université PARIS-SACLAY

Identifying women at risk of or with reproductive tract infections in low- and middle-income countries with a focus on asymptomatic women

Identification des femmes à risque ou ayant des infections de l'appareil reproducteur dans les pays à revenus faibles et intermédiaires, en particulier chez les femmes asymptomatiques

Thèse de doctorat de l'université Paris-Saclay

École doctorale n° 570 : santé publique (EDSP)
Spécialité de doctorat : Epidémiologie
Graduate School : Santé Publique.
Référent : Faculté de médecine

Thèse préparée dans l'unité de recherche **CESP** (Université Paris-Saclay, UVSQ, Inserm), sous la direction de **Bich-Tram HUYNH**, Directrice de recherche, la co-direction de **Tania CRUCITTI**, Directrice de l'unité de Bactériologie Expérimentale à l'Institut Pasteur de Madagascar (IPM), le co-encadrement de **Rindra Vatosoa RANDREMANANA**, Directrice de l'unité d'Epidémiologie et de Recherche Clinique à l'IPM

Thèse soutenue à Paris-Saclay, le 25 novembre 2024, par

Camille FORTAS

Composition du Jury

Membres du jury avec voix délibérative

Béatrice BERCOT PU-PH, Université Paris Cité	Présidente
Philippe MAYAUD Professeur, LSHTM, Londres	Rapporteur & Examinateur
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Hervé FERNANDEZ PU-PH, Université Paris-Saclay	Examinateur

THESE DE DOCTORAT

NNT : 2024UPASR024

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université PARIS-SACLAY | ÉCOLE DOCTORALE Santé Publique (EDSP)

Titre : Identification des femmes à risque ou ayant des infections de l'appareil reproducteur (IAR) dans les pays à revenus faibles et intermédiaires (PFRI), en particulier chez les femmes asymptomatiques

Mots clés : Epidémiologie, Infections sexuellement transmissibles, Vaginose bactérienne, Santé de la femme, Pays à revenus faibles et intermédiaires

Résumé : les infections de l'appareil reproducteur causées par *Chlamydia trachomatis* (CT), *Neisseria gonorroheae* (NG), *Trichomonas vaginalis* (TV), *Mycoplasma genitalium* (MG) et la vaginose bactérienne (VB) peuvent entraîner des conséquences graves pour la santé des femmes. La technique de référence pour la détection de ces pathogènes est basée sur les tests d'amplification des acides nucléiques (NAAT) et le score de Nugent pour la VB. Toutefois, dans les PRFI, réaliser des NAAT est coûteux et requiert du personnel qualifié. Par conséquent, la prise en charge des IAR repose généralement sur les signes et les symptômes, connue sous le nom d'approche syndromique. Le principal défaut de cette approche est de ne pas détecter les cas asymptomatiques et par conséquent, de ne pas les traiter.

Dans cette thèse, nous avons d'abord mis en évidence la prévalence élevée des IAR chez les femmes en âge de procréer à Madagascar, un pays pour lequel il existe peu de données épidémiologiques récentes. Nous avons souligné que 40% des femmes infectées n'auraient pas été traitées si l'approche syndromique seule avait été suivie. Nous avons réalisé une revue systématique et une méta-analyse afin de quantifier la proportion de femmes asymptomatiques dans les PRFI, montrant que plus de la moitié des femmes infectées ne présentaient aucun symptôme. Enfin, nous avons développé un score de risque visant à prédire les infections par CT, NG, TV ou VB avec une sensibilité de 80% pour les femmes asymptomatiques. Le pH vaginal jouait un rôle central dans le score développé. Ainisi, le travail présenté dans cette thèse permet de mieux comprendre les IAR asymptomatiques et de proposer une approche complémentaire à l'approche syndromique pour améliorer la prise en charge des infections chez les femmes dans les PRFI.

Title: Identifying women at risk of or with reproductive tract infections (RTIs) in low- and middle-income countries (LMICs) with a focus on asymptomatic women

Keywords: Epidemiology, Sexually transmitted infections, Bacterial vaginosis, Women's health, Low- and middle-income countries

Abstract: *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), *Mycoplasma genitalium* (MG) and bacterial vaginosis (BV) are infections or conditions from the reproductive tract that can have severe health consequences in women. The gold-standard for detecting these pathogens are the nucleic acid amplification tests (NAATs) and the Nugent score for BV. However, in LMICs, performing NAATs is expensive and requires trained personnel. Therefore, the management of RTIs relies on signs and symptoms, known as the syndromic management approach. The main shortcoming of syndromic management is that it does not detect asymptomatic cases. In this thesis, we first reported a high prevalence of RTIs among women of reproductive age in Madagascar, a country for which recent epidemiological data is lacking. We underscored that 40% of infected women would not have been treated had the syndromic approach been applied. In a second piece of work, we conducted a systematic review and meta-analysis to quantify the proportion of asymptomatic women in LMICs. We found that more than half of infected women present no symptom. Finally, we developed a risk score aiming at predicting infection with CT, NG, TV, or BV among asymptomatic women which showed a sensitivity of 80%. Vaginal pH played a central role in all models. Overall, the work presented in this thesis provides a better understanding of asymptomatic RTIs and provide a complementary approach to the syndromic management to improve their management in women in LMICs.

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Prediction tools STI/BV among women aged 18 – 35 in a cross-sectional multi-centric study in Madagascar, South Africa and Zimbabwe.

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- Asymptomatic Infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* among Women in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. (Poster 1)

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List of acronyms

ARV	Antiretrovirals
BIC	Bayesian Information Criterion
BV	Bacterial vaginosis
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CT	*Chlamydia trachomatis*
DALY	Disability-adjusted life-years
DHS	Demographic health survey
FDA	Food and drug administration
FSW	Female sex workers
GIFT	Genital InFlammation test
GUD	Genital ulcer disease
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
IL	Interleukin
IP	Interferon γ-induced protein
IUD	Intrauterine device
LAP	Lower abdominal pain
LGV	Lymphogranuloma venereum
LMIC	Low- and Middle-Income Countries
MG	*Mycoplasma genitalium*
mg	Milligrams
MSE	Mean square error
NAATs	Nucleic Acid Amplification Tests
NG	*Neisseria gonorrhoeae*
PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
PID	Pelvic inflammatory disease
POC	Point of care
POCT	Point of care test
PrEP	Pre-exposure prophylaxis
RDT	Rapid diagnostic test
ROC	Receiver Operating Characteristic
RTI	Reproductive tract infection
STD	Sexually transmitted disease
STI	Sexually transmitted infection
TV	*Trichomonas vaginalis*
VDS	Vaginal discharge syndrome
VMMC	Voluntary medical male circumcision
VVC	Vulvovaginal candidiasis
WHO	World Health Organization

Résumé en français

Les infections de l'appareil reproducteur (IAR) regroupent les infections sexuellement transmissibles (IST), et d'autres infections endogènes telles que la vaginose bactérienne (VB) ou la candidose vulvovaginale (CVV). Ces infections, chez la femme, peuvent donner lieu à des complications sévères, telles que la maladie inflammatoire pelvienne. Celle-ci peut évoluer en infertilité tubaire et augmenter le risque de grossesse ectopique. En cas de grossesse, certaines de ces infections sont associées à un risque d'accouchement prématuré ou un faible poids à la naissance. Par ailleurs, les personnes infectées par une IAR sont plus à risque d'acquisition et de transmission d'une IST ou du virus de l'immunodéficience humaine (VIH). Dans le cadre de cette thèse, nous nous intéresserons en particulier à trois IST qui sont parmi les plus courantes et pour lesquelles il existe un traitement curatif : *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), et *Trichomonas vaginalis* (TV) ; ainsi qu'à la VB.

Ces infections ont des taux de prévalence et d'incidence élevés dans les pays à revenus faibles et intermédiaires (PRFI). De plus, dans une majeure partie des PRFI, la détection et la prise en charge de ces infections repose sur une approche syndromique proposée par l'Organisation Mondiale de la Santé (OMS). Cette approche consiste à détecter et traiter les individus sur la base des symptômes qu'ils présentent. Toutefois, certaines femmes infectées par une IST ou une VB ne présentent pas de symptôme, elles ne sont, par conséquent, pas détectées par l'approche syndromique et ne sont pas traitées. Ceci peut entrainer des conséquences sur leur santé, en raison des complications qu'elles peuvent développer, et elles peuvent également continuer de transmettre ces infections à leurs partenaires.

Cette thèse a pour objectif général de décrire, de comprendre, et d'explorer de nouvelles pistes pour la détection et la prise en charge des IAR chez les femmes dans les PRFI. Elle est composée de trois parties principales :

- L'OMS a identifié comme priorité en recherche sur les IST, la collecte d'informations sur les données épidémiologiques. Certains PRFI manquent de données récentes et de qualité sur la prévalence des IST. C'est le cas de Madagascar, où les dernières données sur la prévalence de CT, NG et TV chez les femmes issues de la population générale ont été collectées il y a près de vingt ans.

  Nous avons donc mis en place une étude pour estimer la prévalence de CT, NG, TV, *Mycoplasma genitalium* (MG), *Candida albicans* (CA), et de la VB et identifier les facteurs de risque associés à une IST (au moins une infection par CT, NG, TV ou MG). Nous avons également estimé le nombre de femmes infectées qui n'auraient pas été traitées si l'approche syndromique de l'OMS avait été suivie, car elles ne présentaient pas de symptôme mais étaient tout de même infectées.

- Nous avons ensuite cherché à quantifier la proportion de femmes asymptomatiques parmi celles infectées et leur prévalence dans les PRFI. Nous avons, pour cela, réalisé

une revue systématique et méta-analyse à partir des études publiées dans les 20 dernières années dans les PRFI.

- Enfin, partant du constat que l'approche syndromique ne permettait pas de détecter les femmes asymptomatiques, et en raison de sa faible sensibilité et spécificité chez les femmes symptomatiques, nous avons développé plusieurs scores de risque d'infection par une IST ou de VB à partir de questions posées à la patiente ou de tests peu invasifs. Ces scores ont pour but d'identifier les femmes asymptomatiques infectées et d'améliorer la prise en charge par l'approche syndromique des femmes symptomatiques.

Ces trois parties sont développées ci-dessous.

Dans une étude transversale, l'étude PrevISTana, nous avons testé des femmes en âge de procréer pour déterminer la prévalence des IST et les facteurs associés. Nous avons pour cela recruté des femmes de 18 à 45 ans qui venaient consulter au service de consultations externes ou de planification familiale du centre hospitalo-universitaire de gynécologie et d'obstétrique de Befelatanana (CHOGOB) à Antananarivo, la capitale.

Nous avons calculé que la taille d'échantillon nécessaire serait de 470 participantes. Les femmes incluses dans l'étude, ont été interrogées par une enquêtrice formée qui a collecté des données socio-démographiques et de comportement, puis le personnel médical a prélevé des échantillons vaginaux et a collecté des informations sur les signes cliniques et les symptômes de la patiente. Les échantillons vaginaux ont été soumis à des tests d'amplification des acides nucléiques (NAAT) pour la détection de CT, NG, TV, MG et CA, tandis que la VB a été diagnostiquée à partir du score de Nugent. L'ensemble des analyses de laboratoires ont été effectuées à l'Institut Pasteur de Madagascar. Nous avons déterminé les prévalences de ces infections avec leurs intervalles de confiance de Wald à 95% (IC 95%) ; puis nous avons réalisé des régressions logistiques univariées et multivariées pour déterminer les facteurs associés à la détection d'une IST (au moins une infection par CT, NG, TV ou MG). Le comité d'éthique de Madagascar a approuvé cette étude et toutes les participantes incluses ont donné leur consentement éclairé.

Au cours du recrutement, 529 femmes éligibles ont été approchées. Quatorze femmes ont refusé de participer et 14 ont été exclues pour diverses raisons. Ce sont donc au total 501 femmes qui ont été incluses dans l'étude. L'âge moyen des femmes était de 27 ans (écart interquartile : 23 – 32 ans). Plus de la moitié d'entre elles avaient été au moins jusqu'au lycée ou plus (321/501, 64%). La majorité était soit mariée (242/501, 48%), soit en couple (191/501, 38%), et seulement 14% (68/501) était célibataire ou divorcée. Parmi les femmes qui vivaient avec leur partenaire depuis plus d'un an, 14% avaient déclaré qu'elles avaient eu d'autres partenaires sexuels au cours des 12 derniers mois.

La prévalence de CT était de 11,8% (IC 95% : 9,0 – 14,6%), de NG 4,2% (IC 95% : 2,4 – 5,9%), de TV 14,8% (IC 95% : 11,7 – 17,9%) de MG 8,2% (IC 95% : 5,8 – 10,6%) et de CA 15,8% (IC 95% : 12,6 – 19,0%). Pour la détection de la VB, 11 prélèvements n'avaient pas assez de

matériel biologique et ne pouvaient par conséquent pas être utilisés pour le score du Nugent. Parmi les 490 échantillons disponibles, la prévalence de la VB était de 39,6% (IC 95% : 35,3 – 43,9%). Nous avons également trouvé que la prévalence de CT diminuait au fur et à mesure que l'âge augmentait, avec une prévalence de 17,8% chez les 18 – 24 ans, 10,3% chez les 25 – 34 ans et de 3,3% chez les 35 – 45 ans (p = 0,004).

L'analyse des facteurs associés aux IST a été réalisée sur les données complètes, soit en excluant les participantes pour lesquelles la présence de VB n'avait pu être déterminée, et les participantes qui n'ont pas répondu ou n'ont pas souhaité répondre à certaines questions. Au total, cette analyse a donc porté sur 463 participantes. Dans l'analyse multivariée, en ajustant sur l'âge, nous avons trouvé que les femmes en couple mais non mariées avaient un *odds ratio* (OR) de 1,86 (IC 95% : 1,16 – 2,98, p = 0,010). De plus, les femmes qui déclaraient avoir des rapports sexuels en échange d'argent, de cadeaux, ou de travail avaient un *odds* quatre fois plus élevé (OR : 4.25, IC 95% : 1,27 – 16,6, p = 0,019). Enfin, les femmes qui avaient une VB ou un microbiote vaginal intermédiaire avaient un *odds* deux fois plus élevé que celles qui avaient un microbiote vaginal optimal (OR : 2,14, IC 95% : 1,34 – 3,47, p = 0,002 et OR : 2,10, IC 95% : 1,19 – 3,72, p = 0,010, respectivement). Nous nous sommes intéressés aux symptômes des femmes infectées par une IST et nous avons trouvé que 40% d'entre elles ne présentaient aucun des symptômes qui constituent les points d'entrée dans l'approche syndromique pour ces IST-ci, à savoir : un écoulement vaginal anormal, une sensation d'irritation, un prurit et des douleurs abdominales basses.

Cette étude a permis de montrer que la prévalence des IST et de la VB étaient élevées chez les femmes en âge de procréer et consultant au CHUGOB, avec près d'une femme sur trois infectée par une IST. Les prévalences trouvées concordent avec la dernière étude menée sur ces IST vingt ans auparavant, bien que cette dernière ait été conduite en milieu rural. Par ailleurs, nous avons constaté que les femmes incluses dans cette étude avaient un niveau d'éducation plus élevé que la moyenne nationale. En supposant que les femmes avec niveau d'éducation plus élevé aillent plus souvent consulter ou soient plus informées sur les IST, il est à envisager que la prévalence des IST chez le reste des femmes dans le pays soit encore plus élevée. En comparant ces résultats aux études menées dans d'autres pays d'Afrique, nous trouvons que ces prévalences restent élevées, et qu'elles se rapprochent plus des prévalences retrouvées chez les femmes à risque telles que les travailleuses du sexe ou chez les femmes plus jeunes, qui sont souvent aussi plus infectées.

Nous avons identifié que le fait d'être en couple sans être mariée était associé à un risque plus élevé d'IST par rapport au fait d'être marié. Cela peut s'expliquer par le fait que les relations concomitantes sont particulièrement plus courantes chez les femmes non-mariées que chez les femmes mariées. Les femmes, et leurs partenaires qui ont aussi des relations concomitantes, sont donc plus exposés et donc plus à risque. Les femmes qui proposent des relations sexuelles en échanges de faveurs sont également plus à risque. Ces femmes avaient généralement un niveau d'éducation plus bas que les autres femmes. De ce fait, elles connaissaient possiblement une plus grande précarité qui les amenaient par conséquent à vendre des services sexuels comme revenu complémentaire. De plus, les femmes avec une VB ou un microbiote vaginal intermédiaire étaient plus à risque d'une IST. Cette dynamique de

co-infection a été déjà documentée, mais il n'a pas encore été déterminé si la VB facilitait l'acquisition d'IST ou l'inverse. Enfin, près de 40% des femmes infectées n'auraient pas été prises en charge si l'approche syndromique avait été appliquée. En conclusion, la prévalence élevée des IST dans cette étude indique un besoin de prévenir et d'informer la population sur la circulation de ces IST, en particulier dans le cas où il existe plusieurs partenaires dans la même fenêtre de temps, ou pour les femmes qui échangent des rapports contre de l'argent, des cadeaux, du travail ou d'autres services. D'autres études sont nécessaires dans le reste du pays où certaines études préliminaires suggèrent des prévalences encore plus élevées. Elles pourraient permettre, ensemble, de guider les futures interventions de santé publique.

Nous avons vu dans l'étude PrevISTana qu'une proportion importante de femmes infectées ne présentait pas de symptômes. Ce constat a été observé dans d'autres études, toutefois, aucune autre étude à ce jour n'avait fait la synthèse ces résultats. Pour pallier cette absence, nous avons mené une revue systématique de la littérature et méta-analyse dans laquelle nous avons cherché à déterminer la proportion d'infections par CT, NG, ou TV qui étaient asymptomatiques et la prévalence de femmes infectées et asymptomatiques dans la population, dans les PRFI.

Nous avons défini la « proportion d'infections asymptomatiques » comme étant le nombre de femmes testées positives pour un pathogène et étant asymptomatiques divisé par le nombre de femmes testées positives ; et la « prévalence d'infections asymptomatiques » comme le nombre de femmes testées positives et asymptomatiques divisé par le nombre de femmes testées pour ce pathogène. Nous avons également défini un « symptôme » comme une manifestation d'une maladie telle que rapportée par la personne atteinte et un « signe clinique » comme une manifestation de cette maladie observée par un individu extérieur.

Nous avons recherché tous les articles publiés sur le sujet dans les bases de données Medline (PubMed), Web of Science, et Scopus. Nous avons également regardé les références des articles retenus. Nous avons sélectionné les études publiées entre 2000 et 2022 en anglais et en français. Seules les études détectant CT, NG, TV avec des NAAT à partir d'échantillons génitaux ou urinaires ont été incluses. Pour TV, une exception a été faite pour conserver les études testant avec le dispositif InPouch® ou réalisant de la microscopie directement après le prélèvement, car ces deux approches présentent une sensibilité et spécificité acceptable et ont longtemps été utilisés en routine dans les PRFI.

Nous avons d'abord réalisé une analyse qualitative des données, en décrivant les régions, le design des études, les sites de recrutement, les populations incluses, le type d'échantillons, et les procédures de laboratoires utilisées. Nous avons estimé les proportions et prévalences de CT, NG et TV asymptomatiques sur toutes les études avec leurs intervalles de confiance. Nous avons ensuite réalisé des analyses en excluant les études portant sur les travailleuses du sexe, les femmes vivant avec le VIH, et les femmes qui consultaient dans les centres spécialisés sur les IST en raison de leur risque accru d'avoir une IST.

Nous avons appliqué un modèle a effets aléatoires en utilisant l'estimateur de variance de DerSimonian et Laird. L'hétérogénéité statistique a été évaluée en utilisant le test de χ^2 avec le Q de Cochran et le I^2. Des analyses de sous-groupes ont été réalisées par région, niveau de

revenu du pays, milieu urbain ou rural, période de l'étude, et nombre de symptômes pris en compte dans l'étude. Le rôle de ces variables a également été exploré dans une méta-régression. Le risque de biais dans les études a été évalué et nous avons conduit des analyses de sensibilité en excluant les études avec un risque de biais élevé. Cet article a suivi les recommandations de PRISMA.

Nous avons retrouvé 1113 articles publiés sur ce sujet, parmi lesquels 547 étaient des doublons. Parmi eux 177 étaient éligibles, et les données ont pu être extraites pour 48 études correspondants à 99 points de données : 41 pour CT, 27 pour NG, et 31 pour TV. Vingt-et-un pays étaient représentés. La majorité des études (92%) étaient des études transversales, et 71% avaient lieu en milieu urbain. Les sites de recrutement les plus fréquents étaient les hôpitaux et les services de soins prénataux. Six études avaient réalisé un échantillonnage aléatoire en communauté. Les études avaient recruté entre 48 et 4812 participantes dont l'âge variait de 10 à 90 ans. La collecte de spécimens génitaux était la plus courante (85%). Pour TV, quatre études avaient utilisé la microscopie et deux InPouch®. Quatre études portaient exclusivement chez les femmes vivant avec VIH, cinq études sur les travailleuses du sexe et quatre chez les adolescentes.

Les proportions d'infections asymptomatiques pour CT, NG et TV étaient respectivement de 60,7% (IC 95% : 50,4 – 70,5%), 53,3% (IC 95% : 37,1 – 69,1%) et 56,9% (IC 95% : 44,6 – 68,9%), en excluant les femmes à risque accru. Ces proportions étaient significativement différentes selon les régions (p = 0,048, p = 0,019 et p = 0,039 pour chaque pathogène, respectivement). La proportion d'infections asymptomatiques la plus élevée se retrouvait en Afrique pour toutes ces IST (CT : 68,6% [IC 95% : 56,4 – 79,8%] ; NG : 67,2% [IC 95% : 51,0 – 81,9%] ; et TV : 64,9% [IC 95% : 52,2 – 76,7%]).

La proportion de femmes infectées par CT, NG et TV et asymptomatiques parmi celles vivant avec le VIH était élevée : 64,7% pour CT (IC 95% : 48,3 – 79,7%), 61.0% pour NG (IC 95% : 42,7–78,0% ; et 70,5% pour TV (IC 95% : 60,7 – 79,6%). A l'inverse ces proportions étaient en dessous de 50% parmi les travailleuses du sexe (CT : 45,5% [IC 95% : 24,8 – 67,0%] ; NG : 38,1% [IC 95% : 3,1 – 82,9%], et TV : 26,7% [IC 95% : 19,6–34,5%]).

Les prévalences d'infections asymptomatiques pour CT, NG et TV étaient de 4,70 pour 100 femmes (IC 95% : 3,39 – 6,20), 3,11 pour 100 (IC 95% : 1,34 – 5,54) et 5,98 (IC 95% : 3,46 – 9,12), en excluant les femmes avec un risque accru d'IST.

Notre étude a mis en évidence que plus de la moitié des femmes infectées par CT, NG ou TV étaient asymptomatiques dans les pays à revenus faibles et intermédiaires et que la prévalence s'étendait de 3% à 6%. Ces chiffres sont particulièrement alarmants car ces femmes n'ont pas nécessairement conscience d'être infectées, et n'iront probablement pas consulter pour une prise en charge. Cela pourra avoir des conséquences sur leur santé en raison des possibles complications, et elles continueront à transmettre ces IST.

Plusieurs hypothèses peuvent expliquer ces estimations hautes. Tout d'abord, le premier symptôme dont se plaignent les femmes qui ont une IST est généralement l'écoulement vaginal anormal. Il est possible que certaines femmes ne distinguent pas l'écoulement vaginal anormal d'un écoulement physiologique. Aussi, certaines normes sociales peuvent

décourager les femmes de déclarer leurs symptômes. Enfin, la stigmatisation des IST peut constituer un frein à rapporter des symptômes en lien avec une IST.

Nous avons observé que la proportion des femmes asymptomatiques était élevée en Afrique, ce qui constitue un fait inquiétant pour deux raisons. La première est que les IST sont associées à risque accru de transmettre et d'acquérir le VIH, or la prévalence du VIH est la plus élevée en Afrique. La seconde est que l'approche syndromique (qui fait donc que les IST sont traitées à partir des symptômes dont se plaint la patiente) est très répandue en Afrique, ce qui fait que ces infections asymptomatiques ne seront pas détectées ni traitées, contribuant ainsi à la transmission des IST et du VIH.

Parmi les femmes vivant avec le VIH, la proportion d'IST asymptomatiques était élevée. Cette observation a également été faite chez les hommes ayant des relations sexuelles avec les hommes mais les mécanismes biologiques sous-jacents ne sont pas élucidés à ce jour.

Enfin, nous avons retrouvé une plus faible proportion de femmes asymptomatiques chez les travailleuses du sexe. Cela pourrait s'expliquer par une meilleure capacité à reconnaître les symptômes d'IST et de les considérer en tant que tels.

En conclusion, ces proportions et prévalences élevées représentent un problème de santé publique important. Des alternatives à l'approche syndromique sont nécessaires, avec la possibilité de dépister les femmes asymptomatiques également. Pour cela, des tests de diagnostic rapide au chevet des patientes (« *point-of-care tests* », POCT) sont à développer. Dans un premier temps, des outils de prédiction des IST ou de la VB pourraient permettre d'identifier les femmes, asymptomatiques ou non, présentant une probabilité élevée d'avoir une IST ou une VB, ce qui permettrait de minimiser les coûts de dépistage.

Dans cette optique, nous avons élaborés plusieurs scores de risque visant à prédire une probabilité élevée d'infection par une IST ou une VB. Pour cela, nous avons utilisé des données du projet GIFT. Le projet GIFT comporte cinq *work packages* visant à développer un test de détection de l'inflammation génitale (*Genital InFlammation Test*, GIFT). Ce test détecte des marqueurs solubles de l'inflammation génitale que sont les cytokines et chimiokines (IL)-1α, IL-1β et IP-10. Ces biomarqueurs sont également présents chez les femmes asymptomatiques. Ce test a fait l'objet d'une étude diagnostique conduite à Madagascar, au Zimbabwe et en Afrique du Sud et nous avons utilisé les données de cette étude pour construire nos scores de risque.

Dans cette étude, des femmes âgées de 18 à 35 ans, sexuellement actives et non enceintes ont été recrutées dans des centres de planification familiale. Elles ont été interrogées par des enquêtrices formées, sur des questions socio-démographiques, leur comportement sexuel, leur hygiène intime, les antécédents médicaux et reproductifs, leurs traitements et leurs symptômes. Un clinicien, une sage-femme ou une infirmière de l'étude a ensuite rapporté les signes cliniques et a réalisé un examen gynécologique. Des écouvillons vaginaux ont été prélevés pour la détection des IST et de la VB, ainsi que pour le test GIFT.

La détection des IST a été réalisée par des NAAT tandis que le diagnostic de la VB a été réalisé à partir du score de Nugent.

Nous avons dans un premier temps décrit et comparé les caractéristiques des femmes incluses dans cette étude, par pays. Comme notre objectif était d'identifier les femmes à risque pour une IST/VB avant qu'elles ne fassent testées ou examinées, nous avons sélectionné des variables correspondant à des procédures peu ou pas invasives. Par conséquent, toutes les variables en lien avec l'examen physique ou cliniques ont été exclues. Nous avons en revanche conservé le pH vaginal car il a été précédemment décrit comme étant pertinent pour la détection de la VB et qu'il peut être facilement mesuré par la patiente, notamment avec du papier pH.

Nous avons utilisé deux méthodes pour construire nos outils de prédiction : les scores de risque et deux techniques de *machine learning*, plus précisément : des forêts aléatoires suivies d'arbres de classifications. Tous ces modèles ont été construits par validation croisée. Nous avons comparé leur performance en termes de sensibilité, spécificités et valeurs prédictives. Nous avons également produit les courbes ROC associées à chaque modèle afin de comparer leurs performances.

Au total, 706 femmes ont été sollicitées et 676 ont été recrutées. Parmi elles, 673 avaient des échantillons biologiques exploitables, permettant ainsi de calculer les prévalences des IST et de la VB. De plus, 39 participantes n'ont pas souhaité répondre à certaines questions retenues pour la construction des modèles et ont été par conséquent exclues de ces analyses. Ainsi, 634 participantes ont été sélectionnées, dont 212 pour Madagascar, 203 pour l'Afrique du Sud, et 219 pour le Zimbabwe.

L'âge médian était de 27 ans (écart interquartile : 22 – 30 ans). De nombreuses différences distinguaient ces trois populations. A Madagascar, la moitié des femmes vivaient sous le même toit que leurs partenaires (50%) ou étaient mariées (39%), alors qu'en Afrique du Sud la majorité des femmes n'étaient jamais mariées (87%) et au Zimbabwe, la majorité étaient mariées (83%). Le plus haut niveau d'éducation atteint était l'école primaire ou en dessous pour 72% des femmes à Madagascar, et le lycée professionnel ou général pour 65% des femmes en Afrique du Sud et 78% au Zimbabwe. L'accès à des tests pour les IST était facile pour 70% des femmes recrutées en Afrique du Sud, 86% au Zimbabwe, mais seulement 16% à Madagascar. Enfin, 18% des femmes des femmes en Afrique du Sud rapportaient que leur dernier test du VIH était positif, contre 6% au Zimbabwe et 0% à Madagascar. Un peu moins de la moitié des femmes ne présentaient aucun symptôme parmi les suivants : écoulement vaginal anormal, démangeaisons, sensation de brûlure, et douleur abdominale basse (48%).

La prévalence des IST et/ou de la VB était de 55,1% (IC 95% : 51,5 – 58,7%). La prévalence de CT était de 16,6% (IC 95% : 13,9 – 19,7%), de NG 6,4% (IC 95% : 4,7 – 8,5%), de TV de 11,7% (IC 95% : 9,4 – 14,4%) et de la VB, 49,4% (IC 95% : 46,1 – 53,8%). Parmi celles positives pour soit une IST, soit une VB, 19.1% n'étaient touchées que par une ou plusieurs IST, 26,4% avaient à la fois une ou plusieurs IST et une VB, et 54,5% n'avaient qu'une VB.

Six modèles ont été construits pour la prédiction des IST/VB dont cinq avec les scores de risque (modèles 1 à 5) et un avec du *machine learning* (modèle 6). Le premier modèle (modèle 1) a été construit à partir de l'ensemble de femmes incluses. Les prédicteurs d'une plus grande probabilité d'IST/VB étaient les suivants : un pH vaginal élevé, un âge à la première

pénétration vaginale plus jeune, la consommation d'alcool (par rapport à une abstention totale) et un écoulement vaginal anormal. Dans un second modèle (modèle 2), nous ne nous sommes intéressées qu'aux femmes asymptomatiques. Les prédicteurs étaient identiques, à l'exception de l'écoulement vaginal anormal (il ne s'agissait que de femmes asymptomatiques, donc, de fait, l'écoulement vaginal anormal ne faisait pas partie du modèle) et leur effet était du même ordre de grandeur.

Nous avons ensuite réalisé des modèles par pays pour les femmes quel que soit leur statut symptomatique/asymptomatique (modèles 3 à 5) pour Madagascar, l'Afrique du Sud et le Zimbabwe, respectivement. Les prédicteurs d'IST/VB pour Madagascar étaient un pH vaginal élevé et un écoulement vaginal anormal ; pour l'Afrique du Sud, un pH élevé uniquement ; et pour le Zimbabwe, un pH élevé, le fait de ne pas utiliser des préservatifs comme moyen de contraception, et le fait d'être actuellement sous PrEP.

Enfin, le dernier modèle (modèle 6) réalisé par *machine learning sur* l'ensemble des femmes, propose une classification selon deux variables : le pH et l'âge au premier rapport sexuel.

Parmi tous ces modèles, le modèle pour les femmes asymptomatiques (modèle 2) et le modèle appliqué à l'Afrique du Sud (modèle 4) avaient la plus grande sensibilité, respectivement de 79,5% et 78,0%. Les modèles pour l'ensemble de femmes avec les scores de risque (modèle 1) et les modèles pour Madagascar et le Zimbabwe (modèles 3 et 5, respectivement) avaient les spécificités les plus élevées (80,9%, 81,2% et 85,0%). Tous les modèles avaient une valeur prédictive positive supérieure à 80%.

Dans cette étude, les prévalences des IST/VB étaient élevées, avec plus de la moitié des femmes affectées, notamment en raison de la prévalence élevée de la VB. Ce schéma se retrouvait dans tous les pays. Il expliquait probablement les valeurs prédictives positives élevées et observées dans tous les modèles.

Le modèle pour les femmes asymptomatiques était celui qui présentait la plus haute sensibilité, de 80%. Le score dérivé de ce modèle se basait uniquement sur 3 variables : le pH vaginal, l'âge au premier rapport, et consommation ou non d'alcool. Ce score, comme tous les autres présentés dans ce travail, doit encore être affiné pour faciliter son utilisation.

Les facteurs retenus pour ce score étaient différents de ceux trouvés par Tyndall *et al*. Leur score de risque pour une IST asymptomatique comportait les variables suivantes : un âge inférieur à 25 ans, être célibataire, et avoir eu plus d'un partenaire sexuel. La sensibilité de ce score était de 60% et sa spécificité de 57%. De même, dans une autre étude conduite en Ouganda, l'âge inférieur à 25 ans et avoir eu plus d'un partenaire étaient associés à une infection asymptomatique. La divergence entre ces facteurs de risque peut être imputée au design de l'étude, notamment sur les variables récoltées, au fait que nous avons ajouté la VB dans notre *outcome* mais aussi à des différences entre les populations étudiées.

Nous avons mentionné le pH vaginal comme variable prédictive d'une STI/VB. Cette variable est ressortie dans tous nos modèles. Il est déjà connu qu'un pH élevé est associé à une VB, cela est une composante du critère d'Amsel. L'augmentation du pH peut s'expliquer par une

diminution des lactobacilles qui produisent de l'acide lactique contribuant à maintenir un environnement vaginal acide. Un pH élevé peut également indiquer une infection par TV.

Les études précédentes qui ont cherché à utiliser le pH dans un score de risque pour la VB ont montré des résultats contrastés. Une étude a montré qu'un pH > 4,5 était fortement associé à une VB, tandis que l'autre n'a pas montré de lien, possiblement en raison d'un seuil plus élevé (à 5). Dans notre modèle 6 basé sur des méthodes de *machine learning*, nous avons trouvé pour les valeurs de pH comprises entre 4,3 et 4,9, une seconde question relative à l'âge était nécessaire pour affiner de classer la femme comme infectée par une IST ou non. Ce résultat suggère que, pour certaines valeurs, le pH n'est pas suffisant pour prédire avec précision les IST/VB et qu'il pourrait être combiné avec d'autres indicateurs.

Il est important de noter que malgré les importantes différences socio-démographiques qui existaient entre les femmes recrutées dans les trois pays, le pH vaginal s'est retrouvé dans tous les modèles spécifiques par pays modèles 3 à 5). Notamment en Afrique du Sud, le pH vaginal seul était suffisant à prédire une IST/VB, et ce modèle était celui qui avait la plus haute sensibilité parmi tous les modèles pays-spécifiques. L'utilisation d'une variable biologique comme prédicteur indique qu'elle pourrait être utilisée de manière universelle. Toutefois, plusieurs études ont monté que le pH vaginal pouvait changer selon l'ethnicité, même après ajustement des données socio-démographiques, de l'activité sexuelle, des IST, du comportement sexuel et de l'hygiène intime. D'autre études sont nécessaires pour déterminer les possibles variations régionales de pH vaginal et éventuellement établir un seuil selon la zone concernée. Des dispositifs d'autotest du pH ont déjà été commercialisés, toutefois d'utilisation du papier pH pourrait être une option plus coût-efficace pour les PRFI.

Pour développer ces modèles, nous avons utilisé deux techniques : la régression logistique et des méthodes de *machine learning*. A notre connaissance, cette étude est la première à utiliser du *machine learning* pour la détection des IST/VB dans les PRFI. Les scores de risque construits avec des modèles de régression logistique avait une sensibilité de 68,7%, relativement similaire à cette trouvé avec les techniques de *machine learning*, de 71,6%. Toutefois, ce dernier était plus facile à interpréter, avec uniquement deux questions et un arbre de décision, ce qui réduit le risque d'erreur.

Cette étude avait plusieurs points forts. D'abord, elle a été conduite sur des populations avec des prévalences d'IST et de VB différentes. Cette hétérogénéité permet de réduire le risque que nos modèles ne soient applicables qu'à une seule population. Par ailleurs, nous avons conduit l'ensemble de nos modèles par validation croisée, ce qui consolide nos estimations de performance et évite le surajustement.

Cette étude avait également des limites. Il est possible que nos modèles prédisent la VB plus qu'ils ne prédisent les IST, en raison de la prévalence élevée de VB. Bien que cela soit une information importante pour les femmes enceintes ou les femmes symptomatiques, la détection d'une VB asymptomatique n'est pas nécessairement prioritaire, dans la mesure où elle n'est pas systématiquement traitée. Ensuite, le pH vaginal a été retrouvé dans tous nos modèles, toutefois nous n'avions pas d'information sur la présence de sperme qui peut augmenter sa valeur.

Cette étude a permis de développer des scores de risque/arbre de décision faciles à utiliser et qui pourraient permettre d'être traitée pour IST/VB sans même présenter les symptômes de point d'entrée dans les recommandations de l'OMS. D'autres études devront confirmer que les variables retenues pour ces modèles sont bien acceptées et comprises à la fois par le personnel de santé ; mais également par les femmes qui se feront dépistées. Plus particulièrement, des études sur l'interprétation du papier pH pourraient également valider ou invalider son utilisation en pratique.

En conclusion, les travaux menés dans le cadre de cette thèse avaient pour objectif d'améliorer notre compréhension des IST et la VB dans les PRFI avec une attention particulière sur les formes asymptomatiques. Ce travail a permis de fournir des données épidémiologiques récentes dans des pays où cela manquait. Il a également montré la prévalence et la proportion importante d'IST asymptomatiques dans les PRFI. Enfin, ces travaux proposent un outil de dépistage peu cher, permettant de détecter les IST et la VB, y incompris pour les formes asymptomatiques. Cet outil, combiné à l'approche syndromique de l'OMS pourrait *in fine* améliorer la santé de ces femmes.

Definitions

Reproductive Tract Infections

Reproductive tract infections (RTIs) are infection of the genital tract, caused by (i) organisms introduced from the outside during sexual contact (referred to as sexually transmitted infections [STIs]), (ii) an overgrowth of organisms normally present in the reproductive tract such as bacterial vaginosis (BV) or vulvovaginal candidiasis (VVC) (referred to as endogenous infections), or (iii) organisms introduced during medical procedures (referred to as iatrogenic infections).[1]

Sexually Transmitted Infections

STIs are infections with bacteria, viruses, or other microorganisms that can be passed from one person to another through blood, semen, vaginal fluids, or other body fluids as a result of a sexual act involving the penis, vagina, mouth, or anus with an infected partner. These infections can also be spread through sharing needles, blood transfusions, breastfeeding, or from an infected mother to an infant during pregnancy and childbirth.[2] Some STIs can be transmitted by skin-to-skin contact with infected areas (mouths, genitals) such as herpes and human papillomavirus (HPV).

Symptoms versus signs

We define symptoms as subjective manifestations of a disease that is apparent to the patient whereas signs are objective features of a disease observed by a health care provider or other person.[3]

Screening

The purpose of screening is, according to the World Health Organization (WHO), to "identify people in an apparently healthy population who are at higher risk of a health problem or a condition, so that an early treatment or intervention can be offered and thereby reduce the incidence and/or mortality of the health problem or condition within the population."[4]

We distinguished the screening test from the screening programs:

- A screening test "sort out apparently well persons who probably have a disease from those who probably do not."[5] It is not intended to be diagnostic and those with positive findings must be referred for diagnosis and treatment.
- A screening program is the systematic process of testing individuals in a population to identify those at risk for a specific condition or disease. It is a public health intervention which may have different aims which include reducing mortality, incidence, or severity of a condition.[4]

Case management

WHO describes that comprehensive STI case management should "provide treatment, obtain cure, reduce infectiveness, reduce the risk of developing complications of STIs and reduce or prevent future risk-taking behavior, including in other biobehavioral interventions, such as HIV pre-exposure prophylaxis (PrEP) and voluntary medical male circumcision, and ensure that sex partners are appropriately treated."[6]

Chapter 1: Introduction

Reproductive Tract Infections (RTIs) are among the most important causes of maternal and perinatal morbidity and mortality.[1] The number of new cases of the three main curable sexually transmitted infections (STIs) (*Chlamydia trachomatis* [CT], *Neisseria gonorrhoeae* [NG] and *Trichomonas vaginalis* [TV]) globally is estimated to be 170 million per year among women of reproductive age, with around 20% of these infections occurring in Africa alone.[7] Among women, serious complications of STIs include pelvic inflammatory disease (PID) which can evolve into tubal factor infertility and increase the risk of ectopic pregnancy.[8,9] Some infections are associated with preterm delivery and low birth weight.[10,11] In addition, STIs are associated with an increased risk of human immunodeficiency virus (HIV) acquisition.[12,13] The prevalence and incidence of STIs/RTIs are particularly concerning in low- and middle-income countries (LMICs),[14] where, additionally, their detection can be challenging. Thus, a majority of patients are treated according to the symptoms they complain about and the clinical signs they present. This approach, known as syndromic management, lacks specificity and, by definition, misses asymptomatic infections.

The main aim of this thesis is to improve our understanding and the identification of STIs and BV among women in LMICs, with particular attention to asymptomatic women. To illustrate the extent of asymptomatic STIs among women of reproductive age, in a country for which data is lacking, I will present the results from a cross-sectional study we conducted in Madagascar in 2022. In this study, I determined the prevalence of RTIs, risk factors associated with having an STI, and the limitations of the syndromic approach owing to asymptomatic infections. In a second piece of work, I conducted a systematic review covering studies published over the past 20 years and meta-analysis where I quantified, in LMICs, the proportion of women missed by the syndromic approach because of being asymptomatic. Finally, in my third project, I will present different STI management risk scores and algorithms that aim at improving the detection of women with an STI and/or bacterial vaginosis (BV) among all women and asymptomatic women.

1.1. Overview of reproductive tract infections among women

1.1.1. Sexually transmitted infections

More than 30 different bacteria, viruses, and protozoa are known to be transmitted through sexual contact. The most common infections are caused by CT, NG, TV, *Treponema pallidum* (causing syphilis), HIV, hepatitis B virus (HBV), herpes simplex virus (HSV), and human papillomavirus (HPV).[15] Four of the infections caused by these pathogens are currently curable: chlamydia, gonorrhea, trichomoniasis, and syphilis. *Mycoplasma genitalium* (MG) is another curable STI that is becoming increasingly common.[16] Symptoms of infection by CT, NG, TV, and MG include among women abnormal vaginal discharge and lower abdominal pain, which are entry points into syndromic management. In this thesis, we will focus on these four STIs as they are the most common worldwide, are all curable, and share the same symptomatology.

In the next sections, we will describe in detail the pathogens, the incubation period, clinical signs and symptoms in women, complications, and treatment options for CT, NG, and TV, as well as MG, discovered forty years ago.

1.1.1.1. Chlamydia trachomatis

Chlamydia trachomatis is an important genital pathogen and the most common bacterial cause of STIs. It is a true obligate intracellular pathogen with a slow growth cycle which tends to make chlamydial infection chronic in nature. Chronic diseases are defined as conditions lasting for one year or more, and CT infections have been documented to persist for 15 months or more.[17,18] Chlamydial infections of the genital tract are primarily caused by serovars D, E, F, G, H, I, J, and K.[19] Not much is known about the specific virulence of the different genital *C. trachomatis* serovars.[20] However, using recent progress in molecular techniques, experts revealed that serovar classification may not be always appropriate and that genovariants and genotypes should be used instead to account for CT's frequent genomic recombination.[21]

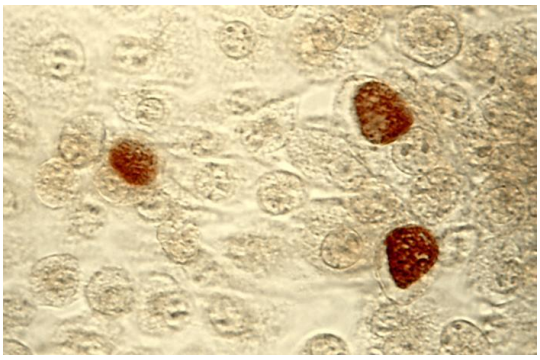


Figure 1.1. Microscopy of *Chlamydia trachomatis*
(Credit: Public Health Image Library, U.S. Centers for Disease Control and Prevention, ID #3802)

The majority of CT genital infections are silent. If symptoms occur, they usually appear within 1 – 3 weeks of exposure. Women who have symptoms may have an abnormal vaginal discharge or dysuria. When the infection spreads from the cervix to the upper genital tract, some women will experience lower abdominal pain, low back pain, nausea, fever, dyspareunia, or bleeding between menstrual periods. Chlamydial infection of the urogenital tract can spread to the rectum by autoinoculation and vice-versa.[22] Infection in the rectum can also be acquired through anal intercourse or after oral sexual exposure.[23] Anorectal symptoms include pain, discharge, or bleeding.[24]

Characteristic clinical features of chlamydial cervicitis are mucopurulent endocervical discharge, edema, hypertrophy or cervical ectopy, and induced mucosal bleeding.[25] Ascending infections can lead to severe complications such as PID which may result in infertility, ectopic pregnancy, and chronic pelvic pain.[8,9] Sometimes, chlamydial PID can cause upper abdominal infection which manifests as perihepatitis. In pregnant women, there is some evidence that CT infection is associated with premature rupture of membranes, spontaneous abortion, preterm labor, small for gestational age, and low birth weight.[26] Mother-to-child transmission can occur during delivery and may result in ophthalmia neonatorum or pneumonitis in the neonate.[27] Postpartum endometritis is also associated with CT infection.[26] Moreover, CT is associated with an increased risk of HIV acquisition and transmission.[12]

Antibiotic therapy successfully clears CT infections among those who seek clinical treatment or are screened for infection. WHO recommends doxycycline 100 mg, orally twice daily for 7

days.[6] A single-dose therapy, azithromycin 1 gram orally, can be a preferred option for less compliant patients, such as adolescents or asymptomatic individuals, and is also recommended for pregnant or breast-feeding women. Effective alternatives include erythromycin or ofloxacin.

1.1.1.2. Neisseria gonorrhoeae

Neisseria gonorrhoeae is a diplococcal bacterium composed of two cells joined by a flattened adjacent side exhibiting a coffee bean-like morphology. This obligate human pathogen has been co-evolving with its human host for centuries. The genome, morphology, and phenotype of NG are closely related to the other *Neisseria* species such as *Neisseria meningitidis*, which occasionally causes fatal septicemia and/or meningitis.[28] NG predominantly infects columnar epithelium-lined mucous membranes as found in the endocervix and the transitional squamocolumnar epithelia but can also attach to the stratified squamous epithelium of the ectocervix.[29]

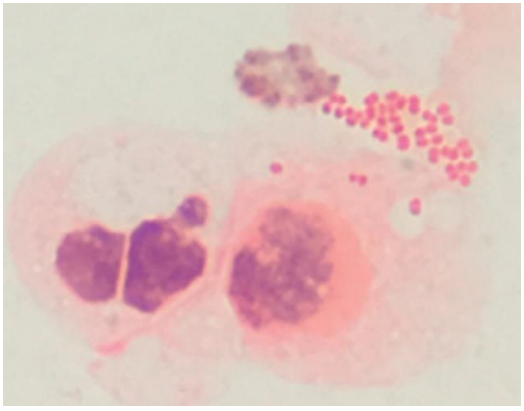


Figure 1.2. Microscopy of *Neisseria gonorrhoeae*
(Credit: Photo Library of Saint Louis Hospital, Paris)

The incubation period ranges from 2 to 8 days. When present, symptoms can appear as a vaginal discharge due to increased exudate from the cervix which may be thin or watery and green or yellow in color, dysuria, or, less commonly, vaginal bleeding between periods, lower abdominal pain or tenderness. However, most women are asymptomatic and are diagnosed

as sexual contact of infected partners or by screening. Pharyngeal gonorrhea is mostly asymptomatic. Infection in the rectum causes discomfort, pain, or discharge.[30]

Clinical examination may be normal, or a mucopurulent discharge may be evident from the cervix, sometimes accompanied with hyperemia and contact bleeding of the cervix.[30]

Infections that are not detected or adequately treated may ascend in the genital tract, causing salpingitis which can result in serious complications and sequelae. These complications and sequelae comprise PID, chronic pelvic pain, ectopic pregnancy, and infertility.[25] During pregnancy, NG is associated with premature rupture of membranes and perinatal mortality in addition to preterm birth and low birth weight.[11] Infected women can transmit the infection to the neonate during delivery causing ophthalmia neonatorum.[11] Conjunctivitis is also observed sporadically.[28] Disseminated gonococcal infections, when the organism invades the bloodstream and causes a systemic infection, are rare but can occur in both adults and neonates. The infection presents then as arthritis with or without septicemia, and a rash. NG is also associated with an increased risk of HIV acquisition.[12]

The treatment of NG is challenging as it has developed or acquired antimicrobial resistance. This antimicrobial resistance is of serious public health concern as the pathogen has become highly resistant to all previously recommended antimicrobials (sulfonamides, penicillins, tetracyclines, fluoroquinolones, early-generation of macrolides, such as erythromycin, and cephalosporins, such as cefuroxime) and resistance to the currently recommended ceftriaxone has emerged.[31] The new recommended first-line treatment by WHO is ceftriaxone 500 mg to 1 g, and, where resistance to cephalosporin is a concern, spectinomycin 2 g (or gentamycin 240 mg) intramuscularly as a single dose plus azithromycin 2 g orally.[32]

1.1.1.3. Trichomonas vaginalis

Trichomonas vaginalis is the etiological agent of trichomoniasis, the most prevalent curable non-viral sexually transmitted infection in women globally.[7] TV is an anaerobic, flagellated protozoan parasite, that can survive in a moist environment.[33] This characteristic may explain documented non-sexual transmission, via fomites or water, which has been described but remains rare.[34–38] The adherence of TV to the epithelial cell surface is a crucial factor in its pathogenesis and typically results in the lysis of the host cell and erosion of the epithelial

layer. The higher likelihood of persistence in the female genital tract has been linked to the availability of iron during menstrual cycles, an essential nutrient.[25]



Figure 1.3. Microscopy of *Trichomonas vaginalis*
(Credit: Department of Health and Human Services, U.S. Centers for Disease Control and Prevention)

Symptoms of trichomoniasis usually develop within 3 to 28 days of infection.[39] Symptoms include a vaginal discharge that may be thick, thin or frothy, yellow-green in color, and which may also have an unpleasant fishy smell; soreness, swelling and pruritus around the vagina, dyspareunia and dysuria. During clinical examination, punctate hemorrhagic spots (colpitis macularis or strawberry cervix) may be present on the vaginal and cervical mucosa but is rare.

TV infection is associated with cervicitis, PID, and infertility.[25] Its association with cervical cancer remains controversial.[40] During pregnancy, TV infection is associated with preterm delivery, low birth weight [10] and premature rupture of placental membranes.[41] After birth, neonatal genital and nasopharyngeal infections have also been reported. In studies reporting hazard ratios, individuals infected with TV are 1.5 times more likely to acquire HIV compared with non-infected individuals (95% Confidence Interval [CI]: 1.3 to 1.7).[13]

WHO recommends as a first-line option for adults and adolescents, including pregnant women, treatment by metronidazole 400 mg or 500 mg, orally, twice daily for 7 days.[42]

1.1.1.4. Mycoplasma genitalium

Mycoplasma genitalium is a flask-shaped organism with a terminal organelle, distinct from other pathogenic mycoplasma species. It was first isolated in 1981 from the urethral exudates

of men with non-gonococcal urethritis. The lack of genes involved in amino acid synthesis makes MG totally dependent on the exogenous supply of amino acids, which explains in part the difficulties in cultivating this microorganism.[25] With the development of molecular methods, this sexually transmitted organism has received increased attention.



Figure 1.4. Microscopy of *Mycoplasma genitalium*
(Credit: Thomas Deerinck, NCMIR, Science Photo Library)

The pathogen adheres to host epithelial cells in the urogenital tract, thus causing an activation of proinflammatory signals ultimately resulting in leucocyte recruitment. Factors leading to immune evasion are unclear.[43] No data on the incubation period is available, however a 2-week period is considered the time after exposure to testing.[44] Although testing usually occur after the onset of symptoms, up to 50% of women with MG may not display any.[45] Women developing symptoms may experience abnormal vaginal discharge, dysuria, abdominal pain, dyspareunia, intermenstrual or post-coital bleeding.[44] Mucopurulent cervicitis and cervical or vaginal discharge are clinical signs indicating laboratory testing. Nevertheless, vaginal discharge may not necessarily occur.[46] A meta-analysis showed that MG infection was associated with an increased risk of PID, preterm birth, spontaneous abortion, and infertility.[47] Evidence of an association between MG and HIV-1 infection has also been documented.[48]

The recommended treatment by WHO is doxycycline 100 mg orally twice daily for 7 days, followed by azithromycin 1g orally for 1 day then 500 mg once daily for 3 days in settings with low or suspected low resistance to macrolides. In settings with high or suspected high resistance to macrolides, WHO suggests doxycycline 100 mg orally twice daily for 7 days,

followed by moxifloxacin 500 mg orally once daily for 7 days.[42] European guidelines recommend moxifloxacin as a second line therapy. Given the lack of natural history studies estimating the risk of sequelae of asymptomatic infections, the European guidelines do not recommend treatment of asymptomatic infections, owing to the risk of antimicrobial resistance and adverse events.[49] Increasing prevalence of mutations associated with azithromycin/moxifloxacin resistance is a major growing concern even though a high heterogeneity across regions has been observed.[50]

1.1.2. Endogenous infections

Other RTIs can impact women's health such as BV or VVC. Both are extremely common conditions. They cause vaginal discharge and itching – symptoms that are shared with STIs – and thus may lead to misdiagnosis and subsequently, inappropriate treatment. The potential to confuse STIs for BV or VVC based on their symptoms and vice versa leads us to briefly describe those two endogenous RTIs.

1.1.2.1. Bacterial vaginosis

The optimal vaginal microbiome is characterized by the dominance of *Lactobacilli spp*. BV occurs when this ecosystem is disturbed and that *Lactobacilli* are replaced by bacteria such as *Gardnerella* spp., *Fannyhessea vaginae*, *Prevotella* spp., *Mobiluncus* spp. and others.[51] The reduction and/or loss of lactic acid-producing Lactobacilli cause the elevation in vaginal pH. The factor triggering this overgrowth of bacteria and the exact agents responsible for BV remain unknown, but *Gardnerella* spp. are considered to have a central role, owing to their propensity to adhere to vaginal epithelial cells and to initiate a biofilm.[52,53] *Gardnerella* spp. and *Prevotella* spp. produce sialidases which degrade the cervicovaginal mucus, and consequently enhance biofilm formation by facilitating the attachment of other BV-associated bacteria.[54,55] This pathogenic process causes signs and symptoms which form the criteria of a common clinical diagnostic method: Amsel criteria (outlined in section 1.3.1.2).[56] The mucinases and sialidases released by BV-associated bacteria occasion vaginal discharge and cause increased exfoliation of vaginal epithelial cells that are visible on

microscopy as “clue cells”. Some BV-associated bacteria produce volatile amines, which are responsible for malodor and are at the origin of the “amine test” or “whiff test”.[57–59] The laboratory reference method for the diagnosis of BV is the Nugent score (described in section 1.3.1.2).

BV is often asymptomatic, but, as described above, symptomatic women present a profuse vaginal discharge, with a characteristic fishy odor, and that may appear after unprotected coitus.[60] Pruritus, dysuria, and dyspareunia are rare.[25]

Serious obstetric and gynecologic complications may arise from BV. In pregnant women, BV was found to be associated with low birth weight, preterm delivery, premature rupture of membranes, and chorioamnionitis, including in sub-Saharan Africa.[61–63] Consequences have also been described among asymptomatic pregnant women for whom BV was associated with preterm delivery, late miscarriages, maternal infections.[64]

In non-pregnant women, BV may be associated with incident PID among women at risk for STIs or vaginal disturbance [65] and with endometritis.[66] Furthermore, it was suggested that BV is associated with infertility and more specifically with tubal infertility but not necessarily with a decreased conception rate.[67] BV was shown to be associated with acquiring HIV infection,[68] and other viral and bacterial STIs.[69–71]

The current first-line option recommended by WHO for BV is metronidazole, 400 or 500 mg orally, twice daily for 7 days. Effective substitutes include clindamycin 300 mg, twice daily for seven days.[6] However, recurrence has been reported in 60% of the women with BV over one year.[72] Reasons for recurrence are unclear and may include the persistence of residual infection, resistance to antimicrobials, and possibly reinfection from either a male or female partner.[73] Persistence may occur due to the formation of a biofilm that protects BV-causing bacteria from antimicrobial therapy and resistance may result from poor adherence to treatment of genitourinary infections. Recommended treatment for recurrent BV consists of a course of metronidazole 500 mg twice daily for 10–14 days. If ineffective, metronidazole vaginal gel 0.75% for 10 days, followed by two times per week for 3–6 months, is an alternate treatment option.[73] The role of probiotics in the treatment for BV remains debated.[74,75]

1.1.2.2. Vulvovaginal candidiasis

Vulvovaginal candidiasis is another major common cause of vaginitis.[76] *Candida albicans* (CA), along with other related *Candida* species, are the primary causative agents of VVC. The opportunistic yeast CA commonly colonizes the vaginal canal.[77] Infection can be asymptomatic or symptomatic. Common symptoms of VVC include vaginal itching, vaginal discharge, burning, and pain.[78] Signs include vulvar edema, fissures, excoriations, redness, and thick curdy vaginal discharge. VVC can also be a recurrent condition,[79] possibly due, in some cases, to the presence of *Candida albicans* in the rectum acting as a reservoir.[80]

WHO recommends the following options:[42]
- fluconazole 150–200 mg orally as a single dose;
- clotrimazole 500 mg intravaginally as a single dose, or 200 mg intravaginally once daily for 3 days, or 10% cream intravaginally once;
- miconazole 1200 mg intravaginally as a single dose or 400 mg intravaginally once daily for 7 days;
- econazole 150 mg intravaginally as a single dose; or
- nystatin 100 000 units intravaginally twice daily for 15 days.

1.1.3. Iatrogenic infections

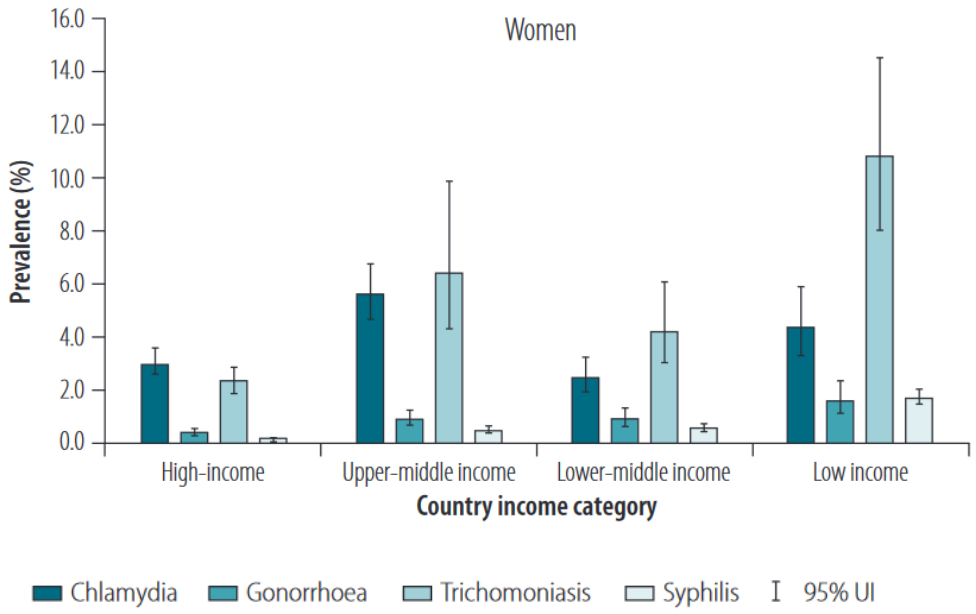
Iatrogenic infections are infections that are passed to the women following examination or intervention during pregnancy or delivery, or through medical procedures. Contaminated needles or other instruments may transmit infection if infection control is poor. Transcervical procedures, such as manual vacuum aspiration, dilatation and curettage or insertion of an intrauterine device (IUD) are an example of medical procedures during which such infections can occur. This risk can be reduced by using appropriate infection prevention procedures and aseptic techniques along with treating existing cervical infection. This thesis will not focus on iatrogenic infections.

1.2. Epidemiology of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Mycoplasma genitalium*, and bacterial vaginosis

1.2.1. *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*

WHO estimated that 367 million new infections occur every year: 129 million with CT, 82 million with NG, and 156 million with TV.[14] The global prevalence in 15-49-year-old women for CT is 3.8% (95% UI: 3.3 – 4.5), for NG 0.9% (95% UI: 0.7 – 1.1) and for TV 5.3% (95% UI: 4.0 – 7.2).[14]

The prevalence of CT is the highest in upper middle-income countries (as classified by the World Bank) and the second highest in low-income countries. For NG and TV, high-income countries had the lowest prevalence while low-income countries had the highest prevalence (**Figure 1.5**).



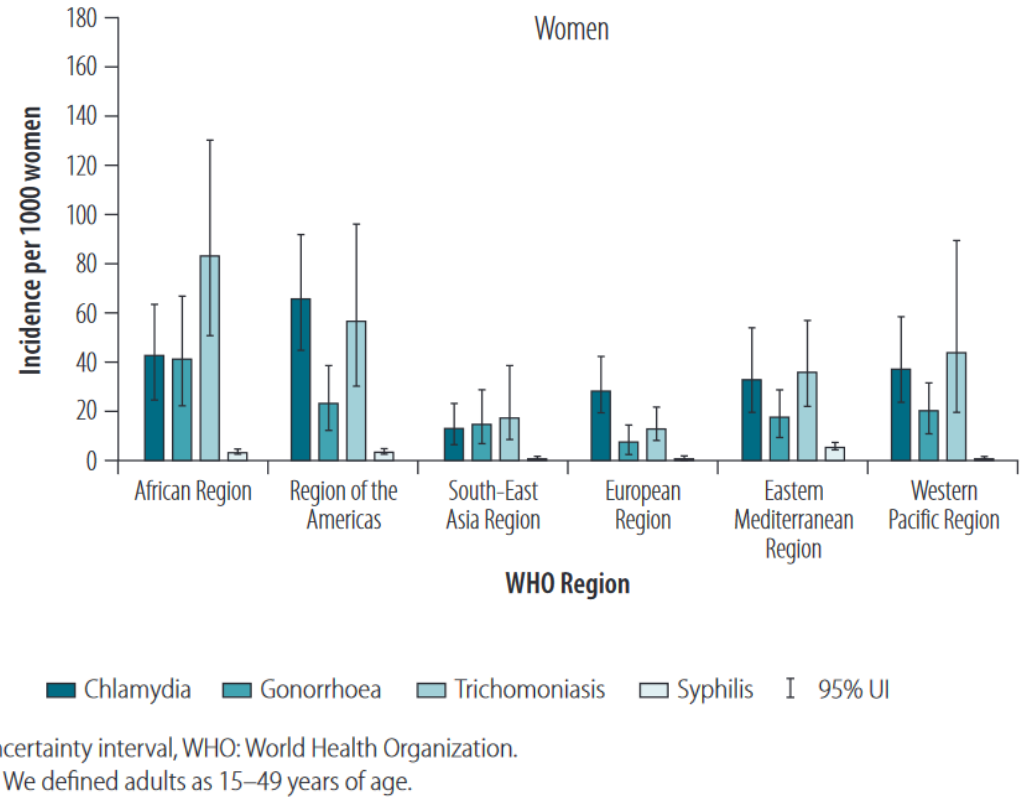
UI: uncertainty interval.
Notes: We defined adults as 15–49 years of age and used year 2016 income classification from the World Bank.[17]

Figure 1.5. Prevalence estimated of CT, NG, TV, and syphilis in women, by World Bank classification 2016 (from Rowley et al., 2019 [14]).

The global incidence rate for CT was 34 cases per 1000 women per year (95%UI: 25 – 45), for NG 20 per 1000 (95%UI: 14 – 28) and for TV 40 per 1000 (95%UI: 27 – 58). Of all regions, the WHO region for Americas had the highest incidence rate for chlamydia in women (**Figure 1.6**). The WHO African region has the highest incidence of NG and TV in women.

Nearly 92% of new infections by CT, NG, TV, and syphilis occur in LMICs: 13.5% (50.8 million) in low-income countries, 31.4% (118.1 million) in lower middle-income, 47.1% (177.3 million) in upper middle-income.

Although, at the global level, the age-standardized incidence rate of CT, NG, TV, syphilis and genital herpes shows a decreasing trend between 1990 and 2019, the change of incident cases remains positive in LMICs (**Figure 1.7**).[81]



UI: uncertainty interval, WHO: World Health Organization.
Note: We defined adults as 15–49 years of age.

Figure 1.6. Incidence rates for CT, NG, TV, and syphilis in women, by WHO regions (from Rowley et al., 2019 [14]).

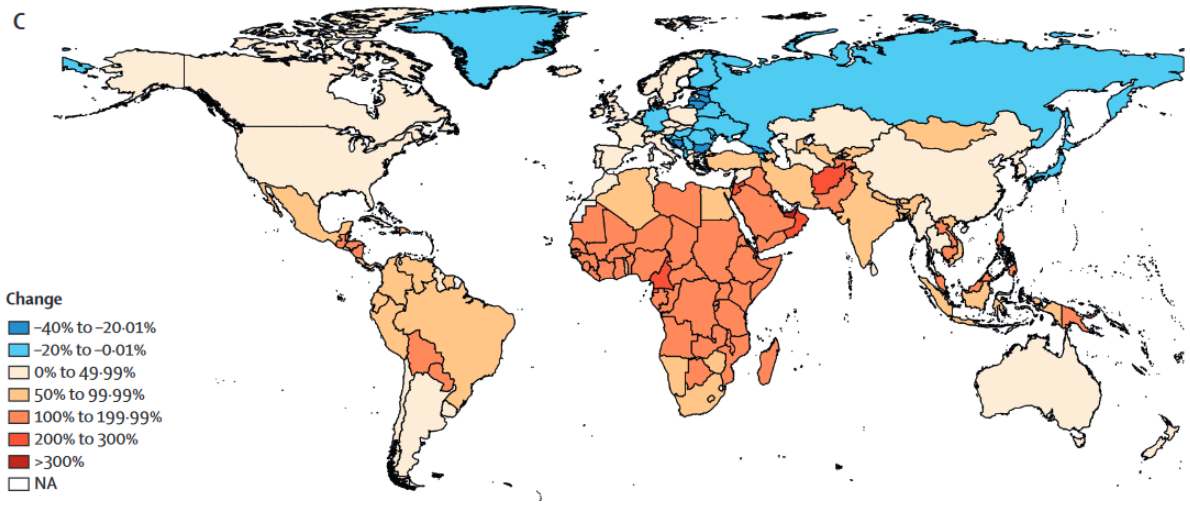


Figure 1.7. Change of incident cases of CT, NG, TV, syphilis, and genital herpes from 1990 to 2019 (from Zheng, 2022 [81]).

NA = not applicable

Furthermore, it is worth noting that adolescents have a higher STI prevalence than other age groups and that in LMICs, the population is generally younger which may explain the increased burden.

1.2.2. *Mycoplasma genitalium*

Data on MG epidemiology is scarce. A systematic review and meta-analysis found that the summary average prevalence in adults aged 16 to 44 was 1.3% (95%CI: 1.0 – 1.8) in countries with higher levels of development, 3.9% (95%CI: 2.2 – 6.7) in countries with lower levels (represented in this meta-analysis by only three countries: Honduras, Vietnam, and Tanzania). Moreover, there is growing evidence that the prevalence of MG is higher among younger women and lower among older women.[16,45]

1.2.3. Bacterial vaginosis

BV prevalence has been shown to vary across ethnicity within countries. Studies in the US or in Canada demonstrated that BV prevalence was highest in Blacks, and lowest in Whites and Asians, and intermediate in Hispanics. Ethnic differences have also been observed in the UK, Canada, China, Iran and Peru. In addition, BV prevalence correlates with HIV prevalence. Thus, BV prevalence tends to be highest in sub-Saharan Africa where it ranges from 25% to nearly

60%, with the exception of few studies. By opposition, BV prevalence was the lowest in Asia, Oceania and Western Europe where only few studies had BV greater than 20%.[82]

In sub-Saharan Africa, regional differences were also found with a prevalence of BV of 42.1% (95%CI: 35.6 – 49.0%) in South Africa, and 35.2% (95%CI: 27.7 – 43.6%) in Southern and Eastern Africa (excluding South Africa) in women aged 15 to 24.[83]

We have seen in this section that the prevalence and incidence rate of CT, NG, TV, MG, and BV are notably high in LMICs. However, epidemiological data on STIs in some areas lack attention despite experts' recommendations.[84] With no data published over the past 20 years on the prevalence of CT, NG, TV and MG in the general population, Madagascar represents one those countries where the epidemiology of STIs is poorly documented. In this thesis, we determined the prevalence of CT, NG, TV, MG, CA, and BV and the factors associated with having an STI among women of child-bearing age in Antananarivo, the capital city of Madagascar (**Chapter 3**).

1.2.4. Burden of complications associated with these conditions

Pelvic inflammatory disease is an important complication associated with STIs/BV. It is an inflammatory process of the upper genital tract which may include the uterus, fallopian tubes, and related pelvic organs. It could lead to salpingitis and tubal adhesion which increase the possibility of ectopic pregnancy. The Global Burden of Disease study conducted in 2019 collected data on the prevalence and incidence of PID and ectopic pregnancy in 204 countries. It revealed that 1.05 million women between 15-49 years had an active PID globally (95%UI: 0.77, 1.43 million). The age-standardized rate of PID prevalence was 53.2 per 100,000 women (95%UI: 33.0, 88.6). Concurrently, the number of incidence cases for ectopic pregnancy was 6.68 million (95%UI: 5.21, 8.58 million) and the incidence rate was estimated to be 342.4 per 100,000 women.[85]

1.3. Detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, and bacterial vaginosis

1.3.1. The reference standards

1.3.1.1. Sexually transmitted infections

The gold standard for the detection of CT, NG, TV, and MG relies on the direct detection of microorganisms using quality-assured molecular laboratory tests. Nucleic acid amplification tests (NAATs) are the most sensitive of molecular diagnostic tests.[86] The commercial amplification technologies sold by different manufacturers are polymerase chain reaction (PCR), transcription mediated amplification, and strand displacement amplification. In the two field studies we conducted, and which are described in this thesis, we used real-time PCR-based assays. Contrary to conventional PCR, real-time PCR monitors and measures the amplification of DNA during the amplification process, and not at its end. This reduces considerably the analytical time and avoids amplicon contamination. In addition, it may give an estimate of the amount of microorganisms' DNA present in the biological sample.

For a NAAT to be successful, specimens need to be appropriately sampled, stored and transported. Nucleic acid extraction/purification, amplification and detection are other critical stages. When properly conducted, NAATs have a high sensitivity permitting the detection of pathogens at low concentrations and from asymptomatic infections or at an early phase of infection. However, the high sensitivity can also be problematic because even low levels of contamination can lead to false-positive results. In addition, this technique also detects genetic material of dead organisms, meaning that a positive result does not necessarily mean an ongoing infection.

Multiple barriers hamper the use of NAATs in resource limited settings. Equipment and reagents can be expensive. The process of NAAT testing is considered to be a technique of high complexity and generally requires well-trained personnel and sophisticated laboratory setting. Operating these techniques, interpreting the results, and ensuring quality control often require specialized training and expertise, which may be lacking in settings with limited access to education and training programs. In addition, the procurement of reagents and

consumables may be challenging due to unreliable supply chain and difficulties in transportation. Electricity, temperature-controlled environments and specialized equipment are necessary both for sample collection, storage, and for running NAATs and may not be available. In addition, access to maintenance and servicing may not always be available or if available, it is at a high cost. Finally, test turnaround time requires patients to return to the clinic for results which can lead to significant loss to follow-up. These requirements limit access to diagnosis and treatment. Therefore, providing testing near the patient or at the point-of-care (POC) is essential.

1.3.1.2. Bacterial vaginosis

The detection of BV can be done using a clinical approach or a laboratory approach.

In clinical approach, known as the **Amsel criteria,** BV is diagnosed if at least three of the following four criteria are present:

- Homogenous white-grey adherent discharge,
- A vaginal fluid pH > 4.5,
- The release of a fishy amin odor from the vaginal fluid when 10% potassium hydroxide solution is added (known as whiff or sniff test),
- “Clue cells” visible on microscopic examination.

Clue cells are squamous epithelial cells covered with numerous small coccobacillary organisms with a granular appearance.

BV is characterized by an increase in diversity and load of certain bacteria, the laboratory approach relies on semi-quantifying different morphologies of bacteria by microscopical examination of a Gram-stained vaginal smear, a technique known as the **Nugent score**.[87] The score uses a weighted combination of the following organisms: large Gram-positive rods (Lactobacilli), Gram-negative or Gram-variable rods (e.g. *G. vaginalis*); and curved Gram-negative or Gram-variable rods (e.g. *Mobiluncus* spp.). Semi-quantifications of the morphotypes per high-power field are averaged from 5 representative fields. The weights of the averaged counts are given as outlined in **Table 1.1**.

The sum of these three scores provides the Nugent score where the criteria are:

- 0 – 3 is considered an optimal microbiota
- 4 – 6 is considered an intermediate microbiota
- 7 – 10 is diagnostic of BV

Table 1.1: Nugent's scoring system.

Score	Gram-positive rods (lactobacilli)	Gram-negative and Gram-variable rods	Curved rods
0	> 30	0	0
1	5 – 30	< 1	1 – 5
2	1 – 4	1 – 4	> 5
3	< 1	5 – 30	
4	0	> 30	

1.3.2. Point-of-care tests

1.3.2.1. Definition

A consensus definition proposed for a provider-based POC test (POCT) is:

"*A test to support clinical decision making, which is performed: (i) by qualified staff nearby the patient; (ii) during or very close to the time of consultation; (iii) to help the patient and clinician to decide upon the most appropriate approach; and (iv) for which result should be known at the time of clinical decision making*".[88,89]

This definition encompasses a variety of formats such as lateral flow tests referred to as rapid diagnostic tests (RDTs) or molecular test as long as they allow treatment decision at the time of patient encounter. To meet this definition, a POCT requires certain characteristics.

1.3.2.2. Characteristics of a point-of-care test

The ideal POCT should meet the following criteria represented by the acronym "ASSURED":

A = Affordable
S = Sensitive
S = Specific
U = User-friendly
R = Robust and rapid (stored at room temperature and results available in <30 min)
E = Equipment-free or minimal equipment (solar- or battery-powered)
D = Deliverable to those who need them

Two additional criteria have been recently proposed: real-time connectivity (R) and ease of specimen collection (E), leading to the acronym "REASSURED". Real-time connectivity includes transmission of test data via mobile phone for instance but also aims at improving quality assurance and allow for real-time decision making.[90] However, in areas of LMICs where access to internet can be limited, this criterion is not adapted.

1.3.2.3. Advantages of point-of-care tests

POCT offer multiple advantages.[86] The most important one is that it reduces the time of notification of result and therefore patients can be treated immediately. Asymptomatic infections may also be treated, and presumptive treatment can be avoided if the test can detect the causative organisms, consequently overtreatment with antibiotics is reduced. Having immediate result also offer an opportunity for patient counselling and improved partner treatment or tracing. It may reduce onwards transmission and progression of disease. Finally, POCT may allow for self-testing, which increase privacy and convenience in comparison to provider-collected samples, thus it may improve patient acceptability to be tested.[91]

A decision analysis framework found that, in settings where a proportion of patients do not return to collect their treatment after a positive test, less sensitive POCT could lead to the treatment of more patients and be more cost-effective.[92]

1.3.2.4. Overview of point-of-care tests for sexually transmitted infections

Molecular POCT for CT, NG, TV, or MG have a higher sensitivity than POCT and, therefore, should be chosen in priority when available. However, they are not yet equipment-free and generally too expensive for routine implementation in LMICs where RDTs are preferred. Most

RDT are lateral flow tests, where infection-specific antibody or antigen is used. Test results can generally be read visually after 10 – 30 minutes. Results are most often read by human eye which can make the interpretation of the result subjective and lead to false-positive and false-negative results. To overcome this issue and to support quality assurance, a diagnostic test reader can be used.

Currently, RDTs for HIV, syphilis and dual HIV/syphilis detection exhibit adequate performance and acceptability.[93–97] For TV, the antigen detection-based OSOM® Trichomonas test (Sekisui Diagnostics), which has a turnaround time of 10 minutes, has shown reasonable performance (sensitivity : 83 – 86%) when compared to NAATs.[98] However, many current RDTs for CT and NG suffer from low diagnostic accuracy, particularly low sensitivity. The enzymatic BioChekSwab CT Rapid Test (EnZtek Diagnostics, Rio Vista, California, USA) had a sensitivity of 0% (95% CI: 0, 33.6%) and a specificity of 96.9% (95% CI: 92.2, 99.1%).[99] Sensitivity for CT antigen detection RDTs reached 37% for vaginal swabs and 53% for cervical swab.[100] Studies evaluating lateral flow assays or other non-molecular test for point-of-care detection of NG among women are scarce. A study that included 1500 non-pregnant women evaluated that the sensitivity of NG of the antigenic test ACON CT/NG Duo was 12.5% compared with NAATs.[101] A novel lateral flow detecting NG surface exposed antigen, tested on 200 symptomatic women with vaginal discharge using vaginal swab specimens in South Africa, showed promising performance, with a sensitivity of 91.7% (95% CI: 78.2% to 97.1%). However, this test has not been commercialized yet.[102] RDTs at a reasonable cost are not available for MG.[103]

The OSOM® BVBlue® for the detection of the sialidase enzyme to diagnose BV shows a sensitivity of 82 – 96% when compared with Nugent score [104–106] with the exception of one study which found a sensitivity of 38%.[107] In a population of asymptomatic pregnant women, the POCT showed a sensitivity of 81% when compared with Gram staining-confirmed BV.[108] The manufacturer declares a sensitivity of 93% compared with Gram staining-confirmed BV.[109] The test costs between 10 and 25€, a price that remains excessive for most people living in LMICs. It is worth noting that one study showed that pH > 4.5 alone displayed a sensitivity of nearly 80%, and pH > 4.5 and/or positive whiff test, a sensitivity of 83% compared to Nugent score.[110] Another POCT exists, BD Affirm™ VPIII that displays a sensitivity of 80%,[111] but requires equipment, electricity and is relatively expensive.

VGTest™ ion motility spectrometry (sensitivity: 83%) and FemExam™ (sensitivity: 91%) have been formerly tested but are no longer commercialized.[112]

1.3.3. The syndromic management of STIs

To accommodate contexts in which laboratory facilities or POCT are not available, WHO developed guidelines on the use of a syndromic approach for the management of some STIs and has advocated its use since the mid-1980s.

1.3.3.1. Description

The syndromic case management enables all trained first-line service providers to recognize an STI syndrome and treat accordingly. The objectives are to obtain cure, reduce infectiveness, reduce the risk of developing complications of STIs, reduce or prevent future risk-taking behavior, and ensure that sex partners are appropriately treated. The management relies on utilization of flow charts, or algorithms, for each syndrome, in addition to education and counselling on prevention, infection and treatment. The WHO guidelines for the management of symptomatic STIs remain the reference,[6] however, countries may have adapted those guidelines based on the available treatments and antimicrobial susceptibility data on their geographical areas.

1.3.3.2. Entry points and flow charts of the WHO guidelines

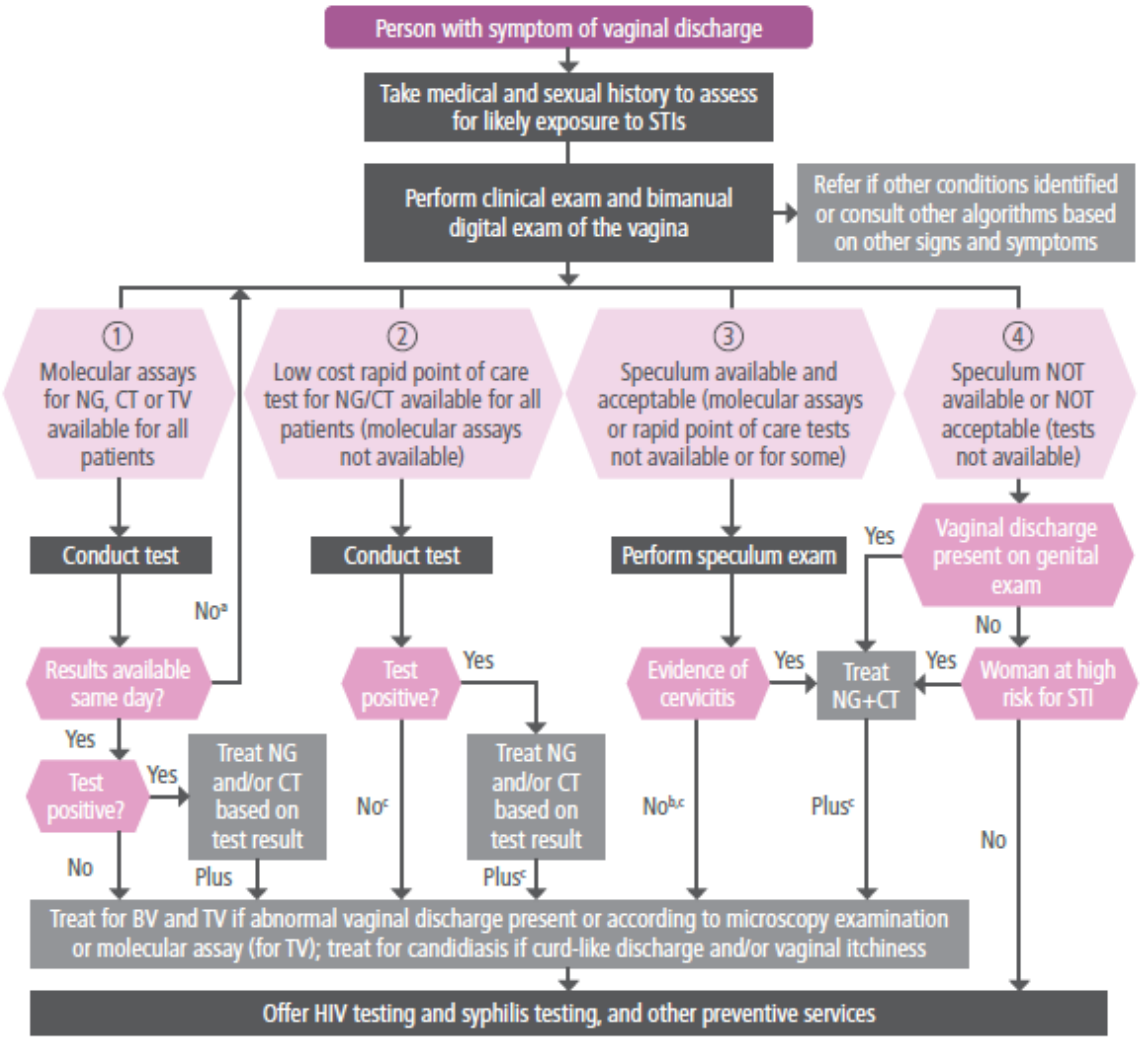
Before any consideration on symptoms, the guidelines stress the importance of conducting a risk assessment and taking medical and sexual history. The risk may be increased for sexually active women younger than 25 years of age, who had new sexual partner in the three months preceding the visit or had more than one sex partner in the three months preceding the visit. In addition, investigation of PID, assessment of surgical conditions or pregnancy, vulvovaginal visualization of any lesion, overt genital discharge, erythema or excoriations is recommended good practice. Adding to these considerations, the WHO guidelines developed five syndromes that serve as entry points into the management of STIs:

- urethral discharge syndrome,

- vaginal discharge syndrome (VDS),
- lower abdominal pain (LAP) syndrome,
- genital ulcer disease (GUD) syndrome, and
- anorectal discharge.

Several syndromes fall outside of the main scope of this thesis and will therefore not be discussed. This is the case **for urethral discharge syndrome** which relates to males only. The causative organisms for **GUD syndrome**, which include HSV-2, HSV-1, *T. pallidum* (syphilis) and *C. trachomatis* serovars L1–L3 causing lymphogranuloma venereum (LGV), are not the focus of this thesis. **Anorectal discharge** is prevalent among men who have sex with men, female sex workers (FSW), transgender people and heterosexual women who engage in anal sexual intercourse. This syndrome will be touch upon briefly.

Vaginal discharge syndrome includes a vaginal discharge that is perceived by the woman as abnormal (in terms of quantity, color, and odor), vulval irritation or itching. In settings with limited access to molecular assays or RDT, WHO suggests performing a speculum examination, and treating for CT and NG if there is evidence of cervicitis and for BV and TV otherwise and based on the results of microscopy, if available. If the speculum examination is not feasible or not acceptable, WHO suggests treating for CT and NG all people at high risk of STIs and all women who have vaginal discharge on genital examination. Moreover, WHO recommends treating for candidiasis if the discharge is curd-like and/or if the patient complains of itchiness (**Figure 1.8**).



NG, *N. gonorrhoeae*; CT, *Chlamydia trachomatis*; TV, *Trichomonas vaginalis*; BV, bacterial vaginosis.

[a] If molecular assay was performed and results were not available on same day, revise the syndromic treatment initially provided according to the test results when available

[b] perform rapid point of care test or molecular assay if available to confirm NG/CT and treat if positive; if negative do not treat and ask woman to return if symptoms recur

[c] if woman complains of recurrent or persistent discharge refer to a centre with laboratory capacity

Figure 1.8. Flow chart for health-care providers to manage vaginal discharge according to local availability of resources and preferences (reproduced from the Guidelines for the management of symptomatic sexually transmitted infections. Geneva: World Health Organization; 2021 [6]).

Lower abdominal pain is described for sexually active women with either cervical motion tenderness or lower abdominal tenderness. In settings where no tests are available, WHO suggests treating for PID on the same visit and to schedule follow-up assessment three days later to assess for clinical improvement, and if the women has not improved refer for further assessment. Urgent referral is recommended for women with missed/overdue period, recent

delivery/abortion/miscarriage, abdominal guarding and/or rebound tenderness, abnormal vaginal bleeding, abdominal mass, or detection of a suspected cervical lesion (**Figure 1.9**).

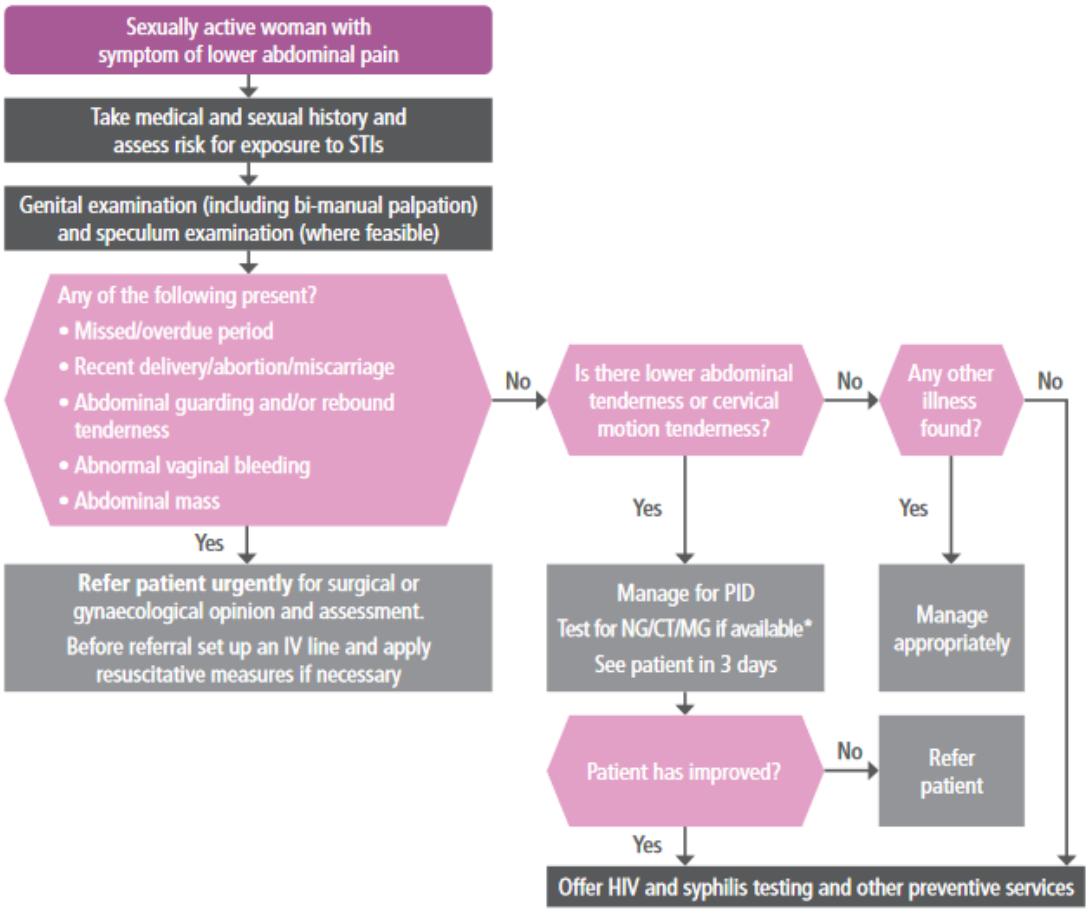


Figure 1.9. Flow chart for the management of lower abdominal pain (reproduced from the Guidelines for the management of symptomatic sexually transmitted infections. Geneva: World Health Organization; 2021 [6]).

1.3.3.3. Limitations of the syndromic management approach

Despite providing an alternative in settings where access to STI testing is constrained, syndromic management faces multiple limitations. Most importantly, it fails to detect asymptomatic cases. In **Chapter 4**, we will describe the prevalence and proportion of these asymptomatic STI cases in LMICs, who are missed by this approach. Furthermore, symptoms are often non-specific, which can result in inappropriate treatment (e.g., treating for NG when

the infectious agent is in fact CT) or overtreatment (treatment of non-infected women), illustrating the low sensitivity and specificity of this approach.

1.4. Development of a RDT to improve the detection of RTIs: the Genital InFlammation Test (GIFT) study

Alternatives to the syndromic approach, with a focus on detecting asymptomatic infections, are being explored. One of these alternatives involves detecting markers that indicate genital inflammation caused by STIs.

A team at the University of Cape Town in South Africa conducted a preliminary validation study, which determined that three cytokines, interleukin (IL)-1α, IL-1β and interferon γ-induced protein (IP)-10, were associated with the presence of STIs and BV.[113] In a subsequent study, they evaluated the performance of these three cytokines across different cohorts, reporting sensitivities ranging from 70% to 77% and specificities between 71% to 85%.[114] Specifically, one analysis showed a sensitivity of 77% (95% CI: 71-83%) and a specificity of 71% (95% CI: 59-81%). Furthermore, in an earlier study, they showed that women with asymptomatic STIs and BV had comparable elevated levels of genital inflammation to women with symptomatic conditions.[115] This suggests that these cytokines may be useful in detecting STIs and BV among asymptomatic women, addressing a limitation of syndromic management. As a next step, the team developed a prototype lateral flow device called the Genital InFlammation Test (GIFT), designed for the immediate detection of these inflammation markers. The performance of the GIFT is being evaluated in a multicenter prospective observational diagnostic trial conducted in Madagascar, South Africa, and Zimbabwe, where Madagascar has a low HIV prevalence, unlike South Africa and Zimbabwe.[116] However, previous studies conducted in Madagascar suggested a high STI and BV prevalence. The study from chapter 3 was conducted as part of the GIFT project, to confirm the elevated STI and BV prevalence and to ensure the feasibility of the GIFT diagnostic trial in Madagascar.

Moreover, in **Chapter 5** of this thesis, we developed different models to identify in particular asymptomatic women at risk of STIs and BV and who should be given the GIFT device in priority. At present, the performance of the prototype has not yet been determined. The management, including treatment, of women with a positive GIFT result will be defined once the test's performance is evaluated.

Chapter 2: Thesis objectives

This thesis aims at studying the identification of women with, or at risk for, CT, NG, TV, or MG, with a particular but not exclusive focus on asymptomatic women in settings with limited access to STI testing. Three important knowledge gaps are addressed in this work.

First, as prevalence data on CT, NG, TV, and MG in Madagascar had not been documented for 20 years, my objectives were (i) to determine the prevalence of and risk factors associated with the infection with these pathogens among women attending a major hospital of Antananarivo, the capital city of Madagascar; (ii) to describe the symptoms that women experienced, by STI and BV status, and factors associated with asymptomatic infections among the population of infected women; and (iii) to evaluate the performance of the WHO syndromic approach (**Chapter 3**).

Second, while it is widely acknowledged that a majority of STIs in women are asymptomatic, no study to date quantified the extent of the problem across different settings, especially in areas in which the syndromic approach prevails. To address this gap, my objective was to conduct a systematic review where I selected published data since 2000 in all LMICs. I completed this review by a meta-analysis in which I calculated the prevalence of asymptomatic women infected by CT, NG, or TV in the population and the proportion of asymptomatic women among those infected (**Chapter 4**).

Third, in the context of resource-limited settings, with the perspective to limit costs, my objective will be to prioritize women who should receive GIFT by developing an algorithm or risk score to guide the allocation of these tests. Country-specific risk scores and scores for asymptomatic women only will be presented (**Chapter 5**).

Finally, these works are discussed in the context of their collective contributions to the scientific literature and relevance to public health, by characterizing populations who may be the target of public health interventions (**Chapter 6**).

Chapter 3: Sexually transmitted infections and bacterial vaginosis in women of childbearing age in Antananarivo, Madagascar: prevalence and risk factors from a cross-sectional study (the PrevISTana study)

3.1. Introduction

Women in the African region are predominantly affected by STIs, with more than 34 million prevalent cases of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV).[7] Moreover, the prevalence of BV reaches 25% in sub-Saharan Africa.[117]

However, epidemiological data on the general population remains scarce in certain countries, such as Madagascar.

Madagascar is an island country located off the southeastern coast of Africa, characterized by a sub-tropical to tropical maritime climate. Covering 592 800 squared kilometers, it is the world's fourth-largest island after Greenland, New Guinea, and Borneo. Its population of 30.3 million inhabitants is composed of 18 major Malagasy ethnic groups of which the Merina is the largest. The population is notably young with 43% under the age of 15 and only 4% over 65. Life expectancy averaged 65 years.[118] Christianity is the most widely practiced religion.[118] Poverty remains an important issue with 80.7% of the population living below the income poverty line of 2.15 USD per person per day.[119] Access to basic amenities is limited: only 48% of households have access to drinking water and 33% have electricity (73% in urban areas and 24% in rural areas).[118]

Health care facilities, including dispensaries, health centers, and hospitals can be found throughout the country, but they are concentrated in urban areas. Medical expenses are unaffordable for many people leading them to rely on traditional healers.[120] In addition, the availability of trained medical professionals remains extremely low with only 1.6 doctors for 10 000 inhabitants [121] and there are approximately three hospital beds per 10 000 people.[122]

Regarding STIs, a few studies on syphilis and HPV have been recently published.[123–125] Yet, to the best of our knowledge, the latest study on CT, NG, TV, MG, and BV was published in 2005 on a study conducted in 2002,[126] more than two decades ago. This study was conducted on 310 men and 333 women aged 15 – 49 years living in a rural area and was derived from a cross-sectional morbidity study of urogenital schistosomiasis. The authors found that 27% of men and 38% of women were infected by CT, NG, TV, MG, or *Treponema pallidum*. In women, young age (15 – 24 years) as compared with older age (25 – 49) was associated with STIs, however, social, behavioral, or biological factors were not explored.

In this context, we aimed to provide recent estimates on the prevalence of CT, NG, TV, MG, and BV among women of childbearing age attending a hospital in Antananarivo. Obtaining these recent estimates was essential for the GIFT project to understand the epidemiology of STIs and BV in this setting, as well as to validate the site's suitability for the upcoming diagnostic evaluation study of the GIFT device. Additionally, we investigated the socio-demographic and behavioral factors associated with the presence of at least one of the four tested STIs. We also explored the symptoms presented by the women included in the study and we determined the performance of the WHO syndromic approach.

3.2. Methods

3.2.1. Study design

We conducted a cross-sectional study between March and November 2022 at the outpatient and family planning departments of the Gyneco-Obstetrics Befelatanana University Hospital (CHUGOB) in Antananarivo, the capital city of Madagascar. We chose this University's teaching hospital since it serves as the primary referral center for gynecology and obstetrics. This study was a feasibility study for a larger trial on the evaluation of a rapid diagnostic test, the Genital InFlammation Test (GIFT), which aims to detect genital inflammation in women. This study was named the "PrevISTana study", which stands, in French, for "**Prév**alence des **IST** à **Tana**narive" (Translation: *Prevalence of STIs in Antananarivo*).

3.2.2. Study procedures

Our inclusion criteria were women aged 18 to 45 years. We excluded pregnant women. Women with metrorrhagia or bleeding during examination or swabbing were also excluded because the presence of blood on the swab could interfere with pathogen detection. Menstruating women or women who had their last day of menstruation in the past three days were invited to return at a later date to the hospital for enrolment. Finally, we excluded women who had taken antibiotics in the past 30 days as they would have cleared the pathogen but fragments of pathogens' DNA could still be detectable and would yield false positive cases.

Participants answered a questionnaire in the Malagasy language administered by a nurse or a midwife experienced in research in a dedicated consultation room. We collected socio-demographic data, sexual history, and risk behavior information. Following the interview, a gynecologist or midwife inquired about symptoms, conducted a genital examination, and documented clinical signs.

Clinicians followed established procedures and prescribed treatment for suspected STIs. If the clinician did not initially suspect an STI but the laboratory (Experimental Bacteriology Unit [UBEX], Institut Pasteur in Madagascar [IPM]) later identified it on samples, participants were notified by phone to collect treatment at the hospital for free. Testing and enrollment did not involve any charges or incentives for the participants.

The gynecologist or the midwives collected three vaginal specimens with a speculum in place. One cotton and two Copan flocked® swabs (Copan, Brescia, Italy) were used to collect vaginal fluid from the lateral vaginal wall. The two flocked swabs were introduced simultaneously to detect CT, NG, TV, MG, BV, and the fungal infection by *Candida albicans* (CA). The national STI/AIDS program offered HIV testing with capillary blood.[127]

We used the cotton swab to prepare immediately after collection a vaginal smear for Nugent scoring. We heat-fixated the vaginal smear for 1 minute on a heating plate at 40°C and stored it at room temperature until transport to UBEX at IPM. We kept the Copan flocked swabs at 2-8°C until transport. The transport of swabs to UBEX was done on the same day using a cool box and icepacks, where they were stored at -20°C until testing.

3.2.3. Laboratory methods

After thawing one flocked swab per participant, we eluted the vaginal secretion for at least 15 seconds in 1.2 ml of diluted phosphate-buffered saline (dPBS) (pH 7.4 - 1:9, PBS: saline). We extracted the DNA from an aliquot of 200 µL using the QUICK-DNA HMW magbead kit (Zymo Research, USA) according to the manufacturer's instructions. We used Presto CT/NG (Goffin Molecular Technologies, Beesd, The Netherlands) for the detection of CT and NG, according to the manufacturer's procedure. The performance of the Presto assay has been previously evaluated and has shown a sensitivity and specificity of 93.3% (95%CI: 68.1, 99.8%) and 99.4% (95%CI: 96.8, 100%) for CT; and 100% (95%CI: 76.8, 100%) and (98.8% (95%CI: 95.8, 99.9%) for NG.[128] This technique was selected for economic reasons, because it could be used on different platforms, and because of its specific procedure for the detection of CT/NG coinfection. We used previously published methods for the detection of TV, MG, and CA.[129–131] We utilized the CFX platform (Bio-Rad, California, USA) for all amplifications.

We Gram-stained the fixated vaginal smear and examined it using the Nugent score. BV was defined by a Nugent score of above or equal to 7, an intermediate microbiota by a score between 4 and 6, and optimal vaginal microbiota by a score below 4.

Macrolide and fluoroquinolone resistance determinants were assessed in MG by PCR and sequencing of resistance-associated regions of the *parC* gene and 23S rRNA. The mutations investigated in *parC* were: G241A, A247C, A247T, G248T, G248A, T249G, G259A, G259T, and

A260G, and in 23S rRNA: A2058T, A2058C, A2058G, A2059G, and A2059C according the *E. coli* numbering. We did not culture NG, consequently we could not perform antimicrobial susceptibility testing using Etest. In addition, we did not explore fluoroquinolone resistance in NG using molecular techniques because a previous study performed by UBEX showed that all tested NG isolates were resistant to fluoroquinolones using Etest, which was confirmed using whole genome sequencing.[132]

3.2.4. Statistical methods

We determined the sample size using the prevalence of CT, NG, TV, MG, and BV from former studies conducted in Madagascar.[126,133] For each pathogen, we employed a precision of 0.05 for prevalence rates equal to or exceeding 10% and a precision of 0.025 for rates below 10%, using a two-sided 95% confidence interval. We retained the largest required sample size, 470, and to accommodate potential attrition or sample loss, we augmented it to 515.

We determined the prevalence using Wald 95% confidence intervals (CI). We defined the outcome as having an STI, i.e., a positive test for either CT, NG, TV, or MG. We described the socio-demographic characteristics, behaviors, and medical features of participants. We compared those infected by an STI and those with no STIs using Chi-2 or, in the case the application conditions were not met, the Fisher test. We created three age groups using standard categories for ease of comparison with other studies: 18-24, 25-34, and 35-45.[126,134,135] We utilized bivariate analyses to explore the associations between socio-demographic and behavioral characteristics of the participant and the outcome. We performed multivariable logistic regression to examine associations with STI infection, adjusting for age. We included the variables with $p \leq 0.2$ in the bivariate analyses to build the final model using a manual backward approach. The statistical significance of variables in the final model was assessed at the 0.05 level. In addition, we conducted analyses using the same approach to identify, among infected women, factors associated with (i) having two or more STIs, and (ii) having an asymptomatic form of STI. For all the logistic regressions conducted, our measures of effect were odd ratios (OR) in bivariate analysis and adjusted odd ratios (aOR) for multivariable analysis.

Finally, we described symptoms or absence of symptoms by STIs. We evaluated the performance of the 2021 WHO syndromic flowcharts. Since the RTIs we tested for do not

cause genital ulcer disease, we only assessed the performance of the vaginal discharge syndrome (VDS) and lower abdominal pain (LAP) flowcharts. We compared the number of women who would be diagnosed positive under either the VDS flowchart (path 3, with a speculum available) or under the LAP flowchart. We also evaluated the performance of the entry points in each algorithm (i.e. the symptoms: abnormal vaginal discharge, irritation, or pruritus for the vaginal discharge syndrome algorithm and lower abdominal pain for the eponymous algorithm). Sensitivity, specificity, and predictive values were calculated using the laboratory results as the reference. Detailed methodology is available in Annex A. We used R (version 4.1.1) to perform the statistical analyses.

This study followed the STROBE statement.

3.2.5. Ethical consideration

The ethical committee (Comité d'Ethique de la Recherche Biomédicale, CERBM) in Madagascar approved the study protocol (N° 124-MSANP/SG/AMM/CERBM). All participants gave their informed consent before joining the study.

3.3. Results

Between March and November 2022, 529 eligible women attended the CHUGOB. Among them, 14 did not consent to participate to the study, five bled during vaginal swabbing, three were positive for pregnancy after testing, three were lost to follow-up between the interview and sampling, and three withdrew consent, resulting in 501 included women (**Figure 3.1**). Two-thirds of the women were recruited at the outpatient department (339/501, 68%), and a third were recruited at the family planning service (162/501, 32%).

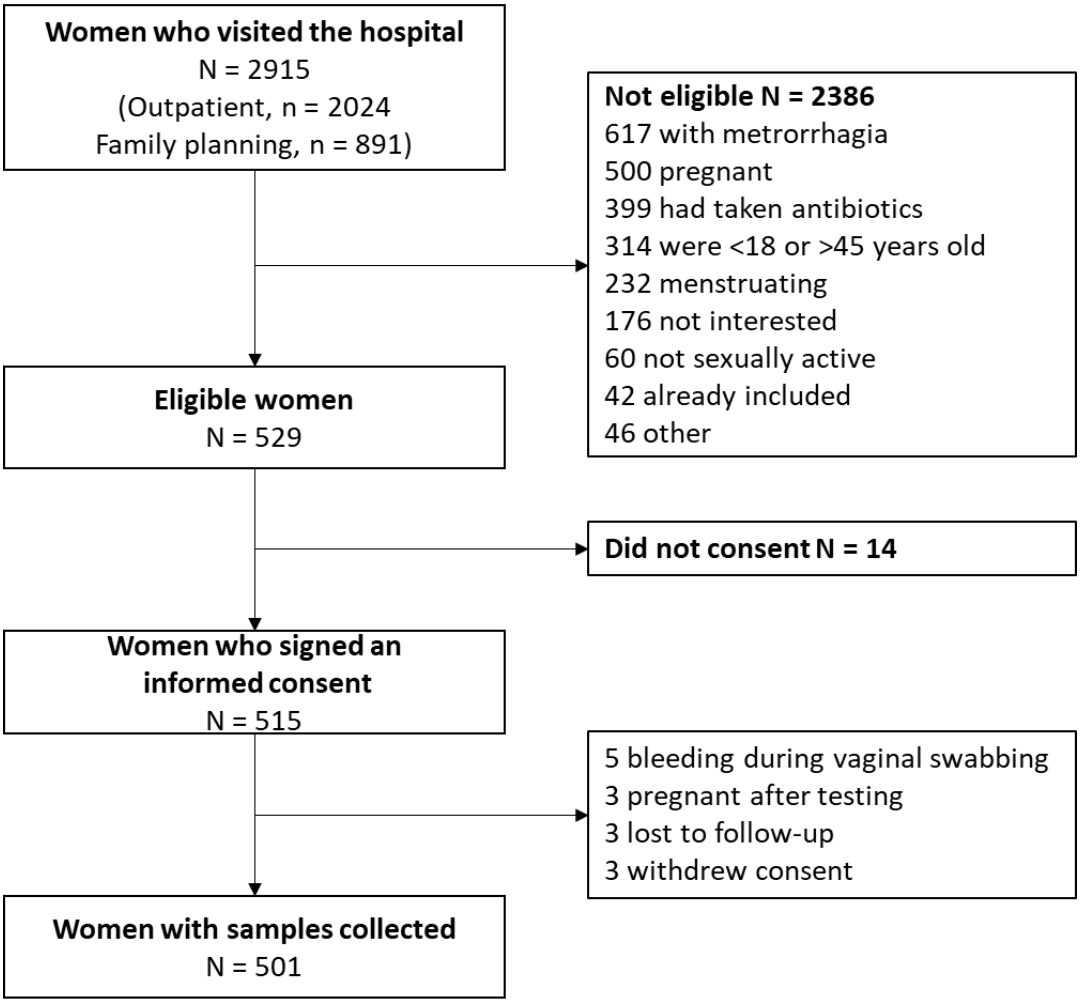


Figure 3.1. Number and selection of women attending CHUGOB.

3.3.1. Study participants' characteristics

The median age was 27 years (interquartile range (IQR): 23-32). More than half of the participants went at least to high school (132/501, 26%) or had higher education (189/501, 38%) (**Table 3.1**). A third of the participants (160/501, 32%) were employed by the public service or companies, whereas 151/501 (30%) owned their own businesses. The majority of them were either married (242/501, 48%) or in a relationship (191/501, 38%). In contrast, 68 (14%) were single or divorced. Most never used condoms (355/501, 71%). Although the majority of the women declared they had a permanent partner (471/501, 94%) and lived with them for more than a year (444/501, 89%), concomitant partners were not uncommon with 61/444 (14%) of the latter stating they had other partners in the past 12 months. Similarly, 127/444 (28%) indicated that their permanent partner had other partners in the past 12 months. Circumcision among permanent partners was extremely common (460/471, 98%). Previous STIs were reported by 46/501 (9%) of the participants. Noticeably, 74/501 (15%) of the women declared having ever experienced forced sexual intercourse.

Table 3.1. Characteristics of infected and uninfected women

Characteristics	**No STIs §** (N = 349)	**At least one STI** (N = 152)	**Overall** (N = 501)	**p-value ¶**
Socio-demographic				
Age				0.004
18 – 24	115 (33%)	70 (46%)	185 (37%)	
25 – 34	159 (46%)	65 (43%)	224 (45%)	
35 – 45	75 (21%)	17 (11%)	92 (18%)	
Education level				0.953
No schooling or primary school	43 (12%)	19 (13%)	62 (12%)	
Middle school	80 (23%)	38 (25%)	118 (24%)	
High school	92 (26%)	40 (26%)	132 (26%)	
Higher education	134 (38%)	53 (35%)	189 (38%)	
Marital status				<0.001
Single / Divorced	44 (13%)	24 (16%)	68 (14%)	
In a relationship	116 (33%)	75 (49%)	191 (38%)	
Married	189 (54%)	53 (35%)	242 (48%)	
Activity				0.775
Owner of their own business	105 (30%)	46 (30%)	151 (30%)	
Civil servant / Private sector	116 (33%)	44 (29%)	160 (32%)	
Jobless / Housewife / On leave	79 (23%)	39 (26%)	118 (24%)	
Student	49 (14%)	23 (15%)	72 (14%)	

Characteristics	No STIs § (N = 349)	At least one STI (N = 152)	Overall (N = 501)	p-value ¶
Behavior / Exposure to STI/HIV				
Personal hygiene				0.392
Wash vulva	244 (70%)	100 (66%)	344 (69%)	
Wash vulva and vagina	104 (30%)	52 (34%)	156 (31%)	
Prefer not to say†	1 (<1%)	0 (0%)	1 (<1%)	
Has a permanent partner				0.025
No	17 (4.9%)	7 (4.6%)	24 (4.8%)	
Yes, not living with partner	69 (20%)	46 (30%)	115 (23%)	
Yes, living with partner	261 (75%)	95 (63%)	356 (71%)	
Prefer not to say†	2 (<1%)	4 (2.6%)	6 (1.2%)	
Other partner(s) in the past 12 months				0.009
No	302 (87%)	113 (74%)	415 (83%)	
Yes, one other partner	34 (9.7%)	24 (16%)	58 (12%)	
Yes, two or more other partners	11 (3.2%)	11 (7.2%)	22 (4.4%)	
Prefer not to say†	2 (<1%)	4 (2.6%)	6 (1.2%)	
Ever had sex in exchange for money, goods, or job				0.004
No	345 (99%)	143 (94%)	489 (97%)	
Yes	4 (1.1%)	9 (5.9%)	13 (2.6%)	
Frequency of condom use				0.868
Always	5 (1.4%)	2 (1.3%)	7 (1.4%)	
From times to times	94 (27%)	45 (30%)	139 (28%)	
Never	250 (72%)	105 (69%)	355 (71%)	
Permanent partner had other partner(s) in past 12 months				0.028
No	137 (39%)	39 (26%)	176 (35%)	
Yes	88 (25%)	51 (34%)	139 (28%)	
Prefer not to say†	0 (0%)	1 (<1%)	1 (<1%)	
Not concerned*	19 (5.4%)	11 (7.2%)	30 (6.0%)	
Do not know	105 (30%)	50 (33%)	155 (31%)	
Current partner(s) reported STI symptom				0.136
No	253 (72%)	98 (64%)	351(70%)	
Yes	92 (30%)	50 (33%)	142 (28%)	
Do not know†	4 (1.5%)	4 (2.6%)	8 (1.6%)	
Biological testing				
Candida albicans	66 (18.9%)	13 (8.6%)	79 (15.8%)	0.009
Bacterial vaginosis (BV)‡	120/341 (35.2%)	74/149 (49.7%)	194/490 (39.6%)	
Intermediate microbiota	59/341 (17.3%)	36/149 (24.2%)	95/490 (19.4%)	0.003

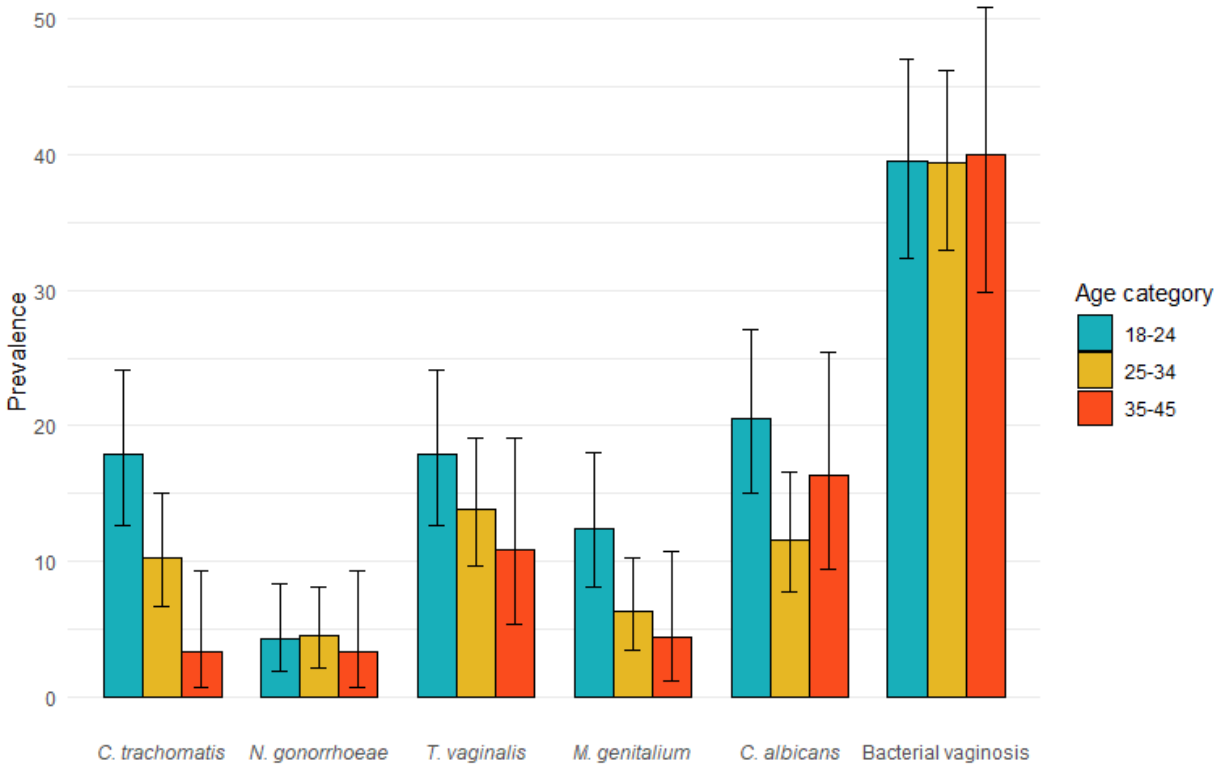
* No permanent partner or did not want to say if they had one
† Excluded from the Chi² or Fisher tests
‡ 11 women could not be tested for BV
§ STIs refer to the detection of either CT, NG, TV, or MG
¶ Using Chi² test or Fisher test when appropriate

3.3.2. Prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, bacterial vaginosis, and vaginal *Candida albicans*

The prevalence of CT was 11.8% (59/501, 95% CI: 9.0, 14.6), for NG 4.2% (21/501, 95%CI: 2.4, 5.9), for TV 14.8% (74/501, 95%CI: 11.7, 17.9), and for MG 8.2% (41/501, 95%CI: 5.8, 10.6).

For BV, 11 vaginal smears contained too little material; consequently, the Nugent score could not be interpreted. Of the available samples, the prevalence was 39.6% (194/490, 95%CI: 35.3, 43.9). The prevalence of vaginal CA was 15.8% (79/501, 95%CI: 12.6, 19.0). Due to a shortage of HIV tests, only the first 281 women were tested, and among them, two were HIV seropositive, resulting in an HIV prevalence of 0.7% (95%CI: 0.1, 2.5). We identified that 152/501 (30.3%) women had at least one infection by CT, NG, TV, or MG.

When stratifying by age category, we observed that as age increased, CT prevalence decreased (p=0.004). A similar but not significant trend was observed for TV and MG (**Figure 3.2**).



	CT	NG	TV	MG	CA	BV
18-24 (n=185)	17.8 (12.6, 24.1)	4.3 (1.9, 8.3)	17.8 (12.6, 24.1)	12.4 (8.0, 18.1)	20.5 (15.0, 27.1)	39.6 (32.4, 47.1)*
25-34 (n=224)	10.3 (6.6, 15.0)	4.5 (2.2, 8.1)	13.8 (9.6, 19.1)	6.3 (3.5, 10.3)	11.6 (7.7, 16.5)	39.4 (32.9, 46.3)†
35-45 (n=92)	3.3 (0.7, 9.2)	3.3 (0.7, 9.2)	10.9 (5.3, 19.1)	4.3 (1.2, 10.8)	16.3 (9.4, 25.5)	40.0 (29.8, 50.9)‡

* Denominator is 182 for BV in 18-24 age group
† Denominator is 218 for BV in 25-34 age group
‡ Denominator is 90 for BV in 35-45 age group

Figure 3.2. Prevalence of CT, NG, TV, MG, CA, and BV by age groups, with 95% confidence intervals.

Prevalence of RTIs in the outpatient department and at the family planning services did not differ except for *Candida albicans*, which was more than twice as frequent in the outpatient department than at the family planning department (**Figure 3.3**).

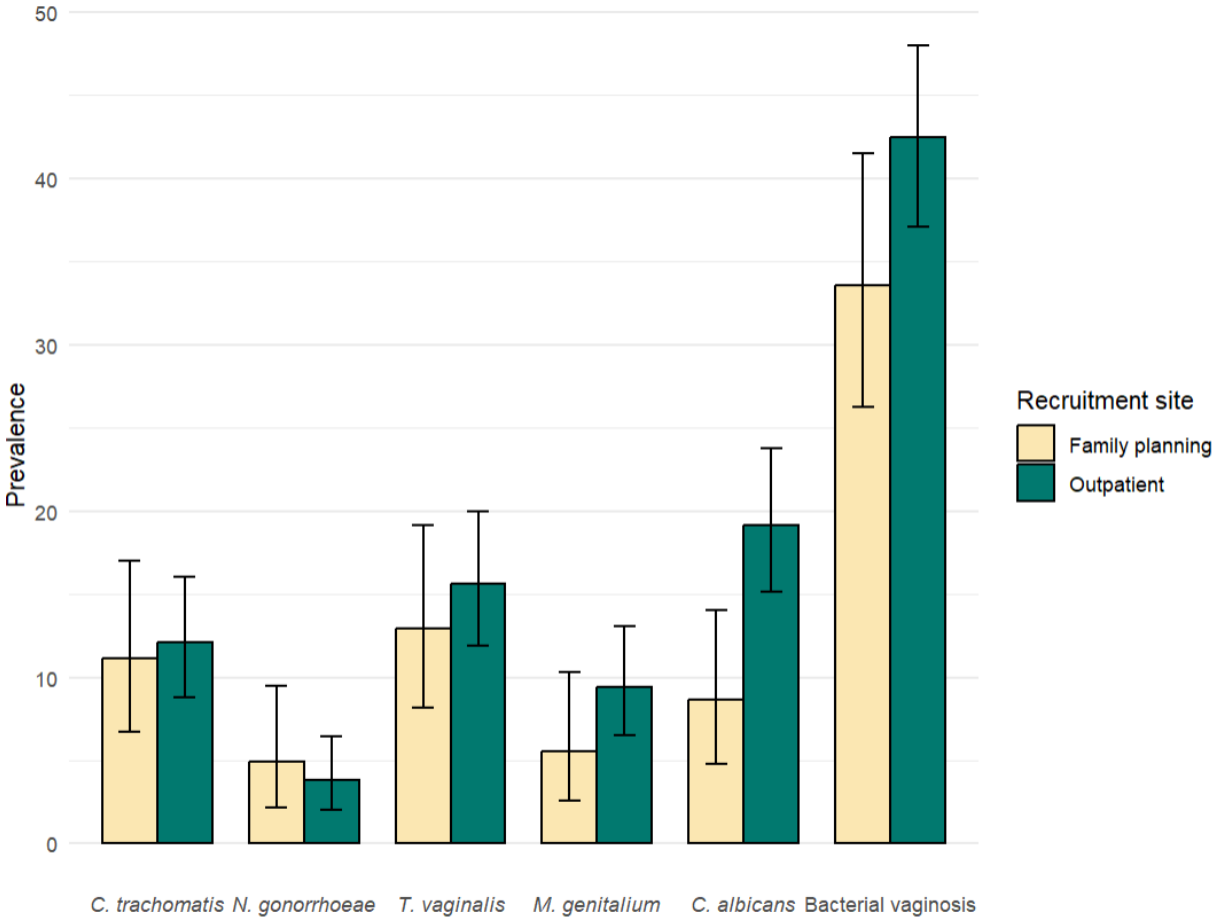


Figure 3.3. Prevalence of CT, NG, TV, MG, CA, and BV by recruitment site with 95% confidence intervals

3.3.3. Factors associated with sexually transmitted infections

We conducted the analyses on complete data (n=463), excluding participants with missing BV results (11/501, 2.2%) and those who preferred not to answer or did not know the response to specific questions (27/501, 5.4%). We compared age, education, marital status, activity, and STI status between those excluded and included in the risk factors analysis. We found no statistical evidence that the excluded group differed from those included.

In multivariable analysis, adjusting for age, we found that women in a relationship but not married had 86% increased odds of having an STI than married women (aOR: 1.86, 95%CI: 1.16,

2.98, p=0.01) (**Table 3.2**). Women who had sex in exchange for favors had 4-fold increased odds of having an STI (aOR: 4.25, 95%CI: 1.27, 16.6, p=0.019). Finally, we found that the odds of having an STI were 2.14 higher in those with BV compared to those with an optimal microbiota (95%CI: 1.34, 3.47, p=0.002). Likewise, women with intermediate microbiota had twice the odds of having an STI (aOR: 2.10, 95%CI: 1.19, 3.72, p=0.010).

Table 3.2. Factors associated with at least one infection with CT, NG, TV, or MG among 463 women attending CHUGOB, in Antananarivo, Madagascar.

		Bivariate analyses			Multivariable analysis		
Characteristic	**N**	**OR**	**95%CI**	**p-value**	**aOR**	**95%CI**	**p-value**
Age category				0.005			0.100
18-24	172	2.71	1.46, 5.27	0.002	2.05	1.06, 4.15	0.039
25-34	204	1.95	1.06, 3.79	0.038	1.69	0.89, 3.34	0.12
35-45	87	-	-		-	-	
Marital status				0.001			0.034
Married	226	-	-		-	-	
In a relationship	177	2.25	1.46, 3.51	<0.001	1.86	1.16, 2.98	0.010
Single or divorced	60	1.67	0.88, 3.11	0.110	1.34	0.68, 2.58	0.4
Permanent partner				0.063			
Yes, living with partner	337	-	-				
Yes, not living with partner	103	1.74	1.09, 2.77	0.019			
No permanent partner	23	0.97	0.34, 2.41	> 0.9			
Participant had other partners*				0.035			
No	392	-	-				
Yes, one	50	1.80	0.97, 3.29	0.058			
Yes, two or more	21	2.45	1.00, 5.98	0.047			
Partner had other partners*				0.052			
No	167	-	-				
Yes	131	1.97	1.18, 3.29	0.009			
Do not know	142	1.68	1.02, 2.81	0.044			
Not concerned	23	1.24	0.42, 3.23	0.7			
Ever had sex in for favors							
No	451	-	-		-	-	
Yes	12	5.05	1.56, 19.2	0.007	4.25	1.27, 16.6	0.019
Vaginal microbiota				<0.001			0.003
Optimal	194	-	-		-	-	
Intermediate	90	2.30	1.32, 3.54	0.003	2.10	1.19, 3.72	0.010
Bacterial vaginosis	179	2.21	1.39, 3.54	<0.001	2.14	1.34, 3.47	0.002

* In the past 12 months

3.3.4. Among infected women, factors associated with two or more sexually transmitted infections

In our study population, 117/501 (23.4%) had a mono-infection and 35/501 (7.0%) had a co-infection (**Figure 3.4**). Co-infections represented 23.0% (35/152) of all infections, with the combinations of CT-NG and CT-TV being the most common co-infections (8/35 each).

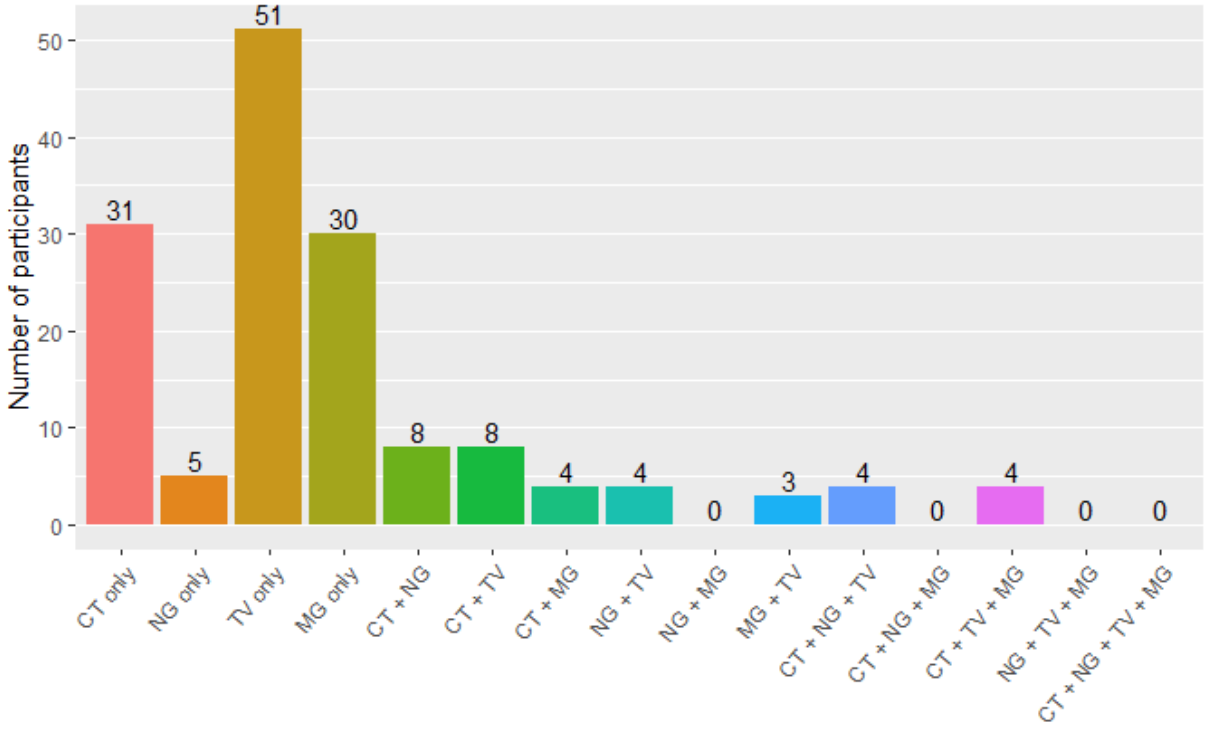


Figure 3.4. Counts of mono-infections and co-infections

In this section, we explored the factors associated with a co-infection among women infected with CT, NG, TV, or MG. As in the previous analysis, women with missing information were removed, thus one hundred and thirty-six women were included in this analysis. In the multivariable analysis, we found that women performing external and internal wash of their genitalia were at increased risk of co-infection in comparison to women performing only external wash (aOR: 2.52, 95% CI: 1.05, 6.14, p=0.039). In addition, women whose primary source of income was their partner faced a lower risk of co-infection compared to those whose income primarily came from themselves (aOR: 0.32, 95%CI: 0.12, 0.86, p=0.025) (**Table 3.3**).

Table 3.3. Factors associated with a co-infection among women infected by CT, NG, TV, or MG

		Bivariate analyses			Multivariable analysis		
Characteristic	**N**	**OR**	**95%CI**	**p-value**	**aOR**	**95%CI**	**p-value**
Main source of income				0.032			0.051
Participant	36	-	-		-	-	
Partner	72	0.29	0.11, 1.73	0.010	0.32	0.12, 0.86	0.025
Other	28	0.59	0.19, 1.73	0.3	0.83	0.25, 2.65	0.8
Personal hygiene				0.012			0.039
External wash	90	-	-		-	-	
External and internal wash	46	2.90	1.26, 6.74	0.012	2.52	1.05, 6.14	0.039

3.3.5. Symptomatic and asymptomatic forms of reproductive tract infections

We described the symptoms women reported after prompting for each RTI (**Figure 3.5**): vaginal discharge, pruritus, lower abdominal pain, and dyspareunia were the most common symptoms. When defining asymptomatic by the absence of vaginal discharge, irritation, pruritus, and lower abdominal pain, the entry points of the syndromic management, we found that nearly half of the infected women were asymptomatic. We found a higher proportion of asymptomatic women among the women infected by NG [57.1% (12/21)] whereas the lowest proportion of asymptomatic was found among TV-infected women [36.5% (27/74)]. Noticeably, 25% of the women with abdominal pain were infected by TV.

Among women with an STI, 92/152 (60.5%) had at least one symptom that corresponded to the entry points of the WHO syndromic management algorithms for the investigated STIs: vaginal discharge syndrome (which includes: abnormal vaginal discharge, irritation, or pruritus) or lower abdominal pain,[6] and 60/152 (39.5%) were asymptomatic.

In addition, we compared abnormal vaginal discharge from the perception of the gynecologist vs the patient. Gynecologists observed abnormal vaginal discharge in 80 women, both infected and uninfected. Among these women, 62 (70%) reported abnormal vaginal discharge, while 27 (30%) did not. Moreover, 13 women reported abnormal vaginal discharge which had not been detected by the gynecologists. Agreement over abnormal vaginal discharge was moderate between the patient and the gynecologist (Cohen's Kappa coefficient: 0.48, $p<0.001$).

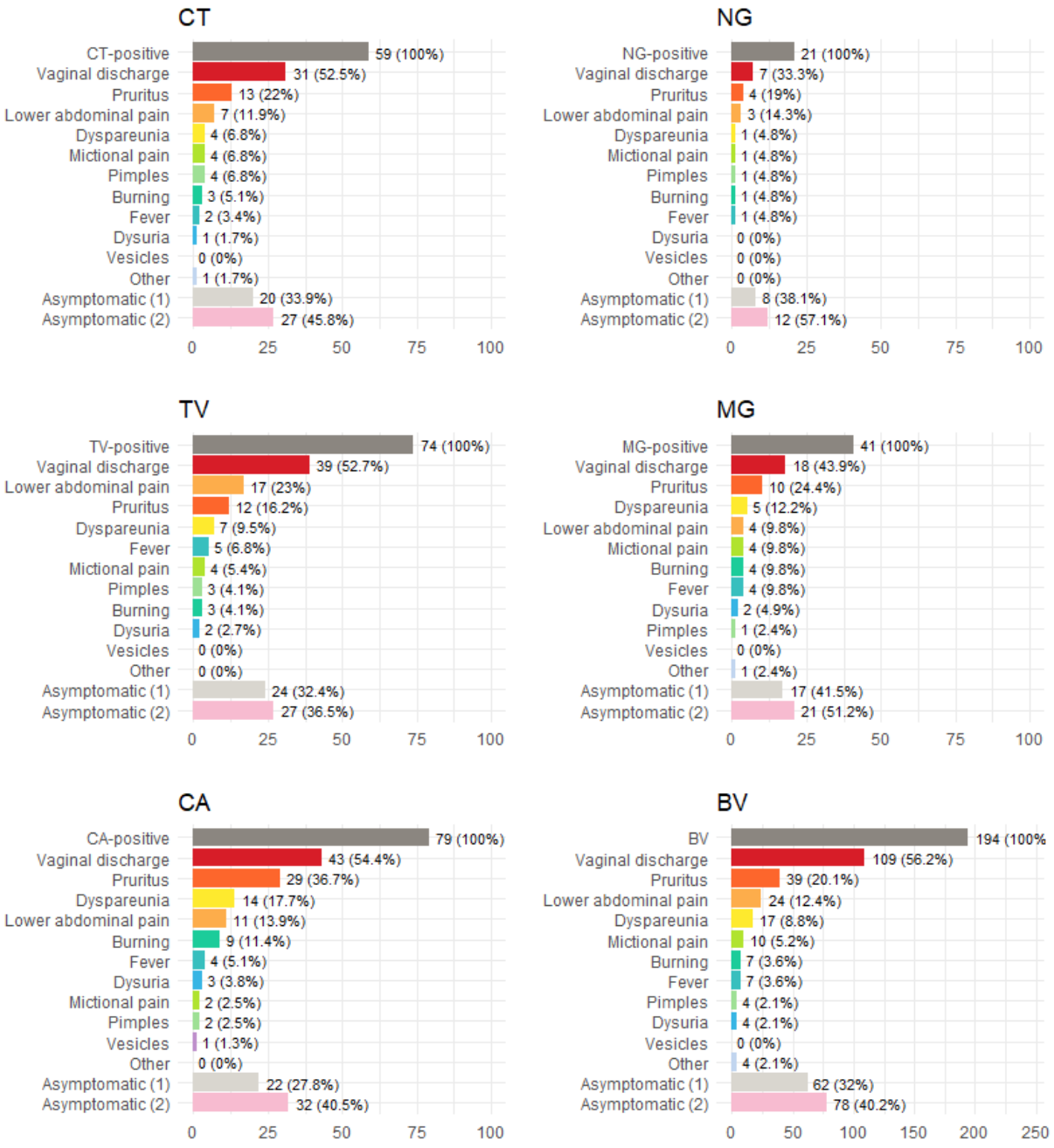


Figure 3.5. Symptoms and asymptomatic form of infections by CT, NG, TV, MG, CA, and BV among women of child-bearing age, in Antananarivo, Madagascar.

Asymptomatic (1) corresponds to the absence of all symptoms listed in the graphs
Asymptomatic (2) corresponds to the absence of vaginal discharge, burning, itching, and lower abdominal pain, the entry points of the syndromic management.

3.3.6. Performance of the syndromic approach

Overall, the performance of the syndromic approach was poor, and the sensitivity did not exceed 62.7% (**Table 3.4**). Despite relatively high prevalences, the probability of being infected when presenting the symptoms (i.e. the PPV) was very low. As a consequence, if all women

were treated according to their symptoms and/or signs, a large proportion of the study population would have been overtreated (i.e. the false positive), especially if only the entry points (i.e. the symptoms) are considered. Using the full WHO flowchart instead of symptoms only would reduce overtreatment (increased specificity) but also reduces the sensitivity.

Moreover, up to half of those infected will not be treated because they are not detected by the syndromic approach.

Table 3.4. Performance of the syndromic approach on the detection of CT, NG, TV, MG, CA, and BV.

	Gold standard	True positives	False positives	False negatives	True negatives	Se	Sp	PPV	NPV
Full WHO VDS and LAP algorithms with speculum use									
C. trachomatis	59/501 (11.8%)	33/501 (6.6%)	194/501 (38.7%)	26/501 (5.2%)	248/501 (55.9%)	55.9	56.1	14.5	90.5
N. gonorrhoeae	21/501 (4.2%)	7/501 (1.4%)	220/501 (43.9%)	14/501 (2.8%)	260/501 (51.9%)	33.3	54.2	3.1	94.9
T. vaginalis	74/501 (14.8%)	32/501 (6.4%)	199/501 (39.7%)	42/501 (8.4%)	228/501 (45.5%)	43.2	53.4	13.9	84.4
M. genitalium	41/501 (8.2%)	21/501 (4.2%)	225/501 (50.9%)	20/501 (4.0%)	205/501 (40.9%)	51.2	44.6	7.6	91.1
C. albicans	79/501 (15.8%)	45/501 (9.0%)	166/501 (33.1%)	34/501 (6.8%)	256/501 (51.1%)	57.0	60.7	21.3	88.3
BV	194/490 (39.6%)	101/490 (20.2%)	131/490 (26.1%)	93/490 (18.6%)	165/490 (32.9%)	52.1	55.7	43.5	64.0
Entry points of WHO VDS and LAP algorithms									
C. trachomatis	59/501 (11.8%)	37/501 (7.4%)	253/501 (50.5%)	22/501 (4.4%)	189/501 (37.7%)	62.7	42.8	12.8	89.6
N. gonorrhoeae	21/501 (4.2%)	11/501 (2.2%)	279/501 (55.7%)	10/501 (2.0%)	201/501 (40.1%)	52.4	41.9	3.8	95.3
T. vaginalis	74/501 (14.8%)	43/501 (8.6%)	230/501 (45.9%)	31/501 (6.2%)	197/501 (39.3%)	58.1	46.1	15.8	86.4
M. genitalium	41/501 (8.2%)	23/501 (4.6%)	267/501 (53.3%)	18/501 (3.6%)	193/501 (38.5%)	56.1	42.0	7.9	91.5
C. albicans	79/501 (15.8%)	49/501 (9.8%)	224/501 (44.7%)	30/501 (6.0%)	198/501 (39.5%)	62.0	46.9	17.9	86.8
BV	194/490 (39.6%)	125/490 (25.0%)	156/490 (31.1%)	69/490 (13.8%)	140/490 (27.9%)	64.4	47.3	44.5	67.0

VDS: Vaginal discharge syndrome
LAP: Lower abdominal pain
BV: Bacterial vaginosis
Se: Sensitivity
Sp: Specificity
PPV: positive predictive value, NPV: negative predictive value

3.3.7. Among infected women, factors associated with an asymptomatic form of infection

In this analysis, 134 had available data for logistic regressions. Of them, 80/134 (59.7%) were symptomatic and 54/134 (40.3%) were asymptomatic. We defined "asymptomatic" as the absence of patient-reported symptoms of abnormal vaginal discharge, burning, itching, and lower abdominal pain. We explored factors associated with an asymptomatic form of infection in bivariate and multivariable models (**Table 3.5**). In the univariate analysis, we found an association between women aged 35 – 45 years and an increased odds of being asymptomatic in comparison with the 18 – 24 age group, with limited evidence of this association (OR: 2.96, 95% CI: 0.93, 10.5, p=0.073). In addition, higher education was associated with a decreased odds of being asymptomatic (or an increased odds of declaring symptom) in comparison to the group whose highest education was middle school or below (OR: 0.28, 95% CI: 0.11, 0.65, p=0.004). Finally, being currently under hormonal contraception was associated with an increased risk of being asymptomatic (OR: 2.61, 95%CI: 1.26, 5.47, p=0.009). In the multivariable analysis, adjusting for age, the adjusted OR was 0.38 (95% CI: 0.15, 0.95, p=0.040) for infected women with a higher education in comparison to those did not go further than middle school and 2.19 (95% CI: 0.97, 5.01, p=0.059) among women under hormonal contraception.

Table 3.5. Factors associated with an asymptomatic form among women infected with CT, NG, TV, or MG.

		Bivariate analyses			Multivariable analysis		
Characteristic	**N**	**OR**	**95%CI**	**p-value**	**aOR**	**95%CI**	**p-value**
Age category				0.067			0.13
18-24	62	-	-		-	-	
25-34	57	0.74	0.35, 1,56	0.4	0.63	0.28, 1,40	0.3
35-45	15	2.96	0.93, 10.5	0.073	2.17	0.63, 8.19	0.2
Education				0.008			0.073
Middle school or below	47	-	-		-	-	
High school	37	0.75	0.31, 1.77	0.5	0.91	0.36, 2.30	0.8
Higher education	50	0.28	0.11, 0.65	0.004	0.38	0.15, 0.95	0.040
Currently under hormonal contraception							
Yes	47	2.61	1.26, 5.47	0.009	2.19	0.97, 5.01	0.059
No	87	-	-		-	-	

3.3.8. Presence of resistance-associated mutations in *Mycoplasma genitalium*

Amplification of resistance genes could be performed for *parC* in 23/41 (56%) of the samples and 16/41 (39%) for 23S rRNA. Mutation in *parC* (position G248T) was detected in 1/23 (4.35%, 95%CI: 0.11% to 22.0%) and in 23S rRNA in 0/16 (0%, 95%CI: 0% to 20.6%).

3.4. Discussion

We identified a high prevalence of STIs in women of child-bearing age attending CHUGOB in Antananarivo. A third were infected with at least one STI, among them, one in five was infected with at least two pathogens simultaneously.

We estimated the prevalence of CT and NG to be 11.8% (95%CI: 9.0, 14.6) and 4.2% (2.4, 5.9), respectively. Our findings are similar to those determined in rural Madagascar (for which we calculated the 95%CI): 9.3% (95%CI: 6.4, 13.0) and 5.4% (3.2, 8.4), respectively.[126] In our study, the prevalence of MG exceeded that reported in this study (8.2% [5.8, 10.6] compared to 2.7% [1.2, 5.1]). Conversely, the prevalence of TV was lower in our investigation (14.8% [11.7, 17.9]) compared to the cited study (23.4% [19.0, 28.3]). Yet, these estimates must be compared with caution since the two studies were conducted 20 years apart, and one was in the capital city and the other in rural areas. BV prevalence in our study (39.6% [35.3, 43.9]) was consistent with findings among women in rural Madagascar (33.0% [28.0, 38.4])[126] and very similar to estimates found in the rest of Africa.[83]

In a meta-analysis that estimated STI prevalence among women in different African regions, our figures were closer to the estimates found among young women in East Africa and at high risk, i.e., recruited from recreational facilities or engaged in transactional sex.[83] CT prevalence in our study was similar to the estimate in this high-risk group: 10.3% (7.4, 14.1). Likewise, we found a TV prevalence of 14.8% (11.7, 17.9), comparable to the 12.7% (7.5, 20.6) estimate among women at risk. Our results raise concern as the participants of our study are drawn from the community, a population at lower risk, and indicate a substantial circulation of STIs among Malagasy women of child-bearing age.

The prevalence of MG in our study was 8.2% (5.8, 10.6) and was comparable to that in pregnant women in sub-Saharan Africa (13.5% [4.0, 27.2]).[136] However, it was lower than estimates from adolescents and young women (<25 years) in South Africa (22.1% [19.3, 25.2])[137] but more than six times higher than in high-income countries (1.3% [1.0, 1.8]).[16]

The prevalence of TV appeared to decrease with age: it was 10.9% (5.3, 19.1) for women aged 35-45 versus 17.8% (12.6, 24.1) for those aged 18-24. This result contrasts with other studies that have shown that TV infection is associated with an older age.[138,139] However, the

confidence intervals of our estimates overlap which suggests that this observation may be caused by sampling fluctuations.

Among factors related to having an STI, we found that being in a relationship was associated with increased odds of having an STI in comparison to being married. Marriage is officially monogamous in Madagascar; therefore, married women would have only their husband as a sexual partner which decreases their exposure to STIs and thus could explain the lower odds of acquiring an STI. This was supported by our data, which shows that 5.7% of the married women had other partners in the past 12 months, while 20.3% of the women in a relationship but not married were in this situation. Relationships that overlap in time (or concurrent relationships) are not uncommon among unmarried young individuals in Madagascar[140] and have been shown to be associated with an increased risk of STIs compared to single relationships.[141]

Although sex in exchange for favors concerned only less than 3% of the participants, the magnitude of this association was the highest among all explored risk factors. The proportion of women who declared having ever exchanged sex for favors in our study was comparable to another study conducted in Antananarivo among the 15-24-year (5.9%).[140] The proportion of women who exchanged sex in our study is consistent with demographic health surveys (DHS) data in Africa summarized in a meta-analysis where 4.34% (3.16, 5.51) of the women were concerned.[142] Analyses of the features of those women in our data showed an association with low education, a factor also stressed in the meta-analysis.[142] Women with low education may have difficulty finding a job or have jobs with low pay, and therefore, they may turn to selling sex as a complementary income.

Lastly, we found that the women with BV or intermediate microbiota had a doubled odds of having an STI compared to women with an optimal microbiota. This was also found Allsworth *et al*.[143] It remains unclear whether BV facilitates STI acquisition or the reverse. Several studies have demonstrated that an optimal microbiota produces antimicrobial compounds that are protective against STIs.[144] One prospective cohort study examined the temporal precedence and determined an increased risk of acquiring NG, but not CT, among women with BV.[145] However, another longitudinal study has shown that other STIs may increase the risk of acquiring BV.[146] Further research is needed to elucidate these dynamics and inform targeted interventions for improving women's reproductive health.

Our study revealed other findings. Women aged 18-24 years were more likely to have a CT infection than those aged 35-45. This asymmetric burden among people below 25 years has also been documented in national surveillance data in the United States[147] and a large study in China.[135] This can be explained by behavioral factors such as a higher number of sexual partners, but also biological factors such as cervical ectopy.[148] Moreover, it has been hypothesized that untreated CT infection might confer some immunity against further infection.[149] Women aged 35 to 45 may be more likely to have been already exposed to CT, and spontaneously cleared the infection without treatment, and as a result may have developed such immunity.

In our study, we also observed that condoms were rarely used, a finding shared with other studies conducted in Madagascar.[150,151] Low condom use was justified by steady relationships; however, in our study, we did not find a difference in condom use between women married and non-married women. According to the DHS, condom uptake with a partner that was neither the spouse nor the person they were living with increases with education: it ranges from 1.2% among those who have never been to school to 12.6% among those who went to university.[118] When comparing with other African countries, a study pointed out that, out of 18 countries, Madagascar has the lowest condom uptake at last intercourse among men aged 15-29 years and the lowest percentage of men who know about condoms.[150] Historically, in Malagasy society, which has been influenced by Christian values, condom use was not favorably regarded. This attitude may have endured over time, shaping cultural perceptions and impacting sexual health practices.[152]

Paradoxically, despite high proportions of infections and co-infections, the prevalence of HIV in our study was low (0.7%) and consistent with the estimate found in a study conducted between 2010 and 2015 in Madagascar (0.4%).[124] Low HIV prevalence could be explained by the isolated island status of Madagascar and the fact that most men are circumcised. While evidence that circumcision is protective against HIV has been established, a recent review found mixed evidence against the acquisition and transmission of CT or NG and no evidence against MG.[153]

We reported that 7% of the women were co-infected by two STIs or more. This result is much lower than reports from Tanzania (19%)[154] and South Africa (18%).[155] When we explored the factors associated with co-infections among the infected women, we found an increased

risk among women who practiced vaginal douching compared to women who washed only their external genitalia. This association has been previously described.[156–158] However, Joesoef *et al.* reported that douching with water was not associated with STIs, but douching with water and soap, betel leaf or a commercial product substantially increased this risk.[156] In our study, we did not collect information on the type of product used for douching or the occasion (e.g. after sex, during menses). This information could enhance our analysis and help tailor future health promotion messages to local practices. Underlying biological factors may explain this association. Douching may alter the vaginal microbiota which can in turn facilitate STIs.[159] Additionally, since douching is associated with BV [160] and BV with STIs, an indirect effect mediated by BV may also be at play.

Moreover, we found that when the partner was the main source of income, there was a protective effect against co-infections compared to when the main source was the participant herself was the main source. This result suggests that financial dependence on a partner could lead to a more stable relationship, reducing the likelihood of exposure to multiple infections. In contrast, women who are financially independent may experience increased freedom and autonomy, which could lead to more sexual encounters or relationships that might expose them to higher risks of multiple STIs.

Consequences of co-infection remain unclear. The simultaneous presence of STIs can complicate diagnosis and treatment, and the potential effects on long-term health outcomes have yet to be studied.

We underscored that nearly 40% of the women with an STI did not present with symptoms of the WHO syndromic management guidelines. Moreover, 41 to 53% of the women presented either a vaginal discharge syndrome or lower abdominal pain but had no STIs. These results indicate that a substantial fraction of infected women would not have received appropriate treatment while a large number of uninfected women would have been unnecessarily treated under the syndromic approach. Our findings are consistent with those of Verwijs *et al.* who determined the same proportion of false positives among women in Rwanda and a similar performance of the WHO syndromic approach.[161] These results raise concern over the high number of women who may be overtreated and the small fraction of infected women who may not be treated and therefore may continue to transmit STIs and may develop complications.

We also noted that among women with lower abdominal pain, one in four were infected by TV. Other studies have also reported and association between TV and lower abdominal pain.[36,162] However, the WHO guidelines recommend treatment only for CT, NG, bacteria associated with BV, and MG in case of lower abdominal pain.[6] The importance of lower abdominal pain in TV infection warrants further investigation.

In this study, we found marginal evidence of associations between education and hormonal contraception with asymptomatic infections, possibly owing to a lack of statistical power. The association between higher level of education and decreased odds of asymptomatic infection, may be explained by the likelihood that more educated women were better able to identify their symptoms and/or felt safer disclosing them.

The association between hormonal contraception and asymptomatic infection is less clear. Estrogens seem to play a role in promoting the growth of lactobacilli by stimulating the accumulation of glycogen in the vaginal mucosa.[163] The predominance of lactobacilli might maintain stability of the microbiota and reduce STI bacterial load. Reduced bacterial loads may decrease the level of genital inflammation and therefore the intensity of the symptoms. However, further studies are needed to confirm this association and this explanatory hypothesis.

Finally, among women infected with MG, we detected one mutation in *parC* associated with antimicrobial resistance to fluoroquinolones in 41 samples (0.2%). A recent systematic review of antimicrobial resistance in NG and MG in sub-Saharan Africa mentioned two studies exploring resistance in MG, both conducted in South Africa.[164] One of these studies detected one mutation in *parC* in 266 samples (0.4%), no mutation in *gyrA*.[165] The other found mutations in *parC* in 19 of 43 samples, and none in *gyrA*.[166] None of these studies detected macrolide resistance. Currently, there is a paucity of studies on MG resistance in Africa and the heterogeneity between Malagasy and South African context precludes drawing conclusions for the region as a whole. However, another study showing little NG resistance in Madagascar[132] suggests that the country may be preserved from the introduction of resistant strains, possibly due to its geographical isolation.

Our study had several limitations. First, we conducted this study in the capital city, and the results may not be representative of the women in Madagascar. In our study population, 88%

of the participants went to middle school or above, whereas at the national level, only 31% of girls do.[118] As the DHS shows that knowledge on HIV prevention and condom uptake increases with the level of education,[118] women included in our study may be less at risk for STIs. Thus, our estimates, which were already high, may be even higher in the Malagasy female population.

Second, our questionnaire contained sensitive questions. This caused social desirability bias that may have led to an underestimation of the strength of the associations in our analysis.

Nevertheless, this study has several strengths. It is the first study on CT, NG, TV, and MG in a non-female sex worker population since the community-based study conducted twenty years ago.[126] Additionally, we had no missing data for determining the prevalence rates except for BV, for which the inability to analyze the samples was due to random events. We found no difference in characteristics between the 95% of participants with complete data and the 5% with incomplete data. Thus, we believe our results are strongly reliable. Finally, the laboratory team was rigorously trained, and emphasis was made on the quality of the procedures using the most recent recommendation for the detection of the pathogens.

In conclusion, the high prevalence of STIs in this urban, educated female population showed a need for intervention and prevention, such as the promotion of and negotiation skills on condom use for both men and women who have occasional partners, especially when they engage in transactional sex. Young people should be targeted in priority due to their higher likelihood of being infected and their misconceptions about condoms.[167]

Further studies on the prevalence of STIs and HIV in other cities and rural areas should be considered, especially in Southern regions where polygamy and extramarital relationships are far more common. Similarly, STI diffusion could follow a different pattern in coastal cities where sex tourism is common.[168] A comprehensive picture of the epidemiology of STIs, including HIV, at the country level, is needed to serve as a guide for future public health prevention and intervention programs.

3.5. Role in the study

The PrevISTana study was conducted jointly with the “Institut Pasteur de Madagascar” (IPM). I wrote the study protocol and developed the questionnaires, under the supervision of Bich-Tram Huynh, Tania Crucitti and Rindra Vatosoa Randremanana. Both were reviewed by Aina Harimanana and Tsinjo Rasoanaivo, epidemiologists at IPM, with special attention related to sexual activity and cultural acceptance. Dr Solange Bienvenue, the principal investigator at the hospital (CHUGOB) also reviewed the protocol and the questionnaire.

As I relocated to Madagascar just weeks before the study commenced, I remained there for over a year to contribute to the coordination of the study. I presented the study protocol to the doctors and midwives at the hospital. STIs are a sensitive subject and the field investigators at IPM had not worked on this topic before. Thus, they received a training which I attended.

I participated in the verification of the electronic entry forms, data management, data cleaning, and conducted all the statistical analyses. After all samples were analyzed by the UBEX laboratory team, I presented the study results to the hospital personnel involved in the study.

Lastly, I obtained a grant from IPM to test for the presence of resistance-associated mutations in MG. Lala Fanomezantsoa Rafetrarivony conducted the analyses for the detection of the mutations.

Chapter 4: Asymptomatic infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* among women in low- and middle-income countries: a systematic review and meta-analysis

4.1. Introduction

In the previous chapter, we underscored that nearly half of CT, NG, and TV infections did not present vaginal discharge syndrome or lower abdominal pain and would have been missed if the syndromic approach had been used. The limitations of the WHO guidelines on the syndromic management of STIs have been stressed in multiple studies.[115,169–175]

Not capturing asymptomatic STI cases is a concern, as it leaves a fraction of those infected undiagnosed and untreated. As mentioned in the introduction, in women, untreated infections caused by CT, NG, and TV can result in serious complications such as pelvic inflammatory disease (PID), infertility, ectopic pregnancy, preterm birth or low birth weight.[8–11] These STIs are also associated with a higher risk of acquiring and transmitting other STIs, including HIV.[12,13] Lastly, untreated infections sustain the transmission.

To date, no study has provided pooled estimates of the burden of asymptomatic infections of the three most common curable STIs.

In this perspective, we conducted a systematic review and a meta-analysis to estimate: (i) the proportion of asymptomatic women among those infected by CT, NG, and TV and, (ii) the prevalence of asymptomatic CT, NG, and TV infections in the female population in LMICs.

4.2. Methods

4.2.1. Search strategy

We searched MEDLINE, Web of Science, and Scopus databases. Search terms combined pathogen and disease terms, terms related to the absence of symptoms and countries ever classified as LMICs by the World Bank between 2000 and 2022[176] (full search terms in **Annex B, supplementary Text 1**). We also screened references for eligible articles. When the estimates were collected but not presented, we contacted the authors up to three times.

This study was registered with PROSPERO, CRD42022286673.

4.2.2. Definitions

We defined "proportion of asymptomatic infection" as the number of women who tested positive for a pathogen and were asymptomatic divided by the number of women positive for that pathogen. The definition of the "prevalence of asymptomatic infection" was the number of women who tested positive for a pathogen and were asymptomatic divided by the number of women tested for that pathogen. Each prevalence rates, therefore, relate to the context of the study setting from which they are derived and may be specific to its context (e.g. hospital, or primary healthcare settings).

We defined symptoms as manifestations of a disease that were reported by the participant, while signs were considered features of a disease observed by a healthcare provider or other person.

4.2.3. Selection criteria

We selected studies published between 1 January 2000 and 31 December 2022, in English and French. We included studies which tested for STIs using genital or urine specimens. We kept studies that used NAATs for the diagnosis of CT, NG, and TV.[177] For TV, we also included articles that used InPouch® or performed wet mount microscopy immediately after collection, as these techniques showed fair sensitivity and specificity[178] and remain common detection methods in LMICs.[179]

We selected studies where the investigator actively asked about at least one symptom, or where participants self-reported symptoms. We excluded articles in which authors assumed the absence of symptoms in the entire study population without collecting such information. Finally, we excluded articles when "asymptomatic" was defined as the absence of clinical signs as reported by the healthcare provider rather than by the women as we intended a participant-centred approach.

BTH, TC, RR, and CF conducted an initial screening of titles and abstracts in pairs and inclusion conflicts were resolved through consensus. CF performed data extraction, and BTH, TC, and RR ensured exactness and validity. When multiple publications on the same study population and outcomes were found, we prioritized the most comprehensive and recent data. Extracted data included the number of asymptomatic positive women, total women tested, and women positive for a specific STI. Other variables we extracted were: first author, publication year, study year, country, recruitment site, number and type of reported symptoms, definition of asymptomatic women, rural/urban setting, specific populations (female sex works (FSWs), adolescents, women with HIV, adolescents, infertile women), age range, specimen type, other STI pathogens tested for, and detection techniques.

4.2.4. Data analysis

We described regions, study designs, settings, recruitment sites, specific populations, number of symptoms, sampling methods, and laboratory procedures of the selected studies.

Proportion and prevalence of asymptomatic CT, NG, and TV infections were estimated first for all studies and excluding studies on FSWs, women with HIV, and women attending an STI clinic as they are not representative of the general population and have a higher risk of having an STI. We referred to these groups as "populations with an increased risk of STIs". Separate estimations were conducted for FSWs, women with HIV as well as other specific populations such as pregnant women, adolescents, and infertile women.

Random-effect models were applied for each of the above analyses using the DerSimonian and Laird variance estimator. As some figures were near 0 or 1, the Freeman-Tukey double arcsine transformation was applied. Statistical heterogeneity was evaluated using the χ^2 test with Cochran's Q and I^2. Analyses and forest plots were conducted using *meta* and *metafor*

packages in R software (version 4.1.1). Proportions and prevalence maps were generated using qGIS (version 3.22).

Subgroup analyses were performed by region, country income level, rural/urban setting, study period, and the number of symptoms assessed in the study. The role of these study variables in the heterogeneity of the estimates were explored in meta-regressions.

The risk of bias was assessed using the appraisal tool for cross-sectional studies (AXIS) using >70% of validated items as a cut-off to qualify studies with a low risk of bias.[180] We conducted a sensitivity analysis to assess the impact of excluding studies with a high risk of bias on our overall estimates.

Publication bias was evaluated by Funnel plots and the Egger test.

This article was written following the PRISMA checklist (**Annex B, supplementary Table 1**).

4.3. Results

We identified 1113 articles, among which 547 were duplicates. We eliminated 468 articles based on title and abstract and screened 173 full texts and their citations. We found 4 additional relevant citations among selected articles. In total, 177 articles were eligible. Of those, 42 had available and extractable data and 45 reported having data according to our criteria but did not present the numbers. We were able to contact 40 of those 45 authors and retrieved data from six articles. In total, we used data from 48 studies corresponding to 99 data points for both the prevalence and the proportion: 41 for CT, 27 for NG, and 31 for TV (**Figure 4.1**, **Table 4.1**).

Twenty-one countries were represented in our study: eight in Africa, seven in Asia, five in Latin America, and one in Oceania. Most studies were cross-sectional (44/48, 92%) and few were cohort (2/48, 4%) or randomized controlled trials (2/48, 4%). Almost three-quarters of the studies took place in urban settings (34/48, 71%), 21% (10/48) in rural settings, and 8% (4/48) in mixed settings. Hospitals and antenatal care were the most frequent recruitment sites (14/48, 29% and 11/48, 23%, respectively), followed by primary or secondary health care centres (9/48, 19%), locations where people with a high risk of STI can be found such as FSW venues, HIV centres or STI clinics (8/48, 17%). Six studies performed random sampling within the community (6/48, 13%).

Some studies focused on specific populations: 15/48 (31%) were on pregnant women, 5/48 (10%) on FSWs, 4/48 (8%) on adolescents, 4/48 (8%) on women with HIV, and 4/48 (8%) on infertile women exclusively. Studies had enrolled between 48 and 4812 participants, and their age ranged from 10 to 90 years old although this information was unavailable for 14 studies.

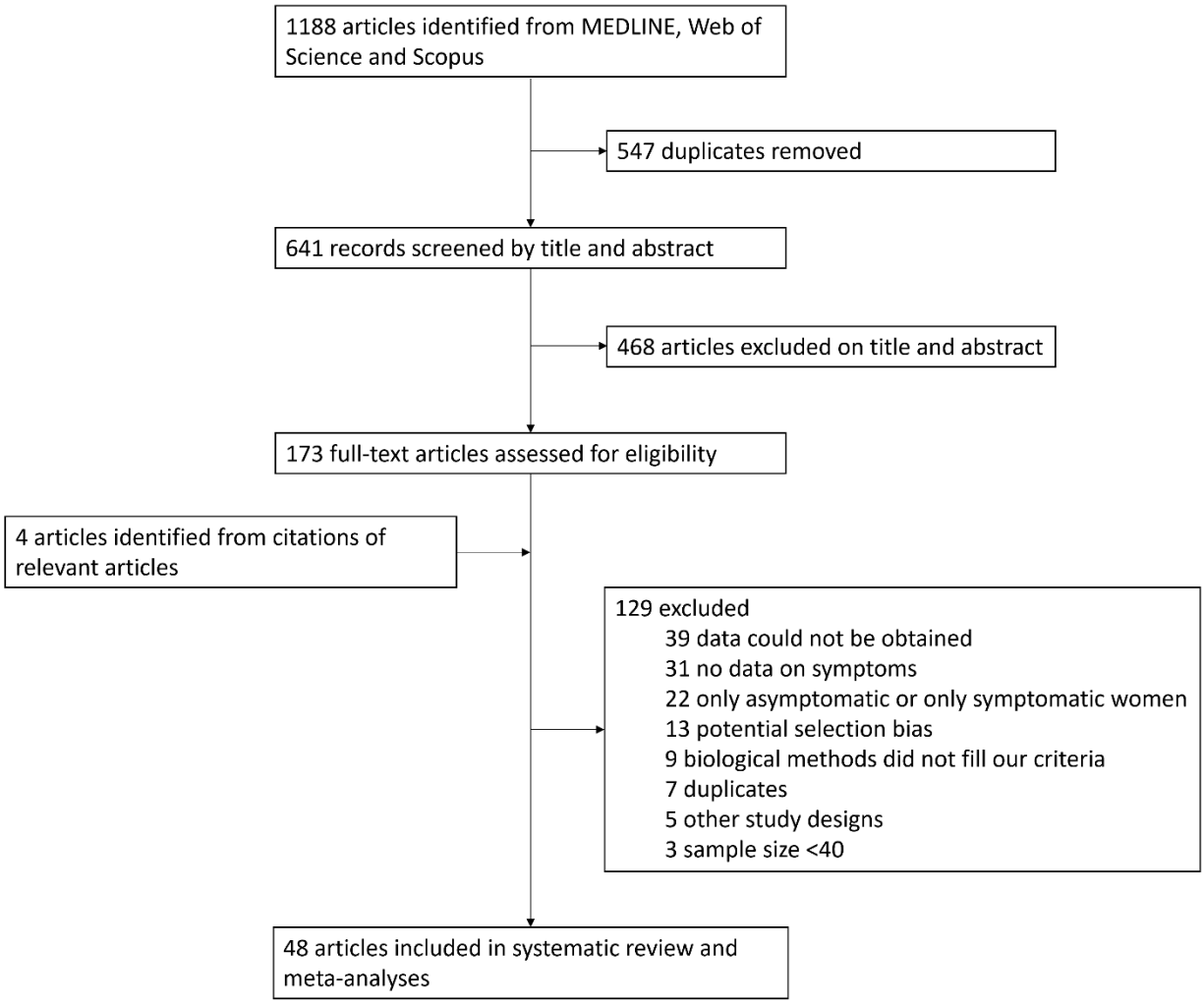


Figure 4.1. Study selection.

The number of recorded symptoms ranged from one to 14, with nearly half of the studies (23/48, 48%) exploring 1–4 symptoms.

Genital specimens (41/48, 85%) were the most common specimens, and few studies used urine (7/48, 15%). All techniques used for the laboratory diagnosis of CT (n=41) and NG (n=27) were NAATs, as per research criterium. For TV, NAATs were used in 25/31 studies, wet mount microscopy in 4/31, and InPouch® in 2/31 studies.

The estimates summarised below are presented in **Tables 4.2 – 4.5, Figures 4.2 and 4.3.** The proportion of asymptomatic CT, NG, and TV infections, excluding populations with an increased risk of STIs, was 60.7% 95%CI [50.4; 70.5] (33 data points, 26 268 participants), 53.3%

[37.1; 69.1] (21 data points, 16 950 participants), and 56.9% [44.6; 68.9] (23 data points, 10 824 participants), respectively.

The proportion of asymptomatic CT, NG, and TV infections (excluding populations with an increased risk of STIs) was significantly different across regions (p=0.048, p=0.019, and p=0.039, respectively for each pathogen). Africa presented the highest proportion of asymptomatic infections (CT: 68.6% [56.4; 79.8], 14 data points, 12 368 participants; NG: 67.2% [51.0; 81.9], 13 data points, 7 681 participants; TV: 64.9% [52.2; 76.7], 12 data points, 6 152 participants). Latin America had the lowest proportion of asymptomatic women infected with CT (30.9% [11.2; 54.8], five data points, 2 850 participants) and with NG (14.0% [0.0; 67.5], two data points, 1 546 participants). The lowest proportion of asymptomatic TV infection was found in Asia (17.3% [0.0; 49.5], four data points, 2 606 participants).

When stratifying by country income level, the proportions of asymptomatic cases were the highest in middle-income countries (CT: 63.2% [50.2; 75.1], 11 data points, 14 887 participants; NG: 60.9% [41.1; 79.2], 14 data points, 10 793 participants; TV: 61.7% [45.4; 77.1], 17 data points, 6 688 participants), and the lowest in low-income countries (CT: 55.5% [37.3; 73.1], 11 data points, 11 381 participants; NG: 37.7% [18.4; 58.8], seven data points, 6 157 participants; TV: 45.4% [29.7; 61.6], six data points, 4 136 participants). The proportion of asymptomatic infected women was also the highest in rural settings (CT: 71.6% [55.6; 85.3], ten data points, 9 150 participants; NG: 70.4% [46.3; 90.3], 9 data points, 4 464 participants; TV: 75.0% [49.8; 94.0], six data points, 2 619 participants), and the lowest in urban settings (CT: 55.6% [43.8; 67.1], 20 data points, 15 276 participants; NG: 44.4% [26.5; 62.9], 11 data points, 12 124 participants; TV: 58.9% [46.5; 70.8], 15 data points, 7 544 participants).

Analyses on specific populations showed that, in women with HIV, the proportion of asymptomatic CT, NG, and TV infections exceeded 50% (CT: 64.7% [48.3; 79.7], three data points, 739 participants; NG: 61.0% [42.7; 78.0], three data points, 739 participants; TV: 70.5% [60.7; 79.6], four data points, 965 participants). In FSWs, those proportions were below 50% (CT: 45.5% [24.8; 67.0], five data points, 1 890 participants; NG: 38.1% [3.1; 82.9], three data points, 1 051 participants; TV: 26.7% [19.6; 34.5], two data points, 546 participants).

In studies conducted in the community, the proportion of asymptomatic CT infections was higher (63.0% [29.7; 91.3]) than in studies conducted at the primary, secondary, or tertiary

health facility level (58.6% [46.0; 70.7]). The difference between the sampling sites was even greater for NG, where 78.9% [53.5; 97.0] infections were asymptomatic in the community versus 44.9% [27.9; 62.3] in health facilities. However, the proportion of asymptomatic infections was similar for TV, with 52.6% [34.4; 70.5] in the community and 52.9% [37.3; 68.2] in health facilities.

Table 4.1. List and characteristics of included articles.

Study	Country	Income level	Study design	Setting	Method for pathogen detection	No. women tested	No. women positive	No. women positive and asymptomatic	Proportion of asymptomatic infection (%)	Prevalence of asymptomatic infection (per 100 women)	Risk of bias
Chlamydia trachomatis											
Badman, 2016 [181]	Papua New Guinea (PNG)	LM	Cross-sectional	Urban	Molecular (PCR)	125	25	19	76.0	15.20	Low
Bruce, 2010 [182]	PNG	L	Cross-sectional	Urban	Molecular (PCR)	129	30	3	10.0	2.33	Low
Cabeza, 2015 [183]	Peru	UM	Cross-sectional	Urban	Molecular (PCR)	600	60	8	13.3	1.33	Low
Chang, 2020 [184]	China	UM	Cross-sectional	Urban	Molecular (PCR)	4812	404	138	34.2	2.87	High
Chen, 2020 [135]	China	UM	Cross-sectional	NS	Molecular (PCR)	5006	44	44	100	0.88	Low
Chen, 2005 [185]	China	LM	Cross-sectional	Urban	Molecular (PCR)	505	296	228	77.0	45.15	Low
Claeys 2002 [186]	Nicaragua	L	Cross-sectional	Urban	Molecular (PCR)	1185	40	3	7.5	0.25	Low
Conde-Ferráez, 2017 [187]	Mexico	UM	Cross sectional	Urban	Molecular (PCR)	233	15	11	73.3	4.72	High
Das, 2013 [188]	India	LM	Cohort	Urban	Molecular (PCR)	417	47	18	38.3	4.32	Low
Davey, 2019 [189] *	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	135	28	19	67.9	14.07	Low
Davey, 2019 [189] †	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	107	21	10	47.6	9.35	Low
Frohlich, 2007 [190]	South Africa	LM	Cross-sectional	Rural	Molecular (PCR)	48	4	4	100	8.33	Low
Frohlich, 2007 [190]	South Africa	LM	Cross-sectional	Rural	Molecular (PCR)	226	20	20	100	8.85	Low
Ghebremichael, 2014 [191]	Tanzania	L	Cross-sectional	Urban	Molecular (PCR)	1440	2	1	50.0	0.07	Low
Gokral, 2005 [192]	India	L	Cross-sectional	Urban	Molecular (PCR)	100	34	29	85.3	29.00	High
Hazel, 2014 [193]	Namibia	UM	Cross-sectional	Rural	Molecular (PCR)	209	24	23	95.8	11.00	Low
Hoffman, 2019 [155]	South Africa	UM	Cross-sectional	Rural	Molecular (PCR)	251	52	33	63.5	13.15	Low
Hokororo, 2015 [154]	Tanzania	L	Cross-sectional	Rural	Molecular (PCR)	403	46	11	23.9	2.73	High
Jenab, 2010 [194]	Iran	LM	Cross-sectional	Urban	Molecular (PCR)	80	17	6	35.3	7.50	High
Kalsom, 2020 [195]	Malaysia	UM	Cross-sectional	Urban	Molecular (PCR)	137	15	13	86.7	9.49	Low
Karim, 2021 [196]	Morocco	LM	Cross-sectional	Urban	Molecular (PCR)	809	13	7	53.8	0.87	High
Kerubo, 2016 [197]	Kenya	L	Cross-sectional	Rural	Molecular (PCR)	507	13	9	69.2	1.78	Low
Lan, 2008 [198]	Vietnam	L	Cross-sectional	Rural	Molecular (PCR)	1012	43	24	55.8	2.37	Low
Li, 2021 [199] ‡	China	UM	Cross-sectional	Urban	Molecular (PCR)	881	46	39	84.8	4.43	Low
Li, 2021 [199] §	China	UM	Cross-sectional	Urban	Molecular (PCR)	595	35	18	51.4	3.03	Low
Li, 2021 [199] ¶	China	UM	Cross-sectional	Urban	Molecular (PCR)	254	15	9	60.0	3.54	Low
Lowe, 2019 [200]	Zimbabwe	L	Cross-sectional	Urban	Molecular (Xpert®)	385	8	6	75.0	1.56	High
Mahafzah, 2008 [201]	Jordan	LM	Cross-sectional	Urban	Molecular (PCR)	1275	7	1	14.3	0.08	High
Mania-Pramanik, 2012 [202]#	India	L	Cross-sectional	Urban	Molecular (PCR)	174	19	5	26.3	2.87	High
Mania-Pramanik, 2012 [202]¶	India	L	Cross-sectional	Urban	Molecular (PCR)	264	30	17	56.7	6.44	High
Mbizvo, 2001 [203]	Zimbabwe	L	Cross-sectional	Urban	Molecular (LCR)	393	15	8	53.3	2.04	Low
Moodley, 2015 [204]	South Africa	UM	Randomised controlled trials (RCT)	Urban	Molecular (PCR)	1459	259	148	57.1	10.14	High
Mudau, 2018 [205]	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	247	91	64	70.3	25.91	Low

Study	Country	Income level	Study design	Setting	Method for pathogen detection	No. women tested	No. women positive	No. women positive and asymptomatic	Proportion of asymptomatic infection (%)	Prevalence of asymptomatic infection (per 100 women)	Risk of bias
Nessa, 2004 [206]	Bangladesh	L	Cross-sectional	Urban	Molecular (PCR)	400	174	80	46.0	20.00	Low
Nessa, 2005 [207]	Bangladesh	L	Cross-sectional	Urban	Molecular (PCR)	439	77	42	54.5	9.57	Low
Obasi, 2001 [208]	Tanzania	L	Cross-sectional	Rural	Molecular (PCR)	4726	113	89	78.8	1.88	Low
Peters, 2014 [209]	South Africa	UM	Cross-sectional	Rural	Molecular (PCR)	589	97	70	72.2	11.88	Low
Rocha, 2014 [210]	Brazil	UM	Cross-sectional	Urban	Molecular (PCR)	361	23	13	56.5	3.60	Low
Silveira, 2020 [211]	Brazil	UM	Cross-sectional	Both	Molecular (PCR)	498	34	8	23.5	1.61	Low
Wangnapi, 2015 [212]	PNG	LM	RCT	Both	Molecular (PCR)	362	39	8	20.5	2.21	Low
Warr, 2019 [213]	Kenya	L	Cohort	Rural	Molecular (PCR)	1221	65	28	43.1	2.29	Low
Neisseria gonorrhoeae											
Badman, 2016 [181]	PNG	LM	Cross-sectional	Urban	Molecular (PCR)	125	14	9	64.3	7.20	Low
Bruce, 2010 [182]	PNG	L	Cross-sectional	Urban	Molecular (PCR)	129	48	4	8.3	3.10	Low
Chang, 2020 [184]	China	UM	Cross-sectional	Urban	Molecular (PCR)	4812	43	7	16.3	0.14	High
Chen, 2005 [185]	China	LM	Cross-sectional	Urban	Molecular (PCR)	505	191	144	75.4	28.51	Low
Claeys, 2002 [186]	Nicaragua	L	Cross-sectional	Urban	Molecular (PCR)	1185	5	0	0.0	0.00	Low
Das, 2013 [188]	India	LM	Cohort	Urban	Molecular (PCR)	417	43	15	34.9	2.96	Low
Davey, 2019 [189] *	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	135	3	2	66.7	0.04	Low
Davey, 2019 [189] †	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	107	9	7	77.8	1.19	Low
Frohlich, 2007 [190]	South Africa	LM	Cross-sectional	Rural	Molecular (PCR)	48	2	2	100	0.23	Low
Frohlich, 2007 [190]	South Africa	LM	Cross-sectional	Rural	Molecular (PCR)	226	15	15	100	2.52	Low
Ghebremichael, 2014 [191]	Tanzania	L	Cross-sectional	Urban	Molecular (PCR)	1440	24	17	70.8	1.39	Low
Hazel, 2014 [193]	Namibia	UM	Cross-sectional	Rural	Molecular (PCR)	209	150	138	92.0	9.58	Low
Hoffman, 2019 [155]	South Africa	UM	Cross-sectional	Rural	Molecular (PCR)	251	39	27	69.2	27.00	Low
Hokororo, 2015 [154]	Tanzania	L	Cross-sectional	Rural	Molecular (PCR)	403	27	9	33.3	2.49	High
Kalsom, 2020 [195]	Malaysia	UM	Cross-sectional	Urban	Molecular (PCR)	137	0	0	..	0.00	Low
Karim, 2021 [196]	Morocco	LM	Cross-sectional	Urban	Molecular (PCR)	809	113	83	73.5	22.93	High
Kerubo, 2016 [197]	Kenya	L	Cross-sectional	Rural	Molecular (PCR)	507	3	2	66.7	0.16	Low
Lan, 2008 [198]	Vietnam	L	Cross-sectional	Rural	Molecular (PCR)	1012	7	4	57.1	2.30	Low
Lowe, 2019 [200]	Zimbabwe	L	Cross-sectional	Urban	Molecular (Xpert®)	385	7	4	57.1	3.74	High
Mahafzah, 2008 [201]	Jordan	LM	Cross-sectional	Urban	Molecular (PCR)	1275	14	4	28.6	0.31	High
Mbizvo, 2001 [203]	Zimbabwe	L	Cross-sectional	Urban	Molecular (LCR)	393	7	2	28.6	0.25	Low
Moodley, 2015 [204]	South Africa	UM	RCT	Urban	Molecular (PCR)	1459	93	49	52.7	0.98	High
Mudau, 2018 [205]	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	247	17	9	52.9	1.78	Low
Peters, 2014 [209]	South Africa	UM	Cross-sectional	Rural	Molecular (PCR)	589	60	41	68.3	2.85	Low
Rocha, 2014 [210]	Brazil	UM	Cross-sectional	Urban	Molecular (PCR)	361	5	2	40.0	4.17	Low
Wangnapi, 2015 [212]	PNG	LM	RCT	Both	Molecular (PCR)	362	32	3	9.4	0.74	Low
Warr, 2019 [213]	Kenya	L	Cohort	Rural	Molecular (PCR)	1221	29	22	75.9	10.53	Low
Trichomonas vaginalis											
Adeoye, 2007 [214]	Nigeria	L	Cross-sectional	Urban	Microscopy	544	18	4	22.2	0.74	High
Badman, 2016 [181]	PNG	LM	Cross-sectional	Urban	Molecular (PCR)	125	47	32	68.1	25.60	Low
Bruce, 2010 [182]	PNG	L	Cross-sectional	Urban	Molecular (PCR)	129	53	12	22.6	9.30	Low

Study	Country	Income level	Study design	Setting	Method for pathogen detection	No. women tested	No. women positive	No. women positive and asymptomatic	Proportion of asymptomatic infection (%)	Prevalence of asymptomatic infection (per 100 women)	Risk of bias
Bruni, 2019 [215]	Brazil	UM	Cross-sectional	Urban	Molecular (PCR)	499	21	4	19.0	0.80	High
Das, 2013 [188]	India	LM	Cohort	Urban	Molecular (PCR)	417	85	25	29.4	6.00	Low
Davey, 2019 [189] *	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	135	5	4	80.0	2.96	Low
Davey, 2019 [189] †	South Africa	UM	Cross-sectional	Urban	Molecular (Xpert®)	107	16	12	75.0	11.21	Low
de Waaij, 2017 [216]	South Africa	UM	Cross-sectional	Rural	Molecular (PCR)	604	75	38	50.7	6.29	High
Frohlich, 2007 [190]	South Africa	LM	Cross-sectional	Rural	Molecular (PCR)	48	10	10	100.0	20.83	Low
Frohlich, 2007 [190]	South Africa	LM	Cross-sectional	Rural	Molecular (PCR)	226	54	54	100.0	23.89	Low
Ghebremichael, 2014 [191]	Tanzania	L	Cross-sectional	Urban	Molecular (PCR)	1440	152	94	61.8	6.53	Low
Hoffman, 2019 [155]	South Africa	UM	Cross-sectional	Rural	Molecular (PCR)	251	81	50	61.7	19.92	Low
Kalsom, 2020 [195]	Malaysia	UM	Cross-sectional	Urban	Molecular (PCR)	137	1	1	100.0	0.73	Low
Kerubo, 2016 [197]	Kenya	L	Cross-sectional	Rural	Molecular (PCR)	507	12	6	50.0	1.18	Low
Lan, 2008 [198]	Vietnam	L	Cross-sectional	Rural	Microscopy	1012	10	3	30.0	0.30	Low
Lopez-Monteon, 2013 [217]	Mexico	UM	Cross-sectional	Urban	Molecular (PCR)	210	48	45	93.8	21.43	Low
Lowe, 2019 [200]	Zimbabwe	L	Cross-sectional	Urban	Molecular (Xpert®)	385	31	19	61.3	4.94	High
Mabaso, 2020 [218]	South Africa	UM	Cross-sectional	Urban	Molecular (PCR)	362	47	28	59.6	7.73	High
Mahafzah, 2008 [201]	Jordan	LM	Cross-sectional	Urban	InPouch®	1275	9	1	11.1	0.08	High
Mbizvo, 2001 [203]	Zimbabwe	L	Cross-sectional	Urban	Microscopy	393	60	40	66.7	10.18	Low
Miranda, 2014 [219]	Brazil	UM	Cross-sectional	Both	Molecular (PCR)	299	23	16	69.6	5.35	High
Moodley, 2015 [204]	South Africa	UM	RCT	Urban	Molecular (PCR)	1459	223	118	52.9	8.09	High
Mucci, 2016 [220]	Argentina	UM	Cross-sectional	Urban	Microscopy	210	3	0	0.0	0.00	High
Mudau, 2018 [205]	South Africa	UM	Cross-sectional	Urban	Molecular (PCR)	247	59	43	72.9	17.41	Low
Paul, 2012 [221]	India	LM	Cross-sectional	Urban	Molecular (PCR)	198	10	7	70.0	3.54	High
Rassjö, 2006 [222]	Uganda	L	Cross-sectional	Urban	Molecular (PCR)	199	16	7	43.8	3.52	Low
Rocha, 2014 [210]	Brazil	UM	Cross-sectional	Urban	Molecular (PCR)	361	46	21	45.7	5.82	Low
Shehabi, 2009 [223]	Jordan	LM	Cross-sectional	Urban	Molecular (PCR)	182	1	0	0.0	0.00	High
Tann, 2006 [173]	Uganda	L	Cross-sectional	Urban	InPouch®	250	244	13	5.3	5.20	Low
Tavakoli Oliaee, 2017 [224]	Iran	UM	Cross-sectional	Urban	Molecular (PCR)	150	24	2	8.3	1.33	High
Wangnapi, 2015 [212]	PNG	LM	RCT	Both	Molecular (PCR)	362	73	15	20.5	4.14	Low

L: Low-income country
LM: Lower middle-income country
UM: Upper middle-income country
PNG: Papua New Guinea
RCT: Randomised controlled trial

* Pregnant and without HIV
† Pregnant and with HIV
‡ Obstetric ward
§ Gynaecology ward
¶ Infertility ward
# Antenatal care

Table 4.2. Proportion and prevalence of asymptomatic CT, NG, and TV infections among women in LMICs.

	Proportion of asymptomatic infections (per 100 infected women) [95%CI]			Prevalence of asymptomatic infections (per 100 tested women) [95%CI]		
	CT	NG	TV	CT	NG	TV
Overall						
Excluding populations with an increased risk of STI*	60.7 [50.4; 70.5]	53.3 [37.1; 69.1]	56.9 [44.6; 68.9]	4.70 [3.39; 6.20]	3.11 [1.34; 5.54]	5.98 [3.46; 9.12]
Including populations with an increased risk of STI	58.8 [50.4; 67.0]	52.2 [38.5; 65.6]	53.6 [43.0; 64.1]	6.05 [4.26; 8.12]	3.70 [1.79; 6.23]	5.84 [3.75; 8.34]
Continent*						
Africa	68.6 [56.4; 79.8]	67.2 [51.0; 81.9]	64.9 [52.2; 76.7]	4.99 [2.70; 7.90]	5.67 [2.15; 10.66]	9.06 [5.71; 13.07]
Asia	66.2 [47.2; 83.0]	27.7 [9.0; 50.9]	17.3 [0.0; 49.5]	5.55 [3.40; 8.19]	0.13 [0.03; 0.28]	0.08 [0.00; 0.31]
Latin America	30.9 [11.2; 54.8]	14.0 [0.0; 67.5]	50.1 [16.4; 83.8]	1.89 [0.56; 3.91]	0.12 [0.00; 1.16]	4.54 [0.52; 11.9]
Oceania	47.4 [2.7; 95.0]	32.7 [0.0; 88.3]	43.3 [4.8; 87.5]	7.22 [0.00; 24.73]	3.12 [0.00; 12.25]	12.77 [0.06; 40.12]
Country income level*						
Low-Income	55.5 [37.3; 73.1]	37.7 [18.4; 58.8]	45.4 [29.7; 61.6]	3.55 [1.87; 5.70]	0.57 [0.17; 1.17]	3.12 [0.73; 7.02]
Middle-Income	63.2 [50.5; 75.1]	60.9 [41.1; 79.2]	61.7 [45.4; 77.1]	5.34 [3.59; 7.39]	5.21 [1.77; 10.22]	7.28 [3.72; 11.88]
Setting*						
Rural	71.6 [55.6; 85.3]	70.4 [46.3; 90.3]	75.0 [49.8; 94.0]	5.36 [3.11; 8.15]	6.80 [1.39; 15.60]	10.27 [2.41; 22.46]
Urban	55.6 [43.8; 67.1]	44.4 [26.5; 62.9]	58.9 [46.5; 70.8]	4.83 [2.90; 7.19]	1.33 [0.29; 3.02]	4.81 [2.25; 8.21]
Study year*						
1998 - 2011	61.8 [48.1; 74.7]	55.9 [32.9; 77.8]	57.3 [41.0; 73.0]	4.41 [2.37; 6.99]	3.72 [0.98; 7.99]	5.94 [2.86; 10.00]
2011 - 2022	57.7 [42.1; 72.7]	48.9 [26.9; 71.1]	61.1 [41.3; 79.5]	4.09 [2.88; 5.49]	2.42 [0.47; 5.63]	6.99 [2.09; 14.30]
Number of symptoms assessed*						
Between 1 and 4	57.4 [41.6; 72.4]	40.6 [13.8; 70.4]	55.1 [37.7; 71.9]	6.53 [3.46; 10.45]	4.39 [0.46; 11.76]	5.68 [2.97; 9.17]
Five and more	69.0 [51.7; 84.2]	71.8 [56.6; 85.2]	63.6 [43.3; 81.9]	4.29 [2.48; 6.53]	3.50 [1.27; 6.68]	7.66 [2.94; 14.24]
Specific populations†						
Pregnant women	55.3 [40.6; 69.6]	45.7 [27.9; 63.9]	58.8 [43.9; 73.0]	7.50 [4.31; 11.44]	2.60 [1.27; 4.32]	7.96 [4.73; 11.91]
Female sex workers	45.5 [24.8; 67.0]	38.1 [3.1; 82.9]	26.7 [19.6; 34.5]	13.63 [2.76; 30.72]	9.49 [0.04; 30.73]	7.02 [4.16; 10.52]
Adolescents	57.4 [17.9; 92.2]	35.9 [15.0; 59.2]	46.4 [27.6; 65.7]	1.89 [1.55; 2.27]	1.12 [0.01; 3.59]	2.05 [0.33; 4.98]
Women with HIV	64.7 [48.3; 79.7]	61.0 [42.7; 78.0]	70.5 [60.7; 79.6]	10.11 [0.13; 31.39]	3.09 [0.68; 6.96]	6.42 [1.89; 13.23]
Infertile	74.7 [59.9; 87.2]	..	100 [5.5; 89.2]	11.76 [4.33; 22.07]	0.00 [0.07; 3.40]	0.73 [0.04; 4.60]
Sampling						
In the community	63.0 [29.7; 91.3]	78.9 [53.5, 97.0]	52.6 [34.4; 70.5]	2.10 [0.80; 3.99]	8.55 [0.06; 28.0]	2.00 [0.00; 7.48]
At the health facility	58.6 [46.0; 70.7]	44.9 [27.9; 62.3]	52.9 [37.3; 68.2]	4.97 [3.08; 7.27]	1.94 [0.42; 4.45]	5.10 [1.75; 10.0]

* These analyses excluded populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

† These analyses were conducted exclusively on these populations and did not consist of a sub-group analysis. "Pregnant women" and "Women with HIV" are not mutually exclusive

Table 4.3. Proportion and prevalence of asymptomatic CT infections: number of studies, number of participants, and I^2.

	Number of asymptomatic	Number of positive	Study population	Number of data points	Number of countries	Pooled proportion estimates in % [95% CI]	Heterogeneity I^2 for proportion	P-value for subgroup analysis	Pooled prevalence (per 100 women) estimates [95% CI]	Heterogeneity I^2 for prevalence	P-value for subgroup analysis
Overall											
Excluding populations with an increased risk of STI*	910	1718	26 268	33	17	60.7 [50.4; 70.5]	93.2%	..	4.70 [3.39; 6.20]	96.1%	..
Including populations with an increased risk of STI	1361	2462	28 897	41	18	58.8 [50.4; 67.0]	93.2%	..	6.05 [4.26; 8.12]	97.7%	..
Continent*											
Africa	470	751	12 368	14	6	68.6 [56.4; 79.8]	87.8%	0.048	4.99 [2.70; 7.90]	97.3%	0.068
Asia	370	731	10 563	12	6	66.2 [47.2; 83.0]	94.6%		5.55 [3.40; 8.19]	95.5%	
Latin America	43	172	2 850	5	4	30.9 [11.2; 54.8]	89.4%		1.89 [0.56; 3.91]	89.6%	
Oceania	27	64	487	2	1	47.4 [2.7; 95.0]	95.0%		7.22 [0.00; 24.73]	95.7%	
Country income level*											
Low-Income	251	442	11 381	11	6	55.5 [37.3; 73.1]	91.8%	0.482	3.55 [1.87; 5.70]	96.0%	0.184
Middle-Income	659	1 276	14 887	22	11	63.2 [50.5; 75.1]	94.0%		5.34 [3.59; 7.39]	95.6%	
Setting*											
Rural	311	477	9 150	10	5	71.6 [55.6; 85.3]	91.0%	0.106	5.36 [3.11; 8.15]	95.2%	0.746
Urban	539	1 124	15 276	20	14	55.6 [43.8; 67.1]	90.9%		4.83 [2.90; 7.19]	97.0%	
Study year*											
1998 - 2011	478	789	13 987	17	12	61.8 [48.1; 74.7]	90.8%	0.727	4.41 [2.37; 6.99]	97.4%	0.782
2012 - 2022	403	895	12 181	15	9	57.7 [42.1; 72.7]	94.2%		4.09 [2.88; 5.49]	90.6%	
Number of symptoms assessed*											
Between 1 and 4	398	734	6 917	13	10	57.4 [41.6; 72.4]	93.6%	0.345	6.53 [3.46; 10.45]	96.9%	0.267
Five and more	265	420	8 137	15	8	69.0 [51.7; 84.2]	90.9%		4.29 [2.48; 6.53]	94.6%	
Specific populations**											
Pregnant women	377	708	5 749	12	7	55.3 [40.6; 69.6]	92.0%	..	7.50 [4.31; 11.44]	95.7%	..
Female sex workers	371	624	1 890	5	4	45.5 [24.8; 67.0]	96.0%	..	13.63 [2.76; 30.72]	98.8%	..
Adolescents	109	172	5 596	3	2	57.4 [17.9; 92.2]	95.4%	..	1.89 [1.55; 2.27]	0%	..
Women with HIV	80	120	739	3	2	64.7 [48.3; 79.7]	47.7%	..	10.11 [0.13; 31.39]	98.0%	..
Infertile	81	111	755	4	3	74.7 [59.9; 87.2]	58.2%	..	11.76 [4.33; 22.07]	92.0%	..
Sampling								0.833			0.031
In the community	154	255	8 452	6	5	63.0 [29.7; 91.3]	93.4%		2.10 [0.80; 3.99]	94.9%	
At the health facility	628	1 212	15 122	19	11	58.6 [46.0; 70.7]	95.0%		4.97 [3.08; 7.27]	96.8%	

* Excludes populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

** "Pregnant women" and "Women with HIV" are not mutually exclusive

Table 4.4. Proportion and prevalence of asymptomatic NG infections: number of studies, number of participants, and I^2.

	Number of asymptomatic	Number of positive	Study population	Number of data points	Number of countries	Pooled proportion estimates in % [95% CI]	Heterogeneity I^2 for proportion	P-value for subgroup analysis	Pooled prevalence (per 100 women) estimates [95% CI]	Heterogeneity I^2 for prevalence	P-value for subgroup analysis
Overall											
Excluding populations with an increased risk of STI*	423	685	16 950	21	13	53.3 [37.1; 69.1]	92.3%	..	3.11 [1.34; 5.54]	98.2%	..
Including populations with an increased risk of STI	606	1 000	18 740	27	14	52.2 [38.5; 65.6]	92.8%	..	3.70 [1.79; 6.23]	98.3%	..
Continent*											
Africa	394	565	7 681	13	6	67.2 [51.0; 81.9]	90.1%	0.019	5.67 [2.15; 10.66]	98.4%	<0.001
Asia	15	64	7 236	4	4	27.7 [9.0; 50.9]	59.7%		0.13 [0.03; 0.28]	12.6%	
Latin America	2	10	1 546	2	2	14.0 [0.0; 67.5]	62.2%		0.12 [0.00; 1.16]	80.6%	
Oceania	12	46	487	2	1	32.7 [0.0; 88.3]	92.8%		3.12 [0.00; 12.25]	91.7%	
Country income level*											
Low income	41	102	6 157	7	5	37.7 [18.4; 58.8]	69.2%	0.116	0.57 [0.17; 1.17]	82.9%	0.002
Middle income	382	583	10 793	14	8	60.9 [41.1; 79.2]	93.9%		5.21 [1.77; 10.22]	98.7%	
Setting*											
Rural	245	332	4 464	9	5	70.4 [46.3; 90.3]	91.9%	0.093	6.80 [1.39; 15.60]	98.8%	0.059
Urban	175	321	12 124	11	10	44.4 [26.5; 62.9]	85.8%		1.33 [0.29; 3.02]	97.0%	
Study year*											
1998 - 2011	277	414	8 552	12	9	55.9 [32.9; 77.8]	93.2%	0.675	3.72 [0.98; 7.99]	98.6%	0.554
2012 - 2022	146	271	8 398	9	7	48.9 [26.9; 71.1]	89.4%		2.42 [0.47; 5.63]	97.5%	
Number of symptoms assessed*											
Between 1 and 4	240	376	5 548	8	7	40.6 [13.8; 70.4]	96.2%	0.079	4.39 [0.46; 11.76]	99.0%	0.806
Five and more	170	247	4 954	10	6	71.8 [56.6; 85.2]	70.1%		3.50 [1.27; 6.68]	95.6%	
Specific populations**											
Pregnant women	97	226	4 106	9	4	45.7 [27.9; 63.9]	79.4%	..	2.60 [1.27; 4.32]	85.1%	..
Female sex workers	163	282	1 051	3	3	38.1 [3.1; 82.9]	97.9%	..	9.49 [0.04; 30.73]	98.6%	..
Adolescents	11	30	910	2	2	35.9 [15.0; 59.2]	7.2%	..	1.12 [0.01; 3.59]	84.3%	..
Women with HIV	20	33	739	3	2	61.0 [42.7; 78.0]	0%	..	3.09 [0.68; 6.96]	80.8%	..
Infertile	0	0	137	1	1	..	..	..	0.00 [0.07; 3.40]	..	..
Sampling								0.312			0.260
In the community	161	184	3 166	3	3	78.9 [53.5, 97.0]	76.3%		8.55 [0.06; 28.0]	99.5%	
At the health facility	215	379	11 264	11	10	44.9 [27.9; 62.3]	88.2%		1.94 [0.42; 4.45]	98.0%	

* Excludes populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

** "Pregnant women" and "Women with HIV" are not mutually exclusive

Table 4.5. Proportion and prevalence of asymptomatic TV infections: number of studies, number of participants, and I^2.

	Number of asymptomatic	Number of positive	Study population	Number of data points	Number of countries	Pooled proportion estimates in % [95% CI]	Heterogeneity I^2 for proportion	P-value for subgroup analysis	Pooled prevalence (per 100 women) estimates [95% CI]	Heterogeneity I^2 for prevalence	P-value for subgroup analysis
Overall											
Excluding populations with an increased risk of STI*	642	1 111	10 824	23	13	56.9 [44.6; 68.9]	91.4%	..	5.98 [3.46; 9.12]	97.3%	..
Including populations with an increased risk of STI	761	1 394	12 684	31	15	53.6 [43.0; 64.1]	91.3%	..	5.84 [3.75; 8.34]	96.6%	..
Continent*											
Africa	504	829	6 152	12	6	64.9 [52.2; 76.7]	91.5%	0.039	9.06 [5.71; 13.07]	95.7%	<0.001
Asia	5	21	2 606	4	3	17.3 [0.0; 49.5]	15.3%		0.08 [0.00; 0.31]	7.2%	
Latin America	86	141	1 579	5	3	50.1 [16.4; 83.8]	93.1%		4.54 [0.52; 11.9]	96.6%	
Oceania	47	120	487	2	1	43.3 [4.8; 87.5]	96.4%		12.77 [0.06; 40.12]	97.5%	
Country income level*											
Low income	160	295	4 136	6	6	45.4 [29.7; 61.6]	92.8%	0.174	3.12 [0.73; 7.02]	96.9%	0.108
Middle income	482	816	6 688	17	7	61.7 [45.4; 77.1]	81.7%		7.28 [3.72; 11.88]	97.4%	
Setting*											
Rural	198	280	2 619	6	3	75.0 [49.8; 94.0]	93.2%	0.090	10.27 [2.41 22.46]	98.5%	0.252
Urban	413	735	7 544	15	11	58.9 [46.5; 70.8]	86.0%		4.81 [2.25; 8.21]	97.0%	
Study year*											
1998 - 2011	460	817	7 872	13	8	57.3 [41.0; 73.0]	93.5%	0.797	5.94 [2.86; 10.00]	97.6%	0.759
2012 - 2022	178	276	2 408	9	7	61.1 [41.3; 79.5]	85.2%		6.99 [2.09; 14.30]	97.0%	
Number of symptoms assessed*											
Between 1 and 4	355	650	4 763	11	9	55.1 [37.7; 71.9]	91.1%	0.487	5.68 [2.97; 9.17]	95.0%	0.518
Five and more	265	406	4 425	10	7	63.6 [43.3; 81.9]	91.9%		7.66 [2.94; 14.24]	97.7%	
Specific populations**											
Pregnant women	291	549	3 598	11	5	58.8 [43.9; 73.0]	88.1%	..	7.96 [4.73; 11.91]	92.5%	..
Female sex workers	37	138	546	2	2	26.7 [19.6; 34.5]	0%	..	7.02 [4.16; 10.52]	41.3%	..
Adolescents	13	28	706	2	2	46.4 [27.6; 65.7]	0%	..	2.05 [0.33; 4.98]	73.7%	..
Women with HIV	73	105	965	4	3	70.5 [60.7; 79.6]	0%	..	6.42 [1.89; 13.23]	92.0%	..
Infertile	1	1	137	1	1	100 [5.5; 89.2]	..	..	0.73 [0.04; 4.60]	..	..
Sampling								0.997			0.296
In the community	103	174	2 959	3	3	52.6 [34.4; 70.5]	51.6%		2.00 [0.00; 7.48]	98.0%	
At the health facility	359	621	5 882	11	7	52.9 [37.3; 68.2]	87.6%		5.10 [1.75; 10.0]	98.0%	

* Excludes populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

** "Pregnant women" and "Women with HIV" are not mutually exclusive

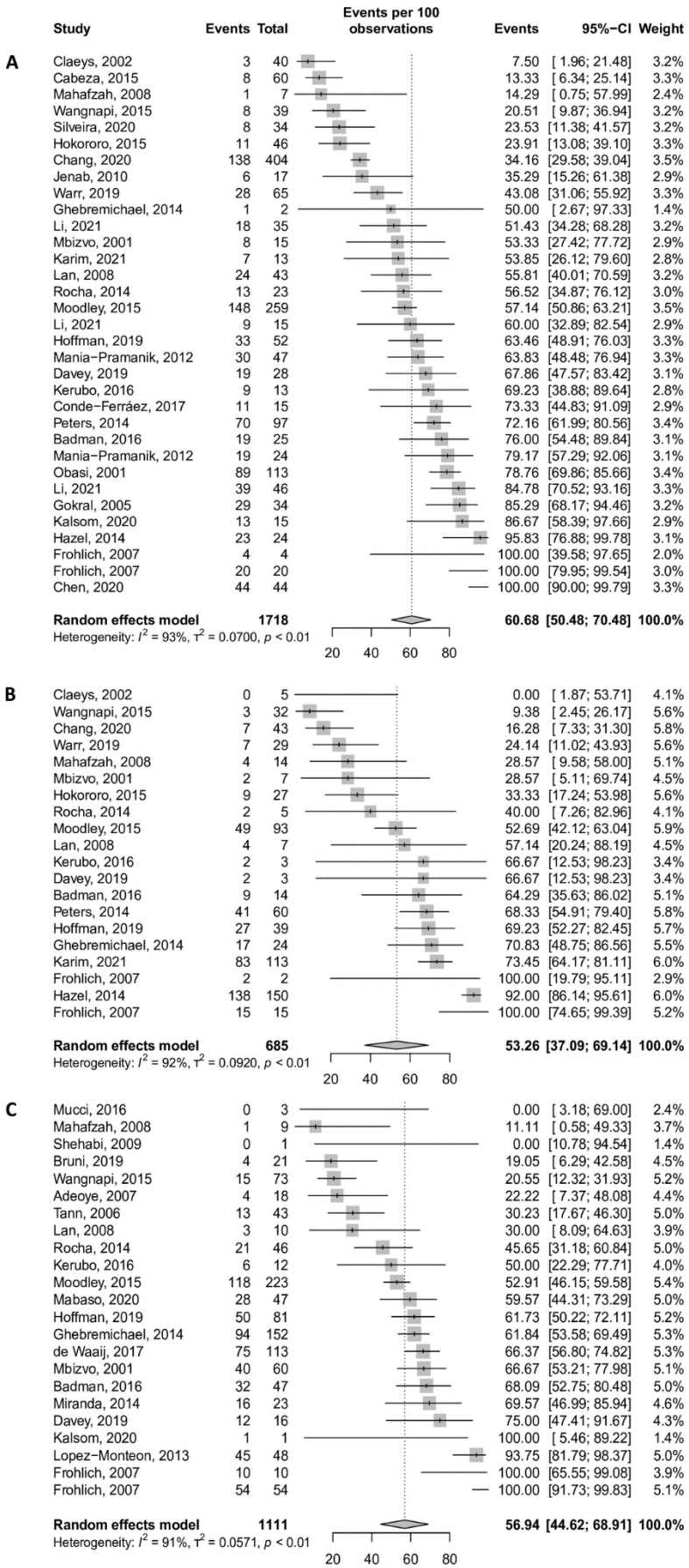


Figure 4.2. Forest plots of the proportion of asymptomatic CT (A), NG (B), and TV (C).

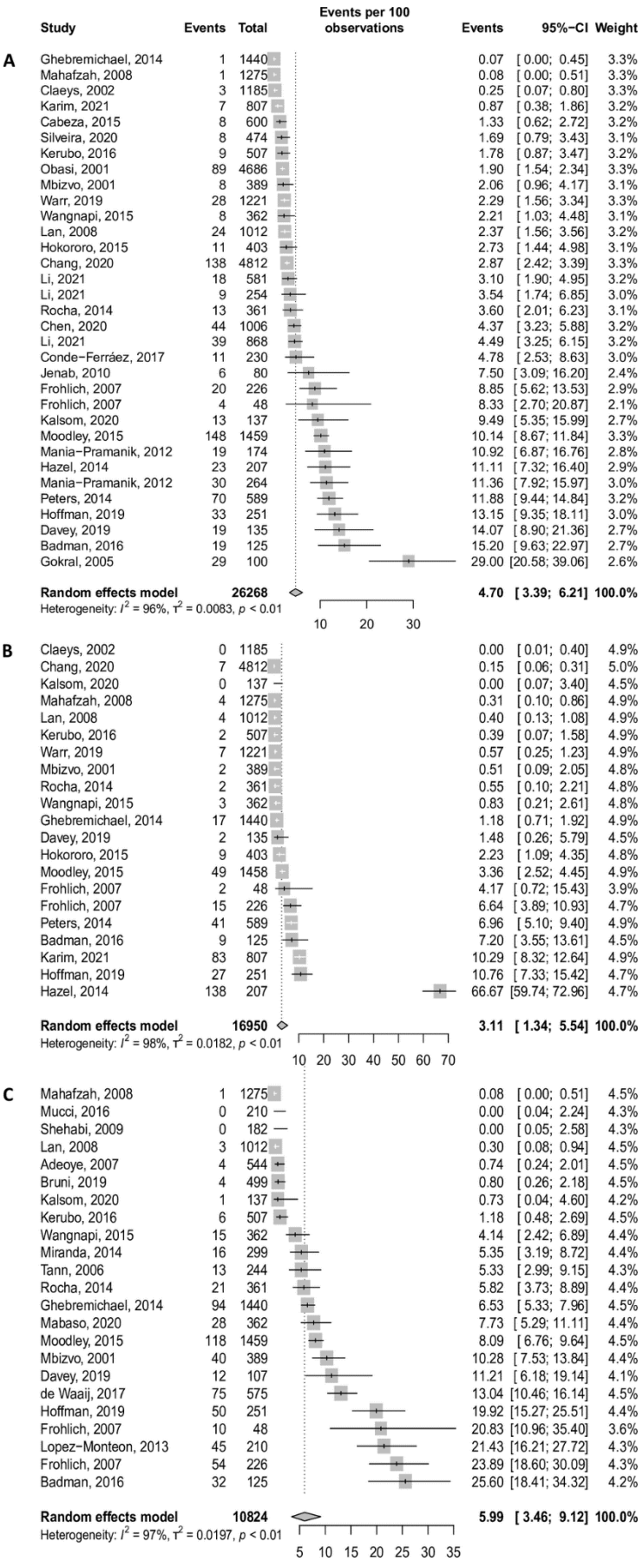


Figure 4.3. Forest plots of the prevalence of asymptomatic CT (A), NG (B), and TV (C).

The prevalence of asymptomatic women infected with CT, NG, or TV was 4.70 per 100 women [3.39; 6.20] (33 data points, 26 268 participants), 3.11 [1.34; 5.54] (21 data points, 16 950 participants) and 5.98 [3.46; 9.12] (23 data points, 10 824 participants), respectively, excluding populations with an increased risk of STI infection.

The prevalence of asymptomatic NG and TV infections differed significantly across regions ($p<0.001$). The highest prevalence was found in Africa and Oceania (NG: 5.67 [2.15; 10.66] and 3.12 [0.00; 12.25], TV: 9.06 [5.71; 13.07] and 12.77 [0.06; 40.12], respectively) and the lowest in Asia and Latin America (NG: 0.13 [0.03; 0.28] and 0.12 [0.00; 1.16], TV: 0.08 [0.00; 0.31] and 4.54 [0.52; 11.9], respectively). A similar pattern was observed for CT, but there was weak evidence of this difference ($p=0.068$).

For NG only, the prevalence of asymptomatic infection was significantly higher in middle-income countries versus low-income (5.21 [1.77; 10.22] vs 0.57 [0.17; 1.17], $p=0.002$) and this difference was nearly significant between rural and urban settings (6.80 [1.39; 15.60] vs 1.33 [0.29; 3.02], $p=0.059$).

Relative to the other specific populations, the prevalence of asymptomatic CT, NG, and TV was low among adolescents (CT: 1.89 [1.55; 2.27]; NG: 1.12 [0.01; 3.59]; TV: 2.05 [0.33; 4.98]) and high among FSWs (CT: 13.6 [2.76; 30.72]; NG: 9.49 [0.04; 30.73]; TV:7.02 [4.16; 10.52]).

Proportion and prevalence by country are presented in **Figure 4.4** and **Table 4.6**.

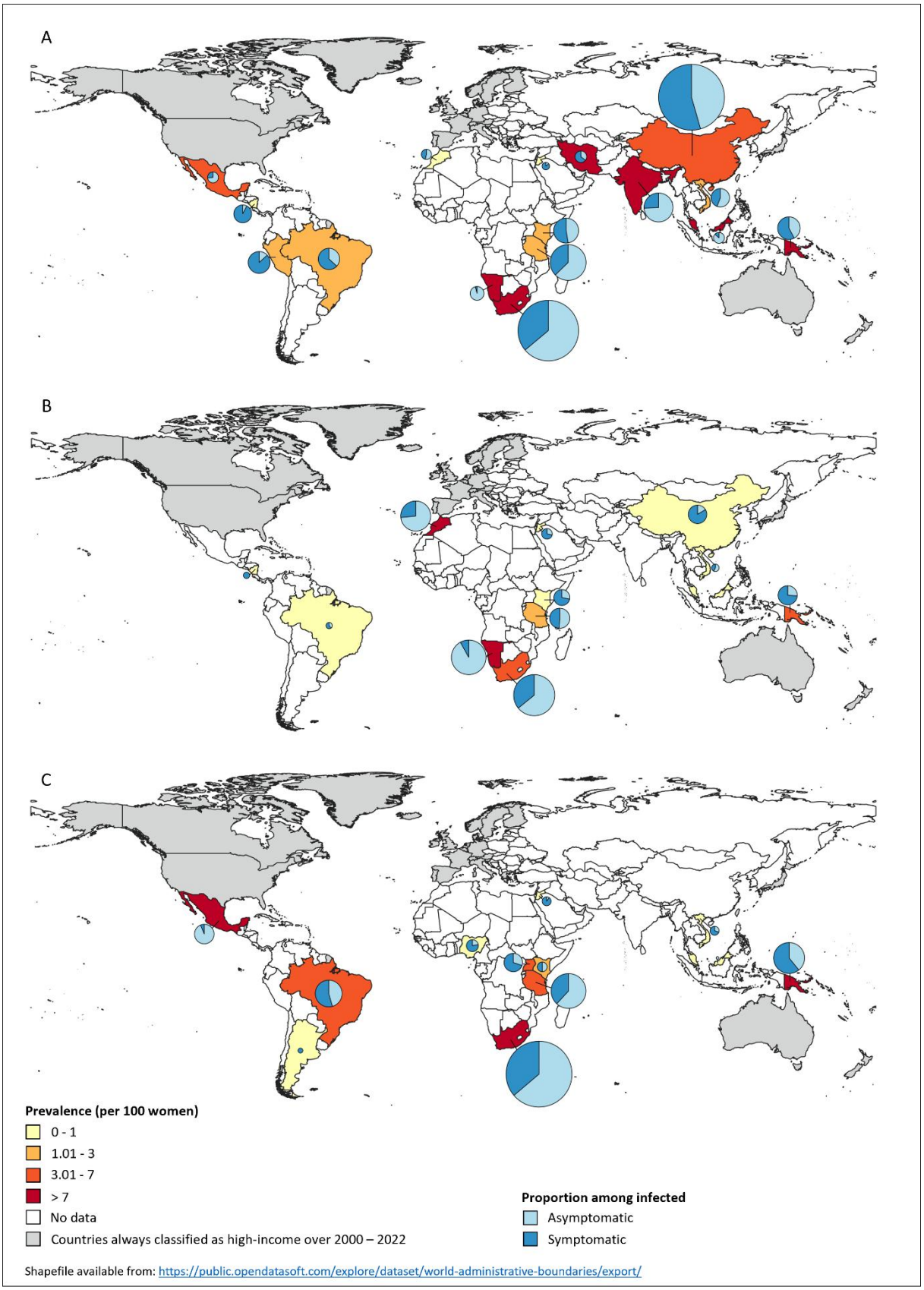


Figure 4.4. Maps of asymptomatic CT (A), NG (B) and TV (C) infections in women, in LMICs, by country.

Table 4.6. Proportion and prevalence of asymptomatic CT, NG, and TV, by country (data used for the map).

	Asymptomatic CT					Asymptomatic NG					Asymptomatic TV				
Country	**N**	**Proportion**	**95% CI**	**Prevalence**	**95% CI**	**N**	**Proportion**	**95% CI**	**Prevalence**	**95% CI**	**N**	**Proportion**	**95% CI**	**Prevalence**	**95% CI**
Argentina	..	..	..	..	..	..	..	..	..	..	1	0.0	[0.0; 50.0]	0.00	[0.00; 0.82]
Brazil	2	38.8	[10.5; 71.8]	2.51	[0.96; 4.73]	1	40.0	[1.9; 86.2]	0.55	[0.01; 1.66]	3	44.5	[19.8; 70.5]	3.50	[0.67; 8.28]
China	5	70.6	[34.5; 96.4]	3.57	[2.78; 4.47]	1	16.3	[6.5; 29.0]	0.15	[0.05; 0.28]	..	..	..	..	..
India	3	76.0	[61.5; 88.0]	15.98	[7.72; 26.44]	..	..	..	..	..	..	..	..	..	..
Iran	1	35.3	[14.0; 59.8]	7.50	[2.57; 14.46]	..	..	..	..	..	..	..	..	..	..
Jordan	1	14.3	[0.00; 51.7]	0.08	[0.00; 0.34]	1	28.6	[7.4; 55.4]	0.31	[0.07; 0.71]	2	2.5	[0.0; 36.0]	0.01	[0.00; 0.22]
Kenya	2	52.9	[28.5; 76.7]	2.13	[1.49; 2.87]	2	33.2	[2.4; 73.4]	0.51	[0.21; 0.92]	1	50.0	[21.6; 78.4]	1.18	[0.39; 2.35]
Malaysia	1	86.7	[63.8; 99.7]	9.49	[5.08; 15.03]	..	..*	..	0.00	[0.00; 1.25]	1	100	[0.0; 100]	0.73	[0.00; 3.11]
Mexico	1	73.3	[47.7; 93.2]	4.78	[2.35; 7.97]	..	..	..	..	..	1	93.8	[84.7; 99.2]	21.43	[16.12; 27.25]
Morocco	1	53.8	[26.1; 80.5]	0.87	[0.32; 1.65]	1	73.5	[64.9; 81.2]	10.29	[8.28; 12.48]	..	..	..	..	..
Namibia	1	95.8	[83.0; 100]	11.11	[7.16; 15.79]	1	92.0	[87.1; 95.9]	66.7	[60.08; 72.94]	..	..	..	..	..
Nicaragua	1	7.5	[1.0; 18.1]	0.25	[0.03; 0.64]	1	0.0	[0.0; 31.7]	0.00	[0.00; 0.15]	..	..	..	..	..
Nigeria	..	..	..	..	..	..	..	..	..	..	1	22.2	[5.5; 44.8]	0.74	[0.16; 1.67]
Peru	1	13.3	[5.7; 23.3]	1.33	[0.55; 2.43]	..	..	..	..	..	..	..	..	..	..
PNG	2	47.4	[2.7; 95.1]	7.22	[0.00; 24.73]	2	32.7	[0.0; 88.3]	3.12	[0.00; 12.25]	2	43.3	[4.8; 87.5]	12.77	[0.06; 40.12]
South Africa	6	76.2	[61.6; 88.4]	10.72	[9.47; 12.03]	6	76.8	[57.7; 92.2]	5.29	[2.95; 8.21]	7	76.6	[59.0; 90.6]	13.97	[9.50; 19.12]
Tanzania	3	51.8	[4.9; 96.9]	1.19	[0.06; 3.58]	2	52.0	[17.2; 85.9]	1.53	[0.66; 2.74]	1	61.8	[54.0; 69.4]	6.53	[5.31; 7.86]
Uganda	..	..	..	..	..	..	..	..	..	..	1	30.2	[17.3; 44.9]	5.33	[2.82; 8.54]
Vietnam	1	55.8	[40.7; 70.4]	2.37	[1.52; 3.41]	1	57.1	[18.6; 91.9]	0.40	[0.08; 0.90]	1	30.0	[5.0; 62.5]	0.30	[0.04; 0.75]

N represents the number of point estimates
PNG: Papua New-Guinea

* Only one study represented Malaysia and no women were tested positive for NG in this study

After excluding studies with a high risk of bias, our sensitivity analyses showed no significant change in our findings (**Annex B, supplementary Tables 2–4**). Results of the meta-regression showed that the continent and the country income level explained a substantial part of the variation in our estimates (**Table 4.7**). Results from the Funnel plots and Egger's tests showed no evidence of publication bias (**supplementary Figure 1**).

Table 4.7. Meta-regression estimates and 95% confidence intervals for the proportion and the prevalence of asymptomatic CT (A), NG (B), and TV (C).

A

	Proportion of asymptomatic CT		**Prevalence of asymptomatic CT**	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Continent				
Africa	Ref	-	-	-
Asia	0.022	(-0.022; 0.264)	0.014	(-0.064; 0.091)
Latin America	-0.328 *	(-0.639; -0.016)	-0.102 *	(-0.205; 0.001)
Oceania	0.210	(-0.307; 0.728)	0.216 *	(0.030; 0.403)
Country income level				
Low income	Ref	-	-	-
Middle income	0.246 *	(0.044; 0.448)	0.115 ***	(0.049; 0.181)
Setting				
Rural	Ref	-	-	-
Urban	-0.156	(-0.405; 0.093)	-0.068 *	(-0.147; 0.011)
Study year				
1998 - 2011	Ref	-	-	-
2012 - 2022	-0.206 *	(-0.404; -0.008)	-0.017	(-0.082; 0.049)
Number of symptoms assessed				
<5	Ref	-	-	-
5 and more	-0.023	(-0.228; 0.183)	-0.102 **	(-0.170; -0.035)

B

	Proportion of asymptomatic NG		Prevalence of asymptomatic NG	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Continent				
Africa	Ref	-	-	-
Asia	-0.468 **	(-0.750; -0.185)	-0.155	(-0.312; 0.002)
Latin America	-0.469 *	(-0.885; -0.052)	-0.124	(-0.346; 0.099)
Oceania	0.045	(-0.411; 0.502)	0.107	(-0.194; 0.408)
Country income level				
Low income	Ref	-	-	-
Middle income	0.254 *	(0.051; 0.458)	0.202 **	(0.075; 0.328)
Setting				
Rural	Ref	-	-	-
Urban	-0.187	(-0.394; 0.019)	-0.145 *	(-0.280; -0.010)
Study year				
1998 - 2011	Ref	-	-	-
2012 - 2022	-0.327 **	(-0.543; -0.111)	-0.064	(-0.193; 0.065)
Number of symptoms assessed				
<5	Ref	-	-	-
5 and more	0.302 **	(0.075; 0.530)	-0.076	(-0.204; 0.052)

C

	Proportion of asymptomatic TV		Prevalence of asymptomatic TV	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Continent				
Africa	Ref	-	-	-
Asia	-0.361	(-0.790; 0.067)	-0.275 ***	(-0.404; -0.145)
Latin America	-0.148	(-0.583; 0.287)	-0.085	(-0.242; 0.072)
Oceania	0.104	(-0.588; 0.797)	0.261	(-0.002; 0.525)
Country income level				
Low income	Ref	-	-	-
Middle income	0.183	(-0.158; 0.524)	0.120	(-0.001; 0.241)
Setting				
Rural	Ref	-	-	-
Urban	-0.233	(-0.591; 0.125)	-0.101	(-0.230; 0.028)
Study year				
1998 - 2011	Ref	-	-	-
2012 - 2022	-0.046	(-0.363; 0.271)	-0.049	(-0.159; 0.061)
Number of symptoms assessed				
<5	Ref	-	-	-
5 and more	-0.086	(-0.409; 0.237)	-0.059	(-0.174; 0.055)

4.4. Discussion

Our study reveals a significant public health challenge: more than half of the women infected with CT, NG, and TV were asymptomatic in LMICs. The prevalence of asymptomatic infections ranged from 3 to 6%. When cumulating the prevalences of each pathogen, accounting for the rarity of co-infections,[196,211] the prevalence could reach up to 13%. These high proportions and prevalence of asymptomatic infections are particularly worrisome since those women remain untreated and thus may develop serious complications. Additionally, they constitute an unseen reservoir that continues to fuel the STI epidemic.

Several hypotheses can be proposed to explain these high estimates. First, women may have difficulties in distinguishing physiological from abnormal vaginal discharge. For instance, in Tanzania, a study found that more than two-thirds of women with abnormal discharge did not report any symptoms.[225] In rural areas of Madagascar, anthropologists reported that genital symptoms are thought to be normal,[152] and women may not report them, even after probing.

Alternatively, social norms may prevent women from declaring their symptoms. They may correctly identify genital symptoms but may be unwilling to communicate them to healthcare professionals due to the stigma attached to such symptoms or fear of being judged.[226]

The notably high proportions of asymptomatic CT, NG, and TV infections in Africa are concerning. STIs are associated with an increased risk of transmitting and acquiring other STIs or HIV and Africa bears amongst the highest STI and HIV prevalence in the world.[7,227] The wide use of syndromic approach in Africa means that asymptomatic women are often left undiagnosed and untreated, posing a risk of transmission to their partners and contributing to adverse health outcomes.

We observed a high proportion of asymptomatic STIs among women with HIV. While the limited number of studies limits definitive conclusions, similar findings have been reported among men with HIV who have sex with men.[228] In this study, detectable plasma HIV RNA, social factors, and sexual practices were associated with asymptomatic STIs. Further research could also explore the potential immunological mechanisms underlying this phenomenon.

Our study revealed an elevated prevalence of asymptomatic STIs among FSWs, suggesting an important circulation of STIs in this population likely due to their important number of sexual partners. However, we observed a relatively lower proportion of asymptomatic women, which could be explained by FSWs' increased awareness and recognition of STI symptoms.[229]

We found a low prevalence of asymptomatic STIs among adolescents, possibly due to the lower STI prevalence in this group and the inclusion of sexually inactive participants in some studies. However, despite the lower prevalence, adolescents remain vulnerable to STI acquisition due to their sexual behavior and physiological susceptibility.[230] Implementing comprehensive sexual education programs during early adolescence could effectively maintain a low STI occurrence in this specific population.[231]

This study has several limitations. First, the majority of the studies were conducted in healthcare facilities which may introduce bias, as women could attend these facilities because of genitourinary symptoms, potentially underrepresenting asymptomatic cases compared to the general population. Consequently, while our estimates are already substantial, the true proportion and prevalence could be even higher. Furthermore, our search yielded only six articles conducted in community settings, indicating a gap in community-based research. Such studies are pivotal for accurately assessing the proportion and prevalence of asymptomatic STIs in the general population. Another limitation pertains to the varying number of symptoms used to determine symptomatic status, ranging from one to fourteen. Studies examining more symptoms were more likely to identify symptomatic cases and fewer asymptomatic cases. However, our comparison of estimates from studies assessing one to four symptoms versus those assessing five or more symptoms did not show any significant differences. Establishing a uniform definition in future research could potentially reduce the heterogeneity among different studies.

Another limitation of our review is that 39 studies appeared to have collected the data we needed but we were unable to obtain these figures. However, we did not find any temporal or spatial pattern among them. In addition, due to our language restrictions, ten studies conducted in Latin America were excluded although they seemed to be relevant.

Nevertheless, our study has several strengths. To the best of our knowledge, this is the first meta-analysis providing comprehensive estimates on asymptomatic STIs in LMICs among

women. Our strict inclusion and exclusion criteria, particularly regarding pathogen identification techniques minimized the risk of including false positive and false negative cases and ensured that the study's findings were based on high-quality and reliable data. We also provided estimates for specific populations of pregnant women, FSWs, adolescents, women with HIV, and infertile women. Further research is needed. Within this large reservoir of asymptomatic STIs, it is important to estimate what percentage of women will develop complications such as PID as well as what percentage will spontaneously clear the pathogen. It is also critical to identify characteristics associated with developing complications or sequelae in order to treat those women at an early stage.

We showed that the prevalence and proportion of asymptomatic STIs among women in LMICs represent a significant public health concern. An alternative to the syndromic management could be the development of an easy-to-use and affordable point-of-care test (POCT) that would rapidly and accurately detect STIs .[232] A POCT to screen women with suspected STIs among asymptomatic women would be more cost-effective than POCT testing for each pathogen on all of them. Currently, a POCT detecting inflammation caused by any STI is being tested.[114] In addition to providing confidential healthcare services and destigmatizing STIs as defined in the global health strategies on STIs by the WHO for 2022–2030,[233] such a device could be used by health care providers to screen asymptomatic women in order to prevent adverse consequences for reproductive and overall health.

4.5. Contributions to the study

Together with my PhD supervisors, I developed the study design and formulated the research question. I also conducted the literature search with the support from an information specialist, Catherine Cecilio working at the Institut Pasteur library. I searched the three databases and divided the retrieved titles and abstracts into three sets. In pairs (one of my PhD supervisors and myself), we independently screened these sets for relevant articles, blinded to each other's selection. We then compared our selections and resolved discrepancies altogether. I extracted the data which my PhD supervisors verified. I conducted all the analyses and drafted the manuscript.

Chapter 5: Prediction of STI/BV among women aged 18 – 35 in a cross-sectional multi-centric study in Madagascar, South Africa and Zimbabwe.

5.1. Introduction

In settings where access to RTI testing is non-existent or expensive, case management relies on symptoms and signs. A specific set of symptoms and signs constitutes a syndrome and indicates a certain type of infection. A probabilistic treatment is then prescribed against the most common organism(s) that cause(s) this syndrome. This approach is called syndromic management and is presented in the form of flowcharts or algorithms which are easily implemented into primary health care and do not require highly trained specialists. Flowcharts have been developed for the diagnosis of vaginal discharge syndrome (VDS), lower abdominal pain (LAP) and genital ulcer disease (GUD), which include the most common spontaneously reported symptoms.[234] In this thesis, we are specifically interested in the algorithms for VDS and LAP, since they include the symptoms associated with the RTIs CT, NG, TV, MG, CA and BV. Entry points into the VDS and LAP flowcharts are respectively: patient complains of vaginal discharge, vulval itching, or burning; and lower abdominal pain.

WHO issued multiple guidelines on the syndromic management of patients with STIs/RTIs since the first one in 1991.[235] Several studies assessed their effectiveness and showed mixed results. In a review of studies published between 1993 and 1998 and using some versions of the WHO 1991 algorithm for vaginal discharge (**Annex D, Figure 1 – 3**), Pettifor *et al*. showed that the sensitivities were between 73% and 93% for women consulting for STI symptoms and 29% to 83% for women from the community. They concluded that there was no evidence that vaginal discharge was an effective indicator of cervical infection, especially in a low-risk population.[236] Along with signs and symptoms, the WHO 1991 algorithm included a risk assessment for one of the three algorithms for vaginal discharge: when vaginal examination and laboratory tests were not possible (**Annex D, Figure 1**). The risk assessment

included the history of high risk such as prostitution, sexual contact with a person with a sexually transmitted disease (STD), history of previous STD, and young age (i.e. <20 years). In a study conducted among pregnant women attending an antenatal clinic, who were assumed to be predominantly asymptomatic, the risk assessment appeared to be feasible and acceptable but showed limited sensitivity.[237] In the same period, several studies tested other risk markers in addition to symptoms and signs for the detection of STIs and improving of the performance of the syndromic approach.[234,238–240]

In 2003, the new WHO guidelines acknowledged that vaginal discharge is a poor indicator of cervical infection but remains a reliable sign of a vaginal infection.[241] To address the limitation in predicting cervicitis, the guidelines introduced the systematic inclusion of risk assessment in all VDS and LAP algorithms (**Annex D, Figures 4 – 7**). While the guidelines did not specifically define how risk assessment should be conducted, they emphasized that it needed to be tailored to the local social, behavioral, and epidemiological contexts. Some commonly used items for risk assessment are: being younger than 21 years old; having had more than one sexual partner in the previous three months, and having had a new partner in the previous three months.[241] Importantly, in the WHO 2003 guidelines the entry points into the flowcharts, and by extension into STI care, were still the symptoms. Risks were assessed after entry into the algorithms.

The WHO 2003 guidelines were evaluated and a new systematic review of these studies found that the sensitivities for cervical infection (CT and NG) performed poorly across countries. Missed treatment occurred in up to 76% of cases and overtreatment in up to 65%.[175] The algorithms for vaginal infection (TV and BV) performed better with sensitivities between 53% and 93%.[175]

The latest published guidelines for managing symptomatic sexually transmitted infections were released in 2021.[6] WHO recognizes that the syndromic approach has its limitations but it remains the standard of care in many resource-limited settings. A significant update in these guidelines was the guidance on the use of rapid diagnostic tests, which have been incorporated into the revised algorithms (Figure 1.8, Figure 1.9).

Overall, the major criticisms of the syndromic approach are that (1) it does not allow the detection of women who are asymptomatic and (2) there are risks of incorrect prescription,

including over, under, and inappropriate prescription.[242] Reasons for incorrect prescriptions were identified to be the lack of specificity of signs and symptoms of STIs, the wide range of presentations of STIs, and simultaneous infections, sometimes involving more than one site.[243] Moreover, the low quality of collecting medical history or clinical examination, in part owing to the lack of essential equipment (such as light), has also been blamed.[242]

As entry points into syndromic management did not seem to provide satisfactory performance and the approach did not allow identification of infected asymptomatic women,[236] there has been a growing interest in designing other predictive tools, usually under the form of risk scores. Whether risk scores are aimed at screening (i.e. sorting out patients who are more likely to have an RTI) or diagnosis, they offer the possibility to use questions or minimally invasive procedures to investigate risk for RTIs. Women identified as being at risk may undergo clinical examination and RTI testing. In resource-limited settings several risk scores have been developed but are usually tailored to specific context and populations, such as adolescents and young adults,[222,244–246] symptomatic women,[247] or asymptomatic women.[248] Additionally, these tools have typically focused on either STI or only BV.[249,250] No study, however, has aimed at detecting both STI and BV in all women. Dual detection of STI/BV is of interest since both are often simultaneously present.[251]

In this study, we aimed to develop a risk assessment tool using data from different countries and populations with different demographics to specifically identify asymptomatic women with an increased risk of STIs and/or BV before undertaking the GIFT test. Two different techniques were used to develop the tools: logistic regression, a classical approach for the creation of risk scores, and machine learning.

5.2. Methods

5.2.1. Study design and population

This study was part of a large project involving a multi-country consortium. The project supports the development of a Genital InFlammation Test (GIFT), a lateral flow device prototype conceived to detect genital inflammation characterized by the immune soluble biomarkers: IL-1α, IL-1β and IP-10, which are cytokines and chemokines associated with STIs/BV (**Figure 5.1**). The project includes (1) the overall project management, (2) a section on device optimization and manufacturing, (3) the validation of the GIFT device in a diagnostic trial conducted among women in Madagascar, South Africa and Zimbabwe, (4) the integration of the GIFT device into care and (5) commercial market testing.[116]



Figure 5.1. The Genital InFlammation Test (GIFT)

The integration into care is composed of four parts:

(i) user experiences and/or perceptions of the GIFT device involving qualitative focus group discussions and in-depth interviews with key stakeholders;

(ii) discrete choice experiments;

(iii) development of management algorithms; and

(iv) economic evaluation of defined management algorithms.

This work presents the first section of part (iii) development of management algorithms. We used data collected during the diagnostic trial to develop the prediction tools.

In the diagnostic trial, women were recruited at family planning services in Madagascar, South Africa, and Zimbabwe. Women were included irrespective of whether they were symptomatic or asymptomatic. They were between the ages of 18 and 35 years, sexually active, and non-pregnant. Those who were treated for any STI/BV in the past 30 days were not eligible. Enrolled women were interviewed using a structured questionnaire with questions on demographics, sexual behavior, hygiene habits, current medical and reproductive history, medication, symptoms and clinical signs. The clinician, midwife, or study nurse performed a physical examination including a gynecological examination. Vaginal swabs were collected by a midwife or a nurse for STI/BV detection, testing with the GIFT device, and other biological analyses.

5.2.2. Detection of STI/BV

CT and NG were tested in each of the laboratory's study site using NAATs (CT/NG GeneXpert® and Presto CT/NG). NAATs were used to test for TV in two sites (TV GeneXpert and in-house qPCR) and RDT (OSOM®) was used in one site. BV was diagnosed by the laboratory at each of the study sites using the Nugent score.

5.2.3. Statistical analysis

5.2.3.1. Outcomes and variables

Characteristics of included women were described by country and compared using χ^2 tests or Fisher's exact tests for categorical variables and Kruskal-Wallis' rank sum tests for continuous variables.

Our prediction outcomes were:

- Any RTI (CT, NG, TV, or BV),
- STIs (CT, NG, or TV) vs no STIs,
- BV vs no BV,
- CT/NG in the absence of TV and BV compared to women with no STI and no BV,
- TV/BV in the absence of CT and NG compared to women with no STI and no BV.

As our objective was to identify women at risk for STIs before further diagnostic testing, we selected variables that minimize physical invasiveness to build our model. Therefore, we excluded variables related to physical and gynecological examination, i.e., all clinical signs, however we included a series of questions and vaginal pH. Vaginal pH has been documented as a relevant marker for the detection of BV,[252] and can be conveniently self-collected by the patient.

More specifically, the included questions concerned: age, marital status, education, level material comfort (e.g. possession of a phone, a car, etc.), access to free STI testing, access to STI treatments, first menstrual age, number of pregnancies, age at first vaginal penetration, number of sexual partners in a lifetime, sex in exchange for favors in the past three months, condom use, currently taking PrEP, suspicion that partner has an STI, type of, timing and frequency of liquid products inserted to wash the vagina, ever treated for STIs, HIV status at last testing (self-reported), past or current schistosomiasis, cigarette and alcohol consumption, vaginal pH, abnormal vaginal discharge, lower abdominal pain, abnormal smell, dysuria, frequent need to urinate, blood in urine, genital or anorectal pruritus, dyspareunia, missed or overdue periods and presence of sores, blisters, ulcers or vesicles

5.2.3.2. Statistical methods

We used two techniques to build the prediction tool: risk scores and random forest followed by classification trees, and we compared their performance in terms of sensitivity, specificity and predictive values.

Risk scores

First, we ran logistic regressions using both forward and backward models and we selected the model with the smallest Bayesian Information Criterion (BIC). Then, the coefficients of the variables retained in the final model were multiplied by 10 to generate weights. Weighted coefficient multiplied by their attributes were summed up to form a risk score. Sensitivity and specificity were calculated at each threshold. We selected the threshold at which the geometric mean between the sensitivity and specificity was the highest.

We developed a risk score for infection with STI/BV among all included women and conducted sub-analyses, among asymptomatic women only, and by countries. We defined asymptomatic women as those who did not report abnormal vaginal discharge, itching, irritation nor lower abdominal pain. All analyses were conducted using R (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria).

Machine learning techniques: random forests (RF) and classification and regression trees (CART)

Classification trees are a supervised learning method from the field of machine learning used to predict a categorical outcome, known as the target variable.[253] In these trees, the endpoints, called "leaves," represent class labels, which are the possible categories or outcomes (e.g., "Presence of any STI" or "Absence of any STI"). The connecting paths, known as "branches," represent decisions based on different characteristics, referred to as features (e.g., "Is the person older than 25?" with branches for "Yes" and "No"). The goal is to create a model that can predict an outcome based on several input variables. Decision trees are particularly useful for creating triage algorithms due to their simplicity and ease of interpretation.

Nevertheless, decision trees are highly sensitive to variations in the data, meaning that small changes can lead to significant alterations in the tree's structure and, consequently, in the final predictions. This sensitivity arises from the tendency of decision trees to overfit the training data, capturing noise and fluctuations rather than underlying patterns. To address this issue, we preselected variables using a more robust method: random forests.

In random forests, multiple deep classification trees are trained on different subsets of the dataset, and their results are averaged.[254] This approach improves stability by reducing variance and minimizing overfitting, which enhances performance in the final model. Random forests also provide a more reliable estimate of variable importance by evaluating the impact of each variable across numerous trees, resulting in more stable and generalizable models.

However, in resource-limited settings, such as primary health care settings, implementing random forests can be challenging due to the need for substantial computational resources and a large set of variables. Therefore, in this context, random forests are primarily used to

rank the importance of variables predicting STI/BV. We used mean squared error (MSE) to select the important variables.

Unlike logistic regression, these techniques allow the inclusion of correlated variables and non-linear distribution of continuous variables.

Selected variables were then used as input for a classification tree model and the tree was pruned to form the final algorithm. Moreover, a weight was added in the model in order to maximize the detection of true positive women, thus maximizing the model's sensitivity, an important performance characteristic for prediction tools that may later aim at screening.

For both risk scores and random forest followed by CART, we performed 10-fold cross-validation 20 times and model predictions and performances were averaged and their 95% confidence intervals obtained. We fitted a Receiver Operating Characteristic (ROC) curve to compare how models performed at different thresholds and compare models between themselves. We selected sensitivity and specificity at the highest geometric mean.

The same approaches were reiterated for the prediction of STIs alone, BV alone, CT/NG and TV/BV. Results of these analyses are presented in **Annex E**.

5.3. Results

5.3.1. Study population

A total of 706 women were invited to participate, among which 30 did not meet the eligibility criteria. Three participants had unusable biological samples, so prevalences for STI (any of CT and/or NG and/or TV) and BV were calculated on 673 participants. In addition, 39 participants declined to respond to the questions used to explore the association with STI/BV (e.g. number of sexual partners in a lifetime) were excluded from the prediction models. Thus, 634 participants were selected, among which 212 were from the site in Madagascar, 203 in South Africa, and 219 in Zimbabwe (**Figure 5.2**).

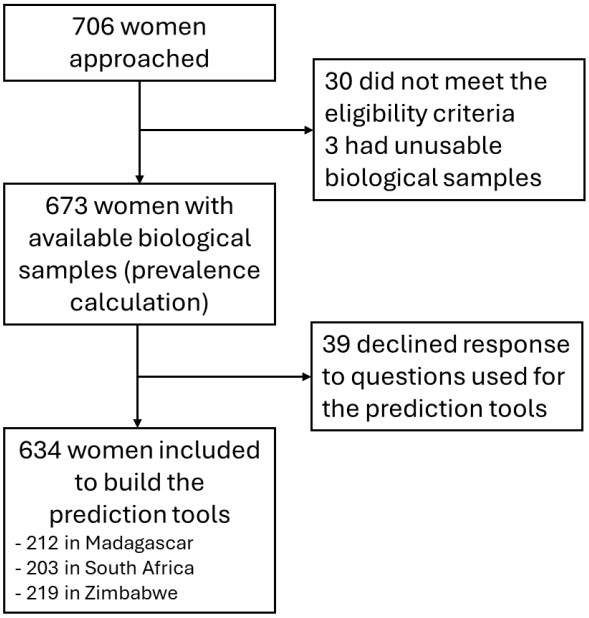


Figure 5.2. Selection of women aged 18 to 35 in the GIFT study for the creation of a prediction tool.

The calculated median age across the three countries was 27 (IQR: 22 – 30). Women recruited in Madagascar were most often living with their partners as if married (50%), or married (39%), women in Zimbabwe were mostly married (83%), whereas women in South Africa were mostly never married (87%). The highest level of education attained by the women recruited in Madagascar was, for 72% of them, middle school and below, while in South Africa and Zimbabwe, the majority went to high school or vocational school (65% and 78% respectively). Access to free or affordable STI testing was easy for 70% of the women in South Africa, 86% in Zimbabwe, but only 16% in Madagascar. Sixty-three percent of the women recruited in Madagascar said they were either unaware of or unconcerned about their ability to access free or affordable STI tests. In South Africa, 36 out of 203 (17.7%) women reported that their last HIV test was positive while in Zimbabwe, 12 out of 219 (5.5%) reported the same. In Madagascar, none of the women (0 out of 212, 0%) reported a positive HIV test (**Table 5.1**). Almost half of the women (307/634, 48.4%) were asymptomatic.

Table 5.1. Characteristics of the women selected for the prediction tool.

	Madagascar N = 212	South Africa N = 203	Zimbabwe N = 219	Total N = 634	p-value
Age, median [IQR]	26 22-29	24 21-29*	28 24-32	27 22-30	<0.001
Marital status, n (%)					<0.001
Never married	13 (6.1)	177 (87)	22 (10)	212 (33)	
Living together as if married	106 (50)	11 (5.4)	0 (0)	117 (18)	
Married	83 (39)	13 (6.4)	182 (83)	278 (44)	
Divorced, separated or widow	10 (4.7)	2 (1.0)	15 (6.8)	27 (4.3)	
Highest level of education, n (%)					<0.001
Middle school and below	153 (72)	18 (8.9)	6 (2.7)	177 (28)	
High school or vocational	46 (22)	131 (65)	170 (78)	347 (55)	
Higer education	13 (6.1)	54 (27)	43 (20)	110 (17)	
Occupation, n (%)					<0.001
Student	6 (2.8)	23 (11)	4 (1.8)	33 (5.2)	
Formal occupation	18 (8.5)	18 (8.9)	46 (21)	82 (13)	
Informal occupation	104 (49)	32 (16)	113 (52)	249 (39)	
Housemaker / No occupation	84 (40)	130 (64)	56 (26)	270 (43)	
Number of items†, n (%)	2 1-3	3 3-4	4 3-5	3 2-5	NA
Access to free or affordable STI testing, n (%)					<0.001
Easy	33 (16)	143 (70)	188 (86)	364 (57)	
Not easy or not possible	45 (21)	59 (29)	31 (14)	135 (21)	
Don't feel concerned or DK	134 (63)	1 (0.5)	0 (0)	135 (21)	
Getting STI treatment, n (%)					<0.001
Easy	32 (15)	159 (78)	196 (89)	387 (61)	
Not easy or not possible	43 (20)	44 (22)	23 (11)	110 (17)	
Don't feel concerned or DK	137 (65)	0 (0)	0 (0)	137 (22)	
Age at first menstruations					<0.001
≤ 11 years old	6 (2.8)	7 (3.4)	28 (13)	41 (6.5)	
12 – 15 years old	151 (71)	135 (67)	159 (73)	445 (70)	
≥ 16 years old	49 (23)	49 (24)	32 (15)	130 (21)	
Don't remember	6 (2.8)	12 (5.9)	0 (0)	18 (2.8)	
Number of pregnancies, median (IQR)	2 1-3	1 0-2	2 1-3	2 1-3	NA
Age at first vaginal penetration, median (IQR)	17 16-18	17 16-18	19 17-21	17 16-19	<0.001
Number of sexual partners, n (%)					<0.001
1	87 (41)	48 (24)	99 (45)	234 (37)	
2	47 (22)	25 (12)	52 (24)	124 (20)	
3	36 (17)	41 (20)	40 (18)	117 (18)	
4 and more	42 (20)	89 (44)	28 (13)	159 (25)	
Sex in exchange for favors,‡ n (%)					0.003
Yes	15 (7.1)	8 (3.9)	28 (13)	51 (8)	
No	197 (93)	195 (96)	191 (87)	583 (92)	
Suspicion that the partner has an STI, n (%)					<0.001
Yes	31 (15)	14 (6.9)	52 (24)	97 (15)	
No	162 (76)	96 (47)	34 (16)	292 (46)	
DK	19 (9)	93 (46)	133 (61)	245 (39)	
Condom use at last vaginal sex, n (%)					<0.001
Yes	4 (1.9)	69 (34)	35 (16)	108 (17)	
No	208 (98)	134 (66)	184 (84)	526 (83)	

	Madagascar N = 212	South Africa N = 203	Zimbabwe N = 219	Total N = 634	p-value
Condom use with casual partner in the past 3 months, n (%)					<0.001
Always	0 (0)	18 (8.9)	14 (6.4)	32 (5.0)	
Sometimes	5 (2.4)	138 (68)	51 (23)	194 (31)	
Never	43 (20)	34 (17)	137 (63)	214 (34)	
No casual partner	155 (73)	3 (1.5)	12 (5.5)	170 (27)	
No intercourse in past 3 months	9 (4.2)	10 (4.9)	5 (2.3)	24 (3.8)	
Use condom as contraceptive, n (%)					<0.001
Yes	0 (0)	24 (12)	6 (2.7)	30 (4.7)	
No	212 (100)	179 (88)	213 (97)	604 (95)	
Liquid products used to wash the vagina, n (%)					<0.001
None	198 (93)	111 (55)	93 (42)	402 (63)	
Water	10 (4.7)	24 (12)	111 (51)	145 (23)	
Water and soap	0 (0)	64 (32)	6 (2.7)	70 (11)	
Other§	4 (1.9)	4 (2.0)	9 (4.1)	17 (2.7)	
Time of washing, n (%)					NA
Wash before sex	1 (0.5)	0 (0)	47 (21)	48 (7.6)	
Wash after sex	1 (0.5)	9 (4.4)	5 (2.3)	15 (2.4)	
Wash before and after sex	6 (2.8)	32 (16)	47 (21)	85 (13)	
Don't wash before or after sex	204 (96)	162 (80)	120 (55)	486 (77)	
Frequency of washing, n (%)					<0.001
Everyday	6 (2.8)	76 (37)	121 (55)	203 (32)	
Don't wash or not everyday	206 (97)	127 (63)	98 (45)	431 (68)	
Ever treated for STIs, n (%)					<0.001
Yes	30 (14)	81 (40)	73 (33)	184 (29)	
No	182 (86)	122 (60)	146 (67)	450 (71)	
HIV status at last testing, n (%)					<0.001
Positive	0 (0)	36 (18)	12 (5.5)	48 (7.6)	
Negative, DK, never tested, PNS	212 (100)	167 (82)	207 (95)	586 (92)	
Currently taking PrEP					<0.001
Yes	1 (0.5)	25 (12)	8 (3.7)	34 (5.4)	
No	211 (100)	178 (88)	211 (96)	600 (95)	
Past or current schistosomiasis, n (%)					<0.001
Yes	1 (0.5)	2 (1.0)	23 (11)	26 (4.1)	
No	205 (97)	192 (95)	196 (89)	593 (94)	
DK	6 (2.8)	9 (4.4)	0 (0)	15 (2.4)	
Cigarette consumption, n (%)					<0.001
Yes	20 (9.4)	44 (22)	1 (0.5)	65 (10)	
No	192 (91)	159 (78)	218 (100)	569 (90)	
Alcohol consumption, n (%)					<0.001
Yes	83 (39)	157 (77)	60 (27)	300 (47)	
No	129 (61)	46 (23)	159 (73)	334 (53)	
Vaginal pH, median (IQR)	4.4 3.6-5.0	4.7 4.1-5.0	4.7 4.3-5.3	4.7 4.1-5.0	<0.001
Abnormal vaginal discharge, n (%)					<0.001
Yes	107 (50)	17 (8.4)	82 (37)	206 (32)	
No	105 (50)	186 (92)	137 (63)	428 (68)	
Lower abdominal pain, n (%)					<0.001
Yes	46 (22)	18 (8.9)	83 (38)	147 (23)	
No	166 (78)	185 (91)	136 (62)	487 (77)	

* In South Africa, only the year of birth was collected, thus age was calculated using January 1st, which has slightly overestimated the age statistics.
† Items included fridge, car, motorbike, bicycle, television, radio, phone, and computer
‡ Sex in exchange for money, drugs, gifts, good or shelter.
§ Other products included lime juice, vinegar, and commercial product specific for genital cleansing.
IQR: Interquartile range, DK: Don't know, PNS: Prefer not to say
NA: Not applicable

5.3.2. STI and BV prevalences and relative proportions

The prevalence of any STI and/or BV was 61.5% (95%CI [57.7, 65.2]). We determined that the overall prevalence for CT was 16.6% (95%CI [13.9, 19.7]); for NG, 6.4% (95%CI [4.7, 8.5]); for TV, 11.7% (95%CI [9.4, 14.4]), and for BV, 49.9% (95%CI [46.1, 53.8]). The prevalences of CT and/or NG (excluding co-infections with TV or presence of BV) was 6.5% (95%CI: 4.8, 8.7), TV and/or BV (excluding co-infections with CT and/or NG) 41.9% (95%CI: 38.1, 45.7), and CT and/or NG and/or TV 28.2% (95%CI: 24.9, 31.8). Prevalences by country are shown in **Figure 5.3**. Of STI/BV-positive women, 18.8% had STI only, 27.1% had both STI and BV and 54.1% had BV only. The prevalence of STI only was 11.6% (95%CI: 9.3, 14.3), both STI and BV 16.6% (95%CI: 13.9, 19.7), and BV only 33.3% (95%CI: 29.7, 37.0).

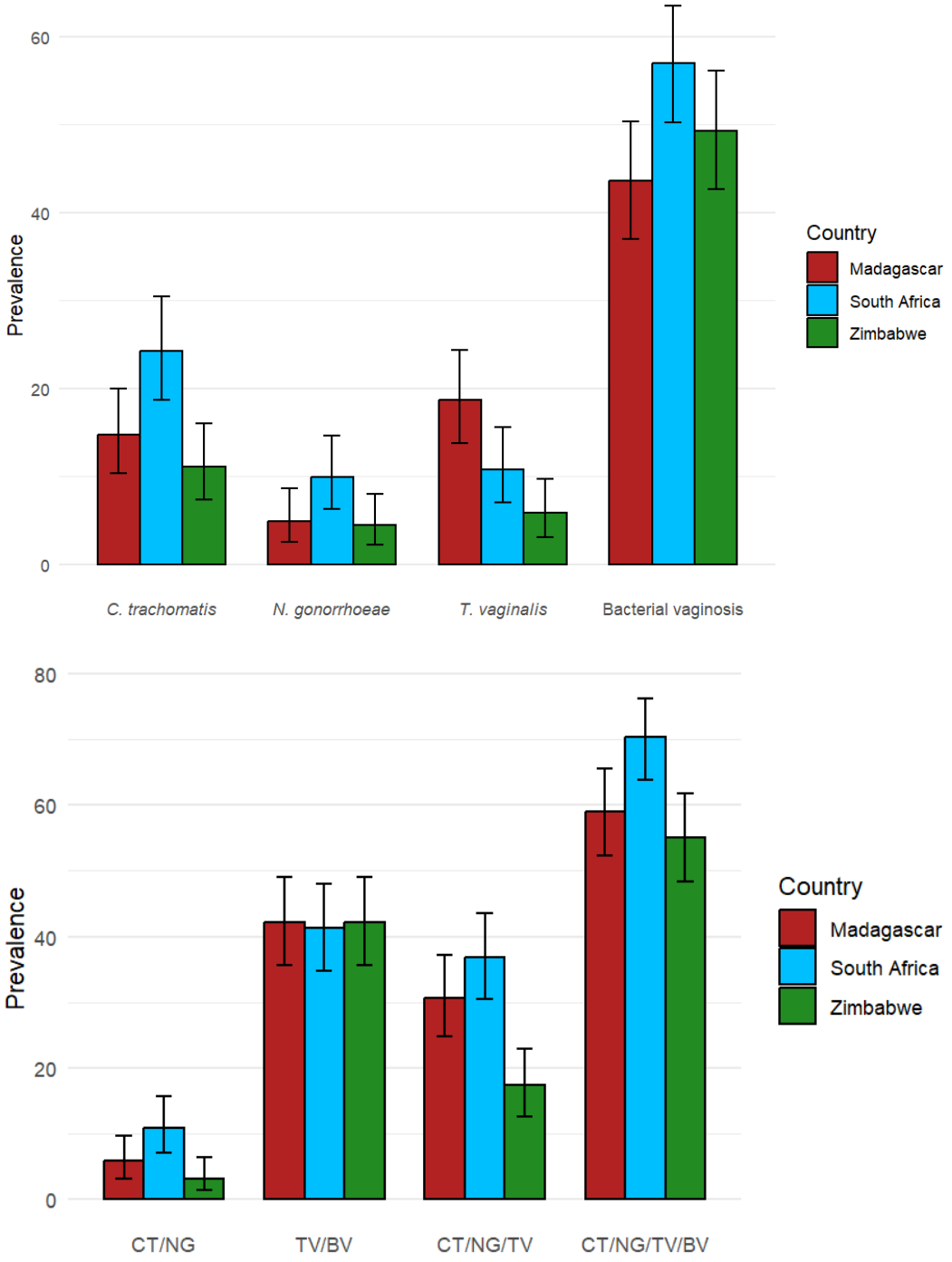


Figure 5.3. Prevalence of CT, NG, TV, BV, CT and/or NG without TV or BV, TV and/or BV without CT and NG, any STI (CT and/or NG and/or TV) and any RTI (CT and/or NG and/or TV and/or BV) among all women aged 18 - 35, by country.

5.3.3. The prediction of STI/BV

The characteristics of women with STI/BV and women with no STI/BV are described in Annex E, section 1, Table 1.

5.3.3.1. Risk scores: models for all women and for asymptomatic women only

First, we computed risk scores using logistic regression models. Factors associated with STI/BV were retained in the models to form risk scores which are presented in **Table 5.2**. We performed a model for the entire study population (model 1) and a model for asymptomatic women only (model 2). Thresholds were obtained for each model. Continuous variables such as age or vaginal pH are multiplied by their respective weighted coefficient and variables representing the presence or absence of a characteristic take the value 0 if absent and the value of the weighted coefficient if present. A score above the threshold (indicated in **Table 5.2**) indicates a high probability of infection whereas a score below indicates a low probability of infection. As an illustration, an asymptomatic woman visits the health care facility and undertakes the risk assessment. She declared her first vaginal sex was at 21, she does not drink alcohol, and her vaginal pH is 4.5, then, her risk score (using model 2 in **Table 5.2**) is:

$$21 \times (-2) + 0 + 4.5 \times 17.7 = 37.65$$

This value is below the threshold 43.65, thus the risk score predicts that the woman has a low probability of being infected by STI/BV. A table such as the one in **Table 5.3** can guide health care professionals in the use of such scores. Table A represents a paper version, but this score could be calculated by a mobile application such as illustrated in Table B.

Table 5.2. Risk scores for the prediction of STI/BV among all women (model 1) and among asymptomatic women only (model 2).

	Model 1 All women		**Model 2 Asymptomatic women**	
	β-coefficient	**Weighting**	**β-coefficient**	**Weighting**
Vaginal pH	1.91	19.1	1.77	17.7
Age at first vaginal penetration	-0.160	-1.6	-0.201	-2
Alcohol consumption	0.620	6.2	0.824	8.2
Abnormal vaginal discharge	0.562	5.6	-	-
Threshold	61.73		43.65	

Table 5.3. Example of the use of the risk score for asymptomatic women for health care personnel (model 2).

A

	Response	**Report value**
How old were you when you had vaginal sex for the first time?	…21… x (-2) = Multiply age by -2	-42
Do you drink alcohol?	☐ Yes (8.2) ☒ No (0)	0
You were given a pH paper for self-testing. According to the scale, what is the value of the pH?	…4.5… x 17.7 = Multiply pH by 17.7	79.65
Total score Sum up the values of all responses		37.65
Is the score above the value 43.65?	☐ Yes ⇨ High probability of STI/BV ☒ No ⇨ Low probability of STI/BV	

B

Example of an interface of the same risk score integrated to a mobile application

Age at first vaginal sex?	
Do you drink alcohol?	
pH value	
Automatic response:	

The AUC of the model among all women (model 1) was 0.812 (95%CI: 0.778, 0.846) and for the model among asymptomatic women only was 0.814 (95%CI: 0.760, 0.863).

5.3.3.2. Risk scores by countries (symptomatic and asymptomatic women)

We repeated this approach for each country. All these models included both the symptomatic and asymptomatic women. In South Africa, vaginal pH alone was sufficient to predict STI/BV (**Table 5.4**). In Madagascar, abnormal vaginal discharge appeared to be another predictive variable. In Zimbabwe, elevated vaginal pH, using condoms as a contraceptive and currently taking PrEP were predictors of STI/BV. Performing country-specific models for asymptomatic women was not possible due to the small sample size composed by each of these groups.

Table 5.4. Risk scores for the prediction of STI/BV among women, regardless of symptoms, in Madagascar (model 3), South Africa (model 4), and Zimbabwe (model 5).

	Model 3 Madagascar		Model 4 South Africa		Model 5 Zimbabwe	
	β coeff.	**Weighting**	**β coeff.**	**Weighting**	**β coeff.**	**Weighting**
Vaginal pH	1.84	18.4	1.65	16.5	2.99	29.9
Abnormal VD	0.954	9.5	-	-	-	-
Not using condom as a contraceptive	-	-	-	-	3.02	30.2
Currently taking PrEP	-	-	-	-	2.43	24.3
Threshold	88.81		74.72		174.8	

VD: vaginal discharge, PrEP: pre-exposure prophylaxis, coeff: coefficient

According to the ROC curves, the model for Zimbabwe and South Africa performed better than the model for Madagascar (**Figure 5.4**). The black dots represented the sensitivity and the specificity selected based on their highest geometric mean. The performance of each model can be found in **Table 5.5**. The model for South Africa had the highest sensitivity (78.0%), whereas the model for Zimbabwe had a slightly lower performance with a sensitivity of 72.3%, and the model in Madagascar had a sensitivity of 63.0%. The probability of predicting no STI/BV when the patient was truly not infected (i.e. the specificity) was 85.0% in Zimbabwe and 81.2% in Madagascar, and 79.0% in South Africa. The AUC for the model in Madagascar was 0.634 (95%CI:0.551, 0.720), in South Africa 0.772 (95%CI: 0.687, 0.847), and in Zimbabwe 0.819 (95%CI: 0.758, 0.874).

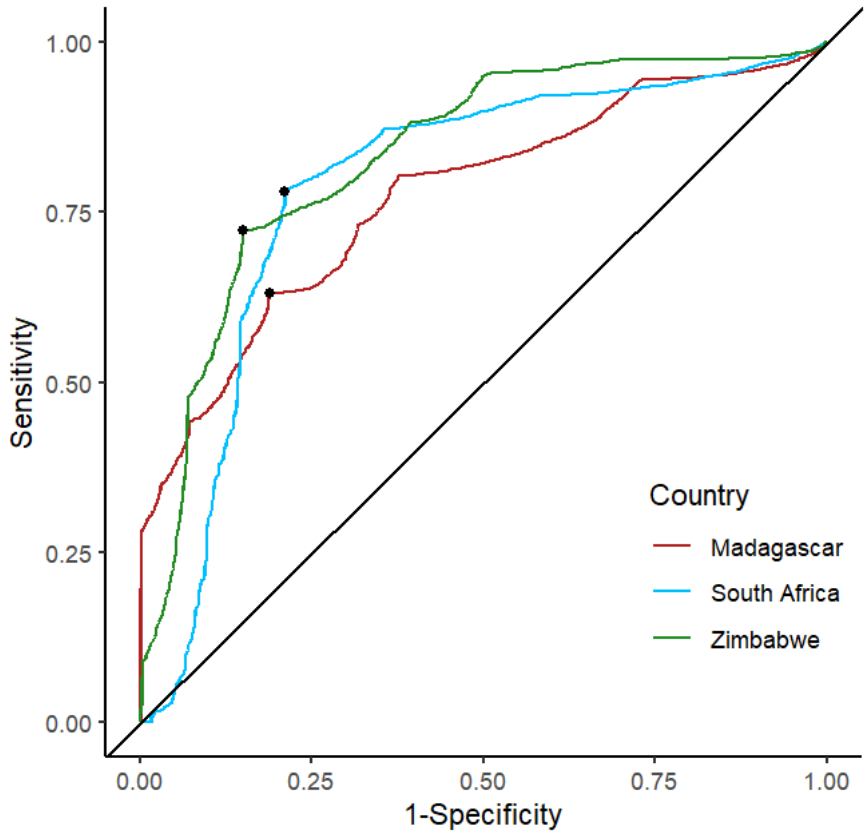


Figure 5.4. ROC curves for the prediction of STI/BV among women, regardless of symptoms, in Madagascar (model 3), South Africa (model 4), and Zimbabwe (model 5).

The black dots represent the selected sensitivities and specificities, based on their highest geometric mean.

5.3.3.3. Risk scores: further analyses

Risk scores were developed to detect the following conditions:

- STIs vs no STIs,
- BV vs no BV,
- CT/NG in the absence of TV and BV compared to women with no STI and no BV,
- TV/BV in the absence of CT and NG compared to women with no STI and no BV.

The results of these analyses are presented in Annex E.

In our models, we found that pH plays a significant role in predicting STIs, BV and TV/BV. However, pH was not retained in the model for predicting CT/NG. The risk score for STI relies on vaginal pH, age, alcohol consumption and access to a free STI test and has a sensitivity of 72.7% (95%CI: 66.1, 79.3) and a specificity of 52.2 (95%CI: 47.6, 56.8). In the risk score for BV, vaginal pH, alcohol consumption and abnormal vaginal discharge were predictors of BV, and

the model has sensitivity of 78.3% (95%CI: 73.7, 82.8) and a specificity of 74.1 (95%CI: 69.4, 78.9). Predictors of CT/NG infection were young age and food security (Se: 70.7% [95%CI: 56.8, 84.7], Sp: 66.8 [95%CI: 60.9, 72.7]), and predictors of TV/BV were vaginal pH and age at first vaginal penetration (Se: 80.1% [95%CI: 75.3, 84.9], Sp: 76.1 [95%CI: 70.8, 81.4]).

5.3.3.4. Random forest and CART

In a second step, we used machine learning techniques to predict STI/BV among all women. We found that variables of importance were vaginal pH, age at first vaginal penetration, age, number of belongings (as a proxy for wealth), alcohol consumption and education ($p<0.1$). When incorporated into a classification tree model, women with a vaginal pH below 4.3 were classified as not having STI/BV, and women with a pH above or equal to 4.9 were classified as having STI/BV. Women with a pH between 4.3 and 4.8 and who had their first vaginal penetration before or at 18 were classified as having STI/BV whereas those who were older were classified as negative for STI/BV.

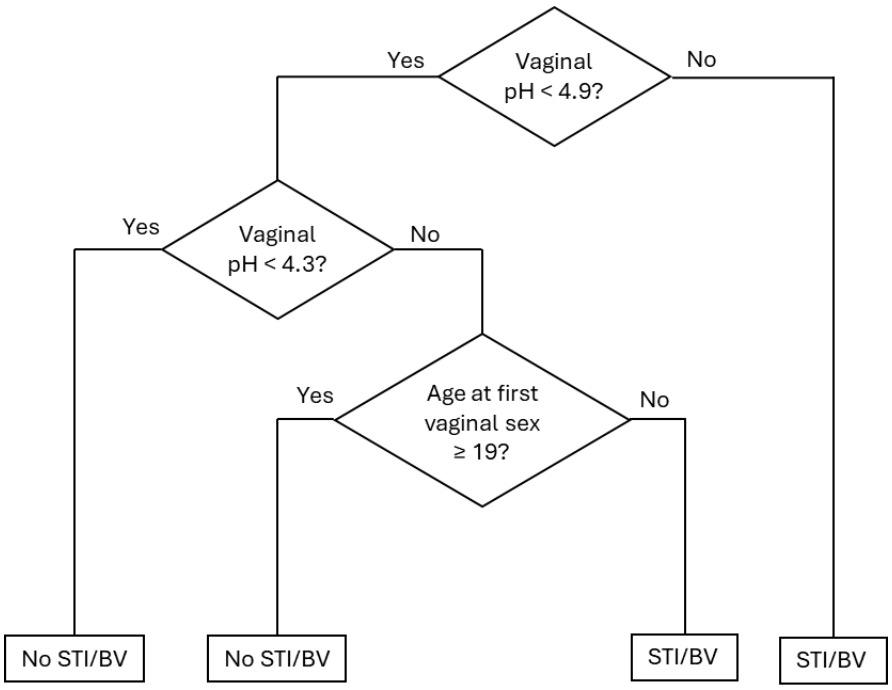


Figure 5.5. Decision tree model for the prediction of women (regardless of symptoms) on STI/BV.

Using the previous example, the woman with a pH equal to 4.5 and first vaginal sex at 21 would fall into the "No STI/BV" prediction.

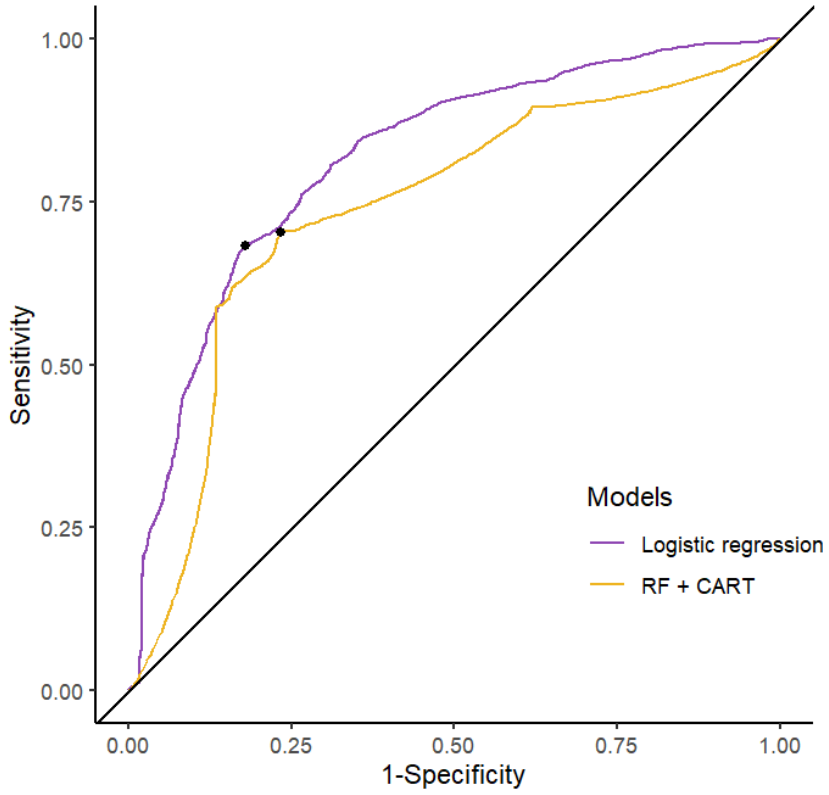


Figure 5.6. ROC curves for the prediction of STI/BV among all women using logistic regression (model 1) and using RF followed by CART (model 6).

The black dots represent the selected sensitivities and specificities, based on their highest geometric mean.

Sub-analyses for asymptomatic women only or by country was not possible due to the small sample size.

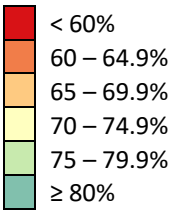
When comparing the model that used generalized linear models (model 1) with the model that used two consecutive approaches from the field of machine learning (model 6), we found that model 1 performed better overall (**Figure 5.6**). However, for the selected threshold, model 6 had a sensitivity of 71.6% whereas model 1 had a sensitivity of 68.7% (**Table 5.5**).

5.3.3.5. Performance of the models for the prediction of STI/BV

The performances of each model along with their 95% CI for Models 1 – 6 are summarized in **Table 5.5**.

Table 5.5. Performances of models 1 to 6 estimated by cross-validation.

	Variables retained in the model	Sensitivity 95% CI*	Specificity 95% CI	PPV 95% CI	NPV 95% CI	AUC 95% CI
Model 1 All women	Vaginal pH Age at first penetration Alcohol consumption Abnormal vaginal discharge (VD)	68.7 [67.2, 70.0]	80.9 [78.9, 82.2]	84.9 [83.6, 85.9]	62.2 [61.0, 63.3]	0.812 (0.778, 0.846)
Model 2 Asymptomatic	Vaginal pH Age at first penetration Alcohol consumption	79.5 [77.5, 80.6]	71.0 [68.7, 72.7]	80.8 [79.6, 81.6]	69.2 [67.3, 70.7]	0.814 (0.760, 0.863)
Model 3 Madagascar	Vaginal pH Abnormal VD	63.0 [61.1, 64.2]	81.2 [79.8, 82.3]	83.3 [82.1, 84.4]	59.5 [57.9, 60.1]	0.634 (0.551, 0.720)
Model 4 South Africa	Vaginal pH	78.0 [76.5, 79.7]	79.0 [77.9, 80.1]	89.4 [87.2, 90.0]	61.2 [59.8, 62.7]	0.772 (0.687, 0.847)
Model 5 Zimbabwe	Vaginal pH Condom as contraceptive Currently taking PrEP	72.3 [70.6, 73.8]	85.0 [83.7, 87.2]	85.1 [83.5, 86.1]	72.0 [70.2, 74.1]	0.819 (0.758, 0.874)
Model 6 RF + CART	Vaginal pH Age at first penetration	71.6 [67.1, 76.1]	73.4 [68.0, 79.0]	80.0 [76.7, 85.1]	62.3 [56.7, 67.9]	0.658 (0.579, 0.742)



PPV: positive predictive value,
NPV: negative predictive value,
RF: Random forest,
CART: Classification and regression trees
* All 95% CI were obtained by bootstrapping

5.4. Discussion

In this study, the prevalence of STIs and BV was elevated, affecting more than half of the women, and was largely due to the high prevalence of BV. This pattern was consistent across the three countries and may explain the high positive predictive values observed in all models.

The model for asymptomatic women (model 2) achieved the highest sensitivity, with nearly 80% of the predictions being accurate. The score derived from this model was based on only three items: vaginal pH, age at first vaginal sex and alcohol consumption, which makes it rapid to use. However, the calculation of the scores may require a calculator or needs further refinement, like all other risk scores in this study.

Risk factors retained in this model differed from those found by Tyndall *et al.* who developed a score for CT/NG detection among asymptomatic women.[248] In their study conducted in Kenya, they explored multiple risk scores. One of their risk scores included the following variable: age < 25 years; being single; and having two or more sex partners in the past year. Its sensitivity was 60%, and specificity 57%. Similarly, in a study conducted in a Ugandan population at risk of HIV, predictors of asymptomatic CT and NG were being under 25 years and having more than one sexual partner.[255] However, this study did not develop any risk score. The divergence between these risk factors and those we found may be due to the inclusion of BV in addition to STIs in our study, as well as differences in study populations, methodologies, or regional sexual health practices and behaviors.

Notably, one of the main predictors included in model 2, vaginal pH, is directly related to BV and was included in all the models developed in this study. An elevated pH is known to be associated with BV and is also a key component of its definition according to the Amsel criteria (pH > 4.5). This increase in pH results from the decrease in *Lactobacilli*, which produce lactic

acid to maintain an acidic vaginal environment. However, an elevated pH can also indicate a TV infection.[256]

Previous studies on BV risk scores have shown mixed results regarding vaginal pH. One study found that a pH > 4.5 was strongly associated with BV,[252] while another found no such association, possibly due to using a higher pH cutoff of 5.[257] In our CART model (model 6), we found that, for pH values between 4.3 and 4.9, a second question was needed to predict the outcome with more precision. This suggests that, for certain values of pH, pH alone may not always be sufficient to accurately predict STI/BV and may need to be combined with other indicators.

Interestingly, although profound demographic differences exist across the recruited women in the three countries, vaginal pH remained a consistent predictor. For South Africa, only pH was selected, and this model yielded the highest sensitivity of all country-specific models. While it has been documented that demographic or behavioral factors are very sensitive to the context and may not be easily derived from one population to another,[258] the use of a biological variable as a prediction tool, such as vaginal pH may facilitate its generalizability. Yet, several studies found that vaginal pH and microbiota differed significantly by ethnicity, even after adjustment for socio-demographics, sexual activity, sexually transmitted diseases, health behavior, and sexual hygiene.[259] This finding suggests that further studies on the variation in vaginal pH across areas may be needed to obtain a country- (or region-)specific cut-off. FDA-approved self-pH-testing devices are commercially available for genital use in women. However, in LMICs, the use of pH paper strip with a precision of one tenth may be a more cost-effective alternative.[260]

Risk score items retained in the model predicting STIs/BV for all women (model 1) and asymptomatic women (model 2) were the same except for abnormal vaginal discharge which was an additional risk factor in model 1. This result suggests that the risk profile of asymptomatic women may not differ from symptomatic women. In addition to pH, two other factors were retained in the model: alcohol consumption and age at first vaginal sex.

The first factor, alcohol consumption, was defined as a positive response to the question “Do you drink alcohol?”, covering from an occasional occurrence to a very frequent occurrence whereas a negative response indicated no consumption of alcohol at all. Alcohol can be

indirectly related to STI/BV through disinhibition or according to the alcohol myopia theory, where individuals under the influence of alcohol focus more on immediate cues (e.g. sexual desire) and less on potential risks (e.g. unprotected sex).[261,262]

The second factor, age at first vaginal sex, was another key predictor. In our study, initiation of sexual intercourse was collected in two manners: age at first sexual experience (including oral, anal, or vaginal) and age at first vaginal sex. We kept the question on vaginal sex only as the two were highly correlated and that it had fewer missing values (data not shown). The association between early sexual life and STI was described in some studies and is viewed as a marker of risky sexual behavior.[263,264] One study showed a stronger link in younger adults, and a diminishing effect in older populations.[265] This suggests that the performance of the risk score may differ whether we ask a younger or an older adult.

The country-specific models revealed that abnormal vaginal discharge was a predictor in Madagascar. While abnormal vaginal discharge has been previously documented as a risk factor for STI/BV in multiple countries,[234,266] it is unclear why this factor was retained only for Madagascar and not for South Africa or Zimbabwe. We hypothesized that, since TV can cause a visible abnormal vaginal discharge and that TV prevalence was higher in Madagascar than in Zimbabwe and South Africa, this variable stood out. We could also explain this result by a difference in perception of an abnormal vaginal discharge across the three populations. Moreover, the model for Zimbabwe retained two other predictors: not using a condom as a contraceptive and currently taking PrEP. PrEP was shown to be associated with an increased incidence of STIs among men who have sex with men,[267] because their protection against HIV leads them to abandon condoms. The same motive could apply to women. PrEP guidelines include recommendations for regular STI testing, but it may not be available in most health care settings in many LMICs. Thus, the incidence of STI in female PrEP users may be high.[268,269]

“Condom use as contraceptive” by comparison with “condom use with an occasional partner” suggests that condoms are used both with their cohabitating partner/husbands and with their occasional partners. This implies that women who use a condom with their main partner may be more protected against STI/BV. This may be explained by the fact that, in Zimbabwe, men have several wives and that 37% of men reported sexual intercourse with a non-marital, non-cohabitating partner, according to the 2015 Zimbabwe’s DHS.[270]

Although BV is not considered an STI, a systematic review showed that condom use appears to be associated with a protective effect against BV.[60] Acting as a barrier, condom minimizes the risk of introducing bacteria altering the vaginal environment.[271] Additionally, condoms reduce the exposure to semen, which can increase the vaginal pH, disrupt the natural balance of bacteria, and potentially lead to BV.[272]

We initially hypothesized that, in the STI/BV prediction model, vaginal pH would be a predictor primarily of BV rather than STIs. However, when we ran a separate model (Annex E) comparing women with STIs to those without, pH remained a strong predictor. This finding may be explained by the high prevalence of TV among women with STIs, highlighting the potential role of elevated pH in TV infections. Another plausible explanation is that many women with STIs also have coexisting BV, making BV a confounder — associated both with STI status and with elevated pH.

In the model exploring factors associated with STIs, the lack of access to free STI testing was a predictor of STI. Although overall access to free testing was better among women in study sites in Zimbabwe and South Africa than in Madagascar, within each country, women who lacked access to or did not feel concerned with free testing consistently showed a higher prevalence of STIs. This finding suggests that free and accessible STI testing may contribute to improved STI control in these settings.

To develop these prediction models, we used logistic regressions but also random forest and CART. To our knowledge, this is the first study using machine learning techniques to predict STI/BV in LMICs. The logistic regression model built and applied on all women (model 1), had a fair sensitivity of 68.7%. The equivalent model built with machine learning techniques (model 6) had a similar sensitivity of 71.6%. However, the CART model is easier to implement: with only two questions and a logical flowchart, it reduces the chances of making errors, contrary to the risk score. Unfortunately, this procedure could not be used for other sub-analyses but the simplicity of its output warrants further development in future studies.

This study was conducted in populations that differed in STI/BV prevalence, demographics, and behavior. This heterogeneity may be an asset for the development of a risk score because it reduces the risk that the prediction tool will be specific to only one population. In addition, model 6 in this study used random forests for the selection of variable, a powerful technique.

Combined with CART, it allowed the machine learning approach to yield a directly usable and understandable output. Finally, we proceeded to cross-validation for the risk scores and CART, which allowed us to provide more reliable estimates of model's performance and prevented overfitting and poor generalization on new data.[273]

This study has several limitations. First, the predictions may primarily reflect the presence of BV or infection with TV, rather than bacterial STIs in general, as pH was not retained in the model for the prediction of CT/NG. However, the high prevalence of BV with STIs merits further discussion. While detection of BV may be beneficial for pregnant or symptomatic women, the value of identifying asymptomatic women with BV is debatable. The benefits of treating asymptomatic BV remain controversial.[274] I argue that further research is necessary to understand vaginal inflammation found among women with asymptomatic BV, particularly in BV persistence, recurrence and treatment failure. Second, our models suggested that pH is a consistent indicator. However, we do not know if other factors influenced the value of the pH such as the presence of semen.[272] Further adjustments in the risk scores may be needed to account for the presence of semen resulting from a recent unprotected vaginal sex. Vaginal pH can also vary during menses, however, as per our selection criteria, we excluded all women who had menses or were a few days after their last day of menses to avoid blood on the samples.

This study offers relatively simple and easy to use risk scores/algorithms for which the entry point is not a sign or symptom, contrary to the syndromic management. Its greatest asset is to account for asymptomatic women. Further evaluation on the acceptability among both the healthcare workers who ask the questions and the women who answer them, in different populations, will be needed. Additionally, studies on the usability of pH paper strip, interpretation of pH values and interrater variability would help to ascertain the validity of this tool.

5.5. Contribution

I contributed to elaborate the questionnaire in the GIFT diagnostic trial and in data cleaning. Before conducting the machine learning techniques, I took a one-week intensive class on this topic. Then, I conducted all the analyses in this section in collaboration with Félix Cheysson, a researcher in statistics at the Centre National de la Recherche Scientifique.

Chapter 6: Contributions, perspectives, and conclusions

6.1. Synthesis of contributions

6.1.1. Contribution to the epidemiological data on prevalence and risk factors of sexually transmitted infections in Antananarivo, Madagascar

In 2024, the WHO research priorities for STIs in the epidemiology domain included (1) estimating the prevalence of CT and NG infections, and (2) evaluating STI antimicrobial resistance; two research areas to which our PrevISTana study contributed.[84]

First, this study was the first on the prevalence of CT, NG TV, and MG in women over the past 20 years in Madagascar. We highlighted an important prevalence of STIs in the capital city of Madagascar, revealing that more than one in three women are infected with either CT, NG, TV, or MG. Additionally, the high prevalence of BV, affecting 40% of women, shows a pattern consistent with other African contexts. These data show the need to implement communication with the public and prevention measures against STIs at country level.

The risk factors analysis we performed may be helpful to target most at risk populations. We found that women with multiple concurrent partners and those engaged in sex in exchange for money, goods or jobs are particularly vulnerable. WHO defines priority target populations as adolescent girls and boys, young adults, sex workers and their clients, men who have sex with men, transgender people, people living with HIV, people who have other STIs, and pregnant women.[233] Sex workers includes by definition women who exchange sex for favors. However, women who have sex in exchange for money, services, shelter, or jobs may not define themselves as sex workers. They constitute an unseen, yet substantial, group at risk that blends into the general population, and that is missed by intervention programs oriented towards FSW. Future public health strategies may reach this group by displaying messages where women in financial precarity may be found such as pupils and students, women in the informal sector, single or adolescent mothers.

In addition, in a country where the usage of condoms achieves extremely low rates, promoting condoms with occasional partners stands as an easily implementable preventive measure against STIs. Finally, this study stressed that 40% of women with an STI did not complain of any symptoms. Along with prevention campaigns, information campaigns on the high circulation of STIs and the possibility of being infected and not displaying symptoms are also needed.

Lastly, we determined that one MG sample out of 41 had a mutation associated with antimicrobial resistance, indicating that resistance to fluoroquinolones against MG may not be a major issue in Antananarivo.

6.1.2. Contribution to the understanding of asymptomatic infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*

Asymptomatic infections are a key obstacle to the control of STIs. In the systematic review and meta-analysis, we gathered all studies documenting asymptomatic women infected with CT, NG, or TV in LMICs. We produced, for the first time, a summary of estimates on asymptomatic infections in LMICs which showed that more than half of the women with an STI are asymptomatic: 60.7% for CT, 53.3% for NG and 56.9% for TV.

In addition, our findings suggest that up to 13% of women in LMICs may have an asymptomatic infection. This prevalence raises concerns as these women, unaware of their infection, are at risk of complications and continue to transmit their STI.

These estimates are important to describe the extent of this “hidden” epidemic. They can be further used in modeling studies, together with estimates on the proportion of these women who will develop complications (or death of their babies), who will acquire HIV or other STIs (and subsequent complications), and the number of partners who will acquire the infection and subsequently infect other concurrent or future partners, who may, in turn, develop adverse events. These models on the dynamic of STI transmission may provide measures of the burden of asymptomatic STI, expressed, for instance, in terms of Quality-Adjusted Life Years (QALYs) or Disability-Adjusted Life years (DALYs). Such projections may inform the need

for a public health intervention, which may take the form of a screening program, provided that certain criteria are met.[275]

6.1.3. Contribution the detection of women with an increased risk of STI/BV, including asymptomatic women and implication for STI control

The development of a low-cost, rapid STI POCT has also been cited by WHO as a key research priority for improving STI diagnosis.[84]

In the third project of this thesis, we developed multiple tools to identify women at risk of STI/BV, with two different approaches, on different sub-populations. Our tools have the advantage of being minimally invasive, they can be easily and rapidly carried out and are relatively inexpensive, the only cost coming from the pH paper strip. In this project, this tool was designed to identify women who are at risk of STI/BV in order to select those who undertake further testing with the GIFT. When applied at a large scale, such as in a screening program, this pre-selection stage has the potential to importantly reduce the cost of screening, due to the reduction in the number of tests that will be given.

Before undertaking further steps, the performance of the combination of the risk score/algorithm combined with the GIFT device will need to be evaluated. To be relevant at the public health level, the evaluation of this combination may not only focus on the sensitivity, specificity, and predictive values, but may include its ability to reduce transmission, the prevalence of STIs/BV and the incidence of complications related to these conditions.

Potential use cases of GIFT may include:

- use in pregnant women,
- as a test of cure,
- to identify women at risk for HIV (since vaginal inflammation increases the risk of HIV) and offer PrEP,
- to rule out inflammation, or
- in combination with the syndromic approach.

Similarly, a comprehensive evaluation of these approaches may be required before upscaling.

Coming back to the third project of this thesis, despite not being our primary objective, this study also provided two interesting findings. First, we obtained epidemiological data on the prevalence of STIs/BV in Madagascar, South Africa and Zimbabwe, contributing to WHO research priorities for STIs. Second, our risk score for Zimbabwe revealed that currently taking PrEP was a risk factor for STIs among women. Although higher acquisition of STIs among PrEP users has been well documented in men who have sex with men in high-income countries, there are only a few studies that support this observation among women in LMICs. Regardless of the country, this finding suggests that women under PrEP, in addition to women who have sex in exchange for favors (from the PrevISTana study), would be a priority population for screening and a prevention messages.

6.2. Perspectives

6.2.1. Should there be an STI screening program?

Decision to have a screening program for STIs first requires a better understanding of their natural history: what percentage of women will develop complications, and what percentage will have a spontaneous resolution? Other important information, not developed here, includes the number of partners infected and the probability of being cured/reinfected after the STI is detected and treated. In the field of screening, asymptomatic infections are often considered separately.

Preamble on asymptomatic infections

In the work conducted in our systematic review and meta-analysis, we underscored the inconsistent definition of "asymptomatic infections". Asymptomatic women were defined as women not presenting a certain number of symptoms based on a pre-defined list relevant to the context or the study in which the definition is used. Defining asymptomatic by the absence of one symptom (e.g. abnormal vaginal discharge) will provide more asymptomatic cases than a definition that uses two or more symptoms. Moreover, the broad range of symptoms and the variations in their presentation across women complicates the use of a uniform definition. In the academic book *Sexually Transmitted Diseases* published in 2000, Rosenthal et al. wrote that "asymptomatic infection does not prompt affected individuals to seek health care and possibly take precautions to avoid transmitting their disease".[276] This description most accurately reflects the situation in practice, because the perception of symptoms can vary between individuals even though other factors, such as financial constraints, may influence the decision to seek healthcare.

In addition, the absence of symptoms may be a transitional phase: in the course of infection, symptoms are usually preceded by an incubation period and may be followed by a second asymptomatic period before the infection disappears. In a mathematical modelling study, the proportion of infections that become symptomatic was estimated to be 6% in women infected with CT, and 14% in women infected with NG.[277]

Some women may never develop symptoms, for biological reasons. For example, some variants of NG establish strong colonization but have limited penetration, leading to asymptomatic infections, thus, they tend to survive more effectively in the cervix.[278]

6.2.1.1. Natural history of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium* and bacterial vaginosis

Natural history studies are helpful to understand the evolution of STIs, in particular finding on the rate of spontaneous clearance and development of complications.

Chlamydia trachomatis

A narrative review of short-term observational studies on the spontaneous clearance of CT, published up to May 2022, found clearance rates from 6 to 26% between initial testing and follow-up for treatment after 2 to 4 weeks, and from 9 to 44% within approximately 10 weeks.[279] A long-term study reported a clearance rate of 54% after 1 year, 82% after 2 years and 94% after 4 years.[280] An untreated infection that resolves spontaneously might confer some immunity against future infections.[281] Treatment for CT may reduce the immune response and make women susceptible to infection again, increasing their risk of repeated infection, a hypothesis which has been called the 'arrested immunity'.[282]

Several studies have examined the risk of sequelae following infection. In a synthesis of evidence from different studies, a mathematical model estimated that the probability of PID following a CT infection is about 16% (95% credible interval 6 – 25),[283] and the probability of tubal factor infertility is near 1%.[284]

Neisseria gonorrhoeae

Studies of the natural history of NG infection in women are limited. A study published in 1946 comparing cure rates after treatment with sulfathiazole or penicillin, reported a sulfathiazole failure rate of 21%.[285] But most importantly, some patients had still positive cultures after 3 months or longer. Another study provided some insight into the presentation of untreated NG. Women were identified through contact tracing of partners of men with gonococcal urethritis. Nineteen were found to be infected with NG, and among them, nine had PID or

tenderness of the adnexa suggestive of inflammation in the upper reproductive tract.[286] Finally, in a prospective study of asymptomatic adolescents, women were screened every three months and self-collected cervicovaginal swabs. NG was detected in these swabs before the quarterly visits in 18 women, suggesting that NG can persist for at least 12 weeks. Three women developed vaginal discharge and dysuria.[287] Taken together, these studies suggested that NG, evades host immune responses, or that reinfection occurred (**Figure 6.1**).

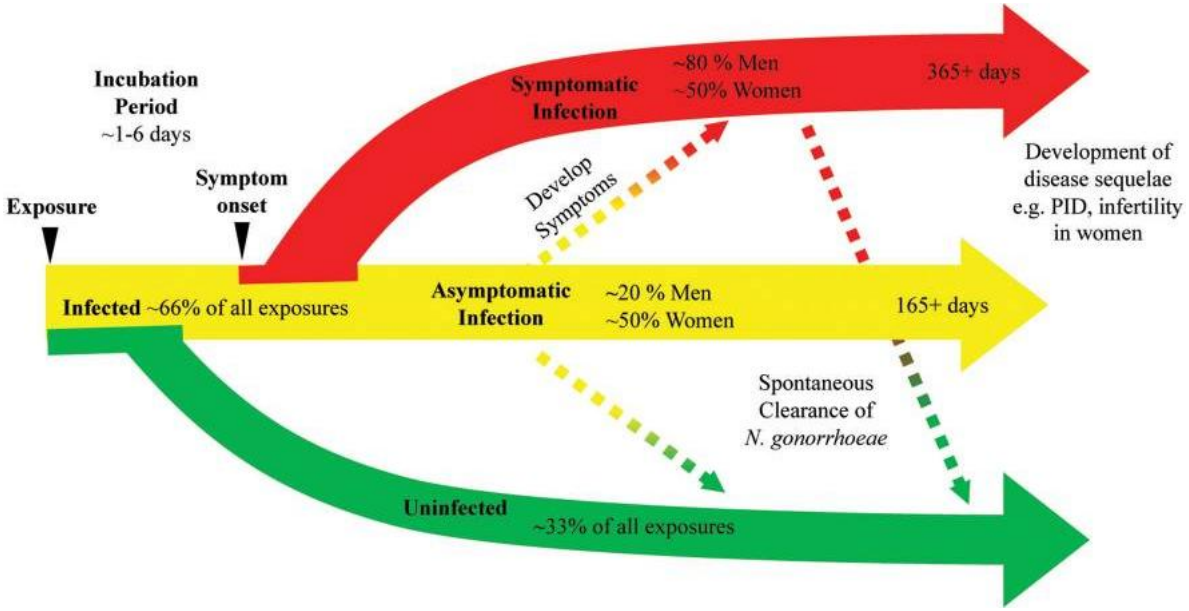


Figure 6.1. Diagram of the natural history of *N. gonorrhoeae* after exposure (reproduced from Lovett & Duncan *[288]*).

Trichomonas vaginalis

We identified two studies on the natural history of TV. In the first study, women 14 – 17 years old were followed up for 27 months.[289] Vaginal samples were obtained during quarterly clinic visits and self-obtained weekly. The weekly samples were tested quarterly. Some weekly samples were positive for up to 12 consecutive weeks. As for NG, this result suggests that clearance of TV is limited or that women were reinfected.

However, this finding is balanced by another study. In a retrospective cohort of Colombian women, a clearance rate of 15 per 100 person/month was determined, and 50% of the women cleared TV after 6 months.[290] Moreover, women having worsened cervical lesion findings had a lower probability of clearance. The persistence rate was 22.7 per 100 person/month.

Factors associated with persistence were older age (35 to 49 years old), having had more than three sexual partners and multiparity.

Mycoplasma genitalium

The natural history of MG has been described in a cohort of FSW in Uganda.[291] In this study, 55% of the women had spontaneously cleared MG within three months, 83% within six, and 93% within 12 months. The clearance rate was 25.7 per 100 person-years. In addition, the authors found that HIV-positive women cleared MG more slowly than HIV-negative women, in particular when their CD4 counts were less than 350/mL.

In a systematic review and meta-analysis, that included the study in Uganda, the authors determined that the median duration of persistence of MG was one to three months in four studies but 15 months in one study. Importantly, the authors mentioned that in all studies, women had opportunities for treatment with antibiotics that may have acted against MG. In addition, they found that the incidence of PID was higher in women infected with MG in comparison to uninfected women (Risk Ratio: 1.73, 95%CI: 0.92, 3.28).

Bacterial vaginosis

In one study in China, researchers described the natural history of asymptomatic BV (Nugent score 7-10) in pregnant women.[292] Among 1014 women, 984 participants completed follow-up over a four-month period. At the end of the study, 42 showed no signs of BV without any treatment. Meanwhile, 433 participants experienced a progression of their condition (defined as self-reported symptoms or diagnosis of another genital infection), while 509 showed no change in symptoms status or Nugent score. Among those who progressed, 196 participants developed symptoms.

Synthesis

Overall, these studies provide some understanding of the course of these infections and the burden they may cause in areas where testing is not always feasible. Clearance may result from the development of host immunity (suggested for CT) but also to antibiotics uptake that has activity against these STIs. On the opposite, persistence in the detection may reflect the actual burden persistence of the pathogen due to the absence of treatment but may also

reflect reinfection from the partner or autoinoculation from an extra-genital biological site. A longer duration of the pathogen in the genital tract may increase the probability to ascend to the upper genital tract, and thus to cause PID.

These studies are usually conducted on a small sample size. Further studies would be needed on the occurrence of complications, especially for TV, MG, and BV, while remaining cautious of the ethical aspects of such studies.

6.2.1.2. Screening in high-income countries: current knowledge and reflection on transposition to LMICs

Although natural history data are important to know whether screening would be relevant, they are not an absolute requirement to develop such programs. In this section, we will describe some screening programs on CT and BV that have been conducted and evaluated in high-income countries.

Chlamydia trachomatis

In high-income countries, the effectiveness of CT screening in clinical trials has been evaluated. The rationale for CT screening is that testing should detect asymptomatic infections in women before they cause PID or other complications. It was shown that screening interventions may have some effect on the reduction in incidence of PID, but with a low quality of evidence.[293] Other studies showed that CT screening did not decrease the CT prevalence.[294,295] Several hypotheses may explain this result, such as the insufficient number of persons covered by the intervention and the insufficient duration of the intervention, but also the low number of treated sexual partners and subsequently the increased risk of reinfection.[296] It is also possible that testing and treatment reduced immunity against chlamydia in the population, leading to increased susceptibility to infection.[282]

In LMICs, these interventions may not be feasible because (1) the test will likely be too expensive for the majority of women, (2) of the limited infrastructure, (3) logistic issues, and (4) competing health priorities (such as malaria, HIV or TB). Because the prevalence of CT in LMICs is high,[7] treating asymptomatic CT in LMICs may be more impactful than in Europe. Moreover, the management of conditions such as PID, ectopic pregnancy and infertility may

require human and material resources which are not always available in LMICs. In addition, LMICs continue to bear an important prevalence of PID and ectopic pregnancy, therefore we may find stronger evidence that treatment of asymptomatic CT reduce these events.[297]

Regarding screening of CT among pregnant women, the guidelines from the United States recommend screening at the first prenatal visit and, for women at risk, retesting during the third trimester.[298] Screening and treating asymptomatic CT or NG-infected pregnant women has been assessed in a non-randomized cluster-controlled trial in Botswana.[299] They found limited evidence that the prevalence of preterm birth or low birth weight was lower in the screen and treat arm than the standard of care. However, the intervention was effective among the nulliparous women. In a cluster randomized cross-over trial conducted in Papua New Guinea, the effect of POC testing and immediate treatment of CT, NG, TV, and BV on preterm birth, low birth weight, and other adverse outcomes was compared with symptom-based treatment without laboratory confirmation. The authors reported no difference in the proportion of preterm birth, low-birth weight or both in the intervention group compared to the control group.[300] Further studies on the benefits and harms of screening and treating asymptomatic CT among women are needed in LMICs.

Aside from screening, other interventions can be leveraged. Mathematical models have shown, in high-income countries, that doubling the efficacy of partner notification has been more cost-effective than increasing CT screening coverage,[301] a finding that suggests that partner notification would have an important potential in STI control in LMICs as well.

A former intervention involved mass treatment with azithromycin against trachoma and was associated with a reduction in the prevalence of genital CT.[302] However, this approach may not be appropriate for chlamydia control due to its potential contribution to resistance in *Treponema pallidum*,[303] NG,[304] and MG.[305]

Bacterial vaginosis

Asymptomatic BV is common, however in many countries, current guidelines do not recommend screening or treatment of asymptomatic BV.[298,306] For asymptomatic pregnant women with BV, a Cochrane review concludes that there is insufficient evidence to recommend routine screening and treatment to prevent preterm birth.[307]

Women with BV have a two-fold increased risk of HIV acquisition,[68] 1.5-2-fold risk of CT[308] and NG and a nine-fold risk of TV.[309] However, to date, there is limited data on whether asymptomatic women with BV face the same risk of acquiring STIs as symptomatic women. In a non-blinded randomized controlled trial (RCT) of women with asymptomatic BV (defined by a Nugent score ≥ 7 and lack of report of a vaginal odor and/or discharge), women randomized to the treatment arm had a significantly longer time to development of incident CT, NG, TV, HSV infections and/or PID than in the observation group.[310] The incidence rate at 6 months was 1.58 per person-year (95% CI: 1.29, 1.87) for the treated women vs 2.29 per person-year (95% CI: 1.95, 2.63) in the observation group. Although additional studies would be needed in other populations, this finding suggests that in areas with a high prevalence of HIV and STIs, treatment of asymptomatic BV may reduce subsequent infections.

Moreover, some asymptomatic BV may evolve into symptomatic BV. Some studies on the natural history of asymptomatic BV found that, in the absence of therapy, 12-18% of women developed symptomatic BV within a month, and 44% at 4 months.[274] In settings like rural Africa, treating the women when they are consulting may be an option as they may not return, and this is for multiple reasons (distance, cost to travel, other priorities) when they develop symptoms.

Lastly, as more and more studies have shown that BV may be sexually transmitted (see next paragraph), an argument in favor of treating BV is to reduce its transmission to the partner(s), who may in turn, transmit it to other women.

6.2.1.3. Important considerations for a successful screening intervention

The population targeted by the intervention should be receptive to the intervention. Receptivity may start by addressing individual-level barriers. In addition, notifying and treating the partner(s) is a key component of STI control and prevention of reinfection.

Education, awareness, and social barriers to STIs testing

Some individuals may not be aware that they have an STI. They may not be aware that infections can be transmitted sexually,[311] may not recognize that their symptoms are related to an STI, or may underestimate the seriousness of their symptoms[312] and finally,

they may not know that they can be infected without presenting symptoms. A study in Iraq found that 30% of undergraduates will ignore their suspicion of having an STI after high-risk behavior if there is no symptom.[313] They may also be in denial or perceive themselves as not at risk or low risk.[314] A study involving focus group discussions in Madagascar illustrates this low-risk perception, where an adolescent reported “I’ve never heard of someone who has STIs in this town; so, I don’t think anyone has them”.[167] Some may be aware of the possibility of an infection, but may still be reluctant to get tested.

Patients have reported the fear of being judged or blamed by healthcare providers.[315] In small communities, concerns about confidentiality are a significant issue.[316]

In addition, infected individuals may fear their partner’s reaction and worry about what an STI diagnosis might imply regarding their partner’s fidelity.[167,317] Anticipation of a physical examination of the genitalia can also be dissuasive.

In some cultures, discussing sexual health can be considered inappropriate, leading to reluctance to seek care. In conservative communities, especially with strong religious or moral values, women may be worried about being judged for their sexual behavior,[317] especially in the context of pre-marital sex.[318]

Addressing these barriers

Information and education campaigns may raise awareness about the risks of infection associated with sexual intercourse. They can also take positive approaches to promoting sexual health and helping individuals recognize the signs and symptoms of STIs. They need to address the importance of seeking treatment and preventing reinfection. An emphasis may be made on the fact that infection can be present even in the absence of symptoms. Finally, it may stress that STIs do not only affect certain groups but can affect any sexually active person.

Media and curriculum-based interventions are effective ways of conveying these messages in information campaigns, some of which have been shown to be successful in LMICs.[319–321] High-quality interventions may require the involvement of relevant stakeholders in both the design and implementation phases, the inclusion of a pilot phase or formative research, the

development of a theory of change, and careful planning of both the intervention and implantation component.[322]

A significant barrier to STI testing is the fear of receiving a positive result (especially for HIV).[323] This fear may be reduced if they are properly informed about existing treatments and the possibility, for some STIs, to be cured. However, for non-curable STIs, they may be informed that treatment can alleviate symptoms.

Addressing the fear of physical examination is important. Providing self-sampling options, such as urine or vaginal swab tests for women, offers a practical and less invasive alternative that has demonstrated high levels of acceptance.[255]

Partner notification and treatment

The stigma attached to STIs makes partner notification difficult, and results in concerns about adverse reaction from the partner. Sometimes, the origin of STI is not well understood, and is thought to come from poor hygiene or female's periods, however, for those aware of the transmission by sexual route, STI-positive diagnosis raise concerns about the partners' fidelity.[324] In a survey conducted in an STI clinic in South Africa, only half of the patients intended to notify their partners.[325] Men had concerns that their partner could react violently against them while women's concerns were that their partner may leave them and refuse to see them again. However, index cases are more likely to notify their primary partners than casual or anonymous partners.[326] Reasons for not informing a casual partner can be explained by the transient nature of the relation where the index patient does not know well the partner or is unable to locate him/her.[326]

Some of the main strategies to improve effectiveness of partner notification include patient referral, expedited partner therapy, provider referral and contract referral. Patient referral is when the patient notifies their partner (simple patient referral), sometimes with verbal or written support (enhanced patient referral). Expedited partner therapy is a clinical option for assuring the treatment of sex partners without needing the partner to come to the healthcare center for a medical examination. The healthcare providers give the visiting patient a treatment to deliver to their sex partners.[327] Provider referral means that the healthcare personnel notifies the partners. In contract referral, the index patient is encouraged to notify

the partner. If the partner does not visit the healthcare center by a certain date, he/she will be contacted by the healthcare provider.

A review that compared the outcomes from trials that used one of the four approaches did not identify an optimal strategy.[328] For STI causing cervicitis, expedited partner therapy could reduce re-infection of the index case in comparison to simple patient referral. Expedited partner therapy, however, was not superior to enhanced patient referral, a finding also found elsewhere.[329] Provider and contract referral could not be evaluated due to the few number of trials.

Partner notification has been shown to be feasible in LMICs, especially when counselling was conducted.[330] In addition, the authors suggested that this intervention is particularly appropriate to LMICs because healthcare providers often deal with a large number of patients so they may not have time for provider-oriented notification. Moreover, costs associated with counselling are generally lower than those of physicians, making this option more accessible in resource-constrained settings.[330] However, this strategy requires to have counselling services, which may be not available everywhere.[331,332]

Is bacterial vaginosis sexually transmitted? Should the partner be treated?

Women who are treated for BV often experience recurrent episodes, and one explanation for recurrence is re-exposure to BV-associated organisms.[333] Current epidemiological and microbiological data provide growing evidence that males carry bacteria that are associated with BV and that these or other agents capable of inducing BV are transmitted to women via the sexual route. In a study conducted among students, BV was not detected in women who had never been sexually active and was rare in women who had only engaged in non-coital sexual activities.[334] By contrast, BV was associated with the practice of penile-vaginal sex. In addition, incident BV has been associated with exposure to a new sexual partner.[335,336] There is also compelling evidence that male circumcision and condom use reduce the risk of BV acquisition and recurrence.[337,338] Further research is needed to fully understand the dynamic of BV and its recurrence. Ultimately, if the role of male partners is confirmed, optimal treatment strategies may require antimicrobial treatment for both women and their partners.

6.2.1.4. Should GIFT be used for screening?

The UK National Committed for Screening has developed criteria for a population screening program.[275] Many of these criteria are currently being investigated such as:

- the robust evidence between the selected cytokines and STIs/BV,
- the distribution of test results in different population and the selected cut-off,
- the test is simple and safe, but evaluation of its precision and validation is ongoing,
- its cost-effectiveness.

A qualitative study has shown that the test is acceptable for women, but questions remain on the next steps if the test is positive (MacWorth-Young, personal communication). There is currently no agreed policy on further diagnosis and on the choices available to individuals tested positive. In this work, we have defined which population should be offered the intervention.

Before using this test in a screening program, we would need evidence that testing with GIFT leads to better outcome than the standard of care, i.e. the WHO syndromic approach is most cases. Randomized controlled trials showing evidence that the screening program reduces mortality and morbidity are needed. More broadly, there should be evidence that the complete program is clinically, socially and ethically acceptable. Lastly, the benefit from such screening program should outweigh harms from overtreatment, especially for NG and MG for which antimicrobial resistance is documented, false positive, false negative, uncertain findings and complications.

These elements indicate that both at the level of the test and as a program, decision on screening with GIFT is premature at this stage.

6.2.2. Prevention before screening

STI prevention can take place upstream of screening efforts. In this section we describe four prevention strategies: one for its importance in terms on the potential or expected high efficacy of the intervention but not yet available (vaccines for CT, NG, TV, or BV), one because

of the wide scale of their implementation (condoms), a relatively recent intervention, for which we observe its lack of efficacy (male circumcision), and an intervention that can be initiated by women, unlike condom use and circumcision (DoxyPreP and PEP).

Vaccination

Vaccination is an appealing preventive measure because it provides long-lasting protection.[339] However, there are currently no vaccines on the market against CT, NG, TV, or MG. In this section, we describe the latest research on vaccine candidates for these STIs.

Chlamydia trachomatis

CTH522, is the first vaccine candidate for CT, trialed in humans since the 1970s. In a Phase I double-blind, randomized, placebo-controlled trial, it induced neutralizing antibodies in all participants with no major safety concerns.[340] In May 2024, two research groups were awarded grants to develop vaccines: the University of North Carolina at Chapel Hill is working on a vaccine candidate that leverages the Chlamydial Protease Activation Factor to induce a strong immune response,[341] and the University of Texas Health San Antonio has developed an oral vaccine names intrOv, derived from an attenuated mutant of chlamydia and aims to move this vaccine into a Phase I clinical trial.[342]

Neisseria gonorrhoeae

Vaccines originally developed to combat infections by *Neisseria meningitidis*, such as MeNZB or 4CMenB (Bexsero) have shown potential to induce immunity against gonorrhoea. Several studies are currently recruiting participants into randomized placebo-controlled trials of the 4CMenB vaccine to assess efficacy against NG infection. A recent randomized trial showed that the adjusted hazard ratio of the incidence of the first episode of gonorrhoea starting from month 3 was 0.78 (95%CI: 0.60, 1.01, p=0.061) in the vaccinated group relative to the non-vaccinated group.[343] Several NG-specific vaccines targeting the outer membrane vesicle are in preclinical or clinical development. Intravaginal or intranasal administration of NGoXIM has induced responses that accelerated the clearance of NG genital tract infection in mice. The dmGC_0817560 vaccine, delivered parenterally, triggered anti-gonococcal serum, vaginal

mucosal IgG and IgA antibodies and gonococcal-specific T-cell responses in the mouse model and immunized mice demonstrated accelerated clearance of genital NG infection. Pre-clinical studies of the intramuscular GMMA vaccines have not been published, however, recruitment for a phase 1/2 study has started, aiming at evaluating the safety, reactogenicity, immunogenicity, and efficacy of this experimental vaccine in randomized, observer-blind, placebo-controlled multi-center study.[344]

Trichomonas vaginalis

To our knowledge, only two vaccines have been tested in humans. Pioneering research on a vaccine in 1963 used heat-killed TV which was shown to improve clinical symptoms of women and 40% elimination of the protozoa.[345] It has been hypothesized that this vaccine was abandoned because of the cumbersome approach which involved intradermal genital injection at 6 to 12 sites.[346] Another vaccine, Solco Trichovac, targeted common antigens between *Lactobacillus acidophilus* and TV. This lactobacillus vaccine was thought to induce antibodies that would cross-react with TV. However no evidence of antigenic similarity was demonstrated.[347] Several vaccine candidates are being investigated in *in silico* studies using proteins,[348] *in vitro* and in the murine model.[346,349–351] One study suggests that extracellular vesicles produced by TV could be exploited for vaccine delivery.[352] However, the work on the development of a TV vaccine primarily focuses now on the screening of potential immunogens.

Mycoplasma genitalium

No vaccine has been developed until now, however *in silico* studies have started to identify putative vaccine target proteins.[353,354]

Condoms

The biological efficacy of condoms has been well established for the prevention of HIV transmission, as demonstrated in sero-discordant couples.[355] However, the impact of condoms depends on how and how often they are used. Past interventions promoting condom use have shown that they achieved higher uptake among female sex workers and

their clients.[356] However, in relationships with a primary partner, condom use was low, except among those who had a known HIV-positive partner, or among women avoiding pregnancy.[356] There was limited evidence of increased condom use in casual relationships indicating that promoting behavior change in these contexts may be more challenging.[356]

In the study we conducted in Madagascar, we found a very low rate of condom use. In addition, a high proportion of women reported concurrent relationships and may not have used condoms with all their partners. These two factors are one of the very plausible explanations for the high prevalence of STIs in Madagascar. The study highlights the urgent need for targeted interventions that tackle the obstacles to condom use, as well as the risks associated with unprotected sex in concurrent partnerships.

Adult voluntary medical male circumcision (VMMC)

Voluntary medical male circumcision is a prevention measure that derived from studies on HIV, where it was shown to reduce the risk of HIV acquisition by 60% in men.[357,358] Several studies have explored the effect of VMMC on female partners' sexual and reproductive health. Recent findings support an effect of VMMC against acquisition and transmission of HSV-2, HPV, syphilis, TV and BV in heterosexual women.[153] Hypotheses explaining this protection were based on biological mechanisms. First, during intercourse, the preputial mucosa is exposed to vaginal and cervical fluids, allowing infections to penetrate through microtears in the mucosa.[359] As the foreskin's mucosal surface is more susceptible to microtears during sexual activity, circumcision would then reduce the risk for men to acquire infection and indirectly, to transmit it to their uninfected female partners. Second, the moist environment of the preputial cavity may be favorable to pathogen survival.[359] With circumcision, the risk of harboring, and thus transmitting infection would be reduced.

In our study conducted in Madagascar, participants reported that 98% of their partners were circumcised, with circumcision usually traditionally performed during childhood. Nevertheless, RTI prevalence was elevated. This example demonstrates that circumcision alone is not sufficient to decrease substantially STIs' prevalence.

Doxy-PrEP and Doxy-PEP

Doxycycline pre-exposure (doxy-PrEP) or post-exposure prophylaxis (doxy-PEP) are novel strategies to reduce the incidence rates of CT, NG (in areas with less tetracycline resistance), and syphilis in populations at high risk of STIs. This preventive intervention consists in taking a single dose of doxycycline 200 mg before or after unprotected sex to prevent infection. Originally intended for men who have sex with men, studies on the efficacy of doxy-PrEP/PEP have shown a decrease in overall STI incidence in studies conducted in this population and among transgender women.[360] To date, only one study has targeted only women. This study, conducted in Kenya among 449 women followed over 12 months, found no evidence of a lower incidence of STIs in the group with doxy-PEP compared to the group with the standard of care.[361] However, the authors reported that this lack of effectiveness could be explained by a low adherence, which was determined by the measurement of doxycycline concentrations in hair samples.

Some adverse events were reported and included mostly nausea among the female participants in the study conducted in Kenya and gastrointestinal events in the studies conducted among men who have sex with men.[360,361]

Much remains unknown about the long-term consequences of doxy-PEP at the individual and populational levels. The frequent use of antibiotics in preventive measures such as doxy-PrEP and doxy-PEP could favor the development of antibiotic-resistant bacterial strains, complicating the treatment of STIs and other infections, and potentially leading to more severe health outcomes.

Moreover, doxycycline can disturb the natural balance of gut microbiota. The long-term consequences of these disruptions remain largely unknown. Comprehensive studies are important to evaluate the effectiveness of doxy-PrEP and doxy-PEP in reducing STIs and their impact. This research will help ensure that the benefits of reducing STI rates do not come at the expense of increased health risks.

6.2.3. Next priorities in research and public health in relation to our findings

Taken together, the findings from this thesis and the current state of research highlight the next priorities for LMICs. Recognizing that economic constraints play a crucial role in these settings, we have aligned these priorities with the economic realities faced by LMICs.

Studies on the natural history of STI infection may provide valuable information on risk factors: it may help identify (1) women that require treatment, because of being at high risk of developing complications, and (2) women with lower priority, because they will clear the pathogen spontaneously. This information, if existing, could be part of the screening and integrated into the risk scores or algorithms.

Further studies on the acceptability and feasibility of a screening score or algorithm are needed, and more specifically, on the (self) use of a pH paper strip. Confirmatory studies on its effectiveness in other countries will be needed. Within the GIFT project, another team will perform the cost-effectiveness of our screening risk scores and algorithms.

The integration of validated screening scores as complementary information to syndromic management will need to be explored and assessed. To avoid specificities from countries, pH may be the most universal component of this risk score but further studies in other countries are needed to confirm this. Evaluation of this score on large and different populations of asymptomatic women would also be required.

Finally, the development of a low-cost, rapid, POCT for CT, NG, TV, and potentially for MG is crucial and will have a pivotal role in settings where the prevalence of HIV is high, among pregnant women and especially for asymptomatic women. This POCT combined with risk scores, may provide a more cost-effective alternative to the syndromic approach, provided both are inexpensive.

In the context of LMICs, where PID and other complications continue to achieve high rates, early and affordable management of STI/BV could lead to significant public health benefits.

6.3. Conclusion

Throughout this thesis, my research aimed at improving our understanding of women STIs and BV in LMICs, with particular attention to asymptomatic infections.

This work has provided epidemiological data in areas that lacked recent data.

I have determined the prevalence and proportion of asymptomatic STIs in women, emphasizing their importance in LMICs.

Additionally, I proposed an inexpensive tool designed to detect STIs and BV, including in asymptomatic women.

This work highlighted the need for innovative and cost-effective strategies to enhance the diagnosis and management of STIs in LMICs, ultimately contributing to improved health outcomes and the prevention of serious complications in these populations.

Annexes

Annex A: Evaluation of the performance of the 2021 WHO flowcharts

The performance of the 2021 WHO flowcharts were evaluated with two approaches:

- Using the full flowcharts for vaginal discharge syndrome (VDS) (Figure 1.8) and lower abdominal pain (LAP) (Figure 1.9)

- Using the entry points into these flowcharts which the symptoms.

Using the full flowcharts of VDS and LAP

As speculums were available, we used path 3 of the VDS flowchart.

Positivity was defined as a positive result either from the VDS flowchart or the LAP flowchart.

CT and NG

A positive result for CT or NG using the VDS flowchart was defined as:

1. Either abnormal vaginal discharge, irritation, or pruritus, as symptoms
AND
2. Cervicitis (cervical discharge or inflammation of the cervix the two variables available in our questionnaire regarding condition)

A positive result for CT and NG using the LAP flowchart was defined as:

1. A spontaneous complaint of lower abdominal pain
AND
2. Pain during palpation of the lower abdomen.

TV

A positive result for TV using the VDS flowchart was defined as:

1. Either abnormal vaginal discharge, irritation, or pruritus, as symptoms
AND
2. Abnormal vaginal discharge as a clinical sign (not curd-like).

TV is not in the LAP flowchart.

BV

A positive result for BV using the VDS flowchart was defined as:

1. Either abnormal vaginal discharge, irritation, or pruritus, as symptoms
AND
2. Abnormal vaginal discharge as a clinical sign (not curd-like).

A positive result for BV using the LAP flowchart was defined as:

1. A spontaneous complaint of lower abdominal pain
AND
2. Pain during palpation of the lower abdomen.

CA

A positive result for CA using the VDS flowchart was defined as:

1. Either abnormal vaginal discharge, irritation, or pruritus, as symptoms
AND
2. Abnormal vaginal discharge as a clinical sign (curd-like) or pruritus.

CA is not in the LAP flowchart.

MG

A positive result for MG using the LAP flowchart was defined as:

1. A spontaneous complaint of lower abdominal pain
AND
2. Pain during palpation of the lower abdomen.

MG does not appear in the VDS flowchart; however, it appears in the VDS treatment. Therefore, only the presence of the entry points (either abnormal vaginal discharge, irritation, or pruritus, as symptoms) was considered for positivity.

Using the entry points of the flowcharts of VDS and LAP

Positivity was defined as a positive entry point into either the VDS flowchart or the LAP flowchart.

Entry points for VDS are: abnormal vaginal discharge, irritation, or pruritus, as symptoms.

Entry point for LAP is: lower abdominal pain, as a symptom.

CT, NG, MG, and BV

A positive result for CT, NG, MG, or BV was defined as:

1. Either abnormal vaginal discharge, irritation, or pruritus, as symptoms

OR

2. A spontaneous complaint of lower abdominal pain

TV and CA

A positive result for TV or CA was defined as: either abnormal vaginal discharge, irritation, or pruritus, as symptoms.

Performance of the flowcharts

Performance of the flowcharts were evaluated using the sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV).

For the definitions of these measures, we used the following terms:

True positive (TP) are women who are positive according to the biological analysis (NAATs for STIs or CA and Nugent score for BV) and positive according to the WHO flowchart (the full flowchart for the first analysis and the entry points for the second analysis).

True negative (TN) are women who are negative according to the biological analysis (NAATs or Nugent score) and negative according to the WHO flowchart.

False positive (FP) are women who are positive according to the WHO flowchart but negative according to the biological analysis (NAATs or Nugent score).

False negative (FN) are women who are negative according to the WHO flowchart but positive according to the biological analysis (NAATs or Nugent score).

Thus, we defined:

Se = TP / (TP+FN)

Sp = TN / (FP+TN)

PPV = TP / (TP+FP)

NPV = TN (TN+FN)

Annex B: Annexes to the systematic review and meta-analysis

Supplementary Text 1: Search terms for citation selection

PubMed

((women[MH] OR female[MH])
OR
(“woman”[TW] OR “women*”[TW] OR “female*”[TW] OR (“adolescent*”[TW] AND “female*”[TW]) OR “girl*”[TW]))

AND

((“asymptomatic infections”[MH] OR “asymptomatic diseases”[MH])
OR
(asymptomatic*[TW] OR “not symptomatic” [TW] OR “absence of symptom*” [TW] OR “without symptom*” [TW] OR “no symptom*” [TW] OR “no sign*”[TW] OR “absence of sign*”[TW] OR “silent infection*”[TW] OR “presymptomatic*” [TW] OR “pre-symptomatic*”[TW]))

AND

((“chlamydia trachomatis”[MH] OR “chlamydia infections”[MH])
OR
(“chlamydia”[TW] OR “chlamydiae”[TW] OR “chlamydia trachomatis” [TW] OR “C. trachomatis” [TW] or (“Chlamydia”[TW] AND “trachomatis” [TW]))

OR

("Neisseria gonorrhoeae"[MH] OR "Gonorrhea"[MH])
OR
("gonorrhea"[TW] OR "gonorrhoea"[TW] OR "gonorrhoeae"[TW] OR “Neisseria gonorrhoeae” [TW] OR “N. gonorrhoeae” [TW] OR (“Neisseria” [TW] AND “gonorrhoeae”[TW]) OR "gonococcal"[TW])

OR

(“Trichomonas vaginalis”[MH] OR “Trichomonas vaginitis”[MH] OR “Trichomonas infections”[MH])
OR
(trichomoniases[TW] OR trichomoniasis[TW] OR “Trichomona*”[TW] OR “Trichomonas vaginalis” [TW] OR “T. vaginalis”[TW] OR (“Trichomonas”[TW] AND “vaginalis”[TW]) OR “Trichomonas vaginali” [TW] OR “T. vaginali”[TW] OR (“Trichomonas”[TW] AND “vaginali”[TW]) OR “trichomonas vaginitides”[TW] OR (“Trichomonas” AND “vaginitides”[TW]))
)

AND

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)

AND (2000:2022[pdat])

Scopus

TITLE-ABS-KEY ("woman" OR "women*" OR "female*" OR ("adolescent*" AND "female*") OR "girl*")

AND

TITLE-ABS-KEY ("asymptomatic*" OR "not symptomatic" OR "absence of symptom*" OR “without symptom*” OR "no symptom*" OR "no sign*" OR "absence of sign*" OR "presymptomatic*" OR "pre-symptomatic*")

AND

TITLE-ABS-KEY ("Chlamydia trachomatis" OR "Chlamydia infection*" OR "chlamydia" OR "chlamydiae" OR "C. trachomatis" OR ("Chlamydia" AND "trachomatis")

OR

"Neisseria gonorrhoeae" OR "gonorrhea " OR "gonorrhoea" OR "gonorrhoeae" OR "N. gonorrhoeae" OR ("Neisseria" AND "gonorrhoeae") OR "gonococcal"

OR

"Trichomonas vaginalis" OR "Trichomonas vaginitis" OR "T. vagilanis" OR ("Trichomonas" AND "vaginalis") OR "Trichomonas vaginali" OR "T. vaginali" OR ("Trichomonas" AND "Vaginali") OR ("Trichomonas vaginitides") OR ("Trichomonas" AND "vaginitides"))

AND

TITLE-ABS-KEY ("Afghanistan" OR "Albania" OR "Algeria" OR "American Samoa" OR "Angola" OR "Antigua" OR "Argentina" OR "Armenia" OR "Azerbaijan" OR "Bangladesh" OR "Barbuda" OR "Belarus" OR "Belize" OR "Benin" OR "Bhutan" OR "Bolivia" OR "Bosnia" OR "Herzegovina" OR "Hercegovina" OR "Bosnia-Herzegovina" OR "Botswana" OR "Brazil" OR "Bulgaria" OR "Burkina Faso" OR "Burundi" OR "Cabo Verde" OR "Cape Verde" OR "Cambodia" OR "Cameroon" OR "Central African Republic" OR "Chad" OR "Chile" OR "China" OR "Colombia" OR "Comoros" OR "Costa Rica" OR "Cote d’Ivoire" OR "Ivory Coast" OR "Croatia" OR "Cuba" OR "Czech Republic" OR "Czechia" OR "Democratic republic of the Congo" OR "Congo" OR "Zaire" OR "DRC" OR "Democratic People’s Republic Korea" OR "North Korea" OR "Djibouti" OR "Dominica" OR "Dominican Republic" OR "Ecuador" OR "Egypt" OR "El Salvador" OR "Equatorial Guinea" OR "Salvador" OR "Eritrea" OR "Estonia" OR "Eswatini" OR "Swaziland" OR "Ethiopia" OR "Fiji" OR "Gabon" OR "Gambia" OR "Georgia" OR "Ghana" OR "Gibraltar" OR "Grenada" OR "Guatemala" OR "Guinea" OR "Guinea-Bissau" OR "Guyana" OR "Haiti" OR "Honduras" OR "Hungary" OR "India" OR "Indonesia" OR "Iran" OR "Iraq" OR "Jamaica" OR "Jordan" OR "Kazakhstan" OR "Kenya" OR "Kiribati" OR "Kosovo" OR "Kyrgyzstan" OR "Kirghizia" OR "Kyrgyz Republic" OR "Kirgizstan" OR "Kirghizstan" OR "Kyrghizstan" OR "Lao PDR" OR "Laos" OR "Lao People*" OR "Latvia" OR "Lebanon" OR "Lesotho" OR "Liberia" OR "Libya" OR "Lithuania" OR "Madagascar" OR "Malawi" OR "Malaysia" OR "Maldives" OR "Mali" OR "Malta" OR "Marshall Islands" OR "Mauritania" OR "Mauritius" OR "Mexico" OR "Micronesia" OR "Moldova" OR "Mongolia" OR "Montenegro" OR "Morocco" OR "Mozambique" OR "Myanmar" OR "Namibia" OR "Nauru" OR "Nepal" OR "Nicaragua" OR "Niger" OR "Nigeria" OR "Northern Mariana Islands" OR "North Macedonia" OR "Macedonia" OR "Oman" OR "Pakistan" OR "Panama" OR

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AND

PUBYEAR > 1999 AND PUBYEAR < 2023

Web of Science

TS=("woman" OR "women*" OR "female*" OR ("adolescent*" AND "female*") OR "girl*")

AND

TS=("asymptomatic*" OR "not symptomatic" OR "absence of symptom*" OR "without symptom*" OR "no symptom*" OR "no sign*" OR "absence of sign*" OR "silent infection*" OR "presymtomatic*" OR "pre-symptomatic*")

AND

(TS=(“chlamydia trachomatis" or “chlamydia infection*" or "chlamydia" or "chlamydiae" or "c. trachomatis" or ("chlamydia" AND "trachomatis"))

OR

TS=("Neisseria gonorrhoeae" OR "gonorrhea" OR "gonorrhoea" OR "gonorrhoeae" OR "N. gonorrhoeae" OR ("Neisseria" AND "gonorrhoeae") OR "gonococcal")

OR

TS=("Trichomonas vaginalis" OR "Trichomonas vaginitis" OR "T. vagilanis" OR ("Trichomonas" AND "vaginalis") OR "Trichomonas vaginali" OR "T. vaginali" OR ("Trichomonas" AND "Vaginali") OR "Trichomonas vaginitides" OR ("Trichomonas" AND "vaginitides")))

AND

ALL=(("Afghanistan" OR "Albania" OR "Algeria" OR "American Samoa" OR "Angola" OR "Antigua" OR "Argentina" OR "Armenia" OR "Azerbaijan" OR "Bangladesh" OR "Barbuda" OR "Belarus" OR "Belize" OR "Benin" OR "Bhutan" OR "Bolivia" OR "Bosnia" OR "Herzegovina" OR "Hercegovina" OR "Bosnia-Herzegovina" OR "Botswana" OR "Brazil" OR "Bulgaria" OR "Burkina Faso" OR "Burundi" OR "Cabo Verde" OR "Cape Verde" OR "Cambodia" OR "Cameroon" OR "Central African Republic" OR "Chad" OR "Chile" OR "China" OR "Colombia" OR "Comoros" OR "Costa Rica" OR "Cote d’Ivoire" OR "Ivory Coast" OR "Croatia" OR "Cuba" OR "Czech Republic" OR "Czechia" OR "Democratic republic of the Congo" OR "Congo" OR "Zaire" OR "DRC" OR

"Democratic People's Republic Korea" OR "North Korea" OR "Djibouti" OR "Dominica" OR "Dominican Republic" OR "Ecuador" OR "Egypt" OR "El Salvador" OR "Equatorial Guinea" OR "Salvador" OR "Eritrea" OR "Estonia" OR "Eswatini" OR "Swaziland" OR "Ethiopia" OR "Fiji" OR "Gabon" OR "Gambia" OR "Georgia" OR "Ghana" OR "Gibraltar" OR "Grenada" OR "Guatemala" OR "Guinea" OR "Guinea-Bissau" OR "Guyana" OR "Haiti" OR "Honduras" OR "Hungary" OR "India" OR "Indonesia" OR "Iran" OR "Iraq" OR "Jamaica" OR "Jordan" OR "Kazakhstan" OR "Kenya" OR "Kiribati" OR "Kosovo" OR "Kyrgyzstan" OR "Kirghizia" OR "Kyrgyz Republic" OR "Kirgizstan" OR "Kirghizstan" OR "Kyrghizstan" OR "Lao PDR" OR "Laos" OR "Lao People*" OR "Latvia" OR "Lebanon" OR "Lesotho" OR "Liberia" OR "Libya" OR "Lithuania" OR "Madagascar" OR "Malawi" OR "Malaysia" OR "Maldives" OR "Mali" OR "Malta" OR "Marshall Islands" OR "Mauritania" OR "Mauritius" OR "Mexico" OR "Micronesia" OR "Moldova" OR "Mongolia" OR "Montenegro" OR "Morocco" OR "Mozambique" OR "Myanmar" OR "Namibia" OR "Nauru" OR "Nepal" OR "Nicaragua" OR "Niger" OR "Nigeria" OR "Northern Mariana Islands" OR "North Macedonia" OR "Macedonia" OR "Oman" OR "Pakistan" OR "Panama" OR "Papua New Guinea" OR "Paraguay" OR "Paulu" OR "Peru" OR "Philippines" OR "Poland" OR "Porto Rico" OR "Republic of the Congo" OR "Romania" OR "Russian Federation" OR "Russia" OR "Rwanda" OR "Samoa" OR "Sao Tome" OR "Principe" OR "Saudi Arabia" OR "Senegal" OR "Serbia" OR "Sierra Leone" OR "Slovakia" OR "Solomon Islands" OR "Somalia" OR "South Africa" OR "South Sudan" OR "Sri Lanka" OR "Saint Kitts" OR "St Kitts" OR "Nevis" OR "St Lucia" OR "Saint Lucia" OR "St Vincent" OR "the Grenadines" OR "Saint Vincent" OR "Sudan" OR "Suriname" OR "Syria" OR "Syrian Arab Republic" OR "Tajikistan" OR "Tadjikistan" OR "Tadzhikistan" OR "Tanzania" OR "Thailand" OR "Timor-Leste" OR "Timor Leste" OR "Tobago" OR "Togo" OR "Tonga" OR "Trinidad" OR "Tunisia" OR "Turkey" OR "Turkmenistan" OR "Tuvalu" OR "Uganda" OR "Ukraine" OR "Uruguay" OR "Uzbekistan" OR "Vanuatu" OR "Venezuela" OR "Vietnam" OR "West Bank" OR "Gaza" OR "Palestine" OR "Yemen" OR "Zambia" OR "Zimbabwe")

The year range was manually selected in Web of Science.

Supplementary Table 1: PRISMA 2020 checklist.

Section and Topic	Item #	Checklist item
TITLE		
Title	1	Identify the report as a systematic review. "Asymptomatic infections with Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis among women in low- and middle-income countries: A systematic review and meta-analysis"
ABSTRACT		
Abstract	2	See the PRISMA 2020 for Abstracts checklist.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of existing knowledge. "To date, no study has provided pooled estimates of the burden of asymptomatic infections of the three most common curable STIs."
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses. "In this perspective, we conducted a systematic review and a meta-analysis to estimate: (i) the proportion of asymptomatic women among those infected by CT, NG, and TV and, (ii) the prevalence of asymptomatic CT, NG, and TV infections in the female population in LMICs."
METHODS		
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses. "We selected studies published between 1 January 2000 and 31 December 2022, in English and French. We included studies which tested for STIs using genital or urine specimens. We kept studies that used NAATs for the diagnosis of CT, NG, and TV. For TV, we also included articles that used InPouch® or performed wet mount microscopy immediately after collection, as these techniques showed fair sensitivity and specificity and remain common detection methods in LMICs. We selected studies where the investigator actively asked about at least one symptom, or where participants self-reported symptoms. We excluded articles in which authors assumed the absence of symptoms in the entire study population without collecting such information. Finally, we excluded articles when "asymptomatic" was defined as the absence of clinical signs as reported by the healthcare provider rather than by the women as we intended a participant-centred approach."
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted. "We searched MEDLINE, Web of Science, and Scopus databases. Search terms combined pathogen and disease terms, terms related to the absence of symptoms and countries ever classified as LMICs by the World Bank between 2000 and 2022 (full search terms in supplementary Text 1). We also screened references for eligible articles. When the estimates were collected but not presented, we contacted the authors up to three times"
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.

Section and Topic	Item #	Checklist item
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process. "BTH, TC, RR, and CF conducted initial screening of titles and abstracts in pairs and inclusion conflicts were resolved through consensus." + Supplementary Text 1
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process. "CF performed data extraction, and BTH, TC, and RR ensured exactness and validity."
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect. "Extracted data included the number of asymptomatic positive women, total women tested, and women positive for a specific STI. "
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information. "Other variables we extracted were: first author, publication year, study year, country, recruitment site, number and type of reported symptoms, definition of asymptomatic women, rural/urban setting, specific populations (female sex works (FSWs), adolescents, women with HIV, adolescents, infertile women), age range, specimen type, other STI pathogens tested for, and detection techniques."
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process. "The risk of bias was assessed using the appraisal tool for cross-sectional studies (AXIS) using >70% of validated items as a cut-off to qualify studies with a low risk of bias."
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results. "Proportion and prevalence of asymptomatic CT, NG, and TV infections were estimated first for all studies and excluding studies on FSWs, women with HIV, and women attending an STI clinic as they are not representative of the general population and have a higher risk of having an STI. We referred to these groups as "populations with an increased risk of STIs". Separate estimations were conducted for FSWs, women with HIV as well as other key populations such as pregnant women, adolescents, and infertile women."
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)). "We selected studies where the investigator actively asked about at least one symptom, or where participants self-reported symptoms."

Section and Topic	Item #	Checklist item
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions. "Random-effect models were applied for each of the above analyses using the DerSimonian and Laird variance estimator. As some figures were near 0 or 1, the Freeman-Tukey double arcsine transformation was applied."
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses. "Analyses and forest plots were conducted using *meta* and *metafor* packages in R software (version 4.1.1). Proportions and prevalence maps were generated using qGIS (version 3.22)."
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. "Proportion and prevalence of asymptomatic CT, NG, and TV infections were estimated first for all studies and excluding studies on FSWs, women with HIV, and women attending an STI clinic as they are not representative of the general population and have a higher risk of having an STI. We referred to these groups as "populations with an increased risk of STIs". Separate estimations were conducted for FSWs, women with HIV as well as other key populations such as pregnant women, adolescents, and infertile women. […] Random-effect models were applied for each of the above analyses using the DerSimonian and Laird variance estimator. As some figures were near 0 or 1, the Freeman-Tukey double arcsine transformation was applied. Statistical heterogeneity was evaluated using the χ^2 test with Cochran's Q and I^2. Analyses and forest plots were conducted using *meta* and *metafor* packages in R software (version 4.1.1)."
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression). "Subgroup analyses were performed by region, country income level, rural/urban setting, study period, and the number of symptoms assessed in the study. The role of these study variables in the heterogeneity of the estimates were explored in meta-regressions."
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results. "We conducted a sensitivity analysis to assess the impact of excluding studies with a high risk of bias on our overall estimates."
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases). "Publication bias was evaluated by Funnel plots and the Egger test."
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome. NA
RESULTS		
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.

Section and Topic	Item #	Checklist item
		"We identified 1113 articles, among which 547 were duplicates. We eliminated 468 articles based on title and abstract and screened 173 full texts and their citations. We found 4 additional relevant citations among selected articles. In total, 177 articles were eligible. Of those, 42 had available and extractable data and 45 reported having data according to our criteria but did not present the numbers. We were able to contact 40 of those 45 authors and retrieved data from six articles. In total, we used data from 48 studies corresponding to 99 data points for both the prevalence and the proportion: 41 for CT, 27 for NG, and 31 for TV (Figure 4.1, supplementary Table 2). "
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded. Figure 4.1
Study characteristics	17	Cite each included study and present its characteristics. Supplementary Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study. Supplementary Table 1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. Supplementary Table 1
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. "Twenty-one countries were represented in our study: eight in Africa, seven in Asia, five in Latin America, and one in Oceania. Most studies were cross-sectional (44/48, 92%) and few were cohort (2/48, 4%) or randomized controlled trials (2/48, 4%). Almost three-quarters of the studies took place in urban settings (34/48, 71%), 21% (10/48) in rural settings, and 8% (4/48) in mixed settings. Hospitals and antenatal care were the most frequent recruitment sites (14/48, 29% and 11/48, 23%, respectively), followed by primary or secondary health care centres (9/48, 19%), locations where people with a high risk of STI can be found such as FSW venues, HIV centres or STI clinics (8/48, 17%). Six studies performed random sampling within the community (6/48, 13%). Some studies focused on specific populations: 15/48 (31%) were on pregnant women, 5/48 (10%) on FSWs, 4/48 (8%) on adolescents, 4/48 (8%) on women with HIV, and 4/48 (8%) on infertile women exclusively. Studies had enrolled between 48 and 4812 participants, and their age ranged from 10 to 90 years old although this information was unavailable for 14 studies."
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. "The proportion of asymptomatic CT, NG, and TV infections (excluding populations with an increased risk of STIs) was significantly different across regions (p=0.048, p=0.019, and p=0.039, respectively for each pathogen). " + Table 4.1, Supplementary Tables 2 – 4
	20c	Present results of all investigations of possible causes of heterogeneity among study results. "Africa presented the highest proportion of asymptomatic infections (CT: 68.6% [56.4–79.8], 14 data points, 12 368 participants; NG: 67.2% [51.0; 81.9], 13 data points, 7 681 participants; TV: 64.9% [52.2; 76.7], 12 data points, 6 152 participants). Latin America had the lowest proportion of asymptomatic women infected with CT (30.9% [11.2–54.8], five data points, 2 850 participants) and with NG (14.0% [0.0–67.5], two data points, 1 546 participants). The lowest proportion of asymptomatic TV

Section and Topic	Item #	Checklist item
		infection was found in Asia (17.3% [0.0–49.5], four data points, 2 606 participants). When stratifying by country income level, the proportions of asymptomatic cases were the highest in middle-income countries (CT: 63.2% [50.2–75.1], 11 data points, 14 887 participants; NG: 60.9% [41.1–79.2], 14 data points, 10 793 participants; TV: 61.7% [45.4–77.1], 17 data points, 6 688 participants), and the lowest in low-income countries (CT: 55.5% [37.3–73.1], 11 data points, 11 381 participants; NG: 37.7% [18.4–58.8], seven data points, 6 157 participants; TV: 45.4% [29.7–61.6], six data points, 4 136 participants). The proportion of asymptomatic infected women was also the highest in rural settings (CT: 71.6% [55.6–85.3], ten data points, 9 150 participants; NG: 70.4% [46.3–90.3], 9 data points, 4 464 participants; TV: 75.0% [49.8–94.0], six data points, 2 619 participants), and the lowest in urban settings (CT: 55.6% [43.8–67.1], 20 data points, 15 276 participants; NG: 44.4% [26.5–62.9], 11 data points, 12 124 participants; TV: 58.9% [46.5–70.8], 15 data points, 7 544 participants). […]"
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. Supplementary Tables 5 – 7
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed. Supplementary Figures 1 – 3
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. NA
DISCUSSION		
Discussion	23a	Provide a general interpretation of the results in the context of other evidence. "Several hypotheses can be proposed to explain these high estimates. First, women may have difficulties in distinguishing physiological from abnormal vaginal discharge. For instance, in Tanzania, a study found that more than two third of women with abnormal discharge did not report any symptoms. In rural areas of Madagascar, anthropologists reported that genital symptoms are thought to be normal and women may not report them, even after probing. Alternatively, social norms may prevent women from declaring their symptoms. They may correctly identify genital symptoms but may be unwilling to communicate them to healthcare professionals due to the stigma attached to such symptoms or fear of being judged. The notably high proportions of asymptomatic CT, NG, and TV infections in Africa are concerning. STIs are associated with an increased risk of transmitting and acquiring other STIs or HIV and Africa bears amongst the highest STI and HIV prevalence in the world. The wide use of syndromic approach in Africa means that asymptomatic women are often left undiagnosed and untreated, posing a risk of transmission to their partners and contributing to adverse health outcomes. We observed a high proportion of asymptomatic STIs among women with HIV. While the limited number of studies limits definitive conclusions, similar findings have been reported among men with HIV who have sex with men. In this study, detectable plasma HIV RNA, social factors, and sexual practices were associated with asymptomatic STIs. Further research could also explore the potential immunological mechanisms underlying this phenomenon. Our study revealed an elevated prevalence of asymptomatic STIs among FSWs, suggesting an important of circulation of STIs in this population likely due to their important number of sexual partners. However, we observed a relatively lower proportion of asymptomatic women, which could be explained by FSWs' increased awareness and recognition of STI symptoms. We found a low prevalence of asymptomatic STIs among adolescents, possibly due to the lower STI prevalence in this group and the inclusion of sexually inactive participants in some studies. However, despite the lower prevalence, adolescents remain vulnerable to STI acquisition due to their sexual behavior and physiological susceptibility. Implementing comprehensive sexual education programs during early adolescence could effectively maintain a low STI occurrence in this key

Section and Topic	Item #	Checklist item
		population.[24]"
	23b	Discuss any limitations of the evidence included in the review. "Firstly, the majority of the studies were conducted in healthcare facilities which may introduce bias, as women could attend these facilities because of genitourinary symptoms, potentially underrepresenting asymptomatic cases compared to the general population. Consequently, while our estimates are already substantial, the true proportion and prevalence could be even higher. Furthermore, our search yielded only six articles conducted in community settings, indicating a gap in community-based research. Such studies are pivotal for accurately assessing the proportion and prevalence of asymptomatic STIs in the general population. Another limitation pertains to the varying number of symptoms used to determine symptomatic status, ranging from one to fourteen. Studies examining more symptoms were more likely to identify symptomatic cases and fewer asymptomatic cases. However, our comparison of estimates from studies assessing one to four symptoms versus those assessing five or more symptoms did not show any significant differences."
	23c	Discuss any limitations of the review processes used. "Another limitation of our review is that 39 studies appeared to have collected the data we needed but we were unable to obtain these figures. However, we did not find any temporal or spatial pattern among them. In addition, due to our language restrictions, ten studies conducted in Latin America were excluded although they seemed to be relevant."
	23d	Discuss implications of the results for practice, policy, and future research. "We showed that the prevalence and proportion of asymptomatic STIs among women in LMICs represent a significant public health concern. An alternative to the syndromic management could be the development of an easy-to-use and affordable point-of-care test (POCT) that would rapidly and accurately detect STIs . A POCT to screen women with suspected STIs among asymptomatic women would be more cost-effective than POCT testing for each pathogen on all of them. Currently, a POCT detecting inflammation caused by any STI is being tested. In addition to providing confidential healthcare services and destigmatizing STIs as defined in the global health strategies on STIs by the WHO for 2022–2030, such a device could be used by health care providers to screen asymptomatic women in order to prevent adverse consequences for reproductive and overall health."
OTHER INFORMATION		
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered. "The study protocol is available on PROSPERO (CRD42022286673)."
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared. "The study protocol is available on PROSPERO (CRD42022286673)."
	24c	Describe and explain any amendments to information provided at registration or in the protocol. No amendments
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.

Section and Topic	Item #	Checklist item
		"This work was supported by the European Union (European and Developing Countries Clinical Trials 2 - EDCTP2 n° RIA2020I-3297, https://www.edctp.org/)."
Competing interests	26	Declare any competing interests of review authors. "We declare no competing interests."
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. "the data dictionary are publicly available with no restriction at https://doi.org/10.57745/FNJZRG"

Supplementary Table 2: Sensitivity analysis – Proportion and prevalence estimates of asymptomatic CT infections excluding studies with a high risk of bias.

	Number of asymptomatic	Number of positive	Study population	Number of data points	Number of countries	Pooled proportion estimates in % [95% CI]	Heterogeneity I^2 for proportion	P-value for subgroup analysis	Pooled prevalence (per 100 women) estimates [95% CI]	Heterogeneity I^2 for prevalence	P-value for subgroup analysis
Overall											
Excluding populations with an increased risk of STI*	510	852	16 664	23	12	64.2 [50.1; 77.3]	93.2%	..	4.23 [2.85; 5.85]	95.1%	..
Including populations with an increased risk of STI	995	1588	18 908	30	14	60.3 [49.8; 70.4]	93.4%	..	6.29 [3.99; 9.05]	97.9%	..
Continent*											
Africa	340	433	9 699	11	5	75.9 [63.0; 87.1]	82.9%	0.001	5.36 [2.76; 8.70]	96.8%	0.044
Asia	147	198	3 858	6	3	77.0 [53.9; 94.2]	91.1%		3.93 [2.78; 5.27]	71.5%	
Latin America	32	157	2 620	4	3	22.3 [7.2; 42.1]	85.3%		1.42 [0.35; 3.18]	88.4%	
Oceania	27	64	487	2	1	47.4 [2.7; 95.0]	95.0%		7.22 [0.00; 24.73]	95.7%	
Country income level*											
Low income	162	291	10 440	7	5	50.1 [25.4; 74.8]	92.6%	0.194	1.29 [0.53; 2.34]	92.9%	0.001
Middle income	348	561	6 224	16	7	69.7 [52.2; 84.8]	93.9%		6.24 [4.25; 8.57]	91.3%	
Setting*											
Rural	300	431	8 747	9	5	76.4 [62.8; 87.9]	86.7%	0.082	5.74 [3.20; 8.92]	95.7%	0.243
Urban	150	304	6 075	11	9	54.7 [32.9; 75.6]	92.0%		3.71 [1.69; 6.43]	95.4%	
Study year*											
1998 - 2011	263	420	10 505	11	8	65.4 [43.5; 84.7]	93.6%	0.925	3.49 [1.64; 5.93]	96.4%	0.343
2012 - 2022	247	432	6 159	12	7	63.3 [43.6; 81.0]	93.8%		4.92 [3.19; 7.00]	91.0%	
Number of symptoms assessed*											
Between 1 and 4	161	355	6 917	8	8	48.2 [24.0; 72.8]	95.3%	0.104	3.92 [1.43; 7.49]	96.3%	0.613
Five and more	247	361	8 137	13	7	73.9 [56.8; 88.3]	89.4%		4.87 [2.69; 7.61]	95.1%	
Specific populations**											
Pregnant women	199	379	3 713	9	5	56.7 [36.4; 76.2]	92.9%	..	7.59 [3.65; 12.7]	95.7%	..
Female sex workers	371	624	1 890	5	4	45.5 [24.8; 67.0]	96.0%	..	13.63 [2.76; 30.72]	98.8%	..
Adolescents	98	126	5 193	2	2	78.6 [70.6; 85.7]	0%	..	1.85 [1.50; 2.24]	0%	..
Women with HIV	74	112	354	2	1	61.4 [38.7; 81.8]	72.8%	..	17.1 [4.27; 35.8]	93.0%	..
Infertile	22	30	391	2	2	74.3 [45.4; 95.6]	60.6%	..	6.01 [1.46; 13.14]	81.8%	..

* Excludes populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

** "Pregnant women" and "Women with HIV" are not mutually exclusive

Supplementary Table 3: Sensitivity analysis – Proportion and prevalence estimates of asymptomatic NG infections excluding studies with a high risk of bias.

	Number of asymptomatic	Number of positive	Study population	Number of data points	Number of countries	Pooled proportion estimates in % [95% CI]	Heterogeneity I^2 for proportion	P-value for subgroup analysis	Pooled prevalence (per 100 women) estimates [95% CI]	Heterogeneity I^2 for prevalence	P-value for subgroup analysis
Overall											
Excluding populations with an increased risk of STI*	271	395	8 195	16	10	58.4 [36.7; 78.7]	91.6%	..	3.46 [1.02; 7,14]	98.1%	..
Including populations with an increased risk of STI	450	703	9 600	21	12	55.3 [38.0; 72.1]	93.1%	..	4.31 [1.61; 8.14]	98.3%	..
Continent*											
Africa	253	332	5 013	10	5	72.3 [50.9; 90.1]	89.1%	0.019	5.98 [1.35; 13.38]	98.6%	0.016
Asia	4	7	1 149	2	2	57.7 [18.6; 91.9]	..		0.23 [0.00; 0.66]	0%	
Latin America	2	10	1 546	2	2	14.0 [0.0; 67.5]	62.2%		0.12 [0.00; 1.16]	80.6%	
Oceania	12	46	487	2	1	32.7 [0.0; 88.3]	92.8%		3.12 [0.00; 12.25]	91.7%	
Country income level*											
Low income	32	75	5 754	6	5	38.6 [14.3; 65.7]	73.6%	0.099	0.42 [0.10; 0.92]	79.6%	0.013
Middle income	239	320	2 441	10	5	70.6 [43.7; 92.4]	93.0%		6.80 [1.00; 16.7]	98.3%	
Setting*											
Rural	236	305	4 061	8	4	75.6 [51.7; 94.1]	90.7%	0.092	7.55 [1.29; 18.06]	99.0%	0.032
Urban	32	58	3 772	7	7	45.8 [21.2; 71.3]	63.4%		0.80 [0.08; 2.06]	87.4%	
Study year*											
1998 - 2011	224	307	5 819	10	8	59.2 [30.8; 85.1]	93.4%	0.838	4.35 [0.71; 10.60]	98.7%	0.433
2012 - 2022	47	88	2 376	6	4	55.3 [29.6; 80.3]	76.4%		2.16 [0.23; 5.64]	93.5%	
Number of symptoms assessed*											
Between 1 and 4	191	283	4 090	7	7	37.6 [6.2; 75.6]	96.7%	0.067	4.56 [0.09; 14.43]	99.1%	0.686
Five and more	78	107	3 744	8	5	78.3 [62.2; 91.5]	49.3%		2.94 [1.00; 5.74]	92.8%	
Specific populations**											
Pregnant women	39	106	2 245	7	3	48.9 [22.9; 75.3]	81.3%	..	2.65 [0.89; 5.18]	84.7%	..
Female sex workers	163	282	1 051	3	3	38.1 [3.1; 82.9]	97.9%	..	9.49 [0.04; 30.73]	98.6%	..
Adolescents	2	30	507	1	1	66.7 [12.5; 98.2]	..	..	0.40 [0.07; 1.58]	..	..
Women with HIV	16	26	354	2	1	62.9 [38.7; 84.4]	27.5%	..	4.56 [2.13; 7.79]	31.5%	..
Infertile	0	0	137	1	1	..	..	..	0.00 [0.07; 3.40]	..	..

* Excludes populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

** "Pregnant women" and "Women with HIV" are not mutually exclusive

Supplementary Table 4: Sensitivity analysis – Proportion and prevalence estimates of asymptomatic TV infections excluding studies with a high risk of bias.

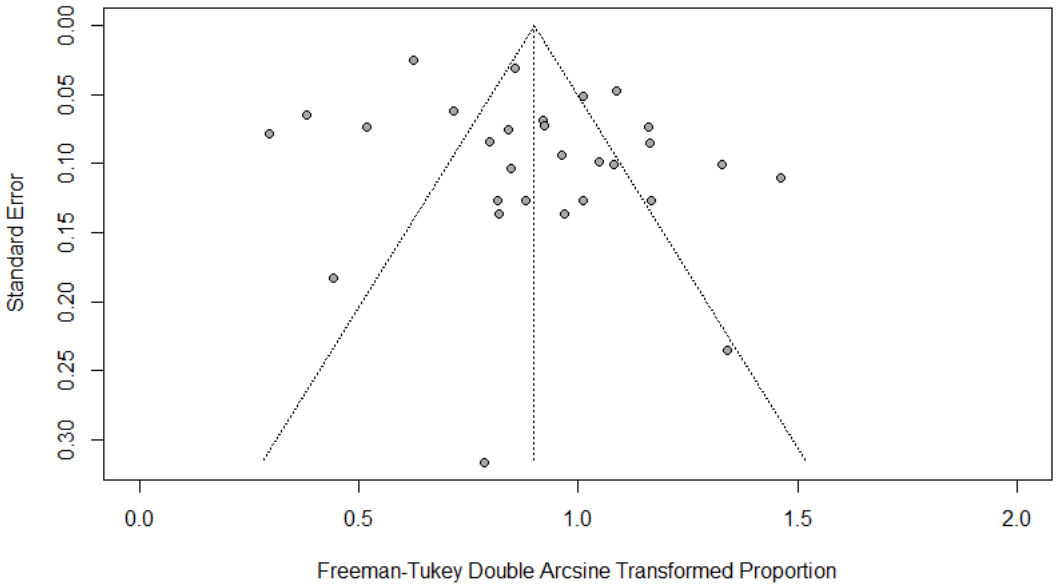
	Number of asymptomatic	Number of positive	Study population	Number of data points	Number of countries	Pooled prevalence (per 100 women) estimates [95% CI]	Heterogeneity I^2 for prevalence	P-value for subgroup analysis	Pooled proportion estimates in % [95% CI]	Heterogeneity I^2 for proportion	P-value for subgroup analysis
Overall											
Excluding populations with an increased risk of STI*	396	653	5 419	14	10	9.02 [4.94; 14.13]	96.9%	..	66.9 [49.4; 82.5]	93.6%	..
Including populations with an increased risk of STI	487	871	6 546	19	11	8.49 [5.23; 12.43]	96.1%	..	61.5 [46.7; 75.5]	93.4%	..
Continent*											
Africa	279	428	3 212	8	5	10.74 [5.71; 17.04]	95.5%	<0.001	72.0 [52.5; 88.2]	93.1%	0.627
Asia	4	11	1 149	2	2	0.23 [0.00; 0.67]	0%		47.0 [0.0; 100]	43.3%	
Latin America	66	94	571	2	2	12.05 [1.54; 31.39]	96.6%		73.3 [20.3; 100]	96.6%	
Oceania	47	120	487	2	1	12.77 [0.06; 40.12]	97.5%		43.3 [4.8; 87.5]	96.4%	
Country income level*											
Low income	156	277	3 592	5	5	3.79 [0.82; 8.70]	97.2%	0.018	50.1 [34.4; 65.9]	79.2%	0.086
Middle income	240	376	1 827	9	5	13.04 [6.89; 20.72]	94.7%		77.9 [50.9; 97.2]	95.4%	
Setting*											
Rural	123	167	2 044	5	3	9.76 [1.29; 24.33]	98.5%	0.948	76.5 [40.8; 99.2]	94.1%	0.549
Urban	258	413	3 013	8	8	9.40 [5.51; 14.17]	92.6%		66.4 [49.5; 81.6]	87.3%	
Study year*											
1998 - 2011	250	448	4 082	8	7	7.70 [3.41; 13.45]	96.9%	0.570	61.1 [36.6; 83.2]	95.8%	0.418
2012 - 2022	146	205	1 337	6	5	10.89 [2.75; 23.27]	97.1%		76.1 [57.1; 91.7]	78.5%	
Number of symptoms assessed*											
Between 1 and 4	114	225	1 342	5	5	7.09 [2.45; 13.79]	93.8%	0.416	59.4 [19.9; 93.9]	95.7%	0.442
Five and more	261	382	3 716	8	5	10.97 [4.40; 19.91]	98.0%		73.6 [54.7; 89.1]	91.3%	
Key population**											
Pregnant women	129	253	1 268	7	3	10.89 [5.31; 18.06]	92.0%	..	63.6 [39.1; 85.2]	92.2%	..
Female sex workers	37	138	546	2	2	7.02 [4.16; 10.52]	41.3%	..	26.7 [19.6; 34.5]	0%	..
Adolescents	13	28	706	2	2	2.05 [0.33; 4.98]	73.7%	..	46.4 [27.6; 65.7]	0%	..
Women with HIV	47	64	382	2	1	9.02 [0.18; 27.51]	95.4%	..	75.1 [62.5; 86.1]	0%	..
Infertile	1	1	137	1	1	0.73 [0.04; 4.60]	..	..	100 [5.5; 89.2]	..	..

* Excludes populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

** "Pregnant women" and "Women with HIV" are not mutually exclusive

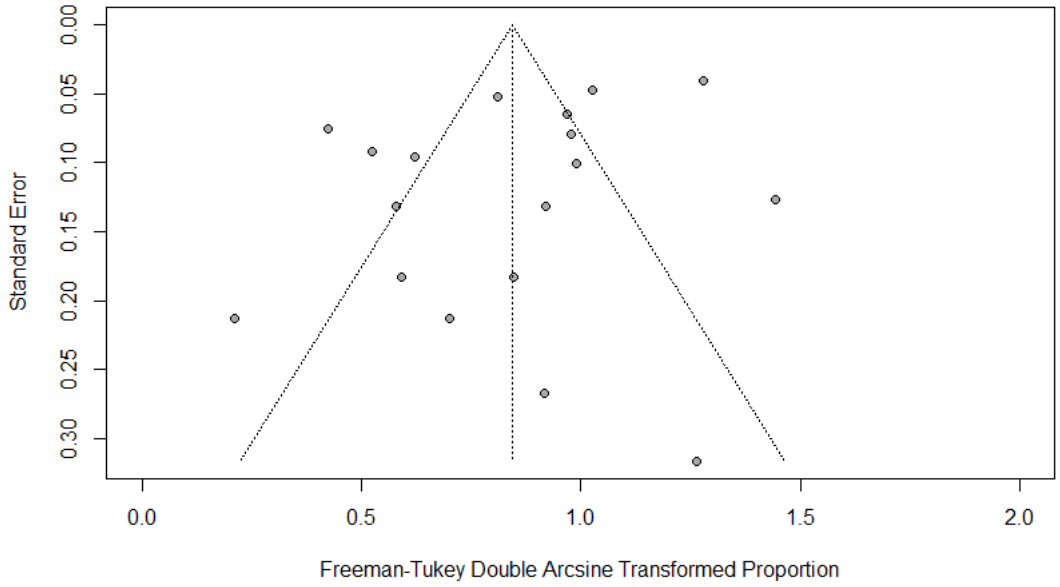
Supplementary Figure 1: Funnel plot and Egger's test for the proportion of asymptomatic CT (A), NG (B), and TV (C).

A



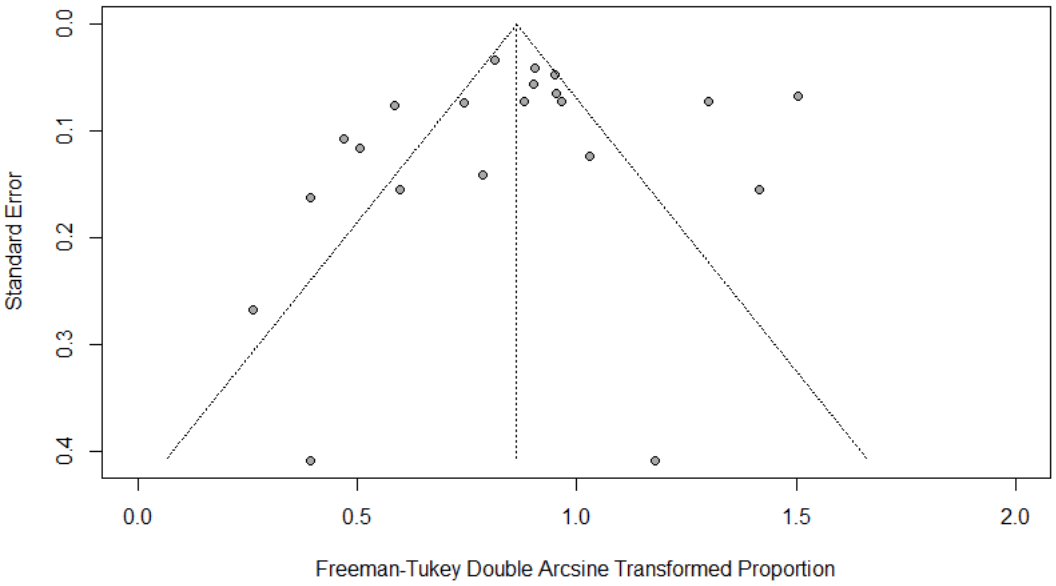
Egger's test: p = 0.070
Eggers' test does not indicate the presence of funnel plot asymmetry.

B



Egger's test: p = 0.118
Eggers' test does not indicate the presence of funnel plot asymmetry.

C



Egger's test: p = 0.575

Eggers' test does not indicate the presence of funnel plot asymmetry.

Annex C: Published article on the systematic review and meta-analysis

PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Asymptomatic infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* among women in low- and middle-income countries: A systematic review and meta-analysis

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Data Availability Statement: Data extracted from the studies (deidentified data) and the data dictionary are publicly available with no restriction at https://doi.org/10.57745/C61VFC.

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Abstract

Syndromic management of sexually transmitted infections (STIs) is common in settings with limited access to diagnostic testing. However, this approach does not capture asymptomatic STIs. Untreated asymptomatic infections may result in serious complications and sequelae in women. We aimed to estimate the proportion and the prevalence of asymptomatic *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) infections among women in low- and middle-income countries. We searched Medline, Scopus, and Web of Science for articles published between 2000 and 2022. We used random effect models to compute the proportion and prevalence estimates and performed subgroup analysis. We evaluated the quality of each article using the Appraisal tool for Cross-Sectional Studies and performed sensitivity analyses. This study was registered with PROSPERO, CRD42022286673. Forty-eight eligible studies were included. The proportion of asymptomatic CT, NG, and TV infections were: 60.7% [95% Confidence Interval (CI): 50.4; 70.5], 53.3% [37.1; 69.1], and 56.9% [44.6; 68.9], respectively. The proportion of women with asymptomatic infections was the highest in Africa for the three pathogens. The pooled prevalence of asymptomatic CT, NG, and TV infection was 4.70 per 100 women [95%CI: 3.39; 6.20], 3.11 [1.34; 5.54], and 5.98 [3.46; 9.12], respectively. More than half of the women infected by CT, NG, or TV were asymptomatic. To avoid undiagnosed and untreated asymptomatic infections leading to complications, alternative approaches to syndromic management urgently need to be considered.

author is articulated in the 'author contributions' section. No additional external funding was received for this study. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

More than one million sexually transmitted infections (STI) are acquired everyday worldwide [1]. Most of the infections by *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) occur in low- and middle-income countries (LMICs) [2].

The gold standard to detect these STIs are the Nucleic Acid Amplification Tests (NAATs); other methods include microscopy and bacterial culture [3]. Whilst high-income countries have developed screening programs [4] and use molecular methods, in most LMICs access to biological methods is hindered by the cost of diagnostics, lack of laboratory facilities and infrastructure, and trained personnel [5].

Therefore, STI detection and treatment in LMICs often rely on the syndromic management [6, 7].However, asymptomatic STI cases are not captured by this approach, leaving a fraction of those infected undiagnosed and untreated.

In women, untreated infections caused by CT and NG can result in serious complications such as pelvic inflammatory disease (PID), infertility, and ectopic pregnancy [8]. Untreated trichomoniasis in pregnant women is associated with preterm birth or low birth weight [9]. These STIs are also associated with a higher risk of acquiring and transmitting other STIs, including HIV [10]. Lastly, untreated infections sustain the transmission.

To date, no study has provided pooled estimates of the burden of asymptomatic infections of the three most common curable STIs.

In this perspective, we conducted a systematic review and a meta-analysis to estimate: (i) the proportion of asymptomatic women among those infected by CT, NG, and TV and, (ii) the prevalence of asymptomatic CT, NG, and TV infections in the female population in LMICs.

Methods

Search strategy

We searched MEDLINE, Web of Science, and Scopus databases. Search terms combined pathogen and disease terms, terms related to the absence of symptoms and countries ever classified as LMICs by the World Bank between 2000 and 2022 [11] (full search terms in S1 Text). We also screened references for eligible articles. When the estimates were collected but not presented, we contacted the authors up to three times.

Definitions

We defined "proportion of asymptomatic infection" as the number of women who tested positive for a pathogen and were asymptomatic divided by the number of women positive for that pathogen. We defined "prevalence of asymptomatic infection" as the number of women who tested positive for a pathogen and were asymptomatic divided by the number of women tested for that pathogen (S2 Text).

We defined symptoms as manifestations of a disease that were reported by the participant, while signs were considered features of a disease observed by a healthcare provider.

Selection criteria

We selected studies published between 1 January 2000 and 31 December 2022, in English and French. We included studies which tested for STIs using genital or urine specimens. We kept studies that used NAATs for the diagnosis of CT, NG, and TV [3]. For TV, we also included articles that used InPouch or performed wet mount microscopy immediately after collection,

as these techniques showed fair sensitivity and specificity [12] and remain common detection methods in LMICs [13].

We selected studies where the investigator actively asked about at least one symptom, or where participants self-reported symptoms. We excluded articles in which authors assumed the absence of symptoms in the entire study population without collecting such information. Finally, we excluded articles when "asymptomatic" was defined as the absence of clinical signs as reported by the healthcare provider rather than by the women as we intended a participant-centred approach.

BTH, TC, RR, and CF conducted initial screening of titles and abstracts in pairs and inclusion conflicts were resolved through consensus. CF performed data extraction, and BTH, TC, and RR ensured exactness and validity. When multiple publications on the same study population and outcomes were found, we prioritized the most comprehensive and recent data. Extracted data included the number of asymptomatic positive women, total women tested, and women positive for a specific STI. Other variables we extracted were: first author, publication year, study year, country, recruitment site, number and type of reported symptoms, definition of asymptomatic women, rural/urban setting, specific populations (female sex works (FSWs), adolescents, women with HIV, adolescents, infertile women), age range, specimen type, other STI pathogens tested for, and detection techniques.

Data analysis

We described regions, study designs, settings, recruitment sites, specific populations, number of symptoms, sampling methods, and laboratory procedures of the selected studies.

Proportion and prevalence of asymptomatic CT, NG, and TV infections were estimated first for all studies and excluding studies on FSWs, women with HIV, and women attending an STI clinic as they are not representative of the general population and have a higher risk of having an STI. We referred to these groups as "populations with an increased risk of STIs". Separate estimations were conducted for FSWs, women with HIV as well as other key populations such as pregnant women, adolescents, and infertile women.

Random-effect models were applied for each of the above analyses using the DerSimonian and Laird variance estimator. As some figures were near 0 or 1, the Freeman-Tukey double arcsine transformation was applied. Statistical heterogeneity was evaluated using the χ^2 test with Cochran's Q and I^2. Analyses and forest plots were conducted using *meta* and *metafor* packages in R software (version 4.1.1). Proportions and prevalence maps were generated using qGIS (version 3.22).

Subgroup analyses were performed by region, country income level, rural/urban setting, study period, and the number of symptoms assessed in the study. The role of these study variables in the heterogeneity of the estimates were explored in meta-regressions.

The risk of bias was assessed using the appraisal tool for cross-sectional studies (AXIS) using >70% of validated items as a cut-off to qualify studies with a low risk of bias [14]. We conducted a sensitivity analysis to assess the impact of excluding studies with a high risk of bias on our overall estimates.

Publication bias was evaluated by Funnel plots and the Egger test.

This article was written following the PRISMA checklist (S1 Table).

Results

We identified 1188 articles, among which 547 were duplicates. We eliminated 468 articles based on title and abstract and screened 173 full texts and their citations. We found 4 additional relevant citations among selected articles. In total, 177 articles were eligible. Of those, 42

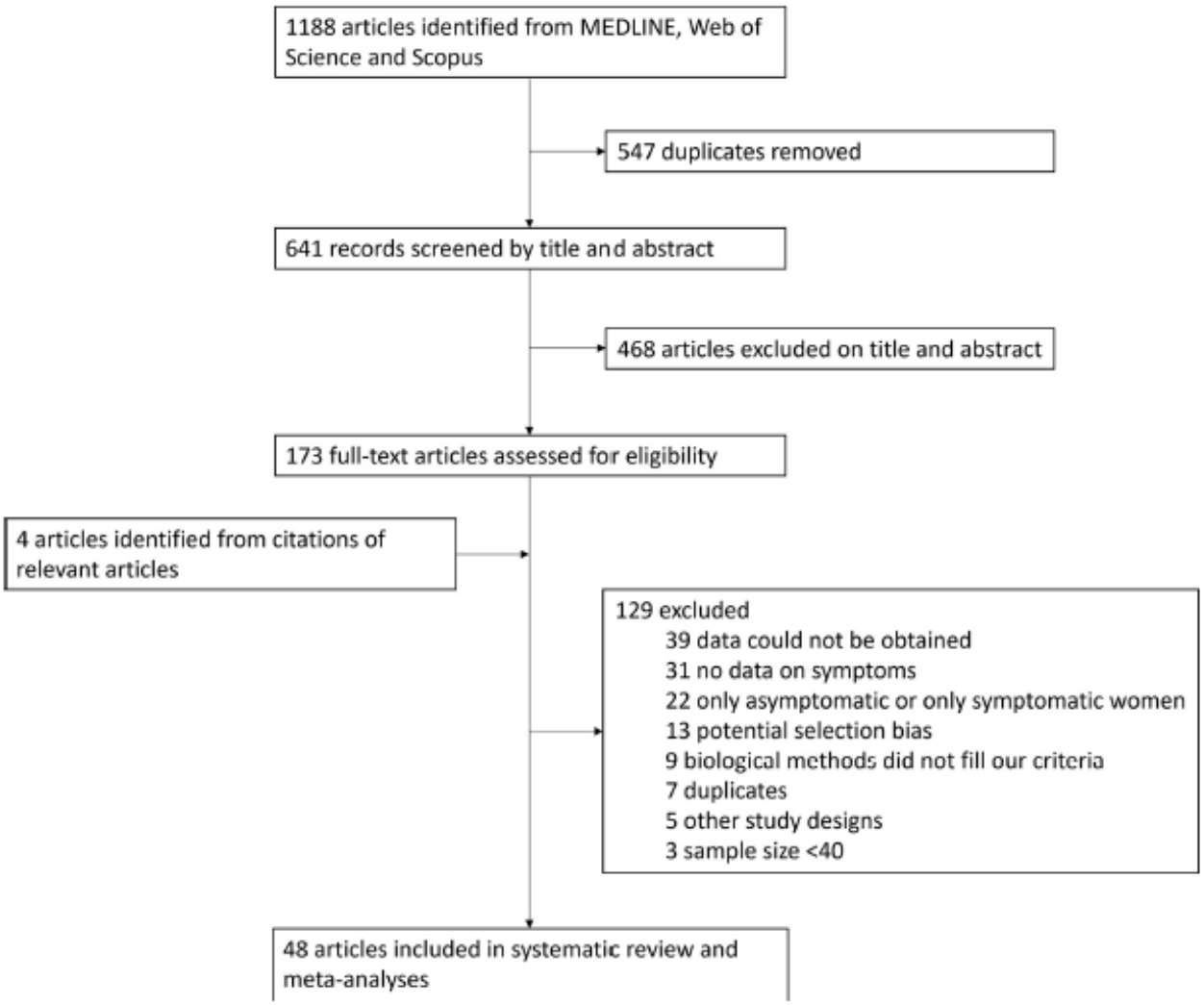


Fig 1. Study selection.

https://doi.org/10.1371/journal.pgph.0003226.g001

had available and extractable data and 45 reported having data according to our criteria but did not present the numbers. We were able to contact 40 of those 45 authors and retrieved data from six articles. In total, we used data from 48 studies corresponding to 99 data points for both the proportion and the prevalence: 41 for CT, 27 for NG, and 31 for TV (Fig 1, S2 Table, S3 Text). Twenty-one countries were represented in our study: eight in Africa, seven in Asia, five in Latin America, and one in Oceania. Most studies were cross-sectional (44/48, 92%) and few were cohort (2/48, 4%) or randomized controlled trials (2/48, 4%). Almost three-quarters of the studies (34/48, 71%) took place in urban settings, 21% (10/48) in rural settings, and 8% (4/48) in mixed settings. Hospitals and antenatal care were the most frequent recruitment sites (14/48, 29% and 11/48, 23%, respectively), followed by primary or secondary health care centres (9/48, 19%), locations where people with a high risk of STI can be found such as FSW venues, HIV centres or STI clinics (8/48, 17%). Six studies performed random sampling within the community (6/48, 13%). Some studies focused on specific populations:

15/48 (31%) were on pregnant women, 5/48 (10%) on FSWs, 4/48 (8%) on adolescents, 4/48 (8%) on women with HIV, and 4/48 (8%) on infertile women exclusively. Studies had enrolled between 48 and 4812 participants, and their age ranged from 10 to 90 years old although this information was unavailable for 14 studies.

The number of recorded symptoms ranged from one to 14, with more than half of the studies (23/48, 48%) exploring 1–4 symptoms.

Genital specimens (41/48, 85%) were the most common specimens, and few studies used urine (7/48, 15%). All techniques used for the laboratory diagnosis of CT (n = 41) and NG (n = 27) were NAATs, as per research criterium. For TV, NAATs were used in 25/31 studies, wet mount microscopy in 4/31, and InPouch in 2/31 studies.

The estimates summarised below are presented in Table 1, Figs 2 and 3, and S3–S5 Tables. The proportion of asymptomatic CT, NG, and TV infections, excluding populations with an increased risk of STIs, was 60.7% 95%CI [50.4; 70.5] (33 data points, 26 268 participants), 53.3% [37.1; 69.1] (21 data points, 16 950 participants), and 56.9% [44.6; 68.9] (23 data points, 10 824 participants), respectively.

The proportion of asymptomatic CT, NG, and TV infections (excluding populations with an increased risk of STIs) was significantly different across regions (p = 0.048, p = 0.019, and p = 0.039, respectively for each pathogen). Africa presented the highest proportion of asymptomatic infections (CT: 68.6% [56.4; 79.8], 14 data points, 12 368 participants; NG: 67.2% [51.0; 81.9], 13 data points, 7 681 participants; TV: 64.9% [52.2; 76.7], 12 data points, 6 152 participants). Latin America had the lowest proportion of asymptomatic women infected with CT (30.9% [11.2; 54.8], five data points, 2 850 participants) and with NG (14.0% [0.0; 67.5], two data points, 1 546 participants). The lowest proportion of asymptomatic TV infection was found in Asia (17.3% [0.0; 49.5], four data points, 2 606 participants).

When stratifying by country income level, the proportions of asymptomatic cases were the highest in middle-income countries (CT: 63.2% [50.2; 75.1], 11 data points, 14 887 participants; NG: 60.9% [41.1; 79.2], 14 data points, 10 793 participants; TV: 61.7% [45.4; 77.1], 17 data points, 6 688 participants), and the lowest in low-income countries (CT: 55.5% [37.3; 73.1], 11 data points, 11 381 participants; NG: 37.7% [18.4; 58.8], seven data points, 6 157 participants; TV: 45.4% [29.7; 61.6], six data points, 4 136 participants). The proportion of asymptomatic infected women was also the highest in rural settings (CT: 71.6% [55.6; 85.3], ten data points, 9 150 participants; NG: 70.4% [46.3; 90.3], 9 data points, 4 464 participants; TV: 75.0% [49.8; 94.0], six data points, 2 619 participants), and the lowest in urban settings (CT: 55.6% [43.8; 67.1], 20 data points, 15 276 participants; NG: 44.4% [26.5; 62.9], 11 data points, 12 124 participants; TV: 58.9% [46.5; 70.8], 15 data points, 7 544 participants).

Analyses on specific populations showed that, in women with HIV, the proportion of asymptomatic CT, NG, and TV infections exceeded 50% (CT: 64.7% [48.3; 79.7], three data points, 739 participants; NG: 61.0% [42.7; 78.0], three data points, 739 participants; TV: 70.5% [60.7; 79.6], four data points, 965 participants). In FSWs, those proportions were below 50% (CT: 45.5% [24.8; 67.0], five data points, 1 890 participants; NG: 38.1% [3.1; 82.9], three data points, 1 051 participants; TV: 26.7% [19.6; 34.5], two data points, 546 participants).

The prevalence of asymptomatic women infected with CT, NG, or TV was 4.70 per 100 women [3.39; 6.20] (33 data points, 26 268 participants), 3.11 [1.34; 5.54] (21 data points, 16 950 participants) and 5.98 [3.46; 9.12] (23 data points, 10 824 participants), respectively, excluding populations with an increased risk of STI infection.

The prevalence of asymptomatic NG and TV infections differed significantly across regions (p<0.001). The highest prevalence was found in Africa and Oceania (NG: 5.67 [2.15; 10.66] and 3.12 [0.00; 12.25], TV: 9.06 [5.71; 13.07] and 12.77 [0.06; 40.12], respectively) and the lowest in Asia and Latin America (NG: 0.13 [0.03; 0.28] and 0.12 [0.00; 1.16], TV: 0.08 [0.00; 0.31]

Table 1. Proportion and prevalence of asymptomatic CT, NG, and TV infections in women in LMICs.

	Proportion of asymptomatic infections (per 100 infected women) [95%CI]			Prevalence of asymptomatic infections (per 100 tested women) [95%CI]		
	CT	NG	TV	CT	NG	TV
Overall						
Excluding populations with an increased risk of STI*	60.7 [50.4; 70.5]	53.3 [37.1; 69.1]	56.9 [44.6; 68.9]	4.70 [3.39; 6.20]	3.11 [1.34; 5.54]	5.98 [3.46; 9.12]
Including populations with an increased risk of STI	58.8 [50.4; 67.0]	52.2 [38.5; 65.6]	53.6 [43.0; 64.1]	6.05 [4.26; 8.12]	3.70 [1.79; 6.23]	5.84 [3.75; 8.34]
Continent*						
Africa	68.6 [56.4; 79.8]	67.2 [51.0; 81.9]	64.9 [52.2; 76.7]	4.99 [2.70; 7.90]	5.67 [2.15; 10.66]	9.06 [5.71; 13.07]
Asia	66.2 [47.2; 83.0]	27.7 [9.0; 50.9]	17.3 [0.0; 49.5]	5.55 [3.40; 8.19]	0.13 [0.03; 0.28]	0.08 [0.00; 0.31]
Latin America	30.9 [11.2; 54.8]	14.0 [0.0; 67.5]	50.1 [16.4; 83.8]	1.89 [0.56; 3.91]	0.12 [0.00; 1.16]	4.54 [0.52; 11.9]
Oceania	47.4 [2.7; 95.0]	32.7 [0.0; 88.3]	43.3 [4.8; 87.5]	7.22 [0.00; 24.73]	3.12 [0.00; 12.25]	12.77 [0.06; 40.12]
Country income level*						
Low-Income	55.5 [37.3; 73.1]	37.7 [18.4; 58.8]	45.4 [29.7; 61.6]	3.55 [1.87; 5.70]	0.57 [0.17; 1.17]	3.12 [0.73; 7.02]
Middle-Income	63.2 [50.5; 75.1]	60.9 [41.1; 79.2]	61.7 [45.4; 77.1]	5.34 [3.59; 7.39]	5.21 [1.77; 10.22]	7.28 [3.72; 11.88]
Setting*						
Rural	71.6 [55.6; 85.3]	70.4 [46.3; 90.3]	75.0 [49.8; 94.0]	5.36 [3.11; 8.15]	6.80 [1.39; 15.60]	10.27 [2.41; 22.46]
Urban	55.6 [43.8; 67.1]	44.4 [26.5; 62.9]	58.9 [46.5; 70.8]	4.83 [2.90; 7.19]	1.33 [0.29; 3.02]	4.81 [2.25; 8.21]
Study year*						
1998–2011	61.8 [48.1; 74.7]	55.9 [32.9; 77.8]	57.3 [41.0; 73.0]	4.41 [2.37; 6.99]	3.72 [0.98; 7.99]	5.94 [2.86; 10.00]
2011–2022	57.7 [42.1; 72.7]	48.9 [26.9; 71.1]	61.1 [41.3; 79.5]	4.09 [2.88; 5.49]	2.42 [0.47; 5.63]	6.99 [2.09; 14.30]
Number of symptoms assessed*						
Between 1 and 4	57.4 [41.6; 72.4]	40.6 [13.8; 70.4]	55.1 [37.7; 71.9]	6.53 [3.46; 10.45]	4.39 [0.46; 11.76]	5.68 [2.97; 9.17]
Five and more	69.0 [51.7; 84.2]	71.8 [56.6; 85.2]	63.6 [43.3; 81.9]	4.29 [2.48; 6.53]	3.50 [1.27; 6.68]	7.66 [2.94; 14.24]
Key population†						
Pregnant women	55.3 [40.6; 69.6]	45.7 [27.9; 63.9]	58.8 [43.9; 73.0]	7.50 [4.31; 11.44]	2.60 [1.27; 4.32]	7.96 [4.73; 11.91]
Female sex workers	45.5 [24.8; 67.0]	38.1 [3.1; 82.9]	26.7 [19.6; 34.5]	13.63 [2.76; 30.72]	9.49 [0.04; 30.73]	7.02 [4.16; 10.52]
Adolescents	57.4 [17.9; 92.2]	35.9 [15.0; 59.2]	46.4 [27.6; 65.7]	1.89 [1.55; 2.27]	1.12 [0.01; 3.59]	2.05 [0.33; 4.98]
Women with HIV	64.7 [48.3; 79.7]	61.0 [42.7; 78.0]	70.5 [60.7; 79.6]	10.11 [0.13; 31.39]	3.09 [0.68; 6.96]	6.42 [1.89; 13.23]
Infertile	74.7 [59.9; 87.2]	..	100 [5.5; 89.2]	11.76 [4.33; 22.07]	0.00 [0.07; 3.40]	0.73 [0.04; 4.60]

* These analyses excluded populations with an increased risk of STI (FSW, women with HIV, and women attending an STI clinic)

† These analyses were conducted exclusively on these populations and did not consist of a sub-group analysis. "Pregnant women" and "Women with HIV" are not mutually exclusive

https://doi.org/10.1371/journal.pgph.0003226.t001

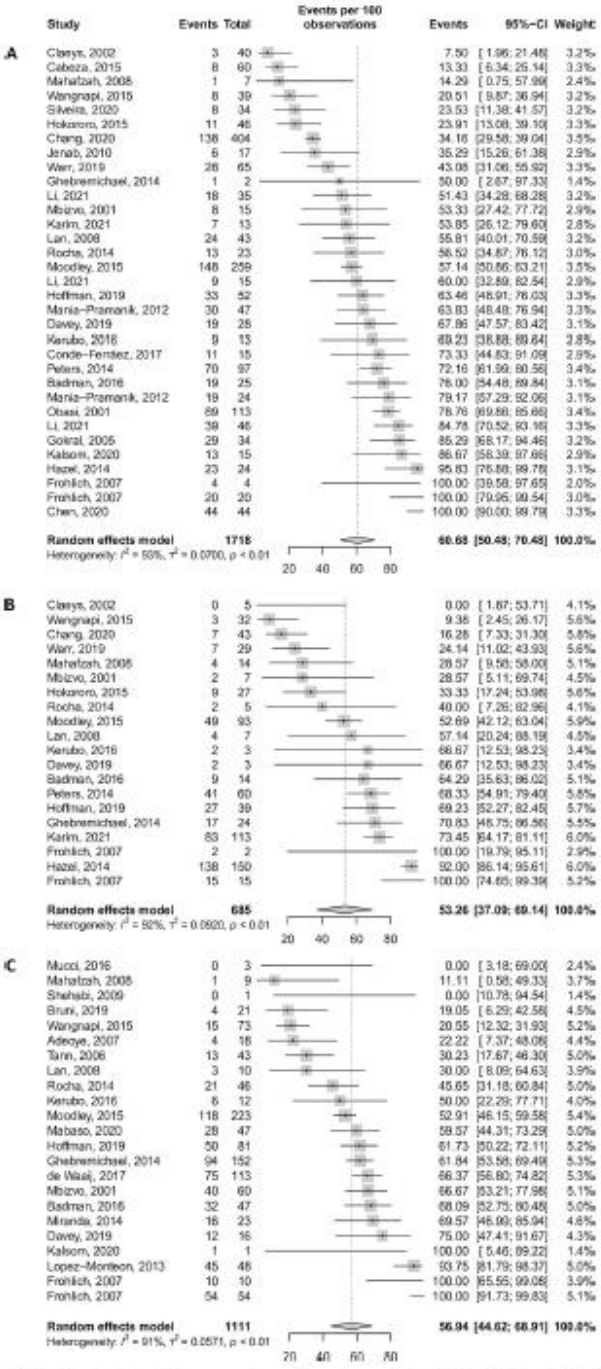


Fig 2. Forest plots of the proportion of asymptomatic CT (A), NG (B) and TV (C), in women, in LMICs.

https://doi.org/10.1371/journal.pgph.0003226.g002

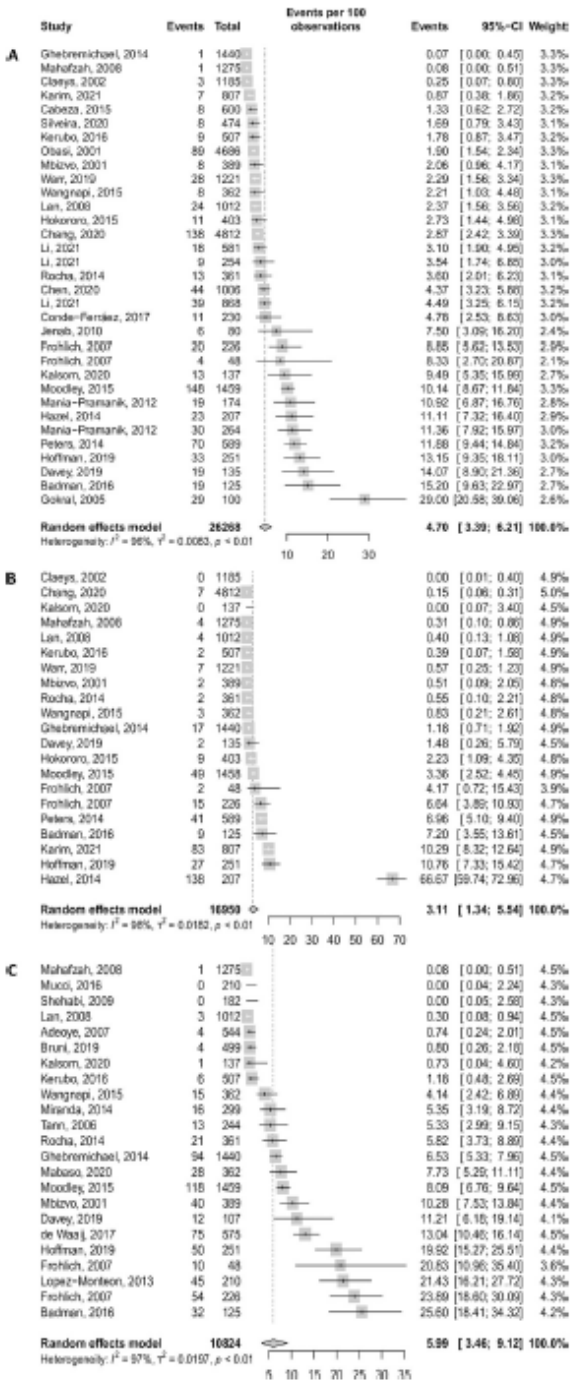


Fig 3. Forest plots of the prevalence of asymptomatic CT (A), NG (B) and TV (C), in women, in LMICs.

https://doi.org/10.1371/journal.pgph.0003226.g003

and 4.54 [0.52; 11.9], respectively). A similar pattern was observed for CT, but there was weak evidence of this difference (p = 0.068).

For NG only, the prevalence of asymptomatic infection was significantly higher in middle-income countries versus low-income (5.21 [1.77; 10.22] vs 0.57 [0.17; 1.17], p = 0.002) and was nearly significantly different between rural and urban settings (6.80 [1.39; 15.60] vs 1.33 [0.29; 3.02], p = 0.059).

Relative to the other key populations, the prevalence of asymptomatic CT, NG, and TV was low among adolescents (CT: 1.89 [1.55; 2.27]; NG: 1.12 [0.01; 3.59]; TV: 2.05 [0.33; 4.98]) and high among FSWs (CT: 13.6 [2.76; 30.72]; NG: 9.49 [0.04; 30.73]; TV:7.02 [4.16; 10.52]).

Proportion and prevalence by country are presented in Fig 4 and S6 Table.

After excluding studies with a high risk of bias, our sensitivity analyses showed no significant change in our findings (S7–S9 Tables). Results of the meta-regression showed that the continent and the country income level explained a substantial part of the variation in our estimates (S10 Table). Results from the Funnel plots and Egger's tests showed no evidence of publication bias (S1–S3 Fig).

Discussion

Our study reveals a significant public health challenge: more than half of the women infected with CT, NG, and TV were asymptomatic in LMICs. The prevalence of asymptomatic infections ranged from 3 to 6%. When cumulating the prevalence of each of pathogen, accounting for the rarity of co-infections, [15, 16] the prevalence could reach up to 13%. These high proportions and prevalence of asymptomatic infections are particularly worrisome since those women remain untreated and thus may develop serious complications. Additionally, they constitute an unseen reservoir that continues to fuel the STI epidemic.

Several hypotheses can be proposed to explain these high estimates. First, women may have difficulties in distinguishing physiological from abnormal vaginal discharge. For instance, in Tanzania, a study found that more than two third of women with abnormal discharge did not report any symptoms [17]. In rural areas of Madagascar, anthropologists reported that genital symptoms are thought to be normal [18] and women may not report them, even after probing.

Alternatively, social norms may prevent women from declaring their symptoms. They may correctly identify genital symptoms but may be unwilling to communicate them to healthcare professionals due to the stigma attached to such symptoms or fear of being judged [19].

The notably high proportions of asymptomatic CT, NG, and TV infections in Africa are concerning. STIs are associated with an increased risk of transmitting and acquiring other STIs or HIV and Africa bears amongst the highest STI and HIV prevalence in the world [2, 20].The wide use of syndromic approach in Africa means that asymptomatic women are often left undiagnosed and untreated, posing a risk of transmission to their partners and contributing to adverse health outcomes.

We observed a high proportion of asymptomatic STIs among women with HIV. While the limited number of studies limits definitive conclusions, similar findings have been reported among men with HIV who have sex with men [21]. In this study, detectable plasma HIV RNA, social factors, and sexual practices were associated with asymptomatic STIs. Further research could also explore the potential immunological mechanisms underlying this phenomenon.

Our study revealed an elevated prevalence of asymptomatic STIs among FSWs. This reflects their increased susceptibility to STIs explained by their frequent engagement in sexual activities with multiple partners. However, we observed a relatively lower proportion of asymptomatic women, which could be explained by FSWs' increased awareness and recognition of STI symptoms [22].

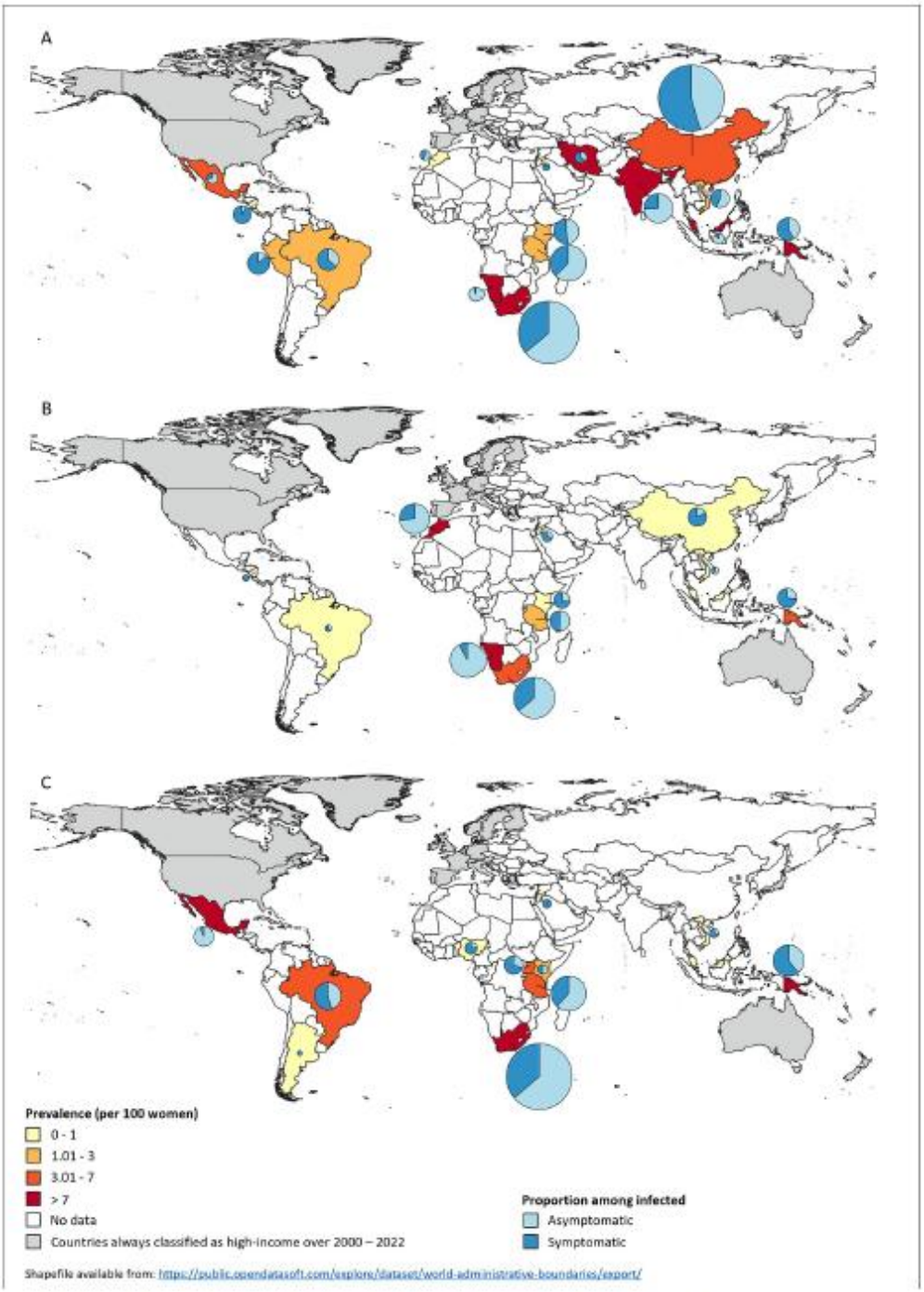


Fig 4. Maps of asymptomatic CT (A), NG (B) and TV (C) infections in women, in LMICs, by country.

https://doi.org/10.1371/journal.pgph.0003226.g004

We found a low prevalence of asymptomatic STIs among adolescents, possibly due to the lower STI prevalence in this group and the inclusion of sexually inactive participants in some studies. However, despite the lower prevalence, adolescents remain vulnerable to STI acquisition due to their sexual behavior and physiological susceptibility [23]. Implementing comprehensive sexual education programs during early adolescence could effectively maintain a low STI occurrence in this key population [24].

This study has several limitations. Firstly, the majority of the studies were conducted in healthcare facilities which may introduce bias, as women could attend these facilities because of genitourinary symptoms, potentially underrepresenting asymptomatic cases compared to the general population. Consequently, while our estimates are already substantial, the true proportion and prevalence could be even higher. Furthermore, our search yielded only six articles conducted in community settings, indicating a gap in community-based research. Such studies are pivotal for accurately assessing the proportion and prevalence of asymptomatic STIs in the general population. Another limitation pertains to the varying number of symptoms used to determine symptomatic status, ranging from one to fourteen. Studies examining more symptoms were more likely to identify symptomatic cases and fewer asymptomatic cases. However, our comparison of estimates from studies assessing one to four symptoms versus those assessing five or more symptoms did not show any significant differences. Establishing a uniform definition in future research could potentially reduce the heterogeneity among different studies.

Another limitation of our review is that 39 studies appeared to have collected the data we needed but we were unable to obtain these figures. However, we did not find any temporal or spatial pattern among them. In addition, due to our language restrictions, ten studies conducted in Latin America were excluded although they seemed to be relevant.

Nevertheless, our study has several strengths. To the best of our knowledge, this is the first meta-analysis providing comprehensive estimated on asymptomatic STIs in LMICs among women. Our strict inclusion and exclusion criteria, particularly regarding pathogen identification techniques minimized the risk of including false positive cases and ensured that the study's findings were based on high-quality and reliable data. We also provided estimates for specific populations of pregnant women, FSWs, adolescents, women with HIV, and infertile women. Further research is needed. Within this large reservoir of asymptomatic STIs, it is important to estimate what percentage of women will develop complications such as PID as well as what percentage will spontaneously clear the pathogen [25] It is also critical to identify characteristics associated with developing complications or sequelae in order to treat those women at an early stage.

We showed that the prevalence and proportion of asymptomatic STIs among women in LMICs represent a significant public health concern. An alternative to the syndromic management could be the development of an easy-to-use and affordable point-of-care test (POCT) that would rapidly and accurately detect STIs [26]. A POCT to screen women with suspected STIs among asymptomatic women would be more cost-effective than POCT testing for each pathogen on all of them. Currently, a POCT detecting inflammation caused by any STI is being tested [27]. In addition to providing confidential healthcare services and destigmatizing STIs as defined in the global health strategies on STIs by the WHO for 2022–2030, [28] such a device could be used by health care providers to screen asymptomatic women in order to prevent adverse consequences for reproductive and overall health.

Data sharing

The study protocol is available on PROSPERO (CRD42022286673). The equations for searching the databases are given in S1 Text. Countries' classifications into income levels are publicly

available from the World Bank [11]. Data extracted from the studies (deidentified data) and the data dictionary are publicly available with no restriction at https://doi.org/10.57745/C61VFC.

Supporting information

S1 Text. Search terms for citation selection.
(DOCX)

S2 Text. Formulae of the calculation of proportion and prevalence of asymptomatic infections. Formula: Calculation of prevalence and proportion of asymptomatic infections.
(DOCX)

S3 Text. References of included articles.
(DOCX)

S1 Table. PRISMA checklist.
(DOCX)

S2 Table. List and characteristics of included articles.
(DOCX)

S3 Table. Proportion and prevalence of asymptomatic CT infections: Number of studies, number of participants, and I^2.
(DOCX)

S4 Table. Proportion and prevalence of asymptomatic NG infections: Number of studies, number of participants, and I^2.
(DOCX)

S5 Table. Proportion and prevalence of asymptomatic TV infections: Number of studies, number of participants, and I^2.
(DOCX)

S6 Table. Sensitivity analysis–proportion and prevalence estimates of asymptomatic CT infections excluding high risk of bias studies.
(DOCX)

S7 Table. Sensitivity analysis–proportion and prevalence estimates of asymptomatic NG infections excluding high risk of bias studies.
(DOCX)

S8 Table. Sensitivity analysis–proportion and prevalence estimates of asymptomatic TV infections excluding high risk of bias studies.
(DOCX)

S9 Table. Proportion and prevalence of asymptomatic CT, NG, and TV by country (data used for the map).
(DOCX)

S10 Table. Meta-regression estimates and 95% CI for the proportion and the prevalence of asymptomatic CT (A), NG (B), and TV (C).
(DOCX)

S1 Fig. Funnel plot and Egger's test for the proportion of asymptomatic CT.
(DOCX)

S2 Fig. Funnel plot and Egger's test for the proportion of asymptomatic NG.
(DOCX)

S3 Fig. Funnel plot and Egger's test for the proportion of asymptomatic TV.
(DOCX)

Acknowledgments

CF, BTH, TC, and RVR conceived the study and research question. CF did the literature search with an information specialist, Catherine Cecilio. CF, BTH, TC, and RVR selected on titles and abstracts the results from the search strategy. CF extracted the data and BTH, TC, and RVR checked extracted data. CF did the analysis and wrote the first draft. BTH, TC, RVR and EDA wrote, edited, and revised the manuscript. CF, BTH, TC, and RVR had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Formal analysis: Camille Fortas, Rindra Vatosoa Randremanana, Tania Crucitti, Bich-Tram Huynh.

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Methodology: Camille Fortas, Rindra Vatosoa Randremanana, Tania Crucitti, Bich-Tram Huynh.

Supervision: Rindra Vatosoa Randremanana, Tania Crucitti, Bich-Tram Huynh.

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Visualization: Camille Fortas, Rindra Vatosoa Randremanana, Tania Crucitti, Bich-Tram Huynh.

Writing – original draft: Camille Fortas.

Writing – review & editing: Elisabeth Delarocque-Astagneau, Rindra Vatosoa Randremanana, Tania Crucitti, Bich-Tram Huynh.

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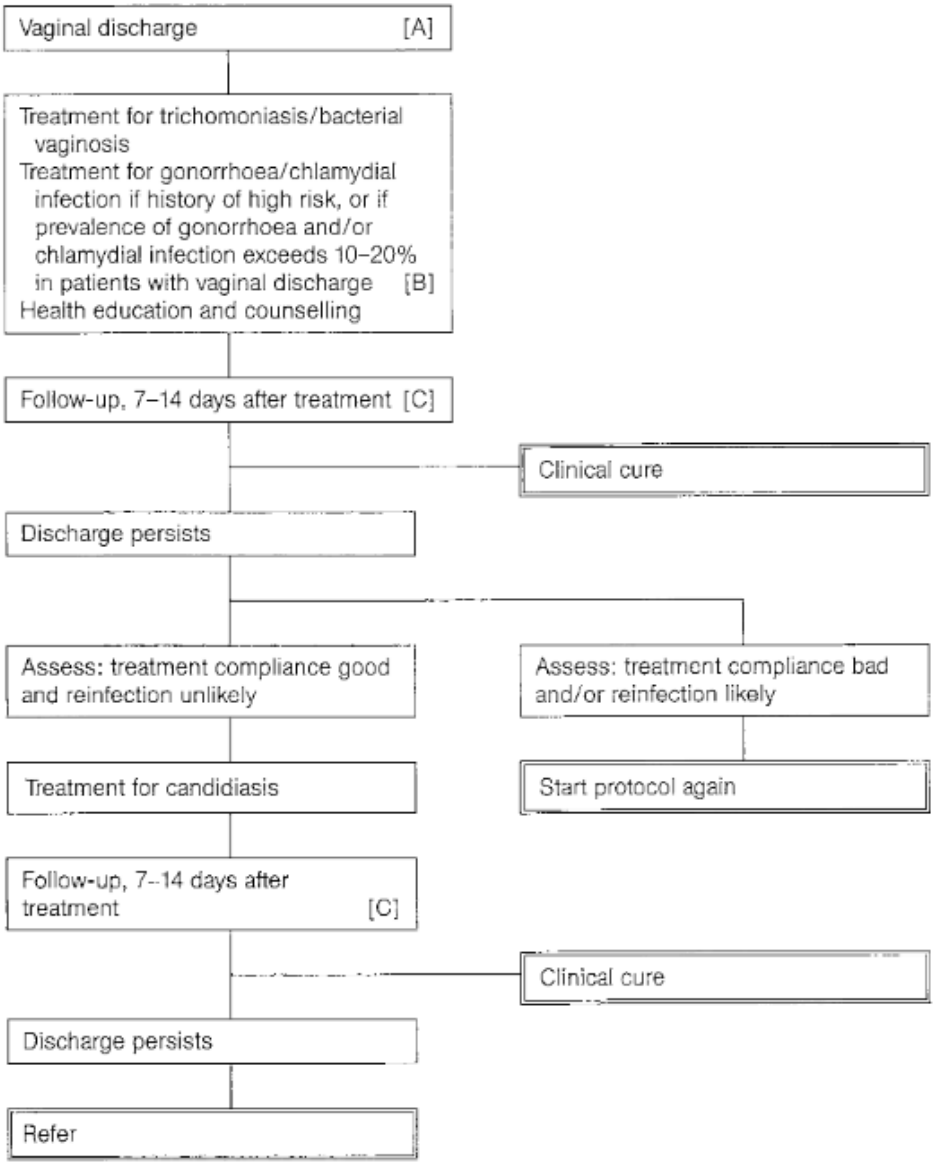
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Annex D: 1991 and 2003 WHO algorithms

Algorithm 3
Vaginal discharge (vaginal examination and laboratory tests not possible)



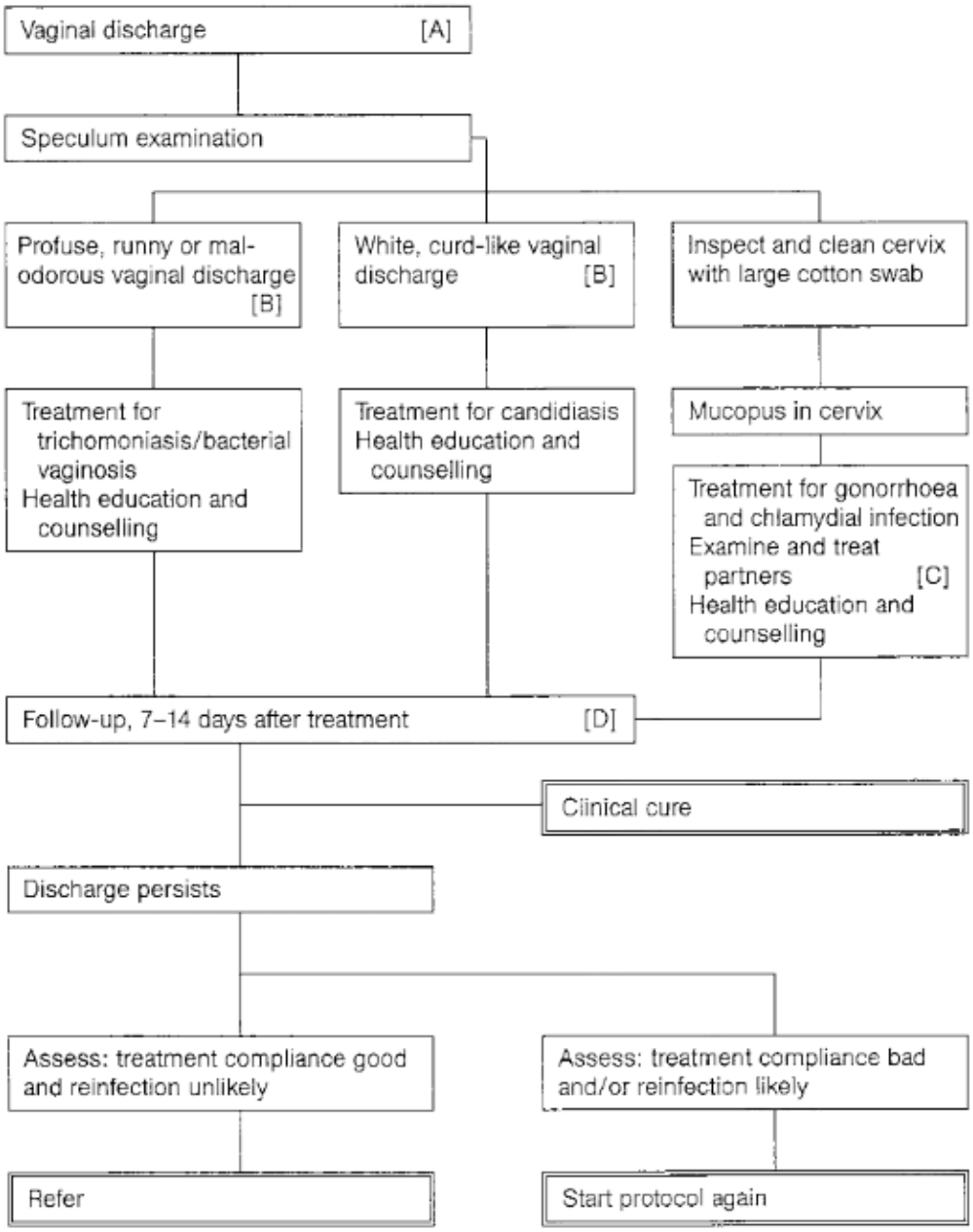
[A] If vaginal discharge is accompanied by lower abdominal pain or pain on moving the cervix, use the appropriate "lower abdominal pain" algorithm (see section 4.3).
[B] History of high risk includes: prostitution; sexual contact with a person with an STD; history of previous STD; young age, i.e., < 20 years. In the case of treatment for gonorrhoea and chlamydial infection, treat and examine partner(s) taking into account local cultural and epidemiological factors.
[C] Patient may be advised to return only if symptoms persist.

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Figure 1: Algorithm for vaginal discharge when vaginal examination and laboratory tests are not possible, from the 1991 WHO guidelines.

Algorithm 4

Vaginal discharge (speculum examination possible, but no laboratory support)



[A] If vaginal discharge is accompanied by lower abdominal pain or pain on moving the cervix, use the appropriate "lower abdominal pain" algorithm (see section 4.3).

[B] In addition, the pH paper test can be used: if pH lower than 4.5, treat for candidiasis; if pH higher than 4.5, treat for trichomoniasis/bacterial vaginosis.

[C] In the absence of a confirmed diagnosis, the decision to notify partner(s) should take into account local cultural and epidemiological factors.

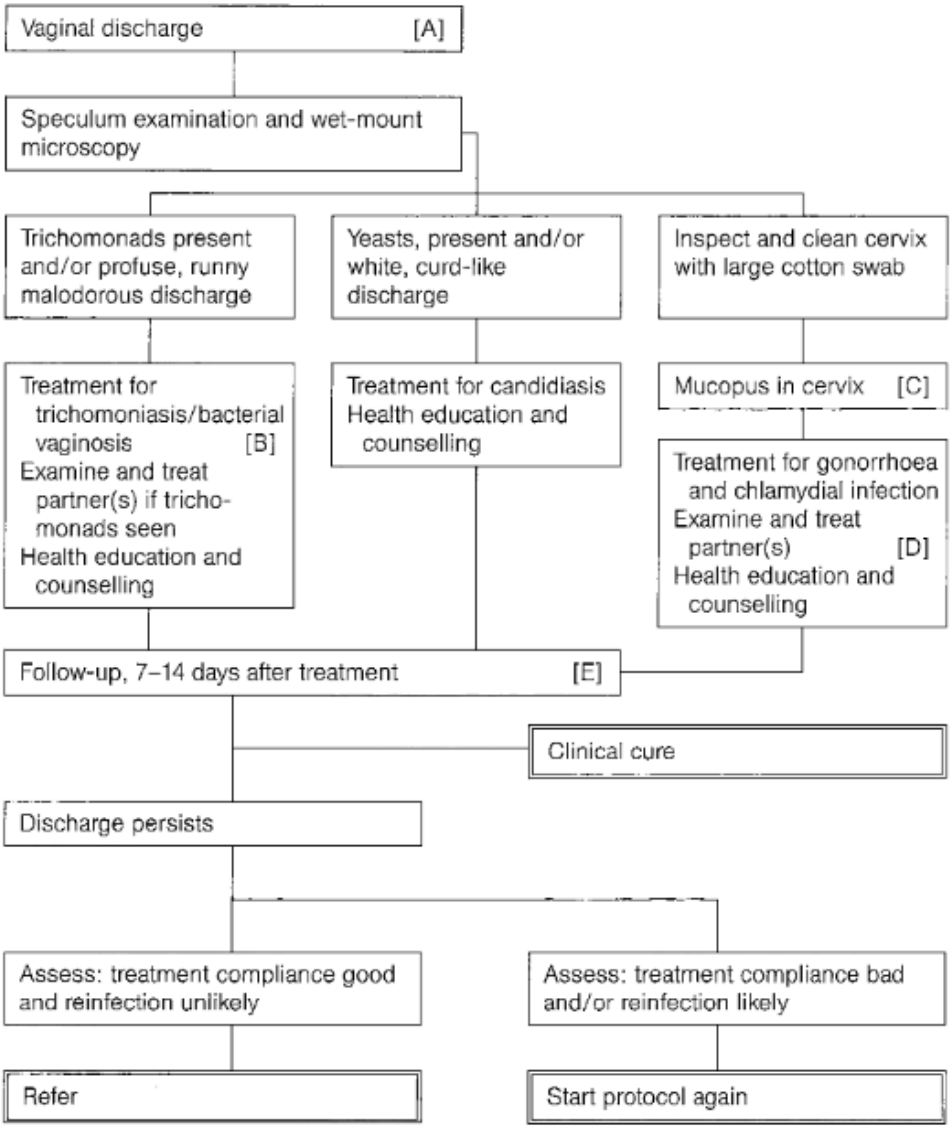
[D] Patient may be advised to return only if symptomatic.

19

Figure 2: Algorithm for vaginal discharge when speculum examination is possible but not microscopy, from the 1991 WHO guidelines.

Algorithm 5

Vaginal discharge (speculum examination and microscopy possible)



[A] If vaginal discharge is accompanied by lower abdominal pain or pain on moving the cervix, use the appropriate "lower abdominal pain" algorithm (see section 4.3).

[B] Bacterial vaginosis has a tendency to recur, but is not considered to be sexually transmitted, or to require partner notification.

[C] Some clinicians may choose to Gram-stain a cervical smear. However, if mucopus is seen exuding from the endocervix, the results of the Gram-stain should not influence treatment decisions.

[D] In the absence of a confirmed diagnosis, the decision to notify partner(s) should take into account local cultural and epidemiological factors.

[E] Patient may be advised to come back only if symptomatic.

20

Figure 3: Algorithm for vaginal discharge when speculum examination and microscopy are possible, from the 1991 WHO guidelines.

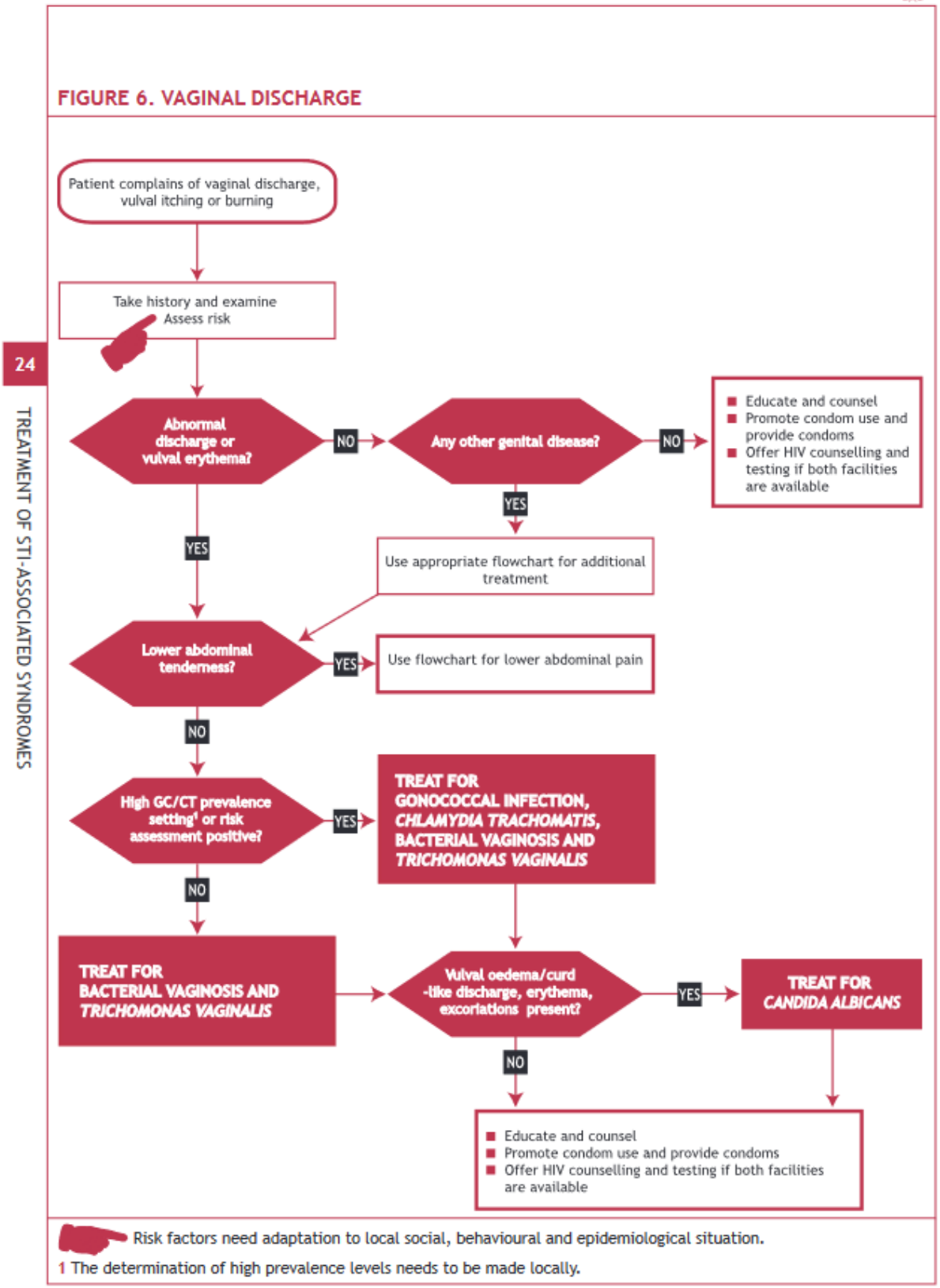


Figure 4: Algorithm for vaginal discharge when speculum examination and microscopy are not possible, from the 2003 WHO guidelines.

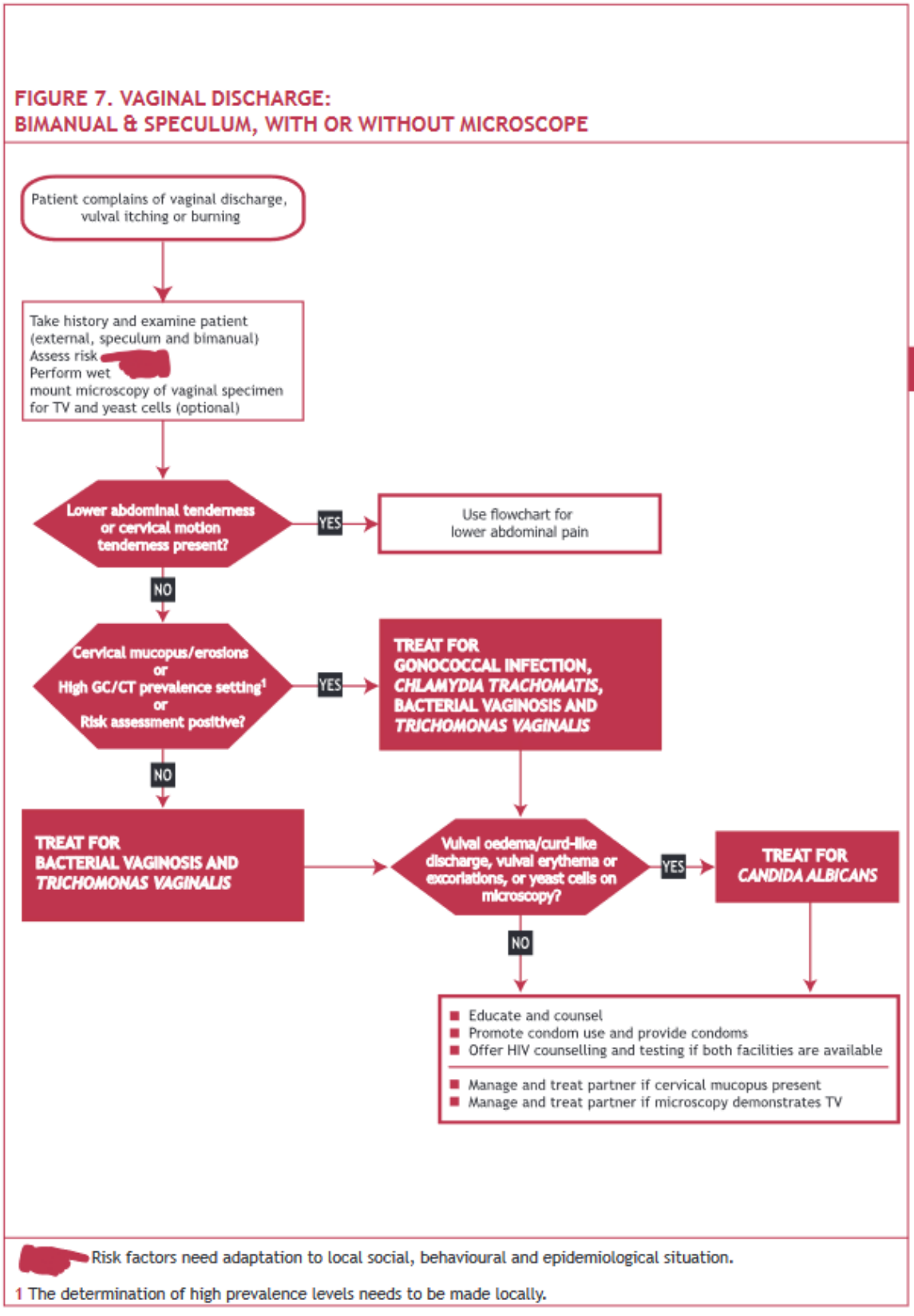


Figure 5: Algorithm for vaginal discharge when speculum examination is possible, with or without microscopy, from the 2003 WHO guidelines.

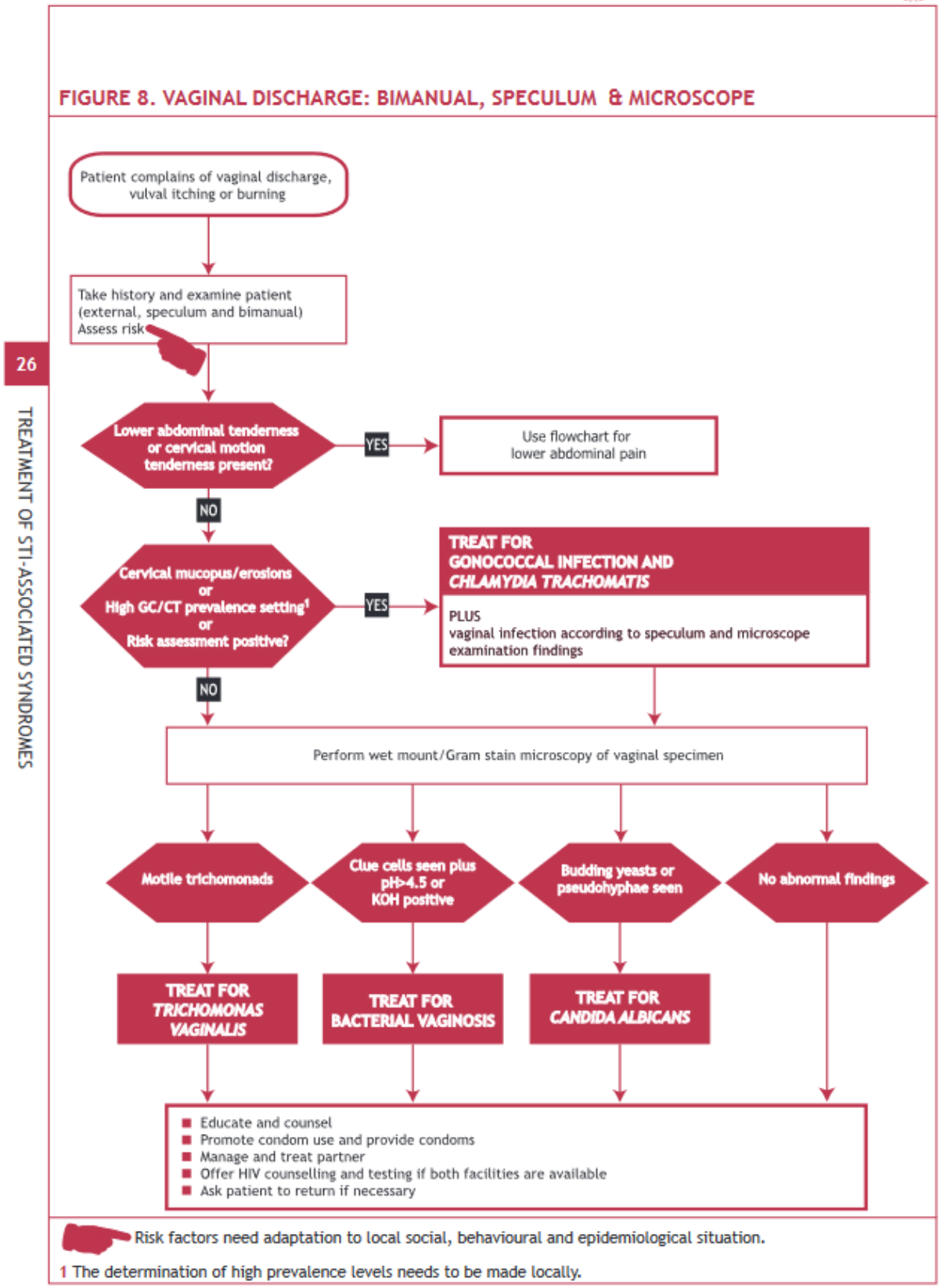


Figure 6: Algorithm for vaginal discharge when speculum examination and microscopy are possible, from the 2003 WHO guidelines.

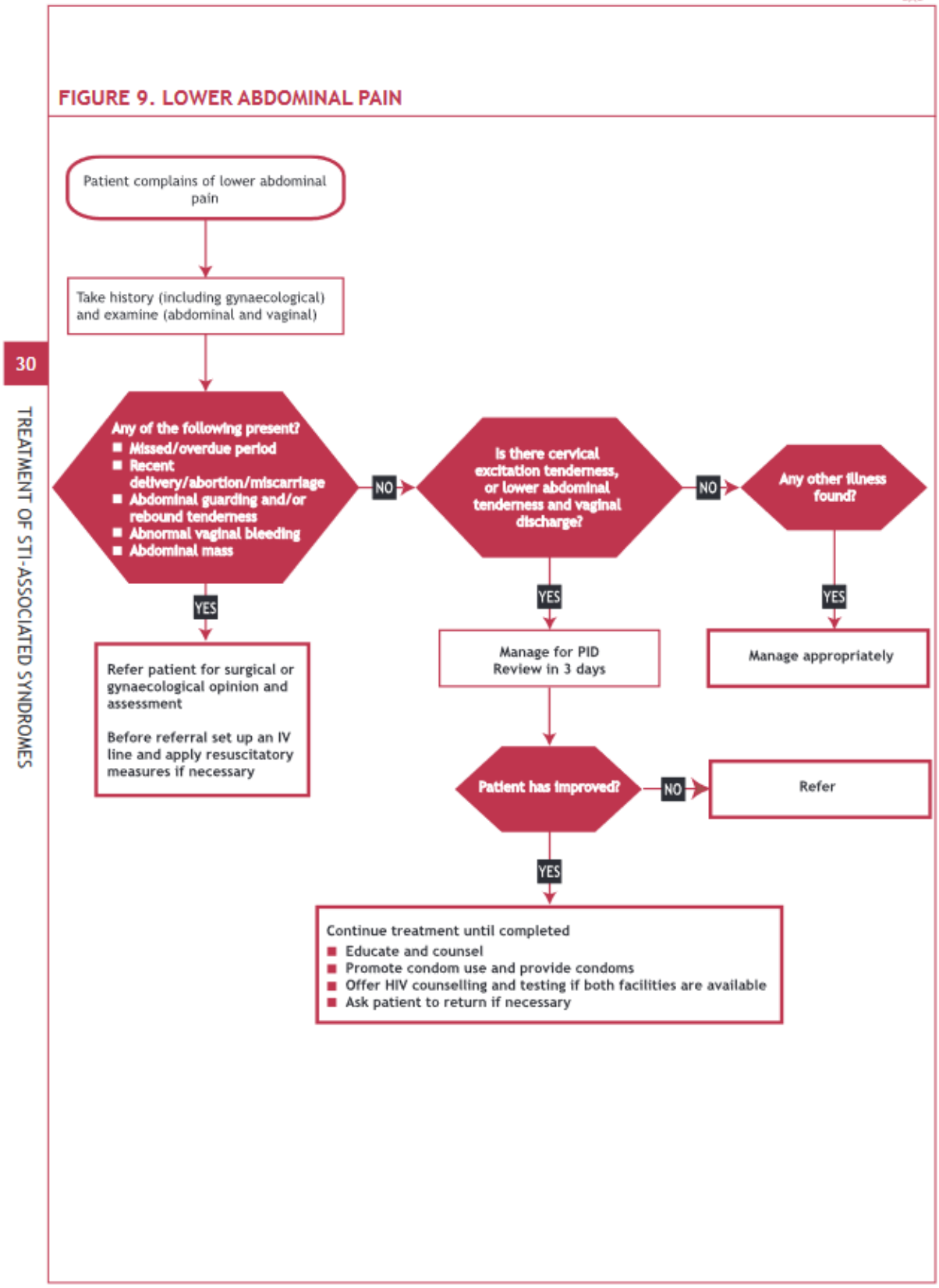


Figure 7: Algorithm for lower abdominal pain, from the 2003 WHO guidelines.

Annex E: Complementary analyses for chapter 5

Section 1: Additional descriptive analysis

Table 1: Characteristics of women with STI and/or BV and women with no STI or BV

	Women with no STI/BV N = 247	Women with STI and/or BV N = 387	Total N = 634	p-value
Age, median [IQR]*	27 22-31	26 22-30	27 22-30	0.2
Marital status, n (%)				0.008
Never married	66 (27)	146 (38)	212 (33)	
Living together as if married	44 (18)	73 (19)	117 (18)	
Married	128 (52)	150 (639)	278 (44)	
Divorced, separated or widow	9 (3.6)	18 (4.7)	27 (4.3)	
Highest level of education, n (%)				0.4
Middle school and below	63 (26)	114 (29)	177 (28)	
High school or vocational	136 (55)	211 (55)	347 (55)	
Higer education	48 (19)	62 (16)	110 (17)	
Occupation, n (%)				0.9
Student	14 (5.7)	19 (4.9)	33 (5.2)	
Formal occupation	34 (14)	48 (12)	82 (13)	
Informal occupation	93 (38)	156 (40)	249 (39)	
Housemaker / No occupation	106 (43)	164 (42)	270 (43)	
Number of items†, n (%)	3 2-5	3 2-4	3 2-5	NA
Access to free or affordable STI testing, n (%)				0.14
Easy	146 (59)	218 (56)	364 (57)	
Not easy or not possible	43 (17)	92 (24)	135 (21)	
Don't feel concerned or DK	58 (23)	77 (20)	135 (21)	
Getting STI treatment, n (%)				0.5
Easy	149 (60)	238 (61)	387 (61)	
Not easy or not possible	39 (16)	71 (18)	110 (17)	
Don't feel concerned or DK	59 (24)	78 (20)	137 (22)	
Age at first menstruations				0.5
≤ 11 years old	15 (6.1)	26 (6.7)	41 (6.5)	
12 – 15 years old	167 (68)	278 (72)	445 (70)	
≥ 16 years old	56 (23)	74 (19)	130 (21)	
Don't remember	9 (3.6)	9 (2.3)	18 (2.8)	
Number of pregnancies, median (IQR)	2 1-3	2 1-3	2 1-3	NA
Age at first vaginal penetration, median (IQR)	18 17-19	17 16-18	17 16-19	<0.001
Number of sexual partners, n (%)				0.13
1	95 (38)	139 (36)	234 (37)	
2	55 (22)	69 (18)	124 (20)	
3	47 (19)	70 (18)	117 (18)	
4 and more	50 (20)	109 (28)	159 (25)	
Sex in exchange for favors,‡ n (%)				0.2
Yes	16 (6.5)	35 (9)	51 (8)	
No	231 (94)	352 (91)	583 (92)	
Suspicion that the partner has an STI, n (%)				0.14
Yes	46 (19)	51 (13)	97 (15)	

	Women with no STI/BV N = 247	Women with STI and/or BV N = 387	Total N = 634	p-value
No	113 (46)	179 (46)	292 (46)	
DK	88 (36)	157 (41)	245 (39)	
Condom use at last vaginal sex, n (%)				0.4
Yes	38 (15)	70 (18)	108 (17)	
No	209 (85)	317 (82)	526 (83)	
Condom use with casual partner in the past 3 months, n (%)				0.7
Always	10 (4)	22 (5.7)	32 (5.0)	
Sometimes	70 (28)	124 (32)	194 (31)	
Never	87 (35)	127 (33)	214 (34)	
No casual partner	70 (28)	100 (26)	170 (27)	
No intercourse in past 3 months	10 (4)	14 (3.6)	24 (3.8)	
Use condom as contraceptive, n (%)				0.3
Yes	9 (3.6)	21 (5.4)	30 (4.7)	
No	238 (96)	366 (95)	604 (95)	
Liquid products used to wash the vagina, n (%)				0.3
None	156 (63)	246 (64)	402 (63)	
Water	64 (26)	81 (21)	145 (23)	
Water and soap	21 (8.5)	49 (13)	70 (11)	
Other§	6 (2.4)	11 (2.8)	17 (2.7)	
Time of washing, n (%)				0.7
Wash before sex	17 (6.9)	31 (8)	48 (7.6)	
Wash after sex	4 (1.6)	11 (2.8)	15 (2.4)	
Wash before and after sex	35 (14)	50 (13)	85 (13)	
Don't wash before or after sex	191 (77)	295 (76)	486 (77)	
Frequency of washing, n (%)				0.7
Everyday	81 (33)	122 (32)	203 (32)	
Don't wash or not everyday	166 (67)	265 (68)	431 (68)	
Ever treated for STIs, n (%)				0.8
Yes	73 (30)	111 (29)	184 (29)	
No	174 (70)	276 (71)	450 (71)	
HIV status at last testing, n (%)				0.4
Positive	16 (6.5)	32 (8.3)	48 (7.6)	
Negative, DK, never tested, PNS	231 (94)	355 (92)	586 (92)	
Currently taking PrEP				0.4
Yes	11 (4.5)	23 (5.9)	34 (5.4)	
No	236 (96)	364 (94)	600 (95)	
Past or current schistosomiasis, n (%)				0.6
Yes	11 (4.5)	15 (3.9)	26 (4.1)	
No	232 (94)	361 (93)	593 (94)	
DK	4 (1.6)	11 (2.8)	15 (2.4)	
Cigarette consumption, n (%)				0.090
Yes	19 (7.7)	46 (12)	65 (10)	
No	228 (92)	341 (88)	569 (90)	
Alcohol consumption, n (%)				<0.001
Yes	94 (38)	206 (53)	300 (47)	
No	153 (62)	181 (47)	334 (53)	
Vaginal pH, median (IQR)	4.1 3.6-4.7	5 4.4-5.3	4.7 4.1-5.0	<0.001
Abnormal vaginal discharge, n (%)				0.021
Yes	67 (27)	139 (36)	206 (32)	

	Women with no STI/BV N = 247	Women with STI and/or BV N = 387	Total N = 634	p-value
No	180 (73)	248 (64)	428 (68)	
Lower abdominal pain, n (%)				0.7
Yes	59 (24)	88 (23)	147 (23)	
No	188 (76)	299 (77)	487 (77)	

* In South Africa, only the year of birth was collected, thus age was calculated using January 1st.

† Items included fridge, car, motorbike, bicycle, television, radio, phone, and computer.

‡ Sex in exchange for money, drugs, gifts, good or shelter.

§ Other products included lime juice, vinegar, and commercial product specific for genital cleansing.

IQR: Interquartile range, DK: Don't know, PNS: Prefer not to say, NA: Not applicable

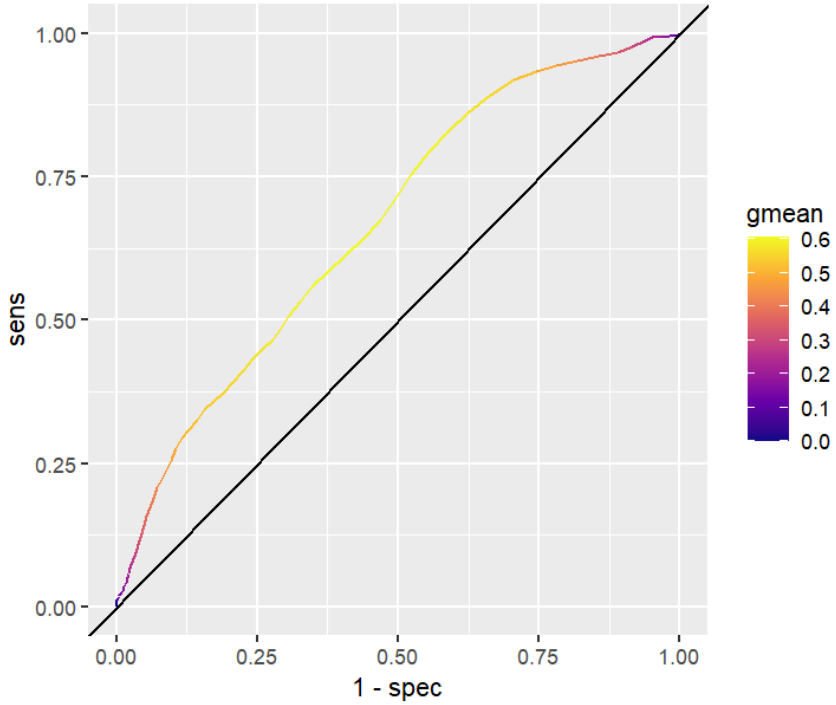
Section 2: Models for the prediction of STI alone, BV alone, CT/NG, and TV/BV

Prediction of STI compared to women with no STI

	Model STI	
	β-coefficient	**Weighting**
Vaginal pH	0.497	5.0
Age	-0.065	-0.7
Alcohol consumption	0.630	6.3
Access to a free test		
Access	Ref.	-
No access	0.947	9.5
Not concerned/DK	0.654	6.5
Threshold	12.75	

Se: 72.7 (95%CI: 66.1, 79.3)
Sp: 52.2 (95%CI: 47.6, 56.8)
PPV: 36.9 (95%CI: 31.8, 42.0)
NPV: 83.3 (95%CI: 79.0, 87.6)

AUC: 0.670 (95%CI: 0.626, 0.716)



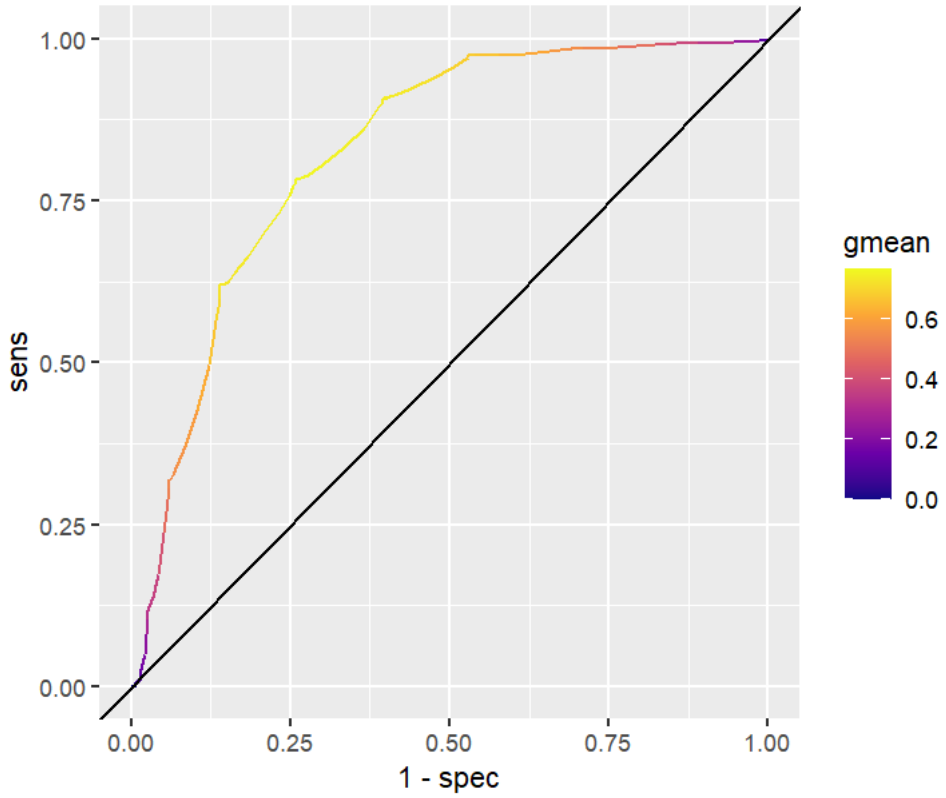
ROC curve for the prediction of CT/NG. The scale on the right represents the geometric mean of the sensitivity and the specificity.

Prediction of BV compared with women with no BV

	Model BV	
	β-coefficient	**Weighting**
Vaginal pH	2.196	22.0
Alcohol consumption	0.579	5.8
Abnormal vaginal discharge	0.624	6.2
Threshold	105.69	

Se: 78.3 (95%CI:73.7, 82.8)
Sp: 74.1 (95%CI: 69.4, 78.9)
PPV: 74.7 (95%CI: 70.0, 79.4)
NPV: 77.8 (95%CI: 73.1, 82.4)

AUC: 0.823 (95CI%: 0.790, 0.856)



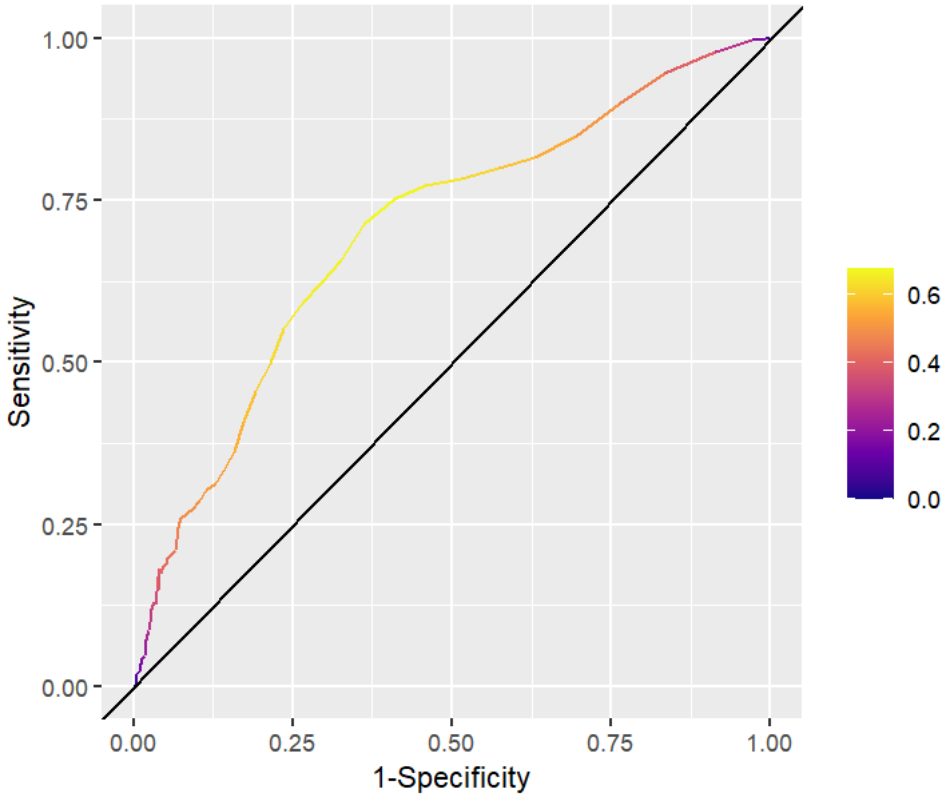
ROC curve for the prediction of BV. The scale on the right represents each threshold values.

Prediction of CT/NG excluding co-infections with BV or TV compared with uninfected women

	Model CT/NG	
	β-coefficient	**Weighting**
Age	-0.131	1.31
Food security	0.339	3.39
Threshold	-25.12	

Se: 70.7 (95%CI: 56.8, 84.7)
Sp: 66.8 (95%CI: 60.9, 72.7)
PPV: 26.1 (95%CI: 18.0, 34.3)
NPV: 93.2 (95%CI: 89.5, 96.9)

AUC: 0.704 (95%CI: 0.615, 0.704)



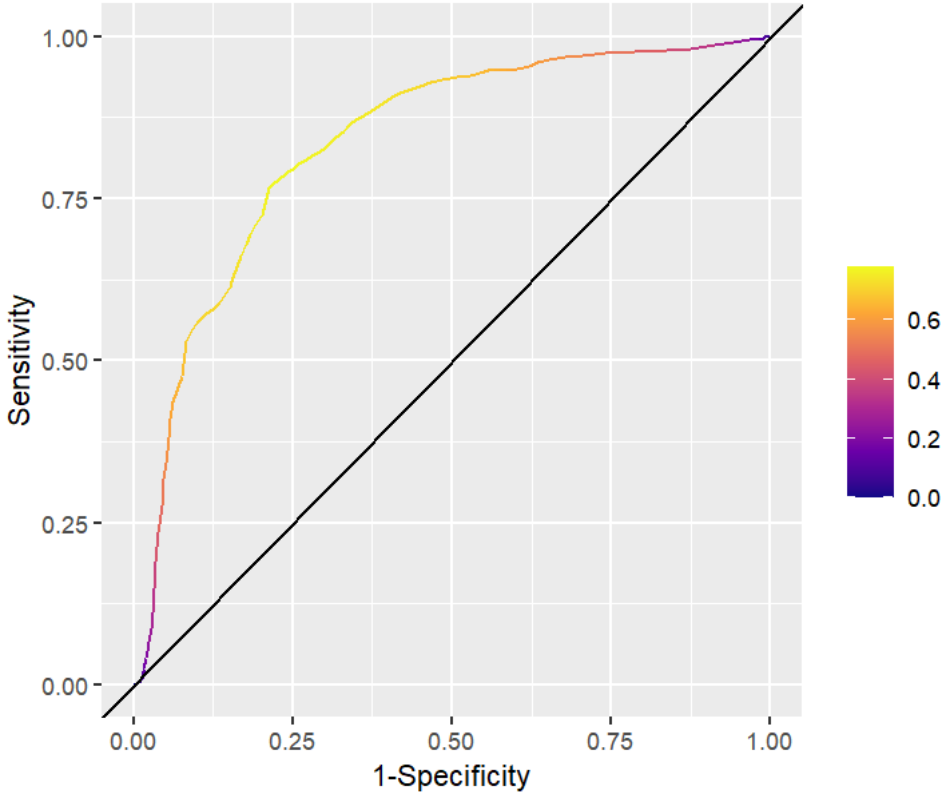
ROC curve for the prediction of CT/NG. The scale on the right represents each threshold values.

Prediction of TV/BV excluding co-infections with CT or NG compared with uninfected women

	Model TV/BV	
	β-coefficient	**Weighting**
Vaginal pH	2.206	22.1
Age at first vaginal penetration	-0.155	-1.6
Threshold	74.63	

Se: 80.1 (95%CI: 75.3, 84.9)
Sp: 76.1 (95%CI: 70.8, 81.4)
PPV: 78.3 (95%CI: 73.4, 83.2)
NPV: 78.0 (95%CI: 72.8, 83.2)

AUC: 0.837 (95%CI: 0.802, 0.871)



ROC curve for the prediction of TV/BV. The scale on the right represents each threshold values.

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