sampling (days)**		
Median	8	
Interquartile	6–12	
Min-max	4–22	

*Among ill pilgrims, **data were not available for the six pilgrims

Clinical features

Table 2 shows the prevalence of gastrointestinal symptoms among pilgrims during the Hajj. A total of 18.6% (70/376) of pilgrims presented at least one gastrointestinal symptom. Diarrhea was the most frequent symptom affecting 13.8% (52/376) of individuals. Among pilgrims suffering at least one gastrointestinal symptom, 12.8% (9/70) reported that they used antibiotics against gastrointestinal symptoms. All nine pilgrims took quinolone (ciprofloxacin) and one also took metronidazole. Anti-diarrheal drug and other compound use were not documented. The median time between departing from Marseille and the onset of symptoms was 14 days, interquartile = 10–16 days. The median time between onset of symptoms and post-Hajj sampling was 8 days, interquartile = 6–12 days (Table 2).

Detection of enteric pathogens by qPCR

Figure 1 shows the prevalence of enteric pathogens which were positively found among 376 pilgrims before and after the Hajj. Carriage of at least one enteric pathogen was higher post-Hajj compared with pre-Hajj (42% vs 24.7%, $p < 0.0001$), and 36.4% of pilgrims acquired at least one enteric pathogen during the pilgrimage. EPEC and EAEC were the pathogens most frequently acquired by pilgrims with acquisition rates of 17.6% and 14.4%, respectively. In addition, some pilgrims acquired EHEC, *Shigella/EIEC/ETEC*, and *Salmonella*, at lower rates. No pilgrim was positive for norovirus pre-Hajj, but 2.4% were positive post-Hajj. The acquisition of adenovirus and rotavirus was rare. A small proportion of pilgrims (6.4%) acquired more than one enteric pathogen (Fig. 1). No sample (pre- or post-Hajj) was positive for astrovirus, *E. histolytica*, *Cryptosporidium* spp., *C. jejuni*, or *Vibrio* spp.

Risk factors for gastrointestinal symptoms

Tables 3 and 4 show univariable and multivariable analysis results for factors associated with gastrointestinal symptoms during the Hajj. Pilgrims who acquired EHEC (aOR = 4.35, 95%CI [1.52–12.46]) were four times more likely to report at least one gastrointestinal symptom. Female gender was positively associated with reporting at least one gastrointestinal symptom (aOR = 2.38, 95%CI [1.32–4.28]) (Table 4). EHEC acquisition was associated with a four-fold higher risk

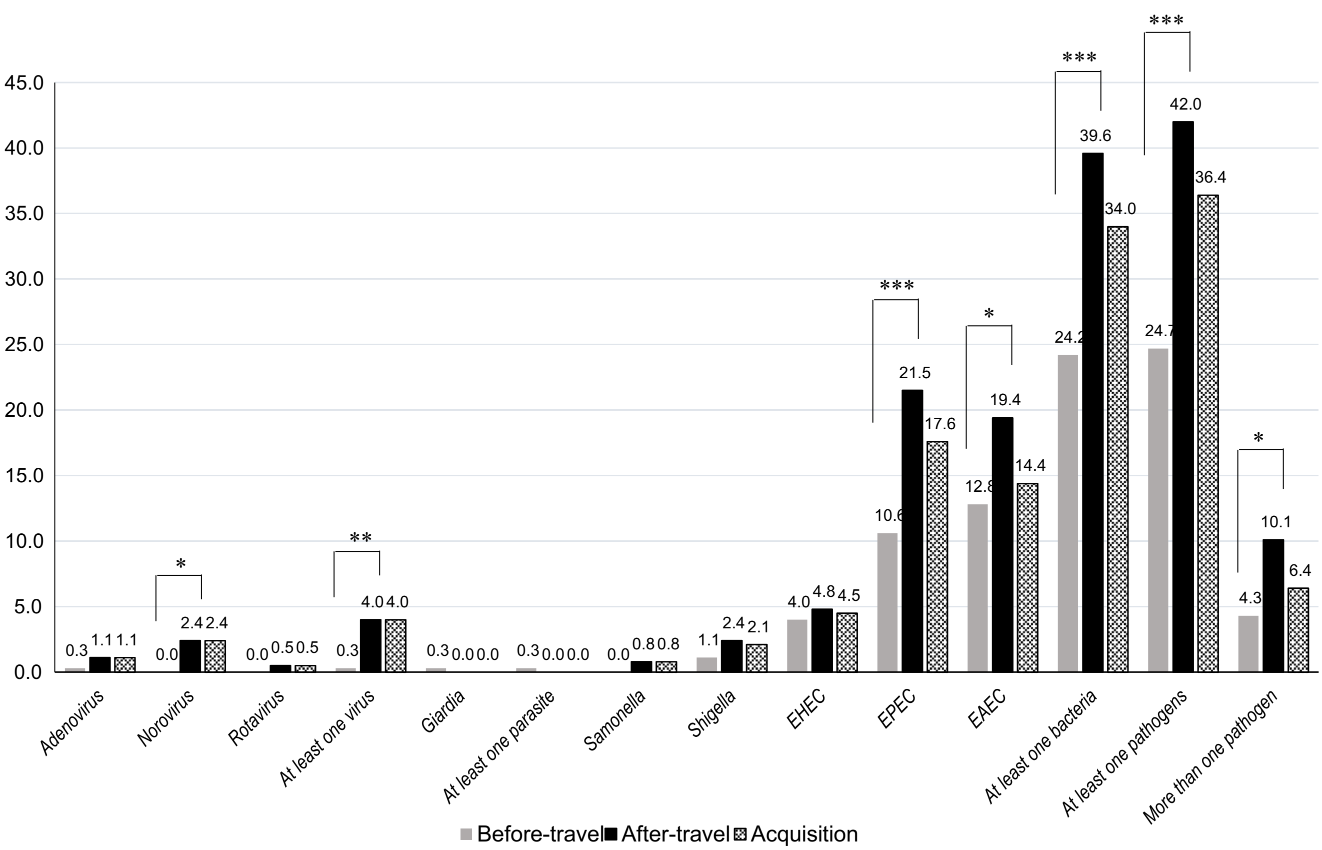

Fig. 1 Enteric pathogens carriage among 376 French pilgrims during the Hajj from 2016 to 2018 (*$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$). Astrovirus, *Entamoeba histolytica*, *Cryptosporidium* spp., *Campylobacter jejuni*, and *Vibrio* spp. were tested, but no sample was positive. EHEC: Enterohemorrhagic *Escherichia coli*, EPEC: Enteropathogenic *E. coli*, EAEC: Enteroaggregative *E. coli*.

Table 3 Risk factors for gastrointestinal symptoms (univariate analysis)

	At least one gastrointestinal symptom			Diarrhea		
	n (%)	OR [95%CI]	*p* Value	*n* (%)	OR [95%CI]	*p* Value
Sociodemographic variables						
Year of travel						
2016	17 (15.7)	Ref	Ref	6 (5.6)	Ref	Ref
2017	22 (15.0)	0.94 [0.47–1.87]	0.86	16 (10.9)	2.07 [0.78–5.49]	0.14
2018	31 (25.6)	1.84 [0.95–3.56]	0.07	30 (24.8)	5.60 [2.23–14.08]	< 0.001
Female gender	50 (23.5)	2.19 [1.25–3.86]	0.006	17 (10.4)	0.59 [0.32–1.10]	0.09
Age ≥ 60 years	45 (19.9)	1.24 [0.72–2.13]	0.43	32 (14.2)	1.07 [0.59–1.96]	0.23
Chronic conditions						
Diabetes mellitus	19 (19.4)	1.07 [0.60–1.92]	0.82	15 (15.3)	1.18 [0.61–2.25]	0.62
Hypertension	25 (24.0)	1.60 [0.92–2.77]	0.10	18 (17.3)	1.46 [0.79–2.73]	0.23
Chronic respiratory diseases	8 (18.2)	0.97 [0.43–2.18]	0.94	6 (13.6)	0.98 [0.39–2.45]	0.97
Chronic heart disease	6 (23.1)	1.34 [0.52–3.47]	0.55	5 (19.2)	1.53 [0.55–4.27]	0.41
Chronic kidney disease	0 (0)	NA	NA	0 (0)	NA	NA
Immunodeficiency	0 (0)	NA	NA	0 (0)	NA	NA
Body mass index						
Normal	12 (11.9)	Ref	Ref	8 (7.9)	Ref	Ref
Overweight	32 (19.2)	1.75 [0.86–3.60]	0.12	27 (16.2)	2.24 [0.98–5.14]	0.06
Obese	26 (24.1)	2.35 [1.11–4.96]	0.02	17 (15.7)	2.17 [0.89–5.28]	0.09
Hand washing more often than usual	50 (22.7)	2.00 [1.13–3.52]	0.02	38 (17.3)	2.11 [1.10–4.06]	0.02
Disinfectant gel use	44 (21.7)	1.56 [0.92–2.67]	0.10	29 (14.3)	1.09 [0.60–1.96]	0.78
Enteric viral acquisition						
Adenovirus	1 (25.0)	1.46 [0.15–14.28]	0.74	1 (25.0)	2.10 [0.21–20.56]	0.53
Norovirus	2 (22.2)	1.26 [0.26–6.18]	0.78	1 (11.1)	0.77 [0.09–6.23]	0.81
Rotavirus	0 (0)	NA	NA	0 (0)	NA	NA
At least one virus	3 (20.0)	1.10 [0.30–4.00]	0.89	2 (13.3)	0.96 [0.21–4.37]	0.95
Enteric bacterial acquisition						
Salmonella spp.	1 (33.3)	2.20 [0.20–24.64]	0.52	1 (33.3)	3.16 [0.28–35.45]	0.35
Shigella spp./EIEC/ETEC	1 (12.5)	0.62 [0.07–5.11]	0.66	1 (12.5)	0.88 [0.11–7.37]	0.91
EHEC	7 (41.2)	3.29 [1.20–8.97]	0.02	6 (35.3)	3.71 [1.31–10.52]	0.01
EPEC	11 (16.7)	0.85 [0.42–1.72]	0.65	6 (9.1)	0.57 [0.23–1.41]	0.22
EAEC	9 (16.7)	0.86 [0.40–1.84]	0.69	7 (13.0)	0.92 [0.39–2.15]	0.84
At least one bacteria	25 (19.5)	1.09 [0.64–1.88]	0.74	18 (14.1)	1.03 [0.56–1.91]	0.93
At least one pathogen	26 (19.0)	1.04 [0.61–1.78]	0.89	19 (13.9)	1.01 [0.55–1.85]	0.99
More than one pathogen	5 (20.8)	1.16 [0.42–3.22]	0.77	4 (16.7)	1.27 [0.42–3.87]	0.68

of reporting at least one gastrointestinal symptom and diarrhea (aOR = 3.68 and 95%CI [1.28–10. 57] and aOR = 3.96 and 95%CI [1.31–11.91], respectively). Pilgrims who suffered gastrointestinal symptoms and diarrhea were more likely to wash their hands more often (aOR = 1.84 and 95%CI [1.04–3.29] and aOR = 2.07 and 95%CI [1.07–4.03], respectively). Finally, being overweight or obese were associated with increased risk of diarrhea (aOR = 2.71 and 95%CI [1.15–6.41] and aOR = 2.51 and 95%CI [1.01–6.28], respectively).

Discussion

Diarrhea and gastroenteritis, notably cholera outbreaks, have been a threat at the Hajj in the past [6, 12]. However, a decreasing prevalence of gastrointestinal infections among pilgrims has been observed in recent decades [2, 4, 13]. This very likely reflects improved sanitary conditions at religious sites and stricter measures to ensure food and water quality at the Hajj. Nevertheless, diarrhea and gastrointestinal infections

Table 4 Risk factors for gastrointestinal symptoms (multivariate analysis)

	At least one gastrointestinal symptom		Diarrhea	
	Adjusted OR* [95%CI]	*p* Value	Adjusted OR* [95%CI]	*p* Value
Female gender	2.38 [1.32–4.28]	0.004		
Hand washing more often than usual	1.84 [1.04–3.29]	0.04	2.07 [1.07–4.03]	0.03
EHEC acquisition	3.68 [1.28–10.57]	0.01	3.96 [1.31–11.91]	0.01
Body mass index				
Normal			Ref	Ref
Overweight			2.71 [1.15–6.41]	0.03
Obese			2.51 [1.01–6.28]	0.05

*Only significant results are presented in this table

still occur among pilgrims, and continuous surveillance of these diseases is part of the public health response for the Hajj.

Cohort studies conducted among Hajj pilgrims from various countries of origin between 2002 and 2013 showed that the prevalence of diarrhea varied according to nationality [6]. Five percent of pilgrims from Riyadh developed diarrheal symptoms during the 2009 Hajj [9]. This proportion was higher (21%) among Iranian female pilgrims during the 2011 Hajj (21.0%) [14] and among French pilgrims in 2013 (23.3%) [15]. During a Hajj-associated gastroenteritis outbreak in 2007, rice served by a catering company were identified as the source of infection [16]. Diarrhea and gastroenteritis are also frequent at other religious MGs. With about 100 million participants, the Kumbh Mela in India is the largest MG in the world. Moreover, cholera epidemics during the event have been also reported in the past [17]. During this event in 2013, 412,703 patients consulted at hospitals, and diarrheal diseases accounted for 16% of clinical presentations at regional hospitals [18]. The risk of gastrointestinal disease at the Kumbh Mela is considered to be linked to the potential contamination of water and food [19]. Between 2009 and 2010, gastrointestinal symptoms accounted for 14% of consultations at local healthcare centers during the Moussem of Moulay Abdellah Amghar, an 8-day annual gathering in Morocco [20]. During the 2010 anniversary of the death (*urs*) of Baba Farid, an annual MG in Pakpattan, Pakistan, 26% of 5918 participants seen at healthcare facilities had gastrointestinal illnesses [21].

Unlike the syndromic surveillance data mentioned above that lacked reliable identification of the responsible pathogen. *Salmonella enterica* serotype typhimurium was responsible of 64 cases of gastrointestinal illness among 9000 participants at a Christian religious festival in Hamilton County, Ohio, in 2010. The consumption of pulled pork prepared in sold at the festival and in a private house was associated with this outbreak [22]. The Grand Magal is both a religious pilgrimage and a festival in Senegal with about 4–5 million participants each year. In 2017, 14.6% pilgrims presented with gastrointestinal symptoms and 4.6% reported diarrhea. The acquisition of gastrointestinal parasites and viruses was low, while bacterial acquisition ranged from 2.2% (for *Campylobacter jejuni*) to 33.3% (for EPEC) [11].

To our knowledge, no study on the risk factors for Hajj-related diarrhea and other gastrointestinal symptoms has been performed to date. In our survey, we confirm that French pilgrims frequently report gastrointestinal symptoms during their stay in KSA (18.6%), notably diarrhea (13.8%). We also confirm that *E. coli* acquisition and notably that of EPEC (17.6%), EAEC (14.4%), and EHEC (4.5%) is frequent. Our results showed that female pilgrims were more exposed to report gastrointestinal symptoms and pilgrims who were overweight or obese were at higher risk of developing diarrhea. Finally, we found a strong association between gastrointestinal symptoms and diarrhea and acquisition of EHEC.

Our results are in line with previous studies realized in general populations. In a randomly selected population study on demographic determinants of acute gastrointestinal symptoms in Canada, being female was associated with a two-fold risk of presenting such symptoms (aOR = 2.46, $p < 0.001$) [23]. This may be due to an increased exposure to gastrointestinal symptoms via foodborne or interhuman transmission because females generally do more food preparation than males and incur greater exposure to pathogens. Obesity is usually associated with metabolic diseases. However, it is also a direct cause of different gastrointestinal illnesses, and when obesity is a risk factor, it could interact with other pathogenetic mechanisms [24]. Several studies showed that obesity was increased risk of diarrhea, compared with normal-weight controls [24]. In a survey study including 2660 participants, obese individuals had a three-fold higher risk of diarrhea with OR = 2.7 and $p = 0.01$ [25]. Furthermore, in an epidemiological study conducted on 35,447 French adults, being a woman with a high BMI was a significative risk factor for diarrhea (OR = 1.05, $p < 0.001$) [26]. The higher prevalence of diarrhea in obese individuals could be attributed to several associated potential mechanisms including accelerated colonic transit

[25] or increased levels of fecal calprotectin resulting in intestinal inflammation [27]. In view of the high prevalence of obesity among Hajj pilgrims [12, 28–34], it would be of interest to conduct large cohort surveys aiming at investigating the relationship between obesity and infectious diseases during the Hajj.

Viruses and *Shigella* that are typically involved in human-to-human-transmitted gastrointestinal infections [35, 36] were rarely detected in our work, and we observed that pilgrims suffering from diarrhea reinforced their hand hygiene practices. By contrast, gastrointestinal bacteria like *E. coli* that are frequently associated with traveler's diarrhea [6, 35] due to the consumption of contaminated food and drink were frequently found in pilgrims. EHEC is a pathotype of *E. coli* capable of producing the Shiga toxin, which is an essential virulence factor. EHEC colonizes the intestinal tract of ruminants, and humans are usually infected by consuming contaminated water or food such as meat, fruit, or vegetables [37]. This pathogen was responsible for several recent food poisoning outbreaks worldwide [38–41]. Notably, in 2011 one of the largest outbreaks of a foodborne infection caused by EHEC serotype O104:H4 was reported in Germany, with 2987 cases, and the mortality was 0.6% [41]. In this German study, EHEC shedding of this pathogen was reported for an extensive period of up to 8 months [41]. The high prevalence of EHEC in pilgrims' post-Hajj rectal swabs could be explained by the longer shedding time of this bacterium compared with other pathogens.

Hand washing and the use of hand sanitizers have been recommended by the Saudi Ministry of Health for the prevention of diarrheal diseases among Hajj pilgrims [42], and these measures appear to be well accepted by pilgrims based on our results. Nevertheless, even compliance with individual preventive measures against diarrhea remains, unfortunately, ineffective at efficiently preventing the disease [43]. Our results showed that pilgrims who suffered GI symptoms and diarrhea were more likely to wash their hands more often. It is not that hand hygiene increases the risk of symptoms, but it is very possible that symptomatic pilgrims were more prone to wash their hands frequently after each use of the toilet given their medical status. Ensuring strict measures regarding food and water quality in Hajj, adherence to preventive measures such as good personal hygiene and environmental management and compliance with regulations asking pilgrims not to cook or bring their own food to the Hajj are required to help reduce the burden of gastrointestinal illnesses at the event.

Our study had some limitations. The study was conducted on a small population and only among French pilgrims who traveled together, staying in the same hotels and tents during their stays and eating the same food during their time in Mina and Arafat. The results cannot, therefore, be generalized to all pilgrims. qPCR which was used to detect enteric pathogens does not distinguish between dead and living micro-organisms. Furthermore, microbiological sampling of food and drink of pilgrims during the Hajj was not carried out. In addition, we did not investigate food and drink characteristics (street food versus restaurant food and non-sterilized water versus bottled water) during the Hajj as risk factors of gastrointestinal symptoms. The onset of gastrointestinal symptoms including diarrhea occurred early during travel, while sampling was performed before departure from the Hajj, several days later (median = 8 days). It could be that the potential acquisition of enteric pathogens linked with diarrhea was partially (or fully) cleared by the time of sampling. Sampling at the onset of symptoms and the culture of enteric pathogens should be conducted in future studies to better identify the pathogens responsible for gastrointestinal symptoms during the Hajj. Nevertheless, our study included pilgrims spanning three Hajj seasons, giving it a stronger statistical power. A number of risk factors have been identified to recognize pilgrims at increased risk of gastrointestinal symptoms, including diarrhea, encountered in this setting.

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Compliance with ethical standards

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

Ethics approval The protocol was approved by the Aix-Marseille University institutional review board (July 23, 2013; reference no. 2013-A00961-44). The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants provided their written informed consent.

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Article 10 :

Lack of Vibrio cholerae among French pilgrims during the 2017 and 2018 Hajj.

Hoang VT, Dao TL, Ly TDA, Belhouchat K, Chaht KL, Drali T, Yezli S, Alotaibi B, Raoult D, Parola P, Fournier PE, Pommier de Santi V, Gautret P.

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Lack of *Vibrio cholerae* among French pilgrims during the 2017 and 2018 Hajj

Dear Editor

Diarrhoeal disease has been a serious public health problem in the context of the Hajj, but data on the etiology of diarrhoeal disease at the Hajj are very limited. After the 2016 pilgrimage, 43.5% of French pilgrims acquired at least one gastrointestinal pathogen, with Enteropathogenic *Escherichia coli* and enteroaggregative *E. coli* being the most frequent [1]. Also, during the 2011–2013 Hajj seasons, nearly 42% of 544 faecal samples collected from diarrheal pilgrims from 40 countries were positive for at least one pathogen. *Salmonella* and enterotoxigenic *Escherichia coli* were detected the most frequently (34.7%) [2].

Cholera continues to cause deadly outbreaks around the world, both in focal locations in endemic areas and in epidemics. Cholera was the main cause of mortality and morbidity among Hajj pilgrims in the 19th century with about 20,000 pilgrims who died from cholera during the 1821 Hajj season [3]. Around 15,000–30,000 pilgrims died from cholera during the 1865 Hajj, accounting for 17–33% mortality rates with a worldwide spread from Mecca through returning pilgrims, killing an estimated 200,000 individuals [3]. Due to improved sanitary conditions in Saudi Arabia in general and at religious sites, no additional cholera outbreaks among pilgrims have been recorded over the last three decades. There is currently no mandatory cholera vaccine for pilgrims arriving from countries with cholera outbreaks. However, cholera continues to cause outbreaks around the globe, including in countries sending pilgrims. The 2016–2018 cholera outbreak in Yemen, with 3928 deaths recorded was considered to be a high risk for a potential outbreak at the Hajj, given that about 20,000 Yemenis per year are granted a visa for the Hajj [4]. In 2018, a smaller cholera outbreak was declared in northern Algeria. A total of 217 cases with cholera-like symptoms were hospitalised and two of the patients died. Of the cases, 83 were confirmed as *V. cholerae* serogroup O1 Ogawa [5].

These cholera epidemics call for extreme caution and particular attention must be paid to prevention, surveillance, and control measures. Furthermore, about 80% of individuals infected with *V. cholerae* show no symptoms and remain undetected but are infectious [4]. Due to outbreaks of cholera in recent years, we conducted a study aiming to screen for *V. cholerae* among French pilgrims during the 2017 and 2018 Hajj.

At total of 268 pilgrims were included. The sex ratio was 1:1.2 and the median age was 61 years (interquartile = 56–66 years, min = 22, max = 86 years). One hundred and sixteen (43.3%) pilgrims were born in Algeria, 55 (20.5%) were born in Morocco, 54 (20.2%) were born in Tunisia, 29 (10.8%) were born in France and 14 (5.2%) were born in other countries. Of them, 128/268 (47.8%) pilgrims reported washing their hands more often than usual and 132/268 (49.3%) used hand gel. None of the pilgrims reported being vaccinated against cholera. In total, 88/268 (32.8%) of the pilgrims presented at least one gastrointestinal symptom during their time in Saudi Arabia. Diarrhoea (16.8%) and constipation (16.8%) were the most frequent, followed by abdominal pain (7.5%), nausea (3.4%) and vomiting (3.0%). Only one (0.4%) pilgrim was hospitalised in KSA and 10/88 (11.4%) pilgrims with gastro-intestinal symptoms were still symptomatic on return.

Rectal swabs were obtained from each pilgrim prior to leaving France and Saudi Arabia. The toxR gene (toxR-F1: 5′-CCGAATAACCA CCCTGATCTTT-3′, toxR-R1: 5′-ACCTGTGGCAATGACTTCTATC-3′, toxR-Pr: CY5 -ACTGGCTACCGTCAATCGAACTGT- BHQ2) was used to screen for all isolates for *V. cholerae* using quantitative real-time PCR method. All rectal swabs tested negative for *V. cholerae*.

Our results are based on a relatively small number of individuals and are therefore not representative of all pilgrims participating in the Hajj. However, using a sensitive method, we noted the absence of *V. cholerae* among French pilgrims before and after the Hajj. This suggests that cholera did not play a major role in the epidemiology of Hajj-associated gastro-intestinal infections in 2017–2018, despite 17% of pilgrims experiencing diarrhoea during their stay in Saudi Arabia. Our results corroborate the fact that no cholera outbreak was reported at the Hajj by Saudi medical authorities in 2017–2018. With emphasis on public health surveillance based on proactive surveillance, the implementation of rapid control measures and early detection, cholera is under constant surveillance by the Saudi authorities.

Hand washing and the use of hand sanitiser should be recommended for the prevention of diarrhoeal diseases among Hajj pilgrims [6]. During the Hajj, pilgrims practice hand washing regularly as part of a ritual purification. Ablution is, in most cases, performed by Muslims before the five daily obligatory prayers and consists of the methodical washing of the hands, mouth, nose, face, forearm, head, ears, and feet with running water. Consequently, hand washing compliance of Hajj pilgrims during their stay in KSA is usually quite good [6].

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Declaration of competing interest

Van-Thuan Hoang, Thi-Loi Dao, Tran Duc Anh Ly, Khadidja Belhouchat, Kamel Larbi Chaht, Tassadit Drali, Saber Yezli, Badriah Alotaibi, Didier Raoult, Philippe Parola, Pierre-Edouard Fournier, Vincent Pommier de Santi, and Philippe Gautret declare that they have

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no conflict of interest.

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Partie 2:

Etude environnementale des pathogènes respiratoires lors du Hajj

Préambule

Pendant le Hajj en 2016 et 2018, nous avons collecté 142 échantillons environnementaux, dont 75 prélèvements à La Mecque, 48 à Mina, 8 à Arafat et 11 à Médine (article 11). Au total, 70/142 (49,3%) prélèvements étaient positifs pour au moins un pathogène respiratoire. Parmi les échantillons positifs, *K. pneumoniae* était la bactérie la plus fréquemment retrouvée (57,1%), suivie de *S. pneumoniae* (12,9%), *S. aureus* (10,0%) et *H. influenzae* (7,1%). De plus, 32,9% des échantillons ont été testés positifs pour le rhinovirus et 1,4% pour le coronavirus. Aucun échantillon n'était positif pour le virus de la grippe A et B ou parainfluenza virus. Les surfaces avec les taux les plus élevés d'échantillons positifs à *K. pneumoniae* étaient les tables de cuisine (80,0%), les réserves de glace (76,9%), le bord du couvercle des refroidisseurs d'eau (62,5%) et les équipements de salle de bain et d'ablution (60,0%). Les surfaces avec les taux les plus élevés d'échantillons positifs à rhinovirus étaient des poignées de porte (40,9%), tandis que celles avec les taux les plus élevés d'échantillons positifs à *S. pneumoniae* étaient des parties de tente (40,0%) et des robinets de fontaine (60,0%).

En comparant la distribution des agents pathogènes respiratoires par zone géographique où les échantillons ont été prélevés, les taux de positivité de Mina étaient significativement plus élevés pour la plupart des agents pathogènes ; notamment, tous les échantillons positifs pour *S. pneumoniae* ont été prélevés à Mina (article 11).

Article 11 :

Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018.

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Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018

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A R T I C L E I N F O

Keywords:
Hajj
Respiratory pathogens
Environmental sampling
Human rhinovirus
Klebsiella pneumoniae
Streptococcus pneumoniae

A B S T R A C T

Background: Respiratory tract infections are common in the context of the Hajj pilgrimage and respiratory pathogens can be transmitted via contact with contaminated surfaces. We sampled surfaces during the Hajj to detect the presence of respiratory bacteria and viruses.
Methods: Frequently touched surfaces at Mecca, Mina, Arafat and Medina were sampled. The common respiratory pathogens were tested by qPCR.
Results: 70/142 (49.3%) environmental samples collected were positive for at least one respiratory pathogen. Among the positive samples, *Klebsiella pneumoniae* was the bacterium most frequently tested positive (57.1%), followed by *Streptococcus pneumoniae* (12.9%), *Staphylococcus aureus* (10.0%) and *Haemophilus influenzae* (7.1%). 32.9% positive samples tested positive for rhinovirus and 1.4% for coronavirus. Surfaces with the highest rates of positive samples were kitchen tables (100%), water fountain faucet (73.3%) and edge of water coolers lid (84.6%). Samples collected in Mina were the most frequently contaminated with 68.8% being positive for at least one pathogen and 18.8% positive for a combination of multiple pathogens.
Conclusion: These preliminary results indicate that respiratory pathogens are common in environmental surfaces from areas frequented by Hajj pilgrims. Further larger-scale studies are needed to better assess the possible role of environmental respiratory pathogens in respiratory infections in Hajj pilgrims.

1. Introduction

The Hajj or Muslim pilgrimage to Mecca, Saudi Arabia, is the “Fifth Pillar of Islam”. Muslims who are financially and physically able are required to perform Hajj once during their life. Hajj is one of the largest annual religious mass gatherings in the world. This event gathers from two to three million participants from more than 180 countries [1]. It takes place for 6 days beginning on the eighth and ending on the thirteenth day of Dhu al-Hijjah, the last month of the Islamic calendar. Because the Islamic calendar is lunar and the Islamic year is about eleven days shorter than the Gregorian year, the Gregorian date of Hajj changes from year to year.

The Hajj must be performed in three main locations in Mecca, in Mina and Arafat, which are respectively at 5 and 18 km from Mecca. Most pilgrims sleep in hotels in Mecca and in large tent camps in Mina during the Hajj. Due to overcrowding, pilgrims move slowly between sites, by foot or by bus for hours [2,3]. Most pilgrims also travel to Medina in order to visit the tomb of the Prophet Muhammad.

Respiratory tract infections (RTIs) are a leading cause of hospitalization during the Hajj in Saudi hospitals. Acute upper respiratory tract infection is the most common clinical presentation. The overall prevalence of RTIs, when evaluated among cohorts of pilgrims from different origins, varied from 50 to 93% [4].

The bacterial pathogens most frequently isolated from pilgrims with

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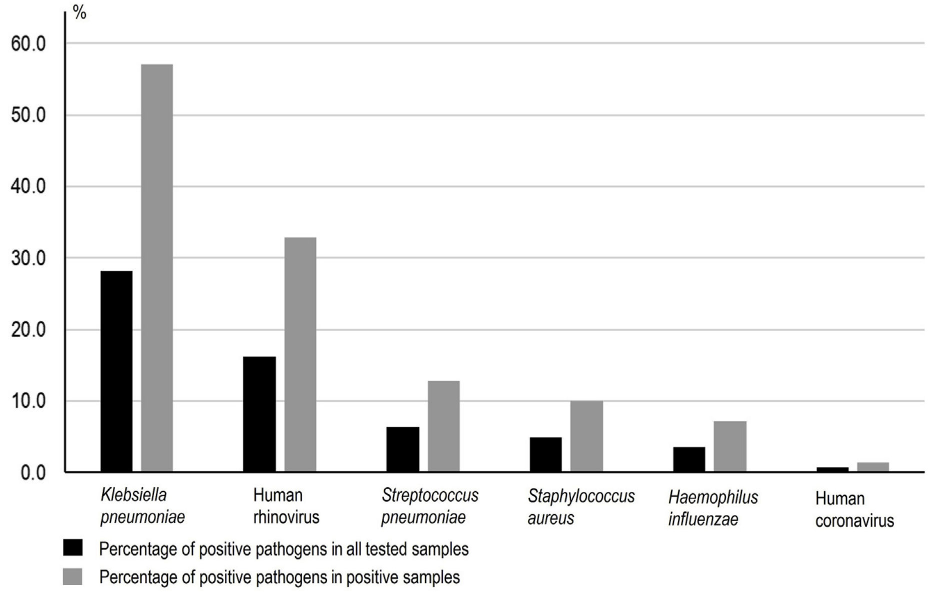

Fig. 1. Distribution of pathogens in all tested environmental samples (N = 142) and in positive samples (N = 70).

respiratory diseases are *Haemophilus influenzae, Klebsiella pneumoniae, Streptococcus pneumoniae* and *Staphylococcus aureus* [4]. The most common viral pathogens are human rhinovirus (HRV), non-MERS coronaviruses (HCoVs) and influenza viruses [4–6].

Many pilgrims are elderly people with co-morbidities that could increase the risk of RTIs [3,7]. The high rate of RTIs among Hajj pilgrims are likely due to inter-human transmission, given the overcrowding conditions at the Grand Mosque in Mecca with close contact between pilgrims during rituals (up to 8 persons per m^2) and in hotels or other infrastructures in the city, and during the accommodation in tents in Mina with an average of 50–100 people per tent [8–10].

In addition, the environment may play an important role in the transmission of respiratory pathogens through the air or via contact with contaminated surfaces. Many studies conducted in hospitals and/or other health care facilities showed that environmental pathogens are frequently responsible for occupational and nosocomial respiratory infections [11–14].

To assess the potential role of contaminated surfaces in the transmission of respiratory pathogens at the Hajj, we sampled environmental surfaces at various locations along pilgrim route during the Hajj 2016 and 2018 for the molecular detection of common pathogens responsible for Hajj-associated RTIs in pilgrims.

2. Materials and methods

2.1. Surface sampling

Surface swabbing was conducted from 6 to 19 September 2016 (the Hajj took place from 10 to 15 September) and from 16 to 24 August 2018 (the pilgrimage took place from 19 to 24 August), using a commercial collection and transferred to Sigma-Virocult® medium and stored at −80 °C. Swabs were used to sample 25 cm^2 (5 × 5 cm) areas of frequently touched surfaces in facilities used by French pilgrims in Mecca (hotel, commercial center, restaurant), in Mina (tent camp), in a bus from Mina to Mecca, in Arafat (tents and ablution facilities) and in Medina (hotel). Facilities that were investigated were those used by French pilgrims enrolled in prospective cohort surveys that focused on RTIs and respiratory pathogen human carriage.

2.2. Identification of respiratory pathogens

DNA and RNA were extracted from the samples using the EZ1 Advanced XL (Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendations. All quantitative real-time PCR were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA).

The pathogens tested were based on their frequency in respiratory samples obtained from Hajj pilgrims [4,7,15].

Real-time PCR amplifications were carried out using LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. The *SHD* gene of *H. influenzae*, *phoE* gene of *K. pneumoniae*, *nucA* gene of *S. aureus* and *lytA CDC* gene of *S. pneumoniae* were amplified with internal DNA extraction controls TISS, as previously described [16].

HCoV and human para-influenza virus (HPIV) were detected by one-step duplex quantitative RT-PCR amplifications of HCoV/HPIV-R Gene Kit (REF: 71-045, Biomérieux, Marcy l'Etoile, France), according to the manufacturer's recommendations. One-step simplex real-time quantitative RT-PCR amplifications were performed using Multiplex RNA Virus Master Kit (Roche Diagnostics, France) for influenza A, influenza B, HRV and internal controls MS2 phage [17].

Negative control (PCR mix) and positive control (DNA from bacterial strain or RNA from viral strain) were included in each run. Positive results of bacteria or virus amplification were defined as those with a cycle threshold (CT) value ≤ 35.

3. Results

We collected 142 environmental samples (66 and 76 samples in the Hajj 2016 and 2018 respectively, 75 samples at Mecca, 48 at Mina, 8 at Arafat and 11 at Medina) (Supplementary data). A total of 70 samples (49.3%) were positive for at least one respiratory pathogen (Supplementary data). Among the positive samples, *K. pneumoniae* was the most common bacterium that tested positive (57.1%), followed by *S. pneumoniae* (12.9%), *S. aureus* (10.0%) and *H. influenzae* (7.1%). In addition, 32.9% positive samples tested positive for HRV and 1.4% for HCoV. No sample was positive for influenza virus A and B or HPIV (Fig. 1). Overall, the prevalence of positive surfaces was 28.2% for *K. pneumoniae*, 16.2% for HRV and 6.3% for *S. pneumoniae.* Of the 70 positive samples, 12 were positive for more than one pathogen, including 9 positive samples for *K. pneumoniae* and one or two other mostly bacterial pathogens and 3 samples with various associations of bacteria other than *K. pneumoniae* (Supplementary data).

Surfaces with the highest rates of *K. pneumoniae* positive samples

Table 1
Distribution of environmental samples positive for *Klebsiella pneumoniae*, Human rhinovirus and *Streptococcus pneumoniae* by surface type (N = 142).

Surface	N tested	*Klebsiella pneumoniae* n (%)	Human rhinovirus n (%)	*Streptococcus pneumoniae* n (%)
Door handles	22	0 (0)	9 (40.9)	0 (0)
Toilets	18	5 (27.8)	1 (5.6)	1 (5.6)
Bathroom and ablution equipments	15	9 (60.0)	2 (13.3)	0 (0)
Handrails	11	2 (18.2)	2 (18.2)	0 (0)
Ice reserves	13	10 (76.9)	3 (23.1)	0 (0)
Elevator buttons	11	0 (0)	1 (9.1)	0 (0)
Air conditioners	9	0 (0)	1 (11.1)	0 (0)
Restaurant devices	9	2 (22.2)	1 (11.1)	0 (0)
Edge of water coolers lid	8	5 (62.5)	0 (0)	2 (25.0)
Faucet of water fountains	5	0 (0)	1 (20.0)	3 (60.0)
Fridges	5	2 (40.0)	0 (0)	0 (0)
Tent parts	5	0 (0)	0 (0)	2 (40.0)
Kitchen tables	5	4 (80.0)	0 (0)	1 (20.0)
Various[a]	6	1 (16.7)	2 (33.3)	0 (0)
Total	**142**	**40 (57.1)**	**23 (32.9)**	**9 (12.9)**

[a] Hotel door card, hotel room telephone, vendor machine button, hotel lobby table.

were kitchen tables (80.0%), ice reserves (76.9%), edge of water coolers lid (62.5%) and bathroom and ablution equipments (60.0%). Surfaces with the highest rates of HRV positive samples were door handles (40.9%), while those with the highest rates of *S. pneumoniae* positive samples were tent parts (40.0%) and water fountain faucets (60.0%) (Table 1).

By comparing the distribution of respiratory pathogens by geographical area where samples were collected, Mina positivity rates were significantly higher than in other areas for most pathogens and including multiple contaminations by several pathogens. Of note, all samples positive for *S. pneumoniae* were collected at Mina ($p < 0.0001$) (Table 2).

4. Discussion

The microbial contamination of surfaces in both health care and community settings is not uncommon, and pathogens are capable of surviving for prolonged periods on such surfaces and can be transmitted to humans via direct contact with contaminated surfaces [18,19]. Most gram-positive bacteria survive for months on dry surfaces. Many gram-negative species can also survive for months. A few others, such as *Bordetella pertussis*, or *Haemophilus influenzae*, however, persist only for days [20]. However, the human rhinovirus, human coronavirus and influenza virus have all been found to survive in the external environment, for only a matter of hours and occasionally for a day or two [21]. Few studies on the presence of environmental pathogens that may be responsible for human respiratory infections have been conducted in the context of Hajj. Angelakis et al. conducted a study investigating viable bacterial populations in air samples collected around slaughterhouses during the 2012 Hajj season by culture methods. *Bacillus* and *Staphylococcus* spp. were commonly isolated [22]. To our knowledge, only one study aiming at detecting the presence of respiratory pathogens by PCR, was conducted in the context of the Hajj and was based on air and surface samples obtained in Jeddah airport in 2013 [23]. In this survey, pathogens detected on surfaces were adenovirus (n = 3/40, 7.5%), HCoV (n = 3/40, 7.5%), *H. influenzae* (n = 1/40, 2.5%) and *Moraxella catarrhalis* (n = 1/40, 2.5%). No sample was positive for HRV and *S. pneumoniae*. *S. aureus* and *K. pneumoniae* were not tested [23]. Our study showed that respiratory bacteria, notably *K. pneumoniae* and *S. pneumoniae* and HRV were frequently recovered from environmental surface samples in areas frequented by pilgrims. Particularly high levels of contaminated surface were observed in Mina's camp, particularly from the surfaces of the collective kitchen, including tables, drinking sources and the ice supply. Hotel bathroom and ablution area equipments in Mecca were also highly contaminated. Among the French pilgrims surveyed in 2016 [15] and 2018 (unpublished data) who have stayed in the places investigated in the present survey, 16.3% acquired coronaviruses, 16.4% *S. aureus,* 17.3% *K. pneumoniae,* 25.5% *S. pneumoniae,* 26.7% HRV and 30.3% acquired *H. influenzae* during their stay in Saudi Arabia (Fig. 2). High rates of contamination of surface samples by *K. pneumoniae* and HRV suggest a possible source of contamination for pilgrims (Fig. 2). The acquisition of influenza A and influenza B virus was low among pilgrims (2.2% and 0.4% respectively) and no surface sample was positive for these viruses. It is likely that the living conditions in Mina encampment in relative promiscuity and sharing of cooking, food, hygiene and sanitary facilities are responsible for the high rate of environmental contamination by respiratory pathogens from ill pilgrims with possible subsequent transmission to pilgrims through contact with fomites. Although most pilgrims have onset of respiratory symptoms shortly after their arrival in Saudi Arabia (likely resulting from inter-human transmission of respiratory pathogens in various crowed infrastructures in the city), we observed a bimodal pattern of clinical respiratory symptoms with a lower secondary peak occurring in Mina [24]. Our results suggest that the source of contamination for respiratory pathogens might differ in Mecca and Mina, with a possible additional role for contaminated fomites in Mina, besides air-transmission.

K. pneumoniae can be transmitted by person-to-person contact (for example, from patient to patient via the contaminated hands of healthcare personnel, or other persons) or by contamination of the

Table 2
Distribution of environmental samples positive for *Klebsiella pneumoniae*, Human rhinovirus and *Streptococcus pneumoniae* by geographical area (N = 142).

Respiratory pathogens		Mecca		Arafat		Mina[a]		Medina		Total		p-value[b]
		n = 75	%	n = 8	%	n = 48	%	n = 11	%	N = 142	%	
Streptococcus pneumoniae	Yes	0	0	0	0	9	18.8	0	0	9	6.3	< 0.0001
	No	75	100	8	100	39	81.2	11	100	133	93.7	
Klebsiella pneumoniae	Yes	14	18.7	2	25.0	24	50.0	0	0	40	28.2	< 0.0001
	No	61	81.3	6	75.0	24	50.0	11	100	102	71.8	
Human rhinovirus	Yes	11	14.7	1	12.5	5	10.4	6	54.6	23	16.2	0.01
	No	64	85.3	7	87.5	43	89.6	5	45.4	119	83.8	
At least one pathogen	Yes	28	37.3	3	37.5	33	68.8	6	54.6	70	49.3	0.005
	No	47	62.7	5	62.5	15	31.2	5	45.4	72	50.7	
≥2 pathogens	Yes	3	4.0	0	0	9	18.8	0	0	12	8.4	0.03
	No	72	96.0	8	100	39	81.2	11	100	130	91.6	

[a] 5 samples collected in the bus from Mina to Mecca.
[b] Test Fisher's exact.

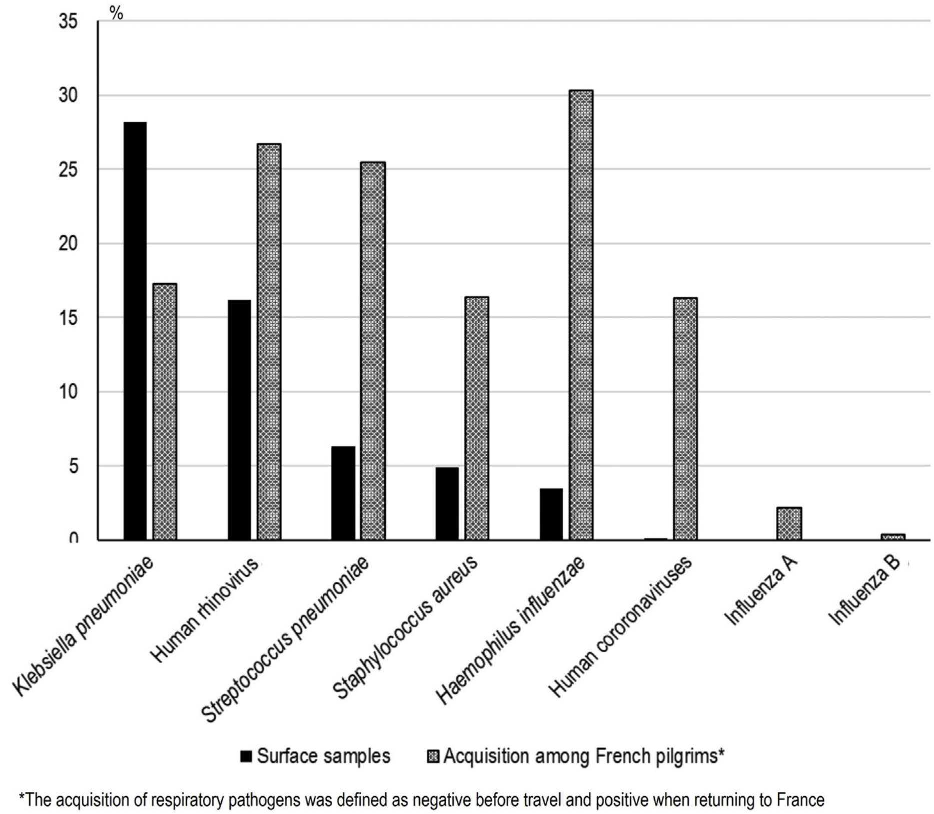

Fig. 2. Positivity rates of environmental samples in comparison to acquisition rates as assessed in respiratory samples among French pilgrims surveyed in 2016 [15] and 2018 [unpublished data].

environment. The bacteria do not spread in the air [25]. In a study conducted between June 2009 and June 2010 on 750 environmental surface samples of bathroom, wash basin taps, wash basin, drains, doors and handles in the Outpatient Department at the Al-Azhar University Hospital in Assuit, Egypt, 13.6% samples were positive for *K. pneumoniae*. Wet surfaces of bathrooms, wash basin taps and wash basins were those most frequently found positive, followed by doors and handles, suggesting that environmental surfaces are potential reservoirs for pathogens such as *K. pneumoniae*. [25]. Humans are the main host for *S. pneumoniae*, but direct person-to-person contact is not required for bacteria transmission, thus indicating that environmental reservoir could be involved [26]. In addition, higher rates of pneumococcal transmission are known to occur in overcrowded environments [27,28].

HRV and HCoV can survive on surfaces for at least a few hours. Their transmission by contact with such surfaces has been described [18,29,30]. HCoV has been detected in hospitals and apartment buildings contaminating various inanimate surfaces, such as telephones, computer mice and toilet handles [18,31]. Also, transmission hand-to-hand and by contact with contaminated surface of HRV was identified [32]. Ikonen et al. conducted a recent study on the presence of respiratory viruses on frequently touched surfaces at Helsinki-Vantaa airport, Finland, detected by qPCR. A total of 9/90 (10%) surface samples were positive for at least one respiratory virus with 4/90 (4.4%) HRV, 3/90 (3.3%) HCoV OC43, 3/90 (3.3%) adenovirus and only 1/90 (1.1%) influenza A virus [33]. Another study that focused on surface samples collected from homes and hospital rooms of patients infected with influenza A H1N1, showed that 33/671 samples were positive for this virus [34].

Our study has some limitations. First, we did not collect air samples. Secondly, we tested only 9 respiratory pathogens (based on their higher frequency in French Hajj pilgrims). Additionally, because the detection was based on molecular techniques only, we cannot prove that the detected pathogens were alive and potentially infective. Finally, our sample size is relatively small. Despite these limitations, we demonstrated that DNA and RNA of respiratory pathogens are present at high rates on surfaces in the environment of pilgrims during the Hajj, notably in Mina. Memish et al. hypothesized that, in the absence of good hand hygiene, there is a possibility that pilgrims could pick up environmental pathogens on their hands from contaminated surfaces and self-inoculate or further transmit these pathogens [23]. Respiratory tract infections during Hajj continue to exert a heavy burden on pilgrims [4]. Besides early identification of pathogenic bacterial or viral clusters for faster mitigation of outbreaks and better understanding of disease etiology, we believe that further larger-scale studies are needed to better evaluate the possible role of environmental respiratory pathogens in causing respiratory infections among Hajj pilgrims. In the interim, reinforced hand hygiene and improved cleaning and disinfection of frequently touched surfaces at various locations during the pilgrimage should be recommended with a priority for wet surfaces, kitchen tables and door handles.

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Declaration of competing interest

Van-Thuan Hoang, Doudou Sow, Khadidja Belhouchat, Thi-Loi Dao, Tran Duc Anh Ly, Florence Fenollar, Saber Yezli, Badriah Alotaibi, Didier Raoult, Philippe Parola, Vincent Pommier de Santi, Philippe Gautret declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2019.101500.

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Supplementary data

Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018

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PCR detection of potential respiratory pathogens on surfaces in Mecca, Mina, Arafat and Medina during the 2016 and 2018 Hajj.

ID	Type of surface	*S. aureus*	*S. pneumoniae*	*H. influenzae*	*K. pneumoniae*	Human coronavirus	Human rhinovirus	At least one pathogens
	Hotel collective areas - Mecca							
1	Entry door exterior handle							
2	Entry door exterior handle						■	■
3	Entry door exterior handle						■	■
4	Entry door exterior handle							
5	Surface of a large table in the lobby				■			■
6	Women's toilet door handle (floor A)						■	■
7	Edge of chair N°1 in men's toilets (floor A)				■			■
8	Edge of chair N°2 in men's toilets (floor A)				■			■
9	Tap handle in men's toilets (floor A)						■	■
10	Shower handle in women's toilets (floor A)				■			■
11	Tap handle N°2 in women's toilets (floor A)							
12	Tap handle N°1 in women's toilets (floor A)							
13	Tap handle N°3 in women's toilets (floor A)							
14	Tap handle N°1 in men's toilets (floor A)							
15	Door handle of men's toilets (floor B)	■						■
16	Handrail of elevator							
17	Handrail of elevator							
18	Buttons inside elevator N°1			■				■
19	Buttons inside elevator N°2							
20	Buttons outside the elevator							
21	Buttons outside the elevator							
22	Buttons outside the elevator							
23	Water supply system						■	■
	Hotel rooms - Mecca							
24	Air conditioning vent (room A1)							
25	Exterior door handle (room A2)							
26	Air conditioning vent (room A2)							

27	Bathroom sink tap (room A2)				■	■		■
28	Interior surface of the bathroom sink (room A2)				■			■
29	Exterior door handle (room A3)							
30	Bathroom sink tap (room A3)							
31	Interior surface of the bathroom sink (room A3)				■			■
32	Exterior door handle (room A4)							
33	Bathroom sink tap (room A4)				■			■
34	Interior surface of the bathroom sink (room A4)				■			■
35	Exterior door handle (room A5)							
36	Air conditioning vent N°1 (room A5)							
37	Air conditioning vent N°2 (room A5)							
38	Bathroom sink tap (room A5)							
39	Interior surface of the bathroom sink (room A5)	■			■			■
40	Toilet bowl (room A6)							
41	Shower handle in toilets (room A6)							
42	Toilet bowl (room A6)	■						■
43	Front door (room A7)							
44	Closet handle (room A7)						■	■
45	Fridge handle (room A7)				■			■
46	Front door handle (room A8)						■	■
47	Closet handle (room A8)						■	■
48	Fridge door (room A8)							
49	Inside of the fridge (room A8)							
50	Door handle, bathroom (room A8)							
51	Shower handle in toilets (room A8)							
52	Outside door handle (room A9)						■	■
53	Door handle, bathroom (room A9)							
54	Outside door handle (room A10)							
55	Light switcher (room A10)							
56	Closet handle (room A10)							
57	Door handle, bathroom (room A10)							
58	Pilgrim personal non-invasive ventilation machine							
59	Pilgrim personal non-invasive ventilation machine							
	Shopping center - Mecca							
60	Escalator handrail N°1(shopping center A)							
61	Escalator handrail N°2 (shopping center A)							
62	Escalator handrail (shopping center B)	■			■			■
63	Stair railing (shopping center B)							
64	Stair railing (shopping center B)							

65	Bank note (shopping center B)						■	■
	Restaurant - Mecca							
66	Table surface in the dining room							
67	Tongs used for cucumbers				■			■
68	Tongs used for tomatoes				■			■
69	Cutlery						■	■
70	Olive ladle							
71	Bread tray							
72	Cup handle							
73	Cup edge							
74	Napkins							
75	Hot water faucet							
	Tent camp - Arafat							
76	Central pole, men's tent							
77	Water cooler faucet button N°1, men's tent							
78	Water cooler faucet button N°2, men's tent							
79	Edge of the water cooler N°1, men's tent							
80	Faucet, men's ablution area				■			■
81	Faucet, men's ablution area						■	■
82	Faucet, men's ablution area							
83	Faucet, toilets				■			■
	Tent camp - Mina							
84	Faucet, women's ablution area	■			■			■
85	Faucet, women's ablution area						■	■
86	Faucet, women's ablution area				■			■
87	Faucet, women's ablution area							
88	Surface of main table, dining room		■	■	■			■
89	Surface of table N°1 for breakfast preparation, dining room				■			■
90	Surface of table N°2 for breakfast preparation, dining room				■			■
91	Kitchen counter				■			■
92	Edge of the water cooler lid, kitchen		■		■			■
93	Fridge handle N°1, kitchen							
94	Fridge handle N°2, kitchen				■			■
95	Faucet of water fountain N°1, camp N°1	■	■	■				■
96	Faucet of water fountain N°2, camp N°1		■					■
97	Faucet of water fountain N°3, camp N°2		■	■				■
98	Faucet of water fountain N°4, camp N°2							
99	Inside of ice tray lid (day 1), kitchen						■	■
100	Inside of ice tray lid (day 2), kitchen							

101	Tent curtain							
102	Edge of ice tray lid, kitchen				■			■
103	Edge of ice tray lid, kitchen				■			■
104	Edge of ice tray lid, kitchen				■			■
105	Edge of ice tray lid, kitchen				■		■	■
106	Edge of ice tray lid, kitchen				■			■
107	Edge of ice tray lid, kitchen				■		■	■
108	Edge of ice tray lid, kitchen				■			■
109	Edge of ice tray lid, kitchen							
110	Edge of ice tray lid, kitchen				■			■
111	Edge of ice tray lid, kitchen				■			■
112	Edge of ice tray lid, kitchen				■			■
113	Edge of front door, men's tent N°1	■	■					■
114	Edge of front door, men's tent N°2		■					■
115	Edge of front door, men's tent N°3							
116	Air conditioning vent, men's tent N°1							
117	Air conditioning vent, men's tent N°2						■	■
118	Edge of the water cooler lid N°1, men's tent				■			■
119	Edge of the water cooler lid N°2, men's tent		■	■	■			■
120	Edge of the water cooler lid N°1, women's tent				■			■
121	Edge of the water cooler lid N°2, women's tent				■			■
122	Toilet bowl							
123	Shower handle women's toilets				■			■
124	Shower handle, men's toilets							
125	Shower handle, men's toilets		■					■
126	Edge of chair, men's toilets							
	Bus from Mina to Mecca							
127	Air conditioning vent N°1 of bus from Mina to Mecca							
128	Air conditioning vent N°2 of bus from Mina to Mecca							
129	Air conditioning vent N°3 of bus from Mina to Mecca							
130	Support handle in the bus from Mina to Mecca							
131	Support handle of the window in the bus from Mina to Mecca				■			■
	Hotel - Medina							
132	Handle of the main door						■	■
133	Elevator buttons							
134	Elevator buttons							
135	Elevator buttons							
136	Elevator buttons							
137	Elevator buttons						■	■

138	Elevator buttons							
139	Stair railing							
140	Door handle (room A11)							
141	Door card (room A11)							
142	Telephone (room A11)							

Negative Positive

Partie 3 :

Utilisation d’antibiotique et acquisition de bactéries multi-résistantes et de gènes de résistance par les pèlerins du Hajj

Préambule

Nos données de 2012 à 2017 ont montré que 47,6% des pèlerins ont utilisé un antibiotique pour des infections respiratoires pendant leurs séjours en Arabie Saoudite (article 12). Selon les recommandations françaises, seulement 39,6% des pèlerins ayant utilisé un antibiotique relevaient d'une indication de prescription. En revanche, 28,8% pèlerins avec une probable infection respiratoire basse n'ont pas reçu d'antibiotique bien qu'ils en aient eu l'indication. En outre, les pèlerins qui ont pris un antibiotique pendant leur séjour étaient significativement plus susceptibles de présenter des symptômes persistants d'infection respiratoire basse, après le Hajj.

Nous avons aussi conduit une étude microbiologique recherchant la présence de bactéries respiratoires et digestives multirésistantes aux antibiotiques et présentant des gènes de résistance à la colistine, en 2017 et 2018, sur 268 pèlerins (article 13). Le résultat a montré que 8,6% des pèlerins portaient au moins une bactérie multirésistante avant le Hajj, contre 19,4% après le Hajj. Tous les pèlerins avec des échantillons positifs post-Hajj ont acquis ces bactéries en Arabie Saoudite. Au total, 81 souches de bactéries multirésistantes ont été isolées. *E. coli* et *K. pneumoniae* étaient significativement plus fréquentes dans les échantillons post-Hajj que dans les échantillons pré-Hajj. La plupart des souches (80,2%) ont été isolées dans des échantillons rectaux.

La recherche de bactéries porteuses de gènes de résistance à la colistine sur les prélèvements nasopharyngés et rectaux a permis d'identifier 23 écouvillons positifs (tous concernant des prélèvements rectaux post-Hajj) qui ont été séquencés avec succès (19 pour mcr-1 gène et 4 pour mcr-4 gène). Ainsi, la prévalence d'acquisition de bactéries porteuses de gènes de résistance à la colistine chez les pèlerins de Marseille était 8,6%. De plus, nous rapportons ici pour la première fois, la circulation de bactéries porteuses de gènes mcr-4 au Hajj.

<u>Article 12 :</u>

Antibiotic use for respiratory infections among Hajj pilgrims: A cohort survey and review of the literature.

Hoang VT, Nguyen TT, Belhouchat K, Meftah M, Sow D, Benkouiten S, Dao TL, Anh Ly TD, Drali T, Yezli S, Alotaibi B, Raoult D, Parola P, Pommier de Santi V, Gautret P.

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Antibiotic use for respiratory infections among Hajj pilgrims: A cohort survey and review of the literature

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A R T I C L E I N F O

Keywords:
Hajj
Pilgrims
Antibiotic
Respiratory tract infections
Recommendation
Determinants

A B S T R A C T

Background: To evaluate the occurrence and determinants associated with antibiotic use for respiratory tract infections (RTIs) among Hajj pilgrims.
Methods: Prospective cohort surveys were conducted among French pilgrims from 2012 to 2017. We also conducted a systematic review about available evidence for antibiotic consumption in relation with RTIs during the Hajj.
Results: 783 pilgrims were included in the survey. During the Hajj, 85.3% presented respiratory symptoms and 47.6% used antibiotics. Pilgrims with productive cough or fever were three times and twice as likely to have used antibiotics. Dry cough, sore throat and voice failure were also associated with increased antibiotic use. 26.3% of pilgrims presented symptoms compatible with a lower tract respiratory infection. According to the French recommendations, only 39.6% of pilgrims who used an antibiotic actually had an indication for it. Antibiotic intake was associated with an increased frequency of persistent symptoms post-Hajj (aRR = 1.31, 95%CI [1.04–1.66]).

The review included 14 articles. The use of antibiotic for respiratory tract infections during the Hajj varied from 7% to 58.5%. In 9 studies, the antibiotic consumption rate was > 30%.
Conclusion: Respiratory tract infections are common during the Hajj, leading to high prevalence of inappropriate antibiotic intake.

1. Introduction

The Muslim Hajj pilgrimage in Saudi Arabia, is among the largest annual religious mass gatherings on earth. Each year, it welcomes more than 2 million pilgrims from more than 180 countries, a third of whom come from outside Saudi Arabia. The Hajj takes place in three main places in the Mecca area, which are the Grand Mosque in the Holy city of Mecca, the Mina valley and the plain of Arafat (about 5 and 18 km from Mecca respectively) [1]. A large proportion of pilgrims are elderly people, many with chronic diseases [2]. Furthermore, the presence of many pilgrims from different countries around the world and overcrowding considerably increase the risk to contract infectious diseases, particularly respiratory and gastrointestinal infections, resulting in a considerable demand for antibiotic use [3,4]. The predominance of bacterial pathogens in Hajj-related gastrointestinal infections poses a major public health risk due to the potential emergence and transmission of antimicrobial resistant bacteria [5]. Antibiotic resistant gastrointestinal and respiratory organisms have been frequently isolated from

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Table 1
Characteristics of the study population, Hajj pilgrims (N = 783).

Variables		n	%
Pilgrimage years	2012	169	21.6
	2013	129	16.5
	2014	98	12.5
	2015	119	15.2
	2016	117	14.9
	2017	151	19.3
Gender	Male	328	41.9
	Female	455	58.1
Age	Median	62	
	Interquartile	54–68	
	Min-max	21–96	
Comorbidities	Diabetes mellitus	216	28.0
	Hypertension	227	29.4
	Chronic respiratory disease	75	9.7
	Chronic heart disease	56	7.3
	Chronic kidney disease	7	0.9
	Immunodeficiency	3	0.4
Duration of stay in KSA (mean ± SD) (days)		22.9 ± 1.7	

KSA; Kingdom of Saudi Arabia.

Hajj pilgrims [6–11]. Although a prescription has been required in order to receive antibiotics in Saudi Arabia for more than 30 years [12], many pilgrims use antibiotic without a prescription [13]. To date, few studies on the appropriateness of antibiotic use among pilgrims were conducted. The objective of this study is to assess the prevalence of antibiotic use during Hajj over a period from 2012 to 2017 among French pilgrims and to study the demographic and clinical determinants associated with antibiotic use during this event. We also conducted a systematic review on available evidence for antibiotic consumption in relation to respiratory tract infections (RTIs) during the Hajj.

2. Methods

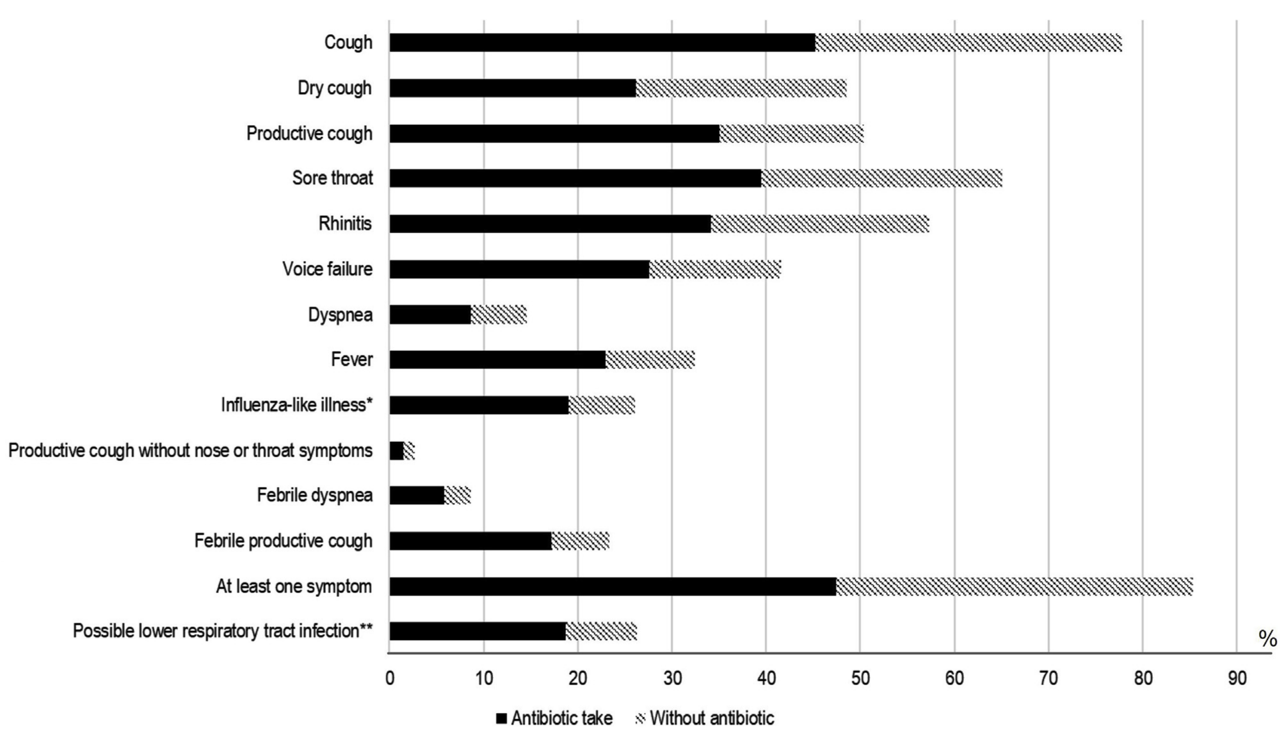
2.1. Prospective cohort

2.1.1. Participants and study design

Pilgrims from Marseille, France participating in the Hajj from 2012 to 2017 were recruited at a specialized travel agency organizing trips to Mecca. Potential adult participants were invited to participate in the study. They were recruited and followed-up by a medical bilingual (Arabic and French) doctor who traveled with the group. The participants were interviewed using a standardized pre-Hajj questionnaire that collected information on demographic characteristics and medical conditions before departing from France. A post-Hajj questionnaire that collected clinical data and information on the use of antibiotics was completed two days before the pilgrims' return to France. Influenza-like illness (ILI) was defined in the current study as the presence of subjective fever, sore throat and cough [14]. Possible pulmonary involvement requiring antibiotic therapy was suspected based on the presence of the following symptoms: productive cough without nasal or throat symptoms; febrile productive cough; dyspnea or febrile dyspnea, according to expert consensus statements [15,16]. Streptococcal pharyngitis was suspected based on the presence of fever and sore throat without cough or runny nose [17]. The protocol was approved by the Aix-Marseille University institutional review board (July 23rd, 2013; reference no. 2013-A00961-44). The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants provided a written consent.

2.1.2. Statistical analysis

Statistical analysis was conducted using STATA software version 11.1 (Copyright 2009 StataCorp LP, http://www.stata.com). Differences in the proportions were tested by Pearson's chi-square or Fisher's exact tests when appropriate. Unadjusted associations between multiple factors and prevalence of antibiotics use for respiratory symptoms were examined by univariate analysis. The results were presented by percentages and risk ratio (RR) with 95% confidence



*ILI: cough, sore throat and fever, **Possible lower respiratory tract infection: productive cough without nasal or throat symptoms; febrile productive cough; dyspnea or febrile dyspnea

Fig. 1. Antibiotic consumption according to respiratory tract infections during the Hajj 2012–2017 (N = 783).

Table 2
Associated factor to antibiotic consumption during the Hajj (N = 754).

Variables		Univariate analysis				Multivariate analysis	
		ATB use = Yes n (%)	ATB use = No n (%)	RR [95%CI]	P-value	aRR [95%CI]	P-value
Socio-demographic characteristics							
Gender	Male	123 (38.8)	194 (61.2)	0.72 [0.61–0.84]	$< 10^{-4}$		
	Female	236 (54.0)	201 (46.0)				
Age*	≥60 years	200 (46.1)	234 (53.9)	0.93 [0.80–1.08]	0.35		
	< 60 years	157 (49.5)	160 (50.5)				
Comorbidities							
Diabetes mellitus*	Yes	96 (45.5)	115 (54.5)	0.94 [0.79–1.11]	0.46		
	No	262 (48.5)	278 (51.5)				
Hypertension*	Yes	100 (45.2)	121 (54.8)	0.93 [0.78–1.10]	0.39		
	No	258 (48.7)	272 (51.3)				
Chronic respiratory disease*	Yes	41 (56.2)	32 (43.8)	1.20 [0.97–1.49]	0.13		
	No	317 (46.8)	361 (53.2)				
Chronic heart disease*	Yes	30 (54.5)	25 (45.5)	1.16 [0.90–1.49]	0.29		
	No	328 (47.1)	368 (52.9)				
Respiratory symptoms							
Cough	Yes	341 (58.1)	246 (41.9)	5.34 [3.47–8.38]	$< 10^{-4}$		
	No	18 (10.8)	149 (89.2)				
Dry cough	Yes	198 (54.1)	168 (45.9)	1.30 [1.12–1.52]	5.10^{-4}	1.56 [1.23–2.00]	$< 10^{-4}$
	No	161 (41.5)	227 (58.5)				
Productive cough	Yes	264 (69.5)	116 (30.5)	2.74 [2.27–3.29]	$< 10^{-4}$	2.97 [2.28–3.88]	$< 10^{-4}$
	No	95 (25.4)	279 (74.6)				
Dyspnea	Yes	65 (59.1)	45 (40.9)	1.29 [1.08–1.54]	0.01		
	No	294 (45.7)	350 (54.3)				
Sore throat	Yes	298 (60.7)	193 (39.3)	2.62 [2.08–3.30]	$< 10^{-4}$	1.52 [1.18–1.95]	10^{-3}
	No	61 (23.2)	202 (76.8)				
Voice failure	Yes	208 (66.2)	106 (33.8)	1.93 [1.66–2.46]	$< 10^{-4}$	1.41 [1.08–1.83]	0.01
	No	151 (34.3)	289 (65.7)				
Rhinitis	Yes	257 (59.5)	175 (40.5)	1.88 [1.57–2.24]	$< 10^{-4}$		
	No	102 (31.7)	220 (68.3)				
Fever	Yes	173 (70.9)	71 (29.1)	1.94 [1.69–2.24]	$< 10^{-4}$	2.01 [1.53–2.63]	$< 10^{-4}$
	No	186 (36.5)	324 (63.5)				
ILI	Yes	143 (72.6)	54 (27.4)	1.87 [1.63–2.14]	$< 10^{-4}$		
	No	216 (38.8)	341 (61.2)				
Possible lower respiratory tract infections							
Productive cough without nasal or throat symptoms	Yes	11 (55.0)	9 (45.0)	1.16 [0.77–1.74]	0.50		
	No	348 (47.4)	386 (52.6)				
Febrile dyspnea	Yes	44 (67.7)	21 (32.2)	1.48 [1.23–1.78]	7.10^{-4}		
	No	315 (45.7)	374 (54.3)				
Febrile productive cough	Yes	130 (73.9)	46 (26.1)	1.86 [1.63–2.13]	$< 10^{-4}$		
	No	229 (39.6)	349 (60.4)				

RR: risk ratio, aRR: adjusted relative risk, ATB: antibiotic.
*: N = 751, data missing for 3 subjects.

interval (95%CI). Results with a p value ≤ 0.05 were considered statistically significant. Only the variables with a prevalence ≥5.0% were considered for statistical analysis. Variables with p values < 0.2 in the univariate analysis were included in the multivariate analysis. Log-binomial regression was used to calculate adjusted risk ratios regarding antibiotic consumption [18].

2.2. Review of antibiotic use during the Hajj

2.2.1. Search strategy and selection criteria

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) guidelines (http://www.prisma-statement. org). The following databases were investigated in an attempt to identify all relevant studies published on: PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Google Scholar (http://scholar.google.fr/). The most recent search was conducted on November 12, 2018. The topic search terms used for searching the databases were the following:

#1 : “Hajj” OR “pilgrims”
#2 : “antibiotic” OR “antibacterial” OR “antimicrobial” OR “treatment”
#3 : #1 AND #2

Only articles published in English were included. For inclusion, articles had to fulfil three criteria: (1) be related to Hajj, (2) be conducted among group of pilgrims independently to the presence of symptoms and (3) report the use of antibiotic. Studies that recruited only sick pilgrims from hospitals or outpatient departments, case reports and reviews were excluded. Reference lists of selected articles were screened to identify studies that might have been missing from the research.

Two researchers (GP and HVT) independently performed the screening of the abstracts. Any discordant result was discussed in a consensus meeting. After screening the abstracts, the full texts of the articles were assessed for eligibility by the same two researchers and selected or rejected for inclusion in the systematic review.

2.2.2. Data collection process

The following data (if available) were extracted from each article: year, countries of origin of pilgrims, study design, sample size of attendees, prevalence of symptoms and ratio of antibiotic use.

2.2.3. Data synthesis and analysis

As a result of the nature of the studies and the heterogeneity in patient populations, a formal meta-analysis was not possible. Therefore, the study results were summarized to describe the main outcomes of

Table 3
Associated factors to persistence of respiratory symptoms at return from the Hajj (uni- and multi-variate analysis (N = 646 ill pilgrims).

Variables		Persistence of respiratory symptoms				
		n (%)	RR [95%CI]	P-value	aRR [95%CI]	P-value
Socio-demographic characteristics						
Gender	Male	148 (46.7)	1.01 [0.86–1.18]	0.92		
	Female	202 (46.3)				
Age*	≥60 years	214 (56.6)	1.10 [0.95–1.28]	0.18		
	< 60 years	136 (51.3)				
Comorbidities						
Diabetes mellitus*	Yes	95 (52.2)	0.95 [0.81–1.14]	0.51		
	No	254 (55.1)				
Hypertension*	Yes	114 (58.8)	1.12 [0.97–1.30]	0.13		
	No	235 (52.3)				
Chronic respiratory disease*	Yes	41 (59.4)	1.11 [0.90–1.37]	0.36		
	No	308 (53.7)				
Chronic heart disease*	Yes	29 (58.0)	1.07 [0.84–1.38]	0.58		
	No	320 (54.0)				
Antibiotic consumption						
Antibiotic intake	Yes	209 (58.4)	1.18 [1.02–1.37]	0.02	1.31 [1.04–1.66]	0.02
	No	142 (49.3)				
Beta-lactamine	Yes	158 (59.8)	1.18 [1.03–1.36]	0.02		
	No	193 (50.5)				
Macrolide	Yes	47 (54.7)	1.01 [0.82–1.24]	0.95		
	No	304 (54.3)				

RR: risk ratio, aRR: adjusted relative risk.
*: data of 3 subjects missing.

interest (i.e. the prevalence of antibiotic use). When possible, percentages not presented in the articles were calculated from the available data.

3. Results

3.1. Cohort survey during the Hajj 2012–2017

3.1.1. Characteristics of study participants

A total of 783 of the 803 pilgrims contacted (97.5%) agreed to participate in our study and completed the questionnaire required before the trip. Of them, 754 (96.3%) also responded to the post-Hajj questionnaire. 328 pilgrims were male (41.9%) with a gender ratio of 1:1.4. The median age was 62 years of age (interquartile = (54–68), min = 21, max = 96 years). Hypertension (28.0%) and diabetes (29.4%) were the most common comorbidities (Table 1).

3.1.2. Clinical features and antibiotic intake during the Hajj

Fig. 1 shows the prevalence of respiratory symptoms and antibiotic use among pilgrims during the Hajj. Most frequent symptoms were cough (77.8%, 587/754), rhinitis (57.3%, 432/754) and sore throat (65.1%, 491/754). 26.1% (197/754) of pilgrims had ILI and 26.3% (198/754) had a possible pulmonary involvement. Only 2 pilgrims presented with symptoms of possible streptococcal pharyngitis and both used antibiotics. In total, 3 patients were hospitalized. Two days prior to leaving Saudi Arabia, 46.6% (351/754) of pilgrims were still symptomatic. A total of 47.6% (359/754) of pilgrims took antibiotics during their pilgrimage, representing 55.6% of ill pilgrims. Beta-lactams were the most commonly used antibiotic (35.0%), followed by macrolides (11.4%), cephalosporines (2.3%), quinolones (1.5%) and sulfonamides (0.1%).

3.1.3. Determinants associated with antibiotic consumption during the Hajj and impact of antibiotics on respiratory symptoms

Table 2 shows results of univariate and multivariate analysis about determinants associated with antibiotic consumption. Pilgrims suffering from productive cough or fever were three times and twice as likely, respectively, to have used antibiotics than others (aRR = 2.97, 95%CI [2.28–3.88], $p < 0.0001$ and aRR = 2.01, 95%CI [1.53–2.63], $p < 0.0001$ respectively). Dry cough, sore throat and voice failure were also associated with increased antibiotic use. Of note, antibiotic intake was not significantly increased in individuals with a possible pulmonary involvement compared to others (18.7% versus 28.9% respectively) (Table 2).

With regard to the persistence of respiratory symptoms post-Hajj, no socio-demographic factors and no chronic conditions was associated. Antibiotic intake was associated with increased frequency of symptom persistence (aRR = 1.31, 95%CI [1.04–1.66], p = 0.02) (Table 3). This increase was only observed in pilgrims with symptoms of LRTI (RR = 1.86, 95%CI [1.32–2.61], p = 0.0002) but not in those with symptoms of URTI (RR = 0.93, 95%CI [0.75–1.15], p = 0.50) (data not shown).

3.1.4. Review on antibiotic consumption at the Hajj

A total of 157 articles were identified in the database search and 5 additional articles were found through the manual search. After screening the titles and abstracts, 14 articles were eventually retained for full-text assessment. All 14 articles were included in the qualitative synthesis of the systematic review (Supplementary Fig. 1). Studies were conducted during the Hajj seasons from 1999 to 2016 and included a total of 7774 pilgrims originating from various countries, including Pakistan, Malaysia, Ireland, US, Singapore, UK, Iran, Australia and India. Most studies were conducted based on a prospective cohort follow-up design and some used a cross-sectional survey design. RTIs were common among pilgrims and hospitalization rates were low. The use of antibiotic for RTIs during the Hajj varied by nationality, from 7% in a Singaporean survey in 2001 to 58.5% among Iranian pilgrims in 2012. In 9 studies out of 14, the antibiotic consumption prevalence was > 30% [13,19–31] (Table 4).

4. Discussion

Our study confirms that RTIs are common during the Hajj with a high proportion of antibiotic use of 47.6%. The multivariate analysis showed that upper respiratory tract infection (URTI) symptoms (dry cough, sore throat and voice failure), productive cough and fever were independent factors associated with increased antibiotic use. In this study, only 26.3% of pilgrims reported clinical symptoms, suggesting a

Table 4
Review of literature on antibiotic consumption during the Hajj.

Pilgrimage year	Study design	Number of pilgrims	Prevalence of respiratory symptoms	Prevalence of antibiotic intake	Reference
2013	Cross-sectional study conducted among Australian Hajj pilgrims in Mina and Mecca, Saudi Arabia	1162	Not documented	34.9%. The reason for antibiotic use was: RTIs in 83.9% cases	14
1999	Prospective cohort study conducted among Pakistani Hajj pilgrims enrolled in Pakistan before the Hajj based on identification numbers attributed by the Pakistani government	2070	ILI (sore throat and cough or temperature ≥ 38 °C) 47.9% URTI (cough or sore throat or rhinitis or myalgia or headache) 72.2% Hospitalization 0.3%	26.6%	19
2008	Prospective cohort study conducted among Irish Hajj pilgrims recruited at a travel clinic.	167	Sore throat or cough or ILI 79% per-Hajj and 15% post-Hajj Hospitalization 1.2% with 1 case of pneumonia and 1 case of tonsillitis.	31%	20
2013	Cross-sectional study conducted among Malaysian Hajj pilgrims on returning to Malaysia	246	RTIs 93.4%, ILI (cough and fever and sore throat) 78.2% hospitalization 1.9%	57.7% population studied (61.8% symptomatic pilgrims)	21
2001	Prospective cohort study conducted among American Hajj pilgrims enrolled at JFK International Airport, New York on departing to Jeddah, Saudi Arabia.	844	Sore throat 53%, fever 21.2% and cough 59.2%	44.8%	22
2001	Prospective cohort study conducted among Singaporean Hajj pilgrims recruited at a vaccination center in Singapore	171	Cough 56%, sore throat 44%	41%	23, 24
2001	Prospective cohort study conducted among Singaporean Umrah pilgrims recruited at a vaccination center in Singapore	160	Cough 13%, sore throat 8%	7%	24
2002	Prospective cohort study conducted among Singaporean Hajj pilgrims recruited at a vaccination center in Singapore	193	Cough 70%	52.9%	25
2002	Prospective cohort study conducted among English Hajj pilgrims recruited at a London Mosque	174	Not documented	21% among pilgrims with RTIs.	26
2003	Prospective cohort study conducted among Iranian Hajj pilgrims recruited at health centers before travel to Mecca.	797		58.2% (because of RTIs)	27
2012	Cross-sectional study conducted among returning Iranian Hajj pilgrims	422	Not documented	58.5%	28
2013	Cross-sectional survey conducted among Hajj pilgrims from 13 countries at Jeddah airport	468	ILI 62%	45.5%	29
2014	Prospective cohort survey conducted among Australian Hajj pilgrims recruited on returning to Australia	93	Not documented	17.2%	30
2016	Prospective multisite cohort study conducted among Indian pilgrims recruited from 4 cities in India	807	76% pilgrims had at least one respiratory symptom	29.4%	31

ILI: influenza like illness, URTI: upper respiratory tract infection, RTI: respiratory tract infections lower respiratory tract infection (LRTI) that may require antibiotic use according to the French recommendations [15]. The antibiotic consumption, however, was lower in patients with a possible pulmonary involvement compared to others presenting with symptoms of URTI. According to the French recommendations [15], only 142/359 (39.6%) of pilgrims who have used an antibiotic had an indication for their use. By contrast, 57/198 (28.8%) pilgrims with a possible LRTI did not receive antibiotic although they had an indication for them. Furthermore, pilgrims who took antibiotics during their stay were significantly more likely to present with persisting symptoms of LRTI, post-Hajj. A possible explanation for this finding is that the sicker patients were more likely to take antibiotics and also more likely to have persistent symptoms. Alternatively, antibiotics may have altered either the underlying flora or the immune responses in ways that impeded recovery.

The high prevalence of antibiotic use at the Hajj is not observed among French pilgrims only. Our review shows that a high proportion of pilgrims of different nationality used antibiotics during their pilgrimage because of RTIs. In a survey conducted among 1162 pilgrims from 13 different countries in 2013, at the Jeddah airport, 62% ILI was observed after the Hajj and 45.5% received antibiotic [29]. Most RTI cases during the Hajj are URTIs, while pneumonia is uncommon among pilgrims [19–21,32]. Most URTIs are due to viruses with no formal need for antibiotic treatment [33]. Antibiotic resistance of bacteria in Saudi Arabia has recently emerged for several reasons, including self-prescribing of antibiotics by patients, irrational or over-prescription by medical staff, sub-therapeutic doses of antimicrobial agents and poor case management by unsuitable combinations and, non-compliance with prescribed treatments by patients. In one study conducted at one hospital in Jeddah, 59.3% *Klebsiella pneumoniae* respiratory isolates were resistant to ampicillin and piperacillin [34]. In another study, conducted at one hospital in Taif, 30.5% gram-negative bacteria isolates had an extended spectrum β-lactamases phenotype [35]. Recent publications have shown that antibiotic-resistant bacteria acquisition at the Hajj is frequent [36]. Among French pilgrim cohorts sampled in 2013 and 2014 a significant acquisition of extended spectrum β-lactamases-, carpabenemase-producing bacteria or mcr-1-positive isolates was reported [8–11]. It is therefore likely that antibiotics used by French pilgrims in the present study, might have been at least partially ineffective in treating LRTI symptoms.

The assessment of the bacterial origin of RTI in pilgrims would necessitate taking a sputum sample before starting treatment for identification and sensitivity testing, since antibiotic intake prior to pneumonia diagnosis can impair the detection of the causative agent [37]. However, most of ill pilgrims are seen in outpatient clinics or by medical missions. In this context, paraclinical diagnosis such as radiology and the culture of respiratory pathogens is difficult and the prescription of antibiotics is often based on the clinical evaluation of the patient. Therefore, antibiotic prescription should be restricted to patients presenting with symptoms of LRTI. Access to antibiotic without prescription is another cause of high prevalence of antibiotic use during the Hajj. A recent knowledge, attitude and practice survey showed that 66.6% pilgrims accessed antibiotics without prescription through a pharmacist. Over 87% of them used non-prescribed antibiotics. A proportion of 79.2% used multiple sources to access antibiotics. Only 12.7% of respondents indicated that in the event of illness, they would visit a clinic and only take the medications prescribed by a doctor. A proportion of 26.5% used antibiotics prescribed to them by a doctor for a previous illness and 10% antibiotics prescribed by doctors for their relatives [38].

In a study conducted during the Grand Magal de Touba, in Senegal, in 2017, 41.8% pilgrims reported respiratory symptoms and only 2.7% received antibiotics [39]. We are not aware about studies documenting the antibiotic intake among participant to other mass gatherings. Such studies would be of interest.

Our study was based on questionnaires and has some limitations including notably the lack of data about clinical examination of participants, the lack of radiological and microbiological documentation of RTIs and the lack of duration of antibiotic treatment. Also we did not differentiate self-prescription of antibiotics and antibiotics prescription by the accompanying doctor.

5. Conclusion

RTIs are common during the Hajj and antibiotic use is frequent in this context. It is therefore necessary to follow the recommendations for antibiotic use based on clinical symptoms in pilgrims in order to ensure rational consumption of antibiotics during Hajj. A pilgrim education strategy on Hajj-related pathologies and indications of antibiotics and the promotion of influenza and pneumococcal vaccination is needed. In particular, it is important to organize information sessions before travel to Mecca, or to use documents (flyers) or information online. In addition, it is important to control the delivery of antibiotics in the countries of origin of pilgrims and to enforce the legislations of KSA government [38]. Rapid identification tests for respiratory pathogens could help medical staff in charge of pilgrims to rationalize their prescriptions for antibiotics.

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Conflicts of interest

Van-Thuan Hoang, Thi-Thu-Thuy Nguyen, Khadidja Belhouchat, Mohammed Meftah, Doudou Sow, Samir Benkouiten, Thi-Loi Dao, Tran Duc Anh Ly, Tassadit Drali, Saber Yezli, Badriah Alotaibi, Didier Raoult, Philippe Parola, Vincent Pommier de Santi, Philippe Gautret declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2019.06.007.

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Supplementary data

Antibiotic use for respiratory infections among Hajj pilgrims: a cohort survey and review of the literature

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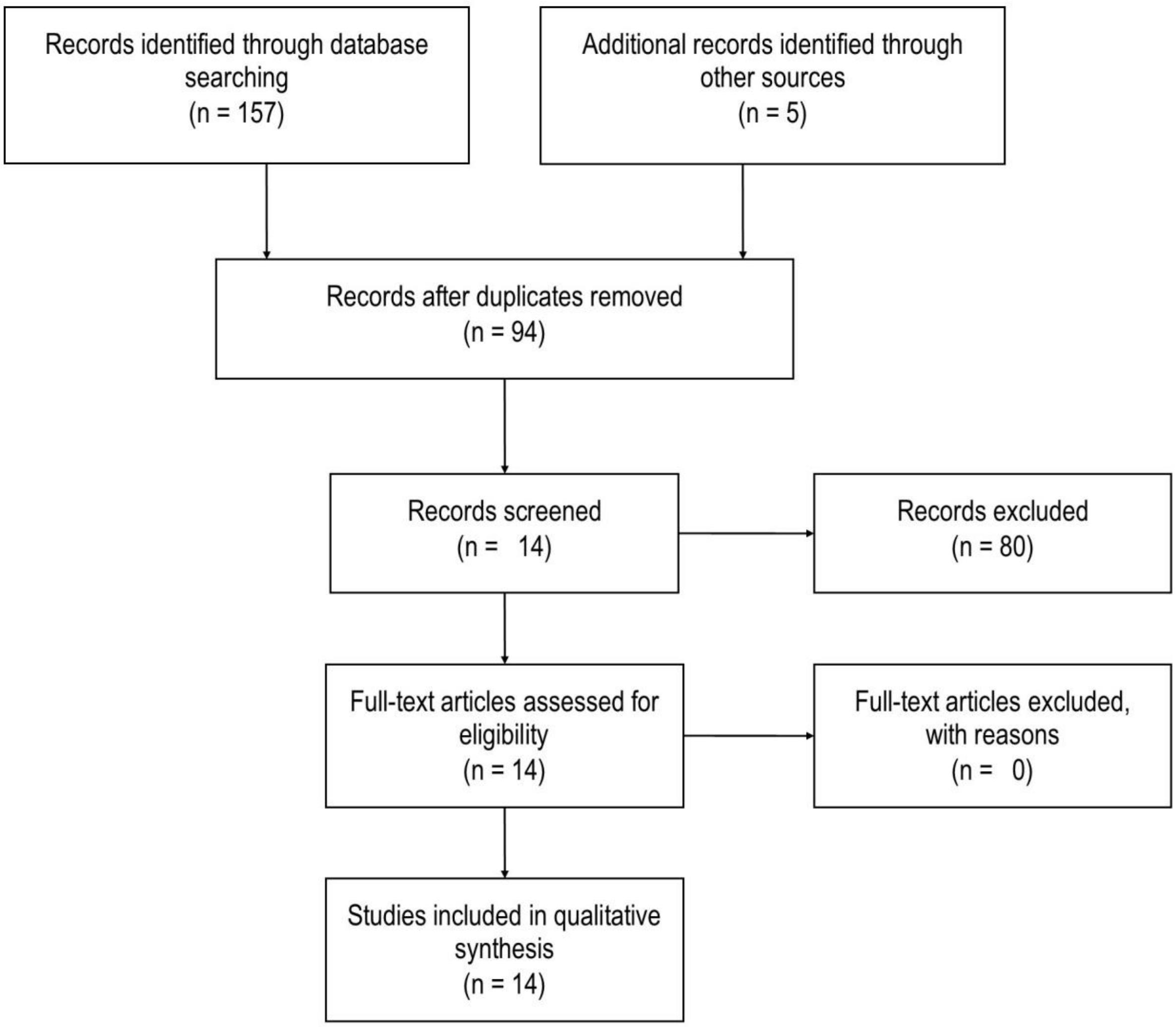
Supplementary figure



<u>Article 13 :</u>

Acquisition of multi-drug resistant bacteria and colistin-resistance genes among French pilgrims during the 2017 and 2018 Hajj

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ORIGINAL ARTICLE

Acquisition of multidrug-resistant bacteria and encoding genes among French pilgrims during the 2017 and 2018 Hajj

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Abstract

The objective of this study is to determine the acquisition of multidrug-resistant (MDR) bacteria and antibiotic resistance-encoding genes by French Hajj pilgrims and associated risk factors. Pilgrims traveling during the 2017 and 2018 Hajj were recruited. All pilgrims underwent two successive systematic nasopharyngeal and rectal swabs, pre- and post-Hajj. Specific culture media were used to screen for MDR bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant bacteria, and extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E). qPCR was used to identify antibiotic resistance-encoding genes from cultured isolates. Direct screening of genes encoding for colistin resistance (*mcr*-1, 2, 3, 4, 5, and 8) from nasopharyngeal and rectal swabs was performed using qPCR, and positive qPCR results were simultaneously tested by sequencing. There were 268 pilgrims included. The percentage of pilgrims acquiring MDR bacteria during the Hajj was 19.4%. A total of 81 strains were isolated (1 carbapenem-resistant *Acinetobacter baumannii*, 12 MRSA, and 68 ESBL-E). ESBL-E strains were found in rectal samples of 6.0% pilgrims pre-Hajj and of 16.4% pilgrims post-Hajj. Only 0.4% pilgrims were positive for CARB post-Hajj and 1.9% carried nasal MRSA pre- and post-Hajj. In addition, 23 (8.6%) post-Hajj rectal swabs were positive for *mcr* genes (19 *mcr-1* gene and 4 *mcr-4* gene). No significant association was found between co-factors and acquisition of MDR bacteria or *mcr* genes. MDR bacteria and genes are acquired by pilgrims during the Hajj mass gathering. Rationalization of antibiotic consumption and implementation of measures to prevent transmission of bacteria among pilgrims during the event are of paramount importance.

Keywords Hajj · Pilgrims · Multidrug resistant bacteria · Risk factors · Colistin · MRSA · ESBL-E

Introduction

The Hajj, the annual Muslim pilgrimage to Mecca, Kingdom of Saudi Arabia (KSA), is one of the largest annual religious mass gatherings worldwide. Each year an increasing number of people travel to the KSA for the Hajj pilgrimage, coming from more than 180 countries [1]. A total of 2,489,406 pilgrims participated in the 2019 Hajj season, with 1,855,027 foreign pilgrims [2]. This event presents major challenges for global health, notably with inter-human transmission of infectious diseases, such as respiratory tract infections (RTIs) and diarrheal infections [1, 3]. Most RTIs are due to viruses [3], and overuse of antibiotics in Hajj patients with such infections has been reported [4].

Circulation of multidrug-resistant (MDR) bacteria has been reported in Hajj pilgrims, Hajj workers, or local patients attending hospitals in Mecca, Mina, and the Medina area [5].

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The concern about spreading MDR bacteria to the community in home countries and increasing the global challenge of MDR bacteria is undeniable.

Our research group conducted several prospective cohort studies aimed at specifically investigating the acquisition of MDR bacteria by French Hajj pilgrims. A two-fold increase in extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriage was documented in French pilgrims following their participation in the 2013 Hajj [6]. Acquisition proportions of CTX-M genes among French pilgrims after the 2013 and 2014 Hajj were 31.0% and 34.8%, respectively [7]. Another study conducted in French pilgrims in 2014 evidenced a bla_{OXA-51} carriage proportion of 23.3% and 38.9% in post-Hajj respiratory and rectal samples, while no pre-Hajj sample tested positive [8]. In addition, carbapenem-encoding resistance genes bla_{OXA-72} and bla_{NDM-5} were detected in two returned pilgrims [8]. Finally, the plasmid-mediated colistin resistance gene *mcr-1* was acquired by 9% of French pilgrims investigated during the 2013 and 2014 Hajj seasons [6].

Despite several studies having been conducted, the risk factors for acquiring resistant bacteria among pilgrims remain poorly understood. In addition, previous studies were performed on a discrete group of multidrug bacteria on a small sample size of pilgrims. In the present study, we aimed to document the acquisition of MDR bacteria (including methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase-producing *Enterobacteriaceae*, glycopeptide-resistant *Enterococci*, and carbapenemase-producing *Enterobacteriaceae*) and antibiotic resistance-encoding genes by French pilgrims during the 2017 and 2018 Hajj and to investigate associated risk factors. We also specifically investigated the acquisition of colistin resistance genes and associated risk factors.

Materials and methods

Participants and study design

The participants and study design were detailed elsewhere [9]. In summary, pilgrims traveling from Marseille, France, to Mecca, KSA, during the 2017 and 2018 Hajj were recruited through a private specialized travel agency. They were included and followed up by two bilingual (French and Arabic) medical doctors traveling with the group. All participants departed for the KSA on the same date, were housed in the same accommodation during their stay, and performed the rituals together. Pre-Hajj and post-Hajj samples were collected 1 week before departing and 2 days before the pilgrims' return, respectively. Pilgrims were considered to have been immunized against influenza when they had been vaccinated within the past year and up to 10 days before the date of travel to the KSA. Pilgrims were considered immunized against invasive pneumococcal disease (IPD) when they had been vaccinated in the previous 5 years [10–13]. Influenza-like illness (ILI) was defined as the presence of cough, sore throat, and subjective fever [14]. Diarrhea was defined as at least three loose or liquid stools per 24 h.

Based on the WHO classification, "underweight" was defined as having a body mass index (BMI) below 18.5; "normal" corresponded to a BMI between 18.5 and 25; "overweight" corresponded to a BMI ≥ 25; and "obese" referred to those with a BMI ≥ 30 [15].

Respiratory and gastrointestinal specimens

All pilgrims underwent two successive systematic nasopharyngeal and rectal swabs: pre-travel (pre-Hajj) and just prior to leaving the KSA (post-Hajj). The nasopharyngeal sampling was done by the doctors accompanying the group [16]. Rectal auto-sampling was done by the pilgrims, in a standardized manner [9].

Nasopharyngeal and rectal swabs were transferred to Sigma-Transwab® media and stored at − 80 °C during 72 h after being collected. The time delay between sampling and processing was estimated to 3 months maximum.

Identification of multidrug-resistant bacteria

Supplementary Fig. 1 shows the identification procedure for MDR bacteria. MRSA agar (bioMérieux, Marcy l'Étoile, France) was used for identifying MRSA. MacConkey agar (bioMérieux, Marcy l'Étoile, France) was used to isolate ESBL-E, carbapenem-resistant *Acinetobacter baumannii* (CRAB), and *Pseudomonas aeruginosa* resistant to ceftazidime or cefepime. SMART agar and VRE agar (bioMérieux, Marcy l'Étoile, France) were used to screen for CPE (carbapenemase-producing Enterobacteriaceae) and glycopeptide-resistant *Enterococcus* spp., respectively.

MDR bacteria were isolated in cultures for identification and antibiogram. Strains were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Microflex; Bruker Daltonic, Bremen, Germany). The disk diffusion method was used for an overview of antibiotic-resistant bacteria in pilgrims and was interpreted using EUCAST (European Committee on Antimicrobial Susceptibility Testing) version 6.0 guidelines (http://www.eucast.org) and French recommendation when EUCAST guidelines were not available (https://www.sfm-microbiologie.org/wp-content/uploads/2019/02/CASFM-V2.0.Mai2017.pdf). ESBL-E isolates were tested against 13 antibiotics (amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefepime, ceftriaxone, ertapenem, imipenem, fosfomycin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, doxycycline, and amikacin). CRAB isolates were tested against 14 antibiotics: ticarcillin, ticarcillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, doripenem, fosfomycin, fusidic acid, sulfamethoxazole-trimethoprim, tobramycin, ciprofloxacin, colistin, and amikacin. MRSA isolates were tested against the following 13 antibiotics: oxacillin, rifampicin, clindamycin, erythromycin, pristinamycin, gentamicin, vancomycin, teicoplanin, doxycycline, fosfomycin, ciprofloxacin, fusidic acid, and sulfamethoxazole-trimethoprim. Enterococcus isolates were tested against the following 11 antibiotics: penicillin G, rifampicin, clindamycin, erythromycin, pristinamycin, gentamicin, vancomycin, teicoplanin, doxycycline, fosfomycin, and fusidic acid.

Detection of antibiotic resistance-encoding genes

Detection of antibiotic resistance-encoding genes was conducted on isolates recovered by culture. DNA was extracted from the isolates using the QIAamp DNA Mini Kit (Qiagen) using the EZ1 Advanced XL (Qiagen) according to the manufacturer's recommendations.

The *mecA and mecC* genes were used to identify resistance-encoding genes in MRSA isolates [17, 18]. The $bla_{\text{CTX-M-A}}$ (CTX-M-1 and –M-9), $bla_{\text{CTX-M-B}}$ (CTXM-2, -M-8, and -M-25), bla_{SHV}, and bla_{TEM} genes were used to detect resistance-encoding genes in ESBL-E and *P. aeruginosa* isolates [19]. The bla_{OXA23}, bla_{OXA24}, bla_{OXA58}, and bla_{NDM} genes were used to detect resistance-encoding genes in *A. baumannii* [20]. The bla_{Oxa48}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{KPC} genes were used for screening for resistance-encoding genes for CPE [8].

Negative controls (PCR mix and sterile H_2O) and positive controls (plasmid DNA extracted from a colony of cultured *E. coli* or *K. pneumoniae*) were included in each run. Positive results for encoding genes were defined as those with a cycle threshold (CT) value ≤ 26.

Identification of colistin resistance genes

We screened for colistin resistance genes in nasopharyngeal and rectal swabs, pre- and post-Hajj, by real-time PCR.

Extracted DNA from each samples were combined into pools of DNA. DNA pooling was performed as previously described [21, 22].

All quantitative real-time PCR (qPCR) reactions were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, USA) with the ready-to-use reaction mix ROX qPCR Master according to the manufacturer's recommendations. Negative control (single PCR mix and sterile H2O) and positive control templates (plasmid DNA extracted from a colony of cultured *Escherichia coli* or *Klebsiella pneumoniae*) were included in each qPCR experimental run. Results were considered positive when the cycle threshold value of real-time PCR was < 35. Individual retesting of each specimen was carried out from positive pools. The qPCR amplification was used to confirm the presence of *mcr-1*, *mcr-2* (including the detection of *mcr-6*), *mcr-3*, *mcr-4*, *mcr-5*, and *mcr-8* genes by using primers previously described [23, 24].

To better characterize the above genes, only positive qPCR results were simultaneously tested by standard PCR. Purified PCR products were sequenced using specific primers and the BigDye Terminator® version 1.1 cycle sequencing ready reaction mix (Applied Biosystems, Foster City, CA, USA). All primers used in this study have previously been described [25, 26].

Sequencing was performed on Applied Biosystems 3130 platform (ABI PRISM, PE Applied Biosystems, USA). Obtained sequences were edited and assembled using Chromas Pro 1.77 (Technelysium Pty Ltd., Australia) and were then aligned with reference genes by GenBank.

These sequences are available in GenBank at accession numbers MT463292-MT463310 (*mcr-1* gene) and MT463311-MT463314 (*mcr-4* gene).

Statistical analysis

STATA software version 15.1 (Copyright 1985-2017 StataCorp LLC, http://www.stata.com, College Station, TX, USA) was used for statistical analysis. Differences in the proportions were tested by Pearson's chi-square or Fisher's exact tests when appropriate. Acquisition of MDR bacteria was defined by the detection of MDR bacteria after travel in individuals who were negative before travel. When individuals were positive pre- and post-Hajj, the species, anatomical sites of sampling, antibiotic susceptibility, and genetic characterization of isolates allowed distinction between acquisition and persistent carriage. Acquisition of a colistin resistance gene was identified if the pilgrim was negative pre-Hajj but positive post-Hajj. In order to evaluate the potential acquisition of at least one MDR bacteria and of colistin resistance genes, we used McNemar's test to compare their prevalence before leaving France and after travel in Saudi Arabia. Unadjusted associations between multiple factors and EBLS-E and colistin resistance gene acquisition were examined by univariable analysis. The results were presented by percentages and risk ratio with 95% confidence interval (95% CI). Variables with p values < 0.2 in the univariable analysis were included in the multivariable analysis. Logistic regression was used to estimate adjusted risk ratios for factors regarding MDR bacteria and colistin resistance gene acquisition. The test was considered statistically significant when the p value was < 0.05.

Ethics statement

The protocol was approved by the Aix-Marseille University institutional review board (July 23, 2013; reference No. 2013-A00961-44).

The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments.

All participants provided their written informed consent.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [PG], upon reasonable request.

Results

Characteristics of study participants

Our study enrolled 268 pilgrims who completed both pre- and post-travel questionnaires and provided paired nasopharyngeal and rectal swabs during the 2017 and 2018 Hajj seasons. Only 21 pilgrims refused to participate in our study. Hence, the acceptation rate to participate in the study was 92.7%. The study population had a median age of 61.0 years (interquartile = (53–66 years), min = 21, max = 88 years), and the male/female ratio was 1:1.4. Hypertension (82/268, 30.6%) was the most common chronic comorbidity reported, followed by diabetes (70/268, 26.1%), chronic respiratory disease (34/268, 12.7%), chronic heart disease (23/268, 8.6%), and chronic kidney disease (6/268, 2.2%). A total of 188 (70.1%) pilgrims had an indication for IPD vaccination [10–13].

Regarding individual preventive measures against infectious diseases, 70/268 (26.1%) pilgrims reported influenza vaccination in the past year. Only 25/188 (13.3%) pilgrims with an indication for IPD had been vaccinated against pneumococcal disease (PCV-13) in the past 5 years, according to French recommendations [10–13]. A total of 105 (39.2%) pilgrims reported using a face mask and 215 (80.5%) using disposable tissues during their pilgrimage. A proportion of 50.0% (134/268) and 65.3% (175/268) of pilgrims declared using hand gel and washing their hands more often than usual during the Hajj, respectively.

Clinical features

Table 1 shows the prevalence of respiratory and gastrointestinal symptoms among pilgrims during the Hajj. A proportion of 86.9% (233/268) and 33.2% (89/268) of pilgrims presented at least one respiratory and at least one gastrointestinal symptom, respectively. A total of 81/268 (30.2%) pilgrims had both at least one respiratory and gastrointestinal symptom during their pilgrimage. Overall, 52.2% (140/268) of all pilgrims used antibiotics during the Hajj, with 42.2% using penicillins (including combination with beta-lactamase inhibitors), 4.8% a macrolide, 3.7% a quinolone, and 3.7% a cephalosporin (Table 1).

Table 1 Respiratory and gastrointestinal symptoms and antibiotic use among 268 pilgrims pre- and post-Hajj

Variables	n	%
Respiratory symptoms		
At least one respiratory symptom	233	86.9
Cough	211	78.7
Dyspnea	27	10.1
Voice failure	108	40.3
Sore throat	156	58.2
Rhinitis	156	58.2
ILI*	40	14.9
Gastrointestinal symptoms		
At least one gastrointestinal symptom	89	33.3
Diarrhea	46	17.2
Nausea	10	3.7
Vomiting	8	3.0
Constipation	45	16.8
Abdominal pain	21	7.8
Antibiotic intake overall among pilgrims during the Hajj		
At least one antibiotic	140	52.2
Antibiotic for respiratory symptoms	135	50.4
Antibiotic for gastrointestinal symptoms	9	3.4
Multiple antibiotic use	6	2.2
Antibiotic groups		
Penicillin	113	42.2
Cephalosporin	10	3.7
Macrolide	13	4.8
Quinolone	10	3.7
Metronidazole	1	0.4

*ILI: influenza-like illness

Detection of MDR bacteria by culture and antibiotic resistance-encoding genes by qPCR

Supplementary Fig. 1 and Table 2 detail the number of MDR bacteria strains isolated among 268 pilgrims before and after the 2017 and 2018 Hajj. Twenty-three (8.6%) pilgrims were carriers of at least one MDR bacteria pre-Hajj, compared with 52/268 (19.4%) post-Hajj ($p < 0.001$). All 52/268 (19.4%) pilgrims with positive post-Hajj samples acquired these MDR bacteria in KSA.

A total of 81 strains of MDR bacteria were isolated. *E. coli* and *K. pneumoniae* were significantly more frequent in post-

Table 2 MDR bacteria isolated from Hajj pilgrims

	Pre-Hajj		Post-Hajj		Acquisition		*p* value
	n	%	*n*	%	*n*	%	(pre-Hajj versus post-Hajj)
Pilgrims positive for MDR bacteria							
Pilgrims positive for at least one MDR bacteria at any anatomical site	23[1]	8.6	52[2]	19.4	52	19.4	*< 0.001*
Pilgrims positive at nasopharyngeal site for							
MRSA	5	1.9	5	1.9	4	1.5	1.0
ESBL-E	2	0.7	3	1.1	3	1.1	0.65
Pilgrims positive at rectal site for							
MRSA	2	0.7	0	0	0	0	0.16
CRAB	0	0	1	0.4	1	0.4	0.32
ESBL-E	16[3]	6.0	44[4]	16.4	44	16.4	*< 0.001*
Strains of MDR bacteria isolated at:							
Nasopharyngeal site							
E. aerogenes	2	0.7	1	0.4	1	0.4	0.56
K. pneumoniae	1	0.4	2	0.7	2	0.7	0.56
S. aureus	5	1.9	5	1.9	4	1.5	1.0
Rectal site							
E. coli	14	5.2	28	10.4	28	10.4	*< 0.01*
K. pneumoniae	2	0.7	17	6.3	17	6.3	*< 0.001*
E. cloacae	0	0	1	0.4	1	0.4	0.32
S. aureus	2	0.7	0	0	0	0	0.16
A. baumannii	0	0	1	0.4	1	0.4	0.32

Italic: statistically significant result

MDR bacteria multidrug-resistant bacteria, *MRSA* methicillin-resistant *Staphylococcus aureus*, *ESBL-E* extended spectrum beta-lactamase-producing *Enterobacteriaceae*, *CRAB* carbapenem-resistant *Acinetobacter baumannii*, *E. aerogenes Enterobacter aerogenes*, *K. pneumoniae Klebsiella pneumoniae*, *S. aureus Staphylococcus aureus*, *E. coli Escherichia coli*, *E. cloacae Enterobacter cloacae*, *C. koseri Citrobacter koseri*, *A. baumannii Acinetobacter baumannii*

[1] Three pilgrims were positive for two strains each

[2] One pilgrim was positive for three strains; one pilgrim was positive for two strains

[3] Two pilgrims were positive at both nasopharyngeal and rectal sites

[4] One pilgrim was positive at both nasopharyngeal and rectal site

Hajj than in pre-Hajj samples, with $p < 0.01$ and $p < 0.001$, respectively (Table 2). Most strains (65/81, 80.2%) were isolated in rectal samples.

Regarding antimicrobial resistance patterns and encoding genes from 81 strains, the only strain of CRAB that was isolated was resistant to imipenem, doripenem, piperacillin-tazobactam, fosfomycin, sulfamethoxazole-trimethoprim, ciprofloxacin, ticarcillin, ticarcillin-clavulanic acid, and tobramycin. This strain was positive for the bla_{Oxa23} gene.

Among 12 MRSA strains isolated, 8 (66.7%) were also resistant to fusidic acid and 1 (7.7%) to erythromycin. All MRSA strains were sensitive to the other tested antibiotics. All 12 strains (100%) were positive for *mecA*.

Supplementary Fig. 2 shows the antibiograms of the 68 ESBL-E strains. All strains were resistant to penicillin, and 57.8%, 57.3%, and 95.5% were resistant to ampicillin-clavulanic acid, cefepime, and ceftriaxone, respectively. Only 8 ESBL-E strains carried bla_{SHV} and/or bla_{TEM} alone. However, all of these strains were resistant bacteria, confirmed by antibiogram. The proportion of $bla_{CTX\text{-}A}$, bla_{SHV}, and bla_{TEM} in 22 *K. pneumoniae* strains was 95.4%, 86.3%, and 63.6%, respectively (supplementary Fig. 2).

Screening for colistin resistance genes

Overall, by qPCR, 27/268 rectal swabs were positive for colistin resistance genes (22 for the *mcr-1* gene and 5 for the *mcr-4* gene). None of the rectal swabs were positive for *mcr-2* (including the *mcr*-6 group), *mcr-3*, *mcr-5*, or *mcr-8* genes. All positive swabs were post-Hajj samples. No nasopharyngeal swab tested positive for any of the colistin resistance genes.

Of the 27 genes detected, 23 were successfully sequenced. The sequences obtained were successfully assembled and aligned with reference genes (19 for *mcr-1* gene and 4 for *mcr-4* gene). The prevalence of colistin resistance gene acquisition among the 268 French Hajj pilgrims was 8.6%.

Looking at the association between colistin resistance gene carriage and the presence of MDR bacteria in each individual, only 2 pilgrims positive for the *mcr-1* gene were positive for ESBL *E. coli* and *K. pneumoniae*. One pilgrim positive for the *mcr-4* gene was positive for ESBL *E. coli*.

Risk factors for acquisition of rectal MDR bacteria and colistin resistance genes

No factor was significantly associated with acquisition in univariate or multivariate analysis (supplementary Tables 1 and 2). Male gender tended to be associated with increased risk for acquisition of rectal ESBL-E (OR = 1.79, 95% CI [0.93–3.42], $p = 0.08$). Interestingly, overall antibiotic intake and association of antibiotic intake were associated with the acquisition of ESBL-E. But these associations were not statistically significant.

Frequent hand washing and use of disinfectant gel tended to be associated with decreased risk for acquisition of colistin resistance genes (OR = 0.45, 95% CI = [0.19–1.07], $p = 0.07$ and OR = 0.86, 95% CI [0.21–1.23], $p = 0.13$, respectively).

Discussion

Our results show that more than 8/10 French pilgrims had RTIs and one third had gastrointestinal symptoms during their stay in KSA. As a result, half of them took antibiotics during their trip, using mostly penicillin, and 19.4% of participants acquired at least one antibiotic-resistant bacterium during their stay in KSA. These were mostly ESBL-E, including *E. coli* and *K. pneumoniae* isolated in a rectal sample. We also observed an 8.6% acquisition rate of colistin resistance genes among pilgrims post-Hajj.

The present work confirms results obtained in French pilgrims in 2013 and 2014 with regard to acquisition of ESBL-E [6, 7], suggesting that circulation of such MDR bacteria at the Hajj is common. We found acquisition of one CRAB in one pilgrim only, in line with our previous study conducted during the 2014 Hajj season [8]. Finally, we found that only 1.5% pilgrims acquired MRSA. This result is in line with those obtained by others [27, 28].

In ESBL-E strains, the resistance rate to sulfamethoxazole-trimethoprim was 58.8% and that to ciprofloxacin was 20.6%. Several studies showed that ESBL-E acquired by travelers may be co-resistant to non-beta-lactam antibiotics, such as quinolone, sulfamethoxazole-trimethoprim, and doxycycline [29–31]. In a study by Kantele et al., the resistance rate of ESBL-E strains to these antibiotics was higher than in our cohort, with 73.0% and 53.0%, respectively. Co-resistance to non-beta-lactam antibiotics complicates the treatment of potential ESBL-E infections and adds to the toll of resistance at hospitals [32].

Our results clearly show an overuse of inappropriate antibiotics by French pilgrims regarding both their indication and the choice of antibiotic class. That may increase the risk of acquisition of MDR bacteria among pilgrims. In fact, in our study, overall antibiotic intake and association of antibiotic intake were associated with increased acquisition of ESBL-E, but this association was not statistically significant, probably due to the small sample size of the studied population. Hajj pilgrims come from over 180 countries, including countries where antibiotics are available and a medical prescription to dispense an antibiotic is not mandatory [4]. The availability of antibiotics over the counter is known to significantly contribute to increasing resistance [33]. Overuse of antibiotics has been thought to contribute to the emergence of MDR bacteria [34, 35]. In a cohort of 783 French pilgrims, overall, 47.6% took at least one antibiotic during their pilgrimage. Only 39.6% of pilgrims who used an antibiotic actually had an indication for it [4]. A review of 14 articles showed that the use of antibiotics for respiratory tract infections during the Hajj varied from 7 to 58.5%. In 9 studies, the antibiotic consumption rate was > 30% [4]. Nevertheless, our study showed that antibiotic use was not significantly associated with acquisition of MDR bacteria. In a previous work conducted in French pilgrims in 2013–2014, use of a β-lactam was associated with increased CTX-M gene acquisition in univariate analysis, but not in multivariate analysis, suggesting that other factors likely contribute to facilitating the acquisition of ESBL-E and related resistance genes by pilgrims [7].

Diarrhea was identified as a major risk factor for ESBL-E acquisition in other studies [36, 37], and in previous work conducted in French pilgrims in 2013–2014, diarrhea was an independent risk factor for CTX-M gene acquisition [7].

In our study, compliance with hand hygiene did not significantly influence MDR bacteria acquisition, in line with other studies conducted among international travelers [30, 31, 36, 38].

During the 2013 and 2014 Hajj, 9% of French pilgrims acquired *mcr-1* genes after their pilgrimage [39], which is similar to our results. The above suggest that circulation of *mcr-1* genes at the Hajj is not uncommon. Since the first description of an *mcr*-1 gene in a plasmid carried by *E. coli* isolated in China in April 2011 during routine surveillance of food animals [40], this gene has been reported from many different sources to occur worldwide in bacteria, including in foods, environments, animals, and humans [41]. The first isolation of this gene in humans was from *E. coli* in Latin America. A recent study showed that the *mcr*-1 gene has been reported in human isolates in 29 countries, in environmental samples in 4 countries, and in animals in 28 countries [42, 43]. Possible transmission of colistin-resistant bacteria between human and animals has been reported [44, 45].

In addition, we report here for the first time the presence of *mcr-4* genes at the Hajj. This gene was described for the first time in a sample obtained from the caecal contents of a pig slaughtered in Italy in 2013 and in *E. coli* strains collected during routine diagnosis of post-weaning diarrhea in pigs from Spain and Belgium in 2015 and 2016 [46]. To our knowledge, no report of *mcr-4* gene detection has been previously described in the KSA. Its presence at the Hajj may have resulted from its introduction by foreign pilgrims or acquired from a common food or water sources.

We did not identify any significant risk factors associated with acquisition of colistin resistance genes in this study, which may be due to the small sample size. Cienfuegos et al. conducted a case-control study, enrolling all inpatients infected with colistin- and carbapenem-resistant *K. pneumoniae* between June 2012 and June 2014 in 4 tertiary care centers in Medellin-Colombia [47]. The authors showed that factors significantly associated with colistin-resistant *K. pneumoniae* were infection by CG258 strains (OR = 17.57) and previous use of colistin (OR = 7.21) [47].

The entire group of French pilgrims traveled together, participated in the rituals together at the same time and locations, shared accommodations in the same hotels and tent camps, and consumed food from the same restaurants [16]. We found no association between acquisition of colistin resistance genes and acquisition of MDR bacteria in our study. This suggests that the sources of contamination were different. MDR bacteria and colistin resistance genes detected in pilgrims may come from multiple sources, including direct and indirect transmission. Inter-human transmission of pathogens, including MDR bacteria, is promoted by the spatial and temporal concentration of people. Social distancing and contact avoidance are difficult measures to implement in the context of mass gatherings such as the Hajj. DNA from gram-negative bacteria was identified at high rates on surfaces in the pilgrim environment, suggesting a potential environmental source of contamination for humans [48]. Indeed, environmental bacteria, especially from water sources, appear to be the main reservoir and source of the colistin-resistant bacteria [49].

Our work has some limitations. The detection of colistin resistance genes directly from samples did not allow identification of the bacteria carrying the colistin resistance genes. Using PCR to detect colistin resistance genes does not distinguish between a dead and living micro-organism. For ESBL-E isolates, we identified only the most frequent resistance-encoding genes which do not allow identifying the sequence types of bacteria. In addition, information on travel to highly antimicrobial-resistant endemic regions in the 6–12 months before their pilgrimage was not investigated. Furthermore, rectal auto-sampling was used for ease of patient acceptability, but it could have influenced the results. Finally, this work was conducted among 268 French pilgrims only and cannot be generalized to all pilgrims. But our study evidenced a significant acquisition of MDR at the Hajj, notably ESBL-E co-resistant to non-beta-lactam antibiotics. These MDR bacteria may spread in the community when pilgrims return to their home countries. Given the results of our study, screening stool samples from larger cohorts of pilgrims is warranted, since more than 10 million pilgrims worldwide embark to the KSA for the pilgrimage each year [1]. Individual preventive measures against infectious disease are recommended during the Hajj, but their effectiveness remains uncertain [50]. Provision of safe water and food supplies with rigorous quality control is likely the best way to limit transmission of MDR bacteria in the context of the Hajj. Pilgrims should be advised against bringing antibiotics from the home country. Rational use of antibiotics should be promoted among health workers, including pilgrim medical missions.

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Data availability The datasets generated during and/or analyzed during the current study are available from corresponding author [P.G] on reasonable request.

Compliance with ethical standards

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

Ethical approval The protocol was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments and approved by the Aix-Marseille University institutional review board (July 23, 2013; reference No. 2013-A00961-44).

Consent to participate Written informed consent was obtained from all individual participants included in the study.

Consent to publish NA.

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Supplementary data

Acquisition of multi-drug resistant bacteria and encoding genes among French pilgrims during the 2017 and 2018 Hajj

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Supplementary table 1: Univariable analysis of risk factors for acquisition of rectal ESBL-E

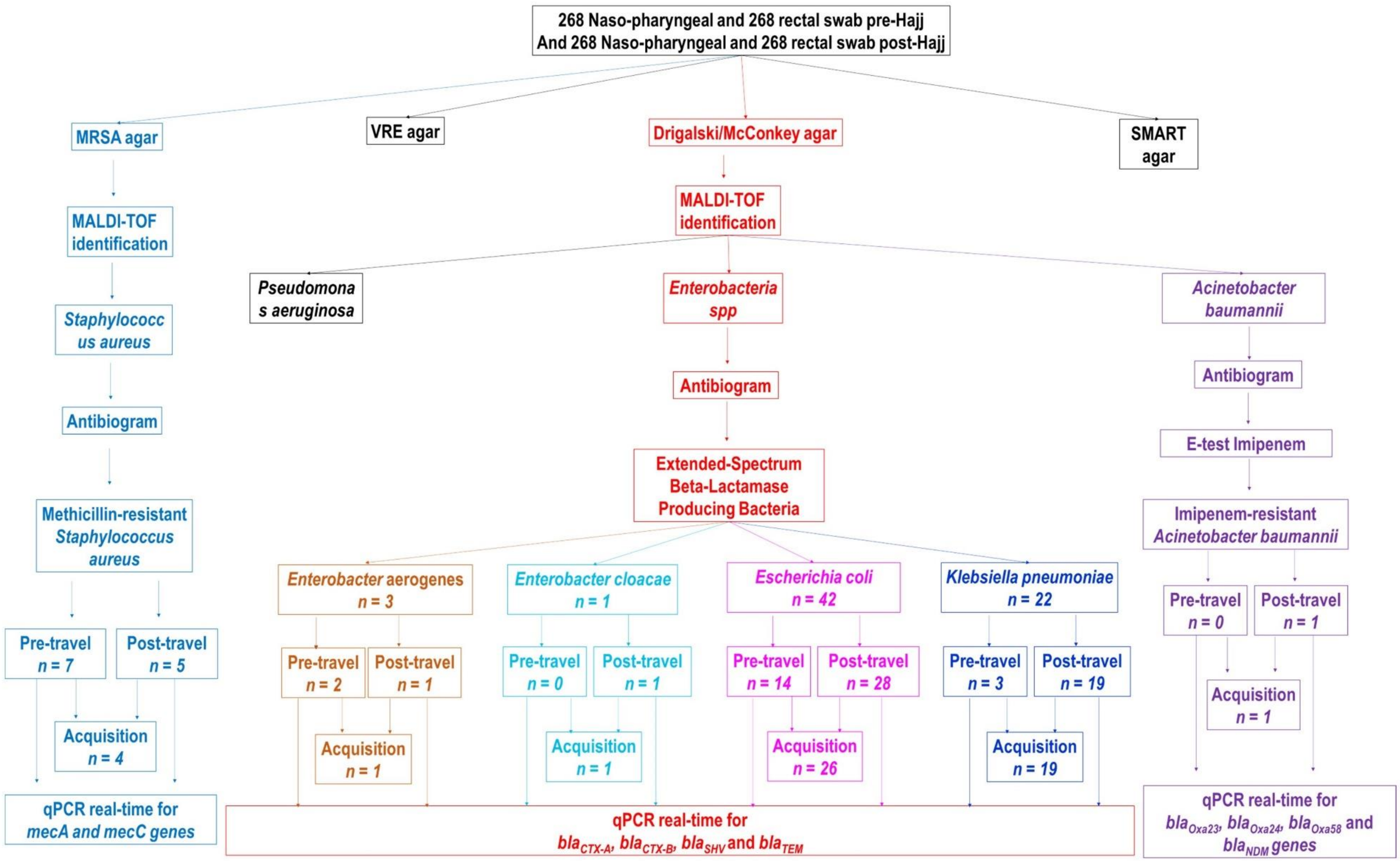
	Acquisition of rectal ESBL-E				
	n	**%**	**OR**	**[95%CI]**	**p-value**
Year					
2018	17	14.0	ref	ref	ref
2017	27	18.4	1.38	0.71 – 2.67	0.34
Gender					
Female	20	13.0	ref	ref	ref
Male	24	21.0	1.79	0.93 – 3.42	0.08
Age					
< 60 years	23	20.4	ref	ref	ref
≥ 60 years	21	13.6	0.61	0.32 – 1.17	0.14
Chronic comorbidities					
Diabetes					
No	34	17.2	0.80	0.37 – 1.73	0.58
Yes	10	14.3			
Hypertension					
No	30	16.1	1.07	0.53 – 2.14	0.85
Yes	14	17.1			
Chronic respiratory disease					
No	37	15.8	1.38	0.56 – 3.40	0.48
Yes	7	20.6			
Chronic heart disease					
No	39	15.9	1.47	0.51 – 4.19	0.47
Yes	5	21.7			
Body mass index					
Normal	11	15.9	ref	ref	ref
Overweight	22	17.3	1.10	0.50 – 2.43	0.80
Obese	11	15.3	0.85	0.38 – 2.46	0.91
Individual preventive measures					
Hand hygiene					
As usual	12	12.9	1.51	0.74 – 3.09	0.26
More frequent	32	18.3			
Disinfectant gel					
No	23	17.2	0.90	0.47 – 1.71	0.74
Yes	21	15.7			
Gastrointestinal symptoms					
No	28	15.7	1.17	0.60 – 2.30	0.64
Yes	16	18.0			
Diarrhea					
No	37	16.7	0.90	0.37 – 2.16	0.81
Yes	7	15.2			
Antibiotic intake					
Overall antibiotic intake					

No	20	15.6	1.12	0.58 – 2.14	0.74
Yes	24	17.1			
Combined antibiotic intake					
No	42	16.0	2.62	0.46 – 14.76	0.48
Yes	2	33.3			
Penicillin					
No	27	17.4	0.84	0.43 – 1.63	0.60
Yes	17	15.0			
Cephalosporin					
No	41	15.9	2.27	0.56 – 9.13	0.25
Yes	3	30.0			
Macrolide					
No	41	16.1	1.56	0.41 – 5.94	0.51
Yes	3	23.1			
Quinolone					
No	41	15.9	2.27	0.56 – 9.13	0.25
Yes	3	30.0			
Imidazole					
No	43	16.1	NA	NA	0.16
Yes	1	100			

Supplementary table 2: Univariable analysis of risk factors for acquisition of colistin-resistance gene

	Acquisition of colistin-resistance genes				
	n	**%**	**OR**	**[95%CI]**	**p-value**
Gender					
Female	13	8.4	ref	ref	ref
Male	10	8.8	1.04	0.44 – 2.47	0.92
Age					
< 60 years	15	9.7	ref	ref	ref
≥ 60 years	8	7.1	0.46	0.57 – 3.44	0.46
Chronic comorbidities					
Diabetes					
No	17	8.6	1.00	0.38 – 2.64	0.10
Yes	6	8.6			
Hypertension					
No	15	8.1	1.23	0.50 – 3.03	0.65
Yes	8	9.8			
Chronic respiratory disease					
No	21	9.0	0.63	0.14 – 2.83	0.55
Yes	2	5.9			
Chronic heart disease					
No	20	8.2	1.69	0.46 – 6.17	0.43
Yes	3	13.0			
Body mass index					
Normal	8	11.6	ref	ref	ref
Overweight	10	7.9	0.65	0.24 – 1.74	0.39
Obese	5	6.9	0.57	0.18 – 1.83	0.34
Hand washing					
As usual	12	12.9	ref	ref	ref
More frequent	11	6.3	0.45	0.19 – 1.07	0.07
Disinfectant gel					
No	15	11.2	0.50	0.21 – 1.23	0.13
Yes	8	6.0			
Gastrointestinal symptoms					
No	16	9.0	0.86	0.34 – 2.18	0.76
Yes	7	7.9			
Diarrhea					
No	19	8.6	1.02	0.33 – 3.14	0.98
Yes	4	8.7			
Overall antibiotic intake (for respiratory tract infections and/or gastrointestinal infections)					
No	11	8.6	1.00	0.42 – 2.35	1.00
Yes	12	8.6			
Combined antibiotic intake					
No	22	8.4	2.18	0.24 – 19.51	0.48
Yes	1	16.7			

Supplementary figure 1: Study results (no sample was positive by culture in VRE, SMART agar and for *Pseudomonas aeruginosa)*



Supplementary figure 2: Antimicrobial resistance pattern and encoding-gene of ESBL strains

Red: resistance, yellow: intermediate, green: sensitive, white: not tested, vertical dash: natural resistance, *E. cloacae: Enterobacter cloacae*, *K. pneumoniae: Klebsiella pneumoniae, E. coli: Escherichia coli*, AMX: amoxicillin, AMC: amoxicillin-clavulanic acid, TAZ: piperacillin-tazobactam, CEF: cefepime, CTX: ceftriaxone, FOM: fosfomycin, IMP: imipenem, ETP: ertapenem, AMK: amikacin, GTM: gentamicin, DOX: doxycycline, STX: sulfamethoxazole-trimethoprim and CIP: ciprofloxacin

Identification	Time sampling	Acquisition	Origine	Germe	AMX	AMC	TAZ	CEF	CTX	FOM	IMP	ETP	AMK	GTM	DOX	STX	CIP	Genes
1	Post-Hajj	Yes	Rectal	*E. cloacae*														SHV
2	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
3	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
4	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
5	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV
6	Post-Hajj	Yes	Rectal	*E. coli*														SHV, TEM
7	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, TEM
8	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, TEM
9	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV
10	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
11	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
12	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
13	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
14	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, SHV
15	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
16	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
17	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
18	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
19	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
20	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A
21	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
22	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
23	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
24	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
25	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV
26	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV
27	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
28	Pre-Hajj	No	Rectal	*E. coli*														TEM
	Post-Hajj	Yes	Rectal	*E. coli*														TEM
29	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
30	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
31	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A
32	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV
33	Post-Hajj	Yes	Rectal	*E. coli*														TEM
34	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
35	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
36	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
37	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A
38	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
39	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A
40	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
41	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
42	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
43	Post-Hajj	Yes	Rectal	*E. coli*														CTX-A, TEM
44	Pre-Hajj	No	Nasopharyngeal	*K. pneumoniae*														CTX-A, SHV, TEM
	Post-Hajj	Yes	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
45	Pre-Hajj	No	Rectal	*K. pneumoniae*														CTX-A, SHV, TEM
	Post-Hajj	Yes	Nasopharyngeal	*K. pneumoniae*														CTX-A, SHV, TEM
46	Post-Hajj	Yes	Nasopharyngeal	*E. aerogenes*														CTX-A
47	Post-Hajj	Yes	Nasopharyngeal	*K. pneumoniae*														SHV
48	Pre-Hajj	No	Rectal	*E. coli*														CTX-A, TEM
49	Pre-Hajj	No	Rectal	*E. coli*														CTX-A, TEM
50	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
51	Pre-Hajj	No	Rectal	*E. coli*														SHV, TEM
52	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
53	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
54	Pre-Hajj	No	Rectal	*E. coli*														CTX-A, TEM
55	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
56	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
57	Pre-Hajj	No	Rectal	*K. pneumoniae*														CTX-A, SHV
58	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
59	Pre-Hajj	No	Rectal	*E. coli*														CTX-A
60	Pre-Hajj	No	Nasopharyngeal	*E. aerogenes*														CTX-A
	Pre-Hajj	No	Nasopharyngeal	*E. aerogenes*														TEM

<u>Partie 4 :</u>

Epidémiologie des infections respiratoires et gastrointestinales chez les pèlerins du Grand Magal de Touba

Préambule

En 2017, nous avons conduit une étude prospective sur les pèlerins du Grand Magal de Touba, au Sénégal, avec 110 participants provenant de deux villages du sud du pays (article 14). La durée du séjour à Touba a été courte (3 jours). La plupart des participants (61,8%) provenaient de groupes familiaux de huit concessions principales différentes, avec quatre personnes ou plus par concession participant au Magal. L'âge médian était de 20 ans (min = huit mois, max = 75 ans, interquartile [15- 31 ans]). Vingt-neuf (26,4%) participants étaient des enfants de moins de 15 ans et cinq (4,5%) avaient plus de 60 ans. La maladie respiratoire chronique était la comorbidité la plus fréquente (8,2%).

Au cours de la période d'étude, 46 (41,8%) pèlerins ont signalé au moins un symptôme respiratoire, la plupart des individus présentant des symptômes au cours de leur séjour à Touba (20,0%) ou peu après leur retour (10,9%). Seuls sept participants (6,4%) ont déclaré souffrir de symptômes respiratoires avant de se rendre à Touba. Le délai médian entre l'arrivée à Touba et le début des symptômes était de 3 jours. Seulement 2,7% ont reçu des antibiotiques pour une infection des voies respiratoires. Au total, 42/46 (91,3%) personnes étaient encore symptomatiques à leur retour.

Des écouvillons nasopharyngés pré- et post-Magal ont été collectés. La prévalence des rhinovirus, des coronavirus et des adénovirus a augmenté après le Magal avec l'acquisition de 13,0, 16,7 et 4,6% respectivement. Le portage de bactéries était élevé, variant de 34,5% pour *S. pneumoniae* à 77,3% pour *H. influenzae*. Le portage de *S. pneumoniae*, *S. aureus* et *K. pneumoniae* a diminué à la suite de la participation au Magal, tandis que celui d'*H. influenzae* a augmenté de manière significative. Malgré la diminution globale du portage bactérien, l'acquisition de bactéries variait de 3,7% pour *S. pneumoniae* à 26,9% pour *H. influenzae*. Nous n'avons trouvé aucune différence significative dans la prévalence des symptômes respiratoires et dans l'acquisition d'agents pathogènes respiratoires selon la concession et l'emplacement du logement à Touba.

Au total, 16 (14,6%) pèlerins ont signalé des symptômes gastro-intestinaux, principalement pendant leur séjour à Touba (7,3%) ou peu après leur retour (3,6%). Seuls quatre participants (3,6%) ont déclaré souffrir de symptômes gastro-intestinaux avant de se rendre à Touba. Seulement 2/110 (1,8%) ont reçu un antibiotique pour des symptômes gastro-intestinaux. Au total, 7/16 (43,8%) des pèlerins étaient encore symptomatiques à leur retour dans les villages.

Soixante (54,5%) participants ont fourni un échantillon de selles avant et 69 (62,7%) après Magal, avec 45 (40,9%) échantillons appariés collectés. Les virus les plus courants étaient l'adénovirus (9,5%) et le norovirus (2,4%). Une proportion de 10,7% des individus ont été testés positifs pour *G. lamblia*. Le portage de bactéries était élevé, de 1,2% pour *C. jejuni* à 78,6% pour EAEC. Il convient de noter que 8,3% de tous les participants étaient positifs pour *T. whipplei*. L'acquisition de virus et de parasites gastro-intestinaux était faible, tandis que l'acquisition bactérienne variait de 2,2% pour *C. jejuni* à 33,3% pour EPEC.

Article 14 :

Respiratory and gastrointestinal infections at the 2017 Grand Magal de Touba, Senegal: A prospective cohort survey.

Hoang VT, Goumballa N, Dao TL, Ly TDA, Ninove L, Ranque S, Raoult D, Parola P, Sokhna C, Pommier de Santi V, Gautret P.

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Respiratory and gastrointestinal infections at the 2017 Grand Magal de Touba, Senegal: A prospective cohort survey

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A R T I C L E I N F O

Keywords:
Respiratory tract infections
Gastrointestinal infection
PCR
Grand magal
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Pilgrims

A B S T R A C T

Background: The Grand Magal of Touba is the largest Muslim pilgrimage in Senegal with a potential for infectious disease transmission.
Methods: Clinical follow-up, adherence to preventive measures and qPCR-based respiratory and gastrointestinal pathogens carriage pre- and post-Magal, were assessed.
Results: 110 pilgrims from South Senegal were included. The duration of stay in Touba was 3 days. 41.8% and 14.5% pilgrims reported respiratory and gastrointestinal symptoms. Most individuals having the onset of symptoms during their stay in Touba, or soon after returning. The acquisition of rhinoviruses, coronaviruses and adenovirus was 13.0, 16.7 and 4.6% respectively and that of *Streptococcus pneumoniae* and *Haemophilus influenzae* was 3.7% and 26.9%.

Acquisition of gastrointestinal viruses and parasites was low, while bacterial acquisition ranged from 2.2% for *Campylobacter jejuni* to 33.0% for enteropathogenic *Escherichia coli*.
Conclusion: This preliminary study confirms that Grand Magal pilgrims are likely to be exposed to communicable disease risk as observed in other pilgrimage settings. Further study including larger numbers of pilgrims are needed to investigate potential risk factors for respiratory and gastrointestinal infections at the Grand Magal.

1. Introduction

The Grand Magal is at once a religious ceremony and a festival. About 4–5 million Mourid participate to the celebration each year, coming from across Senegal and the surrounding countries, as well as from countries outside Africa, making this event the largest Muslim religious mass gathering in West-Africa. The two central events of the Magal are visits to the Great Mosque of Touba, which involves a partial circumambulation of the mosque, and to the mausoleum of Cheikh Ahmadou Bamba. Pilgrims also visit the mausoleums of several other important Mouride leaders who were descendants of the Cheikh. The Mosque area is a secluded place surrounded by walls, within the city. The access to the Mosque area is allowed to everyone (including tourists) but flows are regulated by security staffs. In addition, pilgrims visit places in Touba that are associated with the holy life of the Cheikh, including the "Well of Mercy," a spring which sacred water is reputed to be able to heal all sorts of illnesses and misfortune. A visit to the central library of Touba which contains the writings of the Cheikh and other influential Mouride theologians, and to the Mouride University are among the other places visited by pilgrims. Finally, pilgrims visit their personal Mouride spiritual guides, or Marabouts who receive their followers in their personal residence in the city. Recitation of the Koran and spiritual introspection complete these religious activities. During the Grand Magal period, in addition to the religious celebrations, intense cultural activity takes place including conferences, seminaries, and debates between representatives of various Muslims communities

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from Senegal and from the diaspora. Recitation of poems by Ahmadou Bamba, and collective hymn-singing sessions are organized at night, in the streets, under large open tents. Informally, pilgrims also visit Touba's large temporary marketplace, one of the biggest in the country where a wide range of products can be found with prices lower than those of any other market in Senegal.

During this period, pilgrims are accommodated in private housing structures since there are no hotels in Touba. These houses can be residences of local inhabitants, where family members living in different parts of Senegal or who have emigrated outside the country, come together during the Magal period, or Marabout houses with a capacity that can be up to hundreds of individuals. Many pilgrims sleep on carpets, on the floor, into the houses or on outside terraces.

Food is prepared collectively by family members or by Marabout followers. Slaughtering of animals is mostly done in the streets, in front of housing structures. Different community associations of Mouride disciples (dahiras) are responsible for feeding the pilgrims through free provision of food in the streets and along the roads to Touba. The Senegalese government also provides some technical support.

Medical care is provided free of charge during the Grand Magal. Most of the available local public medical infrastructures are involved in the medical preparation, surveillance and response during the event [1]. As an example, three tertiary care and four secondary care hospitals (total bed capacity of 481) and about 300 primary healthcare centers and 50 private structures in Touba and surrounding cities were mobilized in 2015. The vast majority of the medical staffs taking care of patients during the Grand Magal are nurses, midwives and volunteer community health agents. Overall, 78 medical doctors were mobilized during the Grand Magal 2015, many of them coming from Dakar to temporarily reinforce the local staff.

Based on the registers of consultations carried out in the public health structures of the region, we evaluated the number and the profile of morbidity observed during the pilgrimage of 2015 among 32,229 in and out-patients [1]. The most frequent complaints were fatigue (12%) followed by trauma (11%) and heatstroke (11%). Infectious diseases have also been highly reported, including febrile systemic illnesses (5%) and rapid-test confirmed malaria (3%), diarrheal diseases (5%), respiratory tract infection symptoms (5%) and ear, nose and throat infection symptoms (5%). Microbiological investigation of these infections was not conducted in most cases.

The objective of the present preliminary descriptive study was to evaluate the prevalence of respiratory and gastrointestinal symptoms and the carriage of pathogens commonly associated with these symptoms among pilgrims before and after their stay in Touba.

2. Materials and methods

The complementary Fig. 1 details the procedure of this study.

2.1. Study population

A convenience sample of pilgrims participating in the Grand Magal 2017 was surveyed by a longitudinal prospective cohort study that was conducted from November 4th to November 23rd, 2017. Inhabitants of two villages: Dielmo (13°43′ 22.07″ N, 16°24′40.09″ W) and Ndiop (13°41′08.01"N, 16°23′01.01″ W), located in Fatick region, South Senegal were included. Villages are divided in concessions where individuals from a same family live in close contact. The population in the villages was 991 (510 Ndiop and 481 Dielmo) in 2017, including about 50% Mourides, part of whom did not travel to Touba because of limited financial resources. All pilgrims participating to the 2017 Magal in these villages were invited to participate in our study and were identified by the nurses in charge of the primary health care centers in both villages. At inclusion, the pilgrims were questioned using a standardized questionnaire. This questionnaire addressed demographics, chronic medical conditions and vaccination status against influenza. In Touba, pilgrims were housed in different concrete houses; three groups in three family houses and others in different Marabout houses. Health issues occurring during and after the Magal, were recorded by a nurse who travelled with the group of pilgrims. Housing location in Touba and use of preventive measures (face mask, disposable handkerchiefs, hand washing with soap hand disinfectant) were investigated by questionnaires during the stay in Touba. The consumption of antibiotics was also documented through post-travel questionnaires. Influenza like illness (ILI) was defined as the association of cough, sore throat and fever [2]. Diarrhea was defined by at least three loose or liquid stools per day.

2.2. Sample collection

The procedure included systematic throat, nasal and stool swabs, 1–3 days before departing from the villages (pre-Magal specimens) and 3–6 days following return (post-Magal specimens). Respiratory samples were collected with commercial rigid cotton-tipped swab applicators (Medical Wire & Equipment, Wiltshire, UK) that were inserted in the anterior nose or in the oro-pharynx, and then placed in viral transport media (Sigma Virocult®). Participants provided stools samples in sterile containers. Investigators secondary collected small amounts of stools using swab applicators, placed in viral transport media (Sigma Virocult®). This standard procedure was previously explained to the pilgrims by the investigators. Swabs were kept at 4 °C before being transported to the Dakar laboratory for storage in a −80 °C freezer within 48 h of collection and subsequently transferred to Marseille on dry ice for being processed.

2.3. Identification of respiratory and gastrointestinal pathogens

2.3.1. Respiratory specimens

The EZ1 Advanced XL (Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) was used for the DNA and RNA extraction from the respiratory samples according to the recommendation of the manufacturer. All quantitative real-time PCR were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA). Positive results of bacteria or virus amplification were defined as those with a cycle threshold (CT) value ≤ 35. Negative control (PCR mix) and positive control (DNA from bacterial strain or RNA from viral strain) were included in each run.

2.3.2. Identification of respiratory virus

Human coronavirus (HCoV) and human para-influenza virus (HPIV) were detected by one-step duplex quantitative RT-PCR amplifications of HCoV/HPIV-R Gene Kit (REF: 71-045, Biomérieux, Marcy l'Etoile, France), according to the manufacturer's recommendations. The Multiplex RNA Virus Master Kit (Roche Diagnostics, France) was used to detect influenza A, influenza B, human rhinovirus, human enterovirus, metapneumovirus, respiratory syncytial virus, adenovirus and internal controls MS2 phage by one-step simplex real-time quantitative RT-PCR amplifications [3].

2.3.3. Identification of respiratory bacteria

Real-time PCR amplifications were carried out by using LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. The SDD gene of *Haemophilus influenzae*, phoE gene of *Klebsiella pneumoniae*, nucA gene of *Staphylococcus aureus*, lytA gene of *Streptococcus pneumoniae* [4], P1 gene of *Mycoplasma pneumoniae* [5], ctrA gene of *Neisseria meningitidis* [6] and Toxin gene of *Bordetella pertussis* [7] were amplified with internal DNA extraction controls T4, as previously described.

2.3.4. Gastrointestinal specimens

Stool swabs were centrifuged at 4000 × g for 10 min. The RNA were extracted from 200 μL clear supernatant using the EZ1 Advanced XL

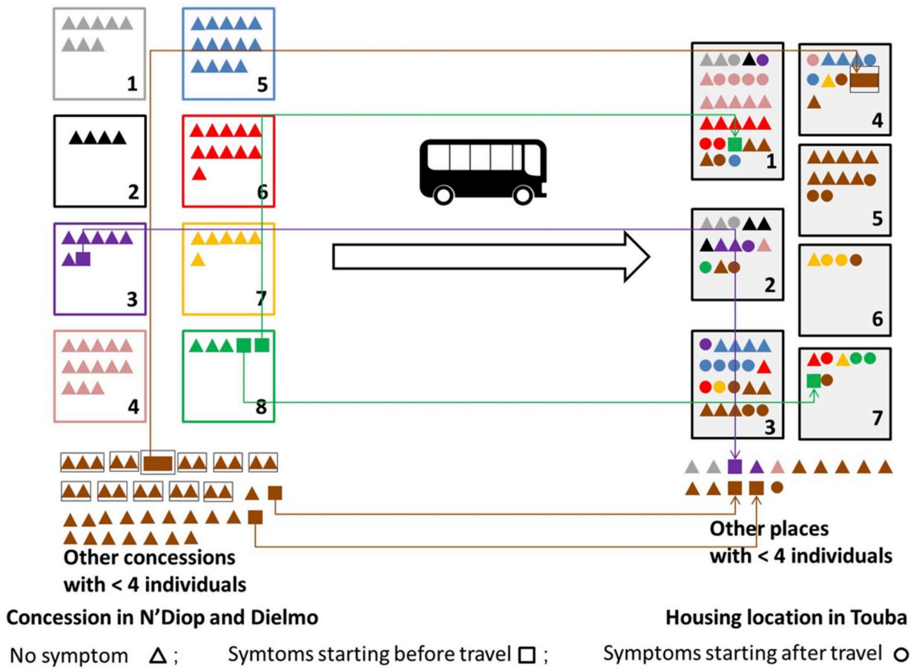

Fig. 1. Distribution of pilgrims according to concession in villages and to housing location in Touba.

(Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendation.

The DNA was extracted by semi-automated extraction. 200 µL of rectal swabs was added to 350 µL of G2 lysis buffer (Qiagen) and glass powder in a tube, then disrupted in a FastPrep BIO 101 apparatus (Qbiogene, Strasbourg, France) at maximum power for 40s. The incubation was at 100 °C for 10 min to allow for complete lysis. Then, the tubes were centrifuged at 10,000 g for 1 min. Subsequently, 200 µL of supernatant was collected in other tubes and enzymatically digested using 20 µL of proteinase K (20 mg/mL, Qiagen), and incubated overnight at 56 °C. The automated procedure using the EZ1 Advanced XL (Qiagen, Hilden, German) with the DNA Tissue Kit (Qiagen) according to the manufacturer's recommendation [8].

2.3.5. *Identification of gastrointestinal virus*

The Multiplex RNA Virus Master Kit (Roche Diagnosis, France) was used for detecting hepatitis A and E virus, adenovirus, rotavirus, norovirus, astrovirus and internal controls MS2 phage by one-step simplex real-time quantitative RT-PCR amplifications [9–11].

2.3.6. *Identification of gastrointestinal bacteria and parasites*

Real-time PCR amplifications were carried out using the LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. The mapA gene of *Campylobacter jejuni*, ipaH gene of *Shigella spp*/EIEC *(enteroinvasive Escherichia coli)* [12], gene invA of *Salmonella spp*, gene stx1 and stx2 of enterohemorrhagic *E. coli,* EAF and eae gene of enteropathogenic *E. coli* (EPEC) and pCVD432 gene of enteroaggregative *E. coli* (EAEC) [13] were amplified with internal DNA extraction controls TISS. The 18S gene was used to detect *Entamoeba histolytica* and *Giardia lamblia*. Finally, gene Hsp70 was amplified to test *Cryptosporidium spp.* [8].

All quantitative real-time PCR to detect respiratory and gastrointestinal pathogens were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA). Negative control (PCR mix) and positive control (DNA from bacterial strain or RNA from viral strain) were included in each run. A cycle threshold (CT) value ≤ 35 was used to assess positive results of bacteria or virus amplification.

2.4. *Statistical analysis*

Pearson's chi-square or Fisher's exact tests were used for statistical analyses. The acquisition of respiratory and gastrointestinal pathogens was defined as negative before travel and positive when returning from Touba. Prevalence of respiratory and gastrointestinal pathogens carriage before and after the Grand Magal, were compared using the McNemar's Test. Percentages and comparisons were carried out using STATA 11.1 (Copyright 2009 StataCorp LP, http://www.stata.com). A p value of ≤ 0.05 was considered significant.

2.5. *Ethics*

Pilgrims were invited to participate on a voluntary basis. Participants (or their parents when minors) were asked to sign a written consent form. The protocol was approved by the National Ethic Committee for Health Research in Senegal (SEN17/62). It was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments.

3. Results

3.1. *Study population*

A total of 123 pilgrims agreed to participate to the survey. Among them, 110 participated to the Grand Magal and fully completed the study and 13 participants cancelled their trip to Touba after inclusion in the study. Most participants (61.8%) were from family groups from 8 different main concessions including 4 or more persons per concession participating to the Magal, while 38.2% remaining participants travelled in small groups from a same concession with 3 persons (2.7%) or 2 persons (18.2%) or alone (17.3%) (Fig. 1). Forty-eight pilgrims were male (43.6%) and 62 female (56.4%) with a gender ratio of 0.77. The median age was 20 years (min = 8 months, max = 75 years, interquartile (15, 31 years)). Twenty-nine (26.4%) participants were children < 15 years and 5 (4.5%) pilgrims were over 60 years of age. Most participants were from N'Diop village (90/110, 81.8%). Chronic respiratory disease was the most common comorbidity (8.2%) (Table 1).

The duration of the stay in Touba was short (3 days) and most of

Table 1
Characteristics of the population.

Variables		n (N = 110)	%
Village	Dielmo	20	18.2
	Ndiop	90	81.8
Concession in village	1	8	7.3
	2	4	3.6
	3	7	6.4
	4	13	11.8
	5	14	12.7
	6	11	10.0
	7	6	5.5
	8	5	4.5
	Others[a]	42	38.2
Median duration of stay in Touba (interquartile) (days)		3 (3, 4)	
Gender	Female	62	56.4
	Male	48	43.6
Age	Median age (interquartile) (years)	20 (15, 31)	
	Age ≤ 15 years old	29	26.4
	Age > 15–45 years old	67	60.9
	Age > 45–60 years old	9	8.2
	Age > 60 years old	5	4.5
Chronic diseases	Diabetes mellitus	1	0.9
	Hypertension	2	1.8
	Chronic respiratory disease	9	8.2
	Chronic heart disease	2	1.8
	Others	14	12.7
Housing location in Touba	1	28	25.5
	2	13	11.8
	3	20	18.2
	4	11	10.0
	5	12	10.9
	6	4	3.6
	7	7	6.4
	Others[a]	15	13.6

[a] Less than 4 individuals per concession in villages or housing location in Touba.

pilgrims (86.4%) were housed in 7 different locations with some overlapping between concessions (Fig. 1).

Regarding preventive measures, 46.4% of pilgrims reported washing their hands more often than usual during the Grand Magal, 63.6% used hand gel frequently. 32.3% and 2.8% pilgrims reported using frequently disposable handkerchiefs and face mask, respectively during the pilgrimage. Only one pilgrim was vaccinated against influenza (Table 2).

3.2. Clinical features and detection of respiratory and gastrointestinal pathogens

3.2.1. Respiratory symptoms and pathogens

During the study period 46 (41.8%) pilgrims reported at least one respiratory symptom with most individuals having the onset of symptoms during their stay in Touba (20.0%) or soon after returning (10.9%). Only 7 participants (6.4%) reported suffering from respiratory symptoms before traveling to Touba. Median time between arrival in Touba and onset of symptoms was 3 days. Rhinitis was the most frequent symptom (30.0%), followed by cough (24.6%), sore throat (10.0%), expectoration (4.6%) and voice failure (4.6%). However, 10.0% ill pilgrims reported fever and 1.8% ILI. Only 2.7% received antibiotics for respiratory tract infection and none of the participants was hospitalized. A total of 42/46 (91.3%) individuals were still symptomatic on returning to Ndiop and Dielmo.

Pre- and post-Magal nasopharyngeal swabs were collected from all 110 and 108 (98.2%) participants, respectively. The most common virus detected was human rhinovirus (18.2%) and human coronavirus (18.2%), followed by adenovirus (7.3%) and influenza B virus (2.7%) (Table 4). The global carriage of bacteria was high, ranging from 34.5% for *S. pneumoniae* to 77.3% for *H. influenzae*. The prevalence of rhinoviruses, coronavirus and adenovirus increased post-Magal with acquisition of 13.0, 16.7 and 4.6% respectively. This increase was statistically significant for coronaviruses, $p = 0.0001$. Carriage of *S. pneumoniae, S. aureus* and *K. pneumoniae* decreased following a participation to the Magal, while that of *H. influenzae* significantly increased, $p = 0.03$. Despite the overall decrease in bacterial carriage, the acquisition of bacteria ranged from 3.7% for *S. pneumoniae* to 26.9% for *H. influenzae* (Table 3). We found no significant difference in the prevalence of respiratory symptoms and in the acquisition of respiratory pathogens according to concession and housing location in Touba (Supplementary Tables S1 and S2).

3.2.2. Gastrointestinal symptoms and pathogens

At total of 16 (14.6%) pilgrims reported gastrointestinal symptoms mostly during their stay in Touba (7.3%) or soon after returning (3.6%). Only 4 participants (3.6%) reported suffering gastrointestinal symptoms before traveling to Touba. Vomiting was the most frequent symptoms (9.1%) followed by diarrhea (4.6%), constipation (1.8%) and nausea (0.9%). None of participants was hospitalized and only 2/110 (1.8%) received antibiotic for gastrointestinal symptoms. A total of 7/16 (43.8%) of the pilgrims were still symptomatic upon their return to the villages.

Sixty (54.5%) participants provided stool sample pre- and 69 (62.7%) post-Magal, with 45 (40.9%) paired specimens collected. Most common viruses were adenovirus (9.5%) and norovirus (2.4%). 10.7% individuals tested positive for *G. lamblia*. The global carriage of bacteria was high ranging from 1.2% for *C. jejuni* to 78.6% for EAEC. Of note, 8.3% of all participants were positive for *T. whipplei*. Acquisition of gastrointestinal viruses and parasites was low, while bacterial acquisition ranged from 2.2% for *C. jejuni* to 33.3% for EPEC (Table 4).

Table 2
Prevalence of adherence with preventive measures during the stay in Touba.

Preventives measures		n (N = 110)	%
Influenza vaccination		1	0.9
Face mask	No	104	94.6
	Sometimes	3	2.7
	Often	3	2.7
Hand washing	As usual	59	53.6
	Much more	51	46.4
Disinfectant gel	No	10	9.1
	Sometimes	30	27.3
	Often	70	63.6
Disposable handkerchiefs	No	44	40.0
	Sometimes	30	27.3
	Often	36	32.7

4. Discussion

Religious mass gatherings like the Hajj pilgrimage in Mecca, Saudi Arabia have been associated with outbreaks of gastrointestinal infections, respiratory infections and meningitis [14], mostly due to crowding conditions favoring the inter-human transmission of pathogens. The Grand Magal pilgrimage in Touba Senegal qualifies for the WHO mass gathering definition [15]. This is the first report addressing the morbidity in a cohort of pilgrims at the Grand Magal. Publications about Grand Magal associated diseases are scarce. A cholera outbreak involving 12,135 patients in the Grand Magal area occurred in 2004–2006, with a total of 29,556 cases reported over an 18-month in the country [16]. In 2018, a dengue outbreak was associated to the Grand Magal [17,18]. As described in the introduction, our group provided overall figures of syndromic surveillance data during the 2015 Grand Magal. The most frequent reasons for consultation were trauma, followed by fatigue and heatstroke. Infectious diseases were also prevalent with, notably, a high rate of febrile systemic illnesses and malaria, diarrheal diseases, and respiratory tract infections [1].

Table 3
Prevalence of respiratory pathogens.

Respiratory pathogens	Pre- and/or post-travel N = 110		Acquisition N = 108		Pre-travel N = 110		Post-travel N = 108		P[a] (Post-travel versus pre-travel)
	n	%	n	%	n	%	n	%	
Respiratory virus									
Influenza A	0	0	0	0	0	0	0	0	NA
Influenza B	3	2.7	1	0.9	2	1.8	1	0.9	0.56
Metapneumovirus	0	0	0	0	0	0	0	0	NA
Respiratory syncytial virus	0	0	0	0	0	0	0	0	NA
Human rhinovirus	20	18.2	14	13.0	6	5.5	14	13.0	0.07
Enterovirus	0	0	0	0	0	0	0	0	NA
Adenovirus	8	7.3	5	4.6	3	2.7	6	5.6	0.25
Coronavirus	20	18.2	18	16.7	2	1.8	19	17.6	0.0001
Coronavirus HKU1	0	0	0	0	0	0	0	0	NA
Coronavirus 229E	4	3.6	3	2.8	1	0.9	3	2.8	0.32
Coronavirus NL63	10	9.1	9	8.3	1	0.9	10	9.3	0.003
Coronavirus OC43	9	8.2	9	8.3	0	0	9	8.3	0.003
Parainfluenza virus	0	0	0	0	0	0	0	0	NA
Respiratory bacteria									
Staphylococcus aureus	80	72.7	15	13.9	65	59.1	50	46.3	0.03
Streptococcus pneumoniae	38	34.5	4	3.7	34	30.9	22	20.4	0.01
Haemophilus influenzae	85	77.3	29	26.9	56	50.9	69	63.9	0.03
Klebsiella pneumoniae	66	60.0	7	6.5	59	53.6	30	27.8	< 0.0001
Neisseria meningitidis	0	0	0	0	0	0	0	0	NA
Bordetella pertussis	0	0	0	0	0	0	0	0	NA
Mycoplasma pneumoniae	0	0	0	0	0	0	0	0	NA

[a] *McNemar's test on 108 paired samples.*

In our study, the population was characterized by the young age of participants with 26.4% children and only few elderly pilgrims, in marked contrast with the characteristics of Hajj pilgrims [19]. Indeed, the Grand Magal is a familial event and many adult pilgrims travel with their children. As a consequence of the young age of participants, the prevalence of chronic conditions was low, as opposed to Hajj pilgrims [19–23]. Also, in comparison with the Hajj pilgrimage, where pilgrims stay in Saudi Arabia for around 3 weeks [19], we note that the duration of stay in Touba during the Grand Magal is shorter (3 days) (Table 5).

The vaccination rate against influenza was dramatically low. The use of face masks was rare, while 2/3 of pilgrims declared using hand sanitizer. The overall prevalence of respiratory symptoms was high, affecting 42% participants and a 4.8 fold increase was observed following the participation to the Grand Magal. This result is in line with studies conducted at the Hajj, where 50–93% pilgrims presented respiratory symptoms [24]. Gastrointestinal symptoms were less frequent, affecting 15% individuals, but three times more frequent after participation in the Grand Magal. Overall, these symptoms were relatively mild leading to a low proportion of antibiotic consumption (12.5% ill pilgrims) and no pilgrim was hospitalized. However, 91.3% and 43.8% of pilgrims still had respiratory and gastrointestinal symptoms, respectively when they returned home, which can be a source of infection for their family members. Acquisition of respiratory viruses and *H. influenzae* at the Grand Magal was common, as observed in the setting of the Hajj pilgrimage [24]. We found no evidence for increased transmission of respiratory infections between individuals from a same concession or from a same housing location in Touba which suggests that respiratory infections may have resulted from contacts with the

Table 4
Prevalence of gastrointestinal pathogens.

Gastrointestinal pathogens	Pre- and/or post-travel N = 84		Acquisition N = 45		Pre-travel N = 60		Post-travel N = 69		P* (Post-travel versus pre-travel)
	n	%	n	%	n	%	n	%	
Gastrointestinal virus									
Hepatitis A virus	0	0	0	0	0	0	0	0	NA
Hepatitis C virus	0	0	0	0	0	0	0	0	NA
Adenovirus	8	9.5	2	4.4	6	10.0	3	4.3	0.56
Astrovirus	0	0	0	0	0	0	0	0	NA
Rotavirus	0	0	0	0	0	0	0	0	NA
Norovirus	2	2.4	1	2.2	0	0	2	2.9	0.32
Gastrointestinal bacteria									
Entamoeba histolytica	0	0	0	0	0	0	0	0	NA
Giardia lamblia	9	10.7	1	2.2	8	13.3	2	2.9	0.18
Cryptosporidium spp	0	0	0	0	0	0	0	0	NA
Salmonella	5	6.0	1	2.2	1	1.5	4	5.8	1
Shigella/EIEC	10	11.9	2	4.4	5	8.3	9	13.0	0.16
EHEC	29	34.5	8	17.8	10	16.7	25	36.2	0.13
EPEC	52	61.9	15	33.3	25	41.7	38	55.1	0.22
EAEC	66	78.6	11	24.4	40	66.7	50	72.5	0.13
Campylobacter jejuni	1	1.2	1	2.2	0	0	1	1.5	0.32
Tropheryma whipplei	7	8.3	0	0	6	10.0	6	8.7	1

(*) McNemar's test on 45 paired sample.

Table 5
Main characteristics of the Grand Magal in comparison with the Hajj pilgrimage [1,18–23].

	The Grand Magal pilgrimage	The Hajj pilgrimage
Number of attendees in recent years	4-5 Million/year	2-3 million/year (10 million/year if including the Umrah)
Frequency	Annual	Annual
Geographical location	Touba, Senegal, Africa	Mecca, Mina and Arafat, Saudi Arabia, Middle-East
Duration of the event	1 day (18th of Safar)	5 days (8th to 12th of Dhu al-Hijjah)
Main religious sites visited by attendees for praying and main rituals	Touba Great Mosque, mausoleums, Wall of Mercy spring, Central library, Mouride University. Animal sacrifice.	Mecca Great Mosque (Kaaba circumnanbulation and walking between the hills of Safa and Marwah), Zam-Zam spring, Mount Arafat, Muzdalifah (gathering of pebbles), Mina plain and Jamarat pillars (throwing of pebbles). Animal sacrifice. Shaving head (mens)
Satellite activities of attendees	Visit to personal Marabout, family meeting, cultural activities, hymn-singing sessions, visit to the market	Visit of the Mosque and of the Prophet tomb in Medina
Origin countries of attendees	Mostly Senegal and surrounding countries (Côte-d'Ivoire, Gabon, Guinée-Bissau and Mauritania). Diaspora from France, Belgium, Italy, Spain, Portugal, US and Canada	More than 180 countries in the Middle-East, South-East Asia, Europe, Africa, America and Australia.
Demographics of attendees	Mostly young population with children	Mostly middle aged and elderly population
Comorbidities of attendees	Rare	Frequent (diabetes, cardiovascular and chronic respiratory diseases, walking disability)
International published official health recommendations	No	Yes (pre-travel health regulation and advice about vaccination, health check and specific vaccination at the port of entry, vaccination requirements for issuance of travel visas)
Local media communication and health education campaigns during the event	Yes	Yes
Recent and current common reported public health complications	Last cholera outbreak (2004-2006 – 12135 persons), dengue outbreak (2018), diarrhea, respiratory tract infections, heat stress, road accidents Malaria endemic.	Last cholera outbreak (1989–102 persons), invasive meningococcal disease (1987 and 2000–2001), diarrhea, respiratory tract infections, heat stress, stampedes. Malaria at elimination stage since 2008.
Estimated mortality	0.2/100.000 in 2015	2/100.000 in 2013 among Indonesian pilgrims 1.2/100.000 in 2016 among Indian pilgrims
Housing	Private houses and marabout houses in Touba and surrounding cities and villages	Hotels in Mecca (and Medica), tent camps in Mina
Food	House food and free street food	Restaurants and street vendors
Sanitary conditions (safe water and food provision, waste management; vector control)	Control of quality of drinking water and food items, inspection of main kitchens and food areas, treatment of sewage water. Cleaning and disinfection of areas highly frequented by pilgrims. Disinfection of garbage sites. Insecticide spraying of religious sites and houses of main Marabouts.	Strict microbiological control of quality of drinking water sources and food items, microbiological testing of kitchen workers and kitchen environments, treatment of sewage water. Prohibiting of bringing fresh food or agricultural products from outside the country. Routine and widespread insecticide spraying included aerially.
Number of hospitals and beds available during the event	7 hospitals, 481 beds	25 hospitals, 5000 beds including 500 for critical care
Information technology and surveillance	Paper-based consultation registers	Web-based electronic health surveillance systems for rapid communication
WHO center for mass gathering medicine in the hosting country	No	Yes
Number of papers according to PubMed.gov over the last 5 years (key words = Magal [tiab] and Hajj [tiab]), search done on 25/04/2019)	4	287

overall population in Touba in overcrowding areas, in and around the Mosque rather than from intra-familiar or intra-domiciliar transmission. Our survey has several limitations, including its small sample size and the fact that most pilgrims came from a single village. Also, the short time between inclusion and post-Magal questionnaire administration and testing makes it difficult to establish a formal link between symptoms and pathogen acquisition and pilgrimage participation. Another limitation is that we did not investigate *Mycobacterium tuberculosis* prevalence in our study. However, given the long incubation time of tuberculosis and the short duration of stay of pilgrims in Touba, PCR-based detection of *M. tuberculosis* in relation to the event would not have been feasible. Finally, the proportion of participant who provided paired samples of stool specimen was low. Further study including larger numbers of pilgrims are needed to investigate potential risk factors for respiratory and gastrointestinal infections at the Grand Magal. Nevertheless, this preliminary study confirms that Grand Magal pilgrims are likely to be exposed to communicable disease risk, as observed in other pilgrimage settings, despite the relatively short duration of the event.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2019.04.010.

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Supplementary data

Respiratory and gastrointestinal infections at the 2017 Grand Magal de Touba, Senegal: a prospective cohort survey

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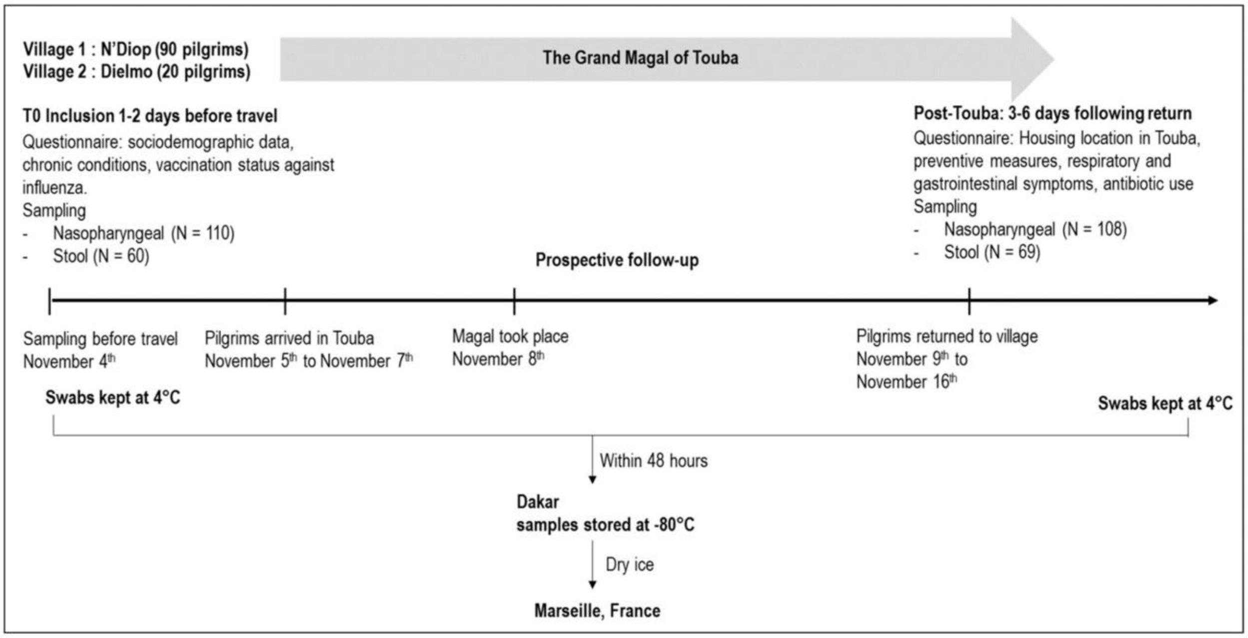
Supplementary figure: Procedure of study



Partie 5 :

Rassemblement de masse et COVID-19

Préambule :

Fin 2019, une nouvelle épidémie d'infection respiratoire aigüe (officiellement nommée COVID-19) a émergé à Wuhan, en Chine, causée par nouveau coronavirus (SARS-CoV-2). Cette épidémie s'est rapidement étendue dans le monde entier. L'OMS a déclaré l'état de pandémie le 11 mars 2020. Jusqu'au 12 avril 2021, 219 pays et territoires sont touchés avec plus de 135 millions de cas rapportés. Cette maladie a tué près de trois millions personnes.

Au vu de la situation du COVID-19 dans les pays hôtes, les rassemblements de masse ont été suspendus ou limités. Afin de contrôler la propagation de l'épidémie de COVID-19, les Jeux olympiques de Tokyo 2020 ont été reportés à 2021. Compte tenu de la forte contagiosité de la maladie et de l'épidémiologie du COVID-19 au Japon, cette décision était appropriée et importante afin de protéger les athlètes et le public.

Le risque de transmission de virus respiratoires, y compris du SARS-CoV-2, est particulièrement élevé en raison des conditions de surpeuplement pendant le Hajj et l'Omra. Des études antérieures ont montré que les pèlerins du Hajj sont âgés et présentent de multiples comorbidités. Leur profil correspond à celui des individus à risque de COVID-19 sévère. Afin d'éviter une épidémie de COVID-19 susceptible de se propager dans de nombreux pays par le retour des pèlerins, l'Arabie Saoudite a suspendu l'Omra et imposé des conditions très strictes au déroulement du Hajj 2020. La participation aux rituels du Hajj a été limitée à seulement 1000 personnes résidant dans le Royaume d'Arabie Saoudite présentant un test COVD-19 négatif. Les personnes âgées de 65 ans et plus et celles souffrant de pathologies chroniques n'ont pas pu y participer.

Article 15 : The Tokyo Olympic Games and the Risk of COVID-19

Hoang VT, Al-Tawfiq JA, Gautret P.

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MASSIVE GATHERING EVENTS AND COVID-19 (M ALMUTAIRI, SECTION EDITOR)

The Tokyo Olympic Games and the Risk of COVID-19

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Abstract

Purpose of Review We reviewed the occurrence of outbreaks at past Olympics and discuss the threat of the COVID-19 pandemic at the Tokyo Games.

Recent Findings Evidence for large respiratory tract infection outbreaks at past Olympics is scant. Nevertheless, in order to control the spread of the COVID-19 outbreak, the Tokyo 2020 Olympics were postponed for 2021. Given the high contagiousness of the disease and the epidemiology of COVID-19 in Japan, this decision was appropriate and important in order to safeguard athletes and the public. However, it is a major problem for Japan, involving massive financial losses and a lost opportunity for athletes, coaches, and instructors.

Summary Up-to-date epidemiological data is needed on which to base an appropriate decision regarding the Tokyo 2021 Olympics. The actual effect of cancellations of such events in reducing the spread of COVID-19 needs to be determined.

Keywords COVID-19 · SARS-CoV-2 · Mass gatherings · Olympics · Paralympics · Athletes

Introduction

Mass gatherings (MGs) are defined as a "concentration of people at a specific location for a specific purpose over a set period of time which has the potential to strain the planning and response resources of the country or community" [1]. MGs can be spontaneous or programmed and irregular or recurrent [1]. Sporting, religious, and cultural events such as the Olympic Games, the Hajj, and music festivals are programmed MGs [1]. MGs pose considerable public health challenges to health authorities and host governments, not only in regard to transmissible disease but also noncommunicable disease, such as trauma or injuries. In addition, illness related to the use of drugs and alcohol and environmental effects are well described as public health problems during certain types of MGs [1]. Infectious disease is one of the major challenges at MGs, as it affects the attendees during the event and can increase the load on the health system in the host country. In addition, infected participants may spread the disease on a large scale upon return to their home countries. In fact, several MGs have been identified as the source of infectious diseases that have spread globally [2•]. Among the infectious diseases, respiratory tract infections (RTIs) are particularly frequent at MGs, due to the inevitable overcrowding and nature of its mode of transmission [2•, 3, 4].

Recently, a novel coronavirus named SARS-CoV-2 emerged in Wuhan city, Hubei province, China, causing an outbreak of a respiratory infectious disease (COVID-19). The outbreak has spread rapidly and widely throughout the world. The World Health Organization (WHO) declared the event a Public Health Emergency of International Concern (PHEIC) on January 30, 2020, and a pandemic on March 12, 2020 [5]. At the time of writing, the COVID-19 pandemic has accounted for 20,812,367 confirmed cases and 747,327 deaths in 213 countries and territories around the world [6].

This article is part of the Topical Collection on *Massive Gathering Events and COVID-19*

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As part of efforts to control the spread of the COVID-19 pandemic, several important MGs events have been cancelled worldwide since early March 2020, including international and national sporting, religious, cultural, and other MGs. Many prominent sporting events with millions of participants have been cancelled or postponed, such as the Formula 1 Grand Prix in China, the Euro 2020 football championship, the Six Nations rugby championship in Italy and Ireland, the Mobile World Congress in Barcelona, and Olympic boxing qualifying events [7]. The 2020 Summer Olympics and Paralympics in Japan were originally scheduled to take place from July 24 to August 9, 2020, and from August 25 to September 6, 2020, respectively. Finally, it was halted in March 2020 because of the COVID-19 pandemic [8]. This international multisport event is now planned for July 23 to August 8, 2021 (Olympics), and August 24 to September 5, 2021 (Paralympics) in Tokyo, Japan. As with many other MGs events, the Tokyo 2020 Olympics would have been a significant opportunity for dispersing pathogens, especially SARS-CoV-2. We review the occurrence of infectious diseases at past Olympic Games, with a special focus on the threat of the COVID-19 pandemic at the Tokyo games.

An Overview of Infectious Diseases at the Olympic Games

Infectious diseases have been frequently reported in religious events like the Hajj, one of the largest religious MGs worldwide, and music festivals. But no strong evidence for a significant increase of infectious disease outbreak during sporting events has been identified [9]. Only a few RTI and gastrointestinal infection outbreaks were found in a review of Gautret et al., which included the Summer and Winter Olympics from 1984 through 2015 [9]. In the 2002 Winter Olympics in Salt Lake City, 36 cases of influenza among participants were recorded [10]. During the Vancouver 2010 Winter Olympics in Canada, an epidemic with 82 cases of measles occurred [11]. No major public health incidents occurred during the London 2012 Olympic Games. Only a few cases of RTIs and gastrointestinal illness were reported during this event, but no food-borne illness was directly linked to a Games venue [12]. During the London 2012 Olympics, a total of 47 sexually transmitted infections were diagnosed in 289 visitors, including 8 chlamydia and 15 nonspecific genital infections. There were no new cases of HIV or syphilis diagnosed [13]. During the Sochi 2014 Winter Olympics in Russia, 249 illnesses were reported among the 2788 athletes (the incidence was 8.9 illnesses per 100 athletes). Of those, 58% were caused by infectious diseases. RTIs were the most frequent (63.9%), followed by gastrointestinal symptoms (11%) [14].

The Rio de Janeiro 2016 Olympic Summer Games in Brazil included 11,274 athletes from 207 countries. During this event, 613 illnesses were reported. Infectious diseases affected 56% of ill individuals. RTIs and gastrointestinal symptoms were the most common and occurred in 202 (47%) and 131 individuals (21%), respectively [15]. Although the Rio de Janeiro 2016 Olympics took place during the time of a Zika outbreak, no cases of Zika virus were detected among the athletes and attendees [16, 17].

In 2018, the Pyeong Ghang Winter Olympics in South Korea had the highest number of athletes (2920) and participating countries (92) in the history of the Winter Olympics [18•]. During this event, the most common cause of illness was RTIs [18•, 19•]. A total of 1639 athletes consulted polyclinics, including 1402 (85.5%) visits for illness, with 107 cases of upper RTIs. Common cold was also observed in 42 of 112 members of the Finland team [20]. The etiology of the RTIs was detected in 30 of 42 patients by multiplex-PCR, with 9 different respiratory viruses identified. Human coronavirus (HCoV) 229E (11 cases) was the most frequent, followed by HCoV NL63, influenza B, human rhinovirus, respiratory syncytial virus type A, and metapneumovirus (5 cases). The survey showed that the viral infections spread easily within the same sport discipline or the team. In addition, co-infections were also common [20]. Also, during the Pyeong Ghang 2018 Winter Olympics, a norovirus outbreak emerged a few days before the event began. This outbreak affected 172 volunteers staying at hostels but only 4 athletes [18•].

Large RTI outbreaks were rarely reported at previous Olympic Games. This can be explained by the fact that the participants move to other locations at the end of an event and do not live on site, contrary to large religious MGs. Furthermore, the duration of each sport event is short (less than 1 day) [9–17, 18•].

Olympic Game Massive Gatherings and Risk Assessment for Dissemination of COVID-19

Unlike the case of Zika virus during the 2016 Summer Olympics in Rio de Janeiro, the SARS-CoV-2 virus is transmitted by the respiratory route and can be easily transmitted between humans. The reproduction number R (R0) of SARS-CoV-2 is estimated up to 4.1 [21–23]. In comparison with the SARS-CoV and influenza virus, SARS-CoV-2 is more transmissible [24•]. In addition, the strong infectivity of SARS-CoV-2 and rapid transmission even from asymptomatic carriers during the long incubation period have been previously described [25]. Because the incubation period of the virus is long (up to 14 days) [26], controlling viral dispersion seems to be difficult. The Tokyo Olympic Games were supposed to receive 20 million non-residents visitors from 204 countries and regions [27••, 28], and the stadiums would have been overcrowded. The relatively close contact between participants, including athletes and staff, spectators, and journalists, could increase the spread of COVID-19. Moreover, there was a high risk of globalization of virus transmission by travellers. Screening at airports is feeble, and nearly 46% of infected travellers cannot be identified [29]. Body temperature is ineffective for screening SARS-CoV-2 among young adult travellers; only 18% of COVID-19 cases present a fever of 38 °C [30].

Currently, the risk of a rebound of COVID-19 in the near future is worrisome in several countries [21], including Japan. Figure 1 shows the two waves of COVID-19 in Japan. The number of new daily cases in the second wave tends to be higher than the first wave. COVID-19 new daily cases varied from 853 to 1998 during the planned periods of the Tokyo 2020 Olympics (from July 24 to August 9, 2020) (Fig. 1) [6].

Regarding athletes, they are viewed as being fit and healthy, but this might not be accurate [31]. The overtraining syndrome and high glycemic diet are often associated with chronic diseases. Those with chronic diseases are more susceptible to SARS-CoV-2 infection and aggravation or complications of COVID-19 [5]. Athletes are exposed to a higher risk of infection because of their compromised immune system [31–33]. Pedersen et al. conducted a study on the relationship between immune depression and infection among athletes [34]. After exercise, athletes have a transitory "open window" effect of immune depression lasting 3 to 72 h. This period makes the host more susceptible to infection from several microorganisms and potentially increases the risk for SARS-CoV-2 infection [35••].

Paralympics athletes are exposed to more risks for infectious disease, including RTIs [35••]. During the Rio 2016 Paralympics season, athlete delegations were approximately 60% physical-motor deficient, 25% visually impaired, and 5% intellectually disabled [35••]. They presented twice the amount of total illness in comparison to the Olympic athletes, and RTIs were the most frequent. Controlling disease in Paralympic athletes is relatively more complex. It is estimated that between 10 and 20% of these athletes are at risk of aggravating symptoms if they are infected by COVID-19 [35••].

Impact of the Cancellation of the Tokyo 2020 Olympic Games

In order to take measures to contain the spread of the COVID-19 pandemic, several national and international MGs, including sporting events, have been cancelled or postponed worldwide since early March 2020 [7]. The question of stopping the Tokyo 2020 Olympic was raised early [8]. On April 28, 2020, the President of the International Olympic Committee confirmed that the 2020 Olympic Games should be cancelled and postponed until 2021 [36]. The name of the event will remain unchanged: "Tokyo 2020 Olympic and Paralympic Games." In the past, several Olympic Games have been cancelled by world wars, including the 1940 Tokyo Summer Olympics due to the onset of World War II [36]. Some Olympic Games have been held successfully despite global

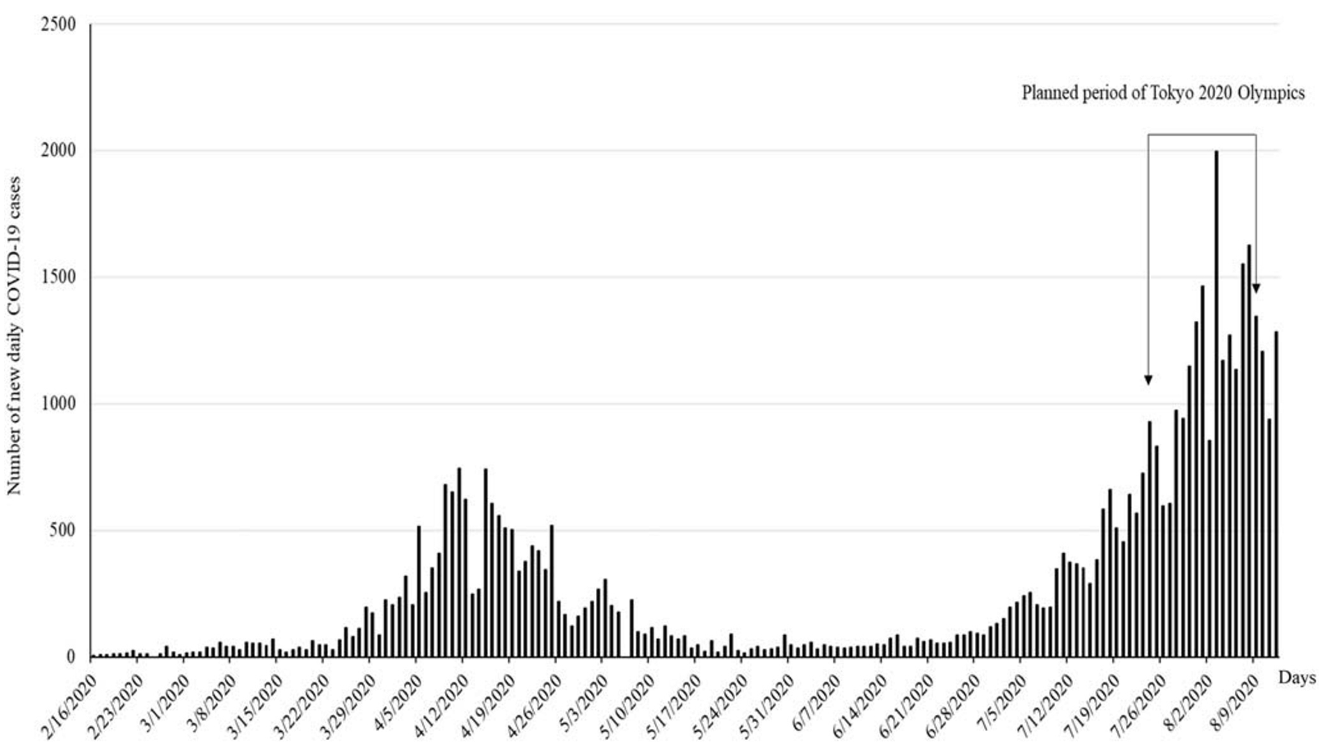

Fig. 1 COVID-19 pandemic in Japan [6]

health crises, such as the Vancouver 2010 Winter Olympics during the H1N1 influenza pandemic and the Rio 2016 Olympics during the outbreak of Zika virus. This is the first time in the 124-year history of the modern Olympic Games that they have been postponed due to a medical issue.

The decision to postpone the Tokyo 2020 Olympic and Paralympic Games to 2021 was appropriate and important in order to safeguard the athletes as well as the public [37]. However, it is a major problem not only for the host country in terms of massive financial losses but also for the 11,000 Olympic and 4400 Paralympic athletes who were due to take part in this important sporting event mostly because of loss of opportunity [38••].

The cancelling of this event cost Japan 4.52 trillion yen (41.5 billion USD), based on operating expenses, considering maintenance expenditures for the unused facilities and loss of tourism activity.

Athletes have been directly affected by the decision to postpone the Tokyo 2020 Olympics due to the COVID-19 pandemic. The postponement of the 2020 Olympics automatically meant retirement and the permanent loss of opportunity for some [38••, 39]. Athletes have lost daily, weekly, monthly, and yearly workout routines, which affects their mental and physical state [38••]. The spread of COVID-19 forced nearly every training site to close and forced players to stay home. Social distancing measures have affected not only the Tokyo Olympic and Paralympic games but all other sporting events, including the cancellation of qualifying tournaments. These changes have increased feelings of uncertainty, confusion, and frustration and made it difficult to establish a specific set of goals [38••, 39, 40]. In addition, because of the limited opportunity to leave the house for systematic and intensive training, when players are allowed to train for shorter periods of time, they tend to overwork to maximize impact and that may increase the likelihood of injury. Limiting interactions with teammates, coaches, and others can also make athletes more anxious [38••, 40].

Coaches and instructors were another group affected by the COVID-19 outbreak. They had particular difficulty in coaching athletes. Also, they may not have been able to allocate enough energy and time to focus on the athlete's conditions, because they needed to care for themselves and their families [38••].

Mandatory doping tests were severely limited by the COVID-19 outbreak in early 2020. European anti-doping organizations raised concerns that urine and blood tests could not be performed. Mobilizing the staff necessary to do so before the end of the pandemic may increase health risks. The World Anti-Doping Agency stated that public health and safety was their topmost priority despite the need for extensive testing [36]. Due to the COVID-19 outbreak, the Chinese anti-doping agency temporarily ceased testing in early February 2020. Also, the USA, Great Britain, France, and

Table 1 WHO recommendations to sport event organizers in the context of COVID-19 [43]

WHO recommendations to sport event organizers in the context of COVID-19
Pre- and during event
✓ Ensure availability of handwashing and hygiene facilities at multiple locations in the event
✓ Ensure good hygiene signage across all venues
✓ Provide first aid and medical services
✓ Temperatures should be checked each day and any fever case announced to the event medical staff
✓ Ill travellers should be managed at points of entry
✓ Ensure capacity to isolate suspected cases
✓ Develop and make available risk communication on clinical features of COVID-19 and preventive measures, including hand hygiene practices, physical distancing, practical implications of quarantine and criteria for asking individuals with symptoms
✓ Ensure availability of rubber gloves to team staff and volunteers handling laundry, towels, etc.
✓ Recommend that towels are for single use only
✓ Provide each participant with a clean water bottle
✓ Make handkerchiefs and containers to dispose used tissues with lids available on all buses
✓ Provide each team with a facility for athletes' temperatures check
✓ Determine the areas to care for and isolate the infected individuals
✓ Determine the areas to quarantine the persons in contact with confirmed cases
✓ Determine the areas for training the athletes and team staff if a COVID-19 case is notified
✓ Prepare a place where to receive a large number of quarantined persons
✓ Predetermine emergency contacts with local health authorities
✓ Medical masks should be ready, particularly for medical staff and sick individuals
✓ Provide disinfectant wipes to disinfect touched surfaces in all areas
✓ Consider provision of individual prevention packages for athletes containing thermometer, medical mask, hand sanitizer, disposable tissues, and disposable plastic drinking cups
Pre-event
✓ Proactively and regularly check the health status of anyone participating
✓ Avoid participating in the event if an individual is feeling ill
✓ The team leader should ensure that their teams and volunteers are briefed on the protocols for a suspected and confirmed patient
During event
✓ Participants should be aware of and cooperate with team medical staff at venues in checking their temperatures each day. Any fever above 38 °C need to be reported to the event medical lead
✓ Wash hands often with soap and water or hand hygiene with alcohol-based sanitizer
✓ Hand sanitizer stations should be available throughout the event venue
✓ Practice respiratory etiquette, cover coughs and sneezes with disposable tissues, isolate and seek medical advice if coughing persist.
✓ Avoid contact with sick persons
✓ Avoid contact with anyone if you are ill
✓ Use of gloves when handling towels or laundry in the team environment
✓ Towels should not be shared
✓ Athletes should not share clothing, bar soap, or other personal items
✓ Water bottles should be labelled and washed with dishwasher soap after each practice or game, and officials and staff should have their own water bottles to prevent the transmission of viruses and bacteria
✓ Advise athletes not to touch their own mouths or nose
✓ Avoid shaking hands or hugging
✓ Avoid steam rooms or saunas
✓ Be aware of regular cleaning of frequently touched surfaces

Germany anti-doping organizations had reduced their testing activities by the end of March 2020 [36]. That can significantly increase doubts about fair play in the Olympic Games.

Tokyo 2021 Summer Olympics, What Needs to Be Prepared in the Current State?

The President of the Japan Medical Association has been quoted as saying that the Tokyo Olympic Games can only be organized next year if an effective vaccine against COVID-19 is developed for public use and the pandemic is fully controlled, not only in Japan but worldwide. Regarding the current COVID-19 pandemic, it is certainly too early to predict whether the Tokyo Olympic Games will occur in 2021 or will once again be cancelled. SARS-CoV-2 surveillance should be maintained for a long time after the elimination of the initial pandemic [41]. However, this is an important sporting event in the world that only happens once every 4 years, so everyone hopes that it will take place as planned.

During this event, the movement of participants across frontiers and within the host country would most certainly intensify the spread of COVID-19 from one country to another, since travel is clearly one of the key contributors to disease globalization.

To date, there is no specific evidence available for planning and implementing a MG during the COVID-19 pandemic nor is there evidence for the effectiveness of individual mitigation actions for COVID-19 [42]. In collaboration with global partners involved in MGs health, the WHO made recommendations for sports event organizers regarding planning for MGs in the context of COVID-19 (Table 1) [43]. By complying with the existing WHO guidelines, governments can impose limits and minimize the spread of SARS-CoV-2 [44].

Detection and monitoring of MG-related COVID-19 cases should be carefully considered in the context of surveillance schemes, and enhanced surveillance is necessary. In collaboration with local health authorities, organizers should agree in advance about the circumstances in which risk mitigation measures would need to be reinforced. The host country must be prepared to provide adequate prevention measures and diagnostic capabilities. However, during the Games, there are about 14 million food dishes expected to be delivered to participants [37]. This represents a huge challenge for organizers, particularly as regards health security. And the control of COVID-19 will add to this burden.

Conclusion

Up-to-date epidemiological data is needed on which to base an appropriate decision regarding the Tokyo 2021 Olympic and Paralympic Games. If the pandemic continues and is uncontrolled, not only in Japan, but worldwide, or if there is no effective vaccine developed for public use, these events may remain cancelled. The cancellations have social and economic impacts on individual livelihoods, public morale, and on national economies. The effect of cancellations of these events on reducing the spread of COVID-19 needs to be determined. If a decision is made to proceed with the Olympic Games, risk mitigation measures must be put in place, consistent with WHO guidelines on social distancing for COVID-19. The rationale for the decision should be also clearly explained and communicated to the public.

Compliance with Ethical Standards

Conflict of Interest Van Thuan Hoang, Jaffar A. Al-Tawfiq, and Philippe Gautret declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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Article 16 :

Hajj and Umrah Mass Gatherings and COVID-19 Infection

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Hajj and Umrah Mass Gatherings and COVID-19 Infection

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Abstract

Purpose of Review We discuss the risk of COVID-19 in religious mass gathering events including Hajj and Umrah pilgrimages.

Recent Findings The risk of transmission of respiratory viruses including COVID-19 is particularly high due to the overcrowding conditions at the Hajj and Umrah. The profile of the Hajj pilgrims who tend to be older and with multiple comorbidities corresponds to that of individuals at risk for severe COVID-19. In order to avoid a COVID-19 outbreak with potential spreading to many countries through returning pilgrims, Saudi Arabia suspended the Umrah, and access to the 2020 Hajj was very limited.

Summary A clear relation between early suspension of religious mass gatherings and lower occurrence of COVID-19 transmission in countries that took such measures promptly was noticed. There are lessons to national and international health organizations for other mass gatherings in the context of the pandemic.

Keywords Hajj · Pilgrimage · Umrah · Mass gathering · COVID-19 · SARS-CoV-2 · MERS-CoV

Introduction

The Hajj in Makkah, Kingdom of Saudi Arabia (KSA), is one of the largest annual religious mass gatherings (MGs) in the world with a strong international component. Each year, two to three million Muslims from more than 180 countries around the world flock to Makkah for the Hajj pilgrimage [1•]. This is one of the five pillars of Islam. It is compulsory, at least once in lifetime, for all responsible adult Muslims who have the physical and financial capacities necessary to accomplish it. The Hajj takes place every year between the 8th and the 13th of Dhul Hijjah, the 12th and last month of the Islamic calendar. In contrast, the Umrah is another Islamic pilgrimage to Makkah (shorter than the Hajj) that can be undertaken at any time of the year. The Umrah is not compulsory, but is still highly recommended [2]. Umrah during Ramadan is equal to Hajj in terms of religious value, according to a statement that is attributed to the Prophet Mohammad [3]. Together, the Hajj and Umrah involve over 10 million participants each year [4•].

The presence of a large number of pilgrims from many parts of the world in congested and crowded areas greatly increases the risk of spreading infectious diseases [1•, 5]. Respiratory tract infections (RTIs) are the most frequent infections transmitted between pilgrims [1•, 5]. Most of pilgrims develop RTIs early after their arrival in Makkah with prevalence up to 90% [6].

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In December 2019, an outbreak of respiratory infectious disease (COVID-19) due to a novel coronavirus (officially named SARS-CoV-2) emerged in the city of Wuhan, in the Chinese province of Hubei. The virus is easily transmitted between humans, and the outbreak was declared a Public Health Emergency of International Concern on January 30, 2020, and then a pandemic on March 12, 2020 [7]. This pandemic is posing serious risks to public health worldwide including in the KSA. All possible measures against lethal COVID-19 were applied in the country, but one of the most important questions asked in the Islamic world in the context of COVID-19 was about approaching Umrah and Hajj pilgrimages.

We review viral respiratory infections at the Hajj and Umrah mass gatherings with a focus on COVID-19.

Transmission of Respiratory Viruses During the Hajj and Umrah

Acute RTIs are a major problem of public health that affects over 5 million individuals (more than 15%) of the KSA population in 2013 [8]. Respiratory viruses are the most frequent cause of these infections [9, 10]. The epidemiology of respiratory viruses in KSA is likely affected by the gathering of more than 10 million Muslims in the holy sites of Makkah and Medina during the Umrah and Hajj seasons [11]. In pilgrims, human rhinovirus (HRV), common human coronaviruses (HCoV), and influenza virus were the respiratory viruses most frequently acquired at the Hajj [2, 5, 6, 12–16].

HRV is a highly contagious respiratory virus. It is a frequent cause of RTIs in humans of all age range. Immunosuppressed individuals or persons with congenital heart disease and bronchopulmonary dysplasia are exposed to severe HRV infection [17]. In cohorts of pilgrims sampled after participation in the Hajj, carriage of HRV ranged from 8.4 to 34.4% and was significantly higher before the Hajj [16]. As an example, a paired survey realized among 692 international Hajj pilgrims showed that over 34% pilgrims acquired HRV after the 2013 Hajj season [12]. More recently (2014 to 2017), in a 4-year cohort of 485 French pilgrims, 26.9% participants acquired HRV after their pilgrimage [18]. The dynamic of HRV acquisition during the pilgrimage was recently described in French pilgrims [6]. The authors showed an early increase in HRV carriage during the first days of the pilgrimage with a prevalence 24 times higher than that of pre-travel samples. Then, HRV carriage decreased progressively in subsequent samples but was still eight times higher in post-Hajj samples compared to pre-Hajj [6].

In November 2002, a pandemic due to the novel HCoV named SARS-CoV started in Guangdong, China. The virus spread to several countries with 8096 confirmed cases reported, and the mortality was nearly 10.0% [19]. Ten years later, a SARS-like CoV identified as MERS-CoV was isolated from a Saudi patient. An outbreak quickly spread to neighboring countries, followed by a wider spread to geographically distant countries [20]. As of September 2019, it involved 27 countries with 2494 laboratory-confirmed cases and 858 deaths (mortality rate was 34.4%). The majority of cases occurred in the KSA with 2102 cases reported and 780 deaths (37.1% case fatality rate) [21]. The largest numbers of MERS-CoV cases were reported in 2014 and 2015 with 523 and 452 cases, respectively [11]. However, no cases of SARS coronavirus and MERS coronavirus were documented in Hajj pilgrims until now [22], and only a few cases of MERS have been reported among Umrah pilgrims [16]. By contrast, other common HCoVs were acquired by pilgrims during their pilgrimage. Acquisition rate of HCoV 229E calculated from a large paired cohort survey (692 international pilgrims) in 2013 was 14.6% [12]. In paired cohorts of French pilgrim ($n = 485$) investigated during the Hajj 2014–2017, the overall HCoV acquisition rate was 8.3% (with HCoV 229E the most frequent (6.2%)) [18] with a marked peak during the 2016 Hajj season with a 19.8% HCoV 229E acquisition rate [2, 13–16, 23•].

In April 2009, an outbreak due to a novel H1N1 influenza virus (influenza A(H1N1)pdm09) was first identified in Mexico and became rapidly pandemic with more than 22 million cases reported in with USA [9]. The KSA was one of the countries affected by the virus, with 15,850 laboratory-confirmed cases, including 124 deaths on December 30, 2009 [24]. The first 100 cases in KSA involved travelers at four airports during June 2009 [24]. The 2009 Hajj took place in the last week of November, during the outbreak that had been declared as a global pandemic by the World Health Organization on June 11, 2009 [25]. The acquisition rate of influenza A(H1N1)pdm09 among 305 returning Iranian pilgrims after the 2009 Hajj season was 1.6% [26]. Koul et al. conducted a study among 300 Indian pilgrims returning from the Hajj and Umrah in 2014–2015. Their qPCR result showed that 11% were positive for influenza virus, including 9 cases of influenza A(H1N1)pdm09, 13 cases of influenza A/H3N2, and 11 cases of influenza B [27]. In another study realized among 1600 international pilgrims after the 2010 Hajj season, a total of 7.5% participants were positive for influenza A by qPCR. Of whom, 9 cases were positive for influenza A(H1N1)pdm09 [28].

Overall, these results show that acquisition of respiratory viruses following the Hajj is very frequent with high carriage rates on leaving KSA and a potential for further transmission on returning to home country. This strongly suggests that the Hajj and possibly the Umrah may contribute to the globalization of common respiratory viruses.

COVID-19 Pandemic in the Kingdom Saudi Arabia and the Main Countries That Usually Send Pilgrims

The first COVID-19 case in KSA was detected on March 02, 2020, in the Qatif region among an individual who had traveled to an endemic region in Iran [29•]. The outbreak then began to spread throughout the country in early April 2020. As of August 11, 2020, a total of 3199 death out of 288,947 confirmed cases COVID-19 (8315 cases/1 M population) were reported in KSA [30]. A dynamic epidemiological model estimated that the 2020 Hajj season could coincide with the peak or deceleration leg of the COVID-19 pandemic curve [31]. Interestingly, the COVID-19 epidemic in KSA was partly related to another pilgrimage (Shiite pilgrimage) with Saudi pilgrims returned from pilgrimage sites in Iraq and Iran being an early source of community seeding of SARS-CoV-2 in KSA contributing to global total cases.

The annual number of pilgrims in the last 10 years varied between 1,862,909 and 3,161,573 (Fig. 1) [32]. The incidence of confirmed cases of COVID-19 in top 10 countries which send Hajj pilgrims in 2018 varied from 227/1 M population to 3910/1 M population [30] (Table 1). Thus, it was expected that the Hajj and Umrah will be suspended [33].

The Risk for COVID-19 Among Hajj and Umrah Pilgrims

During the Hajj and Umrah pilgrimages, the risk of transmission of respiratory viruses including COVID-19 is particularly high due to the overcrowding as pilgrims gather in sacred crowded places where rituals take place such as during tawaaf (circumambulating the Ka'ba) or within the housing structures. By example, these is up to 8 persons per m^2 at the Grand Mosque in Makkah during rituals, and 50–100 pilgrims are housed per tent at Mina encampment [34, 35]. Even when the reproduction rate of an outbreak is low, the over density during the Hajj tends to amplify the spread of transmission [36]. The Sri Petaling MG, a Muslim missionary movement with 19,000 participants, including 1500 foreigners from 30 countries, in the suburb of Kuala Lumpur, Malaysia, that took place from February 27 to March 1, 2020, accounted for > 35% of the COVID-19 cases in the country [37••]. In February 2020, 19.2% (712/3711) of the ship's population on the Diamond Princess Cruise Ship were infected by SARS-CoV-2 [38]. The Shincheonji Christian religious group with approximately 200,000 participants gathering in the city of Daegu, South Korea, took an important role in the COVID-19 epidemic in the country. On March 03, 2020, nearly 3000 related cases were reported out of 5621 cases in whole South Korea [39]. A high attack rate of SARS-CoV-2 was observed during a large wedding in Jordan with a total of 76/350 (21.7%) participants tested positive [40]. In February 2020, 8000 pilgrims had returned to different cities of Pakistan from Qom city in Iran where a pilgrimage of Shia Muslims took place. At the end of March 2020, a total of 990 confirmed cases were reported in Pakistan, of whom, 60% were pilgrims returning from Iran [41•]. In early March 2020, a cluster of 48/53 (90.5%) COVID-19 cases was documented in Greek pilgrims after a Christian pilgrimage in Jerusalem, Israel [42]. In late March 2020, six pilgrims who attended a pilgrimage at a masjid in Pakistan were detected positive for SARS-CoV-2 in China. During their 6-month stay, they had close contact with thousands of masjid pilgrims without face mask [43]. Finally, in March 2020, the COVID-19 outbreak started in Arkansas, USA, with two index cases who participated to a Christian event in a rural county [44•]. A total of 35/92 (38%) attendees were confirmed for the SARS-CoV-2, including 3 deaths. In addition, at least 26 additional patients who had contact with these participants were likely infected by them.

Several risk factors for COVID-19 infection and critical COVID-19 cases were described [45•]. Persons with organ transplant, cancers, severe lung condition, and serious heart condition or pregnant woman are at high risk. Older persons, having lung condition that is not severe, heart disease, diabetes, chronic kidney or liver disease, or obesity are at moderate risk [46]. In a meta-analysis of 3027 patients from 13 studies showed multiple factors to be associated with SARS-CoV-2 infection [45•]. Being male and smoking were associated with a twofold increased risk of severe disease. Age older than 65 years was associated with a sixfold increased risk of disease progression in patients with COVID-19 [45•]. In addition, the proportion of chronic diseases was significantly higher in critical and among those who died compared to others, including hypertension (odds ratio (OR) = 2.72),

Table 1 COVID-19 data [30] until August 11, 2020, in top 10 countries which have highest numbers of Hajj pilgrims in 2018

N°	Country	Population total	Number of Hajj pilgrims in 2018	Number of confirmed cases of COVID-19	Number of confirmed cases by 1 million persons
1	Indonesia	273,839,956	210,984	127,083	464
2	Pakistan	221,342,804	200,969	285,191	1288
3	India	1,381,493,158	183,040	2,269,052	1642
4	Bangladesh	164,869,319	133,157	260,507	1580
5	Turkey	84,438,244	116,551	241,997	2866
6	Egypt	102,537,651	98,143	95,666	933
7	Iran	84,109,372	86,452	328,844	3910
8	Nigeria	206,662,307	59,253	46,867	227
9	Iraq	40,323,330	43,075	164,277	4074
10	Sudan	36,958,139	39,714	12,162	277

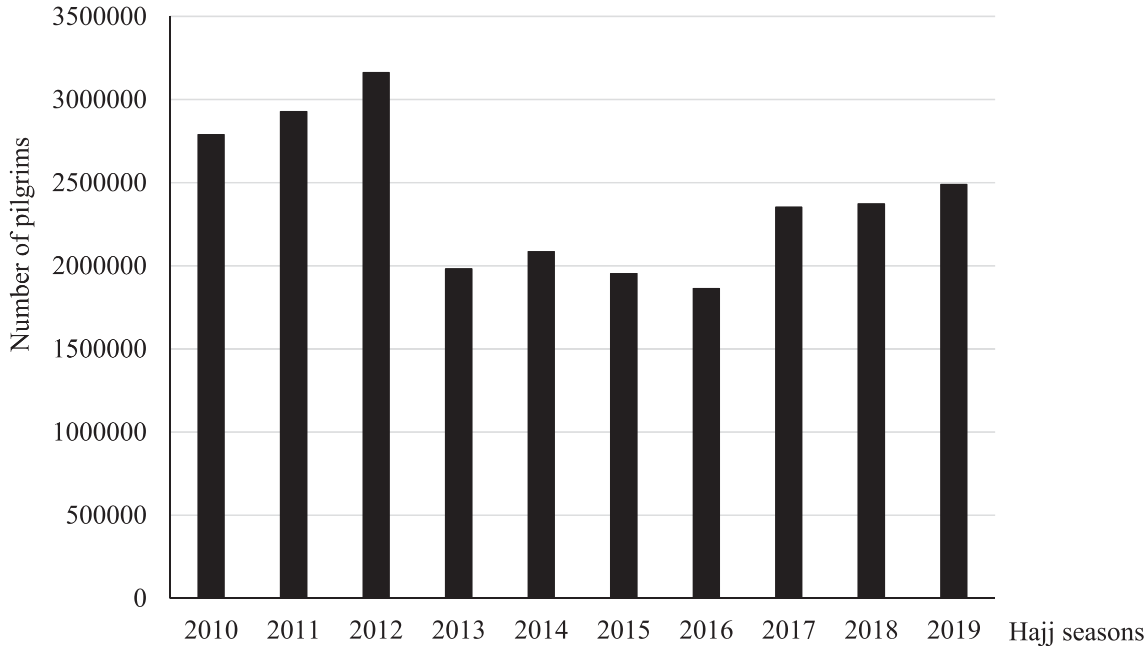

Fig. 1 Number of annual pilgrims to Makkah, Saudi Arabia, in the years 2010–2019 [32]

diabetes (OR) = 3.68), respiratory disease (OR = 5.15), and cardiovascular disease (OR = 5.19) [45•]. The profile of the Hajj pilgrims corresponds to that of individuals at risk for severe COVID. The majority of Hajj pilgrims are elderly and have a high prevalence of chronic diseases [47•, 48]. In a large study on 783 French pilgrims from 2012 to 2017, the median of age of participants was 62 years, and more than 50% have comorbidities. Diabetes and hypertension were the most frequent chronic diseases with prevalence of 29.4% and 28.0% [47•]. In 2013, 87% of Indonesian Hajj pilgrims were aged over 65 years, and 83% met criteria for high risk of health problems [48].

International travel has already been shown to play a central role in the spreading of COVID-19 and international MGs like the Hajj or Umrah, if maintained may well have contributed to the globalization of SARS-CoV-2 through returned participants. [49–51]. A few cases of COVID-19 occurred in Umrah pilgrims before international travel was banned. As an example, the first patient who died of COVID-19 in Pakistan was a returned Umrah pilgrim [52]. In addition, most of Hajj and Umrah pilgrims are from countries with suboptimal disease surveillance or travel health counseling service [53].

Prevention of Respiratory Tract Infections, Including COVID-19, During the Hajj and Umrah

To date, no specific preventive measures or vaccines are available for COVID-19. Furthermore, health systems are still overloaded in many places. The overcrowding during the Hajj and Umrah is inevitable, and it is difficult to prevent the transmission of contagious diseases in this context. The KSA Ministry of Health recommends individual preventive measures such as use of face mask and disposable handkerchiefs and hand hygiene to mitigate the risk of RTIs. However, the efficacy of these measures against RTIs is debated [35], and there have been no reliable controlled studies investigating their efficacy on the incidence of Hajj-related RTIs. The use of face masks may not provide optimal protection from infection, but that may reduce the spread of small-sized saliva droplets around when coughing or sneezing which is the main mode of transmission of most RTIs. Mandatory use of face mask in public places is considered one of the effective measures in controlling the COVID-19 pandemic [54–56], but no investigation has been conducted in the context of MGs so far. In addition, the practice of social distancing, hand hygiene, and contact avoidance was associated with reduced risk of spreading this outbreak [7, 54].

The KSA Ministry of Health annually publishes the recommendations for required immunization such as influenza vaccine and meningococcal vaccine for the Hajj. In 2012, specific individual preventive measures were also recommended against MERS [57].

On May 29, 2020, WHO published the key planning recommendations for mass gatherings in the context of COVID-19 [58]. There are several factors to consider when determining the need to cancel or postpone a mass gathering event. These factors include the number of attendees and the proportion at greater risk of COVID-19 transmission, the density of attendees within a confined place, the level of transmission in the host area, and the community to which the participants will return [58]. WHO also recommended canceling gatherings of more than 10 persons for organizations that serve higher-risk populations or community-wide MGs with more than 250 participants [58]. If a MG is to be held, prevention supplies are needed to be provided to event staff and participants, such as hand sanitizer with at least 60% alcohol, disposable tissues, trash baskets, disposable facemasks, and surface disinfectants (Table 2) [58].

Response of the Kingdom of Saudi Arabia for Controlling the COVID-19 Pandemic at Hajj and Umrah Pilgrimage

Since the KSA has been affected by the COVID-19 pandemic, several measures were applied, including in the holy cities Makkah and Madinah, to protect people from further infection [4•]. In order to prevent the spread of COVID-19 and for avoiding super spreader events, Saudi Arabia suspended the Umrah on March 03, 2020 [59]. The government also closed the Grand Mosque and the Kaaba on March 6, 2020, for over 2 months. The shipping services and all international flights were also suspended [59]. This year, the Hajj took place from July 28, 2020, to August 02, 2020. Because the spread of infection has not been controlled worldwide and in the country, on June 22, 2020, the KSA government announced complete ban of international visitors for the 2020 annual Hajj pilgrimage to Makkah, and access for the domestic population was denied to pilgrims with chronic diseases or aged 65 years and older [60••].

The Hajj pilgrimage has not been canceled since Saudi Arabia's foundation in 1932 [61••]. In addition, the Hajj pilgrimage has faced no significant limits on attendance since the outbreaks of cholera and plague in second half of the nineteenth century [61••]. More recently, during the influenza A(H1N1)pdm09, the population groups with the highest risk of influenza complications, including pregnant women, patients with chronic diseases, and individuals under 12 years or over 65 years of age, were invited to voluntarily refrain from performing the 2009 Hajj to decrease the transmission of the virus [62].

The 2020 Hajj season was successfully ended on August 3, 2020, with no major public health incident [61••]. Although the holy sites in Makkah and Medina remain open, access to the holy sites was limited for no more than 1000 persons (already resident in Saudi Arabia). Wearing of facemasks was mandatory during the pilgrimage. The participants were checked for fever and quarantined if required. Disinfectant measures were also implemented. A social distance of 1.5 m between pilgrims was applied. No pilgrims were allowed to touch the Kaaba. And after the pilgrimage, pilgrims were quarantined for 14 days.

Table 2 WHO's key planning recommendations for mass gatherings in the context of COVID-19 [57]

WHO key planning recommendations for mass gatherings in the context of COVID-19
Planning phase
✓ Establishing direct links of communication between event organizers and health authorities
✓ Ensuring alignment of the event plan with wider national emergency preparedness and response plans
✓ Making provisions for detecting and monitoring event-related cases of COVID-19
✓ Reducing the spread of the virus
✓ Treating ill persons
✓ Disseminating public health messages specific to COVID-19 in culturally appropriate ways and in languages used by participants
✓ Establishing a clear line of command and control and enabling efficient situation analysis and decision-making or developing a risk communication strategy and a community engagement plan for the event
✓ Making provisions for human resources, procurement of personal protective equipment and other medical consumables
Operational phase
• Related to the venue
✓ Hosting the event, at least partially, online/remotely/virtually
✓ Hosting the event outdoors rather than indoors
✓ Adjusting the official capacity of the venue
✓ Ensuring availability of hand washing facilities with soap and water and/or hand rub dispensers
✓ Ensuring regular and thorough cleaning and disinfection of the venue by designated staff
✓ Regulating the flow and density of people entering, attending, and departing the event
• Related to the participants
✓ Advising people to observe physical distancing, respiratory/cough etiquette, and hand hygiene practices
✓ Advising people with higher risk of transmitting COVID-19 that they should not attend the event
✓ Advising people with higher risk of developing severe illness from COVID-19 and individuals in contact with higher-risk patients that they should not attend the event, or making special arrangements for them
• Duration of event
✓ Keeping the duration of the event to a minimum to limit contact among participants
• Risk communication
✓ Ensuring coordination and consistency in crafting and delivering culturally appropriate and language specific messages to participants and the public
✓ Disseminating key messages in line with national health policies
• Surveillance of participants, aimed at detecting and managing individuals developing symptoms during the event
✓ Detection and management of event-related COVID-19 cases should be conducted in accordance with national policies and regulations, within the framework of national health systems
✓ Isolation facilities should be made available at the event site
✓ Arrangements with national and local health authorities regarding diagnosis and treatment of COVID-19 cases identified during the event
Post-event phase
Liaison between event organizers and health authorities, along the following lines:
✓ In case participants or staff develop symptoms during the event, event organizers should liaise with national and local health authorities, as well with those of the participant's home city or country, and facilitate sharing of information
✓ Individuals who develop symptoms upon returning to their home city or country should be advised to contact public health authorities about their potential exposure
✓ Liaison between event organizers and health authorities is required to ensure that systems are in place to detect cases arising

Conclusion

The level of COVID-19 outbreak had been linked to large religious MGs in several countries. A clear relation between early suspension of such events and lower occurrence of COVID-19 transmission in countries that took such measures promptly was noticed [63]. There are lessons to national and international health organizations for other MGs in the context of a pandemic.

The Saudi decision to drastically restrict the Hajj pilgrimage and to cancel the Umrah, two events with super spreader potential, offers impetus and precedence for other stakeholders and countries facing similar challenges amidst the reports of worsening COVID-19 global pandemic. As a global community, in the absence of a vaccine, the political commitment of nations and compliance of communities to effectively use known mitigation tools may help us to overcome the current pandemic. These decisions today will also offer important lessons for future generations.

Compliance with Ethical Standards

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

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CONCLUSIONS ET PERSPECTIVES

Malgré les recommandations sur des mesures préventives individuelles pharmaceutiques et non-pharmaceutiques et leur application, les IRs et IGIs restent encore très fréquentes chez les pèlerins du Hajj et du Grand Magal de Touba lors de leurs pèlerinages.

Nos données suggèrent que les IRs liées au Hajj sont le résultat d'interactions complexes entre de nombreux de virus respiratoires et de bactéries. Des études du microbiote respiratoire avec des outils permettant d'identifier un plus grand nombre d'agents pathogènes seront nécessaires pour mieux élucider ces changements écologiques et leur rôle potentiel dans l'apparition de symptômes respiratoires.

Nos travaux ont confirmé l'efficacité de la vaccination contre la grippe pour réduire les symptômes du syndrome grippal et celle de la vaccination contre les maladies invasives à pneumocoque pour réduire l'acquisition de *S. pneumoniae*.

L'utilisation d'antibiotiques est fréquente et souvent inappropriée pendant le Hajj. Il est donc nécessaire de suivre les recommandations d'utilisation des antibiotiques en fonction des symptômes cliniques chez les pèlerins afin d'en assurer une utilisation rationnelle. Une stratégie d'éducation des pèlerins sur les pathologies liées au Hajj et les indications d'antibiotiques ainsi que la promotion de la vaccination contre la grippe et le pneumocoque sont nécessaires. En particulier, il est important d'organiser des séances d'information avant de se rendre à la Mecque. En outre, il est important de contrôler la délivrance d'antibiotiques dans les pays d'origine des pèlerins et de faire appliquer les législations du gouvernement de l'Arabie saoudite. Par ailleurs, des tests d'identification rapide des pathogènes respiratoires pourraient aider le personnel médical en charge des pèlerins à rationaliser leurs prescriptions d'antibiotiques.

La diarrhée et la gastro-entérite, notamment les épidémies de choléra, ont été une menace au cours du Hajj dans le passé. Cependant, une diminution de la prévalence des IGIs chez les pèlerins a été observée au cours des dernières décennies. Cela reflète très probablement l'amélioration des conditions sanitaires sur les sites religieux et des mesures plus strictes pour assurer la maitrise de la qualité de la nourriture et de l'eau au Hajj. Néanmoins, la diarrhée et les IGIs persistent parmi les pèlerins et la surveillance continue de ces maladies fait partie de la stratégie de santé publique pour le Hajj. Nous avons montré une forte acquisition des pathogènes digestifs chez les pèlerins. Néanmoins, de prélèvements microbiologiques de la nourriture et des boissons des pèlerins pendant le Hajj n'ont pas été effectués. De plus, l'apparition des symptômes gastro-intestinaux dont la diarrhée est survenue tôt au cours du voyage, tandis que le prélèvement a été réalisé avant le départ d'Arabie Saoudite, beaucoup plus tard. Il se peut que l'acquisition potentielle de pathogènes entériques liés à la diarrhée ait été partiellement (ou totalement) éliminée au moment du prélèvement. L'échantillonnage au début des symptômes et la culture des pathogènes entériques devraient être effectués dans les études futures afin de mieux identifier les agents pathogènes responsables des symptômes gastro-intestinaux au cours du Hajj.

Notre étude a aussi mis en évidence une acquisition significative de bactéries multi-résistantes au Hajj, notamment des bactéries productrices de béta-lactamases à spectre élargi. Ces bactéries peuvent se propager dans la communauté lorsque les pèlerins retournent dans leur pays d'origine. Cette menace émergente reste négligée. Il est urgent de prélever des échantillons rectaux provenant de plus grandes cohortes de pèlerins, puisque plus de 10 millions de pèlerins du monde entier se rendent en Arabie Saoudite pour le pèlerinage chaque année.

Des mesures préventives individuelles contre les maladies infectieuses sont recommandées pendant le Hajj, mais leur efficacité reste incertaine. L'utilisation d'un masque facial en présence de symptômes respiratoires et un renforcement de l'hygiène des mains devraient toujours être recommandées pour les pèlerins du Hajj jusqu'à ce que des études contrôlées à grande échelle soient menées pour véritablement évaluer l'efficacité de ces mesures dans le contexte du Hajj. L'usage du mouchoir jetable est, de fait, très fréquent chez les pèlerins malades et permet au moins de diminuer l'acquisition de *S. aureus*. La fourniture d'eau potable et de denrées alimentaires avec un contrôle de qualité rigoureux est probablement le meilleur moyen de limiter les infections digestives et la transmission des bactéries multi-résistantes dans le contexte du Hajj.

Nous avons montré que l'ADN et l'ARN d'agents pathogènes respiratoires sont présents à des taux élevés sur les surfaces de l'environnement des pèlerins pendant le Hajj, notamment à Mina. Il est possible que les pèlerins puissent être infectés par des agents pathogènes environnementaux via leurs mains à partir de surfaces contaminées et s'auto-inoculer ou transmettre ces agents pathogènes en l'absence d'une bonne hygiène des mains. Une hygiène des mains renforcée, un nettoyage et une désinfection améliorés des surfaces fréquemment touchées pendant le pèlerinage devraient être recommandés avec une priorité pour les surfaces humides, les tables de cuisine et les poignées de porte.

Bien que le Grand Magal de Touba soit le plus grand rassemblement religieux musulman en Afrique de l'Ouest, les études sur les problèmes de santé dans ce contexte restent encore très limitées. Notre étude préliminaire confirme que les pèlerins du Grand Magal sont susceptibles d'être exposés au risque de maladies transmissibles, comme observé dans d'autres lieux de pèlerinage, malgré la durée relativement courte de l'événement. Une étude plus approfondie incluant un plus grand nombre de pèlerins et des contrôles est nécessaire pour identifier les facteurs de risque associés aux d'IRs et IGIs durant le Grand Magal. Il est aussi nécessaire de recommander des mesures préventives individuelles et un programme de vaccination visant à diminuer les maladies infectieuses liées à cet événement.

Les rassemblements de masse ont été suspendus ou limités à cause de la pandémie de COVID-19. Une relation claire entre la suspension précoce des rassemblements religieux de masse et une fréquence moindre de transmission du COVID-19 dans les pays qui ont pris rapidement de telles mesures a été observée.

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