Improving Sexually Transmitted Infection diagnosis and control among men who have sex with men in Belgium

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Promotoren **dr. Tania Crucitti | dr. Bea Vuylsteke | prof. dr. Guido Vanham**
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Proefschrift voorgedragen tot het behalen van de graad van doctor in de biomedische wetenschappen
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Het verbeteren van de diagnose en controle van seksueel overdraagbare aandoeningen bij mannen die seks hebben met mannen in België

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Dr. Bea Vuylsteke

Prof. Dr. Guido Vanham

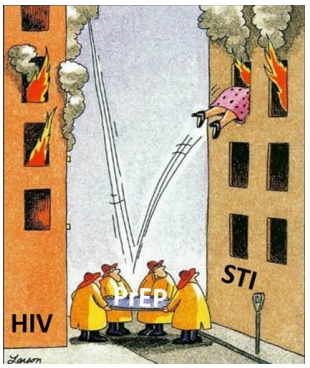
Prof. Dr. Chris Kenyon

DANKWOORD

Eindelijk! De laatste sectie van de thesis die we moeten neerschrijven, maar wel de leukste! Ik begin eigenlijk met plagiaat van Liselotte Hardy (jawel ik heb haar toestemming gevraagd! 😉). ***Ik ging absoluut niet (nooit, never, jamais) aan een doctoraat beginnen*** Die woorden waren dus ook van toepassing bij mij… Te veel werk, ik wil niet alle weekends aan de pc zitten, ik wil bij de kinderen zijn…maar ja… Uiteindelijk heeft een fantastisch collega (Tom Platteau) me laten inzien dat het echt wel dom zou zijn om het niet te doen. Want ik had al een aantal artikels, deed alle research, moest toch nog een aantal publicaties schrijven, dus waarom die weg niet volgen? En ja, tijdens die ene treinreis van het symposium seksuele gezondheid in het Vlaams Parlement (14/12/2018) naar Antwerpen stelde hij nogmaals de vraag nadat ik vermeldde dat er nog een artikel aanvaard was: "jamaar, wat ga je nu doen? Waarom doe je nu toch geen doctoraat?" We zaten op dat moment ook in het labo met een serieuze reorganisatie waardoor het hiv/soa referentielaboratorium werd samengevoegd met het klinisch laboratorium van het ITG. Die verliep niet zo vlot (op zn minst zacht uitgedrukt) en het was volledig onduidelijk waar ik en andere collega's gingen eindigen. Ik wou voor mezelf de weg uitstippelen in plaats van dat anderen die voor mij zouden maken. En dan werd de klik gemaakt. Ja! Ik ga voor dat PhD! Toen ik terug thuiskwam heb ik dan ook gezegd dat ik het toch ging doen. Mijn ouders blij dat ik eindelijk dat licht had gezien maar Simon zei: OE? JIJ GING DAT TOCH NOOIT DOEN? Maar ja eens ik een beslissing heb gemaakt is het moeilijk om erop terug te komen.

De volgende stap was het onderwerp en natuurlijk de promotoren. Maar ook dat was niet moeilijk! Ik was gestart op het ITG als laboratorium coördinator van de FEM-PrEP studie (2010), een PrEP studie bij jonge vrouwen in Kenia, Zuid-Afrika en Tanzania, dus PrEP was geen onbekende. Daarna (2015-2018) werd er onder leiding van Marie Laga de Be-PrEP-ared studie op het ITG opgestart, een PrEP studie bij mannen die seks hebben met mannen of MSM, waarbij ik van in het begin zeer nauw betrokken was.

Vervolgens zijn we eind 2017 ook nog gestart met de coördinatie van het soa gedeelte van de CohMSM-PrEP studie, weer een PrEP studie bij MSM, maar deze keer in 4 West-Afrikaanse landen. Opvolging van soa bij PrEP gebruikers is belangrijk. PrEP wordt gebruikt door HIV negatieve personen ter preventie van HIV, maar zoals je op de cartoon ziet, beschermt PrEP niet tegen soa's, en hierdoor is er de terechte zorg dat er een stijging van verscheidene soa's komt in deze groep. Dus mijn onderwerp werd: het verbeteren van de diagnose en controle van soa bij MSM in België, en dan voornamelijk bij PrEP gebruikers.



Vervolgens de keuze van de promotoren! Natuurlijk kwam **Tania Crucitti** op de eerste plaats. Je bent mijn mentor en ik heb dan ook alles, maar dan ook alles, van u geleerd. Ik kan er u niet genoeg voor bedanken! Dit werk zou er zonder u absoluut niet staan! Ik vind het nog altijd jammer dat we niet meer samen op het ITG werken, en dat ik niet zomaar in je bureau kan binnenvallen om raad te vragen. Alsook dat je door de pandemie spijtig genoeg niet real-life aanwezig kan zijn. Maar dat wil natuurlijk niet zeggen dat we er in de toekomst niet samen op kunnen klinken en geen projecten kunnen opstarten 😉. Wie weet wat de toekomst brengt! Vervolgens **Bea Vuylsteke**, in het begin was het even wennen, maar nu ben jij diegene naar wie ik toe loop als ik met vragen zit over soa. We werken nu sinds 2015 zeer nauw samen en ik vind het fantastisch! We vullen elkaar mooi aan. Je hebt bakken ervaring en ik hang dan ook altijd aan je lippen van zodra je begint te vertellen. Dus die promotoren waren geregeld! Maar…op het ITG heb je iemand nodig die geaffilieerd is aan een universiteit vooraleer je een PhD kan uitvoeren. En gelukkig was **Guido Vanham** nog niet op pensioen. Als er één persoon was die mij kon begeleiden in het PhD traject dan was hij het wel! Vroeger had ik les van hem gekregen, ik keek enorm naar hem op, en miste dan ook geen enkele les van hem (en die was telkens woensdagmorgen, en eerlijk

gezegd was ik niet zo'n goede student, volgde niet zoveel lessen en zat meer in 't kaf te kaarten … sorry mama & papa 😉), maar voor hem stond ik heel graag op! Aangezien onze groep hiv/soa steeds de 10h koffiepauze in de gang van "virologie" nam, kwam ik hem ook regelmatig tegen, dus, de volgende persoon aan wie ik raad ging vragen was hij. Ik stormde dan ook redelijk snel zijn bureau binnen, legde mijn plannen uit (net voor de kerstvakantie), en tot mijn verbazing zei hij bijna onmiddellijk dat hij dat wel zag zitten! Super! Het is een eer dat ik jouw laatste PhD mag zijn! Je hebt me ook op subtiele, en soms ook minder subtiele, manieren geleid in het schrijven van de thesis. Eentje zal ik nooit vergeten: "in een tuin laat je ook niet alles groeien" toen ik de eerste draft van de discussie had doorgestuurd. En ja, ik kon alles terug herschrijven. Of wat minder subtiel…Hier begin ik zelfs nog niet aan 😊 Maar zo heb ik het graag! Heel hard bedankt voor alle raad!

Mijn **THREESOME** was gemaakt! En dan nog wel uit elk departement één, als dat geen **interdisciplinair team** was! Maar…het mocht zo niet doorgaan blijkbaar volgens de ITG regels: Guido ging op pensioen in juni 2019, Bea en Tania waren geen ZAP dus nee, dat ging dan niet (misschien wordt het tijd om deze regel toch maar eens te herzien?). Maar ik wou absoluut niet van hun afwijken, no way, Bea en Tania moesten erop! Gelukkig is er een andere fantastische collega die me uit de nood kwam helpen. **Chris Kenyon**, heel hard bedankt dat je dit voor mij hebt willen doen, je bent een vat vol kennis, en je hebt de fantastische gave om iedereen te overtuigen van je standpunten. Of toch bijna 😉 Als ik je tijdens meetings vol overgave zie/hoor spreken over de gevaren van teveel screenen op soa omwille van antimicrobiële resistentie overtuig je mij bijna dat we moeten stoppen met het regelmatig screenen van soa bij PrEP gebruikers. Maar, vervolgens keer ik dan terug naar mijn bureau, laat alles bezinken, begin me terug te verdiepen in de literatuur, en dan kom ik er toch op terug zoals ik ook aanbeveel in de thesis. Desondanks onze meningsverschillen ben ik heel blij dat je het hoofd van de soa unit op het ITG bent en dat ik zeer nauw met je kan samenwerken.

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Anne Buvé, heel hard bedankt voor het nalezen van de thesis en me klaar te stomen voor de interne verdediging door een aantal kritische vragen op te lijsten! Je bent ook echt één van die personen waar ik gigantisch naar op kijk. Ik vind het jammer dat je op pensioen bent!

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studie. Ik vind het ook zalig om jouw visie te horen en info uit te wisselen. Vervolgens de **Franse & West-Afrikaanse collega's & deelnemers van de CohMSM-PrEP studie.**

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En last but not least: **My three guys**… Wij zijn pas een **TOPTEAM**! **Simon**, heel hard bedankt om mij te blijven steunen, te luisteren naar mn gezaag, de kinderen op te vangen toen ik aan de thesis moest werken. Je brengt me tot rust en soms ook niet 😉. Ik zou niet weten wat ik zonder je zou moeten doen! **Bastian en Victor**, nu zijn de weekends weer voor jullie. Mama moet niet meer werken (althans toch niet in de weekends), YIIIIIIIIEEEEEEHHHHAAAAAAAAAAAAAAAAA! Laat die nu maar komen! En als we op vakantie gaan, en ik de volgende afslag zie, zal ik heel even terugkijken naar deze zware maar zeer leerrijke periode, om vervolgens snel terug te genieten van jullie aanwezigheid en in mn gedachten toch rechtdoor te blijven rijden richting een onbezorgde vakantie!

SUMMARY

The number of bacterial sexually transmitted infections (STIs) among Gay, Bisexual and other Men who have Sex with Men (hereafter called MSM) is dramatically increasing over the last decade. Some have posited that the introduction of several biomedical HIV prevention methods including treatment as prevention (TasP) and Pre-exposure Prophylaxis or PrEP have played a role in these epidemics. They argue that these prevention methods may promote riskier sexual behaviour i.e. increase in condomless anal intercourse (CAI), more seromixing between HIV negative and positive MSM and/or an increase in concurrent sexual partners. Besides leading to an overall increase of STIs among MSM, a dissemination of STIs, previously confined to HIV positive MSM such as syphilis, Hepatitis C and lymphogranuloma venereum or LGV, to PrEP taking MSM engaging in high risk behaviour may occur as well.

The increase in STIs may be particularly fuelled by individuals who are repeatedly infected with STIs (recurrent STIs) since they occupy crucial and central positions in dense sexual networks. Identifying these individuals may therefore be of public health importance to interrupt the chain of transmission of the STI epidemic among MSM. In this thesis, we used epidemiological data of the Be-PrEP-ared study, the Belgian PrEP demonstration project, to scrutinize the STI epidemic among MSM PrEP users and to identify the behavioural factors that are associated with recurrent STIs (**chapter 3**). Among 179 MSM participating in that study, 62% had at least one incident bacterial STI (chlamydia, gonorrhea or syphilis) during 18 months of follow-up, and in fact, more than one in three of the participants experienced recurrent STIs. The most important behavioural characteristic associated with recurrent STIs was sexualized drug use: it was reported in almost 90% of the individuals who were repeatedly infected with STIs. In addition, all LGV infections, except one, were confined to the group that experienced recurrent STIs. This result highlights the importance this group may have on the reported shift of the LGV epidemic from HIV positive to HIV negative MSM. Moreover,

it has been hypothesized that the roll-out of PrEP may further increase the rise of LGV cases among HIV negative MSM. In **chapter 4**, we showed that the increase of LGV cases already occurred before the roll-out of PrEP and that the rise did not accelerate after the introduction of PrEP in Belgium. Nevertheless, the number of LGV cases among PrEP users doubled from 1.2% in 2018 to 2.4% in 2020 at the STI clinic of the Institute of Tropical Medicine. This increase in LGV cases among PrEP users may suggest that the group of individuals with recurrent STIs is enlarging. As such, further surveillance of the LGV epidemic in MSM irrespective of their HIV status is warranted.

Besides the increase in STIs, the scientific community is also concerned about a swift emergence of multi-drug resistant STIs. Indeed, *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG) may evolve to "superbugs" in the not-too-distant future. MG is recently gaining interest as an STI. While certain aspects of the natural history of MG are yet to be determined, it is already evident that the infection usually remains asymptomatic. Indeed, in our Be-PrEP-ared study, MG was the most prevalent STI (17.2%) and most of the infections were asymptomatic (82%). Emergence of macrolide resistant MG, driven by suboptimal macrolide dosage provided for other STIs is reported globally. As a matter of fact, antimicrobial consumption is one of the key drivers of antimicrobial resistance and therefore PrEP users with recurrent STIs may be more at risk to generate multi-resistant STIs, including MG, and may consequently, play a central role in the STI and antimicrobial resistance epidemics. In **chapter 5** we explored the prevalence of resistance-associated mutations (RAMs) to macrolides and fluoroquinolones in MG in the Be-PrEP-ared study and found an astonishingly high prevalence of macrolide RAMs (nearly 90%). In addition, one in four of the MG samples even harboured RAMs to both antimicrobials which are currently used as the first- and second line treatment for MG. Further exploration of the data showed that these high numbers of resistance were driven by the MG cases identified among MSM with recurrent STIs. In these MSM, macrolide RAMs were found in 95% of the MG cases and RAMs to both antimicrobials were found in one out of three. These results strengthen

our evidence on two issues: first, screening and subsequent treatment of asymptomatic MG cases in MSM does more harm than good by increasing the possibility of antimicrobial resistance and second, STIs should not be treated with macrolides wherever possible. Finally, we also reported on the high prevalence of macrolide resistance in the heterosexual population which highlights the need for further antimicrobial stewardship in the general population.

Due to the high prevalence and incidence of STIs found among PrEP users, Belgian PrEP guidelines still recommend quarterly STI detection in MSM including syphilis, chlamydia (CT) and further genotyping to confirm LGV and gonorrhea in three anatomical sites - urethra, anorectum and pharynx. Yet, due to current budget constraints in the healthcare sector and the heavy burden caused by this frequent sampling on healthcare workers, laboratory professionals and PrEP users, it is of general interest to introduce novel, cost-effective STI screening strategies. In **chapter 6** two different pooling strategies to test for CT/NG are documented and in **chapter 7** we explored the use of home-based self-sampling for STI detection among PrEP users. The current Covid-19 pandemic revealed the increasing necessity to adopt novel remote STI screening strategies in case of service interruptions. To facilitate remote care, future mHealth tools tailored for PrEP users need to include home-based self-sampling to test for STIs. These mHealth tools (discussed in the last chapter), may be introduced to complement routine care as soon as they have been found to be acceptable and feasible in large clinical studies.

In conclusion, my thesis delineates the central role individuals with recurrent STIs have in the STI epidemic among MSM. These individuals fuel the STI epidemic through the large sexual networks in which they operate and they may play an important role in the emergence of multi-resistant STIs. While the role of MG as a pathogen is still unclear, multi-resistant NG may have devastating consequences such as infertility in women or even death in very rare cases. Therefore, frequent STI testing is still recommended in

this group. As such, novel STI testing algorithms should be tailored on the individual depending on the risk of experiencing recurrent STIs and reported sexual behaviour.

SAMENVATTING

Het aantal bacteriële seksueel overdraagbare aandoeningen (soa's) onder homo's, biseksuelen en andere mannen die seks hebben met mannen (hierna MSM genoemd) is de laatste tien jaar dramatisch toegenomen. Sommige onderzoekers vermoeden dat de invoering van verschillende biomedische hiv-preventiemethoden, waaronder behandeling als preventie of “Treatment as prevention” (TasP) en preventie voorafgaan aan blootstelling of “pre-exposure profylaxis” (PrEP), een rol hebben gespeeld in deze epidemieën. Zij stellen dat deze preventiemethoden risicovoller seksueel gedrag kunnen bevorderen, d.w.z. een toename van anale seks zonder condoom, meer seromixing tussen hiv-negatieve en hiv-positieve MSM en/of een toename van gelijktijdige seksuele partners. Naast een algehele toename van soa's onder MSM kan er ook een verspreiding optreden van soa’s die voorheen enkel voorkwamen bij hiv-positieve MSM, zoals syfilis, hepatitis C en lymfogranuloma venereum of LGV, naar MSM met een hoog risico gedrag die PrEP gebruiken.

De toename van soa's kan vooral versterkt worden door personen die herhaaldelijk met soa's besmet zijn (terugkerende soa's) aangezien zij cruciale en centrale posities innemen in omvangrijke seksuele netwerken. Om de transmissieketen van de soa-epidemie bij MSM te doorbreken, kan het identificeren van deze personen daarom van belang zijn voor de volksgezondheid. In deze thesis hebben we gebruik gemaakt van epidemiologische gegevens van de Be-PrEP-ared studie, het Belgische PrEP-demonstratieproject, om de soa-epidemie onder MSM PrEP-gebruikers onder de loep te nemen en om gedragsfactoren te identificeren die geassocieerd zijn met het hebben van terugkerende soa's **(hoofdstuk 3)**. Van de 179 MSM die aan die studie deelnamen, had 62% ten minste één bacteriële soa (chlamydia, gonorroe of syfilis) gedurende 18 maanden follow-up, en meer nog: meer dan één op de drie deelnemers had terugkerende soa's. Het belangrijkste gedragskenmerk dat geassocieerd werd met terugkerende soa's was geseksualiseerd drugsgebruik: dit werd gerapporteerd bij bijna

90% van de personen die herhaaldelijk besmet waren met soa's. Bovendien bleven alle LGV-infecties, op één na, beperkt tot de groep met terugkerende soa's. Dit resultaat benadrukt het belang dat deze groep kan hebben op de gerapporteerde verschuiving van de LGV-epidemie van hiv-positieve naar hiv-negatieve MSM. Bovendien werd er gesuggereerd dat de implementatie van PrEP de toename van LGV-gevallen onder hiv-negatieve MSM verder kan vergroten. In **hoofdstuk 4** hebben we laten zien dat de toename van LGV-gevallen in België al plaatsvond voor de implementatie van PrEP, en dat de toename in het aantal LGV gevallen bij hiv-negatieve MSM niet versnelde na de introductie van PrEP. Desondanks is het aantal LGV-gevallen onder PrEP-gebruikers verdubbeld van 1,2% in 2018 naar 2,4% in 2020 in de soa kliniek van het Instituut voor Tropische Geneeskunde. Deze toename van LGV-gevallen onder PrEP-gebruikers kan erop wijzen dat de groep van personen met terugkerende soa's groter wordt. Verdere surveillance van de LGV-epidemie bij MSM, ongeacht hun hiv-status, is daarom nodig.

Naast de toename van het aantal soa's, maken wetenschappers zich ook zorgen over een snelle opkomst van multiresistente soa's. *Neisseria gonorrhoeae* (NG) en *Mycoplasma genitalium* (MG) kunnen in de nabije toekomst ontwikkelen tot "superbugs". MG als soa wint de laatste tijd aan belang. Hoewel bepaalde aspecten van de pathogenese van MG nog moeten worden uitgewezen, is het nu al duidelijk dat de infectie meestal asymptomatisch blijft. In onze Be-PrEP-studie was MG inderdaad de meest voorkomende soa (17,2%) en de meeste infecties waren asymptomatisch (82%). De opkomst van macrolide-resistente MG, gedreven door suboptimale macrolidedosering die voor andere soa's wordt verstrekt, wordt wereldwijd gemeld. Antimicrobiële consumptie is een van de belangrijkste oorzaken van antimicrobiële resistentie en daarom lopen PrEP-gebruikers met terugkerende soa's mogelijk meer risico op het ontstaan van multiresistente soa's, waaronder MG. Daardoor kunnen zij een centrale rol spelen in de epidemieën van soa's en antimicrobiële resistentie. In **hoofdstuk 5** onderzochten we de prevalentie van resistentie-geassocieerde mutaties (RAMs) tegen macroliden en fluoroquinolones in MG in de Be-PrEP-ared studie en

vonden een verbazingwekkende hoge prevalentie van macrolide RAMs (bijna 90%). Bovendien had één op de vier MG stalen zelfs RAM's tegen beide antibiotica die momenteel gebruikt worden als eerste- en tweedelijnsbehandeling voor MG. Bij verdere analyse van de data bleek dat deze hoge resistentiecijfers werden veroorzaakt door de MG-gevallen die vastgesteld werden bij MSM met terugkerende soa's. Bij deze MSM werden, in 95% van de MG-gevallen, RAM's gevonden tegen macrolides en werden RAM's tegen beide antibiotica gevonden in één op de drie van de MG cases. Deze resultaten versterken twee belangrijke bevindingen: ten eerste doet screening en vervolgens behandeling van asymptomatische MG-gevallen bij MSM meer kwaad dan goed doordat de kans op antimicrobiële resistentie toeneemt, en ten tweede moeten soa's waar mogelijk niet met macroliden worden behandeld. Ten slotte rapporteerden we ook over de hoge prevalentie van macrolide resistentie in de heteroseksuele populatie, wat de noodzaak aantoont van het voortzetten van antimicrobieel beheer in de algemene bevolking.

Door de hoge prevalentie en incidentie van soa's bij PrEP-gebruikers, bevelen de Belgische PrEP-richtlijnen nog steeds driemaandelijkse soa-detectie aan bij MSM, inclusief syfilis, chlamydia (CT) en verdere genotypering om LGV te bevestigen, en gonorroe op de drie anatomische plaatsen - urethra, anorectum en farynx. Vanwege de huidige budgettaire beperkingen in de gezondheidszorg en de zware belasting die deze frequente staalafname met zich meebrengt voor gezondheidswerkers, laboratoriummedewerkers en PrEP-gebruikers, is het van algemeen belang om nieuwe, kosteneffectieve strategieën voor soa-screening te introduceren. In **hoofdstuk 6** worden twee verschillende pooling strategieën om te testen op CT/NG gedocumenteerd en in **hoofdstuk 7** hebben we onderzoek gedaan naar "home-based self-sampling" of het thuis zelf afnemen van stalen voor soa detectie bij PrEP gebruikers. De huidige Covid-19 pandemie toont de urgentie aan om nieuwe strategieën voor soa-screening te ontwikkelen in het geval van onderbrekingen in de dienstverlening. Om zorg op afstand te vergemakkelijken, moeten toekomstige

mHealth applicaties op maat van PrEP-gebruikers, “home-based self-sampling” bevatten zodat er getest kan worden op soa's. Deze mHealth tools (besproken in het laatste hoofdstuk), kunnen worden geïntroduceerd als aanvulling op de routine zorg zodra ze acceptabel en haalbaar zijn gebleken in grote klinische studies.

Tot slot toont deze thesis de centrale rol aan die mensen met terugkerende soa's hebben in de soa-epidemie onder MSM. Deze mannen stuwen de soa-epidemie door de grote seksuele netwerken die ze hebben en verder spelen zij mogelijk een belangrijke rol in het ontstaan van multiresistente soa's. Alhoewel de rol van MG als ziekteverwekker nog onduidelijk is, kan multiresistente NG desastreuze gevolgen hebben, zoals onvruchtbaarheid bij vrouwen of, in zeer zeldzame gevallen, tot de dood leiden. Daarom wordt het nog steeds aanbevolen om regelmatig te testen voor soa’s in deze groep. Bijgevolg moeten nieuwe soa test-algoritmes afgestemd worden op het individu, afhankelijk van het risico op terugkerende soa's en het gerapporteerde seksuele gedrag.

THESIS OUTLINE

CHAPTER 1 In this chapter, a general introduction of the global and Belgian STI epidemic will be provided, with a special focus on the evolution of the STI epidemic among gay, bisexual and other men who have sex with men (hereafter called MSM) after the introduction of biomedical HIV prevention methods such as pre-exposure prophylaxis (PrEP). The thesis will then narrow down onto the challenges in the field of STIs diagnosis and control among MSM.

CHAPTER 2 Here, the main objectives of the thesis will be described and how we will contribute to the improvement of the diagnosis and control of STIs among MSM.

CHAPTER 3, 4, 5, 6 and 7 will provide all the published work.

CHAPTER 8 Finally, we will discuss the impact of our results on the field of STI diagnosis and control. In conclusion, we will provide recommendations for future STI guidelines among MSM in Belgium.

TABLE OF CONTENTS

ABBREVIATIONS

(S)AE: (Serious) adverse event

ALT/AST: Alanine transaminase/Aspartate transaminase

AMR: Antimicrobial resistance

ARV: Antiretroviral

CA: Competent Authority

CAI: Condomless anal intercourse

c(ART): combination antiretroviral therapy

CAB: Community Advisory Board

CE: Conformité Européene

CI: Confidence Interval

CT: *Chlamydia trachomatis*

DBS: Dried blood spot

DC: Delta Cycle

DSMB: Data and safety monitoring board

EC: Ethics committee

eCRF: Electronic case report form

EMA: European Medicines Agency

FDA: Food and Drug Administration

FTC: Emtricitabine

FU: Follow-up

GCLP: Good Clinical Laboratory Practices

GCP: Good Clinical Practices

GHB: Gamma-Hydroxybutyrate

HBSS: Home-based self-sampling

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

HPV: Human papilloma virus

HSV-2: Herpes simplex-2 virus

ICH: International Conference on Harmonization

IDI: In-depth interview

IR: Incidence Ratio

IRB: Institutional Review Board

ISO: International Organisation for Standardization

ITM: Institute of Tropical Medicine

LGV: Lymphogranuloma venereum

LMIC: Low and middle income countries

MSM: Gay, bisexual and other men who have sex with men

MSTM: Men and transgender women who have sex with men

MG: *Mycoplasma genitalium*

NAAT: Nucleic Acid Amplification Test

NG: *Neisseria gonorrhoeae*

NRC: National Reference Centre

(a)OR: (adjusted) Odds Ratio

PBS: Phosphate Buffered Saline

PEP: Post-exposure prophylaxis

POCT: Point-Of-Care Test

PrEP: Pre-exposure prophylaxis

PY: Person-years

RCT: Randomized Controlled Trial

RDT: Rapid Diagnostic Test

STI: Sexually Transmitted Infection

SUSAR: Suspected unexpected serious adverse reaction

TasP: Treatment as prevention

TDF: Tenofovir disoproxil fumarate

TFV: Tenofovir

TFV-DP: Tenofovir diphosphate

TP: *Treponema pallidum*

TV: *Trichomonas vaginalis*

UK: United Kingdom

US: United States

WHO: World Health Organization

CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Sexually transmitted infections (STIs) are caused by diverse bacteria, viruses or parasites and are transmitted from one individual to another by primarily vaginal, anorectal or oral sexual contact. In addition, some STIs may also spread through non-sexual blood contacts, orofecal routes of transmission, or via mother-to-child transmission. Whilst there are over 30 different pathogens that can be sexually transmitted, nine are linked to the greatest incidence of STIs: five viral, three bacterial and one parasitic. The five viral STIs can cause severe chronic infections: Herpes simplex virus type I and II (HSV); Human Immunodeficiency Virus (HIV); Human Papilloma Virus (HPV), the primary cause of cervical cancer, and Viral Hepatitis B or C (HBV or HCV). Most of these viral STIs, except for HCV, are incurable and, it is estimated that, in 2016, around 417 million people are living with genital HSV; the overall prevalence of HPV among women was 291 million and around 36.7 million people were living with HIV.[1,2] Furthermore, each day, more than 1 million adults between 15-49 years of age became infected in 2016 with one of the other four STIs: chlamydia including Lymphogranuloma Venereum or LGV (causative agent *Chlamydia trachomatis* (CT)), gonorrhea (*Neisseria gonorrhoeae* (NG)), syphilis (*Treponema pallidum*) and trichomoniasis (*Trichomonas vaginalis* (TV)). [3] Although that the latter four STIs are curable, approximately 376 million new infections were estimated to occur in 2016, and this number is increasing rapidly (Figure 1).[3,4]

Another pathogen that is gaining importance as an STI is *Mycoplasma genitalium* (MG) which is also curable. Despite that figures from the WHO are lacking of this pathogen, it is thought to be exceedingly prevalent.[5] A recent meta-analysis estimated a prevalence of MG of 1.3% in the general population of high-resource countries.[6] Clearly, it should be added to the list as the 10th member of the very prevalent STIs.

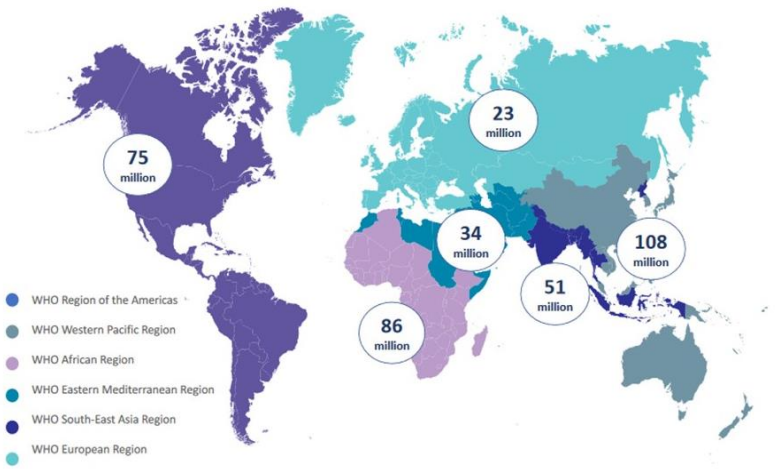

Figure 1: Number of chlamydia, gonorrhea, syphilis and trichomoniasis cases in 2016.[7]

Table 1: 2016 WHO regional prevalence estimates of chlamydia, gonorrhoea, trichomoniasis and syphilis per gender[7]

	Chlamydia	Gonorrhea	Trichomoniasis	Syphilis
Women				
Africa	5.0 (3.8–6.6)	1.9 (1.3–2.7)	11.7 (8.6–15.6)	1.6 (1.2–2.0)
America	7.0 (5.8–8.3)	0.9 (0.6–1.5)	7.7 (5.1–11.5)	0.9 (0.7–1.1)
South-East Asia	1.5 (1.0–2.5)	0.7 (0.4–1.2)	2.5 (1.3–4.9)	0.2 (0.1–0.4)
Europe	3.2 (2.5–4.2)	0.3 (0.1–0.6)	1.6 (1.1–2.3)	0.1 (0.0–0.4)
Eastern Mediterranean	3.8 (2.6–5.4)	0.7 (0.5–1.1)	4.7 (3.3–6.7)	0.7 (0.4–1.0)
Western Pacific	4.3 (3.0–5.9)	0.9 (0.5–1.3)	5.6 (2.7–10.8)	0.2 (0.1–0.4)
Global Total	**3.8 (3.3–4.5)**	**0.9 (0.7–1.1)**	**5.3 (4.0–7.2)**	**0.5 (0.5–0.6)**
Men				
Africa	4.0 (2.4–6.1)	1.6 (0.9–2.6)	1.2 (0.7–1.8)	1.6 (1.2–2.0)
America	3.7 (2.1–5.5)	0.8 (0.4–1.3)	1.3 (0.9–1.8)	0.9 (0.7–1.2)
South-East Asia	1.2 (0.6–2.1)	0.6 (0.3–1.1)	0.2 (0.1–0.5)	0.2 (0.2–0.4)
Europe	2.2 (1.5–3.0)	0.3 (0.1–0.5)	0.2 (0.1–0.3)	0.1 (0.0–0.3)
Eastern Mediterranean	3.0 (1.7–4.8)	0.6 (0.3–1.0)	0.5 (0.3–0.7)	0.7 (0.4–1.0)
Western Pacific	3.4 (2.0–5.3)	0.7 (0.4–1.2)	0.6 (0.2–1.1)	0.2 (0.1–0.4)
Global Total	**2.7 (1.9–3.7)**	**0.7 (0.5–1.1)**	**0.6 (0.4–0.9)**	**0.5 (0.4–0.6)**

In general, STI prevalence is clearly higher in women (estimated to be 3.8 % for CT, 0.9% for NG, 5.3% for TV and 0.5% for syphilis for women and 2.7%, 0.7%, 0.6% and 0.5% respectively for men). However, extreme differences in prevalence are found over the

different world regions (Table 1).[7] Low-income countries have the highest estimated prevalence for gonorrhea, syphilis and trichomoniasis. In contrast, prevalence of chlamydia is the highest in upper-middle income countries, partly due to high estimates in some Latin American countries.[7]

Besides these 10 most important STIs, other STIs also include pubic lice or other infectious diseases that are occasionally transmitted sexually such as *Shigella spp.*, *Giardia intestinalis* through orofecal routes of transmission (for example via rimming). Moreover, although rare, Arboviruses (Zika virus) and Filoviruses (Ebola virus) can also be transmitted sexually.

In this thesis, we will only focus on the following four curable bacterial STIs being CT/NG/MG and *T. pallidum*.

1.1.1 Burden of STIs

Although most STIs usually are not fatal, the global burden of STIs is high.[8,9] The primary infection of many STIs is often asymptomatic and therefore, tends to go unnoticed. Nevertheless, acute symptoms such as cervicitis, urethritis, pharyngitis and proctitis are common for CT/NG and MG, but these STIs can also cause more severe and chronic complications. Indeed, untreated CT/NG and MG may cause serious sequelae such as chronic pelvic pain, pelvic inflammatory disease, postpartum endometriosis and infertility in women; including adverse pregnancy outcomes such as ectopic pregnancy, premature delivery and premature death. In men, these STIs may lead to prostatitis, urethral strictures, ulcerative proctitis and epididymitis, but the overall disease burden is clearly lower in men. Furthermore, rarely and only in the case of NG, disseminated infection is also reported in both genders and may lead to infectious arthritis, endocarditis and meningitis.[8,10] Syphilis can even progress to nervous system and cardiovascular damage and eventually to death.

Any untreated STI among pregnant women may result in mother-to-child transmission and may lead to congenital syphilis, neonatal conjunctivitis in the case of NG and CT and neonatal chlamydia pneumoniae. Syphilis is still the second most common infectious cause of stillbirth globally.[11] Finally, various other STIs can increase the susceptibility to and transmission of HIV due to, for instance, mucosal inflammation or ulceration.[12] Therefore, HIV and STI prevention are intertwined since decades.

Besides these physical conditions, STIs can also result in a decrease of quality of life as STIs are frequently associated with vulnerability, stigma, stereotyping, shame and violence.[7] As a consequence, STIs result in a global burden of morbidity, mortality and financial consequences.[3,13]

Moreover, most STIs do not induce strong protective immunity and therefore recurrent infections may take place. Although investigators still lack a clear understanding of the immuno-pathogenesis of all STIs, it is known that STIs may evade the host immune system by entering epithelial cells (CT, NG, MG) or even neutrophils in the case of NG and further reproduce in them. In addition, MG and NG have very high antigenic variation of their surface-exposed proteins which plays a very important role in immune avoidance.[14,15]Antigenic variation through gene conversion also plays an important role in *T. pallidum* immunopathogenesis.[16] In CT, the presence of protective immunity has been debated, however it can take up to five years to efficiently clear the infection as CT may persist in the host as aberrant bodies which are non-infectious but viable, and which can re-enter the normal development life cycle.[17] Nevertheless, according to the "arrested immunity" hypothesis, immediate treatment reduces the immune response and therefore treated individuals may be more at risk for recurrent CT infections.[18] This hypothesis however requires further study and has not yet been confirmed for other STIs.

The burden of STI sequelae is the highest among women and children in low & middle income countries (LMIC) such as Sub-Saharan African countries. This is mainly due to

the delayed or inadequate diagnosis and treatment which is caused by inequalities and poor accessibility of health care settings, diagnostic testing and availability of drugs and clinic supplies.[19] Furthermore, other sociocultural, political and economic factors may also contribute to the larger STI burden in low-income countries. Despite the major health burden caused by STIs in LMIC, many countries are still applying the syndromic approach, mainly due to limited resources, including lack of trained personnel and laboratory services. The syndromic management of STIs however has many limitations as it does not detect asymptomatic STIs and has a poor positive predictive value, resulting in overuse of antibiotics, which in turn, may increase the development of antimicrobial resistance of several STIs, a phenomenon that will be more elaborated in the section below.[20]

STIs also disproportionally affect key populations that have unprotected sex with multiple partners such as female sex workers and young adults. Another key target population are gay, bisexual or other men who have sex with men (hereafter called MSM).[1] Individual MSM can engage in both insertive and receptive roles and, furthermore, penile-anal, penile-oral (i.e. fellatio) and oral-anal sex (i.e. rimming), all carrying a high risk on STI transmission, are more frequently practiced among MSM than among heterosexuals.[21,22] Besides above-mentioned STI transmission routes, a study showed that even only-kissing was reported to be associated with acquisition of NG among MSM.[23] In that study, of the MSM that reported kissing-only without sex in the previous three months, 3.8% were NG positive at the oropharynx (n=2/52); 2.3% at the anorectum (n=1/44) and 0.0% at the urethra site (n=0/52). Although based on low numbers, the results of this study suggest that kissing alone may be a risk factor for oropharyngeal NG.[23] Furthermore, saliva could operate as a potential medium for transmission in receptive anal sexual practices when used as an anal lubricant, which is a common practice among MSM.[24–26] Such data challenge efficient STI control among MSM. Indeed, while public health interventions focusing on the awareness of condom

use is rather easy to implement, promoting public health messages to avoid kissing to improve NG prevention and control may be more difficult to execute.

Moreover, a proportion of MSM are engaging in high risk behaviour such as condomless anal sex, a higher sexual partner rate and very dense sexual networks.[21] They disproportionally contribute to the STI epidemic among MSM and therefore, this subpopulation of MSM will be the focus of this thesis.

In 2010, MSM accounted for approximately 50% of all syphilis cases in Europe and for 24% of all gonorrhea cases.[27] In 2017, these figures increased to 77% of all syphilis cases and almost half of the gonorrhea cases illustrating the important contribution of MSM to the STI epidemic in Europe.[28,29] Besides these key populations, STIs can also spread to the general population through "bridging populations". Bridging populations such as clients of female sex workers or men who have sex with men and women, are persons who will further transmit the infection to the general or "low-risk" population such as their wives and unborn children or MSM with low risk behaviour.[30–32] In Europe, 47% of MSM reported to ever have had sex with a woman. Of those, 11% reported female sexual contact within one year.[33] These data highlight the importance of those key populations in public health strategies tackling the STI epidemic. **Therefore, in this thesis we will try to contribute to the control and diagnosis of STIs among MSM engaging in high risk behaviour.**

1.1.2 STIs and antimicrobial resistance

Another matter of concern is the fact that this epidemic of STIs runs parallel with a **rapid emergence of resistance to antibiotics of STIs**. Indeed, NG and MG are evolving into "superbugs", which are becoming resistant to almost every antimicrobial introduced for treatment.[8,13,34]

At the time of writing this thesis, all bacterial STIs could still be treated with antibiotics, however antimicrobial resistance (AMR) to several STIs compromises effective

treatment and subsequent control.[13] There are different classes of antimicrobials which bind to essential targets of the bacterium causing microbial cell death or cessation of bacterial growth. The mechanisms of action vary whereby some antimicrobials target the bacterial cell wall or cell membrane, others inhibit DNA/RNA or protein synthesis, and still others inhibit the synthesis of essential metabolites (see Figure 2).[35] Nevertheless, via evolutionary response resulting from the increasing use of these antimicrobials in health care and livestock farming, bacteria started to develop resistance. Antibiotic residues may remain in the meat, milk and eggs which is then consumed by humans. Moreover, resistant micro-organisms can spread between humans and animals. Bacteria can acquire resistance via direct or bystander selection. Direct selection is defined as selection for resistance during exposure to antibiotics intended to treat that particular pathogen whereas bystander selection is defined as selection for resistance during exposure to antibiotics intended for another pathogen.[36] The most important mechanisms of antimicrobial resistance are 1) protective alteration of antibiotic targets, 2) decreased influx of antibiotics into the cell through transport proteins, 3) increased efflux of antibiotics out of the cell via multidrug efflux pumps and 4) expression of antibiotic inactivating enzymes.[15] Figure 2 depicts the modes of action of antimicrobials including the AMR mechanisms used by bacterial STIs. As soon as a resistance mechanism is acquired, a bacterium can transfer the resistance vertically via multiplication or horizontally via mobile genetic elements such as plasmids or naked DNA to other bacteria.

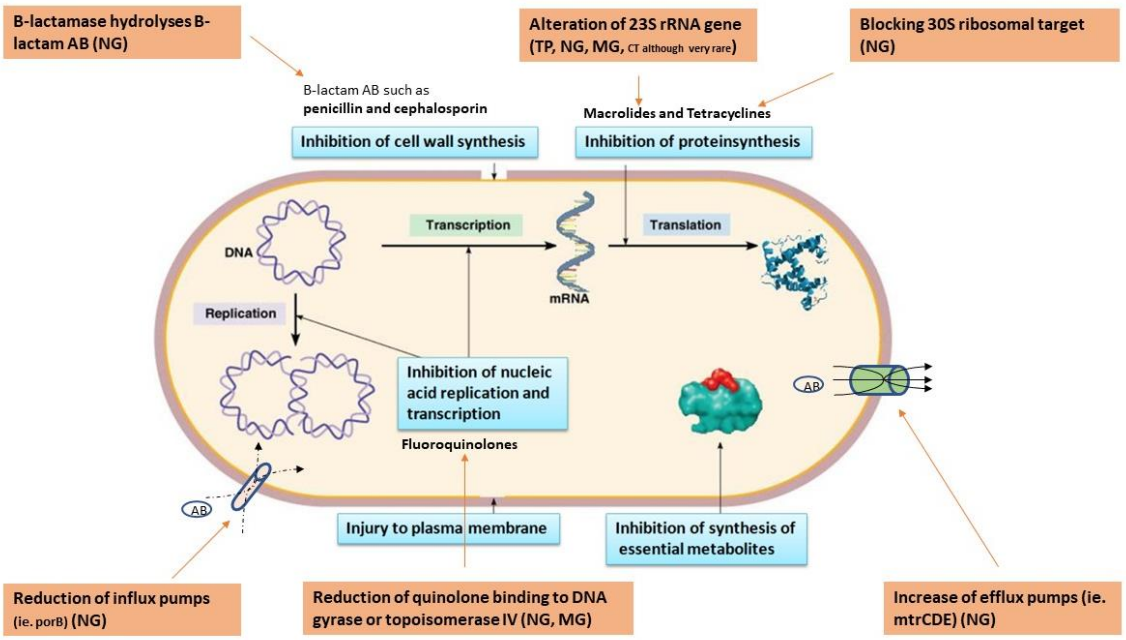

Figure 2: Mechanisms of action of antimicrobials (boxes in blue) used for STI treatment and resistance mechanisms (boxes in orange) by the different bacterial STIs.(adapted from [37])

A major public health concern is the emergence of multi drug resistance in NG and MG. NG and MG are displaying the extra-ordinary capacity to quickly develop and retain resistance despite the absence of antimicrobials. So far, NG has developed resistance to every antimicrobial introduced for treatment. In fact, NG is currently treated with dual antimicrobial therapy to delay resistance to extended-spectrum cephalosporines which is the only remaining first-line antimicrobial monotherapy. Unfortunately, multidrug resistant NG strains to ceftriaxone and azithromycin are starting to emerge.[38] Furthermore gonococci can also develop AMR through gene transfer via transformation with other *Neisseria* species. These commensals inhabit human anatomical sites, particularly the pharynx and are often exposed to antimicrobials.[34] Subsequently, AMR may emerge in these commensal which may then act as a reservoir of AMR genes for NG.[34]

The AMR situation of MG is also a matter of concern. MG is the smallest known self-replicating organism encoding fewer than 500 genes.[39] Due to its small genetic potential it needs to obtain nutrients from the host that it cannot synthetize itself and it is very slow growing. It belongs to the class of the Mollicutes and lacks a cell wall; therefore, antimicrobials targeting the cell wall are not active against MG. Furthermore, doxycycline has a low cure rate of 30-40%. Therefore, the first line treatment is azithromycin (class of macrolides), yet, it only has one gene allele encoding the azithromycin target 23S rRNA which may be the reason underlying the rise in macrolide resistance. Indeed, despite that collected strains from samples before 2003 were 100% susceptible to macrolides, a phenomenal increase in resistant strains has been observed since then.[40] To date, macrolide resistance is exceeding 50% in many European countries.[41] Furthermore, strains exhibiting resistance to moxifloxacin (class of fluoroquinolones), which is the second line treatment of MG, are beginning to emerge.[41] **In chapter 5 of this thesis, we will elaborate on the current antimicrobial resistance status of MG in Belgium against the first- and second line treatment.**

Antimicrobial susceptibility testing of STIs

The gold standard to detect antimicrobial resistance is still based on antimicrobial growth inhibition on agar or in cell-culture. Nevertheless, cultivation of many bacterial STIs is difficult. For example, TP can only be cultivated in live rabbits or in rabbit epithelial cells, CT in cell-lines and MG has a very slow growth up to 1 to 5 months, making it almost impossible to report on antimicrobial resistance via this method.[42] Yet, culture of NG is still the gold standard which enables complete AMR testing of the different antimicrobials.

Therefore, molecular detection methods including sequencing of target genes, such as 23S rRNA, *gyrA* and *parC* to detect macrolide and fluoroquinolone resistance are used to detect resistance associated mutations (RAMs). However the presence of these RAMs cannot ascertain whether or not phenotypic resistance is present. Indeed,

phenotypic antimicrobial resistance may be present in the absence of the described RAMs via other pathways such as novel acquired mutations not yet described, increasing drug efflux or presence of antibiotic degrading enzymes. Moreover, not every RAM will lead to clinical resistance. Therefore RAM results should be interpreted with caution as they can over- or underestimate the prevalence of antimicrobial resistance. Nevertheless, RAMs at position 2058/2059 (*E. coli* numbering) in 23S rRNA gene of MG are strongly associated with clinically reported AMR to macrolides.

Resistance to macrolides – a major problem among STIs

Table 2 lists the current treatment guidelines for the common bacterial STIs including the most important resistance mechanisms to macrolides and fluoroquinolones.

Worryingly, almost every bacterial STI acquired resistance mechanisms to azithromycin which may be due to the widespread use of macrolides to treat STIs (in single or in dual therapy – Table 2) or other infections such as respiratory tract infections. Besides, azithromycin is also used for various other indications including prophylaxis against infection with *Mycobacterium avium* complex in persons with AIDS (acquired immune deficiency syndrome).[43] Therefore its intensive use may have selected for AMR against macrolides in many of the STIs. Indeed, pharmacokinetic studies showed that patients treated with azithromycin have a long duration of suboptimal drug concentrations intra- and extracellularly for at least 2 to 4 weeks following treatment.[44] These subinhibitory concentrations could in fact induce or select for macrolide resistance in groups repeatedly infected with STIs, such as MSM.[44]

The most important resistance conferring mechanism is a single nucleotide polymorphism (SNP) in the 23S rRNA gene encoding 23S rRNA (peptidyltransferase loop of domain V), which presumably results in a reduced affinity for the 50S ribosomal macrolide target. Macrolide resistance of NG, TP and MG is drastically increasing over time: for MG and TP nearly 50% and 100% of macrolide-resistance have been reported

in certain settings.[41,45,46] NG resistance to macrolides is also increasing in Europe from 1.7% in 2014 to 3.6% in 2016.(compiled from data of surveillance Atlas ECDC). In Belgium, a limited study at the STI clinic of ITM, reported 100% of macrolide resistance among 29 syphilis cases in 2014-2015.[45] Resistance of NG to macrolides also steadily increases, from 0.2% in 2013 to 5.2% in 2016 which underscores the necessity of yearly antimicrobial resistance surveillance in order to change treatment guidelines when deemed necessary.[47] In contrast to these STIs, resistance of CT/LGV to macrolides is very rare.[48,49]

Table 2: Treatment guidelines and mechanisms of resistance of bacterial STIs

Antimicrobial class	Antimicrobial resistance mechanism (*E. coli* numbering if not explicitly stated)	Treatment guidelines (according to IUSTI)	
		First-line treatment	**Second-line treatment**
Early syphilis			
Macrolides	**Alterations to the 23S rRNA gene inhibiting binding of macrolides to the 50S ribosomal target** SNP in 23S rRNA (A2058G and A2059G)	Benzathine benzylpenicillin G (BPG) 2.4 million units single dose (IM)	**In case BGP is contra-indicated:** Doxycycline 200 mg daily for 14d (O)
Chlamydia trachomatis (non-LGV). Antimicrobial resistance is very rare			
Macrolides	**Alterations to the 23S rRNA gene inhibiting binding of macrolides to the 50S ribosomal target** SNP in 23S rRNA (A2058C and T2611C) Mutations of *rplD* gene which codes for ribosomal protein L4 leading to disruption of translation	Doxycycline 100 mg twice daily 7d (O) OR Azithromycin 1g single dose (O)	Erythromycin 500 mg twice daily 7d (O) OR Levofloxacin 500 mg once daily 7d (O) OR Ofloxacin 200 mg twice daily 7d (O)
Fluoroquinolones	**Mutations in the quinolone resistance determining regions (QDRD)** *gyrA* SNP S83I		
Lymphogranuloma venereum (LGV)			
See above	See above	Doxycycline 100mg twice daily 21d (O)	Erythromycin 400 mg four times daily for 21 days (O)

Antimicrobial class	Antimicrobial resistance mechanism (*E. coli* numbering if not explicitly stated)	Treatment guidelines (according to IUSTI)	
		First-line treatment	**Second-line treatment**
Neisseria gonorrhoeae (uncomplicated) acquired resistance to every class of antibiotics			
Macrolides	**Alterations to the 23S rRNA gene** -SNP in 23S rRNA gene (C2611T and A2059G) - *erm* genes (*erm*B, *erm*C and *erm*F) that encode rRNA - methylase that methylate nucleotides in the 23S rRNA macrolide target **Overexpression of efflux pumps** - *mtrR* mutations which results in overexpression and increased efflux via the MtrCDE efflux pump - MacAB efflux pump overexpression - *mef*-encoded efflux pump expression	Ceftriaxone 500 mg single dose (IM) **AND** Azithromycin 2g single dose (O)	**In case of Cephalosporin allergy and Fluoroquinolone or Azithromycin resistance are excluded:** Ciprofloxacin 500 mg single dose (O) OR Azithromycin 2g single dose (O)
Fluoroquinolones	**Mutations in the QDRD** *gyrA* SNPs (S91F, D95N and D95G) which reduces quinolone binding to DNA gyrase *parC* SNPS (D86N, S88P and E91K) which reduces binding to topoisomerase IV **Overexpression of efflux pumps** NorM pump overexpression		

Antimicrobial class	Antimicrobial resistance mechanism (*E. coli* numbering if not explicitly stated)	Treatment guidelines (according to IUSTI)	
		First-line treatment	**Second-line treatment**
Mycoplasma genitalium			
Macrolides	**Alterations to the 23S rRNA gene** -SNP in 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, A2059C and A2059T)	Azithromycin 500 mg single dose and then 250 mg once daily 4d (O)	**If Macrolide resistance present:** Moxifloxacin 400 mg once daily for 7-10d (O)
Fluoroquinolones	**Mutations in the QRDR (*M. genitalium* numbering). Only the most important SNPs will be mentioned** - *parC* SNPs which result in alterations in the ParC protein (S83I, D87N, D87Y) which, in turn, reduces quinolone binding to DNA gyrase - *gyrA* SNPs which result in alterations in the GyrA protein (M95I, D99N, D99Y), which in turn, reduces quinolone binding to DNA gyrase		**If resistant to Moxifloxacin and Macrolides:** Pristinamycin 1g four times daily 10 days (O) OR Doxycycline 100 mg twice daily for 14 days (O)

Collated data of [13,34,50–53]. IUSTI: International Union against sexually transmitted infections; O: oral; IM: intra-muscular; SNP: single nucleotide polymorphism; BGP: Benzathine benzylpenicillin; LGV: lymphogranuloma venereum; QRDR: Quinolone resistance-determinining region)

1.1.3 Control of STIs and global response

On the bright side of this grim picture of STIs is the fact that they are preventable and so far, still treatable. Due to the unavailability of effective vaccines for any of these bacterial STIs, early diagnosis of STIs and prevention through treatment are still optimal effective strategies to tackle this epidemic by breaking the chain of transmission. However, since the introduction of highly sensitive and specific molecular amplification techniques, it became clear that asymptomatic STIs were prevalent. Early studies revealed that, in fact, most of the STIs among MSM were of extra-genital origin (53% of the CT infections and 64% of the NG infections) and approximately 85% of the rectal CT/NG infections were asymptomatic.[54] In contrast, urethral CT/NG are mostly symptomatic in men. These data increased the awareness of the high frequency of extra-genital STIs and their potential role in STI control. As a result, targeted behavioural and STI screening strategies among MSM to prevent HIV and STIs including screening for syphilis and CT/NG at all sites of sexual exposure were recommended.[55-57] Given that these infections are underdiagnosed and untreated, they may represent a hidden reservoir contributing to the STI epidemic and may further complicate efforts to reduce transmission.[58] Hence, extensive screening of key populations is essential to curb the STI epidemic. Until now, nucleic acid amplification tests (NAATs) are still the recommended diagnostic method to detect STIs (except for syphilis) due to their high sensitivity and specificity and the unavailability of good point-of-care assays such as rapid diagnostic tests. Nevertheless, performing molecular testing requires a state-of-the-art laboratory and highly trained laboratory professionals. Moreover, triple-site testing of the different anatomical sites (urethra, anorectum and pharynx) is expensive. Therefore, cost-effective methods for appropriate screening for STIs are urgently needed.

The major cornerstones in the prevention of STIs are epidemiological surveillance and screening of key populations, rapid effective antimicrobial treatment, contact tracing,

and last but not least behavioural interventions.[3] Crucial behavioural interventions include education and increasing awareness of STIs, safer sex/risk reduction counselling such as promotion of consistent condom use, reduction in the number of sexual partners, and lastly uptake of testing for STIs. The favoured approach to achieve STI prevention is however a combination of behavioural, biomedical and structural approaches.[3] Therefore, the WHO issued a global health strategy in 2016 with the ambitious goal to end the STI epidemic as a major public health concern. Hereby, they are aiming for a 90% reduction of syphilis and gonorrhea incidence globally by the year 2030 (Figure 3).[3]

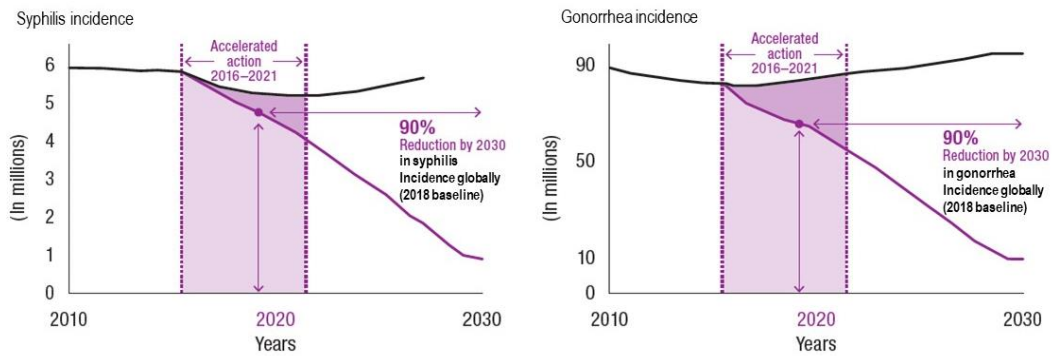

Figure 3: Key targets for the WHO Global Health sector strategy on STIs 2016-2021. (figures from[3])

Hereto, the WHO describes five strategic directions that should be undertaken by all countries.

1. Implementation of etiologic surveillance to identify key populations in order to implement a tailored response among the individuals most at risk.
2. Searching for high-impact interventions to include in STI services.
3. Delivering of STI services (prevention, detection and treatment) to everyone to achieve equity and maximize impact.
4. Identifying sustainable and innovative models for financing of STI responses.
5. Identifying major gaps in knowledge and technologies.

Within the scope of this thesis we will try to contribute to this ambitious goal by delineating parameters to identify individuals who could serve as the motor of the STI epidemic among MSM. Therefore we will describe the Belgian MSM STI epidemic by means of reviewing surveillance data and data of a cohort of HIV negative MSM in Belgium. In addition, we will also contribute to the roll-out of effective screening strategies among MSM in Belgium, which is in fact a major part of the present thesis.

1.2 EPIDEMIOLOGY

1.2.1 Global STI epidemiology

STIs have been reported over the centuries. The rises and falls of the epidemic typically concurs with historical events as illustrated in Figure 4 which depicts the gonorrhea and syphilis epidemic in the United Kingdom (UK) and the United States (US) since 1925 until 2017.[59–61]

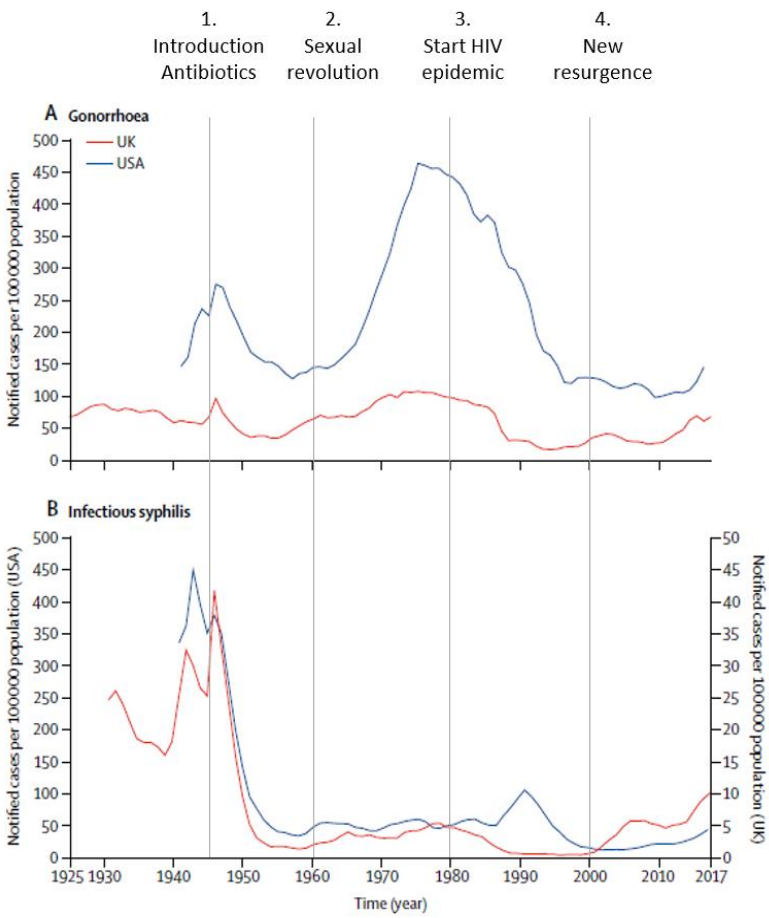

Figure 4: Historical trends for the notified cases of gonorrhea (A) and infectious syphilis (B) in the UK and the USA. (adapted from[55])

1. Introduction of antibiotics

After the World War II, the introduction of antibiotics and their widespread availability (i.e. benzathine penicillin G), led to the plummeting of the number of STI infections in the United States (US) and other industrialized countries.[60–62] This drastic decline was most prominent and durable for infectious syphilis but was less sustained for gonorrhea due to a number of factors including the rapid acquisition of resistance to penicillin.[34]

2. Sexual revolution

In the sexual revolution years 1960-1970, the introduction of the contraceptive pill and concurrent changes in sexual behaviour such as increasing number of partners and condomless sex triggered a new wave of STIs. This rise was most pronounced for gonorrhea, probably due to concomitant antibiotic treatment failures.[34]

3. HIV epidemic

In 1981, HIV was first discovered among MSM in the US.[63,64] It became one of the worst epidemics in the 20th century and due to the increased awareness of the high fatality rate of HIV, campaigns among MSM were initiated to reduce the number of partners and to use condoms.[65] As a result, a decrease in the number of condomless sex was noted, and subsequently, a substantial decline in the number of STIs was reported. By the mid-1990s, the number of syphilis and gonorrhea cases were at their lowest level in many high-income countries which is thought to be due to a combination of a behaviour change among MSM and the high mortality rate of very high-risk individuals by the lethal consequences of AIDS.[60,61]

4. New resurgence

Starting from the new millennium, the STI epidemic began to rise again as seen in Figure 4. This upward trend is clearest for syphilis in many industrialized countries and the rise

in syphilis is almost exclusively seen among men.[60,66–68] Possible mechanisms underlying this resurgence among MSM will be elaborated in section 1.2.3.

1.2.2 STI epidemic in Belgium

Similar to other countries, the number of STIs is continuously increasing in Belgium since 2002 (Figure 5).

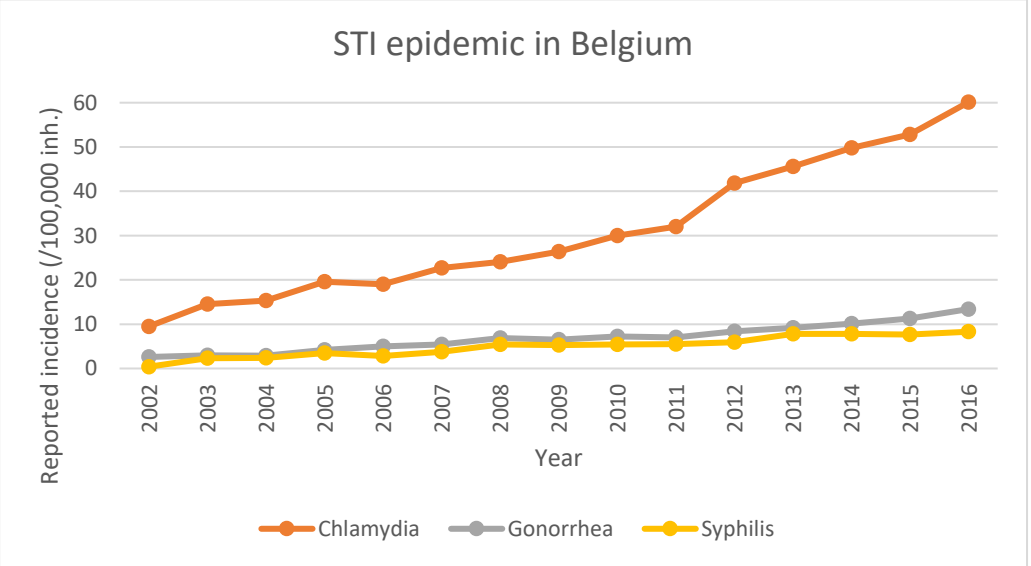

Figure 5: Trends of STI incidence in Belgium 2002-2016.[69]

Chlamydia is the most frequently reported STI in Belgium and increased from 9.5/100.000 inhabitants in 2002 to 60.1/100.000 inhabitants in 2016. Most chlamydia infections were found among young women (age 15 to 29).[70] The second most reported STI is **gonorrhea** and here also the number of cases is steadily increasing. Gonorrhea is mostly detected among men between 20-39 years and increased from 2.6/100.000 in 2012 to 13.4/100.000 in 2016. In line with gonorrhea, an increasing trend in **syphilis** cases is observed among male individuals from 0.4/100 000 inhabitants in 2002 to 8.3/100.000 inhabitants in 2016. Although the increase in STIs in Belgium may be explained partly by an increase in testing frequency, other European countries are also reporting this steadily increase in STIs.[71]

The number of STI cases in Belgium is however probably underestimated due to some limitations of reporting. Sciensano (the public health agency of Belgium) is using data from a voluntary network of laboratories that forward demographic characteristics of each detected case of CT, NG and syphilis to Sciensano to estimate the STI incidence in Belgium. Currently, the representativeness of the presence of the pathogens in the population is guaranteed, however, the stop/start of a large participating laboratory can influence these figures.[70] Whilst the denominator used for the calculation of incidence is the number of tests reimbursed by the federal government (RIZIV), this may also be incorrect as only two molecular assays are reimbursed per year for CT.

Furthermore, Belgium is using a sentinel network of general practitioners to provide additional socio-demographic and behavioural characteristics. Using this network, it is shown that syphilis, followed by gonorrhea is mostly reported among MSM in Belgium (Figure 6).

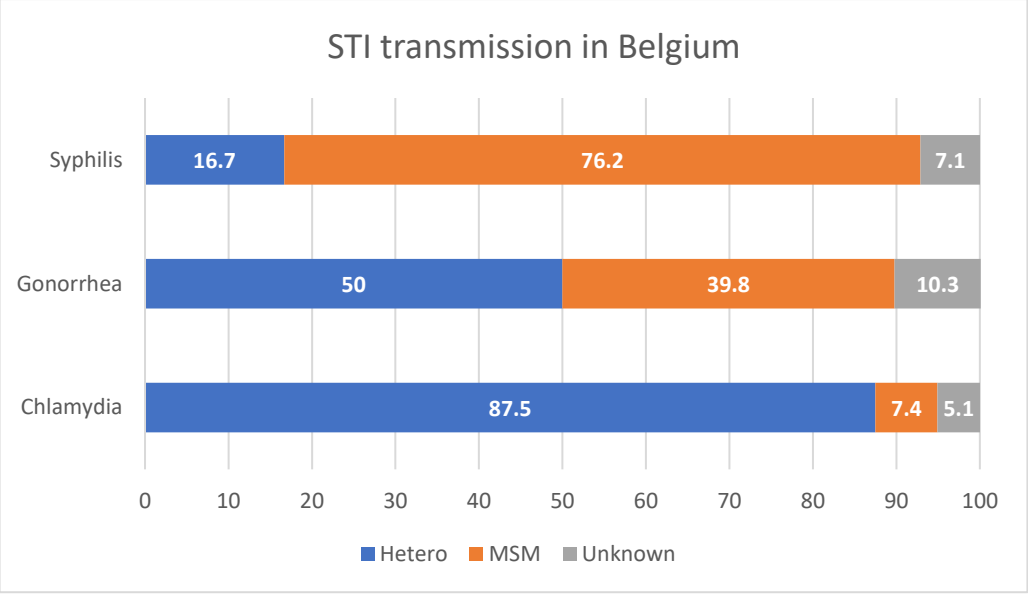

Figure 6: Sexual orientation of individuals with STIs 2017-2019.[70]

Registration of ***M. genitalium*** is not done, and therefore, it is difficult to estimate the incidence of this pathogen in Belgium. Nevertheless, this thesis will try to contribute to the knowledge of this disease by estimating the prevalence and incidence of MG among MSM in Belgium. Furthermore, we will document the antimicrobial resistance of MG in Belgium in chapter 5.

1.2.3 STI epidemic among MSM within the context of HIV prevention strategies

HIV prevention strategies with a focus on pre-exposure prophylaxis

Despite being lethal in the early 80s, the HIV epidemic shifted to a chronic disease after the introduction of combination antiretroviral therapy (cART) in 1996.[72] This resulted in a global change in community perception concerning the fatality and severity of HIV/AIDS, leading to an increase in condomless anorectal intercourse (CAI) among MSM.[73] However, to still stem HIV transmission among MSM, **seroadaptive strategies** such as serosorting (selecting a sexual partners with the same HIV status) and seropositioning (varying their sexual practice preferences depending on the HIV status of the partner) were introduced since 2000 among MSM. These were important harm reduction strategies which were practiced for years.[74–76] Furthermore, the scientific breakthrough that cART could be used to prevent HIV infection opened up new opportunities. **Preventive strategies based on the intake of cART are called biomedical HIV prevention strategies**. The first preventive usage of ART was **PEP or post-exposure prophylaxis**. PEP is the provision of ART after a high-risk exposure and was first used among healthcare workers after percutaneous exposure to HIV-infected blood.[77]

Afterwards, the efficacy of **treatment as prevention or TasP** was confirmed in three randomized controlled trials (RCT) among gay and heterosexual serodiscordant couples.[78–80] In these studies, HIV transmission to the HIV negative partners went almost to zero provided the HIV positive partner was on cART and virally suppressed. Indeed, when taken consistently, cART reduces the plasma viral load to undetectable

levels,[81] apparently abolishing the chances of transmission. While the importance of early treatment to prevent disease progression was recognized earlier, the success of TasP was another argument to stress that HIV positive individuals should receive treatment as soon as possible and widespread campaigns such as U=U (Undetectable = Untransmissible) were launched since 2016.[82]

Concomitantly, RCTs with **PrEP or pre-exposure prophylaxis** were started globally among MSM and transgenders, serodiscordant couples, young African women and injection drug users starting from 2010.[83–87] The concept of PrEP is to protect HIV negative individuals by providing cART orally or topically before and during a risk sex act. The first RCT among MSM and transgender women (iPrEx) in 2010, reported a reduction in HIV acquisition of 44% when using the single tablet combination tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) once daily.[84] In addition, encouraging results (reduction of 75%) of the Partners-PrEP study among heterosexual discordant couples led to the approval of TDF/FTC by the Food and Drug Administration (FDA) as the first regimen to be used to prevent HIV in 2012 among serodiscordant couples and MSM.[85,88] After the approval of PrEP by the FDA, the WHO called for demonstration projects around the world.[89] As a result, the STI/HIV Unit at our Institute (ITM) under the lead of Prof. Dr. Marie Laga received Flemish governmental funding in 2015 to perform the PrEP demonstration project in Belgium in order to assess the feasibility and acceptability of daily or event-driven PrEP among high-risk MSM in Belgium (Be-PrEP-ared). The study protocol, objectives and results of this demonstration project will be discussed in detail in Chapter 3 and Annex 1 & 2.

In 2016, two European PrEP studies among MSM (PROUD and IPERGAY) showed a remarkable reduction of 86% in HIV incidence among MSM. [90–93] As a result, the European Medicine Agency (EMA) recommended to approve the use of TDF/FTC in combination with safer sex practices in adults at high risk in 2016 in the European Union. Consequently, the intake of TDF/FTC to prevent HIV became implemented in

several European countries and was approved in Belgium in June 2017 for high-risk individuals such as female sex workers or MSM. To be able to receive reimbursement, MSM need to report one of the following risk factors: CAI with minimum two partners in the last six months, multiple STI history within the last year, received PEP several times or the use of illicit drugs during sexual activity.[94]

Trends of STIs among MSM after the introduction of PrEP

The above described seroadaptive behaviours to prevent HIV, were associated with a decline in condom use among seroconcordant MSM partners, and may be one of the reasons for the rebound of STIs observed after the year 2000.[27,76,95] Moreover, the advent of biomedical methods, such as PrEP, dramatically improved HIV prevention from 2010 on, but, paradoxically, it may have contributed to a further increase in the incidence of other STIs among MSM due to an increase in risky sexual behaviour. This concept is coined **risk compensation or treatment optimism**. In risk compensation, a preventative intervention leads to a reduced perception of risk since people will feel safer and consequently be more prone to risk-taking behaviours such as increasing the number of condomless sex partners or having more condomless sex[96] while, unfortunately, the current HIV biomedical prevention methods do not prevent other STIs.

Early results of PrEP RCTs did not reveal a change in sexual risk behaviour following PrEP initiation nor an increase in STI incidence. Yet, it should be acknowledged that participants were unaware whether they were using PrEP or placebo. Furthermore, maximum follow-up time in these RCTs was two years which may be too short to observe an alteration in sexual risk behaviour.[97] And finally, they received enhanced risk-reduction counselling at every visit.[24,91,93,98] Therefore, these results may not be generalizable to real life use of PrEP.

Later on, open label studies (studies whereby the participant is aware of the drug that is given to him/her) or behavioural surveillance studies revealed **changes in sexual behaviour among MSM over the years** such as an increase in PrEP use, a decrease in overall condom use accompanied by an increase in the number of condomless partners and a decline in serosorting.[61,96,99,100] For example, in a study describing sexual behaviour of HIV negative MSM in San Francisco, PrEP use increased from 9.8% in 2014 to 43.1% in 2017, having more than five CAI partners in the last six months increased from 1.6% to 12.8% and serosorting declined from 35.7% to 21.4% (Figure 7).[100] In some of the studies, alterations in sexual behaviour have been associated with the introduction of PrEP.[96]

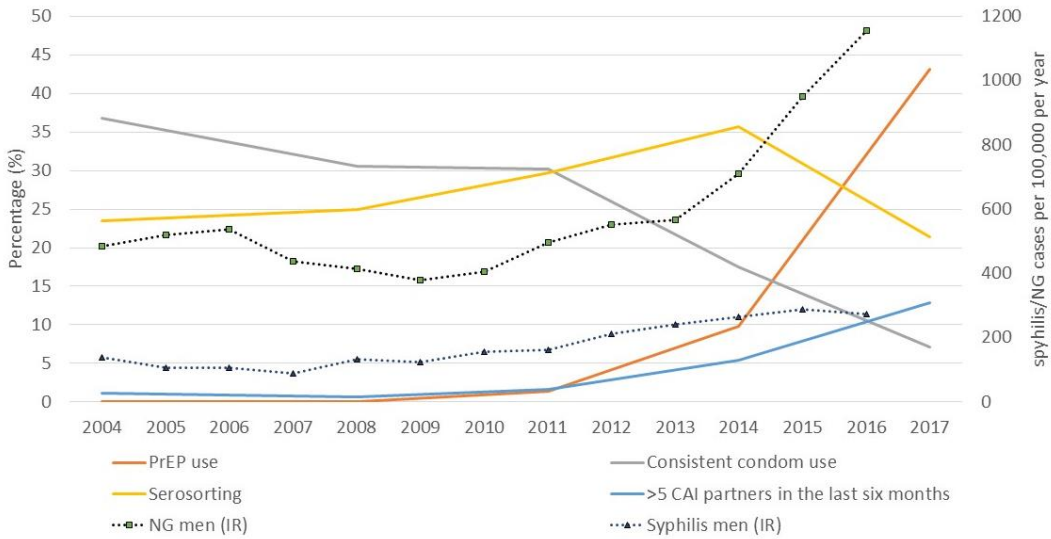

Figure 7: Changes in sexual behaviour among HIV negative MSM in San Francisco and the male incidence ratio (IR) of Neisseria gonorrhoeae (NG) and syphilis over time (2004-2017). Data of NG and syphilis IR are missing for the year 2017. Data from[100,101].

Data on the effect of PrEP on the STI epidemic are however conflicting.[99,102,103] Yet, a global systematic review of PrEP studies, revealed a pooled prevalence of 23.9% and a very high pooled incidence of bacterial STIs (gonorrhea, chlamydia and syphilis) (72.2 per 100 person years).[104] This number of STIs among PrEP users is however not

surprising as one of the factors to be eligible for PrEP is having had a recent STI or being at high risk for sexual acquisition of HIV such as engaging in CAI. This setting highlights that the advent of PrEP provides an opportunity to bring high-risk HIV negative MSM into care for STI testing, treatment and counselling. Furthermore, increased STI screening and monitoring may have important public health benefits by reducing the burden of STIs in this population and consequently, prevent onward transmission of STIs.[104]

Nevertheless, the increasing trend in STIs cannot be attributed to the introduction of PrEP alone. The earliest rises of STIs among MSM preceded the availability of PrEP in several countries, which suggests that PrEP is not the only cause for the observed increase in STI rates in MSM.[74] Decreased HIV concern and increased condom fatigue among MSM was already documented in 2003[105] and in San Francisco, an increase in the number of syphilis and gonorrhea cases was already noted among MSM before 2012 (Figure 7).[101] The TasP strategy was implemented in San Francisco since 2010 and since then, consistent condom use dropped and the number of partners increased among HIV negative MSM. Clearly, the increases in the incidence ratio of NG and syphilis among men may be associated with this gradual change in sexual behaviour and the introduction of any new HIV prevention strategy.

In a wider historical perspective, some STIs, such as syphilis and LGV were almost eradicated in the Western world after the introduction of antibiotics. However, several outbreaks mostly among HIV positive MSM resulted in a resurgence of these STIs.[66,106–108] These infections were again associated with very high risk behaviour i.e. high number of CAI, using drugs before sex, “fisting” contacts, high number of sexual partners, previous STI history and anonymous sexual contacts.[66,106,109] Whilst the majority of these infections were primarily found in HIV positive MSM, increases among HIV negative MSM over the years are noted.[110,111] Following these trends, the scientific community is concerned that the implementation of another biomedical HIV

prevention strategy such as PrEP will lead to a further reduction in conventional HIV prevention strategies such as condom use or serosorting. Consequently, this may lead to an overall increase of STIs among MSM and the dissemination of these previously HIV positive confined STIs to HIV negative MSM taking PrEP with high risk behaviour.[61,74,112]

The rise in STIs among MSM is complex and multifactorial. Other factors besides the use of PrEP or biomedical HIV prevention strategies and the increase in CAI, also contributed to the increase in STIs among MSM as mentioned below.(Figure 8)

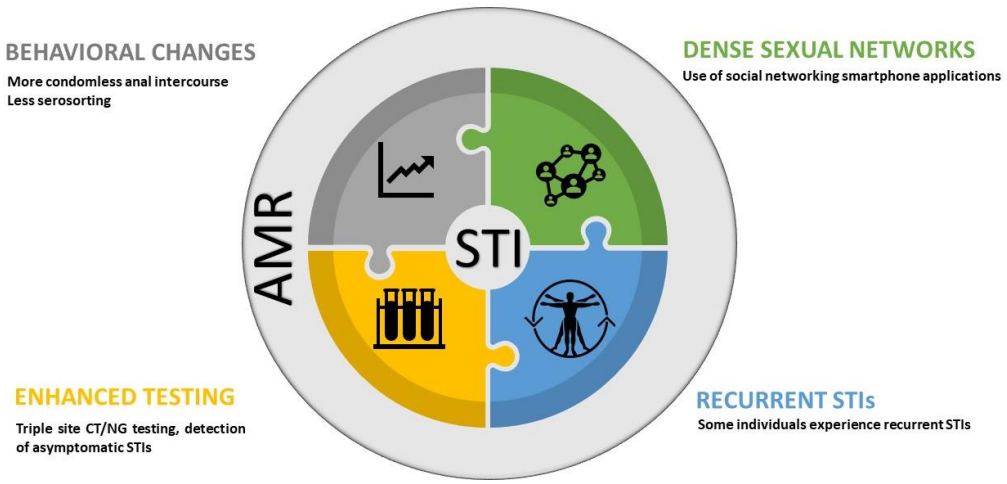

Figure 8: Factors contributing to the rise of Sexually transmitted infections (STIs) in MSM. AMR: antimicrobial resistance; CT/NG: Chlamydia trachomatis/Neisseria gonorrhoeae

1. Firstly, high-risk MSM have **dense sexual networks** and geospatial smartphone applications may be responsible for the enlargement of these networks.
2. Secondly, some MSM are **experiencing recurrent STIs** and may serve, in fact, as the motor of the STI epidemic among MSM.[113] A better understanding of the behaviours underlying recurrent STIs may contribute to the interruption of transmission and thus may contribute to the control of the STI epidemic.

3. Thirdly, the **enhanced STI screening rate** among PrEP users may have contributed to that rise. International guidelines recommend to screen for STIs at least six-monthly in the three anatomical sites of infection being urethra, anorectum and pharynx. Many countries, such as Belgium, urge to test at every follow-up visit, being quarterly. As a consequence many asymptomatic infections, that previously would go unnoticed, are being diagnosed now. Although the increase in STI testing might have influenced the perceived STI epidemic, it is unlikely that testing alone accounts for this steady increase as an increase in symptomatic STIs is also observed.[74,114]
4. Finally, possible treatment failure may be an increasingly important factor due to increasing **antimicrobial resistance** in some STIs.[13] It is possible that acquisition of resistance associated mutations may drive the current rise in several STIs or that AMR is occurring due to the increasing number of STIs. Indeed, it has been acknowledged that increased antibiotic pressure may fuel development of antimicrobial resistance.[36] Those individuals with recurrent STIs may therefore play a pivotal role in the acquisition of antimicrobial resistance.

In the following section, we will discuss in depth above mentioned factors including selected challenges for controlling the STI epidemic among MSM with high risk behaviour and we will address how this thesis will try to contribute to these gaps.

1.3 CHALLENGES FOR CONTROLLING THE STI EPIDEMIC AMONG MSM WITH HIGH RISK BEHAVIOUR

1.3.1 Dense sexual networks

The spread of STIs depends on multiple factors including the prevalence of STIs in the partner network, the duration of infectiousness, frequency of sexual intercourse, the rate of partnership formation and dissolution, the number of sexual partners, length of time of relationships, patterns and dynamic evolution of sexual networks and sexual mixing with other populations.[115]

The importance of concurrent sexual partnerships (having different sexual partnerships at the same time) and dense sexual networks as factors that contribute to community-level STI prevalence has been acknowledged by the scientific community for some time.[116–119] The more concurrent relations in a high connectivity network, the greater the spread of STIs in that network. The effect of concurrent sexual partnerships on network connectivity is depicted in Figure 9: a relatively small increase of people involved in multiple concurrent partnerships (from 55 to 65 %) results in a dramatic increase in connectivity (from 2 to 64 %), which obviously will strongly increase STI transmission rates. It is well known that a proportion of the MSM community has high numbers of concurrent sexual partnerships, which has even increased with the introduction of PrEP as mentioned above. In the UK PROUD study, men allocated to immediate PrEP reported more CAI with ten or more partners in the last three months as compared to the group who did not receive PrEP immediately (21% vs 12%) which suggests tremendously high connectivity networks in the group using PrEP.[90]

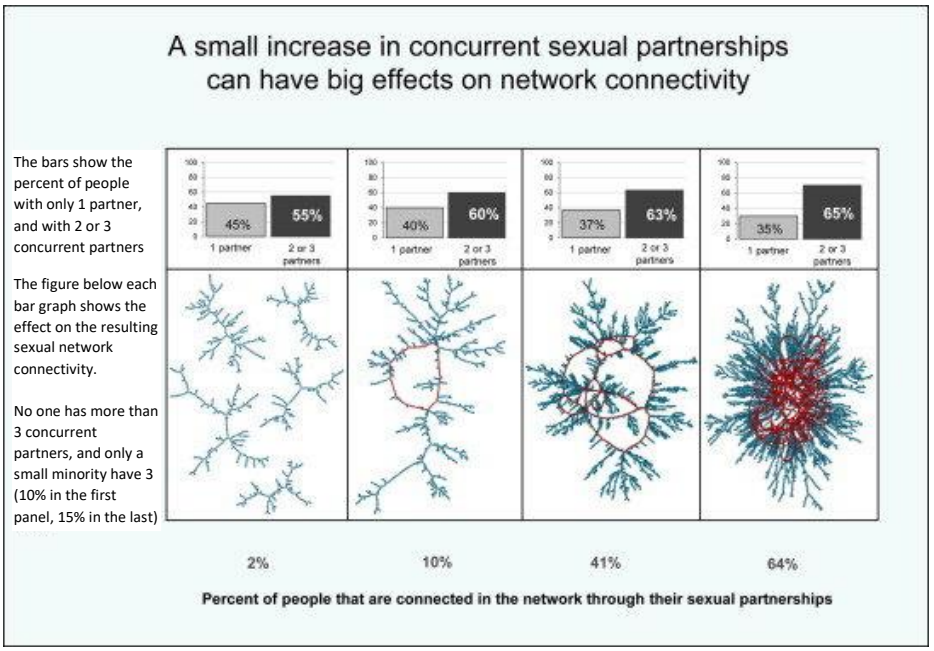

Figure 9: Visualization of the impact of increases in concurrent sexual partnerships on network connectivity in heterosexual individuals.[120]

Seeking sexual partners changed substantially over the years among MSM from personal gay venues in 1990's to online gay websites after 2000. Since the last decade, digital platforms which uses geolocation features, such as social media and geosocial dating applications (i.e. Tindr, Grindr or Hornet) are frequently adopted among MSM to meet anonymous partners.[121] These new developments, had important effects on sexual links, patterns of partner recruitment and sexual mixing, resulting in the enlargement of the sexual networks among MSM.[121] In the last European MSM Internet Survey (EMIS) of 2017, 68% of the respondents met their non-steady sex partners online and this number probably further increased in the last years.[33] The use of these dating applications has been associated with higher risk behaviour such as an increase in sexual partners and a greater frequency of CAI.[122] Furthermore, Grindr users manifested a higher incidence of STIs.[122,123]

Moreover, dense sexual networks may undermine STI prevention methods such as increased screening and effective treatment.[124] Indeed, a treated and cured individual belonging to a dense sexual network, is more prone to reinfection than an individual from a low connectivity network. In addition, while partner notification is one of the pillars to reduce the spread of STIs, it still remains challenging in dense sexual networks with a high number of anonymous sexual partners, but it also opens new intervention opportunities. The more traditional, anonymous physical settings to meetup with partners such as dark rooms and gay sauna's made it difficult to trace back sex partners and to implement partner notification. Now, the online hook-up apps, may provide possible solutions to map sexual networks, to notify recent sexual partners and to intervene in STI transmission.[121] In fact, nearly all men are willing to notify their sex partners they met online using an anonymous partner notification function embedded in hook-up apps.[125] Hence, the surge of such dating apps offers unique opportunities to reach those at risk and to provide additional STI prevention messages. This should be further investigated; however this is not part of this thesis and will therefore not be further addressed.

1.3.2 Core transmitters experiencing recurrent STIs

Whilst it is difficult to shatter the dense sexual networks of MSM, one may try to identify the core transmitters who are taking central positions in these networks, and may therefore, fuel the STI epidemic. Also, the perception of core-MSM concerning curable STIs may be very worrying. In some groups, having a CT or NG infection were described to be 'a rite of passage' as almost everyone in those groups had CT or NG and these infections are perceived to be very easy to cure.[126] These core transmitters with recurrent STIs maintain a longer period of infectivity, have a high number of concurrent partnerships and engage in high risk behaviour such as condomless sex.[127] Previously documented characteristics of core STI transmitters were: being HIV positive, having sex with men and being co-infected with other STIs.[128,129] Since these core STI

transmitters are taking central, crucial positions in sexual networks, it is critical to identify these individuals to target more efficiently interventions.

Research gap to be addressed by this thesis

To the best of our knowledge, no research has yet been done to identify PrEP users that are more prone for recurrent STIs. Therefore, based on data generated from the Be-PrEP-ared study, we will address this knowledge gap by trying to identify the underlying behavioural factors that may lead to recurrent STIs among PrEP users in order to enhance STI control within this core group. The results will be discussed in chapter 3.

1.3.3 Re-emerging STIs

A resurgence of other STIs such as Hepatitis C and LGV among MSM is also noted in the last ten years in Europe and the US.[8,61] **LGV or lymphogranuloma venereum** is caused by the L-serovar of CT (L1 to L3) and in contrast to the serovars D-K, is more invasive, can disseminate via underlying connective tissue and may spread to the regional lymph nodes. It can cause severe ulcerative proctitis or inguino-femoral lymphoadenopathy.[130] LGV infection therefore requires a longer treatment regimen than non-L serovars and is treated for 21 days with doxycycline 100 mg twice daily instead of 7 days which is the regular regime for anorectal non-LGV CT infection.[130]

Classically, LGV can be divided into three different stages. I. Primary painless lesion at the site of inoculation which heals after a few days and tends to go unnoticed; II. Two to six week later, the infection may spread via underlying connective tissue layers and lymphatic tissue. Among MSM, this stage is mostly accompanied with severe proctitis or the typical “buboes”, i.e. formation of severe inguinal lymphadenopathy. Finally, stage III: If LGV is left untreated, chronic lymphadenitis may lead to elephantiasis 1-20 years after the infection. Stage III is very rare among MSM in the Western world.[112] Apart from this “typical” course, the infection may also be asymptomatic or may mimic chronic inflammatory bowel diseases and therefore tends to go unnoticed.[131]

LGV has almost been eradicated in the Western world after the introduction of antibiotics. However, since 2003, outbreaks were reported in Europe and the USA.[112] A first cluster was found in the Netherlands in 2003 among an international sexual network of MSM, which fuelled further transmission and spread of LGV to other Western European countries such as Belgium, Germany, the UK, Spain and France.[106,132] Almost all LGV cases in that network were HIV-positive and reported high-risk sexual practices i.e. high number of CAI, both receptive and insertive, "fisting" contacts and previous STI history.[106] In Europe, the number of LGV cases continues to rise (Figure 10).

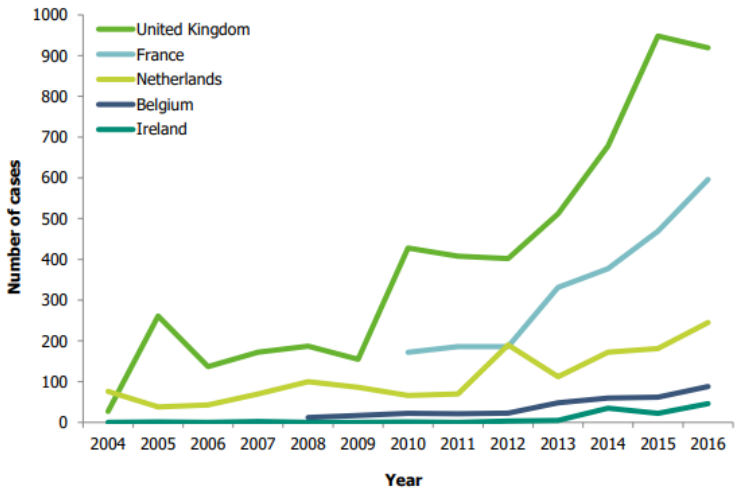

Figure 10: Number of confirmed LGV cases in the five European Member States with the highest numbers of cases in 2016; 2004-2016.[133]

LGV was previously strongly associated with HIV infection.[109] However, a shift in the epidemic is noted as the proportion of HIV negative LGV cases is increasing from 13.0% in 2011 to 30.2% in 2016 (data collated from the surveillance atlas of infectious diseases of ECDC). This may be due to increased CAI and sexual mixing. Furthermore, an increasing number in asymptomatic infections is reported.[134,135] Indeed, currently, about 25% of the anorectal LGV infections are found to be asymptomatic among MSM.[130] The increasing detection in the overall number of cases and asymptomatic

cases may be due to changes in screening practice and previous under diagnosis or rapid detection of LGV cases which are still in the pre-symptomatic phase.[136]

Currently, further LGV identification of all positive anorectal CT samples is recommended among MSM irrespective of their HIV status.[130] However, this was not always the case. Furthermore, not all laboratories are able to distinguish L versus non-L serotype CT. Therefore, the number of LGV cases is probably underestimated. In 2015, in Belgium, the national reference centre of STIs (NRC-STI), was the only laboratory that was able to confirm serotype L. Currently, there are several multiplex NAATs for genital ulcerative disease that also detect LGV, yet, large validations for the detection of LGV in rectal samples are missing.[130] As such, confirmation of LGV is typically done by further discrimination of LGV strains by testing all positive anorectal CT samples using a specific NAAT that targets LGV specific genes such as the polymorphic membrane protein H gene or *pmpH*. In other settings, further sequencing of the *ompA* gene is used for phylogenetic reconstruction.

Nevertheless, data from the Netherlands where all MSM are being tested for anorectal CT and LGV, irrespective of their HIV status or current sexual behaviour, indicate a steady increase in the number and positivity rate of LGV from 2014 to 2018. The author suggests that this may be due to risk compensation followed by the implementation of biomedical HIV prevention strategies.[112]

Research gap to be addressed by this thesis

As compared to neighbouring countries, Belgium seems to be in an early phase of the LGV epidemic. It has been speculated that the introduction of PrEP may be responsible for an increase of LGV among HIV negative individuals. Using data of the NRC-STI, which reports on the LGV epidemic since 2010, and data of the STI/HIV clinic at ITM, we will try to contribute to these knowledge gaps. Firstly, we will describe the Belgian LGV epidemic over time during the pre-PrEP era (2011 until July 2017). In chapter 4 of the

thesis, we will try to explore differences in important behavioural and clinical characteristics over the years such as HIV positivity, transmission and the rate of asymptomatic cases. Secondly, we will assess if the roll-out of PrEP influenced the rectal LGV epidemic in Belgium.

1.3.4 Antimicrobial resistance of STIs

It has been well shown that emergence and transmission of antimicrobial resistant STIs are first found in core groups such as female sex workers and MSM.[137] Although early detection and treatment of STI are crucial for efficient control, researchers are worried that intensive screening for STIs among core groups may select for AMR via direct or bystander selection.[138,139] Indeed, increasing antimicrobial use, even for asymptomatic infections discovered by routine screening, may cause an alarming high antibiotic pressure on these STIs and in addition, may exert an adverse effect on the microbiome and resistomes.[140] For example, the emergence and spread of ciprofloxacin resistance of NG in the US was suggested to occur initially in the MSM population to subsequently spread through the heterosexuals.[141] Moreover, ciprofloxacin resistance of NG was associated with previous antibiotic use.[142] In addition, it has been well acknowledged that resistance of NG to extended spectrum cephalosporines (such as ceftriaxone), may emerge in the pharynx through horizontal gene transfer from non-gonococcal commensal *Neisseria* species to NG isolates.[34] MSM do have a higher prevalence of pharyngeal NG (4.6%) compared to women (2.1%) and men who have sex with women (2.2%).[143] However, it is worth mentioning that MSM are more intensively screened at this anatomical site than other populations and large studies among women are lacking.

The antimicrobial consumption in core MSM is very high due to repeat STIs which are treated according to national guidelines (direct selection). Furthermore, co-infection with other STIs which are not part of the screening program may lead to AMR (bystander selection). The best example of bystander selection among STIs is MG. The

symptomatology of MG and CT are indistinguishable and furthermore, asymptomatic anorectal MG is very common among MSM.[40,144] Nevertheless, the macrolide treatment course for MG, NG and CT differs (see Table 2). This suboptimal dosing for MG due to the treatment for other STIs may exert a significant selection pressure on the emergence of macrolide resistance in MG.

Research gap to be addressed in this thesis

The prevalence of resistance associated mutations conferring AMR of MG to the first- and second line treatment (macrolides and fluoroquinolones respectively) in Belgium has not been documented until now. Chapter 5 will address this major research gap by testing all MG positive samples received by the NRC-STI since 2015 including MG positive samples detected during the Be-PrEP-ared study. Furthermore, we will explore whether certain demographic and epidemiological characteristics are related to the presence of resistance in order to fine-tune future STI guidelines.

1.3.5 Need for enhanced STI screening rate including extra-genital asymptomatic CT/NG infections

In the recent meta-analysis performed among MSM initiating PrEP, the prevalence of STIs was the highest in the rectum, and prevalence of gonorrhea was also higher in the pharynx than in the genital site, which reinforce the importance of testing for STIs at these extra-genital sites among PrEP users.[104] In accordance with other countries, Belgian PrEP guidelines recommend to screen each PrEP user quarterly for STIs including syphilis and CT/NG in the three anatomical sites.[145] As a consequence, PrEP users may thus contribute more to the increasing STI detection rate among MSM and in addition, they may have a greater frequency of recurrent infections.[113]

Importantly, this high number of STI screening visits may put a heavy burden on PrEP users, healthcare workers, and laboratory professionals. PrEP users are mostly healthy and employed, and STI clinics only operate during regular working hours. For some PrEP

users, returning to the clinic for treatment and subsequent related costs of travel and treatment may constitute a barrier to be treated for an asymptomatic STI infection. Therefore, the introduction of different STI screening strategies are required and will be considered in this thesis.

Research gap to be addressed by this thesis

In chapter 6 and 7, we will deal with this need by providing two innovative pre-analytical solutions.

- Firstly, **pooling of the three anatomical sites per individual** (being urethra, anorectum and pharynx) for CT/NG testing is more cost-effective instead of performing three individual assays. It has been estimated that two thirds of the laboratory budget for PrEP was allocated to bacteriological assays with molecular detection of CT/NG being the largest cost.[146] Lowering the number of assays per PrEP user will in turn be cost-saving. In this thesis, we will describe two different sample pooling strategies that can be implemented in different kind of settings. Furthermore, our sample pooling strategies provide the possibility to determine the biological site of infection if deemed necessary by the clinician for treatment purposes.

- Secondly, in most Belgian settings, samples for HIV/STI testing are taken at the time of the visit with the clinician. Results are provided by phone or online one week later. In case of a positive result, the PrEP user should return to the clinic for an additional visit to obtain treatment and further counselling. One way to simplify this testing and treatment cascade is to **self-collect the samples for HIV/STI testing at home** and to ship them by regular mail to the laboratory for certified testing one week before the scheduled visit. In that case, the PrEP user will receive his laboratory results during the visit with the clinician for immediate treatment, thus saving time for all people involved and,

importantly, also limiting time for onward STI transmission. Self-sampling for STIs is well-accepted among MSM.[147,148] This, in turn, may help lowering the burden on the healthcare professionals and PrEP users themselves.

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CHAPTER 2: OBJECTIVES

In the introduction, we summed up the most important challenges of STI control among MSM with high risk behaviour: 1. Gradual changes in sexual behaviour including dense sexual networks with frequent concurrent partnerships; 2. Individuals who experience recurrent STIs; 3. Emergence of STIs previously confined to HIV positive MSM such as lymphogranuloma venereum of LGV; 4. Increasing antimicrobial resistance of MG and NG; 5. The necessity of an enhanced STI screening rate and the additional burden it places on the healthcare system.

The overall aim of this thesis is to address these challenges and therefore, to contribute to the control of the STI epidemic among MSM, and in particular PrEP users, in Belgium by focusing on two major interlinked objectives:

1. **To characterize various important epidemiological and clinical aspects of bacterial STIs among MSM in Belgium (SECTION I)**
    1.1. To document changes in sexual behaviour and bacterial STI incidence among PrEP users (Chlamydia, Gonorrhea, *Mycoplasma genitalium*, and syphilis) **(CHAPTER 3)**
    1.2. To identify potential predictors for STI reinfection among PrEP users in order to uncover the subgroup of MSM most at risk to acquire STIs **(CHAPTER 3)**
    1.3. To document the LGV epidemic of Belgium from 2011-2019 and to explore the influence of the roll-out of PrEP on this epidemic **(CHAPTER 4)**
    1.4. To examine the prevalence of macrolide and fluoroquinolone resistance in *Mycoplasma genitalium* among MSM **(CHAPTER 5)**

2. To evaluate potential improved screening strategies in order to simplify STI testing (SECTION II)

2.1. To develop different sample pooling strategies for molecular testing of *Chlamydia trachomatis/Neisseria gonorrhoeae* as cost-effective intervention of STI screening **(CHAPTER 6)**

2.2. To evaluate home-based sampling for screening of STIs during PrEP follow-up visits **(CHAPTER 7)**

SECTION I: EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF STIS AMONG MSM IN BELGIUM

CHAPTER 3: RESULTS OF A PREP DEMONSTRATION PROJECT: BE-PREP-ARED

The Be-PrEP-ared study started at ITM in 2015 under the lead of Prof. Dr Marie Laga. Together with a multidisciplinary team the study was successfully ended in 2018. I was the clinical coordinator of the study and I wrote the first draft of the study protocol with feedback from the STI/HIV unit of public health, the HIV/STI reference laboratory (which I am part of), the STI clinic and the Clinical Trials Unit of ITM. Furthermore, I ensured the implementation and follow-up of the clinical and laboratory procedures of the study. During my pregnancy leave of Bastian, Vicky Cuylaerts took over for which I am very grateful.

The aim of the study was to evaluate whether PrEP, provided within a comprehensive prevention package, was a feasible and acceptable additional prevention tool for MSM with behaviours increasing their vulnerability to HIV in Belgium. To be able to participate, participants needed to report at least one of the following criteria:

- Reported condomless anal intercourse in the past six months with a casual partner with unknown or HIV positive status
- Reported at least one STI episode in the past six months
- Reported having taken post-exposure prophylaxis in the past six months

A total of 200 participants were included and could opt to use PrEP daily or only during high-risk sexual activity (event-driven). They were followed-up for 18 months with strict three monthly STI testing including syphilis and CT/NG/MG testing at each anatomical site (urethra, pharynx and anorectum). Furthermore HIV serology was performed at every visit. In addition, sexual risk behaviour during the last three months was questioned at every visit using a standardized self-administered electronic

questionnaire. Due to the unavailability of PrEP in Belgium until end of June 2017, participants were able to extend the study until May 2017 in order to avoid loss of PrEP. All details of the procedures and the baseline data can be found in Annex 1 and 2 of the thesis. In short, 200 MSM with behaviours increasing their vulnerability to HIV were included in the study. Bacterial STI prevalence was high at baseline (table 1).

Table 1: Prevalence of Sexually transmitted infections (STI) at baseline in the Be-PrEP-ared study. (Annex 2)

Pathogen		**Baseline prevalence % (n)**
Syphilis		7.5% (5)
Chlamydia (non L and L types)	Total	11.7% (23)
	Urethra	2.5% (5)
	Anorectum	10.0% (20)
	Pharyngeal	1.0% (2)
Lymphogranuloma Venereum (anorectal)		0.5% (1)
Gonorrhea	Total	12.2% (24)
	Urethra	1.0% (2)
	Anorectum	7.0% (14)
	Pharyngeal	7.0% (14)
Mycoplasma genitalium	Total	17.2% (34)
	Urethra	8.5% (17)
	Anorectum	8.5% (17)
	Pharyngeal	0% (0)
Bacterial STI*		39.5% (77)

** Bacterial STI: syphilis, gonorrhea, chlamydia or mycoplasma genitalium*

Self-reported levels of sexual-risk taking in the past three months before enrollment were high. The median reported total number of sexual partners was 12, 71% reported condomless anal intercourse with at least one occasional partner, 64% engaged in group sex, and sexualized drug use was 66% (Annex 2).

The two published papers of this chapter present the longitudinal results of the study (including STI incidence and change in sexual behaviour). In addition, a secondary analysis was performed in order to identify the socio-demographic and risk behaviour factors associated with acquiring recurrent STIs.

- Daily and event-driven pre-exposure prophylaxis for men who have sex with men in Belgium: results of a prospective cohort measuring adherence, sexual behaviour and STI incidence.
- Recurrent sexually transmitted infections among a cohort of men who have sex with men using pre-exposure prophylaxis in Belgium are highly associated with sexualized drug use.

3.1 BE-PREP-ARED LONGITUDINAL DATA

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RESEARCH ARTICLE

Daily and event-driven pre-exposure prophylaxis for men who have sex with men in Belgium: results of a prospective cohort measuring adherence, sexual behaviour and STI incidence

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3.1.1 Abstract

Introduction: Pre-Exposure Prophylaxis (PrEP) is highly effective in reducing the risk for HIV infection among men who have sex with men (MSM) and may have an important impact in slowing down the HIV epidemic. Concerns remain however about low adherence, increased risk behaviour and reduced condom use when using PrEP. The aim of this study was to assess these factors prospectively among MSM using daily and event-driven PrEP in Belgium.

Methods: An open-label prospective cohort study was conducted from October 2017 to May 2018 at the Institute of Tropical Medicine, in Antwerp, Belgium. At enrollment, MSM at high risk for HIV chose between daily or event-driven PrEP. They were allowed to switch regimens or stop taking PrEP at each of their tri-monthly visits. Data were collected on an electronic case report form, web-based diary and self-administered questionnaire. Screening for HIV and other Sexually Transmitted Infections (STIs) was also performed.

Results: Two hundred MSM were followed up for a total duration of 318 person-years. At month 18, 75.4% of the participants were on daily and 24.6% were on event-driven PrEP. The mean proportion of covered sex acts by PrEP for the complete follow-up period was 91.5% for all participants, 96.5% for daily and 67.0% for event-driven PrEP use. The number of casual and anonymous sex partners was significantly higher for daily users, as compared with event-driven users, but did not change over time. In contrast, the mean proportion of condomless receptive anal intercourse with casual and anonymous partners increased significantly during follow-up, for both daily and event-driven use ($p < 0.0001$ for all 4 trends). No new HIV infection was diagnosed during follow-up. The incidence of bacterial STIs was 75.4 per 100 person-years (95% CI 63.8 to 89.1). We did not detect a significant change in *N. gonorrhoeae*/*C. trachomatis* incidence over time. The incidence of hepatitis C was 2.9 per 100 person-years.

Conclusions: PrEP is an effective and well adopted HIV prevention tool for MSM in Belgium. Participants adapted daily and event-driven regimens to their own needs and were able to adapt their PrEP adherence to risk exposure.

KEYWORDS

PrEP; HIV prevention; MSM; Belgium; adherence; STI; event-driven

3.1.2 Introduction

Pre-Exposure Prophylaxis (PrEP) using daily oral tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) is highly effective in reducing the risk for HIV infection among men who have sex with men (MSM).[1–3] In addition, a randomized controlled trial among MSM in France and Canada demonstrated a 86% efficacy of a non-daily, event-driven regimen.[4]

These results underscore the potential impact of including PrEP, daily or event-driven, into a powerful HIV prevention package for the most-at-risk MSM. Concerns remain, however, about "real-life" use of PrEP, including low adherence, increased risk behaviour and reduced condom use, upsurge in sexually transmitted infections (STIs), and long-term drug toxicity.

Adherence is critical for the effectiveness of PrEP and poor adherence may explain incident HIV infections while on PrEP.[5] Studies from other health domains showed that adherence for primary prevention may be more difficult to achieve than for secondary prevention.[6] In addition, PrEP adherence must be understood within the context of variable risk for HIV infection and use of other HIV prevention methods.[7,8] In contrast to anti-retroviral treatment which need lifelong sustained adherence by people living with HIV, PrEP can be taken temporarily during episodes of anticipated increased risk.[9] Non-daily regimens also have the advantage of requiring fewer tablets, thus reducing potential side-effects and cost.[10,11] However, adherence to these regimens should be optimal, as they are less forgiving of missed doses.[12] Although some studies report a lower adherence to non- daily regimens, it remains unclear how good adherence is in a real life situation, when users chose between daily and event- driven PrEP according to their preference.[13]

Another concern raised in relation to PrEP refers to "risk compensation," or a shift towards more risky sexual behaviour triggered by perceptions of decreased HIV risk.[14] A systematic review and meta-analysis of seventeen open-label studies found that PrEP among MSM was associated with a decrease in overall condom use but no increase in HIV.[15] However, these changes in sexual behaviour may increase the chances of acquiring STIs other than HIV.[16] We still lack data on the incidence of STIs and their trends in MSM while on daily and event-driven PrEP.

Belgium is among the countries in the European Union reporting a high HIV incidence, with 7.9 new HIV infections per 100,000 inhabitants in 2017.[17,18] A recent study suggests that ongoing clustered transmission in Belgium is almost exclusively MSM driven.[19] At the time of this study PrEP was not yet approved or reimbursed. Since 1 June 2017, Truvada

can be prescribed and reimbursed as prophylactic medication for people who are at increased risk of HIV acquisition in Belgium.

The aim of this study was to assess adherence, sexual risk behaviour, condom use and STI incidence among daily and event-driven PrEP users in a prospective cohort of Men and Transgender Women who have sex with Men (MTSM) at high risk for HIV in Belgium.

3.1.3 Methods

Study design, setting and participants

Be-PrEP-ared was a single-site, open-label prospective cohort study (EudraCT 2015-000054-37) conducted at the Institute of Tropical Medicine, in Antwerp, Belgium.

We enrolled 200 HIV negative MTSM, aged 18 years or more, who were at high risk of HIV acquisition. Potential participants were recruited through study advertisement at websites and various community-based gay and sexual health organisations. High risk was defined as reporting at least one of the following in the previous six months: unprotected anal sex with a casual partner with unknown or HIV positive status, a STI diagnosis and post-exposure prophylaxis. Exclusion criteria included HIV infection and contraindications for the study drug, as published previously.[20] We calculated a sample size of 200 to estimate the proportion of participants who were non-adherent with a precision of 7% if the proportion of non-adherence was 50%.

Study procedures and data collection

Detailed study procedures have been published before.[20] Eligible participants provided written informed consent and were invited to an enrollment visit, a follow-up visit after one month, and then 3-monthly visits until 18 months. The first enrollment took place on 7 October 2015 and last enrollment on 12 December 2016. The study follow-up period was extended until the end of January 2018 for those participants rolling out before 1 November 2017. The aim of the extension was to cover the window period between the end of the study and the anticipated availability of PrEP in Belgium. Data collection ended on 4 May 2018.

TDF 245 mg/FTC 200 mg was used as PrEP in this study. Participants received detailed information and counselling about the two different PrEP dosing regimens. They then choose between daily PrEP (one pill every 24 2 hours) and event-driven PrEP (a starting dose two to 24 hours before anticipated sexual contacts, and then continuing with the daily regimen until two days after the sexual active episode). The starting dose normally ±

involves two pills unless the most recent dose was taken between one and six days before the starting dose. Participants could stop taking PrEP, or switch regimens at each visit.

Study procedures included a medical history and examination, sexual health counselling, and blood samples for HIV, serum creatinine and syphilis. HSV-2 and hepatitis B virus (HBV) serology were performed at screening and at month 18. We performed serology for hepatitis C (HCV) initially at baseline and at month 18. After discovering two new HCV infections at month 18, we wrote an amendment to extend HCV serology every six months from 23 May 2017 on. Participants self-collected urine and anorectal swabs and a study nurse took pharyngeal swabs for STI testing. We recorded all clinical, laboratory and adherence data on a standardized electronic case report form. At enrollment and at each follow-up visit, participants completed a detailed electronic questionnaire on sexual behaviour from the previous three months, including number of partners, type of sexual contact (insertive or receptive) and condom use per type of partner (steady, casual, anonymous). Casual partners were defined as non- steady, non-anonymous partners with whom the study participant occasionally or regularly had sex.

Participants also completed a personal web-based diary with information on daily pill intake and, if sexual activity took place, the HIV exposure predefined category (low, medium or high). The exposure category was suggested by an algorithm, taking into account type of sexual contact, condom use, type of sexual partner (anonymous, casual or steady), HIV serostatus of the partner and viral load if the partner was HIV positive. High HIV exposure was defined as condomless anal intercourse with a new or occasional partner who is HIV positive or of unknown status, or with a HIV positive steady partner with detectable viral load. Low HIV exposure was defined as consistent condom use during the whole “sex day,” or condomless anal intercourse with a steady partner who is HIV negative or HIV positive with undetectable viral load. Medium exposure was defined on sex-days which did not correspond to “high” neither to “low” exposure.

Laboratory procedures

All laboratory procedures were carried out in the Central Laboratory for Clinical Biology or the HIV/STI Reference Laboratory of the Institute of Tropical Medicine, Antwerp, Belgium. HIV was tested using an on-site point-of-care test (Alere HIV Combo, Alere Inc., Waltham, MA) confirming HIV negativity by two HIV fourth generation enzyme-linked immunosorbent assays including HIV antigen testing according to the algorithm of the ITM.[20] Syphilis was tested through Rapid Plasma Reagin (RPR, Macro-Vue, Becton

Dickinson BD Microbiology Systems, Maryland, USA) and Treponema Pallidum Assay/ Treponema Pallidum passive particle agglutination assay (TPA, Vitros 5600 Ortho-Clinical Diagnostics, Rochester, NY)/SERODIA-TPPA, Fujirebio Inc, Tokyo, Japan). In addition, real-time PCR was used to detect *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) on urine, pharyngeal and anorectal samples according to previously published assays.[20] Samples positive for CT were further tested to distinct L-serovars from non-L serovars using a previously published real-time PCR [21]. HSV-2 antibody testing was done by the Kalon HSV-2 IgG ELISA (Kalon Biological Ltd., United Kingdom). In case a sample was positive on month 18, a look back approach was applied and previous samples were tested until the most recent negative sample. We tested for HCV antibodies, HBsAg, HBsAb, HBcIg and HBcIgM, AST/ALT and creatinine with Vitros 5600 (Ortho-Clinical Diagnostics, Rochester, NY). If a six-monthly sample was positive for HCV, we also looked back at the previous sample to narrow down the time of infection.

Outcomes and definitions

Adherence (based on diary data)

Adherence was estimated by the proportion of anal sex acts covered by PrEP. The proportion of anal sex acts covered by PrEP was calculated as the proportion of "sex-days" (i.e. days when anal intercourse with one or more men occurred as denominator) for which PrEP was correctly taken (numerator). A correct intake of PrEP involved a correct dose of PrEP before, during and after the days on which sexual intercourse took place. A correct dose before involved at least two pills taken on days X (i.e. a sex day) or X-1 (i.e. the day before); or at least one pill on X or X-1 if a pill was taken between day X- 6 and X-1. The last situation occurred when a person was on daily PrEP, or if there was less than one week between two episodes of event-driven PrEP. A correct dose during and after included at least one pill on days X, X + 1 and X + 2.

All information on daily sexual activity and pill intake was extracted from participants' diaries.

Sexually transmitted infections

Participants were considered infected with NG if they tested positive for NG in one of the three biological sites (anorectal, pharynx or urine). The same was done for CT, MG and TV. A diagnosis of syphilis was defined as a positive RPR test with a titre of at least 4 together with a positive TPA or TP-PA test. As for HSV-2, a grey zone ratio of 0.9 up to 1.1 was coded as not interpretable.

Statistical analysis

The study statistician performed all statistical analyses using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.

The analyses of adherence and sexual behaviour characteristics were done by regimen used since the last scheduled visit. In the latter case, if regimens were switched since last scheduled visits, participants were assigned to the regimen he/she took the longest time during this episode. To compare adverse events and STI incidence between the regimens, we took the regimen which was used the longest over the full follow-up.

The proportion of covered sex-days and the proportion of condomless receptive anal intercourse was estimated using a binomial Generalized Linear Model. The number of partners was estimated using a Poisson regression model.

The global incidence rate of STI was calculated with censuring after a positive test. To assess trends in the incidence rate of NG/CT over time, we calculated the number of times a participant had a positive NG or CT test result. A mixed effects Poisson regression model was fitted with visit as a categorical covariate, a random intercept per subject and log (follow-up time) as offset. After each positive lab result, the participant was considered not at risk for 14 days to take the treatment period into account.

Ethical clearance

Ethical approval was provided by the Institutional Review Board of the Institute of Tropical Medicine Antwerp and the Ethics Committee of the Antwerp University Hospital.

3.1.4 Results

General characteristics of the study cohort and choice of PrEP regimens

A total of 197 men and three transgender women participated in the study. A detailed description of the socio-demographic characteristics of the study participants at baseline has previously been published.[22] Briefly, their median age was 38 years (min 22, max 70) and they were predominantly white (89.0%), highly educated (78.5%) and employed (78.5%). After 18 months of follow-up, 89.5% of the 200 participants were still on PrEP. Extension of study participation was taken up by 99 participants. The total follow-up time was 318 person-years.

Figure 1 shows details of the regimen initial choices and switches, discontinuation and lost to active follow-up until M18.

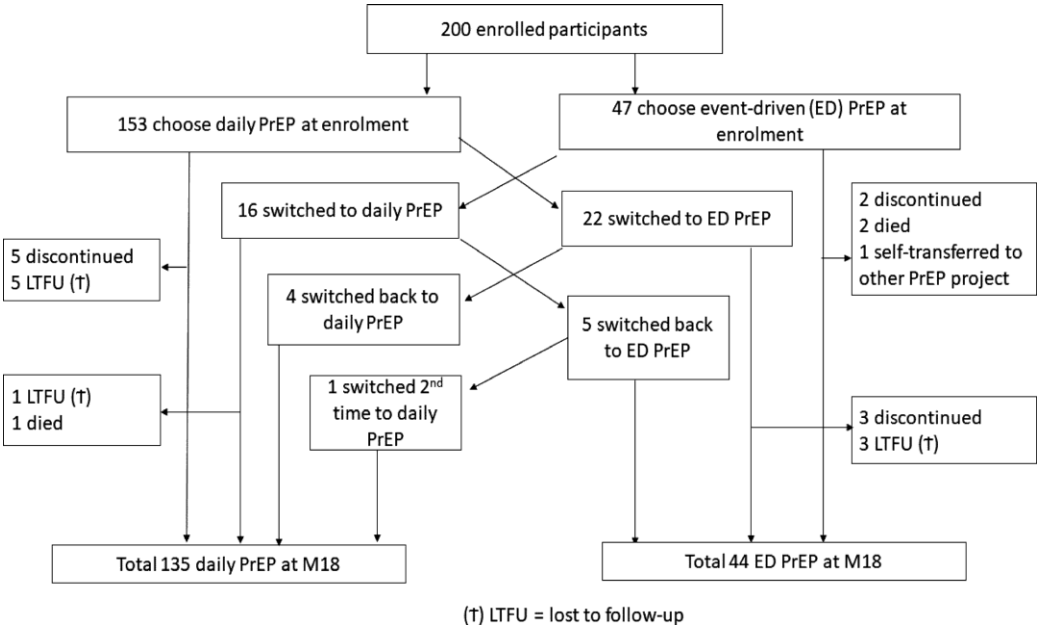

Figure 1: Flow diagram of study participants and PrEP regimen switching patterns.

Thirty-eight participants (19%) switched regimens at least once. At M18, 135 participants were on daily (75.4% of those still on PrEP) and 44 were on event-driven (24.6% of those still on PrEP) regimen. The main reasons for switching to the event-driven regimen (in total 27 switches) included: change in sexual relationship (n = 12) or less sex (n = 4), too many pills to take (n = 6) and illness (n = 4). The main reasons for switching to daily (total 21 switches) included: too difficult to remember to take the pills (n= 15), change in sexual relationship (n = 5) or more frequent sex (n = 2), and convenience or easier to take (n = 3).

During their follow-up, 64 participants interrupted their regimen temporarily, with a total of 95 interruption episodes and a median duration of 14 days (min 1 day to max 169 days). The main reasons for interruption included illness (n = 39), travel (n = 15), running out of pills (n = 12) and side effects of TDF/FTC (n = 11).

Overall, 128 participants (96 in daily and 32 in event-driven regimen) reported 238 adverse events possibly, probably or definitely related to the study drug. Most were gastrointestinal such as flatulence, diarrhoea, nausea and gastric discomfort (76 under daily and 23 under event-driven regimen).

The proportion of participants who expressed the intention to use PrEP in the future was 96% at their last study visit.

Adherence

One participant did not use the diary. The other participants filled in the diary for 95.5% of all follow-up days.

The mean proportion of covered sex-days for the complete follow-up period was 91.5% (95% CI 91.1 to 91.8) for all participants. This proportion was 97.5% (95% CI 97.1 to 97.8) and 95.9% (95% CI 95.4 to 96.3) for daily PrEP and 87.7 % (95% CI 85.7 to 89.5) and 42.1 % (95% CI 39.8 to 44.5) for event-driven PrEP), during days with high and low exposure respectively (mixed effects logistic regression model, all $p < 0.0001$). Figure 2 shows the evolution over time.

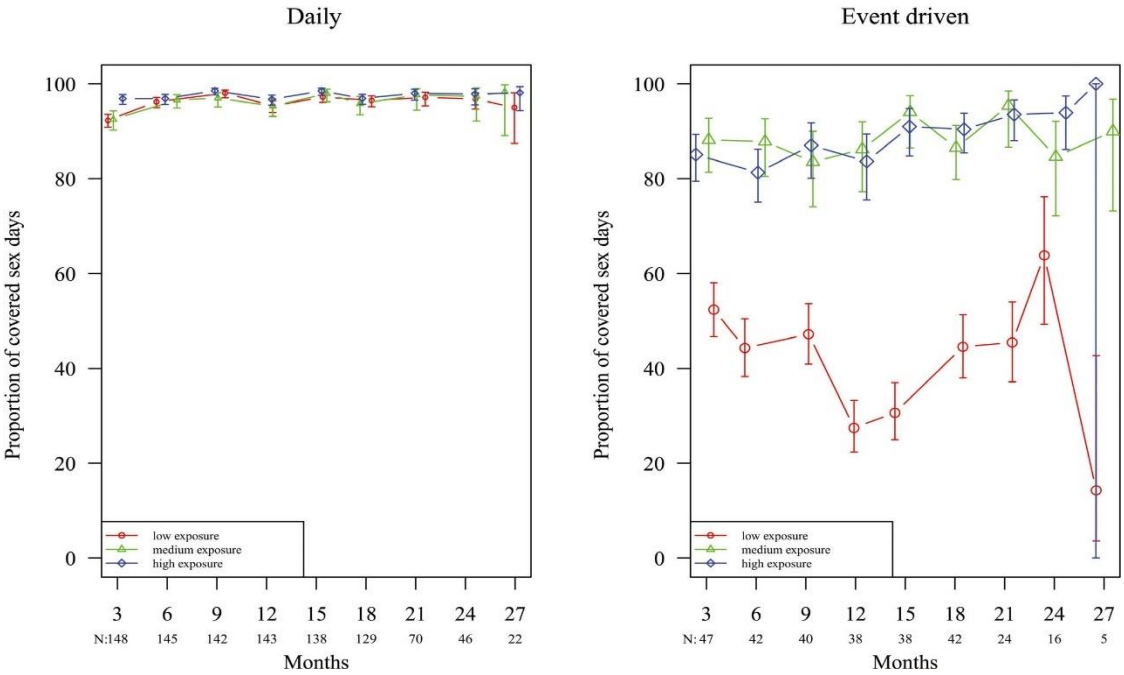

Figure 2: Proportion of covered sex days according to PrEP regimen and per risk exposure.

Sexual risk behaviour

Figure 3 shows the evolution of the number of casual and anonymous sex partners over time. This number was significantly higher for daily, as compared to event-driven PrEP users (joint test of group coefficient and the interaction, $p = 0.044$), but did not change significantly over the study period in both groups (Poisson GEE).

The mean proportion of condomless receptive anal intercourse with casual and anonymous partners increased significantly during follow-up, for both daily and event-driven use (mixed effects logistic regression model, $p < 0.0001$ for all four trends) (Figure 4). Participants' own perception of changing condom-use while on PrEP was assessed in the questionnaire. The proportion answering they use less condoms since starting PrEP was 44.3% on M3, 53.1% on M12 and 61.0% on M18.

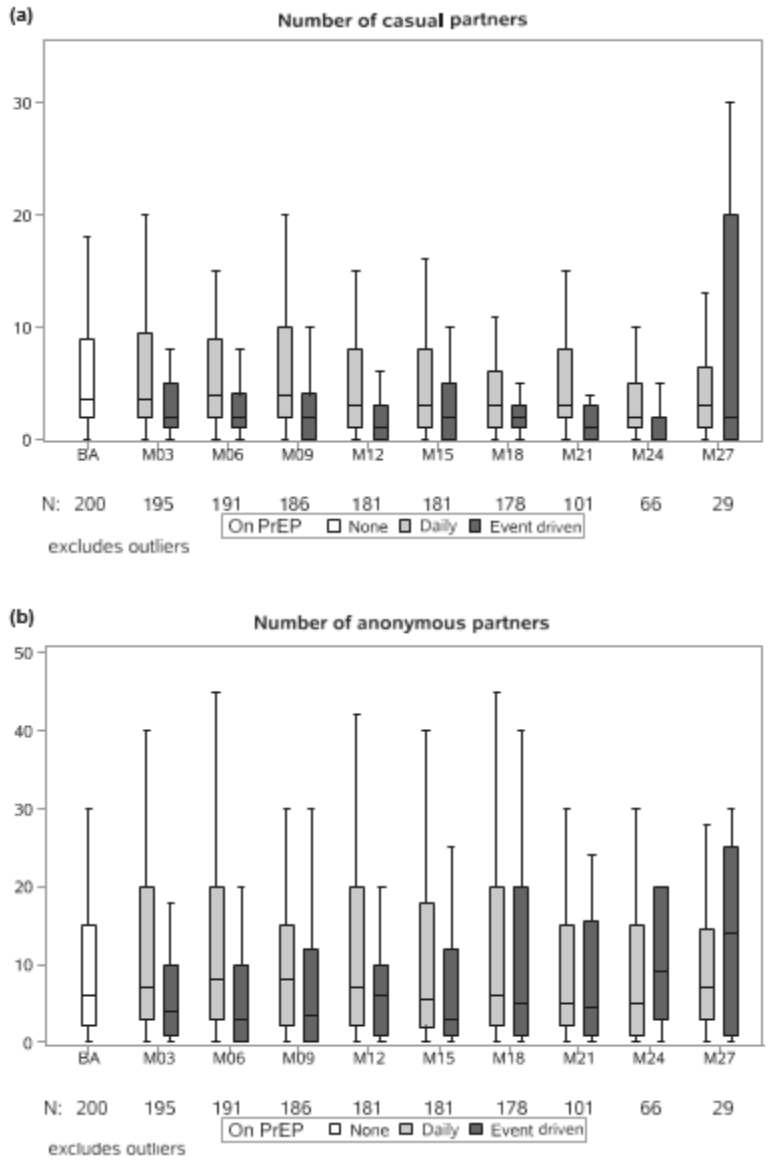

Figure 3: Summary boxplots of the number of casual (a) and anonymous (b) partners in last three months, per drug regimen.

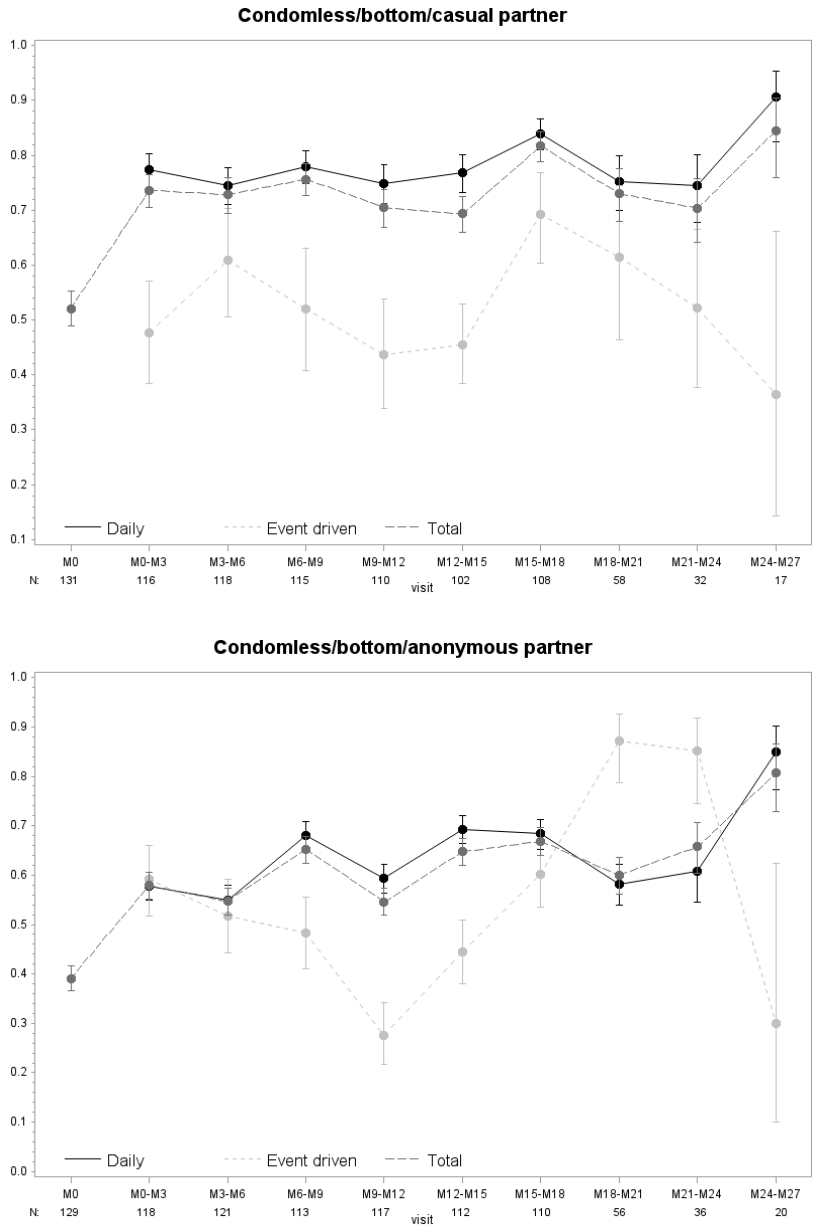

Figure 4: Proportion of condomless receptive intercourse with casual (a) and anonymous (b) partners over time, per PrEP regimen.

HIV/STI incidence

During the 318 person-years of follow-up no new HIV infections were diagnosed. Table 1 summarises incidence rates of the other STIs assessed. The incidence of bacterial STIs

(syphilis or NG or CT or MG) was 75.4 per 100 person-years (95% CI 63.8 to 89.1). A total of nine new hepatitis C infections were reported during follow-up, resulting in an incidence rate of 2.9 per 100 person-years. We did not detect any differences when comparing participants using daily with participants using event-driven PrEP.

Table 1:Incidence rate (IR) of Sexually transmitted infections (STIs).

STI	IR per 100PY	(95% CI)
Syphilis	8.26	5.49 to 12.42
Neisseria gonorrhoeae	38.94	31.78 to 47.72
Pharynx	16.18	12.12 to 21.59
Anal	24.78	19.43 to 31.60
Urine	5.90	3.71 to 9.36
Chlamydia trachomatis (non-LGV)	30.92	24.73 to 38.85
Pharynx	4.54	2.69 to 7.67
Anal	18.72	14.23 to 24.63
Urine	10.20	7.13 to 14.59
Chlamydia trachomatis (LGV)	4.22	2.45 to 7.27
Pharynx	0.00	NA
Anal	4.22	2.45 to 7.27
Urine	0.00	NA
Chlamydia trachomatis (LGV + non-LGV)	34.21	27.59 to 42.42
Mycoplasma genitalium	21.35	6.09 to 28.33
Pharynx	3.50	1.82 to 6.72
Anal	14.24	10.17 to 19.93
Urine	4.73	2.69 to 8.33
Bacterial STI	75.39	63.81 to 89.08
Trichomonas vaginalis	0.95	0.31 to 2.95
Pharynx	0.00	NA
Anal	0.63	0.16 to 2.53
Urine	0.32	0.04 to 2.24
Hepatitis C	2.93	1.53 to 5.64
Hepatitis B	0.00	NA
HSV-2	8.13	4.98 to 13.27
HIV	0.00	NA
Any STI	84.11	71.47 to 98.97

PY: person-years; CI: confidence interval; NA: Not Applicable; LGV: lymphogranuloma venereum; HSV-2: Herpes Simplex Virus-2

We did not detect a significant change of NG/CT incidence over time (Poisson mixed effects model, $p = 0.38$) (Figure 5).

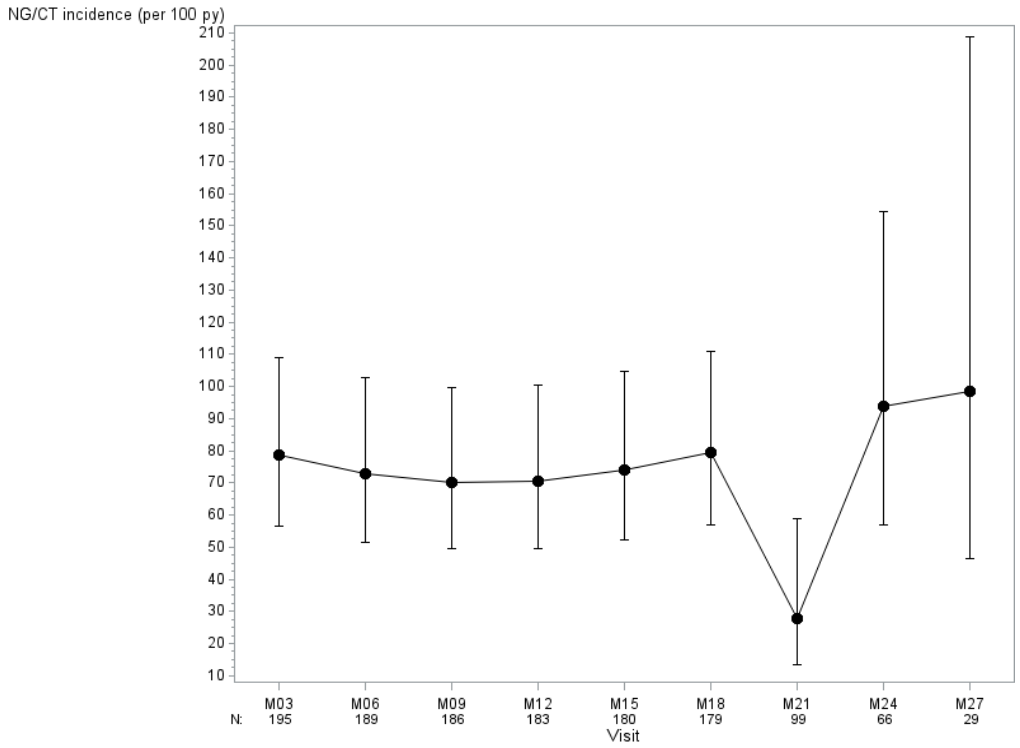

Figure 5: Trend of Neisseria gonorrhoeae (NG)/ Chlamydia trachomatis (CT) tri-monthly incidence over scheduled visits in the Be-PrEP-ared study.

3.1.5 Discussion

This study showed that 200 MTSM at high risk for HIV in Belgium adopted both daily and event-driven PrEP as an HIV prevention method tailored to their sexual life style.

Be-PrEP-ared was one of the first European PrEP demonstration projects among MTSM at high risk for HIV which allowed for choosing between daily or event-driven PrEP, and for switching regimens according to users' preferences. Our results showed a substantial proportion of PrEP users switching regimens during follow-up (19%), which is an indication of users' varying needs and preferences over time. A similar study in Amsterdam found a cumulative proportion of 30% of participants who had switched regimens two years after initiation of PrEP.[23] In addition, 32.0% of our study participants interrupted using PrEP at least once, for various reasons. These results suggest that in real life, "daily" and "event-driven" PrEP may be fluid categories rather than rigid regimens. Rather than proposing daily or event-driven PrEP, it may be more useful to propose PrEP as a daily regimen, which can be interrupted and adapted to short or long episodes of individual protection needs. Given the importance of adherence, emphasis

in patient education and counselling should thus be given on how to safely start and stop PrEP episodes rather than focusing on following a particular regimen.

Event-driven PrEP use may lead to lower adherence compared to daily use, as suggested by other studies.[13,24] In our study, we found a proportion of covered sex days of 67% while on event-driven and 96% while on daily PrEP. In the Adapt study, the proportion covered sex acts was 74% and 85% among MSM in Bangkok and 52% and 75% among women in Capetown, for event-driven and daily PrEP respectively.[13,24] It should be noted however that participants of the Adapt study were randomly assigned to a PrEP regimen, as opposed to our study, where participants self-selected and could switch between daily and event-driven, according their preference, during the entire follow-up period. In the Ipergay Open Label study in France, where participants only had the option of event-driven PrEP, 50% correct doses were taken. The results are hard to compare not only because of differences in study design but also because none of the previous studies reported on the coverage of sex acts in relation to risk exposure. As indicated before, being able to safely use PrEP when it is needed may be of paramount importance, rather than focusing on rigidly following a particular dosing regimen for indefinite period of time. Consistent high adherence during periods of non-exposure is costly.[11] Therefore, Haberer et al. called for a novel paradigm: prevention-effective adherence that proposes the use of PrEP only during periods of heightened risk exposure resulting into effective protection against HIV acquisition.[25] It also includes the use of other effective HIV prevention tools such as condoms and other behavioural strategies.[25] Risk-taking can be seasonal and fluctuations are determined by personal and contextual factors.[26] PrEP adherence will thus depend on users' ability to understand their risk for HIV acquisition, and act upon it. When measuring adherence in that sense (i.e. the proportion of high risk sex acts covered by PrEP), our study participants were highly adherent to PrEP. Adherence was low for men using event-driven PrEP in low exposure situations, e.g. sex with the steady partner, or sex protected by a condom. Low adherence in low-risk situations may be of lesser concern and it fits into an effective "prevention-effective adherence" strategy. Our results show some interesting changes in participants' sexual behaviour: while numbers of casual and anonymous sex partners did not increase, the frequency of condom use with these partners dropped significantly during follow-up. Anonymous partners are usually connected with very short-term sexual interactions and few negotiation of using protection strategies.[27] Our results compare well with results of the Amsterdam PrEP cohort study. In this study, the number of condomless anal sex acts with casual partners increased over time, whereas the number of partners and sex acts were stable.[28] Also in France, the rate of condomless sex at last intercourse increased from 53.3% at baseline to 79% at month 12.[29] In the light of prevention-effective adherence, the

terminology of "risk taking" and hence its assessment may no longer be appropriate for HIV. The high efficacy of PrEP seen in demonstration projects and in the real world suggests that "risk compensation" does not necessarily result in an increased risk for HIV infection in PrEP users.[30]

In contrast to zero new HIV infections, we found a relative high incidence of STI during follow-up, specifically for NG and CT (incidence rate of NG and CT respectively 38.9 and 34.2 per 100 person-years).

We did not find an increasing trend when analysing three-monthly incidences of NG/CT over time. These incidences corroborate other PrEP demonstration studies' results.[31] Neither the PROUD trial nor the Ipergay trial reported increased STI frequency during study follow-up.[1,4] Since our study did not include a control group of highly exposed non-users, we cannot conclude that PrEP use does not increase STI incidence. STI incidence data before PrEP use were also not available. A study in Canada evaluated the impact of PrEP on STI in a cohort of MSM before and after initiation of PrEP, and observed a 72% increase in incidence of STIs in the 12 months after PrEP.[32] In a similar study in Australia STI incidence increase from 69.5 prior to PrEP to 98.4 per 100 person-years during PrEP.[33] The effect of PrEP use on STI incidences remain uncertain and should further be studied. The incidence of hepatitis C among HIV-negative participants was higher than expected in our study, as also observed in the Amsterdam PrEP cohort.[34] These results call for regular screening for STI, including hepatitis C, as an essential component of PrEP delivery.

Some limitations and potential biases should be acknowledged: The original study follow-up duration of 18 months was extended a few months for a limited number of study participants. As the inclusion order in the study was random, a selection bias due to the extension may not apply. However, the number of participants at month 21, 24 and 27 was very small, decreasing the precision of our study results after month 18.

Although reporting bias cannot be excluded, the use of digital tools as opposed to face-to-face interviews may have reduced this bias.[35]

The online diary had its limitations to avoid burdening study participants too much with detailed data provision. For instance, it did not include the timing of sexual intercourse, preventing us from adjusting adherence by the correct timing of the PrEP intake. Furthermore, some participants did not fill it in regularly, or filled it retrospectively after some days, which may have led to a recall bias.

3.1.6 Conclusion

In conclusion, PrEP is an effective, safe and well adopted HIV prevention tool for MTSM who are at high risk for HIV in Belgium. Participants adapted daily and event-driven regimens to their own needs and were able to adapt their PrEP adherence to risk exposure. Monitoring of PrEP use in Belgium is needed to confirm these findings among regular PrEP users outside of the context of a demonstration project.

3.1.7 Acknowledgements

The study medication was donated by Gilead Sciences. Abbott donated the CT/ NG diagnostic kits for Real Time PCR. TR is a postdoctoral fellow of the Research Foundation Flanders. We further thank the Be-PrEP-ared study group, the laboratory staff, the Community Advisory Board and all the study participants.

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3.2 IDENTIFYING INDIVIDUALS WITH RECURRENT STIS

Sexually Transmitted Diseases, Publish Ahead of Print
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Recurrent sexually transmitted infections among a cohort of men who have sex with men using pre-exposure prophylaxis in Belgium are highly associated with sexualized drug use

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3.2.1 Abstract

Background: Men who have sex with men (MSM) experiencing recurrent STIs may play a crucial role in the STI epidemic. However, there is limited understanding of what kind of behaviour leads to recurrent STIs.

Methods: A total of 179 MSM using PrEP were followed up for 18 months and were screened quarterly for chlamydia, gonorrhea, and syphilis from 2015-2018 in Belgium. Participants were stratified into three different groups (no STI; one STI episode; recurrent STI episodes during the study). Socio-demographic and sexual behavioural characteristics were compared between the three groups and significant associations with recurrent STI were explored using multivariate logistic regression models.

Results: A total of 62.0% (n=111/179) of participants experienced at least one STI during the study, and more than one in three became reinfected with an STI at another visit (n=66/179; 36.9%). Participants experiencing recurrent STIs reported the highest frequency of sexualized drug use (86.4%) compared to participants experiencing one (60.0%) or no STI (47.1%). Therefore, sexualized drug use was highly associated with recurrent STIs (adjusted odds ratio (aOR): 4.35). Other factors associated with recurrent STIs were being younger than 40 years old (aOR: 3.29), had a high number (>4) of non-steady partners with whom receptive (aOR: 1.17) or insertive (aOR:1.12) condomless anal intercourse occurred in the last three months

Conclusions: Sexualised drug use was the greatest risk factor for having recurrent STIs. Tailoring prevention and care, including specialised services tackling problematic drug use in a sexual context, may help to curb the STI epidemic among MSM.

KEYWORDS

Sexually transmitted infections, Pre-exposure prophylaxis, Men who have sex with men, recurrent infections

3.2.2 Introduction

The number of bacterial sexually transmitted infections (STIs) continues to rise globally among key populations. Gay, bisexual and other men who have sex with men (further referred to as MSM) are one of the key populations at elevated risk for STIs.[1] For decades, HIV- and STI prevention have been intertwined and primarily focused on behavioural strategies such as promoting safer sex practices, including condom use.[2] However, since 2010, biomedical HIV prevention methods based on the use of antiretroviral therapy for HIV have been introduced, including oral pre-exposure prophylaxis (PrEP).[3] Oral PrEP, if taken correctly, is highly efficacious in reducing HIV risk among high-risk populations, such as MSM, but it does not prevent other STIs. Concerns have been raised that the improved protection against HIV could lead to increased risk-taking (i.e., 'risk compensation'), potentially leading to increases in STIs.[4] Indeed, many PrEP demonstration projects and open-label studies among MSM report a decrease in overall condom use, an increase in the number of condomless contacts, as well as an increase in condomless anal intercourse (CAI).[5–7]

Findings on the impact of PrEP on STI incidence are, however, conflicting. Some studies show an increase in the number of STIs after the start of PrEP, while others do not.[5,6,8–11] Nevertheless, the pooled incidence of chlamydia, gonorrhea and early syphilis in PrEP studies is high (72.2 per 100 person-years).[10] The high number of diagnosed STI infections may be partially explained by frequent screening of asymptomatic PrEP users and by an increasing number of partners and condomless sexual encounters. Furthermore, evidence shows that reinfections may occur more frequently among certain key subgroups, such as men with multiple sex partners and those engaging in condomless sex.[12–15]

Identifying individuals with recurrent STIs is critical as they may be at increased risk for HIV/ STI acquisition and sequelae.[15] Furthermore, these individuals may play a substantial role in the observed increases in reported cases of STIs among PrEP users. Many of the STIs are asymptomatic and these individuals may act as a reservoir of infection, potentially spreading STIs throughout the community through wide sexual networks.[15] Prompt detection and treatment of STIs is key to reducing the individual state of infection, and could be crucial for decreasing community level transmission.[16] A better understanding of what may lead to STI reinfections, could contribute to interrupting this chain of transmission. Better insights into predictors of STI reinfections are therefore needed in order to tailor prevention and care to this sub-population. In studies with different populations, including heterosexuals and MSM, individuals experiencing recurrent STI episodes contributed to 7-23% of all STI diagnoses.[12–14]Reinfection rates among PrEP users are understudied. An Australian PrEP study

reported that 76% of all STIs was attributed to 25% of the participants, and 6% experienced four or more STIs in the first year, accounting for almost one third of all STIs.[11] Thus, differentiating those PrEP users at highest risk for having recurrent STIs may be crucial in curbing the STI infections within this population.

In Belgium, PrEP was first implemented as an open demonstration project that took place from September 2015 to May 2018. A total of 200 MSM taking PrEP daily, or event-driven, were followed up for at least 18 months and were screened quarterly for STIs. In that study, no new HIV infections were detected and although the incidence of bacterial STIs was high (75.4 per 100 person-years), no significant changes in overall *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) incidence was found over time.[6] In June 2017, PrEP became reimbursed in Belgium and approximately 4071 individuals had started taking PrEP before January 2020.[17] PrEP distribution, follow-up and complementary STI testing is partially covered by the healthcare system. It is likely that this number of PrEP users will further increase, leading to a substantial increase in the number of STI tests, and a consequent strain on healthcare providers and public health resources.[18] Targeted screening efforts, such as identifying risk factors for STI reinfection, could improve the cost-effectiveness of periodic STI testing, instead of testing every individual quarterly.

The aim of this study was to assess the magnitude of recurrent STIs in a Belgian cohort of MSM using PrEP, and to identify potential predictors for recurrent STIs. The results of this study could be used to guide clinicians and policy makers in future STI testing policies.

3.2.3 Materials and Methods

Study design and STI testing during the study

We performed a secondary analysis of the Be-PrEP-ared study data, a single-site, open-label cohort study conducted at the Institute of Tropical Medicine, Antwerp, Belgium between September 2015 and May 2018. A total of 197 HIV negative men and three transwomen having sex with men were enrolled. The main methods, including inclusion and exclusion criteria, procedures and results, are documented elsewhere.[6,19,20] For this current analysis, we selected participants who completed at least 18 months of follow-up and we extracted data until month 18. STI-associated symptoms were documented as adverse events. The study was approved by the Institutional Review Board of the ITM and the Ethics Committee of the University of Antwerp, Belgium (reference number: 15/25/255). All participants provided written informed consent.

Comprehensive STI testing was undertaken, including syphilis serology (Treponema Pallidum Assay, Vitros 5600 Ortho-Clinical Diagnostics, Rochester, NY and the Rapid Plasma Reagin of Macro-Vue, Becton Dickinson Microbiology Systems, Maryland, USA) and quarterly nucleic acid amplification testing for CT/NG at the three anatomical sites (anorectum, urethra and pharynx) irrespective of reported sexual behaviour. (Abbot RealTime CT/NG assay, following a confirmation in-house real-time PCR for CT and/or NG using previously published primer sets).[21,22] Further L-genotyping was performed as previously described.[23]

Questionnaire data

Participants self-reported socio-demographic, behavioural and sex-related information on an electronic questionnaire.[19] At each quarterly visit, questions on sexual behaviour covered the previous three months and included their number of casual sex partners ('non-steady, non-anonymous partners with whom the study participant occasionally or regularly had sex') or anonymous sex partners; and the number of condomless anal sexual intercourse (CAI) by partner type and per sexual position (insertive and receptive). To examine the number of non-steady sex partners (with CAI), we calculated the mean reported number of non-steady sex partners with whom CAI occurred (insertive or receptive) over the 18 months of follow-up.

We assessed associations between recurrent STIs and selected characteristics from the previous three months that were only reported at month 9 or month 18 of the study. These characteristics included mental health (presence and severity of depressive symptomatology), and the following sexual behaviour characteristics in the previous three months reported at month 9 or month 18: having participated in group sex activities, transactional sex (having paid someone for sex or having received money for sex), and having sex under influence of alcohol, erection enhancing medication and any recreational drugs (see Table 1 Supplemental Digital Content (SDC) 1).

To assess recreational drug use in the past three months, participants could tick a list of recreational drugs used (Yes/No) including four of the typical "chemsex drugs": GHB (Gamma-hydroxybutyrate), mephedrone, crystal meth, and ketamine.[24]

Mental health was assessed using the 'Patient Health Questionnaire-9'.[25]

Statistical analysis

STI rate during the study

A participant was defined as having an STI when positive for CT, NG or a newly identified case of syphilis (RPR titre ≥1/4 or a ≥4-fold increase in RPR titres from a previous RPR

titre and a positive TPA test on serum). Episodes for CT or NG within 30 days of the previous episode were excluded to eliminate the inclusion of persistent infections. A participant who was positive for more than one STI at one visit was defined as having concurrent STI infections.

Descriptive analyses were used to calculate the prevalence of any STI and each STI separately (CT, NG and syphilis) at every scheduled visit, including CT/NG infection at each of the anatomical sites.

Analysis of risk factors associated with incident recurrent STIs

To identify risk factors associated with recurrent STIs, we stratified our cohort into three groups: participants who had no STI (none) during follow-up, participants who experienced a single STI episode during follow-up (single episode), and participants who had more than one STI episode during follow-up (recurrent STIs). Baseline results were not taken into account.

Socio-demographic and behavioural characteristics, including sexual behaviour were described for each group by calculating frequencies and proportions for categorical variables. We categorised the mean number of non-steady sex partners and the mean number of partners with whom they had CAI (insertive/receptive) with over the last three months as follows: 0-4; 5-9; 10 or more.

Differences in demographic characteristics and sexual behaviour were compared between the three groups using two-sided Fisher's exact test for categorical variables. Differences in continuous variables between participants with recurrent STIs and no recurrent STIs were assessed with Wilcoxon Rank-Sum test.

Covariates found to be independently associated with recurrent STIs in the Fisher's exact test analyses were included in a multivariable likelihood logistic regression model to explore associations between recurrent STIs and behavioural and demographic characteristics. Due to the high collinearity between drug related variables; only sex while using recreational drugs or sexualised drug use was used in the multivariate analyses (Table 2, SDC 1).

The results of the regression models are presented as odds ratios (OR) with 95% confidence intervals (CI). A significance level of $p<0.05$ was used for all analyses.

All statistical analyses were performed using Stata v15.1. (StataCorp LP, College Station, Texas, USA).

3.2.4 Results

A total of 179 participants with a median age of 39 (quartiles 25-75: 33-44) at baseline completed 18 months of study follow-up and provided data for 1278 visits. Only five participants missed one visit during the study.

STI prevalence at each scheduled visit

The prevalence of any STI (CT, NG or syphilis) was 26.8% at baseline (n=48/179) and remained stable between 17.4% and 19.0% during the follow-up visits. Besides the scheduled quarterly visits, participants could visit the study clinic in case of symptoms. An additional 13 STIs were detected during 30 non-scheduled visits. Figure 1 depicts the prevalence of STIs during the quarterly visits, including the CT and NG prevalence per anatomical site.

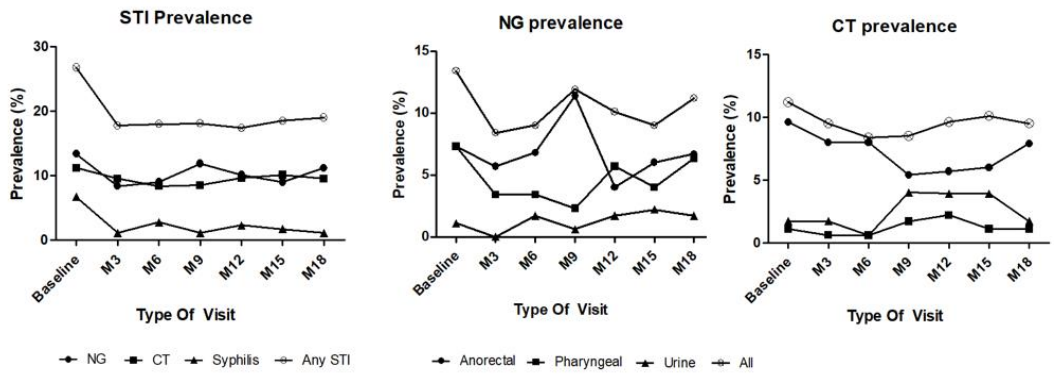

Figure 1: Sexually transmitted infection (STI) prevalence at scheduled follow-up visits

In our study (including baseline), a total of 296 STIs were detected on 255 visits (Table 1).

Table 1: Number of Sexually Transmitted Infections (STIs) found during the study

Single STI	N (total = 296)
Chlamydia	93
Gonorrhea	101
Syphilis	22
Concurrent STIs	
Chlamydia & syphilis	2
Gonorrhea & syphilis	7
Chlamydia & gonorrhea	28
Chlamydia & gonorrhea & syphilis	2

Concurrent STIs were detected at 39 visits (15.3%; n=39/255) Most of the CT or NG infections were of extra-genital origin (85.4%; n=199/233) with a predominance in the anorectum (66.1%; n=154/233), however 26 infections were dual positive in the pharynx increasing the number of pharyngeal infections to 30.5 (n=71/233). A total of 21.9% (n=51/233) CT or NG infections were found in the urethra. Of those infections, 7.3% were dual positive in the urethra and in one of the extra-genital sites (n=17/233).

In total, LGV was detected at thirteen visits in twelve participants (6.7%), only one LGV was detected at the baseline visit (prevalence: 0.6%, 95% CI: 0.1-3.1). A reinfection with LGV was detected in one participant seven months later (results of the L-genotypes can be found in SDC1).

Around one quarter of the total CT or NG infections presented with symptoms (27.9%; n=65/233) and 15.2% (n=5/33) of the syphilis infections presented with signs of primary or secondary stage syphilis.

Recurrent STI infections

During follow-up, 240 STIs were diagnosed in 111 participants (62.0%; 95%CI: 54.5-69.1, n=111/179) (Figure 2). CT or NG infections were primarily found in extra-genital sites (84.0%; n=184/219). About one in four participants was positive for an STI at one single visit (25.1%, n= 45/179; 95%CI: 20.0-32.2) and more than one in three participants, i.e., 36.9%, had an STI at more than one visit during the 18 months of follow-up (n= 66/179; 95%CI: 29.8-44.4) (Figure 2). Most of the incident STI infections were found in this 'recurrent STI' group (77.9%; n=187/240). Seven participants had four or more STI episodes during the 18 months of the study (7/179; 3.9%) accounting for 16.3% of the diagnosed STI infections (two syphilis, 21 CT and 16 NG infections).

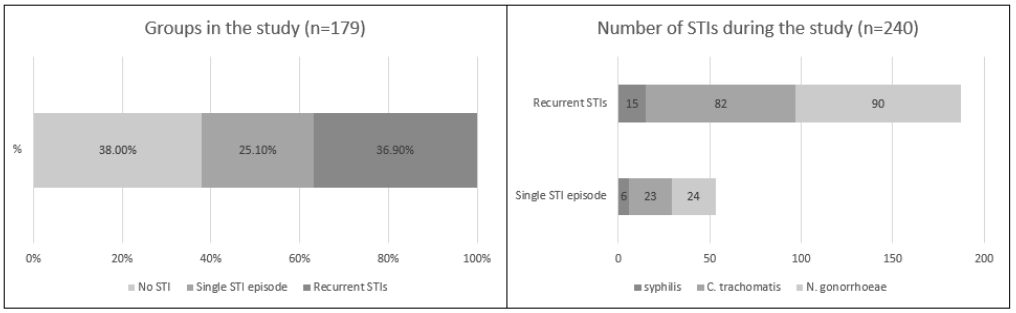

Figure 2: Presentation of the different groups in our study and the number of sexually transmitted infections (STIs) experienced during the study

A total of 21 participants experienced an episode of syphilis during the study. Although most of the syphilis infections (71.4%; n=15/21) were detected in the 'recurrent STI' group, experiencing a syphilis infection during the study was not significantly associated with having recurrent STIs (p=0.220). Furthermore, almost all LGV infections (91.7%; n=11/12) were identified in the recurrent STI group, and experiencing an LGV infection was borderline associated with having recurrent STIs (OR: 7.86; 95%CI: 0.97-63.73; p=0.054). Participants experiencing concurrent STIs at one visit mostly belonged to the 'recurrent STI' group (75.0%; n=24/32) and concurrent CT and NG infection was also more frequently detected among individuals with recurrent STIs compared to participants who tested positive at one visit only (83.3%; n=20/24; p<0.001).

Individuals with recurrent STIs were more frequently symptomatic when having a CT or NG infection compared to the group who had one STI episode only (61/172, 35.5% vs 8/47, 17.0%, n=8/47).(OR: 1.50, 95%CI: 1.13-1.99; p=0.006).

Other factors associated with recurrent STIs

Individuals experiencing recurrent STIs were younger (as compared with those having no or a single STI episode (median age 36; IQR: 31-42 vs 41; IQR: 35-46, p<0.003). Furthermore, they were more likely to report group sex, to report a higher number of sexual partners (median partners 8 vs 21, p<0.0001) and a high number of sexual partners with whom they had CAI with (insertive: median 3 vs 6, p=0.004 and receptive: median 1 vs 6, p<0.0001). The proportion of participants reporting any kind of drug use, and drugs associated with chemsex in particular, was significantly higher among the group with recurrent STIs when compared to participants with no recurrent STIs ((n=47/113; 41.6%; vs n=47/66; 71.2%, p<0.0001). Approximately half of the participants without any STIs reported sexualised drug use (47.1%; n=32/68); while this was more than four out of five among individuals experiencing recurrent STIs (86.4%; n=57/66; p<0.0001, Table 2). Figure 3 shows the proportions of participants per group reporting sexualised drug use, and the number of non-steady partners with whom receptive CAI occurred.

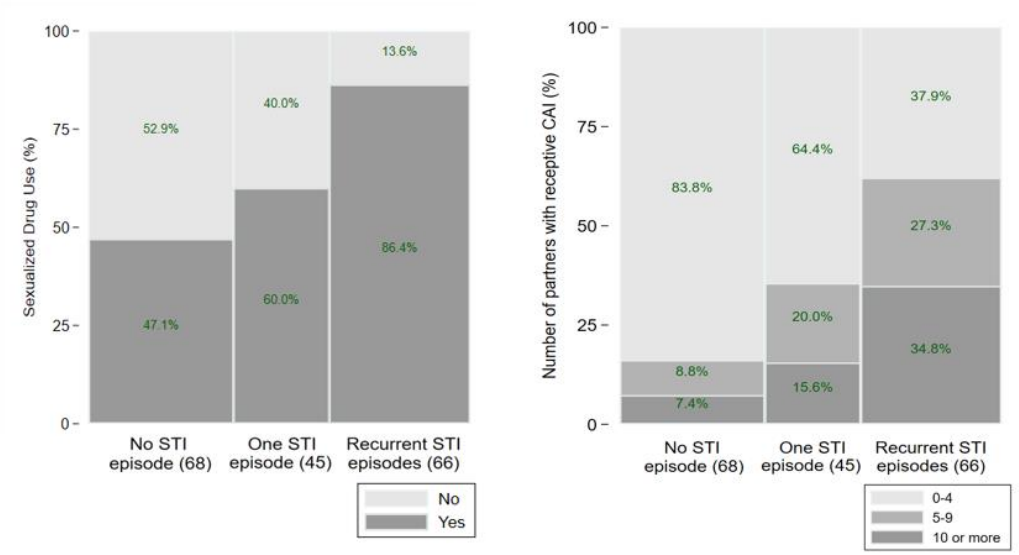

Figure 3: Proportion of participants using drugs in a sexualised context and the proportion of the number of non-steady partners with whom receptive condomless anal intercourse (CAI) occurred over three months; stratified per group

The multivariable model comparing individuals with recurrent STIs with all other participants showed that being younger than 40 years old, sexualised drug use in the past three months and a high number of non-steady partners with whom insertive or receptive CAI occurred (more than four in a period of three months) remained significantly associated with having recurrent STIs over the study period. In the multivariable analysis comparing the recurrent STI group with the “single STI episode” group, sexualised drug use remained associated with having recurrent STIs over the study period. (aOR 3.40; 95%CI: 1.29-9.01; p=0.014) (Table 3).

Table 2: Socio-demographic and selected sexual behaviour characteristics for the three different groups (no STI, a single STI episode or recurrent STIs during the study)

	Variable	All participants n=179	No STI episode n=68 (38.0%)	One STI episode n=45 (25.1%)	Recurrent STIs n=66 (36.9%)	p-value*
Socio - demographic data						
Age	Less than 40y	95 (53.1)	26 (38.2)	24 (53.3)	45 (68.2)	**0.002**
Nationality	Belgian	141 (78.8)	55 (80.9)	35 (77.8)	51 (77.3)	
	Other	38 (21.2)	13 (19.1)	10 (22.2)	15 (22.7)	0.894
Residence	Brussels	58 (32.4)	18 (26.5)	17 (37.8)	23 (34.9)	
	Flanders	110 (61.5)	45 (66.2)	28 (62.2)	37 (56.1)	
	Wallonia	11 (6.2)	5 (7.4)	0 (0.0)	6 (9.1)	0.180
Health Insurance	No	9 (5.0)	3 (4.4)	2 (4.4)	4 (6.1)	
	Yes	169 (94.4)	65 (95.6)	43 (95.6)	61 (92.4)	
	Unknown	1 (0.6)	0 (0.0)	0 (0.0)	1 (1.5)	0.903
Education	No higher education	40 (22.4)	14 (20.6)	15 (33.3)	11 (16.7)	
	Higher education (-3y)	50 (27.9)	23 (33.8)	5 (11.1)	22 (33.3)	
	Higher education (>3y)	89 (49.7)	31 (45.6)	25 (55.6)	33 (50.0)	**0.029**
Average net income	<1200€	20 (11.2)	10 (14.7)	4 (8.9)	6 (9.1)	
	1201-1700€	41 (22.9)	17 (25.0)	9 (20.0)	15 (22.7)	
	>1700€	107 (59.8)	35 (51.5)	28 (62.2)	44 (66.7)	
	Unknown	11 (6.2)	6 (8.8)	4 (8.9)	1 (1.5)	0.334
STI history						
Reported STI 6 months before screening		60 (33.5)	18 (26.5)	14 (31.1)	28 (42.4)	0.143
Any CT/NG or syphilis at screening		48 (26.8)	15 (22.1)	12 (26.7)	21 (32.8)	0.445
Syphilis at screening		12 (6.7)	3 (4.4)	6 (13.3)	3 (4.6)	0.157

Variable		All participants n=179	No STI episode n=68 (38.0%)	One STI episode n=45 (25.1%)	Recurrent STIs n=66 (36.9%)	p-value*
Sexual behaviour in the last three months reported at Month 9 or Month 18						
Had sex while drunk		79 (44.1)	28 (41.2)	20 (44.4)	31 (47.0)	0.797
Had sex while using recreational drugs		116 (64.8)	32 (47.1)	27 (60.0)	57 (86.4)	**<0.0001**
Had sex while using erection enhancers		125 (69.8)	38 (55.9)	32 (71.1)	55 (83.3)	**0.002**
Paid for sex		11 (6.2)	4 (5.9)	5 (11.1)	2 (3.0)	0.250
Received money for sex		12 (6.7)	3 (4.4)	4 (8.9)	5 (7.6)	0.559
Had group sex		132 (73.7)	40 (58.8)	36 (80.0)	56 (85.9)	**0.002**
Used any kind of drugs		143 (79.9)	46 (67.7)	37 (82.2)	60 (90.9)	**0.003**
	Drugs related to chemsex †	94 (52.5)	22 (32.4)	25 (55.6)	47 (71.2)	**<0.0001**
Mental Health in the last three months reported at Month 9 or Month 18						
Mental Health	No or minor depression	141 (78.8)	51 (75.0)	38 (84.4)	52 (78.8)	
	Moderate or major depression	38 (21.2)	17 (25.0)	7 (15.6)	14 (21.2)	0.484
Sexual behaviour in the last three months with non-steady partners						
Number of non-steady sex partners	0-4	36 (20.1)	24 (35.3)	7 (15.6)	5 (7.6)	
	5-9	44 (24.6)	23 (33.8)	13 (28.9)	8 (12.1)	
	10 or more	99 (55.3)	21 (30.9)	25 (55.6)	53 (80.3)	**<0.0001**
Number of non-steady partners with condomless anal receptive intercourse	0-4	111 (62.0)	57 (83.8)	29 (64.4)	25 (37.9)	
	5-9	33 (18.4)	6 (8.8)	9 (20.0)	18 (27.3)	
	10-49	35 (19.6)	5 (7.4)	7 (15.6)	23 (34.9)	**<0.0001**
Number of non-steady partners with condomless anal insertive intercourse	0-4	110 (61.5)	49 (72.1)	30 (66.7)	31 (47.0)	
	5-9	31 (17.3)	10 (14.7)	8 (17.8)	13 (19.7)	
	10-49	38 (21.2)	9 (13.2)	7 (15.6)	22 (33.3)	**0.025**

STI: sexually transmitted infection

Table 3: Predictors of recurrent STIs compared to participants who did not experience recurrent STIs in the 18 months of follow-up and compared to individuals who experienced one STI episode only during the 18 month of follow-up. Bivariable and multivariable logistic regression

	Comparison group: Participants who did not experience a recurrent STI during the 18 months of follow-up				**Comparison group: Participants who experienced one STI episode only during the 18 months of follow-up**			
	Unadjusted Odds Ratio (95%CI)	**p-value**	**Adjusted Odds Ratio (95%CI)**	**p-value**	**Unadjusted Odds Ratio (95%CI)**	**p-value**	**Adjusted Odds Ratio (95%CI)**	**p-value**
Less than 40y	2.70 1.43-5.11	**0.002**	3.29 1.56-6.95	**0.002**	1.88 0.86-4.10	0.155	-	-
Reporting sexualised drug use	5.80 2.62-12.82	**<0.0001**	4.35 1.81-10.43	**0.001**	4.22 1.68-10.62	**0.002**	3.40 1.29-9.01	**0.014**
Reporting sex while using erection enhancers	3.07 1.45-6.51	**0.003**	-	-	2.03 0.81-5.06	0.128	-	-
Reporting group sex	2.73 1.25-5.94	**0.015**	0.80 0.30-2.11	0.645	1.40 0.52-3.78	0.507	-	-
Used any kind of drugs	3.61 1.42-9.23	**0.007**	-	-	2.16 0.69-6.73	0.183	-	-
Used drugs associated to chemsex	3.47 1.81-6.66	**<0.0001**	-	-	1.98 0.89-4.38	0.092	-	-
Number of non-steady sex partners	4.61 1.70-12.55	**0.003**	-	-	2.25 0.67-7.59	0.192	-	-
Number of non-steady partners with receptive CAI	5.22 2.70-10.10	**<0.0001**	1.17 1.06-1.29	**0.001**	2.97 1.35-6.53	**0.007**	1.10 0.99-1.22	0.084
Number of non-steady partners with insertive CAI	2.62 1.40-4.92	**0.005**	1.12 1.02-1.23	**0.018**	2.26 1.03-4.96	**0.042**	1.10 0.99-1.21	0.080

3.2.5 Discussions

In this PrEP demonstration study, we found a high rate of STIs during 1.5 years of follow up, and most of the CT/NG infections were of extra-genital origin (85.4%). In fact, 62.0% of the participants experienced an STI during the study and one in three was positive for an STI at more than one visit (36.9%). Four fifth of all STIs diagnosed in the study were identified among this core group (77.9%). Seven participants were positive for one or more STIs at four or more visits and accounted for 16.3% of all diagnosed STIs. The number of STIs could even be underestimated as we only included STI episodes detected at the clinic site, therefore, some participants could be wrongly included in the group with no or only a single STI episode. Although we cannot exclude treatment failures as no test-of-cure was performed, we anticipate this to be very doubtful as all STIs were treated according to national treatment guidelines, which are, until now, highly efficacious.

An important finding is that being younger than 40 years old, having a high number of non-steady partners with receptive or insertive CAI and sexualised drug use were strongly associated with recurrent STIs. In multivariable analysis, sexualized drug use remained significantly associated with recurrent STIs compared with the group that experienced a single STI episode during 1.5 year of follow-up.

Furthermore, in line with other studies, participants with recurrent STIs had more symptomatic and more concurrent STIs compared to the group who experienced only one STI episode.[12,14] These findings add to the evidence that this group may have higher transmission potential, as symptomatic infections show a higher bacterial load, which is suggestive for increased transmission potential.[26] They may, therefore, form part of core transmission groups that fuel the STI epidemic.

Our data also show that participants who contracted LGV during the study belonged almost exclusively to the group with recurrent STIs. Therefore, concurrent STIs, symptomatic STI or LGV detection merits attention and should stimulate health care providers to offer additional counselling focusing on increasing awareness about the risks of STIs.

Sexualized drug use is very prevalent in our study population (65%, and up to almost 90% when having recurrent STIs) and is amongst the highest ever reported among HIV negative MSM using PrEP in Europe (30-85%).[7,27–30] Of note, sexualized drug use was estimated to be around 11% among the whole MSM population in Belgium.[s31] Substance use is typically associated with condomless sex, having multiple sex partners and group sex and incident STI.[29,s32–35] Our study confirms that sexualised drug use was highly correlated with group sex and a high number of non-steady partners with whom

receptive CAI occurred (see Table 3, SDC1). These results highlight the role of sexualised drug use among PrEP users and warrants attention.

Drug use does not seem to influence adherence to PrEP.[27,28,s36] However, it has been shown that PrEP use may lead to MSM seeking more adventurous sexual encounters, including more CAI, and may include experimenting or engaging in sexualised drug use.[s37] Individuals using drugs in a sexual context may have different attitudes towards the risk of acquiring STIs and it is also likely that they minimise this risk.

Currently, specialised services dealing specifically with problematic sexualised drug use are scarce. Our study further supports the notion that MSM experiencing recurrent STIs during PrEP use should be further counselled. STI risk reduction counselling should include (sexualised) drug use if it is highly prevalent, and if necessary, drug users should be referred to specialised chemsex services (where available). PrEP delivery should thus integrate combination prevention approaches, including PrEP adherence, safer sex counselling including condom use, harm reduction approaches and specialised tailored counselling for those who experience severe psychological problems related to chemsex.[25]

Although it is difficult to estimate the proportion of STI transmissions attributable to this core group, modelling studies show that this group is important and should be a focus for prevention interventions.[s38] However, while rapid treatment can reduce individual STI infectiousness, and hence decrease community prevalence, the underlying determinants of the high rate of STIs in PrEP users need to be tackled, including their dense sexual networks, and the increase in condomless sex.[s39] Indeed, since recurrent STIs primarily stem from reinfections due to an untreated sexual partner, it is important to implement partner notification, which is a major problem given the high number of non-steady partners reported by individuals experiencing recurrent STIs.[s40]

The growing demand for PrEP can put a strain on the workload of integrated sexual health and PrEP clinics.[18] In light of such increasing demand it may be more effective to identify, screen and treat those PrEP users who are more likely to have recurrent STIs. Such a sub-group might also benefit from more specialised care, including additional STI testing, home-based sampling interventions, harm-reduction counselling, and drug-specific counselling, rather than standard care alone.

The proportions of sexual behaviour reported by this study, could be overestimated as the analysis does not take individual behaviour changes between month 9 and month 18 into account. For example, an individual experiencing recurrent STIs at month 12 may have reduced his sexual risk behaviour, such as groupsex or sexualized drug use.

Furthermore, participants reported the number of casual and anonymous partners with whom they had CAI separately. Since for this analysis, the sum of both partners was used, we cannot rule out the possibility that a participant counted a partner twice, as anonymous and casual, but this is unlikely. Finally, participants were asked to report their sexual behaviour in the last three months, which could potentially introduce recall bias.

Nevertheless, the study adds to our understanding of what drives STI reinfection among PrEP users. To our knowledge, prior to this study, sexualised drug use has not been found to be associated with recurrent STIs among MSM PrEP users. Our results show that most of the STIs among PrEP users can be attributed to a relatively small group of individuals. These findings stress the importance of providing tailored risk reduction counselling for such a sub-group, including specific sexualised drug use and harm reduction counselling, sensitisation about the public health consequences of recurrent STIs and increased motivation for partner notification. Another important finding is that PrEP is delivered to a group who benefits most from its objective: people engaging in sexual behaviour with increased risk for HIV acquisition. In times of financial constraints, it is important to use the resources for those most in need. These results will help public health professionals to further refine STI prevention strategies.

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CHAPTER 4: THE BELGIAN LGV EPIDEMIC

The Institute of Tropical Medicine (ITM) harbours an STI clinic which follows-up a large cohort of HIV positive individuals and PrEP users. In addition, the STI reference laboratory located at ITM was selected as the National Reference Centre of STIs (NRC-STI) since 2010 until now. In the first year, the NRC-STI was the only laboratory that could further serotype CT positive samples in order to identify an LGV infection. In addition, laboratories were advised to complete the LGV specific request form to provide additional demographic and behavioral data (Annex 3). Furthermore, since 2011, the NRC-STI was routinely performing LGV detection on each CT positive sample detected at ITM. As such, the NRC-STI has very reliable data in order to scrutinize the LGV epidemic in Belgium.

The following papers will describe the LGV epidemic in Belgium before- and after the introduction of PrEP.

- Lymphogranuloma venereum is on the rise in Belgium among HIV negative men who have sex with men: surveillance data from 2011 until the end of June 2017.
- Did Pre-exposure Prophylaxis Roll-Out Influence the Epidemic of Rectal Lymphogranuloma Venereum in Belgium? Results From the National Surveillance System.

4.1 LGV EPIDEMIC IN BELGIUM FROM 2011 UNTIL THE END OF JUNE 2017

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RESEARCH ARTICLE Open Access

Lymphogranuloma venereum is on the rise in Belgium among HIV negative men who have sex with men: surveillance data from 2011 until the end of June 2017

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4.1.1 Abstract

Background: The number of cases of Lymphogranuloma venereum (LGV) is increasing in Europe. The described epidemic is mostly confined to HIV positive men who have sex with men (MSM). However, dissemination of LGV from HIV positive to HIV negative MSM could take place due to the implementation of pre-exposure prophylaxis (PrEP) and subsequent possible decrease in condom use. We describe here the LGV epidemiology in Belgium before the PrEP-era, starting from 2011 up to the end of the first half of 2017.

Methods: A descriptive analysis of the socio-demographic and clinical characteristics of all LGV cases was performed. Fisher's exact test was used to compare symptomatic to asymptomatic patients. Logistic regression models were used to check for trends over time for: number of LGV cases, HIV status and symptoms.

Results: The number of LGV cases rose by a factor four, from 21 in 2011 to 88 in 2016, and regression models showed a positive trend estimate of 14% increase per half year ($p < 0.001$). LGV decreased among HIV positive cases (odds ratio (OR): 0.79, $p < 0.001$) and increased among HIV negative cases (OR: 1.27, $p < 0.001$). In addition, a rise in the number of asymptomatic LGV cases (6.7%) was observed (OR:1.39, $p = 0.047$). Asymptomatic cases were also less likely to be HIV ($p = 0.046$) or Hepatitis C positive ($p = 0.027$).

Conclusions: The rise of LGV in HIV negative MSM has now been documented. If we aim to halt the epidemic in HIV negative MSM, future public health strategies should include LGV testing of all *Chlamydia trachomatis* positive samples from MSM.

KEYWORDS

Lymphogranuloma venereum, LGV, *Chlamydia trachomatis*, Pre-exposure prophylaxis, Men who have sex with men, MSM

4.1.2 Background

Lymphogranuloma venereum (LGV) is a bacterial sexually transmitted infection (STI) caused by the L serovars of *Chlamydia trachomatis* (CT). Although initially a tropical infection characterized by inguinal buboes, LGV under the form of proctocolitis has become endemic among men who have sex with men in Europe. The CT L serovar is more invasive compared to the other serovar types leading to more severe sequelae and has been suggested to be associated with increased HIV and other STI infection.[1] The number of infections is increasing in many Western and Central European countries where enhanced LGV surveillance has been implemented. In fact, the number of European cases reported in 2014 increased by 32% compared to 2013.[2] However, the increase is probably an underestimate as not all countries have the capacity to identify CT serovar/genotype L and to report LGV. In addition, only suspected LGV cases are tested and recent reports have shown that asymptomatic infections may be higher than initially thought.[3–5]

The LGV epidemic is mostly confined to HIV positive men who have sex with men (MSM).[6] The HIV prevention landscape has changed impressively over the past few years due to the implementation of treatment as prevention and pre-exposure prophylaxis (PrEP). In Belgium, PrEP has been reimbursed since June 2017.[7] PrEP in Belgium can only be prescribed by an HIV specialist working in an AIDS Reference Centre such as the HIV/ STI clinic at the Institute of Tropical Medicine (ITM). According to PrEP guidelines, STIs need to be monitored at least six monthly. Previously, urine sampling was performed for STI screening on a routine basis, however, European guidelines for LGV recommend that MSM who report receptive anal sexual practices in the previous 6 months, are screened twice a year for anorectal CT infection.[8] This recommended biannual STI screening may detect asymptomatic STI infections more frequently and thus prevent further transmission.[9]

To explore differences in behavioural and clinical characteristics of LGV cases over time, we describe here the LGV epidemiology in Belgium including the positivity rate of LGV cases among men attending an Antwerp HIV/STI clinic for the pre-PrEP era, starting from 2011 up to and including the first half of 2017. Repeater and asymptomatic infections were reviewed in depth.

4.1.3 Methods

National surveillance of LGV

In 2011, the ITM was recognized as the National Reference Centre for STIs (hereafter called NRC) and has since then implemented the laboratory surveillance of LGV. Physicians or microbiologists suspecting a case of LGV are urged to send the patient's sample with accompanying socio-demographic and clinical data to the NRC for confirmation of CT genotype L. In addition, health care providers are instructed to send CT positive samples of patients at risk for LGV to the NRC regardless the presence of symptoms.

All biological material (i.e., anorectal-, pharyngeal-, ulcer dry swabs or urine samples) is tested for CT using the Abbott Real-Time CT/NG PCR according to manufacturer's instructions. When testing positive for CT, a confirmation in-house real-time PCR using previously published *pmpH* gene primers/probes is performed to differentiate LGV (L genotypes) from non-LGV (A-K genotypes) strains.[10] In the case of DNA extracts, only the confirmation LGV/non-LGV real-time PCR is performed.

All data from LGV confirmed cases are coded and submitted on a regular basis to the Belgian Institute of Public Health through a secured web portal.

Duplicate records of all LGV infections found by the NRC are removed, based on date of birth, date of consultation, postal code and gender. An LGV re-infection is defined as a separate episode when a new infection is recorded for the same patient at least 3 months after the first episode, to exclude potential treatment failures.[11]

Routine CT detection at the HIV/STI clinic, ITM

Besides being the NRC, the ITM has a large HIV/STI clinic and followed close to 3000 HIV positive patients at the end of 2016. In addition, the HIV/STI clinic is also home to a low threshold clinic that offers free-of-charge screening for HIV and STIs in asymptomatic individuals. In 2016, 2307 individuals, mainly belonging to high risk groups (MSM, transgenders and anyone who engages in unsafe sex), made use of this service. For sexually active MSM, screening for STIs in urine and HIV testing is performed at least twice yearly. In the case of symptomatic individuals, samples are collected from the suspected infection site. The number of CT analyses requested by the HIV/STI clinic from 2011 till 01 July 2017 was retrieved. Due to the fact that no female LGV cases were detected in the clinic, female samples were excluded from the analysis. Results that were invalid due to inhibition, wrong or insufficient volume of

sample or results that could not be confirmed by the LGV/non-LGV real-time PCR were removed from the dataset before analysis.

Statistical analysis

The descriptive analysis was performed using IBM SPSS Statistics version 24. Continuous variables are summarized as medians and interquartile ranges (IQR) and categorical ones as counts and percentages. The longitudinal analysis was performed using R version 3.4.1.[12] Poisson regression models were fitted to the aggregated LGV data by year and by six-monthly period, with the number of cases as the outcome and the respective time variable as the only covariate. Logistic regression models were fitted to the individual data for all the other variables of interest. In all models, time was treated as numerical. The trend estimates are presented as rates for the Poisson models and as odds ratios (ORs) for the logistic regression models with 95% confidence intervals (CIs).

Furthermore, Fisher's exact test was used to compare variables of interest between asymptomatic and symptomatic LGV cases. All tests were two-sided and significance level was set at 5%.

4.1.4 Results

Overall Belgian LGV epidemic

A total of 343 LGV cases were identified by the NRC, of those 186 were from the HIV/STI clinic and 157/765 cases were provided by peripheral laboratories The socio-demographic and clinical data of the 343 cases reported between 2011 and the end of June 2017 are compiled in Table 1. Percentages were calculated excluding missing data.

Table 1: Socio-demographic and clinical data of LGV cases starting from 2011 until end of June 2017.

	2011 n (%)	2012 n (%)	2013 n (%)	2014 n (%)	2015 n (%)	2016 n (%)	S1 2017 n (%)	TOTAL n (%)
Number of LGV cases	21	23	45	58	62	88	46	343
Gender	**343/343 known**							
Male or Trans female	21 (100%)	23 (100%)	45 (100%)	58 (100%)	61 (98%)	88 (100%)	46 (100%)	342 (99,7%)
Female	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (0,3%)
Transmission of all men	**241/343 known**							
MSM	13 (100%)	13 (100%)	26 (100%)	42 (100%)	52 (98%)	55 (98%)	37 (97%)	238 (99%)
HETERO	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	1 (3%)	3 (1%)
Age scale	**343/343 known**							
20–30	3 (14%)	5 (22%)	2 (4%)	10 (17%)	17 (27%)	14 (16%)	6 (13%)	57 (17%)
31–40	7 (33%)	7 (30%)	17 (38%)	28 (48%)	14 (23%)	27 (31%)	17 (37%)	117 (34%)
41–50	8 (38%)	8 (35%)	21 (47%)	15 (26%)	17 (27%)	27 (31%)	14 (30%)	110 (32%)
51–60	2 (10%)	3 (13%)	5 (11%)	4 (7%)	14 (23%)	18 (20%)	8 (17%)	54 (16%)
> 61	1 (5%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	2 (2%)	1 (2%)	5 (1%)
Geographical location	**338/343 known**							
Living in Flemish region	17 (81%)	20 (91%)	37 (84%)	36 (63%)	47 (76%)	61 (69%)	36 (81%)	254 (75%)
Living in French region	2 (10%)	1 (5%)	2 (5%)	3 (5%)	7 (11%)	11 (12%)	3 (7%)	29 (9%)
Living in the capital region	2 (10%)	1 (5%)	5 (11%)	18 (32%)	8 (13%)	16 (18%)	5 (11%)	55 (16%)
Kind of Sample	**337/343 known**							
Anorectal	17 (85%)	22 (96%)	37 (86%)	51 (89%)	56 (92%)	84 (95%)	40 (89%)	307 (91%)
Genital	3 (15%)	1 (4%)	3 (7%)	6 (11%)	2 (3%)	3 (3%)	2 (4%)	20 (6%)
Urine	0 (0%)	0 (0%)	2 (5%)	0 0%)	3 (5%)	1 (1%)	1 (2%)	7 (2%)
Inguinal	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	2 (1%)
Eye fluid	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (0,3%)

	2011 n (%)	2012 n (%)	2013 n (%)	2014 n (%)	2015 n (%)	2016 n (%)	S1 2017 n (%)	TOTAL n (%)
HIV Status	**308/343 known**							
Positive	18 (100%)	20 (100%)	40 (95%)	45 (88%)	45 (75%)	63 (84%)	30 (71%)	261 (85%)
Negative	0 (0%)	0 (0%)	2 (5%)	6 (12%)	15 (25%)	12 (16%)	12 (29%)	47 (15%)
Symptoms	**232/343 known**							
Proctitis	6 (75%)	2 (50%)	19 (63%)	40(89%)	43 (81%)	41 (76%)	25 (66%)	176 (76%)
Inguinal lymphadenopathy or ulcer	0 (0%)	1 (25%)	3 (10%)	0 (0%)	3 (6%)	2 (4%)	3 (8%)	12 (5%)
Other abdominal symptoms[a]	0 (0%)	0 (0%)	3 (10%)	3 (7%)	3 (6%)	0 (0%)	1 (3%)	10 (4%)
Genital Ulcer	2 (25%)	0 (0%)	1 (3%)	1 (2%)	0 (0%)	1 (2%)	1 (3%)	6 (3%)
Urethritis	0 (0%)	0 (0%)	1 (3%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	2 (1%)
Other symptoms[b]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	2 (5%)	3 (1%)
Asymptomatic	0 (0%)	1 (25%)	3 (10%)	0 (0%)	4 (8%)	9 (17%)	6 (16%)	23 (10%)
Co-infections other than HIV	**197/343 known**							
None	6 (40%)	0 (0%)	2 (9%)	7 (25%)	17 (40%)	20 (37%)	10 (33%)	62 (31%)
Only one	6 (40%)	5 (83%)	20 (91%)	21 (75%)	24 (57%)	34 (63%)	15 (50%)	125 (63%)
Two or more	3 (20%)	1 (17%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	5 (17%)	10 (5%)
Gonorrhea	4 (24%)	5 (71%)	11 (41%)	13 (41%)	9 (19%)	17 (28%)	10 (28%)	69 (35%)
Syphilis	2 (12%)	2 (29%)	9 (33%)	4 (13%)	8 (17%)	9 (15%)	6 (17%)	40 (20%)
Hepatitis C	2 (12%)	0 (0%)	4 (15%)	6 (19%)	7 (15%)	10 (17%)	0 (0%)	29 (15%)
Chlamydia A-K	0 (0%)	0 (0%)	0 (0%)	1 (3%)	4 (9%)	0 (0%)	4 (11%)	9 (5%)
Hepatitis B	2 (12%)	0 (0%)	1 (4%)	1 (3%)	0 (0%)	1 (2%)	2 (6%)	7 (4%)
Genital herpes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (4%)	0 (0%)	1 (3%)	3 (2%)
Other[c]	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (5%)	3 (8%)	7 (4%)

% removing unknowns – deviations from 100% can be due to rounding, S Six-monthly period, [a]other abdominal symptoms include rectal ulcers or lesions, fistulas, diarrhoea, constipation, peri-anal pain, [b]other symptoms include conjunctivitis, arthritis or unknown other symptoms [c]other co-infections include shigella, campylobacter, varicella zoster, Mycoplasma genitalium

The mean age was 40.6 years (IQR: 33–48) and LGV episodes were detected in 297 men, two transwomen and one woman. Most of the cases originated from patients living in the Flemish region of Belgium (75%). Of those with LGV for whom data were available, almost all were identified as MSM (98.7%). Three heterosexual transmissions were reported among men (1.2%) albeit that they had rectal LGV infections; two of them were HIV positive and all suffered from symptoms, suggestive of proctitis. The HIV status was known in 90.0% (308/343) of the cases whereby 84.7% (261/308) were HIV positive and 15.3% (57/308) HIV negative. Of the HIV positive men, 73.9% (193/261) were MSM.

Of the samples with known biological origin (337), the majority were anorectal (91.1% - 307/337), followed by genital samples (5.9% - 20/337) and urine (2.1% - 7/337). Two LGV infections were detected in samples of inguinal glands (0.6%) and one swab from eye material was also found to be LGV positive (0.3%). The most reported symptom was proctitis (75.9% - 176/232), followed by inguinal lymphadenopathy or the presence of an inguinal ulcer (5.2% - 12/343). Abdominal symptoms such as diarrhoea, constipation, rectal ulcers, lesions or fistulas and peri-anal pain were reported in 4.3% of the cases (10/232). Other symptoms were genital ulcers (2.6% - 6/232), urethritis (0.9% - 2/232), arthritis (0.4% -1/232) and conjunctivitis (0.4% - 1/232). In 23 cases, LGV infection was found to be asymptomatic (9.9%).

For almost 70% of the LGV patients one (63.5% - 125/ 197) or more (5.1% - 10/197) co-infections other than HIV were reported. Gonorrhea was most frequently reported (35.0% - 69/197) followed by syphilis (20.3% - 40/ 197) and hepatitis C (HCV) (14.7% - 29/197). Chlamydia genotypes A-K, hepatitis B, genital herpes, Mycoplasma genitalium, Shigella, Campylobacter and Human Papilloma Virus were reported less frequently (<5%) and co-infections were absent in 31.5% of the cases (62/197).

Longitudinal data of the LGV epidemic

The number of cases rose by a factor four, from 21 in 2011 to 88 in 2016 (see Fig. 1). In the first half of 2017, 46 cases were identified. Poisson regression models showed a positive trend estimate of 14% increase per half year (95%CI: 1.11–1.17) ($p < 0.001$). Surprisingly, in the second half of 2014, a drop in LGV cases was noted, without any plausible explanation. Piece-wise regression was used to segment the regression in the first half of 2014 and showed that the positive trend estimate rate increased to 30% per 6 month period (95% CI: 1.19–1.43) ($p < 0.001$) for either period before and after the break.

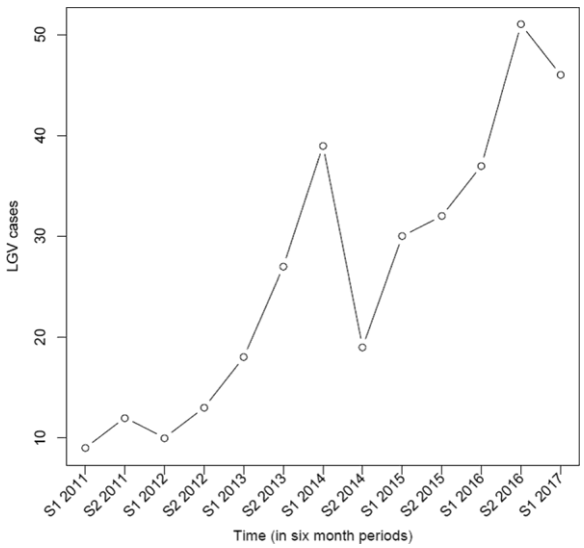

Figure 1: Number of LGV cases over time in Belgium from 2011-S1 2017. S= six months

Patient characteristics over the years with regard to sexual behaviour and demographics were relatively stable over the years. Figure 2 shows the percentage of HIV positive, HIV negative and asymptomatic cases over time. Regression analyses showed that the number of asymptomatic cases increased borderline significantly per year ($p = 0.047$) with an OR of 1.39 (95% CI: 1.02– 1.97). When breaking down the time line per 6 month period, the OR decreased to 1.16 (95% CI: 0.99–1.39), with a p-value of 0.06. A decrease in HIV positive cases (OR 0.79 (95% CI: 0.69–0.88)) and an increase in HIV negative cases (OR 1.27 (95% CI: 1.13–1.45)) over time was, however, highly statistically significant ($p<0.001$). The rates of gonorrhea or syphilis co-infections did not change significantly over time ($p=0.12$ and 0.32, respectively) and are not presented in the figure.

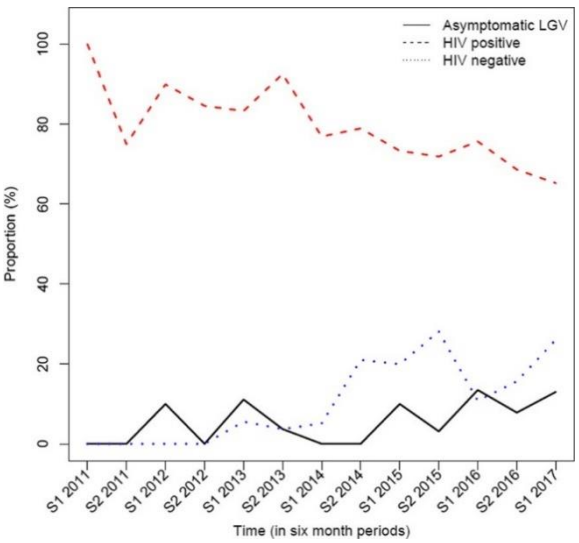

Figure 2: Proportion of HIV-positive, HIV-negative and asymptomatic LGV cases over time. S= six months

Asymptomatic cases

Over the time period, 23 asymptomatic cases were reported: 22 men (all MSM) and one woman (Table 2). The vast majority of asymptomatic LGV infections were anorectal; only two urinary infections were reported (one male, one female).

Table 2: Socio-demographic and clinical data of asymptomatic cases starting from 2011 until end of June 2017.

	2011 n	2012 n	2013 n	2014 n	2015 n	2016 n	S1 2017 n	TOTAL n(%)
Number of asymptomatic LGV cases	0	1	3	0	4	9	6	23
Gender	**23/23 known**							
Male or Trans female	0	1	3	0	3	9	6	22 (96%)
Female	0	0	0	0	1	0	0	1 (4%)
Transmission of all men	**22/22 known[a]**							
MSM	0	1	3	0	3	9	6	22
HETERO	0	0	0	0	0	0	0	0 (0%)
Age scale	**22/22 known[a]**							
31–40	0	1	1	0	0	4	3	9 (41%)
41–50	0	0	2	0	1	4	3	10 (45%)
51–60	0	0	0	0	2	1	0	3 (14%)
Geographical location	**22/22 known[a]**							
Living in Flemish region	0	1	2	0	3	7	5	18 (82%)
Living in French region	0	0	1	0	0	1	1	3 (14%)
Living in the capital region	0	0	0	0	0	1	0	1 (4%)

	2011 n	2012 n	2013 n	2014 n	2015 n	2016 n	S1 2017 n	TOTAL n(%)
Kind of Sample	**22/22 known[a]**							
Anorectal	0	1	3	0	3	8	6	21 (95%)
Urine	0	0	0	0	1	0	0	1 (5%)
HIV Status	**22/22 known[a]**							
Positive	0	1	3	0	2	5	3	14 (64%)
Negative	0	0	0	0	1	4	3	8 (36%)
Co-infections other than HIV	**21/22 known[a]**							
None	0	0	0	0	2	1	2	5 (24%)
Only one	0	0	2	0	1	5	2	10 (48%)
Two or more	0	1	1	0	0	3	1	6 (29%)
Gonorrhea	0	1	2	0	0	6	1	10 (48%)
Syphilis	0	1	2	0	0	2	1	6 (29%)
Chlamydia A-K	0	0	0	0	1	0	1	2 (10%)
Hepatitis B	0	0	0	0	0	1	0	1 (5%)
Mycoplasma genitalium	0	0	0	0	0	2	1	3 (14%)

% removing unknowns – deviations from 100% can be due to rounding; S Six-monthly period; [a]only male cases are presented here

In total, 6.7% (23/343) of all LGV cases were found to be asymptomatic, of those with available data, this percentage increased to 9.7% (21/217) for rectal LGV infections. Seventeen percent (8/47) of the documented HIV negative LGV cases were asymptomatic.

Statistical analysis with Fisher's Exact test revealed that asymptomatic patients were borderline less likely to be HIV positive ($p = 0.046$) and were less likely to have HCV co-infection ($p = 0.027$). Other parameters such as gonorrhea or syphilis co-infection were not significant ($p = 0.144$ and 0.388, respectively).

Re-infections

During this time period, 300 patients were diagnosed with LGV. Of them, 28 were identified as having repeated infections (9.3%).

Time between repeated infections varied from 4 months to 4 years. Most of them (17) were infected twice (4 to 53 months apart), eight reported three episodes, two patients had an LGV infection for the fourth time during this period, and one had five LGV infections (MSM, HIV positive, infection period age range: 35–41 years). The median time to the second episode was 20 months with a range of 4–53 months and 42.9% (12/28) of the repeaters had a second episode within less than 12 months. HIV positivity was confirmed in 25 men (89.3%). Two remained HIV negative and the HIV status is unknown for one patient (all had two episodes within 5 to 15 months). Of the 71 LGV infections found in repeaters, 37 had an STI co-infection (52.1%).

CT epidemic at the HIV/STI clinic

A total of 19,017 valid requests for CT analysis among male patients were received from the HIV/STI clinic including the low threshold centre (Fig. 3). In all, CT was detected in 1019 /19017 cases (5.4%). Genotypes A-K and L were detected in 833 cases (4.4%) and in 186 cases (1.0%), respectively. LGV was the most prevalent in rectal samples (92.5%), followed by genital samples (4.3%). No pharyngeal LGV was detected.

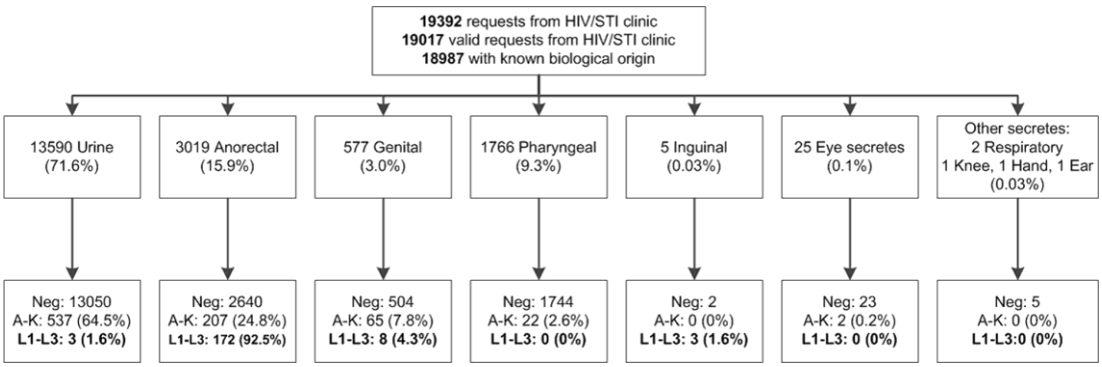

Figure 3: Results of CT testing in men at the HIV/STI clinic of ITM from 2011-S1 2017. S = a six month period

The number of CT analyses is depicted in Fig. 4 and shows an large expansion in the number of test starting in 2015, however the positivity rate for Chlamydia remained the same over the 6 month periods and fluctuated between 4.7–6.7%. The LGV positivity rate did not increase over the months and remained around 1.0% (except for S2 2013 with a peak of 1.8%) (Fig. 4).

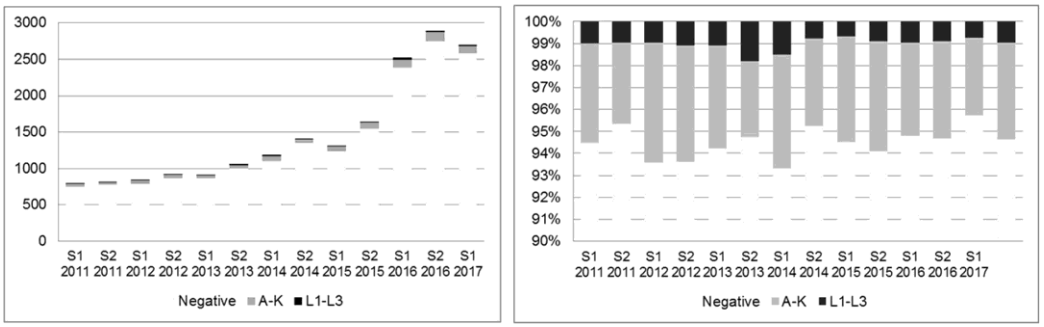

Figure 4: Number of CT analyses among men at the HIV/STI clinic including results over time. The graph on the right hand side represents positivity (%) of A-K and L-types among the samples over time presented in six month periods

4.1.5 Discussion

The number of LGV cases is rising in Belgium, as well as in neighbouring countries.[2] This upward trend might have been influenced by the fact that the Belgian government promoted awareness of LGV among health care providers and microbiologists.[13] Indeed, at the ITM's HIV/STI clinic, the number of requests per half year rose above 1000 during the second half of 2013 and reaches now 2500 requests every 6 months. Ongoing HIV prevention projects with frequent STI screening among high risk populations may have contributed to this increasing testing trend. However, despite enhanced testing the prevalence of LGV at the HIV/STI clinic remained stable during the years we studied and fluctuated at around 1.0%.

The typical LGV patient (HIV positive MSM presenting with proctitis or other abdominal symptoms) was found among 118 men (39.5%) accounting for 135 LGV cases.[14] Hereby rectal LGV (307/337–91.1%) forms the vast majority of the cases, which is in line with previously described epidemics.[4, 15] The mode of transmission of LGV has been questioned and possible urethral and pharyngeal silent reservoirs have been suggested, however, the evidence is sparse.[4,16–18] Being one of the few laboratories that differentiates all positive CT on a routine basis, we had hoped to identify the hidden link in the epidemic. However, none of the pharyngeal samples tested positive for LGV and only a few genital LGV infections were detected (8 out of 577 genital samples). Intriguingly, one case of LGV conjunctivitis was detected in the first half of 2017.

During the reported period, 28 patients had repeated infections (9.3%) which is in line with previous reports.[4,11] Out of these, 26 were HIV positive MSM and co-infections were described in 37 episodes. More worrisome is the observation that three repeaters with gonorrhea as co-infection were asymptomatic. It has been speculated that the central position of LGV repeaters in the sexual network, together with the asymptomatic carriers, may be an important factor in the LGV epidemic.[11,16] Frequent STI screening (e.g. quarterly) among those individuals is therefore recommended.

LGV infection in women was found to be rare.[19–21] Only one woman with LGV was detected over the 6 year period in Belgium and she was screened because her partner was a sexual contact of an LGV case. In addition to this female asymptomatic case, 22 other asymptomatic cases were reported, all among MSM. Fourteen were HIV positive, the other eight were HIV negative. Recent publications show that asymptomatic cases are frequently detected.[3–5] A German study even showed that 42% of 19 documented LGV infections were asymptomatic, but the low number of LGV infections reported in that study limits conclusive interpretations. In that limited study, eight LGV infections were found in HIV negative men, of whom five did not present symptoms (62.5%). We

could not detect such a high number of asymptomatic cases (9.9%) which could imply that the Belgian LGV epidemic is larger than herein described. The number of asymptomatic LGV cases is, however, rising and this increase over time warrants attention. We showed that asymptomatic cases are more likely to be HIV negative (borderline p=0.046) and HCV negative (p=0.027). Almost three quarters (72.7%) of the asymptomatic carriers manifested another STI. It is now well documented that LGV may increase the risk for acquisition and transmission of other STIs, including HIV and HCV.[22,23] Within the group of HIV negative men, we observed a significant rise of LGV ($p < 0.001$). This finding indicates that the spread of LGV is no longer as confined to sexual networks of HIV positive MSM, and screening of high risk HIV negative MSM is therefore warranted.

Availability of PrEP

Due to the availability of PrEP, changes in sexual behaviour could take place. For example, a number of HIV seropositive partners have stated that they would be comfortable engaging in condomless anal sex if their partner was on PrEP, and individuals on PrEP have also experimented with anal receptive intercourse instead of being exclusively anally insertive.[24,25] In fact, the emergence of HCV in high-risk HIV negative MSM using PrEP has also been described.[26,27] The availability of PrEP might therefore initiate the dissemination of LGV from HIV-positive to HIV-negative MSM.

LGV is not notifiable in Belgium, nevertheless, national surveillance is in place for LGV. The NRC is one of the scarce laboratories, and possibly also the only one in Belgium, able to differentiate LGV from non-LGV strains. Consequently, asymptomatic patients will be treated for non-LGV CT infection in other Belgian settings, yet suboptimal treatment, e.g. a one-week course of doxycycline instead of a three-week course, may not prevent onward transmission.[3,28,29] We therefore advocate that all CT positive samples from PrEP users, including those who request PrEP, are referred to the NRC for genotype differentiation and LGV confirmation.

Limitations and weaknesses

Our study has several limitations: First, a major weakness of this investigation was that some of the behavioural parameters were missing. We relied entirely on the information provided by the physicians or microbiologists. The request form documenting the necessary information from LGV patients at the ITM's HIV/STI clinic was not rechecked with the clinic notes for correctness. Second, most of the LGV cases we present here come from the Flemish region of Belgium. Indeed, the HIV/STI clinic is located in Antwerp, which may have introduced a bias regarding the geographical

distribution of the LGV cases. Third, the NRC started with the confirmation of LGV cases in 2011, so the increase over the years could be due to the increased awareness of health care providers but also to ongoing HIV prevention projects at the HIV/STI clinic. However, the rise in HIV negative LGV cases cannot only be attributed to these projects, as cases from HIV negative individuals were also received from other laboratories. Fourth, our sample size over the years is still small and this may have introduced a bias in the regression analyses. Finally, we have not yet performed sequence typing to document the genetic diversity of the LGV cases over recent years because of budgetary constraints. We do, however, advocate that sequence typing should be performed on all samples from the past 2 years to document further the epidemic in Belgium. In addition, discrimination between the L-genotypes could shed more light on why some infections remain asymptomatic.

4.1.6 Conclusions

To conclude, LGV infection is a serious concern for the MSM community in Europe. If we aim to halt the epidemic in HIV negative MSM, future public health strategies should include testing for LGV on all CT positive samples from MSM, irrespective of their HIV status, so that they, and their partners, can be treated correctly.

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4.2 DID PREP ROLL-OUT INFLUENCE THE EPIDEMIC OF RECTAL LGV IN BELGIUM?

EPIDEMIOLOGY

Did Pre-exposure Prophylaxis Roll-Out Influence the Epidemic of Rectal Lymphogranuloma Venereum in Belgium? Results From the National Surveillance System

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Contributions: data collection and clean-up, data analysis, manuscript writing and finalization

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4.2.1 Abstract

Background: An increase of lymphogranuloma venereum (LGV) in HIV negative men who have sex with men is reported in several European countries including Belgium before the implementation of pre-exposure prophylaxis (PrEP).

Setting: The epidemiological characteristics of the male rectal LGV epidemic in Belgium were explored before and after the introduction of PrEP.

Methods: Segmented regression models were used to examine a change in trends before and after the introduction of PrEP in the male rectal LGV epidemic in Belgium and among men attending a large HIV/sexually transmitted infection clinic in Antwerp, Belgium.

Results: Although an increase of 69% was noted in absolute numbers in 2019 compared with 2018 (140 vs 83 cases) in Belgium, models showed that the rate of increase did not change after the introduction of PrEP. More than half of the cases were found in HIV-negative men (56.2%) in 2019, but no difference in the magnitude of the trend was found after the introduction of PrEP. Nevertheless, the data reveal that a statistical significant increase of LGV prevalence was noted among non-HIV-positive men in an HIV/sexually transmitted infection clinic after the implementation of PrEP. Indeed, LGV prevalence in the Antwerp male PrEP cohort increased from 0.8% in 2017 to 2.4% in 2019.

Conclusions: The trend of LGV increase did not accelerate after the introduction of PrEP. Continued surveillance in men who have sex with men irrespective of their HIV status is required for the management and control of the LGV epidemic.

KEYWORDS

Lymphogranuloma venereum, HIV, men who have sex with men, pre-exposure prophylaxis

4.2.2 Introduction

Rectal lymphogranuloma venereum (LGV), a sexually transmitted infection (STI) caused by the L-serovar of *Chlamydia trachomatis* (Ct), is in the western world typically seen in HIV-positive men who have sex with men (MSM).[1] The infection is strongly associated with dense sociosexual networks and a high number of sexual partners.[2,3] Furthermore, the implementation of pre-exposure prophylaxis (PrEP) to prevent HIV is associated with an increase in rectal STIs, and it has been suggested that the use of PrEP might be responsible for the increasing proportion of LGV cases among HIV-negative MSM.[4,5]

The number of cases of LGV continues to rise in Europe, and in 2019, an increase of 45% was seen in the UK (communication through Epidemic Intelligence Information System of European Centre for Disease Prevention and Control). Half of the LGV cases in the UK were found in HIV-negative MSM which may be due to changes in behaviour or a more intensive testing strategy in MSM. A similar increase in LGV cases in HIV-negative MSM has been described in Belgium.[6] Of note, this increase predates the roll-out of PrEP in Belgium in mid-2017 which could be explained by an increasing awareness of symptomatic LGV infection among health care providers and laboratory experts in 2014.[7,8]

In Belgium, LGV confirmation was added to the surveillance activities of the national reference centre (NRC) for STIs in 2011. Additional promotion of LGV by the Belgian government took place in 2014 and in 2018; national STI guidelines were issued which included the recommendation to further test positive anorectal Ct samples of MSM for LGV confirmation irrespective of their HIV status.[9] Yet, this recommendation is not followed by all Belgian laboratories or physicians. The NRC-STI is affiliated to one of the largest Belgian HIV/STI clinics. In this clinic, positive anorectal Ct samples are routinely genotyped to distinguish non-L Ct strains versus LGV strains. We therefore aimed to describe the rectal LGV epidemic in Belgium including the PrEP-era until the end of 2019 and an exploration of the differences in epidemiological characteristics before and after the introduction of PrEP. Furthermore, this article focuses on the LGV epidemic among male patients attending our HIV/STI clinic to better understand the epidemiology of LGV in the HIV and PrEP cohorts of our clinic.

4.2.3 Methods

Study Setting

All male rectal LGV confirmed cases received by the Belgian NRC located at the Institute of Tropical Medicine (ITM) were used to explore the national LGV epidemic before and after the introduction of PrEP.

In addition, the HIV/STI clinic at ITM (Antwerp, Belgium), harbours a large AIDS reference centre which follows a large cohort of MSM in the framework of HIV treatment and HIV/STI prevention. In Belgium, the use of PrEP is reimbursed since July 2017 when prescribed by a physician specialist affiliated to an AIDS reference centre.[7] We reviewed rectal Ct and LGV diagnoses in male patients in this HIV/STI clinic from 2011 until the end of 2019. Reinfections within 3 months of an initial diagnosis were removed from the database to exclude potential persistent infections and treatment failures.

Laboratory Procedures

Molecular testing of Ct was performed using the Abbott Real Time CT/NG molecular assay according to manufacturer's instructions. In case a positive result was obtained, further genotyping was performed by an in-house real time- molecular assay using previously published *pmpH* gene primers/probes to differentiate LGV (L genotypes) from non-LGV (A-K genotypes) strains.[10]

Ethics Statement

No ethical approval or informed consent was necessary because this retrospective study used coded data that was gathered for public health monitoring and infection control. According to ITM's policy, laboratory data of patients can be used for research if the patients' identity is not disclosed to third parties and the patient does not explicitly state his objection.

Statistical Analyses

Overall Belgian Rectal LGV Epidemic

Segmented Poisson regression was performed to explore differences in the number of rectal LGV cases before and after the start of PrEP, for the whole data set and by the HIV status. The second semester (S2) of the year 2017 was used as the cut-off date of the intervention (introduction of PrEP). In addition, logistic regression was performed

to check for associations between the absence of symptoms and HIV status on all received rectal LGV samples since 2011.

Ct and LGV Epidemic in the HIV/STI Clinic in Antwerp, Belgium

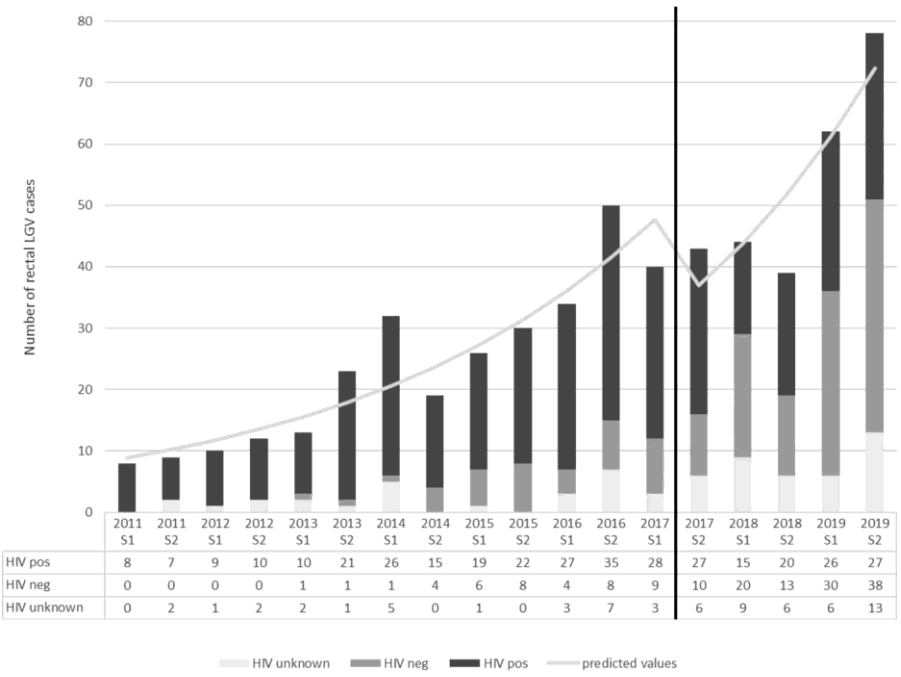
The overall rectal Ct and LGV prevalence was estimated among the male patients of the HIV/STI clinic as well as the male HIV cohort and male HIV negative individuals (HIV negative or HIV status unknown). Since S2 2017, the prevalence of LGV is also presented for PrEP users only. Prevalence per semester is defined as (the number of rectal positive results/the total number of rectal Ct tests performed per year). Segmented logistic regression was performed to explore a change in trend in LGV prevalence in HIV positive individuals and HIV negative individuals.

The results of the regression models are presented as incidence rate ratios (IRR) with 95% confidence intervals (CI) in the case of the Poisson models and as odds ratios (OR) with 95% CI for the logistic regression models. For all analyses, estimates with P values below the 0.05 threshold are considered statistically significant. STATA v15.1 (StataCorp) was used for all statistical analyses.

4.2.4 Results

Overall Belgian Rectal LGV Epidemic

In total, 572 male rectal LGV cases were identified from 2011 till the end of 2019 (Fig. 1). The demographic and epidemiologic data detailing the steady increase in cases until 2016 were previously published.[6] Almost all LGV cases with known sexual behaviour described themselves as MSM (98.9%; n=429/434).



	2011 S1	2011 S2	2012 S1	2012 S2	2013 S1	2013 S2	2014 S1	2014 S2	2015 S1	2015 S2	2016 S1	2016 S2	2017 S1	2017 S2	2018 S1	2018 S2	2019 S1	2019 S2
HIV pos	8	7	9	10	10	21	26	15	19	22	27	35	28	27	15	20	26	27
HIV neg	0	0	0	0	1	1	1	4	6	8	4	8	9	10	20	13	30	38
HIV unknown	0	2	1	2	2	1	5	0	1	0	3	7	3	6	9	6	6	13

Figure 1: Number of rectal LGV cases per semester and per HIV status in Belgium. PrEP was introduced in the second semester (S) of 2017 (vertical line). The grey line represents the predicted values of the segmented regression model

In 2017, 83 LGV cases were reported, and this number remained the same in 2018. An increase of 69% in LGV cases was noted in 2019 (n = 140).

The segmented regression model showed that the number of cases increases with 15% every semester (IRR: 1.15; 95% CI: 1.11 to 1.19; P < 0.0001) or approximately 30% per year. Right after 2017 (introduction of PrEP), there seems to be a significant drop in incidence, but the slope did not accelerate after 2017 (IRR: 1.18; 95% CI: 1.04 to 1.36). Of the LGV cases with known HIV status (n= 505/572), the proportion that was HIV-negative increased from 0.0% in S1 of 2011 and 2012 to 24.3% in S1 of 2017 (n = 9/37; 95% CI: 11.2 to 41.2) just before the introduction of PrEP (Fig. 1). There was a strong increase in this trend over the semesters (IRR: 1.37; 95% CI: 1.22 to 1.53; P <0.0001), but there was no difference in the magnitude of the trend after the introduction of PrEP (P> 0.05). In 2019, 56.2% of the LGV cases with known HIV status were HIV negative (n = 68/121; 95% CI: 46.9 to 65.2).The increase of LGV cases in HIV-positive patients is smaller than in HIV-negative individuals (IRR: 1.13; 95% CI: 1.09 to 1.17). After the introduction of PrEP, no significant difference in magnitude of trend was documented (IRR: 1.05; 95% CI: 0.88 to 1.25; P>0.05).The presence of symptoms and HIV status of 373/572 (65.2%) rectal LGV cases was reported over the 9 years. The proportion of

asymptomatic cases was higher in HIV-negative men (21.4%; 95% CI: 14.2 to 30.2; n = 24/112) than in HIV positive men (7.3%; 95% CI: 4.4 to 11.1; n = 19/261; P<0.0001), and subsequently, the odds of having an asymptomatic LGV infection was higher in HIV-negative individuals (OR: 3.47; 95% CI: 1.81 to 6.65; P<0.0001). Poisson regression, however, did not show a statistically significant increase over the semesters in asymptomatic cases among HIV-positive nor among HIV-negative individuals.

Ct and LGV Epidemic in the HIV/STI Clinic in Antwerp, Belgium

The number of rectal Ct requests increased 10-fold over time: from less than 100 in the semesters of 2011–2013 to over 1000 requests in the semesters of 2019 (Fig. 2). The percentage of rectal swabs that were positive for all serovars of Ct fluctuated between 14.1% and 23.7% in the semesters of 2011–2014. Starting from 2015, the number of rectal Ct requests increased substantially, and subsequently, the prevalence of rectal Ct dropped from 15.1% (n = 23/152; 95% CI:9.8 to 21.7) in the second semester of 2014 to 9.2% in the first semester of 2017 (n = 56/609; 95% CI: 7.0 to 11.8). After S1 2017, the prevalence of rectal Ct increased again and fluctuated between 10.8% and 13.6% between S2 2017 and S2 2019. Although that the prevalence of LGV followed the same trajectory as the prevalence of Ct (high prevalence in the years preceding 2015, followed by a drop in prevalence the next years), an increase in LGV prevalence was only reported in the semesters of 2019 (Fig. 2).

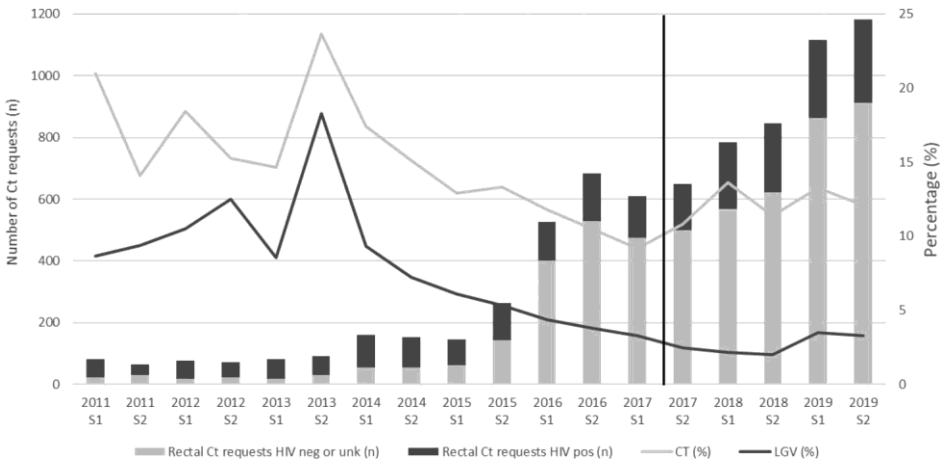


Figure 2: Absolute number of requests (n) for rectal Ct including percent (%) of rectal tests positive for Ct (all types) and LGV in men attending the HIV/STI clinic of ITM, Antwerp, Belgium over the year 2011–2019. The vertical line indicates the introduction of PrEP in Belgium. Neg, negative; pos, positive; S, semester; unk, unknown

LGV Prevalence in the HIV and PrEP Cohort of ITM

Until the second semester of 2014, LGV infections were solely detected in HIV-positive men (Fig. 3). Men not belonging to the HIV cohort were also less frequently sampled (Fig. 2). Starting from the second semester of 2015, more Ct rectal requests were received from HIV- negative men (143 vs 120); and from 2016 until the end of 2019, 3 times more rectal Ct requests were received from HIV-negative men (Fig. 2). The LGV prevalence among HIV-positive individuals was mostly above 10% (11.1%–27.0%) until S2 2017. Starting from S2 2017, prevalence dropped below 10% and fluctuated in between 3.7% and 9.3% until the end of 2019.

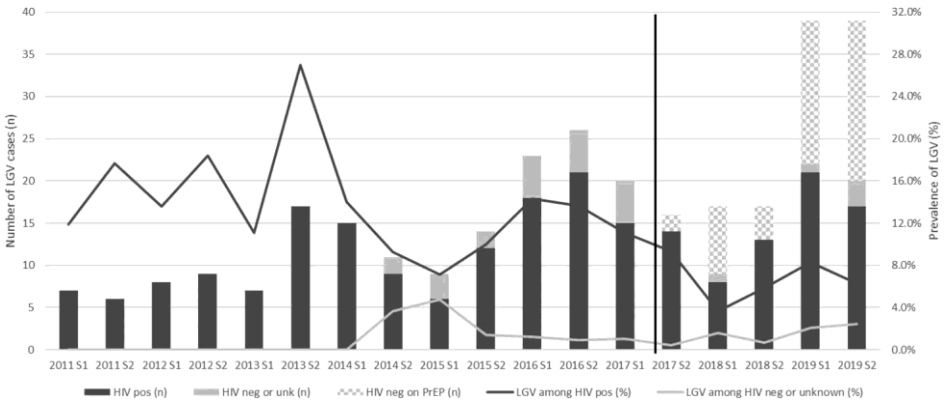


Figure 3: Rectal LGV prevalence among HIV-positive and non–HIV- positive men attending the HIV/STI clinic of ITM, Antwerp over time depicted per semester (S). PrEP was introduced in routine care since the second semester of 2017 (vertical line). neg, negative; pos, positive; unk, unknown.

LGV prevalence of HIV-negative men still remains below 5% (Fig. 3). Since S2 2017, PrEP became reimbursed in Belgium, and over the following semesters, more HIV-negative men visited the clinic in the framework of PrEP visits. Of all the cases of LGV diagnosed at ITM, PrEP users constituted 13.9% (n = 5/36) in 2017 and 46.2% (n = 36/78) in 2019. Among those known to be HIV positive, these figures were 80.6% (n = 29/36) in 2017 and 48.7% (n = 38/78) in 2019.

Segmented logistic regression, however, showed that there was no significant increase in LGV cases in HIV- negative individuals over the semesters before (OR: 1.03; 95% CI: 0.86 to 1.22; *P*>0.05), but an increase was noted after the introduction of PrEP (OR: 1.40; 95% CI: 0.80 to 2.43; *P* = 0.032). Indeed, the prevalence of LGV in the PrEP cohort increased from 0.8% (n = 5/598; 95% CI: 0.3 to 1.9) in 2017 to 2.4% in 2019 (n = 36/1507; 95% CI: 1.7 to 3.3; *P* =0.016). We could not document a change in trend among HIV-positive individuals.

Whereas the size of HIV-negative men who were tested at least once for rectal Ct doubled from 584 individuals in 2017 to 1181 in 2019; most of them were part of the PrEP cohort (360 in 2017 and 938 in 2019). The number HIV- positive patients tested for Ct increased from 221 to 370 over the same time period HIV positive patients were less frequently screened for rectal Ct compared with PrEP users. In 2019, 69% of the HIV cohort was only screened once, 24% twice, and only 7% was screened more than twice. The number of screening tests was considerably higher among the PrEP cohort. Hereby, 56% was screened once, around 31% twice, and 13% was screened more than twice for rectal Ct in 2019.

4.2.5 Discussions

This study shows an increase in Belgian LGV cases of almost 70% in 2019 compared with 2018 which is in line with results reported in the United Kingdom (communication through Epidemic Intelligence Information System of European Centre for Disease Prevention and Control). In the United Kingdom and other European countries, a small decrease in LGV cases was noted in 2017, followed by an increase in 2018 which was possibly linked to a change in testing practice.[11,12] In Belgium, the number of rectal LGV cases remained stable during 2016–2018 but increased noticeably in 2019. Belgium released new national STI guidelines in 2018 that included a recommendation to send Ct positive anorectal samples of MSM to the NRC for LGV genotype determination. This guidance likely increased awareness among health care providers and could, therefore, explain the rise in number of LGV cases in 2019.[8] Furthermore, the increasing proportion of LGV attributed to HIV-negative MSM (56.2% of the LGV cases in 2019) in Belgium, may be related to the more frequent screening for asymptomatic STIs, which remain otherwise undiagnosed, in PrEP recipients compared with HIV-positive individuals. Indeed, our study showed that asymptomatic LGV cases were more likely to be found among HIV-negative (21.0%) than HIV-positive MSM (7.3%) and complements other studies documenting a high asymptomatic LGV rate in HIV-negative MSM (30%–50%).[5,13] Unfortunately, because of underreporting of the presence of symptoms, a change in asymptomatic rate over the years could not be analysed. We can however not exclude that these asymptomatic infections would become symptomatically over time.

In this article, we tried to explore trends in the Belgian LGV epidemic after the introduction of PrEP. Although we found that the number of LGV cases increased among HIV negative individuals over the years, there was no acceleration in this trend after the introduction of PrEP.

To assess a difference in trends of Ct and LGV prevalence over time among HIV-positive and HIV- negative men, we investigated the prevalence in our HIV/ STI clinic. Overall, rectal Ct and LGV prevalence among men in our HIV/STI clinic was high in the years before 2015 (mean prevalence Ct: 17.4% and LGV: 10.6%), subsequently dropped until 2018, but increased again in 2019 (Ct: 12.7% and LGV: 3.4%). Importantly, the data showed an increase in the number of LGV diagnoses and the proportion of tests positive among HIV-negative individuals and in particularly PrEP users (from 0.8% in 2017 to 2.4% in 2019). Interestingly, a Dutch study investigating LGV positivity in rectal samples among HIV-positive and HIV- negative MSM, reported a much lower rectal LGV prevalence in both cohorts (2.5% in HIV positive MSM and 0.3% in HIV negative MSM in 2017) than our study (10.2% and 0.7%) which is probably because of the implementation of universal rectal chlamydia and LGV testing for all MSM since 2015 in the Netherlands.[5] Importantly, they also documented a doubling in LGV prevalence in HIV negatives over time from 0.12% in 2011 to 0.33% in 2017.[5] They, however, could not assess the proportion of LGV among PrEP users, as PrEP was not widely implemented in the Netherlands until 2019.

Although we found an increasing LGV prevalence among PrEP users in our STI clinic, we previously documented that HIV-negative persons constituted an increasing proportion of LGV in Belgium.[6] The current findings suggest that as of 2019, more than half of the LGV infections were found in HIV-negative individuals. In the ITM cohort, 46.2% of LGV diagnoses were in individuals enrolled in our PrEP cohort, and 48.7% were part of the HIV cohort. These findings may reflect less serosorting or simply the dense sexual network that certain MSM are part of, irrespective of the use of PrEP.[11]

Our study has some important limitations. First, the epidemiological data were missing for many cases including the lack of information on the presence of symptoms. Therefore, we could not explore changes of asymptomatic cases by the HIV status over time. Second, every patient given PrEP at least once was categorized as such, regardless of the date of Ct and LGV testing being before, during, or after using PrEP. This may have resulted in overestimation of the number of PrEP users. Furthermore, PrEP users must exhibit high risk behaviour before they are eligible for PrEP acquisition. They are therefore a population already at high risk for STI acquisition. Hence, we cannot generalize the results to HIV-negative MSM that are not taking PrEP. Finally, all rectal male samples of patients not belonging to our HIV cohort were categorized as HIV negative or unknown. Consequently, a few cases might have been erroneously categorized as being not HIV positive.

To conclude, this study showed that the increase in LGV cases among HIV negative men in Belgium started before the introduction of PrEP and that this trend did not increase

after the introduction of PrEP in Belgium. LGV prevalence among PrEP users is however increasing in our STI clinic. Therefore, continued surveillance in MSM irrespective of their HIV status and PrEP use is required for the management and control of the LGV epidemic.

4.2.6 Acknowledgements

The authors thank all laboratory staff of the National Reference Centre of STIs in Belgium including Wendy Thys for the data entry.

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CHAPTER 5: MYCOPLASMA GENITALIUM AND ANTIMICROBIAL RESISTANCE

Epidemiology

ORIGINAL RESEARCH

An alarming high prevalence of resistance-associated mutations to macrolides and fluoroquinolones in *Mycoplasma genitalium* in Belgium: results from samples collected between 2015 and 2018

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Contributions: data collection and clean-up, data analysis, manuscript writing and finalization

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5.1 ABSTRACT

Objectives: The number of reported cases of multiresistant *Mycoplasma genitalium* (MG) is increasing globally. The aim of this study was to estimate the prevalence of macrolide and possible fluoroquinolone resistance-associated mutations (RAMs) of MG in Belgium.

Methods: The study was performed retrospectively on two sets of MG-positive samples collected in Belgium between 2015 and 2018. The first set of samples originated from routine surveillance activities and the second set came from a cohort of men who have sex with men (MSM) using pre-exposure prophylaxis to prevent HIV transmission. Detection of RAMs to macrolides and fluoroquinolones was performed on all samples using DNA sequencing of the 23S ribosomal RNA gene, the *gyrA* gene and the *parC* gene.

Results: Seventy-one per cent of the MG samples contained a mutation conferring resistance to macrolides or fluoroquinolones (ParC position 83/87). RAMs were more frequently found among men compared with women for fluoroquinolones (23.9% vs 9.1%) and macrolides (78.4% vs 27.3%). Almost 90% of the MG infections among MSM possessed a RAM to macrolides (88.4%). In addition, 18.0% of the samples harboured both macrolides and fluoroquinolone RAMs; 3.0% in women and 24.2% in MSM. Being MSM was associated with macrolide RAMs (OR 15.3), fluoroquinolone RAMs (OR 3.8) and having a possible multiresistant MG infection (OR 7.2).

Conclusion: The study shows an alarmingly high prevalence of MG with RAMs to macrolides and fluoroquinolones in Belgium. These results highlight the need to improve antimicrobial stewardship in Belgium in order to avoid the emergence of untreatable MG.

KEYWORDS

***Mycoplasma genitalium*, antimicrobial resistance, Pre-exposure prophylaxis, MSM**

5.2 INTRODUCTION

Mycoplasma genitalium (MG) is an emerging STI and its global burden of disease is not very well known so far.[1] Prevalence varies among different risk populations - and is generally higher in men who have sex with men (MSM) and female sex workers compared with other heterosexual individuals. A systematic review and meta-analysis up to 2017 found the prevalence of MG to be 0.9% in pregnant women, 3.2% in MSM and 15.9% in female sex workers.[2] The prevalence of MG in MSM, is, however, now reaching 10% according to the most recent publications.[3–6]

MG is a cause of non-gonococcal urethritis in men and cervicitis in women, but MG is also frequently found in asymptomatic individuals.[5] The natural history and clinical consequences of asymptomatic MG infections are still poorly understood and including MG in screening programmes among high-risk groups is, therefore, not recommended. Furthermore, along with *Neisseria gonorrhoeae* (NG), MG is one of the STIs that can acquire resistance to different classes of antibiotics at an alarmingly rapid rate.[1] In fact, MG cases with macrolide resistant-associated mutations (RAMs) have been reported globally, and prevalence of these mutations is exceeding 80% in MSM using pre-exposure prophylaxis (PrEP) to prevent HIV.[3 4]

As a result, guidelines mainly suggest testing and treating for MG in cases of non-gonococcal, non-chlamydia urethritis or cervicitis. Moreover, MG-positive samples should be further analysed for the presence of macrolide RAMs to tailor patient treatment and management.[7]

European guidelines recommend the use of moxifloxacin, a fourth-generation fluoroquinolone, in cases where macrolide treatment failure is detected.7 The prevalence of resistance to fluoroquinolones has been noted to be increasing in a number of regions. A recent systematic review from Europe estimated that the prevalence of fluoroquinolone resistance was 5% in this region, but there were considerable gaps in the data, such as from Belgium.[8]

With this study, we aimed to estimate the prevalence of RAMs to macrolides and fluoroquinolones in MG samples received by the Belgian National Reference Centre (NRC) from 2015 to 2018. In addition, we explored risk factors associated with the presence of RAMs in order to inform clinical and testing practice.

5.3 METHODS

Samples

Since 2015, genital, anorectal or urine samples from patients with clinical suspicion of MG could be sent to the NRC, located at the Institute of Tropical Medicine (ITM), Antwerp for MG identification (here- after referred to as surveillance samples). Sociodemographic and clinical data, such as age, postal code, gender, country of birth, sexual orientation, HIV status, presence of symptoms and presence of other STIs is requested for each case.

Samples from a PrEP demonstration study that was ongoing at the ITM from 2015 to 2018 were also included. The Be-PrEP- ared study was a single site, open-label PrEP demonstration study which included 200 HIV-negative MSM.[9–11] A total of 179 HIV-negative individuals were followed up for a period of 18 months and STI screening, including MG detection, was performed quarterly at the three anatomical sites (i.e., urethra, anorectum and pharynx).[10]

For this retrospective study, both surveillance and study samples from 01 January 2015 to 31 December 2018 were included.

Laboratory procedures

MG detection was routinely performed at the NRC with an accredited in-house RT-PCR that targets the *pdhD*-gene until 30 September 2018.[12] After this, MG detection was performed using the S-DiaMGTV multiplex kit, according to manufacturer's instructions (Diagenode Diagnostics, Seraing, Belgium) on the *m*2000rt platform (Abbott Molecular Des Plaines, Illinois, USA). DNA extraction of all samples was performed using the Abbott *m*2000sp instrument and CT/NG extraction kit. Sample remnants and DNA extracts were stored below −20°C.

Sanger sequencing of the 23S rRNA, *parC* and *gyrA* genes was performed on the ABI 36730xl instrument of Applied Biosystems (USA) as described previously to detect the presence of RAMs to macrolides and fluoroquinolones.[13 14]

Mutations found in region V of the 23S rRNA gene, *parC* (nucleotides 164–483) and *gyrA* gene (nucleotides 172–402) are provided according to MG numbering. Samples with a silent mutation (without an alteration in amino acid) are not reported as mutations.

Statistical analysis

All data analyses were performed using STATA V.15.1.

MG was defined as resistant to macrolides if a RAM in the 23S rRNA region V was detected. MG possessing an alteration in ParC at position 83 or 87 was defined as resistant to fluoroquinolones. MG was defined as multiresistant if mutations in both genes (23S rRNA and ParC position 83/87) were detected.

Prevalence of antibiotic resistance is estimated on samples of a new MG episode only. A new MG episode is defined as the first MG episode of a patient or a new MG infection minimum 6 months after the previous MG infection, or in case the patient tested negative for MG in between episodes.

The relationship between relevant sociodemographic and behavioural variables, and the presence of antimicrobial resistance to macrolides, fluoroquinolones or both, was explored on samples with a new MG episode using two-tailed Fisher's exact test.

If a relationship was detected, we report unadjusted and adjusted OR with their corresponding 95% CIs. Variables significantly associated with the resistance of interest were selected for the multivariate logistic regression. For all analyses, a significance level of 0.05 was used.

5.4 RESULTS

Description of the study population

MG infections were found in 212 patients. The sociodemographic characteristics of the patients are described in table 1. One-third of the patients were included in the Be-PrEP-ared study (33.0%; n=70/212). Around one-quarter of the patients had more than one positive MG result over the 4 years (22.6%; n=48/212), most of them (79.1%; n=38/48) were part of the Be-PrEP-ared study.

Table 1: Sociodemographic characteristics of the study population.

	All participants		Women		Men	
	No (n=212)	**%**	**No (n=64)**	**%**	**No (n=148)**	**%**
Median Age (IQR)	32	26-40	26	23-32.5	35	28-42
Source						
Surveillance	142	67.0	64	100	78	52.7
Be-PrEP-ared	70	33.0	0	0	70	47.3
Place of Residence						
Brussels	47	22.2	2	3.1	45	30.4
Flanders	128	60.4	36	56.3	92	62.2
Wallonia	37	17.5	26	40.6	11	7.4
MSM						
No	80	37.7	64	100	16	10.8
Yes	97	45.8	0	0	97	65.5
Unknown	35	16.5	0	0	35	23.7
HIV positive						
No	117	55.2	15	23.4	102	68.9
Yes	15	7.1	1	1.6	14	9.5
Unknown	80	37.7	48	75	32	21.6
Positive for *Mycoplasma genitalium* at more than one visit						
No	164	77.4	63	98.4	101	68.2
Yes	48	22.6	1	1.6	47	31.8
Number of visits						
2	*27*	*12.7*	*1*	*100*	*26*	55.3
3	*7*	*3.3*			*7*	14.9
4	*6*	*2.8*			*6*	12.8
5	*1*	*0.5*			*1*	2.1
6	*1*	*0.5*			*1*	2.1
7	*6*	*2.8*			*6*	12.8

MSM: Men who have sex with men

The 212 patients contributed to a total of 316 MG-positive samples. Around one-fifth of the samples were from women (20.6%; n=65/316) and half of the MG infections (50.9%; n=161/316) were detected in the framework of the Be-PrEP- ared study. Almost 45% of the infections were asymptomatic (44.9%; n=142/316), around one-quarter symptomatic (27.5%; n=87/316) and the presence of symptoms was unknown for the other cases (27.5%; n=87/316). Urethritis was the most prevalent symptom in men (78.9%; n=56/71). Women mostly documented lower abdominal pain, or vaginal discharge (68.8%; n=11/16). Cervicitis symptoms were reported in one-quarter of the symptomatic cases in women (25.0%; n=4/16). STI co-infections were absent in half of the cases (54.4%; n=172/316) and in 86 cases the information was unknown (27.2%; n=86/316). A quarter of the MG cases reported STI-associated symptoms (27.5%; n=87/316) and of those 20 were co-infected with another STI (23.0%; n=20/87).

Presence of possible resistance-associated mutations

Sequencing for both macrolide and fluoroquinolone RAMs was successful for 214 samples. Identified mutations and their resulting amino acid changes are shown in table 2 for the 23S rRNA, gyrA and parC gene.

More than 80% of all MG infections (n=177/214; 82.7%; 95% CI 77.0 to 87.5) presented with a possible RAM to macrolides or fluoroquinolones. This applied to almost 90% of the samples collected from men (87.3%; 95% CI 81.5 to 91.8). Women were more likely to have no RAMs (42.4%) compared with men (12.7%) (OR 5.06; 95% CI 2.03 to 12.3; p<0.0001).

Table 2: Presence of *Mycoplasma genitalium* macrolide and possible fluoroquinolone resistance associated mutations (RAMs) in Belgium based on M. genitalium numbering.

RAMs detected	**All Samples**		**Female Samples**		**Male Samples**	
Polymorphism (mutation)	**No (n=214)**	**% (95%CI)**	**No. (n=33)**	**% (95% CI)**	**No. (n=181)**	**% (95% CI)**
Macrolide RAMS (*Escherichia coli* numbering in parentheses)						
Wild Type	55	25.7 (20.0-32.1)	24	72.7 (54.5-86.7)	31	17.1 (11.9-23.4)
Mutations detected	159	74.3 (67.9-80.0)	9	27.3 (13.3-45.5)	150	82.9 (76.6-88.1)
23SrRNA gene region V						
A2071G(A2058G)	*65*	*30.4*	4	12.1	61	33.7
A2071T(A2058T)	*8*	*3.7*	1	3.0	7	3.9
A2072G(A2059G)	*86*	*40.2*	4	12.1	82	45.3
Possible Fluoroquinolone RAMS						
Wild Type	*129*	*60.3 (53.5-66.7)*	20	60.6 (43.3-75.6)	109	60.2 (52.9-67.1)
Mutations detected	*85*	*39.7 (33.3-46.5)*	13	39.4 (24.4-56.7)	72	39.8 (32.9-47.1)
***gyrA* gene**						
Wild Type	207*	96.7 (93.4-98.7)	32	97.0 (84.2-99.9)	175	92.3 (92.9-98.8)
Missense mutations detected	7	3.3 (1.3-6.6)	1	3.1 (0.77-15.8)	6	3.3 (1.2-7.1)
A96T (G286A)	1†	*0.5*	0	0	1	0.6
V85I (G253A)	2†	*0.9*	1	3.1	1	0.6
M69I (G207A)	*1*	*0.5*	0	0	1	0.6
M95I (G285C)	2†	*0.9*	0	0	2	1.1
A79V (C236T)	*1*	*0.5*	0	0	1	0.6

RAMs detected	All Samples		Female Samples		Male Samples	
Polymorphism (mutation)	**No (n=214)**	**% (95%CI)**	**No. (n=33)**	**% (95% CI)**	**No. (n=181)**	**% (95% CI)**
***parC* gene**						
Wild Type[c]	131‡	61.2 (54.3-67.8)	20	60.6 (42.1-77.1)	111	61.3 (53.8-68.4)
Missense mutations detected	83	38.8 (32.2-45.7)	13	39.4 (22.9-57.9)	70	38.7 (31.5-46.2)
Mutations at 83 or 87	55	25.7 (20.0-32.1)	3	9.1 (1.9-24.3)	52	28.7 (22.3-35.9)
S83I (G248T)	*38*[§]	17.8	1	3.0	37	20.4
S83N (G248A)	4	1.9	1	3.0	3	1.66
S83R (A247C)	1¶	0.5	0	0	1	0.6
D87N (G259A)	4	1.9	1	3.0	3	1.7
D87Y (G259T)	8	3.8	0	0	8	4.4
Missense mutations at other locations in parC						
D82N (G244A)	1¶		0	0	1	0.6
P62S(C184T)	26[**]		9	27.3	17	9.4
A118E (C353A)	1		1	3.0	0	0
Silent mutations	*3*		*1*	*3.0*	*2*	
G75G (G225A)	*1*		*1*		*0*	
H78H (C234T)	*1*		*0*		*1*	
V112V (G336A)	*1*		*0*		*1*	

*CI: confidence interval; *Ten samples were PCR negative and could not be sequenced for the gyrA gene. †Samples presented an additional mutation in ParC: S83I in three of the samples and S83N in two samples. ‡Three silent mutations were not counted as RAM and are categorised as wild-type MG. §Twenty-one samples had an additional silent mutation (H78H (C234T)).¶One sample had an additional mutation P62S (C184T).**Samples presented an additional silent mutation (16 H78H (C234T) and 1 N99N (C297T)).*

Macrolide RAMs

Mutations conferring macrolide resistance at nucleotide position 2071 or 2072 (corresponding to position 2058 or 2059 based on *Escherichia coli* numbering) of the 23S rRNA gene were detected in almost 75% of the samples (74.3%; n=159/214).

All possible fluoroquinolone RAMs

A total of 85 samples (39.7%) presented with possible RAMs in the *gyrA* gene or the *parC* gene (table 2). Only seven samples showed a mutation in the *gyrA* gene, and five of these samples possessed an additional mutation in ParC (amino acid position 83). In total, almost 40% of the samples had a mutation in the *parC* gene (38.8%; n=83/214), and of those, 66.3% (n=55/83) had a mutation at ParC location 83 or 87. Besides these two positions, mutation P62S (C184T) was found in 31.3% (n=26/83) of the MG cases with ParC mutations, and although this alteration was found in both genders, it was more present in women (27.3% vs 9.4%).

Presence of RAMs to both antibiotics

Almost one-third of the samples (31.3%; n=67/214; 95% CI: 25.2 to 38.0) harboured possible RAMs to both macrolides and fluoroquinolones. A2071G/S83I was the most frequent combination (31.3%; n=21/67), followed by A2072G/S83I (19.4%; n=13/67) and A2071G/P62S (16.4%; n=11/67).

Multiple MG episodes in individuals

Online supplementary annex 1 documents the presence of RAMs in 48 individuals that experienced multiple MG episodes over the 4 years including antimicrobial treatment and RAMs if available. (https://sti.bmj.com/content/early/2020/08/07/sextrans-2020-054511) They contributed to 104 additional MG episodes and 23 of them were categorised as a new MG episode.

Six participants (included in the Be-PrEP-ared study) remained positive at every 3 monthly visit for 18 months and therefore none of these MG infections was considered to be new MG episodes. Nevertheless, in three of those participants, infection with different MG genotypes was found.

Microbiological failure (remaining positive to MG within 4 months and harbouring a mutation against the provided therapy) was documented in nine cases in eight individuals: six A2071G; one A2072G and two S83I mutations. We could not detect any de novo mutations.

Prevalence of antimicrobial resistance and associations between antimicrobial resistance and sociodemographic determinants

Due to the unknown relevance of the mutations found in GyrA and ParC, the exploration of risk factors for having fluoroquinolone resistance or multiresistance was conducted on samples which harboured a mutation in ParC at position 83 and 87.

In addition, we only included samples of a new MG episode. Of the 235 MG samples that were categorised as a new MG infection, sequencing for both 23S rRNA and *parC* gene was successful for 167 samples. The prevalence of the different MG genotypes and the socio- demographic characteristics are presented in table 3. The number of macrolide-resistant MG cases were significantly higher in men (78.4%; 95% CI 70.4 to 85.0) than in women (27.3%; 95% CI 13,3 to 45,5; p<0.0001) (table 3).

Table 3: Socio-demographics and presence of the different *Mycoplasma genitalium* genotypes.

	Total		Wild type			Macrolide resistant (23S rRNA)			Fluoroquinolone resistant (ParC pos. 83/87)			Multi-resistant (23S rRNA + ParC pos. 83/87)		
	No.	**%**	**No.**	**% (95% CI)**	**p-value**	**No.**	**% (95% CI)**	**p-value**	**No**	**% (95% CI)**	**p-value**	**No**	**% (95% CI)**	**p-value**
All samples	167		47	28.7 (22.0-36.2)		114	68.3 (60.6-75.2)		35	21.0 (15.1-27.9)		30	18.0 (12.5-24.6)	
Year														
2015-2016*	51	30.5	6	11.8 (4.4-23.9)		45	88.2 (76.1-95.6)		12	23.5 (12.8-37.5)		12	23.5 (12.8-37.5)	
2017	63	37.7	17	27.0 (16.6-39.7)		43	68.3 (55.3-79.4)		16	25.4 (15.3-37.9)		13	20.6 (11.5-32.7)	
2018	53	31.7	25	47.2 (33.3-61.4)	**<0.0001**	26	49.1 (35.1-63.2)	**<0.0001**	7	13.2 (5.5-25.3)	0.239	5	9.4 (3.1-20.7)	0.119
Source of sampling														
Surveillance	94	56.3	40	42.6 (32.4-53.2)		50	53.2 (42.6-63.6)		16	17.0 (10.1-26.2)		12	12.8 (6.8-21.2)	
Be-PrEP-ared study	73	43.7	8	11.0 (4.9-20.5)	**<0.0001**	64	87.7 (77.9-94.2)	**<0.0001**	19	26.0 (16.5-37.6)	0.182	18	24.7 (15.3-36.1)	0.066
Gender														
Women	33	19.8	22	66.7 (48.2-82.0)		9	27.3 (13.3-45.5)		3	9.1 (19.2-24.3)		1	3.0 (0.8-15.8)	
Men	134	80.2	26	19.4 (13.1-27.1)	**<0.0001**	105	78.4 (70.4-85.0)	**<0.0001**	32	23.9 (16.9-32.0)	0.092	29	21.6 (15.0-29.6)	**0.010**
Sexual Orientation														
Hetero	47	28.1	30	63.8 (48.5-77.3)		15	31.9 (19.1-47.1)		4	8.5 (2.4-20.4)		2	4.3 (0.5-14.5)	
MSM	95	56.9	9	9.5 (4.4-17.2)	**<0.0001**	84	88.4 (80.2-94.1)	**<0.0001**	25	26.3 (17.8-36.4)	**0.015**	23	24.2 (16.0-34.1)	**0.002**
Unknown	25	15.0	9	36.0 (18.0-57.5)		15	60.0 (38.7-78.9)		6	24.0 (9.4-45.1)		5	20.0 (6.8-40.7)	
HIV status														
Negative	108	64.7	23	21.3 (14.0-30.2)		84	77.8 (68.8-85.2)		22	20.4 (13.2-29.2)		21	19.4 (12.5-28.2)	
Positive	13	7.8	0	0.0	0.126	12	92.3 (64.0-99.8)	0.299	4	30.8 (9.1-61.4)	0.474	3	23.1 (5.0-53.8)	0.720
Unknown	46	27.5	25	54.4 (39.0-69.1)		18	39.1 (25.1-54.6)		9	19.6 (9.4-33.9)		6	13.0 (4.9-26.3)	
Type of Visit														
Initial Visit	151	71.3	46	30.5 (23.2-38.5)		102	67.6 (59.5-74.9)		30	19.9 (13.8-27.1)		27	17.9 (12.1-24.9)	
Return visit*	16	28.7	2	12.5 (1.6-38.3)	0.157	12	75.0 (47.6-92.7)	0.778	5	31.3 11.0-58.7)	0.332	3	18.8 (4.0-45.6)	1.000
Site of Infection														
Genital	118	70.7	40	33.9 (25.4-43.2)		73	61.9 (52.5-70.6)		24	20.3 (13.5-28.7)		19	16.1 (10.0-24.0)	
Anorectal	45	27.0	7	15.6 (6.5-29.5)	**0.022**	38	84.4 (70.5-93.5)	**0.008**	10	22.2 (11.2-37.1)	0.832	10	22.2 (11.2-37.1)	0.374
Pharyngeal	3	1.8	0	0.0		3	1.00.0		1	33.3 (0.8-90.6)		1	33.3 (0.8-90.6)	
Unknown	1	0.6	1	100.0		0	0.0		0	0.0		0	0.0	

	Total		Wild type			Macrolide resistant (23S rRNA)			Fluoroquinolone resistant (ParC pos. 83/87)			Multi-resistant (23S rRNA + ParC pos. 83/87)		
	No.	**%**	**No.**	**% (95% CI)**	**p-value**	**No.**	**% (95% CI)**	**p-value**	**No**	**% (95% CI)**	**p-value**	**No**	**% (95% CI)**	**p-value**
STI associated Symptoms														
No	65	38.9	12	18.5 (9.9-30.0)		53	81.5 (70.0-90.1)		14	21.5 (12.3-33.5)		14	21.5 (12.3-33.5)	
Yes	53	31.7	15	28.3 (16.8-42.3)	0.271	36	67.9 (53.7-80.1)	0.132	12	22.6 (12.3-36.2)	1.000	10	18.9 (9.4-32.0)	0.820
Unknown	49	29.3	21	42.9 (28.8-57.8)		25	51.0 (36.3-65.6)		9	18.4 (8.8-32.0)		6	12.2 (4.6-24.8)	
STI co-infections														
No STI co-infection	92	55.1	17	18.5 (11.1-27.9)		73	79.4 (69.6-87.1)		21	22.8 (14.7-32.8)		19	20.7 (12.9-30.4)	
Any STI co-infection	25	15.0	3	12.0 (2.5-31.2)	0.560	20	80.0 (59.3-93.2)	1.000	6	24.0 (9.4-45.1)	1.000	4	16.0 (4.5-36.1)	0.779
Unknown	50	29.9	28	56.0 (41.3-70.0)		21	42.0 (28.2-56.8)		8	16.0 (7.2-29.1)		7	14.0 (5.8-26.7)	
Chlamydia	*13*													
Gonorrhea	*11*													
Trichomoniasis	*1*													
Syphilis	*5*													

*Associations were calculated using Fisher's exact test. P values in bold are <0.05 so statistical significant. *All samples from return visits were from MSM.*
†Concurrent STI infections were found in 25 participants. MSM, men who have sex with men.

This difference in gender was also seen in fluoroquinolone- resistant MG cases (23.9% vs 9.1%, p=0.092), however, to a lesser extent and not statistically significant. The prevalence of multiresistant MG cases was remarkably higher in men (21.6%; 95% CI 15.0 to 29.6) than in women (3.0%; 95% CI: 0.8 to 15.8; p=0.010). Furthermore, being MSM increased the prevalence of all antibiotic-resistant MG genotypes. Almost one quarter (24.2%; 95% CI 16.0 to 34.1, p=0.002) of the MG samples from MSM were multiresistant, and almost 9 out of 10 infections among MSM had a mutation in the 23S rRNA gene (88.4%; 95% CI 80.2 to 94.1, p<0.0001). Being of male gender and not MSM markedly decreased the presence of macrolide (53.9%; n=21/39) and fluoroquinolone resistance (18.0%; n=7/39). The number of wild-type MG samples increased to 43.6% (n=17/39), whereby the number of multiresistant MG cases dropped to 15.4% (n=6/39) (data not in table).

Being MSM was the only factor associated with quinolone resistance (OR 3.84; 95% CI 1.20 to 16.09; p=0.013) or having a multiresistant MG infection (OR 7.19; 95% CI 1.63 to 65.16; p=0.0033). Having an MG infection with macrolide resistance was besides being associated with being male and MSM, also associated with an anorectal site of infection. Multivariate analyses however showed that being MSM was the only factor associated with a higher probability of having an MG infection with macrolide resistance (adjusted OR 15.33; 95% CI 3.52 to 66.77; p<0.0001).

5.5 DISCUSSION

A recent systematic review of macrolide and fluoroquinolone resistance in MG found that global macrolide resistance increased to 51.4% by 2017, while fluoroquinolone resistance remained stable at around 8%.[15] In Europe, the fluoroquinolone resistance prevalence is now estimated to be 5%.[8] We found considerably higher rates of resistance in Belgium. In fact, <30% of the MG strains analysed were wild type. Almost 20% (18.0%) had RAMs to both macrolides and fluoroquinolones, and this was particularly prevalent in MSM—24.2%. Previous reports from Belgium reported a lower prevalence of macrolide RAMs among female sex workers (6.9%) and MSM (44%).[6 16] It should be noted that in both studies another technique than the gold standard Sanger sequencing was used.

While MG resistance has been described as occurring in MSM, the prevalence of RAMs in low-risk populations has not often been studied. The present study shows that RAMs to macrolides or fluoroquinolones are also prevalent in lower risk populations, such as women (33.3%) and heterosexual men (56.4%), but to a lesser extent than in MSM (90.5%). This may be explained by the relatively high consumption of both macrolides and fluoroquinolones in the general population in Belgium.[17]

In MSM, almost 9 out of 10 MG infections were resistant to macrolides. An important driver of macrolide use in high STI- prevalence populations, such as PrEP cohorts, are chlamydia and gonorrhea infections,[18] where single dose azithromycin (alone or in combination) is a recommended therapy.[19 20] Individuals with chlamydia or gonorrhea are frequently co-infected with MG (as was the case for 20% in our study), which increases the probability of bystander antimicrobial selection pressure and which, in part, may explain the high prevalence of macrolide RAMs found in MSM.[4 21 22] We could however not detect any de novo mutations, probably due to the already high prevalence of antimicrobial resistance in MSM.

The present study also shows that asymptomatic MG infections are prevalent, but that most of them (81.5%) presented with RAMs for macrolides or fluoroquinolones. One of the most important criteria to screen and treat patients with asymptomatic infectious disease is the availability of an effective treatment to decrease disease prevalence. These results add up to the evidence that screening for MG and treatment of asymptomatic MG cases should be discouraged.

Treatment options for symptomatic infections with possible combined macrolide-resistant and fluoroquinolone-resistant MG are extremely limited. Pristinamycin is listed as third-line treatment in the International Union against STI (IUSTI) treatment guidelines, however, this treatment is difficult to obtain in Belgium. The current European IUSTI MG treatment guidelines advocate azithromycin as first-line therapy.[7] The high prevalence of macrolide RAM in Belgium suggests that this approach is suboptimal. An alternative approach would be to follow the Melbourne protocol of treating MG infections with doxycycline for 7 days while awaiting test results for macrolide resistance.[23] In the absence of macrolide resistance, azithromycin is administered, but if macrolide resistance is found then sitafloxacin/ moxifloxacin is given. While this protocol resulted in high cure rates and very low risk of genesis of macrolide resistance, our study results show that almost all MSM had a mutation conferring macrolide resistance, which is in line with other results.[3–5 24] Therefore, the detection of fluoroquinolone RAMs seems more relevant than the detection of macrolide resistance in this population.

In contrast to the commercial availability of several assays detecting macrolide RAMs in MG, assays detecting fluoroquinolone RAMs are sparse.[25] There are different mutations in the quinolone resistance determining region of the *parC* and *gyrA* gene that have been associated with fluoroquinolone resistance.[25–28] In our study, only seven samples presented with a GyrA mutation and five of them coincided with a ParC mutation at amino acid position 83, which seems to lead to high-level resistance.[29] The two other alterations found (M69I and A79V) have not yet been correlated with clinical

resistance. Due to this low number of mutations, the role of mutations in GyrA seems of less importance in MG. The *parC* gene is more susceptible for mutations; in our study, most of the mutations were found at position 83 and 87, the 'hot spots' for fluoroquinolone resistance (n=55/83; 66.2%).[14] Besides these two positions, an alteration at position 62 (P62S) in the ParC was detected in 32.9% of the samples with fluoroquinolone RAMs. This alteration has been previously described, however, clinical resistance has not yet been determined.[26 27 29]

Our study has several limitations. First, the sampling methodologies may have introduced biases towards populations at higher risk for antimicrobial resistance, such as MSM with higher consumption of antimicrobials. Second, microbiological cure with moxifloxacin despite mutations at position 83/87 are documented, therefore the degree of fluoroquinolone resistance may be overestimated.[30] Third, the estimation of antimicrobial resistance of MG has been made on new MG episodes only. Yet, we document that patients could become infected within 6 months with another MG genotype, which may be either explained by a true re-infection with another MG strain or by a mixed infection with multiple MG strains. Therefore, prevalence mentioned here may be inaccurate. Fourth, clinical data including antimicrobial treatment were lacking for many of the surveillance cases, and finally, no test-of-cure within 14 days of treatment was performed. It is therefore unclear whether the infection was new or persistent. However, we tried to correct for this bias, by only including new MG infections that are detected 6 months after the previous MG infection.

In conclusion, we found high rates of macrolide and possible fluoroquinolone RAMs in MG. These results provide further motivation to promote antimicrobial stewardship in the general population, but particularly among high STI prevalence populations such as PrEP cohorts. New treatment guidelines are urgently required that incorporate genotypic resistance testing for macrolides and fluoroquinolones. Finally, because of the high number of multiresistant cases found in this study, new antimicrobials are urgently needed to treat patients with MG resistance to both macrolides and fluoroquinolones

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SECTION II: TO EVALUATE POTENTIAL IMPROVED SCREENING STRATEGIES IN ORDER TO SIMPLIFY STI TESTING

CHAPTER 6: CT/NG POOLING METHODOLOGIES

To facilitate molecular triple-site testing for *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG), two different pooling methodologies are described using two different molecular platforms: Abbott m2000rt and GeneXpert.

- Take three, test one: a cross-sectional study to evaluate the molecular detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pooled pharyngeal, anorectal and urine samples versus single-site testing among men who have sex with men in Belgium
- To Pool or Not to Pool Samples for Sexually Transmitted Infections Detection in Men Who Have Sex With Men? An Evaluation of a New Pooling Method Using the GeneXpert Instrument in West Africa

6.1 ABBOTT CT/NG POOLING METHODOLOGY

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Take three, test one: a cross-sectional study to evaluate the molecular detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pooled pharyngeal, anorectal and urine samples versus single-site testing among men who have sex with men in Belgium

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Contributions: data collection and clean-up, partly data analysis, manuscript writing and finalization

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6.1.1 Abstract

Objectives: To investigate the efficacy of performing a pooling strategy of triple-anatomical site samples (pharyngeal, anorectal and urine samples) for simultaneous *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) nucleic acid amplification detection.

Methods: A total of 117 specimen sets (pharyngeal, anorectal and urine) were collected from 98 men between 2014 and 2016. Double sampling of pharyngeal, anorectal and urine samples allowed for pooled and unpooled analyses using a multiplex Abbott Real Time CT/ NG assay, together with confirmatory PCR testing in case of CT/NG positivity. Clinical and demographic data were analysed.

Results: The positivity rate for the triple-site pooled testing for CT and NG was 8.5% (10/117) and 6.8%, (8/117), respectively, compared to the single-site testing total positivity rate, which was 9.4% (11/117) and 4.3% (5/117) for CT and NG, respectively. Pooled analysis missed one CT-positive urine sample and one CT-positive anorectal sample could not be confirmed. In addition, less PCR inhibition was reported for the pooled sample (PS) testing and ERV-3 qPCR testing revealed ineffective sampling of self-collected anorectal swabs in two cases. No pharyngeal samples were positive for CT, nor were any urine samples positive for NG.

Conclusion: This small study showed that PS testing is a possible testing strategy for screening high-risk men who have sex with men attending pre-exposure prophylaxis (PrEP) clinics. However, due to the low positivity rate of CT/NG in this study, larger evaluations are needed to confirm the effectiveness of CT/NG screening with multiple-site PS nucleic acid amplification test (NAAT) screening practices

KEYWORDS

***Chlamydia trachomatis*, *Neisseria gonorrhoeae*, diagnostics, screening, pooling**

6.1.2 Introduction

The use of pre-exposure prophylaxis (PrEP) in Men who have Sex with Men (MSM) has been associated with increased diagnoses of Sexually Transmitted Infections (STIs).[1] In fact, a PrEP demonstration study in Belgium showed a high prevalence for *Chlamydia trachomatis* (CT) (11.7%) and *Neisseria gonorrhoeae* (NG) (12.2%). Guidelines for delivering PrEP to MSM include 6- monthly screening for CT/NG.[3,4] High prevalence rates of extra-genital CT and NG have been reported among MSM.[5] Thus, screening for CT and NG should preferably be performed on three sites: pharynx, urethra and anorectum. One way to reduce the cost of biannual three-site testing is to pool the three samples per patient. Using the Aptima Combo 2 assay, Sultan et al[6] have recently demonstrated that pooled three-site testing in MSM has a similar sensitivity to single-site testing for CT (92% vs. 96%) and a slightly poorer sensitivity for NG (90% vs. 99%) in a high-risk population characterized by a high CT (16 %) and NG (27 %) prevalence.

We conducted an exploratory study to evaluate the appropriateness of pooled triple-site (pharyngeal, urethral, anorectal) testing using nucleic acid amplification tests (NAATs) for CT and NG in a cohort of high-risk MSM. In contrary to the method used by Sultan et al., we designed a strategy that allowed individual testing of the samples after pooled testing.

6.1.3 Materials and methods

Design and evaluation of the pooling strategy

The pooling strategy was designed so that individual testing of the three samples after unpooling was still feasible.

Study cohort

Between August 2014 and May 2016, participants were recruited in an observational cohort sub-study-related syphilis diagnostic tests (ClinicalTrials.gov Registration Number: NCT02059525) conducted at the Institute of Tropical Medicine (ITM), Antwerp, Belgium. Inclusion of participants was based on a diagnosis of syphilis. Aside from the systematic screening of all individuals at the 6- month follow-up visit, individuals were additionally tested if they developed symptoms suggestive of a CT or NG infection or reported recent sexual contact with a CT- or NG-infected individual. The Institutional Review Board of ITM and the Ethics Committee of the University Hospital Antwerp approved this study (13/44/426). All participants provided written consent to this sub-study.

Patient involvement

Participants were not involved in the design and conduct of the study.

Clinical specimen collection

The study physician collected two pharyngeal samples by rubbing both tonsillar pillars and the posterior oropharynx for approximately 10 seconds using regular flocked swabs (Copan Flock Technologies S.R. L., Brescia, Italy). Two flocked swabs were provided to the participants for anorectal self-sampling. Participants were verbally instructed to insert the swab 3 cm into the anus and rotate three times. All swabs were stored at −20°C (±5°C) within two hours of sampling. First, void urine collected in a sterile container were aliquoted into 2 mL microtubes within two hours and either refrigerated (2–8°C) and tested within three days for the separate NAAT analysis, or frozen at −20°C for later pooled NAAT analyses, as outlined below.

Single-site sample testing for C. trachomatis and N. gonorrhoeae

One set of urine, pharyngeal and anorectal samples (randomly chosen), henceforth referred to as 'Separate Samples' (SSs), were analysed according to the routine testing algorithm at the ITM. Pharyngeal and anorectal swabs were eluted by adding 1.2 mL of diluted phosphate-buffered saline (dPBS) (pH 7.4–1:9, PBS:saline) directly onto the swab. After vortexing the swab, 500 µL of the sample eluate was immediately analysed using the Abbott Real-Time (RT) CT/NG assay (DNA extraction and sample preparation using Abbott m2000sp and the Abbott m2000rt system for amplification and detection of CT/NG, Abbott Molecular Inc. Des Plaines, IL, USA) according to the manufacturer's instructions. The remainder of the eluates/urine was stored at -20°C. In case of positivity, the same DNA extracts were tested by in-house real-time PCR (RT- PCR) assays both (*Chlamydia trachomatis* and *Neisseria gonorrhoeae*) based on previously published primer sets.[7,8]

We defined 'true positives' when positive in both the Abbott and in-house RT-PCR assay. An initial positive Abbott assay result followed by a negative confirmatory NAAT result was defined as 'not confirmed'. Inhibition of the NAAT was defined as 'inhibition'.

Triple-site pooled sample (PS) testing for C. trachomatis and N. gonorrhoeae

All pooled samples (PSs) were batch-tested according to the same test algorithm as the SSs. Results of the SSs were not known by the performer. In short, pharyngeal

and anorectal swabs were eluted in 600 µL of dPBS. A total of 170 µL from each of the anorectal and pharyngeal swab eluates were added to 170 µL of urine. The total volume of 510 µL was loaded onto the Abbott instrument. Leftover eluates of the swabs and urine aliquots were stored separately at −20°C. When the PS was positive for CT and/or NG, unpooled analysis followed using 500 µL urine and 200 µL of swab eluate diluted with 300 µL dPBS. These samples are referred to as 'unpooled samples' (USs) from hereon and were tested as outlined above.

Data analysis

In case of discrepant results between the SS and PS testing, human DNA concentration was assessed using an ERV-3 quantitative PCR.[9] Values are summarized as medians and interquartile ranges (IQR). Fisher's exact test was used to compare CT and NG positivity rate following the different testing methods. All analyses were performed in Stata 13 (StataCorp LP, College Station, TX, USA).

6.1.4 Results

Study sample collection and cohort characteristics

A total of 120 sample sets from 98 individuals were collected, 3 sample sets were incomplete and excluded from this analysis. The median age of the participants was 40 (IQR 31–47). All except three participants reporting being MSM and 87% (N = 85) were HIV infected. At the time of collection, one patient reported symptoms compatible with CT or NG (dysuria). Twenty-two individuals were screened because one of their recent sexual partners recently tested positive for NG/CT. Sixteen (13.3%) participants reported antibiotic use during the previous 3 months before testing.

Detection of Chlamydia trachomatis

CT was detected in eight anorectal, three urine and no pharyngeal samples (Table 1A). Out of these, PS testing detected seven of the anorectal and two of the urine infections, one CT-positive anorectal sample was missed due to inhibition in the confirmatory RT-PCR. One anorectal CT infection (confirmed by in-house RT-PCR to be LGV) was detected with the pooled but not the SS analysis. An ERV-3 qPCR performed on this SS anorectal swab was negative, indicating the lack of human cells. PCR inhibition was detected in four anorectal SSs; three of these were not inhibited in the PS testing and tested negative. One CT infection in urine was missed by PS testing; however, the SS testing revealed a weak positive value (Delta cycle value of the Abbott assay (DC) = 0.49). There was no statistically significant difference in the overall positivity rates of CT ascertained by SS (9.4%) or PS (8.5%) testing.

Detection of Neisseria gonorrhoeae

In the SS protocol, four anorectal, one pharyngeal and no urine samples tested positive for NG (Table 1B). All of these samples were detected using PS and US analyses. An additional three NG-positive anorectal samples were detected using PS and US testing. Two of them were initially positive in the SS testing by Abbott, but could not be confirmed. One of the anorectal samples was collected from a participant taking amoxicillin treatment for pharyngitis. ERV-3 qPCR testing performed on the SS related to the third PS positive case revealed a low amount of human material (25 cells/PCR vs. 8123 cells/ PCR). Further, of the three anorectal SSs-containing inhibitors, two were reported as negative with the PS testing. One pharyngeal sample was negative in the SS testing but was weakly positive in the PS testing (DC = 0.89), however the NG could not be confirmed by the in-house RT-PCR. The contrary was observed for another pharyngeal sample, which was weakly positive in the SS testing (DC=0.18) but was not further confirmed and which tested negative in the PS testing. There was no statistically significant difference in the positivity rates of NG as determined by SS (4.3%) or PS (6.8%) testing.

Table 1: Results of separate sample/single-site (SS) and pooled triple-site sample (anal/pharyngeal/urine) (PS/US) NAAT analyses for C. trachomatis (table 1A) and N. gonorrhoeae (table 1B) detection among cohort of 98 individuals (N = 117 specimen sets analysed).

1A:		**Pooled Samples (PSs) including unpooled samples (USs) NAAT results**												
		Chlamydia trachomatis (CT)												
		CT+			**CT–**			**Inhibition**			**Not confirmed**			
Separate samples (SS) NAAT results		**A**	**P**	**U**	**A**	**P**	**U**	**A**	**P**	**U**	**A**	**P**	**U**	**Total**
CT+	**A**	7			0			0			**1**			8
	P		0			0			0			0		0
	U			2			**1**			0			0	3
							DC = 0,5							
CT-	**A**	**1**[a]			104			0			0			105
	P		0			117			0			0		117
	U			0			114			0			0	114
Inhibition	**A**	0			**3**			1			0			4
	P		0			0			0			0		0
	U			0			0			0			0	0
Not confirmed	**A**	0			0			0			0			0
	P		0			0			0			0		0
	U			0			0			0			0	0
	Total	8	0	2	107	117	115	1	0	0	1	0	0	351

1B:		Pooled Samples (PSs) including unpooled samples (USs) NAAT results												
		Neisseria gonorrhoeae (NG)												
		NG+			NG−			Inhibition			Not confirmed			
		A	P	U	A	P	U	A	P	U	A	P	U	Total
NG+	**A**	4			0			0			0			4
	P		1			0			0			0		1
	U			0			0			0			0	0
NG-	**A**	**1[a]**			106			0			0			107
	P		0			114			0			**1**		115
	U			0			117			0			0	117
Inhibition	**A**	0			**2**			1			0			3
	P		0			0			0			0		0
	U			0			0			0			0	0
Not confirmed	**A**	**2** **DC = 0,4** **DC = 2,1**			**1** **DC =1,5**			0			0			3
	P					**1** **DC = 0,2**						0		1
	U			0			0			0			0	0
	Total	7	1	0	109	115	117	1	0	0	0	1	0	351

A – Anorectal, P – Pharyngeal, U – Urine, [a]low or no presence of human cells (ERV-3 qPCR), DC = delta cycle value Abbott assay of the separate sample

6.1.5 Discussion

Although larger evaluations are needed to confirm these findings using the Abbott RT-CT/NG assay, the results of this pilot study are concordant with Sultans' et al. large study using the Aptima assay for Chlamydia and a smaller pooling study that was recently performed by Thielemans' et al. using the Abbott assay for CT/NG.[10] In addition, the design of the pooling strategy allowed us to determine with good sensitivity the biological site of infection. No additional samples will therefore need to be taken nor does the participant needs to come for an additional visit in case prescription is send by mail which will return in an additional cost-saving.

We found less NAAT inhibition in the PS strategy. This could in part be explained by the additional freezing step or by the smaller amount of volume, and thus inhibitors, used to prepare the PSs. We used self-sampling for anorectal specimens in this study because of its proven effectiveness.[11] However, our study that requested the collection of two subsequent anorectal samples showed that patient sampling error can occur, as demonstrated by the two SSs that contained no or few human cells and were likely poorly collected.

Limitations to this study include the low sampling number and the overall low CT/NG positivity rates in urine and pharyngeal samples. Furthermore, the SSs were subjected to immediate PCR testing in the context of routine clinical care, whereas the PS/US testing occurred between 1 month and 1 year after sampling, including subjection to a freeze thaw cycle which could cause DNA degradation in addition to elimination of inhibitors.

In conclusion, this small study contributes to the evidence that PS testing works and that it could be an appropriate testing strategy for screening high-risk MSM in PrEP clinics. It will be up to the end users of the testing to decide whether the site of infection provides valuable information for treatment or surveillance purposes and if this should be required.

6.1.6 Acknowledgments

We would like to thank the study participants and the laboratory staff of the HIV/STI Reference Laboratory, Institute of Tropical Medicine, Antwerp, Belgium.

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6.2 GENEXPERT CT/NG POOLING METHODOLOGY

ORIGINAL STUDY

To Pool or Not to Pool Samples for Sexually Transmitted Infections Detection in Men Who Have Sex With Men? An Evaluation of a New Pooling Method Using the GeneXpert Instrument in West Africa

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6.2.1 Abstract

Background: Men who have sex with men (MSM) using preexposure prophylaxis (PrEP) are at risk for sexually transmitted infections (STIs). Therefore, PrEP services should include regular screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) at urethra, anorectum, and pharynx. However, financial and logistic challenges arise in low- resource settings. We assessed a new STI sample pooling method using the GeneXpert instrument among MSM initiating PrEP in West Africa.

Methods: Urine, anorectal, and pharyngeal samples were pooled per individual for analysis. In case of an invalid result only (strategy 1) or a positive result of the pool (strategy 2), samples were analysed individually to identify the infection's biological location. The results of 2 different pooling strategies were compared against the individual results obtained by a criterion standard.

Results: We found a prevalence of 14.5% for chlamydia and 11.5% for gonorrhea, with a predominance of infections being extragenital (77.6%). The majority of infections were asymptomatic (88.2%). The pooling strategy 1, had a sensitivity, specificity and agreement for CT of 95.4%, 98.7%, and 0.93, respectively; and 92.3%, 99.2%, and 0.93 for pooling strategy 2. For NG, these figures were 88.9%, 97.7%, and 0.85 for strategy 1, and 88.9%, 96.7%, and 0.81 for strategy 2.

Conclusions: West African MSM have a high prevalence of extragenital and asymptomatic STIs. The GeneXpert method provides an opportunity to move from syndromic toward etiological STI diagnosis in low-income countries, as the platform is available in African countries for tuberculosis testing. Pooling will reduce costs of triple site testing

KEYWORDS

Sexually transmitted infections, Africa, pooling, pre-exposure prophylaxis, men who have sex with men

6.2.2 Introduction

The incidence of sexually transmitted infections (STIs), including *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG), is increasing globally. Their burden is disproportionally higher in low- and middle-income countries (LMIC) and in key populations, such as men who have sex with men (MSM).[1] Fast detection and treatment of STIs is essential, as they raise serious health concerns, including increased risk of acquiring human immunodeficiency virus (HIV) infection.[2] United Nations Programme on HIV/AIDS recommends a combination strategy of biomedical, behavioural, and structural approaches for HIV prevention.[3]

The use of pre-exposure prophylaxis (PrEP) is an effective new biomedical HIV prevention tool, which is increasingly used among MSM in many high-resource countries.[4,5] However, PrEP may lead to a decrease in condom use and hence enhance STIs.[6] Indeed, PrEP demonstration studies among MSM in high-resource settings reported a high STI prevalence and incidence, whereby most of the STIs were of extragenital origin, that is, pharynx and anorectum. These STIs are frequently asymptomatic.[7–11] Although frequent screening of STIs among MSM in high-resource settings is currently debated,[12] African MSM often report sexual relations with women.[13] This sexual behaviour may contribute to the spread of STIs to the general population. Hence, fast detection and treatment of STI infections is recommended in this population. The World Health Organization (WHO), therefore, advocates the integration of STI testing and treatment in all PrEP services, so that populations at risk have access to both STI prevention and care.[14] Furthermore, triple-site testing is recommended in MSM.[15,16]

To date, nucleic acid amplification tests (NAATs) are the recommended diagnostic methods to detect STIs due to their high sensitivity and specificity. However, this method requires a state-of-the-art molecular laboratory and highly trained laboratory technicians.[17,18] Unfortunately, the screening of STIs using NAATs is hampered or even absent in LMIC due to the lack of adequate laboratory services and limited resources. Because of these barriers, LMIC use a syndromic approach for the diagnosis and treatment of symptomatic STIs.

The GeneXpert platform (Cepheid, Sunnyvale, CA) holds promise as a method to detect STIs in LMIC. This platform is a molecular assay which requires minimal training and yields results within 2 hours. Since 2010, the WHO has recommended the use of the GeneXpert platform for the confirmation of tuberculosis and the detection of rifampicin resistance of *Mycobacterium tuberculosis*. As a consequence, the GeneXpert platform has become widely available throughout Africa.[19] Currently, the Food and Drug Administration

approved a cartridge based CT/NG assay on the GeneXpert system which can be used to test samples of genital, pharyngeal, and anorectal origin.[20] However, the high cost of the GeneXpert CT/NG cartridge hinders its utilization for the diagnosis of CT/NG in Africa.

In addition, testing 1 genital (urine) and 2 extragenital samples (anorectal and pharyngeal) for STI screening in MSM will further increase this cost. Hence, pooling of the 3 collected samples per individual offers potential cost-savings in CT/NG detection in MSM presenting for PrEP.[21] Several pooling methods are described, including 3 using the GeneXpert instrument for STI detection.[21-26] To our knowledge, none of these pooling methods have been implemented in LMIC. In addition, very few are able to determine the biological location of the infection, which could be important for treatment and surveillance purposes.

We evaluated the performance of a new pooling method using the GeneXpert platform for the detection of CT and NG among MSM initiating PrEP in 4 West African countries.

6.2.3 Materials and Methods

Study Setting

The CohMSM-PrEP study is being conducted in 4 sites in West Africa: Ouagadougou, Burkina Faso; Lomé, Togo; Bamako, Mali and Abidjan, Côte d'Ivoire. Its aim is to assess the feasibility of PrEP among a cohort of approximately 500 MSM, including STI prevalence. Samples for CT/NG testing were collected from all participants at their initiation visit and transported to research laboratories (SEREFO, Bamako and Institut Pasteur, Abidjan) or to national reference laboratories for tuberculosis (Laboratoire National de Recherche sur la Tuberculose-TB, Ouagadougou and CHU-SO-LNR-TB, Lomé) where the GeneXpert instrument was available (hereafter called local STI laboratory).

The study has been approved by all applicable ethics committees, and all participants provided written informed consent.

Quality Control

The STI reference laboratory of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, ensured the reliability and quality of the results of the local STI laboratories, including compliance with Good Clinical and Laboratory Practice Standards. This laboratory provided hands-on training in sample col- lection, processing, and storage at study initiation in each site. An external quality control (EQC) panel was tested at study

initiation and quarterly during the study. If DNA contamination was suspected, an environmental control of the laminar flow, bench, pipettes and the surface of the GeneXpert instrument was done.

Specimen Collection

Participants provided first-void urine and a physician took 2 pharyngeal and 2 anorectal samples (Eswab 1 mL, Copan Diagnostics, Brescia, Italy). After collection, samples were stored refrigerated (2–8°C) or frozen (−20°C) on site, depending on the time of transport to the local STI laboratory (2–8°C < 48 hours < −20°C). Transport of samples was performed under temperature-monitored conditions using a cool box and cooling elements. Upon sample receipt in the local STI laboratory, 1 aliquot (1 mL) of urine, 1 anorectal, and 1 pharyngeal Eswab (both randomly chosen from the duplicates) were immediately frozen (−20°C) until shipment on dry ice to ITM for reference testing. Samples for local testing were stored refrigerated (2–8°C) if analysis was performed within 72 hours of collection, otherwise, samples were stored frozen (−20°C).

Laboratory Methods

To provide a direct comparison of the pooling method on the GeneXpert platform in the 4 West African countries with standard molecular testing and to avoid site-specific biases, we compared the results obtained with the GeneXpert platform with the results obtained using a criterion standard performed in a controlled laboratory environment.

Pooling Method

At the local STI laboratory, a volume of 400 µL of the 3 samples (urine, anorectal, and pharyngeal sample) per participant was transferred in a microtube (hereafter called pool). After vortexing, 1 mL of the pool was transferred into the CT/NG Xpert cartridge. When the result of the pool was negative, all samples were considered negative and were not individually tested. When the pool was positive or invalid, individual samples were tested as follows: 400 µL of 1 sample was added to 800 µL diluted phosphate-buffered saline (hereafter called unpooling). After vortexing, 1 mL was transferred into the cartridge and analysed. Sample processing was performed in a laminar flow cabinet.

The CT/NG Xpert assay is not labelled for use on pooled samples nor is it licensed to be used with samples collected with Eswabs. The use of Eswabs on the CT/NG Xpert assay was validated prior to the study, and no decrease in assay performance was detected (Supplemental Digital Content (SDC) 1, http://links.lww.com/OLQ/A497).

Criterion Standard Test Algorithm

All duplicate samples were tested individually at ITM according to the following test algorithm in place: CT/NG was detected using the Abbott RealTime (RT) CT/NG assay (Abbott Molecular, Des Plaines, IL) according to the manufacturer's instructions. DNA extracts of positive samples were tested by in- house RT-PCR assays for CT and/or NG, both based on previously published primer sets.[27,28] The in-house RT-PCR for CT is able to differentiate L-type from non-L types. A sample was considered positive when positive in both the Abbott and the in-house RT-PCR. An initial positive Abbott assay result followed by a negative confirmatory NAAT result was defined as "not confirmed." Inhibition according to the Abbott assay was defined as "inhibition." The Abbott RT CT/NG assay and the in-house RT-PCRs were validated for the use of extragenital samples using Eswabs and no decrease in sensitivity was found (SDC 1, http://links.lww.com/OLQ/A497).

To exclude for sampling errors and confirm the quality of the sample, the presence of human material in the duplicate sample tested at ITM was assessed using a human Endogenous Retrovirus-3 PCR on anorectal and pharyngeal samples that were solely positive on site.[29]

Identification and Validation of the 2 Pooling Strategies

Two different on-site pooling strategies were evaluated. Strategy 1 consisted of triple-site pooling and testing, and unpooling only when the pooled sample result was invalid. Strategy 2 consisted of triple-site pooling and testing, and unpooling when the pooled sample result was invalid or positive for either CT or NG.

The result of the 2 strategies was compared with the infection status according to the criterion standard. A participant was defined as not infected when his 3 samples were all negative according to the criterion standard. A participant was defined as infected if at least 1 sample was positive. In the event that 1 or more sampling site(s) were not confirmed and the other sampling site(s) were negative, the participant infection status was defined as not confirmed.

Statistical Analysis

The sensitivity, specificity, positive predictive value, negative predictive value, with 95% confidence intervals were calculated for strategies 1 and 2, excluding inhibited and not confirmed infection status. In addition, agreement of both strategies with the criterion standard test algorithm was assessed by the Cohen's kappa statistic. All analyses were performed in STATA V15.0.

Cost Analysis

The costs of the 2 different screening strategies were compared against triple-site testing using the GeneXpert CT/NG assay. The obtained prevalence of CT/NG in this study was used to simulate the costs of STI testing in a population of 500 MSM. An invalid rate of 4% was assumed.

6.2.4 Results

Patient Characteristics, Test Results, and Prevalence of CT/NG

The ITM received baseline samples from 503 CohMSM-PrEP study participants. However, because the pooling method was not performed on 6 participants' samples, samples from 497 participants were included in the analysis. All participants were MSM, with a median age of 24 years (interquartile range, 22–28).

Prevalence of STIs According to the Criterion Standard Algorithm

According to the criterion standard test algorithm performed at ITM, the study population had a prevalence of 14.5% CT (72 of 497), 11.5% NG (57 of 497), and 22.1% CT or NG (110 of 497). The anorectal site was the most commonly infected with CT or NG (n = 76; 60.8%); followed by the urethra (n = 28; 22.4%) and pharynx (n = 21; 16.8%). Two participants were positive in 2 biological sites for CT and 11 for NG. All confirmed CT-positive samples were non-L genotypes. Of the 110 infected participants, 97 (88.2%) of them reported no symptoms of STI.

Test Results at the Study Sites

Using pooled samples 131 participants were positive for CT or NG: 21 had a mixed CT/NG infection; 71 were solely CT-infected; and 39 solely NG-infected. A total of 353 participants tested negative and were not further investigated. An invalid result was obtained in 13 participants.

Using strategy 1, 4 additional NG infected participants were detected by unpooling the pools with an invalid result.

Using strategy 2, individual testing of the pooled samples with invalid or positive results decreased the number of infected participants with CT or NG to 128: 26 with a dual CT/NG infection, 60 with CT only and 42 with NG only.

The CT/NG results obtained on site (pooled and unpooled samples) and the results obtained at ITM are available in the SDC 2 Figure 1, http://links.lww.com/OLQ/A498.

Test and Sample Quality

The study sites participated in a quarterly EQC: 1 NG-positive sample was missed; no false-positive results were reported.

The number of samples positive for CT/NG, whatever the biological site, at the study sites was systematically higher as compared with the numbers found at ITM (SDC 2 Fig. 1, http://links.lww.com/OLQ/A498). An environmental check revealed the contamination of the GeneXpert instrument's surface with CT at 1 site. The contamination is probably the cause of overreporting CT in this site. Another site had a large number of falsely detected NG; however, contamination with NG was not detected during the quarterly EQC assessments and the environmental check.

The presence of human DNA was assessed in 43 of 46 individual extragenital samples and in 11 (9 anorectal and 2 pharyngeal) (25.6%) samples, human DNA was not detected.

Performance of the 2 Pooling Strategies Using the GeneXpert Method

Samples from the CT contaminated site collected after August 31, 2018, were excluded from statistical analyses, which limited the number of participants to 448. The SDC 2, http://links.lww.com/OLQ/A498 documents all discordant cases (SDC 2 Tables 1 and 2, http://links.lww.com/OLQ/A498).

The evaluation of the 2 pooling strategies using the GeneXpert against the criterion standard for CT and NG is presented in Tables 1 and 2.

Table 1: Comparison of the 2 Test Strategies to Detect CT and NG.

				Criterion Standard Test Algorithm			
Organism				**Positive**	**Negative**	**Not confirmed**	**Total**
CT	**Strategy 1**	**GeneXpert**	**Positive**	62	5	3	70
			Negative	3	372	3	378
			Total	65	377	6	448
	Strategy 2	**GeneXpert**	**Positive**	60	3	3	66
			Negative	5	374	3	382
			Total	65	377	6	448
NG	**Strategy 1**	**GeneXpert**	**Positive**	48	9	3	60
			Negative	6	381	1	388
			Total	54	390	4	448
	Strategy 2	**GeneXpert**	**Positive**	48	13	3	64
			Negative	6	377	1	384
			Total	54	390	4	448

Strategy 1 will only test the samples individually when the pooled sample result was invalid. Strategy 2 will test the samples individually when the pooled sample result was invalid or positive for either CT or NG. The discordant samples are explained in detail in the Supplementary material.

Table 2: Evaluation of the 2 pooling strategies

		SENSITIVITY % (95% CI)	**SPECIFICITY % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**% AGREEMENT**	**K-COEFF**
CHLAMYDIA	**STRATEGY 1 (unpooling of INV)**	95.4% (87.1-99.0)	98.7% (96.9-99.6)	92.5% (83.8-96.7)	99.2% (97.6-99.7)	98.2%	0.93
	STRATEGY 2 (unpooling of INV & POS)	92.3% (83.0-97.5)	99.2% (97.7-99.8)	95.2% (86.7-99.0)	98.7% (97.0-99.6)	98.2%	0.93
GONORRHEA	**STRATEGY 1 (unpooling of INV)**	88.9% (77.4-95.8)	97.7% (95.7-98.9)	84.2% (73.5-91.1)	98.5% (96.8-99.3)	96.6%	0.85
	STRATEGY 2 (unpooling of INV & POS)	88.9% (77.4-95.8)	96.7% (94.4-98.2)	78.7% (66.2-86.4)	98.4% (96.7-99.3)	95.7%	0.81

INV: invalid; POS: positive

Chlamydia trachomatis

According to strategy 1, 3 CT were missed, resulting in a sensitivity of 95.4%. The Abbott delta cycle values (difference in cycle numbers between the cut-off control and the sample cycle number) of the individual specimens from 2 discordant pools were low, which correlates with a low target concentration. *Chlamydia trachomatis* was falsely detected on site in 5 participants (specificity, 98.7%). Applying strategy 2, the sensitivity decreased to 92.3% and the specificity increased to 99.2%.

Neisseria gonorrhoeae

Six NG infections were missed with strategy 1, yielding a sensitivity of 88.9%. The Abbott delta cycle values for the individual specimens included in the 6 pools indicated a high bacterial load.

Using strategy 1, 9 samples were positive but not confirmed by the criterion standard algorithm (specificity, 97.7%). When applying the second strategy, 1 sample was actually negative, however, 5 additional tests were positive, almost all from 1 study site, suggesting a possible DNA contamination (specificity, of 96.7%).

Cost Analysis

A prevalence of 22% CT/NG, as found in this study, was assumed. Compared with triple testing, a 56% decrease in costs was noted with strategy 1 and 30% with strategy 2 (Table 3).

Table 3: Number of tests required according different strategies and cost simulation using a combined prevalence of CT/NG of 22%

STI screening strategies	No. participants	Calculation of No. tests required	Total No. tests	Assay cost (US $19 per test)	Cost per infection
Triple site testing	500	Every site of sampling = 500 * 3	1500	US $ 28,500	US $ 220
Pooling strategy 1	500	500 pooled samples = 500 individual testing of 20 invalid results = 20*3	560	US $ 10,640	US $ 97
Pooling strategy 2	500	500 pooled samples = 500 individual testing of 20 invalid results = 20*3 individual testing of 110 positive results = 110*3	890	US $ 16,910	US $ 154

6.2.5 Discussion

We are among the first to report on the prevalence of chlamydia and gonorrhea in MSM initiating PrEP in West Africa. The data indicate a high prevalence of chlamydia (14.5%) and gonorrhea (11.5%), mainly in asymptomatic (88.2%) individuals. These asymptomatic infections would not have been treated according to the syndromic approach, which is currently the standard of care in LMIC. In addition, 77.6% of infections were extragenital. These findings reinforce the recommendation that STI services, including triple-site testing, should be integrated in PrEP programs in LMIC. Therefore, we aimed to implement an STI screening strategy using the GeneXpert instrument as its availability throughout Africa will facilitate STI testing. The Xpert CT/NG assay is now put forward as a potential point-of-care assay for STI detection in remote health care settings as it is easy, robust, and has very high analytical performance.[30-32] Previous studies showed that this technology is acceptable in identifying STIs among Sub-Saharan African young women, however, to date, no study has been performed among African MSM.[32-37]

We used a new pooling method with the GeneXpert platform to screen for STIs in genital and extragenital samples among MSM. Although the pooling method was designed as such to identify the origin of infection, we showed that there is no clinical utility. First of all, we did not detect a single case of Lymphogranuloma venereum (LGV) in our study population. LGV is frequently detected in European MSM and requires a 3-week treatment with doxycycline versus 1 week in the event of a regular chlamydia infection. Secondly, all antimicrobials recommended nowadays for the treatment of gonorrhea are equally effective in the 3 biological sites.,[19,20,38] Furthermore, the performance of the pooling method did not improve when unpooling positive samples. Nevertheless, we favour keeping the possibility to test the individual samples in case of an invalid pooled sample result to avoid additional sample collection and subsequent delay in result reporting. Using this strategy would decrease the testing cost with 56% compared with triple-site testing and would further decrease the burden on the laboratory's workload.

Applying strategy 1 resulted in 9 participants not receiving treatment (9 [2.0%] of 448), and 14 participants receiving unnecessary treatment (14 [3.1%] of 448). The number of false positives increased to 16 for the individually tested samples, mainly caused by a probable contamination of NG at one of the sites. On the other hand, the Xpert CT/NG assay can detect as little as 10 NG genome copies per reaction.[32] We cannot exclude the idea that some of the positive NG results solely obtained with the GeneXpert were truly low positive results not detectable by the criterion standard.

However, we also report on a CT DNA contamination of the GeneXpert instrument's surface at another site. The GeneXpert method is a closed system that reduces

contamination to an absolute minimum, yet, due to its very low lower limit of detection, the assay is more prone to target contamination caused by the presence of genetic targets in the work environment or by sample cross contamination.

Although the GeneXpert CT/NG assay can be integrated into remote health care settings, this apparent risk of contamination may lead to erroneous results, which may cause emotional distress, stigma and unnecessary antibiotic pressure. Therefore, we strongly recommend that detection of CT/NG using the GeneXpert instrument is performed under the supervision of qualified laboratory personnel and regular environmental control is integrated into a quality assurance program.

This is, to our knowledge, the first study reporting on a sample pooling method among a large number of African MSM initiating PrEP. Other pooling methods in MSM have been published, including methods using the GeneXpert assay.[21-26] Our results are in accordance with previously described studies that reported a reduced sensitivity for CT (90-94%) and/or NG (89.7-91.7%). Using our pooling method, sensitivity for CT and NG also decreased (95.4% and 88.9% respectively). A possible explanation for these reduced sensitivities is that pooling of samples will additionally dilute samples with low CT or NG bacterial load. To decrease the risk of over dilution, we opted to work with simple urine containers and Eswabs (1 mL transport medium) whereas most pooling methods elute the swabs in a transport medium volume of over 1 mL.

Furthermore, using Eswabs, our pooling method can tackle one of the most important global health priorities set forward by the WHO, namely the surveillance for antimicrobial resistance of NG.[39] Future research will need to show if surveillance for AMR of NG using Eswabs can be implemented in LMIC.

Our study design did not include a direct comparison of pooled and individual sample testing using the GeneXpert assay. Therefore, negative pools were not retested. In addition, the reduced sensitivities may be further explained by the use of Eswabs which are not licensed for use in both molecular techniques, however the use of Eswabs was evaluated on both assays prior to study start. Furthermore, samples tested with the criterion standard algorithm underwent an additional freeze-thawing cycle which may have impaired the DNA in low concentration samples. Finally, even though physicians were trained in sample collection to avoid sampling errors, the present study showed that human DNA was lacking in one quarter of the anorectal and pharyngeal samples which were positive solely using GeneXpert. This error may be due to the fact that 2 samples of each anatomical site were requested. This finding can further clarify the discordant results found in the study.

In conclusion, we showed that MSM initiating PrEP in Africa have high prevalence rates of extragenital and asymptomatic STIs and that African countries can perform an etiological diagnosis of STIs without implementing specialized NAATs.

The availability of PrEP in LMIC is a unique opportunity to strengthen STI services in high risk populations. The momentum is now to move to efficient STI screening and to limit onward transmission. In this new PrEP-era, the WHO, ministries of health and stakeholders at a global level will need to ensure that STI management is integrated in PrEP services, and negotiations with companies to provide the tests at affordable prices are, therefore, essential.

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CHAPTER 7: HOME-BASED SAMPLING FOR STI DETECTION

Open access Research

BMJ Open

Evaluation of the 'Colli-Pee', a first-void urine collection device for self-sampling at home for the detection of sexually transmitted infections, versus a routine clinic-based urine collection in a one-to-one comparison study design: efficacy and acceptability among MSM in Belgium

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7.1 ABSTRACT

Objectives Pre-exposure prophylaxis (PrEP) users are screened bi-annual for sexually transmitted infections (STIs). A novel device, called the Colli-Pee, collects first-void urine in a standardised way and the collector tube can be easily delivered by regular post to a certified laboratory. The aim of the study was a one-to-one comparison between the STI test results obtained with the urine collected in the clinic, versus urine collected at home in a real-life setting by Men who have Sex with Men (MSM) in Belgium. The user-friendliness and acceptability of the Colli-Pee device by the users was also evaluated.

Design A single-site nested sub study in a prospective PrEP demonstration project (Be-PrEP-ared) among MSM in Belgium.

Participants A total of 473 home-based samples from 213 MSM were received with a mean age of 38.5 years.

Interventions: Participants were requested to collect a urine sample at home using the Colli-Pee device and to send it to the laboratory via regular mail.

Primary and secondary outcome measures The presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) was determined using molecular amplification assays. Agreement between test results of samples collected at the clinic and collected at home were evaluated using Cohen's kappa statistic. Results: TV was not detected. A very good to almost perfect agreement was found for CT, NG and MG of k=0.75, 0.87 and 0.85, respectively. Using the Colli-Pee device only one low positive CT and two MG infections were missed, however, three additional CT, two NG and six MG infections were detected.

Conclusions The Colli-Pee device is a feasible and convenient way to collect urine at home for STI testing. This may be particularly relevant for populations that need frequent STI testing, such as PrEP users and patients who prefer home-sampling.

KEYWORDS

***Neisseria gonorrhoeae*, *Chlamydia trachomatis*, screening, urine, preexposure prophylaxis, MSM**

7.2 INTRODUCTION

According to the WHO's Global Health Sector Strategy on sexually transmitted infections (STIs) 2016–2021, early diagnosis and linkage to treatment are one of the key elements for preventing further transmission of STIs.[1] Currently, first-void urine is still favoured as the sample of choice for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in men, using nucleic acid amplification tests (NAATs).[2–4] In general, a regular urine container is used to collect the first-void urine sample, but the collected volume of urine is not standardised. Furthermore, this type of container is less convenient for postal delivery to the laboratory. Another sponged-based device (UriSWAB, Copan Diagnostics, Brescia, Italy) has been suggested as an alternative for postal delivery of urine. However, this device only holds 2 mL of urine and does not guarantee that only first-void urine is collected.[4 5] The Conformité Européene (CE) labelled Colli-Pee device (Novosanis, Belgium), provides a clean and standardised solution to the above-mentioned issues as it efficiently captures first-void urine (20 mL) without interruption of the urine flow and allows the samples to be sent by post (figure 1).

Figure 1: The Colli-Pee device instructions for use.

The Colli-Pee device is currently used for the detection of human papilloma virus and several urological cancers for which collection of first-void urine is essential.[6 7] In the field of STIs, a standardised first-void urine study reported that the organism load of CT is maximal in the first 4–5 mL and that the performance of diagnostic tests improved when using only first-void urine.[8 9]

Although pre-exposure prophylaxis (PrEP) is becoming crucial in HIV prevention, recent reviews of real-world PrEP demonstration studies showed that PrEP is associated with increased diagnoses of STIs in Men who have Sex with Men (MSM).[10 11] Consequently, current guidelines recommend a biannual screening of STIs in PrEP users because of their high-risk behaviour.[12 13]

In order to facilitate the patient flow during follow-up visits by PrEP users, and prompt treatment of STIs, home-based collection of first-void urine could be sent to the laboratory by regular mail for STI detection before the scheduled visit. STI results may then be available at the time of the physician consultation and in the case of a detected STI also immediately treated, limiting the risk of further transmission.

The objectives of this study were to compare the results of the molecular detection of several STIs using the Colli-Pee device versus a sample obtained in the clinic, the use and acceptability of the Colli-Pee device and its convenience for shipment by regular mail. To assess these objectives, a nested sub study was performed among MSM who participated in a Belgian PrEP demonstration cohort.[14]

7.3 METHODS

The evaluation was undertaken as a sub study of Be-PrEP-ared, a PrEP demonstration study among MSM at high risk for HIV in Belgium.

The main study

The Be-PrEP-ared project (EudraCTn°: 5015–00005437) was a phase 3, single-site, open-label prospective cohort study where 200 MSM at high risk of acquiring HIV were asked to participate in the project and to take PrEP daily or event-driven. Detailed study methods are described elsewhere.[14] Participants were tested for NG, CT, *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) at baseline and every 3 months. Detection of these STIs was performed at the three biological sites: urethra, anorectum and pharynx. During each study visit, participants collected urine in two urine containers at the clinic as per the following instructions: urinate in the first container up to the marked line at approximately 20 mL, afterwards complete the second cup with no restrictions. Urine in the first container (hereafter the clinic-based sample) was weighed and thereafter stored refrigerated until analysis that took place within 48 hours. Urine in the second container was used to detect proteinuria.

Laboratory procedures

In the first instance, CT/NG detection was performed using the Abbott Real Time (RT) CT/NG assay (DNA extraction and sample preparation using Abbott m2000sp and the Abbott m2000rt system for amplification and detection of CT/NG [Abbott Molecular Des Plaines, Illinois, USA]) according to manufacturer's instructions. The remainder of the urine and DNA extracts were stored at −80°C. In the case of positivity, the same DNA extracts were tested by in-house real time (RT)-PCR assays for CT and/or NG, both

based on previously published primer sets.[15 16] A sample was considered positive when positive in both the Abbott and the in-house RT-PCR. An initial positive Abbott assay result followed by a negative confirmatory NAAT result was defined as 'not confirmed'. Inhibition of the NAAT according to the Abbott assay was defined as 'inhibition'.

The same DNA extracts were used for further testing. MG was detected and reported using an accredited in-house RT-PCR that targets the *pdhD*-gene[17] and in addition the DiaMGTV multiplex kit (Diagenode diagnostics, Seraing, Belgium) that detects MG and TV simultaneously was used for TV detection. The results for MG of the DiaMGTV multiplex kit were not used for reporting purposes and are only provided for information only. No further confirmation of TV took place.

The Colli-Pee substudy

At the baseline visit of the Be-PrEP-ared study, participants were asked consent to participate in this substudy. After signing the informed consent form, they received a Colli-Pee device and a prepaid envelope. They were instructed to collect first-void urine the next day at home using the Colli-Pee device (the home-based sample), to document the date and time of collection and to send the collector tube filled with urine back to the laboratory by regular post, using the prepaid envelope. On receipt in the laboratory, the urine was weighed, stored refrigerated (2°C–8°C) and CT, NG, MG, TV was detected using the same NAATs within 48 hours. The urine and DNA extracts' remnants were stored at −80°C. The quantity of human cells was measured at baseline using a human Endogenous Retrovirus-3 (ERV-3) quantitative PCR on the paired clinic- and home-based samples.[18]

The lab technicians were blinded for the results obtained for the clinic-based sample. In addition, the result of the home-based sample was not disclosed to the physician or participant.

During the next visit, which took place within 14 days after baseline, participants were asked to complete a small survey (five questions only) on the user-friendliness and willingness to use the Colli-Pee device (acceptability). Two questions documenting their opinion (likes-dislikes) of the Colli-Pee device were open-ended.

At follow-up month 6 and month 18 of the study, Colli-Pee devices were again distributed to those who agreed to participate and the survey was repeated at month 18 (results unreported).

Patient and public involvement

Patients were not involved in the Colli-Pee substudy. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Statistical analysis

The agreement of the results of the molecular assays using each of the two sampling methods was assessed by the use of Cohen's kappa statistic and percent agreement. Samples that were not confirmed were coded as negative samples for the calculation of the agreement. The agreement of volume of urine collected and the agreement of concentration of human DNA in both sampling methods was assessed by using a t-test. A p-value of <0.05 was considered statistically significant. Both analyses were performed using STATA V.15.0.

A descriptive analysis was made of the results of the self-administered questionnaire on the acceptability and user-friendliness of the Colli-Pee device.

7.4 RESULTS

Demographics

The main study took place at the Institute of Tropical Medicine, Antwerp, Belgium from September 2015 until May 2018. Of the 219 participants who were screened for eligibility into the main study, six participants did not consent to the Colli-Pee substudy. All participants who consented to the substudy were MSM and three identified themselves as transwomen.[19] The mean age of the participants was 38.5 years (IQR 32–44). A total of 473 home-based samples from 213 participants were received. Two home-based samples could not be linked to the corresponding clinic-based sample and were therefore excluded, bringing the total number to 471. As shown in table 1, the number of home-based samples received at the laboratory declined over time.

Table 1: Number of clinic and home-based samples received during the study

Kind of visit	Clinic based	Home based (% home- based samples received)
Screening	218	187 (85.8%)
Month 6	191	152 (79.6%)
Month 18	179	132 (73.7%)

Although the participants were instructed to report the urine collection date and hour on the collection device, only 72.8% (343/471) were labelled with collection date. Most

of the home-based samples (79.6%) were taken within 2 days after the clinic-based sample and 3.8% were taken after 20 days (13/343) (median 1 day; min-max: 0–70 days). The median time between the collection of the home-based sample and its reception at the laboratory after postal return was 5 days (min-max: 0–27 days), 72.9% arrived at the laboratory within those 5 days, an additional 25.7% within 10 days and five samples were received after 10 days (11, 13, 15, 17 and 27 days, respectively).

Comparison of weight and concentration of human material between both sampling methods

A total of 455 home-based and 423 clinic-based samples were weighed. The mean net weight of the home-based sample was 19.68g ± 2.14 g (95% CI 19.5 g to 19.9 g and min-max: 6.81 g- to 39.47g) vs 22.87g ± 13.64 g (95% CI: 21.6 g to 24.2g and min-max: 2.88 g to 86.23 g) for the clinic-based sample ($p<0.001$).

The quantity of human cells was analysed at baseline only (n=187). In a total of five home-based and one clinic-based sample, ERV could not be detected and these samples were considered as lacking human material. After removal of the paired samples lacking ERV or containing inhibitors, 182 observations could be paired. The mean quantity of the clinic-based sample was $11.3*10^3$ cells/PCR (95% CI 7.4 to $15.2*10^3$) and for the home-based sample $14.2*10^3$ cells/PCR (95% CI 6.8 to $21.5*10^3$) ($p>0.05$).

STI results and agreement

Of the 471 home-based samples with a matching visit, six home-based and one clinic-based sample gave inhibition and were excluded from the analysis (n=464). The results are shown in table 2.

TV was not detected. Percent agreement (Cohen's kappa coefficient) for CT, NG and MG is 99.1% (0.75); 99.6% (0.87) and 98.3% (0.85), respectively, which indicates substantial agreement for CT and almost perfect agreement for the other two STIs.

Tables 3 and 4 show the discordant results. For some of the home-based samples, the date of collection was unknown so the time between the clinic visit and time of reception at the laboratory is depicted here. A delta cycle (DC) value of the Abbott assay of less than two indicates a low positive infection.

Table 2: Sexually transmitted infections results of the home-based and clinic-based urine samples.

		Clinic-based results		
STI	**Home-based result**	**Negative**	**Positive**	**Total**
Chlamydia trachomatis (non-LGV)	**Negative**	454*	**1**	455
	Positive	**3**	6	9
	Total	457	7	464
Neisseria gonorrhoeae	**Negative**	455	**0**	455
	Positive	**2**	7	9
	Total	457	7	464
Mycoplasma genitalium	**Negative**	431	**2**	433
	Positive	**6**	25	31
	Total	437	27	464
Trichomonas vaginalis	**Negative**	464	**0**	464
	Positive	**0**	0	0
	Total	464	0	464

**Two result were not-confirmed in the clinic-based sample.*

Table 3: Sexually transmitted infections that were not detected in home-based urine samples.

STI	**DC value CT/NG Abbott assay**	**Ct value in-house RT-PCR for CT, NG or MG***	**Ct-value S-Diag MGTV RT-PCR (for information only)**	**Days between collection**	**Days of transport**
CT	1.49	33.19	NA	0	2
MG	NA	32.20	Neg	3	4
MG	NA	32.04	37.44	0	8

**A different in-house RT-PCR assay was used for CT, NG or MG. Ct, cycle threshold; DC, delta cycle; Max, days between clinic visit and reception of the home-based sample at the laboratory.*

Table 4: Sexually transmitted infections that were additionally detected in home-based urine samples.

STI	**DC value CT/NG Abbott assay**	**Ct value in-house RT-PCR for CT, NG or MG***	**Ct-value S-Diag MGTV RT-PCR (for information only)**	**Days between collection**	**Days of transport**
CT	3.99	34.26	NA	1	2
CT	2.68	35.31	NA	1	6
CT	0.27	36.06	NA	6	4
NG	2.93	37.58	NA	8	4
NG	10.08	25.26	NA	2	6
MG	NA	31.28	Neg	Max 6	Max 6
MG	NA	31.96	40.51	1	2
MG	NA	28.66	34.67	Max 3	Max 3
MG	NA	34.58	38.50	9	4
MG	NA	34.23	Neg	1	5
MG	NA	32.04	38.14	Max 3	Max 3

**A different in-house RT-PCR assay was used for CT, NG or MG. Ct, cycle threshold; DC, delta cycle; Max, number of days between clinic visit and reception of the home-based sample at the laboratory.*

7.4.1 Acceptability and user-friendliness of the Colli-Pee device

A total of 164 participants provided feedback regarding the use of the Colli-Pee device at baseline. On a scale of one to five, 87.8% found that the Colli-Pee device was easy to very easy to use. Instructions on how to send the Colli-Pee device were found to be easy by 90.2% of the participants. Four participants found the Colli-Pee difficult to use (2.4%) and four other participants found it difficult to follow the instructions (2.4%). Likes from participants were: the ease of use (54.9%), no interruption of the urine flow (15.9%), hygienic (11.6%) and privacy of the home-based sample collection (11.0%); the dislikes were: nothing (47.0%), not being recyclable (14.6%), not hygienic (10.4%) and being too large (6.1%).

To the question of whether they would order an online STI test, 89.0% answered positively (146/164) and 91.1% (133/146) of those individuals would use the Colli-Pee device in that case. Six participants (4.1%) would not want to use the Colli-Pee device when ordering an online STI test. Participants were also asked how much they would pay for an online STI test with self-sampling. Price indications ranged from 0€ (10 participants) to 60€. Most of the participants (89/164) were willing to pay 10–20 €.

7.5 DISCUSSION

Many studies have reported on male self-collected urine versus urethra clinician-collected sampling for STI screening, but 'real-world' studies, including sending of home-based urine samples for STI detection in men by post, are sparse.[4 5 20 21] In this study, we showed that the Colli-Pee collection device is a valuable and reliable method for collecting first-void urine for STI detection in MSM in Belgium, and that the collector can be shipped by regular post. Compared with the clinic-based sample, a total of three STIs (one CT and two MG infections) were not detected in the home-based sample. However, 11 additional infections were found in home-based samples collected with the Colli-Pee device (3 CT, 2 NG and 6 MG infections). This high number of additional STIs could be explained by the fact that first-void urine contains more DNA/RNA than mid-stream and, as a consequence, should still be used for STI detection.[9] Indeed, we showed that using the Colli-Pee device first-void urine was collected in a more standardised way compared with the clinic-based samples ($p<0.001$). Also, more human cells were collected in the home-based samples, however statistical significance was lacking. The fact that participants could become positive during the time in between sampling points is one of our main limitations and cannot be ignored. Preliminary data of the Be-PrEP-ared study showed high-incidence estimates after

twelve months of the main Be-PrEP-ared study for urethral CT/NG and MG: 11.5, 5.1 and 6.9 incidence rate per 100 person-years, respectively.

Nevertheless, the most important observation is that only one Chlamydia positive result was missed. The DC value of the Abbott assay performed on that clinic-based sample highlighted the low bacterial load of that infection; in addition, transportation at room temperature for 2 days could have induced DNA degradation.

The WHO underlines the importance of integrating point-of-care assays (POCTs) including innovative delivery options such as self-testing.[22] Unfortunately, to our knowledge, current commercial POCTs for the most important STIs such as CT and NG are still of sub-optimal quality and do not meet the ASSURED criteria that were developed by the WHO STI Diagnostics Initiative.[22–25] A solution to the unavailability of qualitative POCTs could be internet-accessed STI testing (e-STI testing) which is increasingly available as an alternative to clinic testing all over the world.[26] E-STI testing includes postal self-sampling test kits that are sent to a certified laboratory and web-based delivery of test results. Swab2Know, an online HIV testing project confirmed that e-HIV testing is accept- able and feasible among MSM in Belgium.[27] Commercial online self-sampling services for STIs are now emerging over the internet, but evaluation of these services is lacking.

The present study is, however, subject to several limitations. First, we only enrolled Be-PrEP-ared participants and the participation level seriously declined during the study. As a result, our number of CT/NG positives is quite low, which precludes firm conclusions. Second, as mentioned above, home-based samples were not taken on the same day as clinic-based sampling and participants could have become positive during that window period. We also do not know whether participants had urinated 1 hour prior to collection. However, recent data show that the time between micturition is not crucial for the detection of Chlamydia in men.[28] Third, we did not monitor the temperature of the transport of home-based samples which could also have an impact on the quality of the samples, however, outside temperature between October 2015 and May 2018 varied between −10°C and 33°C with an average of 11°C.

Fourth, we cannot exclude specimen contamination, however, participants were instructed how to correctly collect the clinic-based and home-based sample. Finally, reporting bias is also not to be excluded. Not all participants who used a Colli-Pee device completed the survey, the additional questions were included at the end of the lengthy main questionnaire of the Be-PrEP-ared study.

Besides PrEP users, e-STI testing has the potential to reach those who are most in need and a recent study showed that some higher-risk groups, such as MSM, were more

likely to use online services.[26 29] Many studies have shown that home-based sampling is well accepted and, in fact, is the preferred approach in these groups for STIs. Reasons for choosing home-based sampling were shorter waiting times for results, convenience and less embarrassment.[30] Participants views regarding ordering an online STI test in this study were very positive, 89% would like to order such a kit. The Colli-Pee device was also found to be easy (90.2%) and although hygiene was one of the likes, it also appeared in the dislikes, probably because the need to detach the collector manually can cause leakage of urine. Participants were also concerned regarding possible ecological consequences, although the plastic material is recyclable and can be incinerated into energy. We demonstrated that postal delivery of home-based collected urine does not influence STI detection and can be used among PrEP users. Subsequently, PrEP users will be able to send first-void urine to the laboratory with the Colli-Pee device 1–2 weeks before their routine PrEP follow-up visit. Results can then be discussed during the physician consultation and followed by treatment and future antimicrobial testing if applicable, decreasing the number of physician visits. Decreasing the number of face-to-face visits will lower the burden on staff workload and healthcare resources. However, future economic evaluations will need to be conducted to prove this statement. E-STI testing could be a promising approach in Belgium to reach patients in hard-to-reach populations and research on this topic should be stimulated. Therefore, future studies to study the acceptability and impact of postal shipment of home-collected material on the performance of STI assays requires additional assessment

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CHAPTER 8: DISCUSSION

This thesis contributed to the knowledge of STIs among MSM with high risk behaviour in Belgium in the era of pre-exposure prophylaxis (PrEP) as a key HIV biomedical prevention method. In the introduction, five different challenges for controlling the STI epidemic among MSM were described:

1. The increase in **riskier sexual behaviour** including dense sexual networks;

2. The phenomenon of **core transmitters** who experience recurrent STIs;

3. The **emergence of particular STIs**, which were previously confined to HIV positive MSM, in the PrEP taking MSM with high risk behaviour;

4. The occurrence of **antimicrobial resistance** among STIs;

5. The need for an **enhanced STI screening rate** including the detection of extra-genital asymptomatic STIs.

To address these challenges, various important epidemiological and clinical aspects of STIs among MSM in Belgium were investigated in section I. Furthermore, in order to facilitate the enhanced STI screening among MSM using PrEP, novel CT/NG screening strategies were reported in section II. The results that address the above mentioned challenges will be shortly summarized in the first part of the discussion. In the second part, recommendations will be formulated for future STI guidelines among MSM in Belgium. Finally, we will assess whether these guidelines could be extrapolated to other populations or settings such as low- and middle income countries.

8.1 RISK BEHAVIOUR AND STI EPIDEMIOLOGY IN THE BE-PREP-ARED STUDY

Most of the results reported in this thesis were observed in the framework of the Be-PrEP-ared study, the Belgian PrEP demonstration study that took place from 2015 to 2018 (chapter 3). The results of that study showed that PrEP was acceptable among Belgian MSM and led to the implementation and reimbursement of PrEP in Belgium. In that study, no HIV infections were found, however, **the STI prevalence and incidence was high** which is in line with other PrEP studies.[1] In fact, prevalence for one or more bacterial STI (syphilis, CT/NG or MG) was 39.5% at baseline, with *Mycoplasma genitalium* infection being the most prevalent (17.2%), followed by gonorrhea (12.2%), chlamydia (11.7%) and syphilis (7.5%). TV was very rare in our cohort which is concordant to other studies.[2] Therefore, it is not clinically relevant to test for TV among MSM.

Also in line with other studies, most of the CT/NG infections were of extra-genital location (85.4%), and only one out of four of these extra-genital CT/NG infections (24.6%) were symptomatic which underscores the need for extra-genital CT/NG testing. Importantly, sexual risk-behaviour was high at baseline. A high partner rate was noted (median of 12 in the last three months) and 64% of the participants reported receptive condomless anal intercourse (CAI) in the previous three months. Longitudinal data of the study revealed that the median number of sexual partners did not change over time, yet, the frequency of condom use dropped significantly. These findings underline the fact that changes in sexual behaviour takes place among MSM PrEP users which, could negatively impact on the STI epidemic. Nevertheless, **despite decreasing condom use, no significant change in CT/NG incidence was found over time** at each scheduled visit (62/100 person years) which may be due to our extensive STI screening. These behavioural and CT/NG prevalence figures are similar to those from the AMPrEP study, the sister PrEP study in the Netherlands.[3] Noteworthily, a subset of individuals had

several recurrent CT/NG infections in our study, which may have a disproportionate effect on the quarterly CT/NG incidences. Therefore, a secondary analysis was performed to scrutinize the number of STIs in our cohort and to identify certain factors that may predict the presence of recurrent STIs.

8.2 INDIVIDUALS EXPERIENCING RECURRENT STIS

In this sub-study (chapter 3.2), limited to the 179 participants who completed the 18 months of follow-up, we noted that 62% of the participants experienced one or more STIs (CT/NG or syphilis). Furthermore, more than one third of the participants experienced recurrent STIs. Almost 80% of all STIs detected in the Be-PrEP-ared study were found in this sub-group underscoring the importance to identify these individuals. Indeed, the number of non-steady sexual partners in the last three months was much higher among individuals experiencing recurrent STIs compared to the other participants which confirms the dense sexual networks among this subset of PrEP users (median 21 vs 8). Moreover, besides the high number of sexual partners, we described a number of other factors that were associated with recurrent STIs. These include age below 40, a higher education, group sex, use of erection enhancing drugs, but most importantly, **sexualized drug use**. As a matter of fact, sexualized drug use was reported in almost 90% of the participants experiencing recurrent STIs compared to 52.2% of the other participants. Moreover, engaging in sexualized drug use was the sole behavioural characteristic that remained statistically significant in the multivariate logistic regression models comparing individuals with recurrent STIs with the individuals that experienced only one STI episode during the study. Sexualized drug use has been previously associated with incident STIs among PrEP users[4], however, to our knowledge, **no study analysed the association between sexualized drug use and the presence of recurrent STIs**. Aside of the fact that certain sexual risk behaviours such as increasing number of partners with CAI, longer sexual sessions, sharing sex toys and fisting are more common among individuals using drugs during sexual activity[5], drug

use may also affect the users' immune responsiveness and hereby may enhance susceptibility to various infectious agents including STIs.[6] Moreover, we showed that almost all LGV infections and the majority of the concurrent CT/NG infections were confined to this core group. Identifying those individuals is important for public health approaches as they may potentially further spread STIs through their dense sexual networks which was also confirmed in this study by the high number of non-steady sexual partners. Therefore, reporting sexualized drug use or the identification of an LGV- or a concurrent CT/NG infection should trigger health care professionals to offer tailored STI risk and harm reduction counselling, including on sexualized drug use and expedited partner notification to further prevent STI transmission in their networks.

In conclusion, we showed that individuals with recurrent STIs were responsible for 4/5th of the STIs and almost all LGV infections in our study. Moreover, sexualized drug use was highly associated with having recurrent STIs.

8.3 RE-EMERGING SEXUALLY TRANSMITTED INFECTIONS

The results of the Be-PrEP-ared study underscored the importance of LGV infections among PrEP users. Moreover, we showed that LGV was mostly found among PrEP users at risk for recurrent STIs. The introduction of PrEP was expected to create a large increase of LGV cases among HIV negative MSM due to a number of factors such as a gradual change in sexual behaviour (enlargement of sexual networks due to social media and geosocial dating applications, less condom use and serosorting), an increase in CT/NG testing and subsequent LGV identification among HIV negative MSM presenting for PrEP with high risk behaviour and finally, an increased awareness of LGV testing among health-care providers. A shift of the LGV epidemic from HIV positive to HIV negative MSM is now noted in numerous European countries, however, **the influence of the roll-out of PrEP on the LGV epidemic was not yet investigated**.[7] To answer that question, we investigated the national surveillance data of rectal LGV (rLGV) cases in Belgium from 2011 until the end of 2019. Although the number of rLGV

cases further increased over the semesters, there was no difference in the magnitude of this trend after the introduction of PrEP amongst either HIV positive or HIV negative individuals. Nevertheless, in 2019, 56.2% of all rLGV cases were found in HIV negative individuals whereas rLGV was exclusively found among HIV positive individuals in 2011. Exploration of the number of rLGV cases in the male ITM cohort however showed a significant increase of LGV cases among HIV negative individuals after the roll-out of PrEP. This increase may be partly explained by the increase in frequency of CT/NG testing among PrEP users and as such, previous underdiagnosis of asymptomatic LGV infections, and moreover, by the increase of the PrEP cohort at ITM. Indeed, whereas 360 MSM were included in the PrEP cohort in 2017, this number increased by a factor 2.5 to 938 in 2019. Noteworthily, the prevalence of LGV among PrEP individuals doubled from 1.2% in 2018 to 2.4% in 2019 which may suggest that more PrEP users are exhibiting more risk behaviour and may belong to the group with recurrent STIs as we have seen in chapter 3.4.

In conclusion, our data now show that, although an increase in LGV cases is noted among HIV negative individuals over the years, the roll-out of PrEP did not accelerate this trend.

8.4 *MYCOPLASMA GENITALIUM* AND ANTIMICROBIAL RESISTANCE

Mycoplasma genitalium is the STI of choice to investigate the emergence of bystander antimicrobial resistance. As discussed above, this pathogen was very prevalent among PrEP users and in addition, many of these infections were asymptomatic (82%). The public health importance of asymptomatic MG infections is still poorly understood and due to the swift emergence of antimicrobial resistance, guidelines are now recommending not to screen for MG.[8] As such, the Be-PrEP-ared team decided to stop treatment of asymptomatic MG infections. During that time, we noted spontaneous

clearance of some MG infections without treatment, whereas conversely some individuals remained positive for 18 months even after treatment. The latter may either be due to reinfection or treatment failure. Additional research on the presence of resistance-associated mutations (RAM) to fluoroquinolones (ParC 83/87) or macrolides (23S rRNA) in the new MG cases found in the Be-PrEP-ared study showed **alarmingly high resistance levels** (see chapter 5). Almost 90% of the MG samples were resistant to macrolides and one fourth of the samples (24.7%) even harboured RAMs to both macrolides and fluoroquinolones which is much higher than previously reported among MSM in Europe.[9–11]

To our knowledge, no study reported on the antimicrobial resistance of MG among MSM with recurrent STIs. Not surprisingly, most new episodes of MG infections in the Be-PrEP-ared study were found among these individuals (57.5% vs 42.5%). **Interestingly, individuals experiencing recurrent STIs had more MG with macrolide or fluoroquinolone RAMs compared to the individuals who did not experience a recurrent STI** (see Figure 1). Mixed-effects logistic regression showed that this was only statistically significant for macrolide resistance (OR: 5.83, 95%CI: 1.12-30.34).

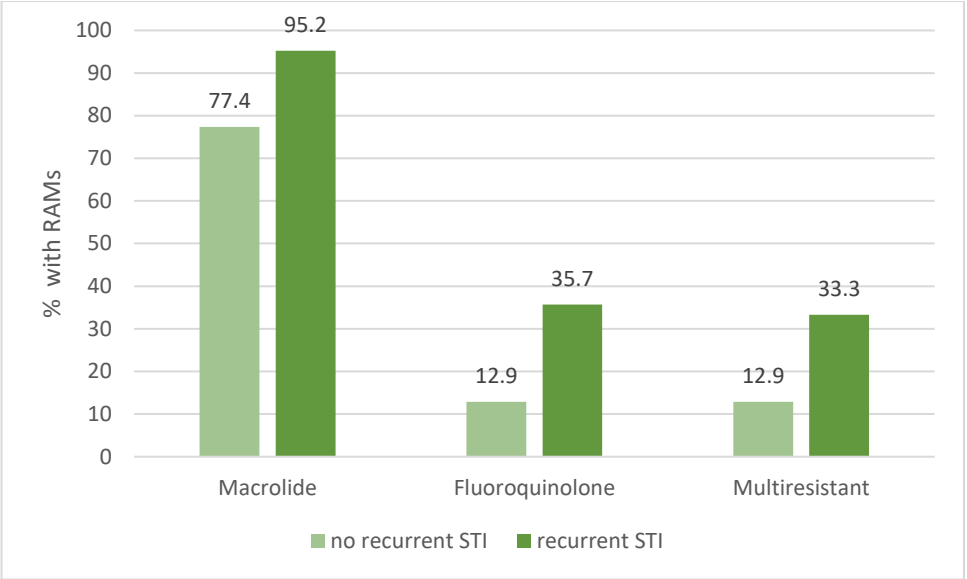

Figure 1: Proportion of resistance associated mutations (RAMs) of MG to macrolides, fluoroquinolones or both antimicrobials of the Be-PrEP-ared cohort stratified by individuals without or with recurrent STIs

Despite our small sample size, these results suggest that AMR in MG is fuelled by antimicrobial consumption for other STIs. Indeed, by experiencing multiple STI infections and subsequent treatment, macrolide resistance figures in MG are now reaching almost 100% among individuals experiencing recurrent STIs. Therefore, we propose that **macrolide usage to treat STIs should be limited**. Furthermore, these data show, once more, **the importance that individuals with recurrent STIs have on the STI epidemic through the high level of antimicrobial resistance** found among this group.

Using the above mentioned epidemiological results of the papers presented in Section I, we can now conclude that individuals with recurrent STIs have a prominent place in the STI epidemic which is fuelled by their sexual behaviour such as a high number of sexual partners, CAI and sexualized drug use (Figure 2). The results further show that individuals experiencing recurrent STIs play a central role in the epidemic of emerging STIs such as LGV and the emergence of antimicrobial resistance. Enhanced testing in this population is therefore recommended.

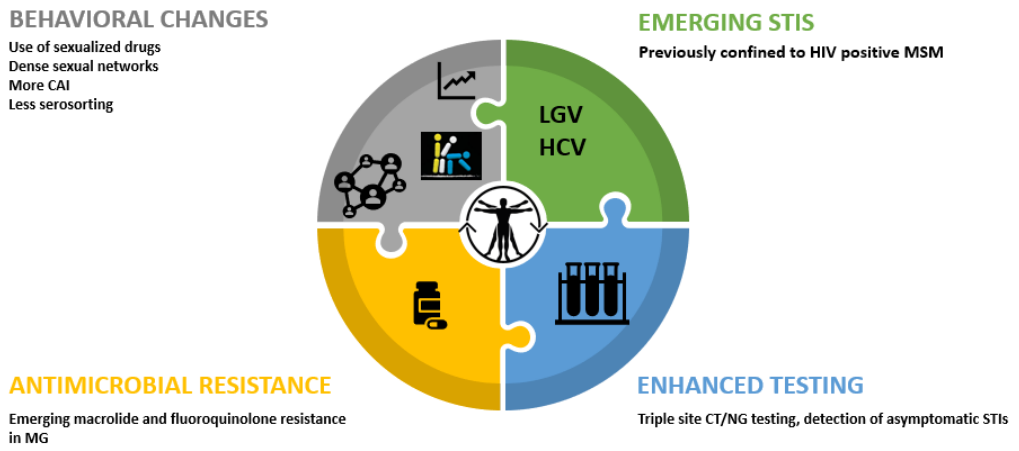

Figure 2: Central position of individuals with recurrent STIs in the STI epidemic. CAI: condomless anal intercourse; LGV: lymphogranuloma venereum; HCV: Hepatitis C virus

Indeed, provided we could approach these individuals with additional STI screening, harm reduction counselling including sexualized drug use and expedited partner notification, we may be able to diminish the STI epidemic among MSM. However, such labour- and resource intensive strategy will place a heavy burden on health care workers, laboratory professionals and PrEP users themselves. Therefore, **in section II of the thesis we presented several simplified CT/NG screening strategies** (ie. home based sampling and sample pooling methodologies) to address some of these issues.

8.5 THE NEED FOR ENHANCED SCREENING TO TEST FOR CT/NG

Before discussing the different CT/NG screening strategies proposed in this thesis, it may be appropriate to argue why enhanced CT/NG screening is necessary among MSM and especially those MSM who are at risk for recurrent STIs. The reproductive number of sexually transmitted infections depends on the number of sexual contacts, the transmission rate and the duration of infection. As mentioned above, the number of partners among PrEP users is high. Table 1 tabulates the estimated transmission rate and duration of infection of asymptomatic CT/NG at the three anatomical sites.

Frequent CT/NG screening and treatment may, thus, lower the duration of infection and consequently will decrease the transmission rate of CT/NG. As such, frequent CT/NG screening will reduce CT/NG incidence among MSM.

Table 1: Estimated duration of infection and transmission probability of asymptomatic *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in the different anatomical sites of infection[12–19]

	Duration of infection	Transmission probability
Neisseria gonorrhoeae		
Urethra	17 weeks	urethra to anorectum: 0.3-0.8
Anorectum	12 to 49 weeks	anorectum to urethra: 0.02-0.5
Pharynx	4 to 19 weeks	pharynx to urethra: 0.08
Chlamydia trachomatis		
Urethra	28 to 71 weeks	urethra to anorectum: 0.2-0.5
Anorectum	82 weeks	anorectum to urethra: 0.3-0.6
Pharynx	95 weeks	unknown to date

Modelling studies may be helpful to understand the infection dynamics of STIs. However, available studies yield contradictory results concerning the effect frequent screening may exert on the incidence. An important modelling study reported that bi-annual screening would avert 42% of NG and 40% of CT infections among MSM after one decade.[19] In contrast, a Belgian modelling study also reviewed the effect of NG screening among MSM on the NG prevalence over 10 years and found a very limited reduction in NG prevalence if 50% of the Belgian MSM were screened once yearly (from 11.9%/14.7% to 10.2%/12.4% at pharyngeal/anorectal site, respectively).[13] In our Be-PrEP-ared cohort, despite screening and treating CT/NG 3-monthly, we did not find a change in CT/NG prevalence over the visits. At baseline, a prevalence of 21.8% was found and this remained stable between 15.7 and 18.4% during the follow-up visits. **Therefore, the effect of PrEP use and the subsequent effect of increased CT/NG testing frequency on CT/NG incidences still remains uncertain and should be further investigated.**

The COVID-19 pandemic provided us a natural study opportunity whereby all non-essential services were closed for a certain period. As such, only symptomatic CT/NG infections were detected and treated. Luckily, this drastic measure was halted after 9

weeks in Belgium. Therefore, the effect of the absence of STI screening for asymptomatic STIs on the STI epidemic could not be assessed. Nevertheless, Jenness et al. published a very interesting modelling study and modelled the effect of interruption of STI services and sexual distancing on the STI epidemic among MSM.[20] An interruption of 18 months of clinical services by 50% in the absence of sexual distancing showed a dramatic increase in CT/NG incidence among MSM (from 19.4 to 48.8 per 100 person years) after 1.5 years (Figure 3).[20]

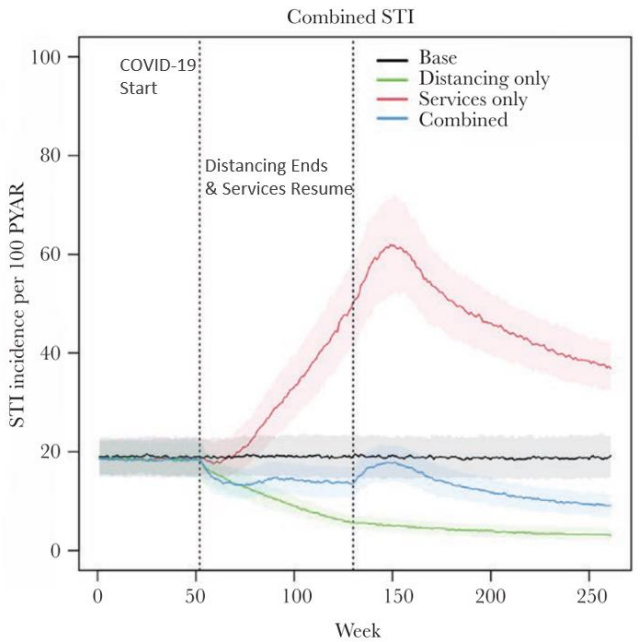

Figure 3: Projected CT/NG incidence among MSM before, during and after an 18 month period of clinical service interruption of 50%.[20]

These results highlight that asymptomatic CT/NG screening among MSM is essential to curb the STI epidemic and should not be interrupted. On the other hand, frequent STI testing and consequently detection of asymptomatic STI cases may increase antimicrobial consumption and resistance as a consequence in this population. Indeed, though extensive screening programs may be able to decrease CT/NG prevalence, this comes at the expense of an increase in antibiotic exposure as we have seen in the

previous section.[21] Currently, there is limited evidence of extended-spectrum cephalosporin resistance in NG due to ceftriaxone treatment. However, we recently reported on the shift towards high minimum inhibitory concentrations of ceftriaxone in commensal *Neisseria* species found among MSM which raises concerns given the swift ease of NG to acquire antimicrobial resistance via horizontal gene transfer from commensal *Neisseria* spp.[22,23] Bridging of NG infections from MSM to the heterosexual population has been documented and NG may result in very serious sequelae such as infertility in women and even death in very rare cases of disseminated gonococcal infection.[24] Therefore, **frequent screening and prompt treatment of NG is still recommended to lower the chance of developing multidrug resistant NG which may be subsequently further transmitted to the heterosexual population**. In order to facilitate the required frequent triple-site CT/NG screening among PrEP users, we evaluated **different CT/NG screening strategies** on which we will elaborate below.

8.5.1 Home-based self-sampling for CT/NG

One of the STI testing strategies that may facilitate this high CT/NG testing rate is home-based self-sampling (HBSS). In this thesis, we assessed whether HBSS was acceptable for PrEP users by using the Colli-Pee™ device to collect first-void urine for STI testing. The results of this study (chapter 7) indicated that **90% of the participants were prepared to use a HBSS kit for CT/NG testing** and almost all CT/NG infections were detected (one low positive CT infection was missed). Although we only tested CT/NG in urine, others have shown that HBSS for other anatomical sites was well received among MSM.[25] Furthermore, self-collected anorectal and pharyngeal samples were as accurate as clinician-collected samples.[26] However, less encouraging results were recorded with mailed-in extra-genital samples. In one study, 30% of the CT/NG infections were missed, but the authors stated that this could be due to different swab types used in that study or due to inadequate self-sampling.[27] Nevertheless, HBSS among MSM is currently implemented in several countries including the UK and the

Netherlands.[28,29] **In Belgium, HBSS for CT/NG as a routine practice is currently an unexplored field and should be urgently implemented**. Moreover, the Covid-19 pandemic revealed the increasing necessity to adopt self-care interventions such as telemedicine, digital health platforms and self-sampling or self-testing in order to avoid crowded waiting rooms and to limit social contacts as much as possible.[30,31] In fact, home-based self-sampling for STIs, ie. self-collection of the biological sample and subsequent shipment by regular mail to a certified laboratory for testing, is recommended by the WHO to increase testing coverage among individuals with high risk behaviour even during this pandemic.[32] Many studies showed that HBSS for STIs achieved higher screening uptake and was accepted by most of the participants.[33–35] As such, **HBSS for STIs among PrEP users may ease the burden for healthcare workers and PrEP users.** First, PrEP users could be asked to self-sample one week before the clinic visit. Next, at the scheduled follow-up visit, the clinician will have the results and if needed immediately treat and/or counsel the patient, hereby limiting onward transmission. An alternative for stable PrEP users is to replace one out of every two physical consultations with remote follow-up including HBSS. However, in the latter scenario, **HBSS will need to be combined with mobile Health applications (mHealth)** to facilitate remote care, including adherence and retention support, result reporting and partner notification in order to ensure best standard of care. Furthermore, HBSS will need to include self-sampling of blood for HIV and syphilis. Encouraging results of pilot studies of mHealth tools among PrEP users showed that such type of remote care is highly acceptable and resulted in a better retention in care.[36–38]

8.5.2 Pooling of samples for CT/NG testing

Another challenge facing CT/NG screening among PrEP users is the **high cost of quarterly triple-site CT/NG screening** (requiring 12 tests per person per year). In Belgium only four CT/NG molecular assays are reimbursed by the social health insurance for PrEP users (two in the case of the general population), thus eight CT/NG

molecular assays cannot be reimbursed. Testing of CT/NG at the three anatomical sites increases the workload and the financial pressure of laboratories. Consequently, the quarterly triple-site CT/NG screening imposes a financial burden on the healthcare system, PrEP users and laboratories.

Hence, there is a need for methods that reduce the number of CT/NG molecular assays to perform. In this context, **two different pooling methodologies** to test for CT/NG using two different testing platforms (ie. Abbott CT/NG RealTi*m*e and Xpert CT/NG assay of Cepheid) were described in this thesis. Both pooling methods were designed to identify the anatomical site of infection without the need to take an additional sample of the patient (see chapter 6). Furthermore, the GeneXpert pooling method deliberately relied on Eswabs™ in order to optimize the surveillance of antimicrobial resistance of NG. Though the Eswab™ allows prolonged transport time (up to 72 hours), the viability of NG decreases over time.[39] However, deferred NG culture using Eswabs™ after molecular detection was previously reported to be feasible within 24-48h.[40] Using the GeneXpert platform, CT/NG results may even be available within 2 to 24 hours increasing the success rate of NG culture.

As recognized by the STI community, the major limitation of pooling samples to test for CT/NG is, due to further sample dilution, the reduced assay sensitivity as compared to single-site testing, implying that low bacterial load samples may be missed. In our pooling methods, the dilution factor was kept to a minimum to avoid unnecessary excessive dilution and consequently false negative results which was found to be acceptable in our methodologies. **Although a reduced sensitivity was noted, the increasing necessity for triple-site testing due to the high burden of extra-genital infections justifies the implementation of pooling strategies for CT/NG testing which will, in addition, lead to significant cost savings.**

8.6 RECOMMENDATIONS FOR FUTURE STI CONTROL AMONG MSM

Taking into account all the previous data and considerations, the following recommendations could be made to further control the STI epidemic among MSM whereby individuals with recurrent STIs are targeted.

First, **screening for STIs among MSM should continue** and particularly MSM taking PrEP. STI screening among MSM should include HCV, syphilis, CT/NG at the three sampling sites including detection of anorectal LGV, but not MG. Laboratories may use **validated pooling CT/NG testing methodologies**.

Second**, STI screening algorithms should be tailored to sexual behaviour and frequency of STIs**. The Belgian PrEP guidelines recommend quarterly CT/NG screening which places an enormous pressure on healthcare services; PrEP is taken for several years. Therefore, alternative algorithms targeting individuals with recurrent STIs could be implemented (Fig 4):

- During the first year of PrEP use, patients are tested quarterly for CT/NG in order to identify the individuals with recurrent STIs.
- After one year depending on the STI results and the patient's sexual behaviour, CT/NG testing frequency will be tailored specifically to the individual PrEP user.
    - In the event no or only one STI (except for LGV, active HCV or active syphilis) was detected in the first year and sexual behaviour was unaltered, CT/NG testing should only be performed once yearly.
    - In case the individual had more than one STI episode, concurrent STIs or an LGV, active syphilis or active HCV infection in the preceding year, and maintained sexual risk behaviour; quarterly or minimum biyearly CT/NG screening should be continued.

- Finally, in case sexual risk behaviour patterns changed, the frequency of CT/NG testing may be reassessed depending on the judgement of the health care provider.

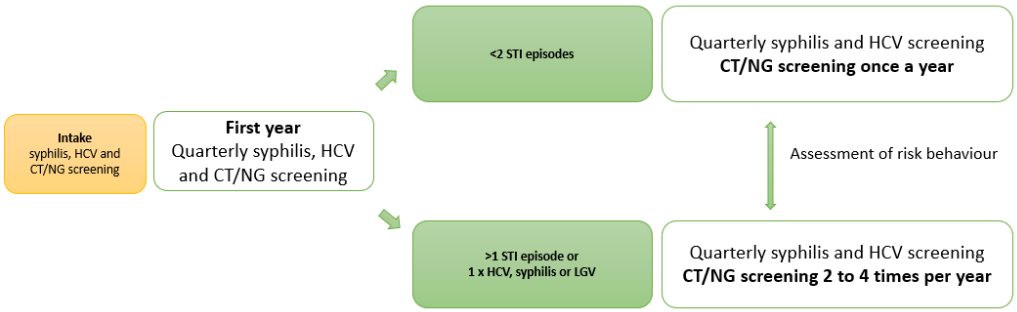

Figure 4: Alternative STI testing algorithm among PrEP users. HCV: Hepatitis C Virus; CT/NG: Chlamydia trachomatis/Neisseria gonorrhoeae

Evidently, such an alternative STI testing algorithm will require prospectively testing in clinical studies before implementation in regular care. However, using retrospective data of PrEP clinics, the ability to lower costs and to avoid missed CT/NG infections by this STI testing algorithm may already be investigated.

Third, **detected STI infections should be immediately treated.**

Fourth, next to early diagnosis and treatment of STIs, **behavioural interventions such as risk-reduction counselling are paramount** to support PrEP users in the most optimal way. Tailored risk reduction counselling should include **a personalized risk assessment including sexualized drug use**, partner notification and, in case of individuals with recurrent STIs, further sensitization about the public health consequences of recurrent STIs.

Finally, the use of **mHealth applications** among PrEP users or other individuals in need of frequent STI screening should be further explored. Ideally, mHealth applications should be part of routine care, therefore, these applications should include **STI home-based self-sampling**, adherence reminders for PrEP or any other HIV/STI related

therapy, an HIV/STI self-risk assessment tool, HIV/STI prevention and harm reduction information, partner notification, linkage to care, and a dynamic map showing the available STI services for treatment. Furthermore, mHealth applications may be used in situations where accessibility to regular care is not obvious such as in the current Covid-19 pandemic.

8.7 EXTRAPOLATION TO OTHER POPULATIONS OR SETTINGS

8.7.1 Low-resource settings

At the start of this thesis (February 2019), the main objective was to optimize the control and diagnostic of STIs in high- and low-resource settings. Since 2018, our team coordinated the STI part of the CohMSM-PrEP study in four West-African countries (including Togo, Côte d'Ivoire, Mali and Burkina Faso). The CohMSM-PrEP study is a demonstration study that assessed the feasibility and acceptability of oral PrEP in West-Africa and baseline CT/NG results are reported in the GeneXpert CT/NG pooling strategy paper (chapter 6.2). CT/NG prevalence figures in West-African MSM were 14.5% for CT and 11.5% for NG (quite similar to Belgium) and most of the CT/NG infections were of extra-genital origin (77.6%). As in Belgium, the majority of the infections were asymptomatic (88.2%). Interestingly, no LGV case was detected. Unfortunately, the global Covid-19 pandemic resulted in the suspension of all international travel including the shipment of biological samples. As such, our focus was reoriented on STI control and diagnosis in Belgium solely. Nevertheless, results of this thesis may help to control STIs among PrEP users in low-resource countries. Indeed, as required by the WHO, all PrEP services should integrate STI control including treatment, detection, and prevention. Moreover, the WHO urges to move away from syndromic approach to treat STIs because of the inherent inability of this strategy to 'treat' asymptomatic infections infections including the risk to overtreat or mistreat your patient.[41] Due to the increase in antimicrobial resistance in NG it is of utmost importance to effectively treat the patient. As such, the WHO endorses the use of

molecular amplification assays for syndromic management of STIs. However, if these assays or future sensitive point-of-care assays are unavailable, the syndromic approach can still be used as it is an essential component of managing people with symptoms of STIs in low resource settings. Furthermore, STI screening among African MSM is increasingly important due to concurrent female relationships. Indeed, in the CohMSM-PrEP study, 54% of African MSM reported to have a female partner in the previous three months.[42] These settings require a cheap, sensitive point of care assay to detect CT/NG. **The GeneXpert pooling methodology may represent an improvement to expensive and laborious molecular based triple-site testing in low-resource settings**. In addition, the widespread availability of the GeneXpert in African settings makes it feasible to implement reliable CT/NG detection into PrEP care in these settings. Unfortunately, few PrEP services in low-resource settings are currently implementing etiological STI screening mainly due to lack of financial resources.[43] Therefore, the alternative STI testing algorithm described above focuses on individuals who will have the highest impact on the STI epidemic and reduces the frequency of STI testing. Further studies of the feasibility of this algorithm and frequency of STI testing in this setting are needed.

8.7.2 Heterosexual men and women

Although pharyngeal NG testing in heterosexual men and women is currently not recommended, there is increasing evidence that they are also common among women and heterosexual men who reported recent sexual contact with a NG index case. In fact, 40% of the gonorrhea cases would have been missed in this population if screening only included urogenital testing.[26,44–46] Furthermore, voices are rising to perform universal anorectal testing among sexually active females.[26,47] As for men, these extra-genital infections are mostly asymptomatic and act as a reservoir which contributes to the overall CT and NG epidemic. Furthermore auto-inoculation from rectum to vagina

and cervix may take place which may lead to severe complications as mentioned in the introduction.

We have described two different pooling methodologies which were validated for MSM (chapter 6). In the meantime a pooling method for female sex workers has been reported by our Belgian colleagues from Gent.[48] In line with ours and other pooling methods a small reduction in sensitivity was reported. Although further CT genotyping is warranted in MSM, our research showed that LGV is very rare among women. Therefore, additional L-genotyping is not needed among asymptomatic women. However, MG is equally present among women and heterosexual men.[49] Moreover, in chapter 5, we reported on macrolide and fluoroquinolone resistance in MG in these populations. Although lower than among MSM, **macrolide resistance in MG was found in this heterosexual group which contributes further promotion of antimicrobial stewardship in the general population, and especially to limit macrolide usage as much as possible.**

Finally, the results obtained in this thesis were based on MSM PrEP users which are at very high risk for STIs. Therefore our results may not necessarily be generalizable to low-risk MSM.

8.8 FUTURE PREVENTION AND TREATMENT OPTIONS

There is now increasing evidence to stop using macrolides in the treatment of STIs. Of note, whereas AMR to macrolides in NG was almost 0 in 2013, it is now reaching 16% in 2020 in Belgium (see Figure 5).[50]

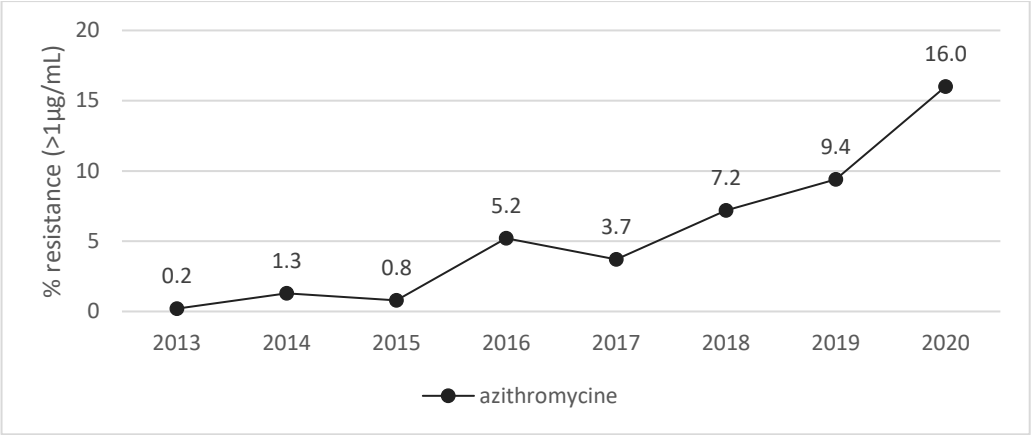

Figure 5: Azithromycin resistance in Neisseria gonorrhoeae isolates in Belgium from 2013-2020[50]

Other novel treatments are paramount in the control of STIs. Currently, chlamydia infections including LGV can be effectively treated with doxycycline. For MG, treatment with doxycycline will lead to microbiological cure in 40% of the cases.[51] Alternative screening algorithms whereby first doxycycline is given and sequential treatment (macrolides or fluoroquinolones) depends on macrolide resistance results, can also be implemented.[51] However, in our PrEP cohort, macrolide resistance reached almost 90%, therefore macrolide resistance-guided treatment is redundant in this population. Otherwise, fluoroquinolones such as moxifloxacin could be given, but as we have reported in our thesis, antimicrobial resistance to fluoroquinolones is also beginning to emerge. Other antimicrobials under investigation for the treatment of MG are sitafloxacin or solithromycin. Yet, in the future, dual antimicrobial therapy may be necessary to treat MG due to the rapid speed of antimicrobial resistance.[52]

Finally, NG treatment guidelines are also currently under review. In North-America, recent NG guidelines removed azithromycin from their treatment regimen and are recommending single use of ceftriaxone.[53] The rationale is based on the severe impact macrolide use has on the microbiome and on other pathogens such as MG, the increasing incidence of macrolide resistance in NG and the continued low incidence of

ceftriaxone resistance. European guidelines are still recommending the dual use of azithromycin and ceftriaxone.[54]

Besides novel antimicrobial treatments, new prevention methods, such as vaccines, are urgently needed. Besides effective vaccines for HPV and HBV, less research is done on bacterial STIs as they are still treatable with antimicrobials. However, due to the emergence of multi-drug resistant NG isolates, the development of NG vaccines, becomes imperative. Unfortunately, due to high levels of surface antigenic variation of NG, no vaccine is yet available.[55] Currently, investigators are looking into the potential utility of meningococcal serogroup B vaccines in eliciting a cross-protective effect against NG. The effectiveness of this vaccine against NG was estimated to be 31%, however larger randomized controlled trials have to be conducted.[55] In the absence of protective natural immunity against recurrent infections and the availability of effective vaccines, control of STIs currently still relies completely on active antibiotic therapy. Other novel antibiotic-sparing methods such as antibacterial mouthwashes or phage therapy are possible alternatives. However, despite intensive research by an Australian group (Chow et al.) and a group in our institute (Kenyon et al.) on the use of oral mouthwashes to control oropharyngeal gonorrhea, early results are rather disappointing.[56–58] The search for possible active phages against NG is ongoing in the unit of Chris Kenyon and other researchers. Furthermore, it has been speculated that healthy microbiota can protect against STIs. Indeed, in women, *Lactobacillus spp.* in the vaginal microbiome hinders the stablishment of infection by pathogens.[59] Studies documenting the protective role of the different microbiota (anorectal, urethral, penile and pharyngeal) in men are however lacking. Taking all results into account, the development of effective vaccines and affordable antimicrobial treatments to treat STIs are extremely paramount. Additionally, other non-pharmaceutical interventions focusing at behavioural changes and condom use are still imperative in STI control.

8.8.1 Pre-exposure prophylaxis for STIs?

The overwhelming effect of PrEP to prevent HIV, prompted investigators to look into the possibility to use doxycycline as Post- or Pre-exposure prophylaxis for STIs.[60–62] Although promising results were obtained with doxycycline-post-exposure prophylaxis to prevent chlamydia or syphilis (70 to 73% relative risk reduction) in MSM PrEP users, no reduction was observed for NG nor MG.[9,60] Moreover, public health experts are extremely worried about the potential harms that prolonged doxycycline PEP/PrEP may have on the individual (adverse events) and on the emergence of antimicrobial resistance. Indeed, while resistance of CT and *Treponema pallidum* to doxycycline is rare, high rates of tetracycline resistance in NG have been reported globally.[62] Furthermore, resistance associated mutations to tetracyclines have been found in MG among MSM PrEP users.[9] These results raise questions on the use of doxycycline-PrEP/PEP to prevent STIs and the effect it may have on the emergence of antimicrobial resistance. Notwithstanding the reluctance of the scientific community to use doxycycline-PrEP/PEP to prevent STIs, a very recent study among PrEP users in the UK, reported that 9% of PrEP users consumed off-the-counter doxycycline PrEP/PEP.[63] To date, data on the use of doxycycline PrEP/PEP in Belgian PrEP users are lacking and should be further investigated.

8.8.2 New diagnostic assays?

Early diagnosis of STIs is an essential pillar of STI control. Although that NAATS are still the gold standard to detect STIs (except for syphilis), there is a high need for qualitative rapid diagnostic tests (RDTs) to test for STIs. Indeed, such tests may have an enormous impact on STI control, however, these tests must adhere to the REASSURED criteria that are outlined in Table 2.[64]

Table 2: The REASSURED criteria for an ideal rapid test

	Description
R	Real-time connectivity (tests are connected and/or a reader is used to read test results to provide required data to decision makers)
E	Ease of specimen collection
A	Affordable
S	Sensitive
S	Specific
U	User-friendly
R	Robust and Rapid (results within 30 minutes)
E	Equipment-free
D	Deliverable to those who need them

RDTs for syphilis do exist, however, they mostly rely on treponemal-antibody detection, remaining positive lifelong, and therefore, cannot be used to distinguish between active and past infections.[65] Good RDTs with dual detection of treponemal- and non-treponemal antibody detection are urgently needed. To date, no qualitative RDT to detect CT/NG is available.[66] However, molecular near point-of-care CT/NG assays with a turnaround time <30 minutes are now becoming increasingly available.[67] Nevertheless, such kind of assays cannot be used as RDTs as they require complex instruments and stable electricity. Molecular detection of STIs also warrants prudence as new variants of the pathogens may in fact evade these techniques by altering the target site of the assay. Indeed, diagnostic selective variants of CT emerged in Sweden and Finland.[68,69] Moreover NG isolates with *porA* pseudogene deletion have also been detected (target which is frequently used in NG confirmatory molecular assays).[70] Although this deletion is very rare (<0.4%), microbiologists should remain vigilant and a sudden decrease in incidence warrants further investigation.

Nevertheless, the WHO demands new innovations including the development of reliable, low-cost, point-of-care assays in order to reach the targets to lower gonorrhea and syphilis incidence with 90% by 2030.[71]

8.9 CONCLUSION AND FUTURE PERSPECTIVES

Accessible and high-quality STI health care in order to rapidly detect and treat STIs is one of the cornerstones of STI control. The results of the Be-PrEP-ared study showed that MSM engaging in high risk sexual behaviour, part of dense sexual networks and at risk for recurrent STIs, are willing to comply with PrEP use combined with strict HIV and STI follow-up. PrEP now provides the opportunity to bring these HIV negative MSM into care. In Belgium, the number of PrEP users is rising yearly. At the end of 2019, around 4000 individuals had started PrEP and this figure is estimated to increase by 40% per year.[72] Since PrEP follow-up visits exert an enormous financial and work burden on the laboratories, health care services and PrEP users, alternative STI testing algorithms are urgently required to allocate the resources to those most in need such as individuals with recurrent STIs. Indeed, we have now shown that these individuals play an important role in the STI epidemic by sustaining the transmission of STIs including LGV within the MSM community. Furthermore, we showed that antimicrobial resistance of MG was higher among individuals with recurrent STIs. Therefore, they should be targeted using novel STI testing algorithms.[73] To this end, we here proposed such an algorithm which now should be further evaluated in clinical studies. Furthermore, the reported CT/NG pooling methodologies may contribute to lower the financial burden. Moreover, we showed that home-based sampling for STIs was acceptable for PrEP users. The current COVID-19 pandemic underscored the need of mHealth services including home-based sampling for PrEP users to ensure an adequate monitoring.

In 2019, almost all PrEP users in Belgium were MSM (98%), only one HIV infection was reported due to inconsistent PrEP use, however, at least one STI was diagnosed among 23% of the PrEP users. Unfortunately, the magnitude of recurrent STIs is not yet

included in the PrEP surveillance. In Australia, the number of individuals experiencing recurrent STIs rose from 16% before PrEP use to 20% after one year of PrEP use.[74] Limiting our data to the first 12 months instead of 18 months, a similar proportion of PrEP users with recurrent STIs was found (21%). However, the number of individuals with recurrent STIs rose to 28% in the last 12 months of the study (data not included in thesis). Thus, we urge Sciensano, the Belgian public health agency, **to set up a surveillance system in order to monitor the number of recurrent STIs among PrEP users**. Such a system is easy to implement in the PrEP surveillance and will provide unique data on the STI epidemic among PrEP users. It will also provide the possibility to assess whether this core group that may fuel the STI epidemic among PrEP users is enlarging and inform health care providers about the importance of these individuals on the STI spread.

To conclude, although our results will aid the effective control of STIs among MSM by implementing new screening strategies such as pooling of samples, home-based sampling and identification of PrEP users at risk for recurrent STIs; case detection and treatment alone will not be able to reduce the incidence of STIs with 90% by 2030 as stated by the WHO.[71] Primary prevention activities such as STI risk-reduction counselling and education about antimicrobial resistance, safer sex promotion such as condom use, expedite partner notification and behavioural changes towards STIs are thus crucial.

8.10 REFERENCES

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ANNEXES

9.1 ANNEX 1 - PROTOCOL PAPER OF THE BE-PREP-ARED STUDY

JMIR RESEARCH PROTOCOLS De Baetselier et al

Protocol

Pre-Exposure Prophylaxis (PrEP) as an Additional Tool for HIV Prevention Among Men Who Have Sex With Men in Belgium: The Be-PrEP-ared Study Protocol

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9.1.1 Abstract

Background: Pre-exposure prophylaxis (PrEP) is a promising and effective tool to prevent HIV. With the approval of Truvada as daily PrEP by the European Commission in August 2016, individual European Member states prepare themselves for PrEP implementation following the examples of France and Norway. However, context-specific data to guide optimal implementation is currently lacking.

Objective: With this demonstration project we evaluate whether daily and event-driven PrEP, provided within a comprehensive prevention package, is a feasible and acceptable additional prevention tool for men who have sex with men (MSM) at high risk of acquiring HIV in Belgium. The study's primary objective is to document the uptake, acceptability, and adherence to both daily and event-driven PrEP, while several secondary objectives have been formulated including impact of PrEP use on sexual behaviour.

Methods: The Be-PrEP-ared study is a phase 3, single-site, open-label prospective cohort study with a large social science component embedded in the trial. A total of 200 participants choose between daily or event-driven PrEP use and may switch, discontinue, or restart their regimen at the 3-monthly visits for a duration of 18 months. Data are collected on several platforms: an electronic case report form, a Web-based tool where participants register their sexual behaviour and pill use, a more detailed electronic self-administered questionnaire completed during study visits on a tablet computer, and in-depth interviews among a selected sample of participants. To answer the primary objective, the recruitment rate, (un)safe sex behaviour during the last 6 months, percentage of reported intention to use PrEP in the future, retention rates in different regimens, and attitudes towards PrEP use will be analysed. Adherence will be monitored using self-reported adherence, pill count, tenofovir drug levels in blood samples, and the perceived skills to adhere.

Results: All participants are currently enrolled, and the last study visit is planned to take place around Q3 2018.

Conclusions: As PrEP is not yet available in Belgium for use, this study will provide insights into how to optimally implement PrEP within the current health care provision and will shape national and European guidelines with regard to the place of PrEP in HIV prevention strategies.

KEYWORDS

Pre-exposure prophylaxis; HIV prevention; MSM; Belgium; daily; event-driven; demonstration project; acceptability; adherence

9.1.2 Introduction

Pre-exposure prophylaxis (PrEP) using Truvada (emtricitabine/tenofovir disoproxil fumarate) is a promising addition to the field of HIV prevention. Several clinical trials among men who have sex with men (MSM) at high risk of HIV infection have shown that daily PrEP is effective in preventing HIV when taken correctly.[1-3] Within the European context, the UK-based PROUD clinical trial examined daily use, whereas the French Ipergay study tested event-driven PrEP (i.e., on demand, before and after anticipated sex). Both trials yielded significant protection effects (86% reduction in incident HIV),[4,5] showing that PrEP is a very promising tool to prevent HIV within this high-risk population.

Daily Truvada use for PrEP was approved by the Food and Drug Administration (FDA) in the United States as early as July 2012.[6] As of September 2015, the World Health Organization (WHO) recommended that people at substantial risk of HIV infection should be offered PrEP as part of combination prevention approaches.[7] The European HIV prevention landscape is also changing rapidly. In November 2015, daily and event-driven Truvada as PrEP, in combination with safer sex practices, was approved in France.[8] After recommendation by the European Medicines Agency (EMA), the European Commission approved once-daily Truvada as PrEP in combination with safer sex practices in August 2016.[9,10] Norway joined France by providing PrEP free of charge to at-risk groups in October 2016, and the United Kingdom will make PrEP available in the context of a large clinical study in mid-2017.[11,12] While European PrEP guidelines are available, they provide little detail and remain general, and large-scale implementation guidelines are lacking.[13] Therefore context-specific experiences with PrEP delivery that can help to shape appropriate recommendations are urgently needed.

The number of MSM in Belgium is estimated to be around 106,000, which is 4.2% of the total male population.[14] As in many western European countries, MSM represent a high-risk population for both HIV and other sexually transmitted infections (STIs) including gonorrhea, syphilis, and chlamydia infection. In 2013, 61% of all registered STIs in men in Belgium were reported among MSM (sentinel surveillance).[15] Since 2002, there is a trend of increasing numbers of new HIV infections among MSM, who represented 50% of the 1001 newly registered HIV infections in Belgium in 2015.[16] A venue-based, cross-sectional study was conducted in 2009-2010 among 649 MSM in 2 Flemish cities in Belgium, Antwerp and Ghent, and revealed HIV prevalence's as high as 14.5% in cruising venues to 4.9% in more general gay venues to 1.4% at younger MSM venues.[17]

PrEP is a potential game-changer for the HIV epidemic among MSM in Western Europe including Belgium, but little is known about how PrEP will be used and how different regimens will influence sexual behaviour and lifestyles of MSM.

The overall aim of this study is to provide the necessary data to shape Belgian and European guidelines with regard to the place of PrEP in strengthening HIV prevention. To this end, the following primary and secondary study objectives have been formulated.

The primary study objectives are as follows:

- To document the current preventive needs of MSM at high risk of acquiring HIV, including the uptake, acceptability, and feasibility of using PrEP daily or event-driven
- To evaluate adherence to the 2 different PrEP regimens

The secondary objectives can be found in Textbox 1.

- To study the impact of pre-exposure prophylaxis (PrEP) use on other preventive strategies such as condom use
- To study the impact of PrEP use on sexually transmitted infection (STI) trends
- To study the safety of daily and event-driven use of PrEP
- To document real-life effectiveness of PrEP use on HIV seroconversion and treatment-related resistance
- To evaluate the feasibility of 3-monthly HIV testing using oral fluid self-sampling testing

Textbox 1: Secondary objectives of the Be-PrEP-ared study

9.1.3 Methods

Study Design

The Be-PrEP-ared project is a single-site, open-label prospective cohort study with a nested qualitative component. It takes place in the HIV/STI clinic of the Institute of Tropical Medicine (ITM), Antwerp, Belgium. A total of 200 MSM at high risk of acquiring HIV were enrolled and are being followed up for 18 months, with 3-monthly follow-up (FU) visits. Truvada is being provided to them as part of a comprehensive HIV prevention package including regular HIV/STI testing and basic adherence and sexual health counselling.

Participants

Eligibility is assessed using the inclusion and exclusion criteria as shown in Textbox 2.

Inclusion criteria:

- Able and willing to provide written informed consent
- Born to male sex (including transgender females)
- Aged 18 years or more
- Had sex with a man in the last 12 months
- HIV negative (confirmed at enrolment)
- Reporting at least 1 criterion for high risk:
    - Condomless anal intercourse in the last 6 months with a casual partner with unknown HIV status or HIV positive status
    - A sexually transmitted infection episode in the last 6 months
    - Having taken post-exposure prophylaxis in the last 6 months
- Able and willing to participate in the project as required by the protocol for 18 months
- Motivated to strengthen prevention efforts, including willingness in starting to use pre-exposure prophylaxis

Exclusion criteria:

- Having symptoms or clinical signs consistent with acute HIV infection
- Being allergic to the active substances or any of the excipients
- Having an estimated creatinine clearance of <60 mL/minute/1.73 m^2 according to the CKD-Epi formula (Chronic Kidney Disease Epidemiology Collaboration)
- Having an active hepatitis B infection
- Taking HIV post-exposure prophylaxis
- Participating in other clinical studies (phase I-III) or another research project related to HIV and antiretroviral therapy

Textbox 2: Selection criteria of the Be-PrEP-ared study.

Sample Size

Given the provided funding, we included 200 MSM at high risk of acquiring HIV. The sample size was determined to estimate the proportion of participants who are nonadherent. Using the normal approximation for the calculation of the 95% confidence

interval and using the worst-case value for proportion of nonadherence of 0.5 with 200 participants, the proportion of nonadherence can be estimated to a precision of 7%.

Participant Recruitment

The project has been advertised by websites of various community-based gay and sexual health organizations and their respective social network sites and person-to-person promotion of the project. Referral to the project is also done by health care providers at the HIV/STI clinic at ITM. Potential participants were invited to preregister on the project website[18] where details about study participation were also provided. Registered candidates were then invited for a screening visit at the clinic in random order so that they were not invited in the same order as they registered with the exception of the last candidates, who were invited on a "first come first served" basis.

Investigational Product

Truvada is used for PrEP in this trial. One daily film-coated tablet contains 200 mg of emtricitabine and 245 mg of tenofovir disoproxil fumarate.

When eligible, participants can self-select between 2 different PrEP dosing regimens:

- A pill every 24 hours (further referred to as daily)
- Starting dose before anticipated sex and 1 pill daily during a sexual active episode (further referred to as event-driven). This is the regimen which has been described by the Ipergay protocol (Figure 1):[4]

    - A dose of 2 pills between 2 and 24 hours before having sex (or 1 pill if the most recent dose was taken between 1 and 6 days ago)
    - A tablet of Truvada every 24 hours (starting when the first 2 tablets are taken) during the period of sexual activity including after the last sexual intercourse
    - Finally, a last dose of 1 tablet of Truvada approximately 24 hours later
    - Tablets need to be taken every 24 hours with a window period of 2 hours before or after the scheduled time.

All participants, irrespective of their regimen, can opt to switch regimen and to discontinue or to (re)start using PrEP at every FU visit at the HIV/STI clinic at ITM. Before being given a new supply, the participant must be confirmed to be HIV negative using HIV point-of-care tests.

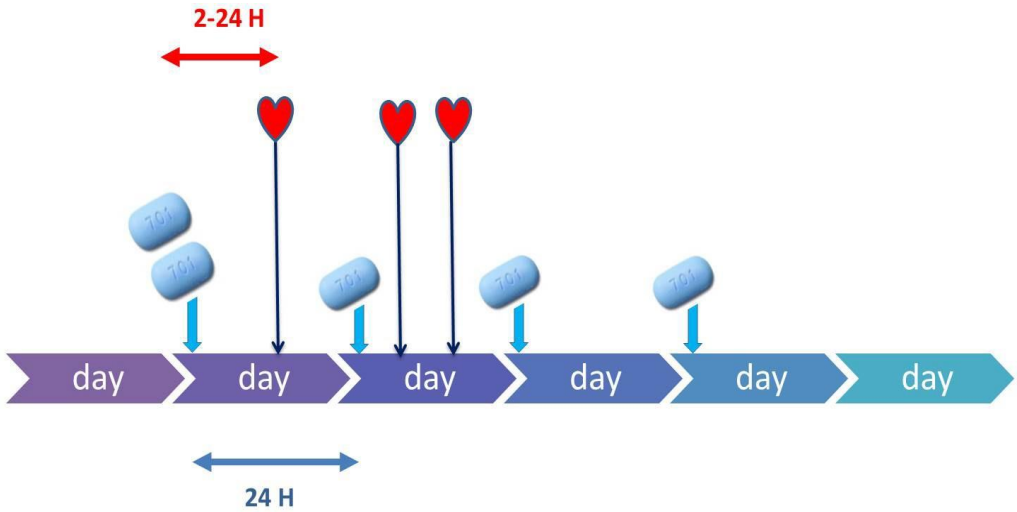

Figure 1: Event-driven scheme (adapted from the Ipergay protocol).

All participants are followed up for 18 months and will undergo a total of 9 prescheduled visits corresponding to screening, enrollment, FU month 1, and 6 3-monthly FU visits. During every prescheduled visit, participants see a social scientist (or social science assistant), a study nurse, and a physician.

Table 1 summarizes the study procedures. At the screening visit after written informed consent is obtained, study staff collects data on basic socio-demographics and current sexual behaviour with special attention to high risk criteria (see Textbox 2); performs a physical examination with special attention to symptoms of an acute HIV infection; collects blood, urine, anal, and pharyngeal samples for kidney, liver, HIV, and STI testing; and performs preventive counselling.

The participant is invited to come back to the clinic within 2 weeks to confirm eligibility, including a reassessment of symptoms of acute HIV infection, relevant medical history, and current medication and recreational drug use. When eligible, the participant receives 1 box of 30 Truvada tablets, and the different PrEP regimens are discussed. Detailed information on PrEP use, adherence counselling, and preventive sexual health counselling is provided. Study staff explains the use of an online diary to collect data on sexual activity and adherence throughout study participation. The participant is also instructed to complete a self-administered questionnaire on a tablet computer.

At every follow-up visit, adverse events and concomitant medication are documented and a physical examination and HIV testing are performed. In addition, participants are screened for STIs every 3 months. Oral fluid samples are taken every 3 months using the Intercept i2 collection device (OraSure Technologies Inc) for future HIV testing.

PrEP-related toxicity is monitored, adherence and prevention counselling is provided, and PrEP use is discussed. The participant takes the leftover Truvada back to the clinic and gets a refill to cover the needs until the next visit, with a maximum of 90 tablets. Intra- and extracellular drug level assessment of tenofovir or tenofovir diphosphate is performed at month 1 and month 3. Afterward, drug level monitoring will only take place for a proportion of daily users and some interesting event-driven users at the end of the study.

At FU month 15, specific counselling will address the impact of discontinuing PrEP use and will support participants in developing personal risk reduction solutions, since PrEP may not be available and reimbursed after individual completion of the study.

Different methods are being used to measure adherence: online diary, pill counts, questionnaire, and drug level assessment. Results of these measurements will be triangulated to assess adherence. When a participant is diagnosed with an STI, treatment will be given according to national guidelines.

Table 1: Schedule of assessments

Procedures	Screening (1-2 weeks prior)	Enrollment (day 0)	FU[a] month 1	FU month 3, 6, 9, 12, 15	FU month 18
Informed consent	X				
Relevant medical history		X			
Current/concomitant medication		X	X	X	X
Adverse events			X	X	X
Diary collection			X	X	X
HIV rapid test	X		X	X	X
HIV antigen test	X		X	X	X
Syphilis	X			X	X
HSV-2[b]	X			X[c]	X[d]
Hepatitis B	X				X
Hepatitis C	X			X[c]	X[d]
Creatinine	X				X
Phosphate	X				X
ALT/AST[e]	X				X
Proteinuria	X			X	X
CT/NG/MG/TV[f] (urine)	X			X	X
Anorectal and pharyngeal swab for CT/NG/MG/TV	X			X	X
Oral fluid collection				X	X
Drug level testing (blood/hair)			X	X	X
Provide Truvada		X	X	X	
IDI[g] (subsample of men)			X	X[h]	X
Questionnaire		X	X	X	X
Preventive sexual health counselling	X	X	X	X	X
Adherence counselling		X	X	X	X

[a]FU: follow-up; [b]HSV-2: herpes simplex-2 virus; [c]Will only be done using a look-back procedure when the final visit is positive to determine the time of infection more accurately, if funding permits; [d]Only when screening visit result was negative; [e]ALT/AST: alanine transaminase/aspartate transaminase [f]CT/NG/MG/TV: Chlamydia trachomatis / Neisseria gonorrhoeae / Mycoplasma genitalium / Trichomonas vaginalis; [g]IDI: in-depth interview; [h]Only month 9.

Online Diary

The participant is asked to complete an online diary with information regarding pill intake, number of rectal and oral sex acts, and an individual risk assessment of acquiring HIV for each day participated in the study. A Web platform is created where participants can log in with a personal account using their smartphone, laptop, or other devices. They are instructed to complete the online diary every day or at least twice a week to limit recall bias. At every visit, and more clearly emphasized at the month 1 visit, the social scientist or designee examines all diaries for completion to allow for optimal data collection and verifies whether participants encountered problems or difficulties or unclear procedures when completing the online diary. Participants also receive an email when they have not completed their diary for more than 7 days. Event-driven users are able to complete the diary only during sexual active periods. In addition, a paper version is available if preferred. Finally, an audit trail is available.

Questionnaire

At the enrollment visit, a detailed electronic self-administered and standardized questionnaire collects data on sociodemographic characteristics, well-being, sexual lifestyle, sexual behaviours, and determinants related to HIV risk, as well as motivations for (not) choosing either PrEP dosing regimen. It was based on similar questionnaires used in other PrEP studies or HIV prevention and sexual behaviour research among MSM. The questionnaire was pilot-tested before study initiation among 7 MSM, and their suggestions were incorporated into the final version. It was translated and back-translated in 3 different languages: Dutch, French, and English. At the FU visits, a shortened version is used assessing adherence, recent sexual behaviour, and reasons for switching PrEP dosing regimen if applicable. At FU month 9, a more comprehensive questionnaire is used reassessing several measures of the enrollment questionnaire (e.g., well-being). At FU month 18, a questionnaire is used similar to FU month 9 that includes questions assessing participant experiences of and attitudes toward using and receiving PrEP, for which the content will be informed by the in-depth interviews (IDIs) and their experiences of study participation such as the collection of oral fluid for HIV testing.

In-Depth Interviews

To explore participant prevention needs, their preferences for and attitudes toward PrEP use (i.e., regimen choice and decision making), user experiences, and perceived influences on their sex lives, 35 to 40 IDIs are conducted throughout the project. All interviews are conducted by a social scientist with expertise in qualitative research after

obtaining informed consent. The data collection and analysis is guided by an inductive approach based on grounded theory.[19,20] The topic guide is developed within the study team and is amended where necessary to improve data collection and account for an iterative qualitative data collection approach without losing consistency.[21] Dutch- or English-speaking participants are purposely selected based on information-rich events (e.g., switching PrEP regimen) and availability. Triangulating the results of the IDIs with other quantitative data from the trial will allow for improving validity of the overall study results.

Laboratory Procedures

Table 2 provides an overview of all laboratory tests that are performed in the study. All testing is performed at ITM except for drug level testing which is performed at the University Hospital of Gent, Belgium. Dry blood spots (DBS) are taken at every visit where blood is taken and stored together with oral fluid and hair samples (when consented) for future HIV testing or drug level testing.

Table 2: Be-PrEP-ared laboratory procedures.

To be tested	Kind of test
HIV	See Figure 2
Syphilis	RPR[a] (Macro-Vue, BD) and TPA (Vitros 5600) /TPPA[b] (Fujirebio)
Hepatitis B	HBsAg/HBsAb[c], HBcIg/HBcIgM[d] (Vitros 5600)
Hepatitis C	Antibody Hepatitis C (Vitros 5600)
Biochemistry: AST/ALT[e], creatinine, and phosphorus	Creatinine clearance calculated using CKD-Epi[f] formula (Vitros 5600)
Proteinuria	Urine dipstick (Siemens Hema-Combistix)
Chlamydia trachomatis (CT)/Neisseria gonorrhoeae (NG)	Abbott Real Time *CT/NG* with confirmation by in-house PCR[g]
Mycoplasma genitalium (MG)/ Trichomonas vaginalis(TV)	In-house PCR for *MG* and Diagenode (S-DiaMGTV) for *TV*
Herpes simplex virus-2 (HSV-2)	Kalon HSV Type 2 IgG
Plasma and upper layer packed cell drug level testing	Thermo Scientific Q-Exactive hybrid quadrupole–Orbitrap mass spectrometer (LC-MS/MS[h] system)
HIV-1 resistance	RNA sequencing
HIV-1 viral load	Cobas 4800 (Roche)

[a]RPR: rapid plasma reagin; [b]TPA/TPPA: Treponema pallidum assay/ Treponema pallidum particle agglutination assay; [c]HBsAg/HBsAb: hepatitis B surface antigen/hepatitis B surface antibody; [d]HBcIg/HBcIgM: total hepatitis B core antibody/hepatitis B core IgM antibody; [e]AST/ALT: aspartate transaminase/alanine transaminase; [f]CKD-Epi: Chronic Kidney Disease Epidemiology Collaboration; [g]PCR: polymerase chain reaction; [h]LC-MS/MS: liquid chromatography coupled with tandem mass spectrometry

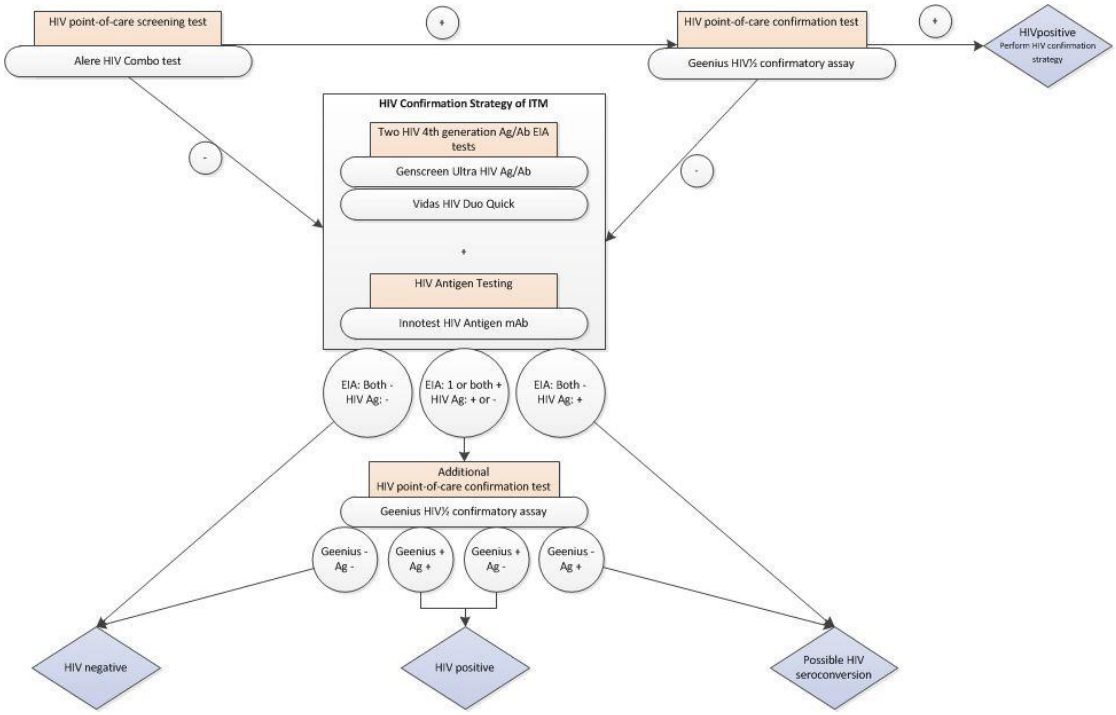

Figure 2: HIV algorithm in the study

HIV Seroconverter Procedures

If a participant becomes HIV positive during the study, he is discontinued immediately from the study but is followed up for safety. In this case, study staff collects all unused pills, conducts the final visit procedures including resistance and viral load testing, discusses the pros and cons of early ARV therapy, and refers the participant to an AIDS reference centre of choice for linkage to HIV care.

Safety

Safety and tolerability of Truvada is evaluated by recording adverse events (AEs) and grading laboratory and vital signs evaluations in the electronic case report form (eCRF) starting from enrollment until the final visit. Severity, causality, and outcome are also assessed by the study physician. Any event that occurred before the enrollment visit is documented as medical history. All AEs are followed up until resolution to the extent possible. An HIV infection is not considered a serious adverse event (SAE). Due to the possible renal adverse effects of Truvada, elevations in serum creatinine are monitored closely. Truvada will be interrupted when creatinine clearance is below 60

mL/minute/1.73 m² and will be permanently discontinued when the clearance stays below 50 mL/minute/1.73 m² after repeat testing.

All SAEs whether or not deemed drug-related or expected are reported within 24 hours (1 working day) to the sponsor (ITM). Line listings of all reported SAEs are sent to the concerned ethics committee (EC) and the Belgian Competent Authority (CA) on a yearly basis. In addition, all fatal or life-threatening suspected unexpected serious adverse reactions (SUSARs) need to be reported to the Belgian CA and to the concerned EC within 7 days. Nonfatal and non–life-threatening SUSARs must be reported within 15 days. Gilead Sciences is notified immediately in case of SUSARs and will receive SAE listings every 2 weeks.

No formal data safety monitoring board (DSMB) has been set up due the fact that this drug is widely used and approved for treatment and prevention of HIV infection by the FDA and EMA. However, an independent data safety monitor has been appointed to review all SAE reports. In case of major safety concerns, this monitor may advise the sponsor to halt recruitment of the trial and/or organize a formal DSMB with a complete overview of the available safety data.

Data Collection

Databases

Due to the project's mixed methods approach, 4 different types of databases have been set up:

- A clinical trial database was programmed and validated prior to project start: an eCRF developed in the Good Clinical Practice (GCP)-compliant clinical trial software MACRO (InferMed, United Kingdom) with CFR 21 Part 11 in-built consistency checks is used.
- An online survey database is used for the questionnaires (using Survey to Go mobile survey software from Dooblo for the development of the online questionnaires).
- The online diary data is stored on a secured and password-protected Web platform that was created for the purpose of this project.
- The interview data from the IDIs is stored using a computer-assisted software program for data storage and analysis (NVivo 10.0, QSR International).

Confidentiality and Security of Trial Participant Data

Private information on trial participants is handled confidentially. Only the participant identification number, initials, and date of birth are captured in the eCRF and all other study documentation. Name and contact data for each participant is kept separately, and

access to them is limited to the authorized study staff. The same confidentiality rules apply for all study documents and electronic files. The computers and eCRFs are only accessible by the study staff with personal username and password. The online diary and survey data are stored on secured servers only accessible to the researchers.

Data Analysis

End Points

The uptake, acceptability, and feasibility of using PrEP will be examined by the end points documented in Textbox 3. A mixed method analysis of quantitative and qualitative end points will be conducted by triangulating the results from both data collection types.

Clinical Data Analysis

The study design is observational, and all analyses will be descriptive. As participants may change regimens, a single participant may be included in different regimens over time and contribute to person-months of daily, event-driven, or no use in the analyses.

Adherence may be dichotomized as adherent/nonadherent and is defined in general as the proportion of pills the participant needed to take that were actually taken. The number of pills actually taken is calculated based on self-reporting through the diary. Different indicators for adherence will be used for each of the regimens and per 3 months:

- Adherence to regimen: number of pills taken/number of pills which should have been taken according to the PrEP dosing regimen.
- Estimated proportion of covered sex acts: number of sex acts covered with PrEP/number of sex acts. This indicator will be calculated separately for high-risk sexual activities and low-risk sexual activities. High-risk sexual activities are defined as anal intercourse without a condom with a partner of unknown HIV status or known to be HIV positive with detectable virus load.

Exploratory analysis of predictors of retention on PrEP or (non)adherence may be performed using regression models (linear, logistic, or Cox-regression). Different groups may be considered for analysis depending on the change of regimen: group remaining on daily use, group remaining on event-driven, group changing from daily to event-driven, and group changing from event-driven to daily use.

All nonserious and serious AEs will be grouped according to a prespecified side-effect coding system and tabulated. The number of subjects experiencing any AE, any SAE, and any drug-related SAE will be summarized by PrEP regimen.

Preventive needs:

- Recruitment rate (number of screened participants/number of registered on study-specific website)
- (Un)safe sex behaviour during the last 6 months
- Percentage of reported intention to use pre-exposure prophylaxis (PrEP) in the future at final visit
- Retention rates in the different regimens
- Attitudes towards PrEP use: satisfaction and motivation for future use

Adherence:

- (In)consistent pill take, percentage of days with no pill taken/days on which a pill should have been taken
- Tenofovir drug levels in blood and/or hair samples
- Perceived skills to adhere, including self-efficacy

Impact of PrEP use on other preventive strategies:

- Number of sex partners
- Self-reported condom use
- Sex under influence (alcohol, drugs)

Impact of PrEP use on sexually transmitted infection (STI) trends (descriptive analyses only):

- STI incidence and trends: *Chlamydia trachomatis* / *Neisseria gonorrhoeae*, *Mycoplasma genitalium* / *Trichomonas vaginalis*, herpes simplex virus-2, syphilis, and hepatitis C

Safety of the different regimens of PrEP use:

- Rate of adverse events related to PrEP

Real-life effectiveness of PrEP use:

- Incidence of HIV infection by regimen
- Genotypic viral resistance

Feasibility of oral fluid self-sampling testing:

- Feasibility

Textbox 3: Endpoints of the Be-PrEP-ared study

Questionnaire Data Analysis

Analysis of the questionnaire data will be mainly descriptive, using uni- and bivariate statistical analyses. Depending on the results, multivariable statistical models and

longitudinal analyses may be used. All computations will be done using SPSS version 23.0 (IBM Corp).

Interview Data Analysis
Interview data will be analysed inductively based on grounded theory principles using multiple, independent coders. They will establish a data-driven codebook. This approach ensures triangulation from different perspectives [19,20] and thus improves data validity.

Ethics and Quality Assurance

The protocol and all study documents were reviewed and approved by the Institutional Review Board (IRB) of the ITM, the EC of the University Hospital of Antwerp, and the CA of Belgium. No study activities were performed before approval from all these bodies. Amendments to the protocol must be approved by the sponsor and by the concerned IRB, EC, and CA. Yearly updates are sent to all of these bodies. The study is carried out according to the principles stated in the Declaration of Helsinki as amended in 2013 and any further updates, all applicable national and international regulations, and according to the most recent International Conference on Harmonization (ICH) and WHO GCP guidelines. All laboratory activities are conducted in accordance with Good Clinical Laboratory Practices (GCLP) and EN-ISO (International Organisation for Standardization) 15189.

The study is monitored in accordance with regulations applicable to clinical trials, including ICH-GCP and GCLP requirements, and sponsor-specific monitoring and source data verification standard operating procedures. A trial management group is in charge of the day-to-day management of the clinical study.

Informed Consent

Before any study procedures took place, participants were asked to provide written informed consent.

Community Advisory Board

A Community Advisory Board (CAB) was set up with representatives from local and regional MSM community and health organizations and Belgian MSM prevention experts to ensure that the demonstration project meets the target group's needs. The researchers consult with this CAB twice yearly or more often if needed. Its main purposes are to assist in recruiting participants; to ensure proper feedback of the project results

to the community; and to assist in safeguarding the community's ethical, social, and cultural norms.

9.1.4 Results

The clinical trial part of the study has been registered in the EudraCT database (EudraCT 2015-000054-37). A total of 200 participants were enrolled on December 12, 2016. The last participant's last visit will take place around Q2 2018.

9.1.5 Discussion

Given EMA's recent approval of PrEP and recent national developments, we assume that PrEP will soon be available and may even be (partially) reimbursable in Belgium. Our study results will be useful for PrEP implementation as part of overall HIV combination prevention (e.g., screening guidelines for PrEP eligibility). Given the mixed method approach and longitudinal data collection, this study will provide insights into factors influencing PrEP use and choice of regimen—as participants are able to switch dosing regimen or to discontinue—and how it relates to user perspectives. Actual PrEP use may shift and be influenced by several factors such as users' self-perceived risk for HIV, actual user experience with PrEP, PrEP adherence, and perceived impact on sexuality. These findings could have important implications for HIV prevention policies and health care expenditure.

To our knowledge, the Be-PrEP-ared study is, together with the AMPrEP project of Amsterdam, the first study investigating the uptake of different PrEP dosing regimens at the choice of the participant.[22] Moreover, the study is novel in longitudinally exploring the preferences for and experiences of using the different regimens or discontinuing and restarting PrEP. Allowing participants to adapt PrEP use and different regimens to better suit periods of perceived high risk of HIV infection is novel and could lead to important insights into the need for PrEP within this high-risk population. Furthermore, such insights are crucial for developing appropriate adherence counselling guidelines and an optimal integration of PrEP within the already existing tools for HIV prevention.

One major strength of this project is its strong mixed methods approach, allowing for methodological triangulating of the various data sources and for exploring MSM's prevention needs in depth. This will lead to an improved understanding of the uptake and acceptability of PrEP use within the current context and greatly improves the validity of the results.[23]

Moreover, the mixed methods approach will lead to important in-depth insights about and knowledge of adherence to PrEP, one of the key outcomes of the project. In our study we combine different methods to assess adherence to PrEP. We test drug levels of tenofovir and tenofovir diphosphate immediately in plasma and upper layer packed cells at FU month 1 and FU month 3. With intracellular tenofovir diphosphate, an assessment of the adherence over the past 2 to 4 weeks can be made and "white-coat adherence" (ie, only taking PrEP just before the study visit) can be detected.[24] In addition, DBS and hair are also stored for future emtricitabine/tenofovir disoproxil fumarate therapeutic drug monitoring testing to reflect longer term windows of exposure when funding is available. Furthermore, the online diary will lead to improved insights into the number and timing of pills taken throughout the study, whereas the questionnaire and online diary will explore attitudes towards adherence to the medication.

Another strength is the participation of and close communication with health care providers and members of the HIV prevention and MSM community in Belgium through the CAB. The use of the CAB not only helps to raise awareness about the study and to more efficiently disseminate the results of the study, it is also crucial in developing study procedures and implementation guidelines that are sensitive to those who will be using PrEP.[25,26]

The number of MSM who registered for participation in the first 3 weeks of launching the registration study website (i.e., 196 in total) shows that there is an interest for PrEP within this population. However, interpreting the registration rate as a measure for PrEP acceptability would remain difficult: we did not overly promote study registration after the initial 3 weeks as it was clear that the desired number of participants would be reached; not all possible candidates may thus have been aware of the study and some may have anticipated not being able to participate in the study due to the promoted consultation hours (i.e., during office hours) or may have been unwilling due to setting-related reasons (e.g., distance to the clinic).

The slow recruitment rate was mainly due to limited staff resources (i.e., limited availability of study physicians) resulting in approximately 4 screenings per week. This could be an important limitation, as those enrolled first could differ from those enrolled last (i.e., as PrEP use is at least 1 year apart) and thus would not be comparable. Media coverage or PrEP availability, changing sexual norms within the community, and related factors may have had a different influence on participants. However, the study may reflect actual willingness to use PrEP and allows for exploring how an evolving HIV prevention landscape including access to PrEP affects individual PrEP uptake and adherence. Moreover, it cannot be excluded that individual differences between the

start and end of the study will be the result of similar influences rather than PrEP use on its own.

Conducting a demonstration project such as Be-PrEP-ared may in itself have an impact on HIV prevention that goes beyond providing ARV medication to participants. It has reinforced collaborations with community organizations and health care providers and can help in increasing PrEP awareness and influence policy on HIV prevention. In the wake of Be-PrEP-ared, various sub studies have already started in Belgium such as a survey among health care providers to assess their PrEP knowledge, attitudes, and willingness to prescribe. New studies are being set up in this evolving field, which will be important to allow for cross-national research. To develop a good understanding of how to optimally implement and provide PrEP integrated into the existing health care structures, new research will be of paramount importance.

In conclusion, results from this study will contribute to a better understanding of PrEP users' experiences including their choices for specific PrEP regimens. The findings will help to inform appropriate delivery strategies for the roll-out of PrEP and for policy makers to consider financial reimbursement of PrEP in Belgium.

9.1.6 Acknowledgments

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9.2 ANNEX 2 - BE-PREP-ARED BASELINE DATA

PREVENTION RESEARCH

Choosing Between Daily and Event-Driven Pre-exposure Prophylaxis: Results of a Belgian PrEP Demonstration Project

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9.2.1 Abstract

Background: Daily pre-exposure prophylaxis and event-driven pre-exposure prophylaxis (PrEP) are efficacious in reducing HIV transmission among men who have sex with men (MSM). We analysed baseline data from a PrEP demonstration project "Be-PrEP- ared" in Antwerp, Belgium, to understand preferences for daily PrEP or event-driven PrEP among MSM at high risk of HIV and factors influencing their initial choice.

Methods: Cross-sectional data from an open-label prospective cohort study, using mixed methods. Participants who preregistered online were screened for eligibility and tested for sexually transmitted infections (STIs). Eligible participants chose between daily PrEP and event-driven PrEP and reported on behavioural data through an electronic questionnaire. In-depth interviews were conducted with a selected subsample. Bivariate associations were examined between preferred PrEP regimens and sociodemographic factors, sexual behaviour, and STIs at screening.

Results: In total, 200 participants were enrolled between October 2015 and December 2016. Self-reported levels of sexual risk-taking before enrollment were high. STI screening revealed that 39.5% had at least 1 bacterial STI. At baseline, 76.5% of participants preferred daily PrEP and 23.5% event-driven PrEP. Feeling able to anticipate HIV risk was the most frequent reason for preferring event-driven PrEP. Regimen choice was associated with sexual risk-taking behaviour in the past 3 months. Almost all participants (95.7%) considered it likely that they would change their dosing regimen the following year.

Conclusion: Event-driven PrEP was preferred by 23.5% of the participants, which better suits their preventive needs. Event-driven PrEP should be included in PrEP provision as a valuable alternative to daily PrEP for MSM at high risk of HIV.

KEYWORDS

PrEP, MSM, HIV prevention, event-driven PrEP, daily PrEP

9.2.2 Introduction

With 1.8 million new HIV infections in 2016 worldwide, HIV prevention remains a public health challenge.[1] In Europe, condomless anal sex between men is the predominant mode of HIV transmission.[2] Almost 40% of all new HIV cases reported in 2016 in Europe were among men who have sex with men (MSM).[3] In Belgium, new HIV infections among MSM represented 52% of all HIV diagnoses in 2016.[4] Although progress has been made in reducing the number of HIV infections worldwide,[1] additional prevention strategies for key populations such as MSM are clearly needed to further reduce the number of new HIV infections.

Pre-exposure prophylaxis (PrEP), with oral emtricitabine and tenofovir disoproxil fumarate (FTC/TDF), is an efficacious biomedical tool for HIV prevention.[5] Its efficacy has been demonstrated in 12 clinical trials within different populations and geographical areas.[6] In 2015, two European clinical trials confirmed PrEP efficacy in reducing the risk of HIV among MSM at high risk of HIV: the PROUD study in England and IPERGAY in France and Canada.[7,8] Both showed PrEP to be safe and to reduce the risk of HIV by 86% among MSM at high risk. To translate clinical trial efficacy into population wide effectiveness, research informing an optimal implementation is crucial.[9] In particular, insights are needed on how to achieve correct use among those at highest risk, when they are at risk.

Individual-level risk factors for HIV acquisition among MSM have been well documented, such as having had condomless anal intercourse (CAI) and having a sexually transmitted infection (STI).[10] They have been successfully translated into PrEP eligibility criteria to maximize public health impact.[11] Less scientific attention has been devoted to the use of different PrEP dosing regimens to reflect different patterns in sexual risk-taking. Tailoring PrEP use to users' needs could increase its effectiveness, but requires a better understanding of personal regimen choices. This could further optimize prevention behaviour, public health impact, and cost- effectiveness of PrEP.[12]

Almost all clinical trials have focused on daily oral PrEP.[6] The IPERGAY study was the first to demonstrate the efficacy of a nondaily regimen among MSM at high risk of HIV, referred to as "event-driven" or "on-demand" PrEP.[8] Event-driven PrEP entails the use of 2 tablets of PrEP between 2 and 24 hours before anticipated sex, continuing with 1 tablet every 24 hours until 2 days after the last sex event. Nondaily regimens have the advantage of requiring fewer tablets, thus reducing potential side-effects and cost, although they are less forgiving of missed doses.[13] We hypothesize that for a subgroup of MSM at high risk of HIV, event-driven PrEP is preferred. However, we do not yet know

what proportion of MSM prefers to take event-driven (versus daily) PrEP when given that choice, what their profile is, and what influences their decision.

In this article, we present baseline data from a PrEP demonstration project among MSM in Belgium: the Be-PrEP-ared study. More particularly, we examine the proportion of MSM preferring daily PrEP or event-driven PrEP, the reasons for their initial choice, and associations with socio-demographic and sexual behaviour factors.

9.2.3 Methods

Design

Cross-sectional baseline data were used from the Be-PrEP-ared study: a single-site, open-label prospective cohort study using a mixed-method approach with an embedded qualitative component (EudraCT 2015-000054-37). The study site is the HIV/STI clinic of the Institute of Tropical Medicine in Antwerp, Belgium. The aim of Be-PrEP-ared is to evaluate whether daily PrEP and event-driven PrEP, provided within a comprehensive prevention package, are feasible and acceptable additional prevention tools for MSM at high risk of HIV acquisition in Belgium. Study procedures and details have been described in the published study protocol.[14] A community advisory board was set up to provide advice throughout the entire research process and to act as a link to local MSM communities.

Study Population

To be included in the study, participants had to be: born as male, test HIV-negative, be aged 18 years and older, be able and willing to provide written informed consent and to participate as required by the protocol, have had sex with another man in the past 12 months, be motivated to strengthen own prevention efforts, and to correspond to at least 1 criterion for 'high risk of HIV' (Textbox 1). Exclusion criteria included the following: symptoms of acute HIV infection, an estimated creatinine clearance of < 60 mL/min/1.73 m^2 according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) formula, an active hepatitis B infection, or taking postexposure prophylaxis or other products containing emtricitabine, tenofovir disoproxil or other cytidine analogues (such as lamivudine), or adefovir dipivoxil.

- Reported condomless anal intercourse in the past 6 months with a casual partner with unknown or HIV positive status;
- Reported (at least) 1 STI episode in the past 6 months;
- Reported having taken postexposure prophylaxis in the past 6 months.

Textbox 1: Criteria for 'high risk for HIV'

Participant Recruitment

The study was advertised through the community advisory board's social media, national media (eg, newspaper and television), and person-to-person promotion. Potential participants could preregister for participation on the study website (www.be-prep-ared.be) between September 9, 2015, and June 18, 2016. Participants who registered before September 25, 2015, were randomized to a ranking number on the list with the screening appointments. Thereafter, the screening list was completed in chronological order of registration, to allow for continuous preregistration and screening of participants until the sample size of 200 was reached.

Enrollment Study Procedures

At the screening visit, written informed consent was obtained, and potential participants were screened for eligibility. Study procedures also included the collection of basic socio- demographic characteristics and current sexual behaviour, a medical examination with special attention to symptoms of an acute HIV infection, and the collection of blood, urine, anal, and pharyngeal samples for kidney, liver, HIV, and STI testing. The screening visit ended with preventive sexual health counselling.

Potential participants were invited to come back to the clinic within 2 weeks to confirm eligibility and enrollment in the study. Symptoms of acute HIV infection were reassessed, and data were collected on relevant medical history, current medication, and recreational drug use. Participants were instructed to complete a baseline questionnaire on an electronic tablet in the waiting room. A counsellor thoroughly informed participants about the PrEP dosing regimens, invited them to choose between daily and event-driven regimens, and provided sexual health and adherence counselling. When asked, participants were explained that both regimens were considered equally efficacious, if taken correctly. Participants then received box with 30 PrEP tablets and started with their preferred regimen.

Laboratory Methods

STI testing was performed at the screening visit. Screening for HIV, hepatitis B, hepatitis C, syphilis, and HSV-2 was performed in blood samples. In addition, real- time polymerase chain reaction was used to test for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* on urine, pharyngeal, and anorectal samples. Details of laboratory procedures are provided in the protocol.[14]

Questionnaire

The self-administered baseline questionnaire was developed by an interdisciplinary research team based on surveys used in other studies related to PrEP or HIV prevention among MSM.[7,8,15,16] The questionnaire included questions on sociodemographic information, sexual preferences, the most recent sexual event (e.g., last time anal sex occurred), sexual behaviour in the past 3 months (e.g., number of anonymous contacts), and sexual risk-taking behaviours (e.g., last time transactional sex occurred). A list of potential reasons for choosing daily PrEP or event-driven PrEP was provided, adapted to the regimen, including an open-ended "other" option. The questionnaire allowed for multiple answers for this question and asked about the most important reason of choice. The questionnaire was available in 3 languages: Dutch, French, and English. Participants were instructed to complete the questions regarding preferences for daily PrEP or event-driven PrEP after the counsellor visit, to ensure that they were properly informed about the regimens.

In-Depth Interviews

The mixed-method design included qualitative research to complement quantitative findings and to get an in-depth understanding of participants' prevention needs, preferences for and attitudes toward PrEP use, user experiences, and perceived impact of PrEP on their sexual life.[14] A preliminary subset of 11 in-depth interviews, conducted by social scientists, was transcribed verbatim and analysed according to content-analytical principles.

Statistical Analysis

Only participants enrolled in the study were included in the analysis. Participants were considered infected with *N. gonorrhoeae* if they tested positive for *N. gonorrhoeae* in 1 of the 3 biological sites (anorectal, pharynx, or urine), and not infected with *N. gonorrhoeae* if all 3 testing sites were negative. If a test result was invalid or not confirmed at 1 of the 3 sites, the final result was considered invalid. The same was

performed for *C. trachomatis*, *M. genitalium*, and *T. vaginalis*. Syphilis was defined as a positive rapid plasma reagin with a titre of at least 4 and a positive *Treponema pallidum* assay or *Treponema pallidum* particle agglutination test. A grey zone result for HSV-2 was coded as invalid.

We examined associations between factors related to sociodemographic background, sexual behaviour factors, and STIs at screening with the preferred PrEP regimen using X^2 or Fisher exact test. If an association was found in an ordinal variable with more than 2 categories, '*P* value for trend' was calculated using the Mantel-Haenszel linear-by-linear association X^2 test. IBM SPSS Statistics 24.0 or SAS 9.4 was used for all computations.

Ethics and Quality Assurance

Ethical approval was provided by the institutional review board of the Institute of Tropical Medicine Antwerp and the ethics committee of the Antwerp University Hospital. The protocol and all relevant information were submitted to the Competent Authority of Belgium. The study is monitored in accordance with regulations applicable to clinical trials, including Good Clinical Practice guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and Good Clinical Laboratory Practice requirements, institutional-specific monitoring, and source data verification standard operating procedures

9.2.4 Results

Screening and Enrollment

We enrolled 200 participants in the study between October 2015 and December 2016 (Fig. 1). In total, 324 persons preregistered for participation, and 300 potential participants were contacted. Of them, 31 could not be reached; 7 reported having become HIV-positive between time of registration and time of contact; and 43 had become unable or unwilling to participate because of changes in occupation, place of residence, or relationship. On screening, 1 person was found HIV-positive, and 18 did not meet other inclusion criteria.

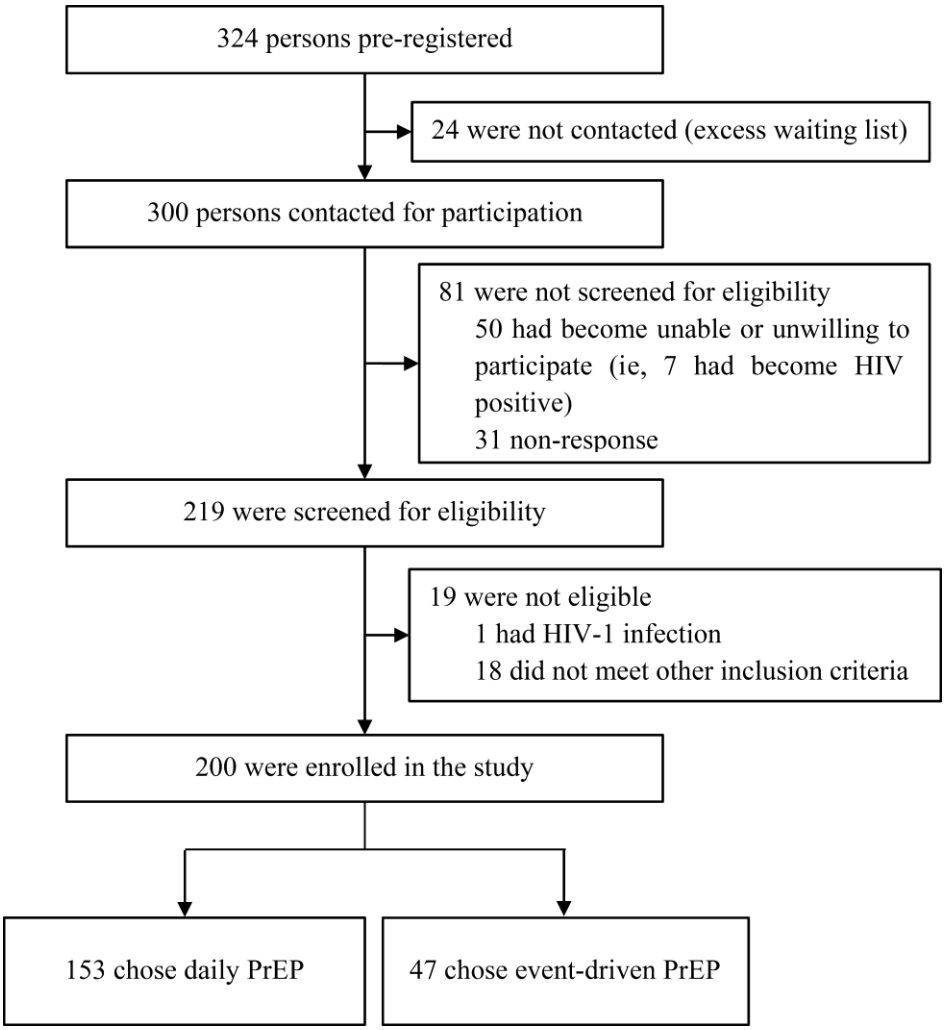

Figure 1: Screening and enrollment of participants.

Among the 200 participants enrolled, 47 (23.5%) chose event-driven PrEP, and 153 (76.5%) preferred the daily PrEP regimen.

Profile of Study Participants

The median age of the study participants was 38 years, with a minimum of 22 years and a maximum of 70 years. Three participants were transgender women. Participants were predominantly white (89.0%), highly educated (77.5%), and employed fulltime, part-time, or were self-employed (78.5%; Table 1). Participants who were not employed and those with a lower average net income were significantly more likely to prefer event-driven PrEP over daily PrEP.

Table 1: Sociodemographic Characteristics of Study Participants, Total and per Preferred PrEP Regimen

	Total	Daily	Event-Driven	
	N = 200, n (%)*	n = 153, %†	n = 47, %‡	*P*§
Age				0.716
18–30	42 (21.0)	22.2	17.0	
31–40	76 (38.0)	37.9	38.3	
41+	82 (41.0)	39.9	44.7	
Sex				0.554
Man	197 (98.5)	98.7	97.7	
Transwoman	3 (1.5)	1.3	2.1	
Racial-ethnic background				0.533
White	178 (89.0)	88.2	91.5	
Other	22 (11.0)	11.8	8.5	
Education				0.644
Primary school	5 (2.5)	2.0	4.3	
Secondary school	40 (20.0)	19.6	21.3	
Higher education	155 (77.5)	78.4	74.5	
Occupation				0.001
Employed full-time, part-time, or self-employed	161 (84.5)	88.9	68.1	
Not employed‖	31 (15.5)	11.1	31.9	
Average monthly net income (€)¶				0.002#
0–1700	70 (37.4)	30.6	60.5	
1700–2950	79 (42.2)	47.2	25.6	
2950+	38 (20.3)	22.2	14.0	
Health insurance**				0.483
Yes	187 (93.5)	94.7	91.5	
Living situation				0.868
Alone	100 (50.0)	49.7	51.1	
With others††	100 (50.0)	50.3	48.9	
Partner status				0.164
Steady partner	90 (45.0)	47.7	36.2	
No steady partner	110 (55.0)	52.3	63.8	
HIV status steady partner‡‡				0.325
HIV-negative	61 (69.3)	67.1	80.0	
HIV-positive	27 (30.7)	32.9	20.0	
Circumcision				0.815
Yes	36 (18.0)	17.6	19.1	

**Frequency and percentage within total. †Percentage within daily PrEP. ‡Percentage within event-driven PrEP. §P value of the x2 or Fisher exact test for associations between chosen regimen and variables. ‖Category includes the following: "unemployed," "student," "retired," or "disabled or long-term sick leave." ¶"Rather not say" (n = 13) was excluded from the analysis. #P value for trend is 0.003. **"Rather not say" (n = 1) was excluded from the analysis. ††Category includes the following: "with parents," "with partner," and "with others." ‡‡HIV status only for "steady partner" (n = 90) and "not sure" or "don't know" (n = 2) was excluded from the analysis*

Reasons for PrEP Regimen Choice

Table 2 shows the most common reported reasons for PrEP regimen choices.

Table 2: Reasons for PrEP Regimen Preferences.

Daily PrEP (N=149)*	**n (%)†**	**Event-driven PrEP** (N=46) ‡	**n (%)†**
Daily PrEP seems to be safer	47 (31.5)	I can anticipate very well when I will be at risk for HIV	19 (41.3)
I think it is easier to take 1 pill a day	31 (20.8)	Event-driven PrEP seems less burdensome for my body	11 (23.9)
I find it difficult to anticipate when I will be at risk of getting HIV	31 (20.8)	I have little risk in acquiring HIV	6 (13.0)
I have a lot of risk of getting HIV	14 (9.4)	I am afraid of adverse events (on long term) when I would take PrEP daily	6 (13.0)
I want to be able to have sex at any given moment without having the risk of getting HIV	11 (7.4)	Event-driven PrEP seems easier to adhere to	2 (4.3)
My steady partner has HIV	8 (5.4)	It is difficult for me to remember to take one pill a day	1 (2.2)
I want to have sex without a condom more often	7 (4.7)	I do not like to take pills daily	1 (2.2)

**"Missing" (n = 2) and "other" (n = 2) were excluded from the analysis.† n: frequency; %: percentage within the chosen PrEP dosing regimen. ‡"Other" (n = 1) was excluded from the analysis*

The most important reasons for choosing event-driven PrEP were as follows: feeling able to anticipate the risk of HIV (41.3%), perceiving it as less burdensome for the body (23.9%), assessing one's own risk of HIV to be low (13.0%), or being afraid of adverse events (13.0%). Triangulation with qualitative findings from the in- depth interviews corroborated these preferences as illustrated by the quotes (Textboxes 2 and 3).

> "Mostly when I have sex, then I do know it beforehand, or I know there will be a chance.[-] It's not that frequent and mostly it's in the weekend that I have sexual intercourses, so then I only [take tablets] in the weekend, and perhaps the days thereafter when sex has occurred"

Textbox 2: Participant preferring event-driven PrEP, 38 years old

> "Yeah, but I have to think about my body [-], that it does not get damaged. So I was thinking like, [-] I'm going to choose so that there is no harm, that I have to take as little [tablets] as possible, that I'm only going to take it when I'm going to have sex."

Textbox 3: Participant preferring event-driven PrEP, 23 years old

By contrast, the most important reasons for choosing daily PrEP were as follows: safety (31.5%), ease of daily pill-taking (20.8%), and difficulties with anticipating HIV risk (20.8%), as illustrated in Box 4: the participant would not be able to take PrEP in advance or to plan it as such, which rendered event- driven PrEP less safe according to him.

> "I have a lot of routine in my life, but I have a hard, stressful and irregular job. [-] I think that daily [PrEP] is most safe, and that's it. If you take the other [regimen] then you really have to have it planned to [take it] in advance, and that's just really something I cannot do."

Textbox 4: Participant preferring daily PrEP, 30 years old

Almost all participants anticipated that the odds were high that they would switch PrEP regimen in the following year: 45 (95.7%) in the event-driven group, and 145 in the daily group (95.4%).

Sexual Behaviour

The median reported total number of sexual partners in the past 3 months before enrollment was 12. Half of the participants reported 4 or more occasional sex partners in the past 3 months, and 64.5% reported 4 or more anonymous sex partners (Table 3). Sexual risk-taking in the past 3 months was high, with 70.5% of the participants reporting CAI with at least 1 occasional partner and 60.0% with at least 1 anonymous partner. Sixty-four percent had participated in group sex, and 61.5% had sex while using recreational drugs.

Preferring daily PrEP was associated with more recent anal sex before enrollment and higher reported number of occasional and anonymous partners in the past 3 months. Participants who preferred daily PrEP were more likely to have had CAI with at least 1 occasional partner, to have participated in group sex, and to have had sex while being drunk in the past 3 months, as compared with event-driven users.

Table 3: Sexual Behaviour Characteristics of Study Participants, Total and per Preferred PrEP Regimen.

	Total	Daily	Event-Driven	*P*§
	N = 200, n (%)*	n = 153, %†	n = 47, %‡	
Sexual attraction to sex				0.045
Only to men	164 (82.0)	85.0	72.3	
Men, sometimes women	35 (17.5)	15.0	25.5	
Men and women	1 (0.5)	0.0	2.1	
Last time anal intercourse				0.039
Within a week	90 (45.0)	49.0	31.9	
More than a week ago	110 (55.0)	51.0	68.1	
No. of steady partners in the past 3 months				0.273
None	85 (42.5)	41.2	46.8	
One	63 (31.5)	30.1	36.2	
More than 1	52 (26.0)	28.8	17.0	
No. of occasional partners in the past 3 months				0.014 ‖
None	17 (8.5)	7.2	12.8	
1–3	83 (41.5)	36.6	57.4	
4–10	67 (33.5)	38.6	17.0	
11 or more	33 (16.5)	17.6	12.8	
No. of anonymous partners in the last 3 months				0.031¶
None	25 (12.5)	9.8	21.3	
1–3	46 (23.0)	20.3	31.9	
4–10	69 (34.5)	37.3	25.5	
11 or more	60 (30.0)	32.7	21.3	
Sexual risk-taking in the past 3 months				
CAI with at least 1 occasional sex partner	141 (70.5)	81.0	63.4	0.018
CAI with at least 1 anonymous partner	120 (60.0)	69.6	64.9	0.584
Participated in group sex	129 (64.5)	68.6	51.1	0.028
Had sex while used recreational drugs	123 (61.5)	63.4	55.3	0.319
Had sex while used enough alcohol to feel drunk	85 (42.5)	51.0	14.9	<0.001
Paid a man for sex	12 (6.0)	7.2	2.1	0.301
Received money, drugs, or something else	21 (11.0)	12.4	4.3	0.171

**Frequency and percentage within total; †Percentage within daily PrEP; ‡Percentage within event-driven PrEP; §P value of the x2 or Fisher exact test for associations between chosen regimen and variables; ‖P value for trend is 0.011; ¶P value for trend is 0.006.*

STI Prevalence

In 39.5% of participants at least 1 bacterial STI was detected at screening, 15 (7.5%) had syphilis, and 3 participants were diagnosed with hepatitis C (Table 4). Among the 24 participants with gonorrhea, 2 cases were detected in urine, 14 in anorectum, and 14 in the pharynx (data not in table). Among the 23 participants (11.7%) with chlamydia infection, 5 cases were detected in urine, 20 in anorectum, and 2 in the pharynx (data not in table). One case of lymphogranuloma venereum was detected in the anorectum, all other chlamydia infections were non–lymphogranuloma venereum strains. Among the 34 participants (17.2%) with mycoplasma infection, 17 cases were detected in urine, 17 in anorectum, and no cases were detected in the pharynx. There was no statistically significant difference in prevalence of STIs between participants choosing daily PrEP or event-driven PrEP.

Table 4: Sexually Transmitted Infections Found at Screening Among Participants, Total and per Preferred PrEP Regimen.

	Total		Daily		Event-Driven	
	n/N	%*	n/N	%†	n/N	%‡
Syphilis§	15/200	7.5	14/153	9.2	1/47	2.1
Neisseria gonorrhoeae	24/196	12.2	18/150	12.0	6/46	13.0
Chlamydia trachomatis	23/196	11.7	14/151	9.3	9/45	20.0
Mycoplasma genitalium	34/198	17.2	27/151	17.9	7/47	14.9
Any bacterial STI‖	77/195	39.5	58/150	38.7	19/45	42.2
Trichomonas vaginalis	0/197	0.0	0/151	0.0	0/46	0.0
Hepatitis C	3/200	1.5	1/153	0.7	2/47	4.3
HSV-2	68/194	35.1	51/149	34.2	17/45	37.8

**Percentage within total. †Percentage within daily PrEP. ‡Percentage within event-driven PrEP. §Positive = rapid plasma reagin , 1/4 and Treponema pallidum assay "positive." ‖Positive for Syphilis, N. gonorrhoeae, C. trachomatis, or M. genitalium.*

9.2.5 Discussion

We showed that MSM coming forward and screened for PrEP in Belgium were at high risk of HIV acquisition, and among those about 1 in 4 preferred event-driven PrEP over daily PrEP. The choice of event-driven PrEP was related to a 'lower risk-profile' and is motivated by feeling able to anticipate the risk of HIV and concerns about side effects.

The Be-PrEP-ared study is one of the first European PrEP demonstration projects among MSM at high risk of HIV in which participants were able to self-select between daily PrEP or event-driven PrEP. In our study, 23.5% of the MSM preferred event-driven PrEP over daily PrEP. An ongoing Dutch PrEP demonstration project (i.e., AmPrEP) also invited participants to choose between these 2 options and found similar results (i.e., 27.4% preferred event-driven).[16,17] The Australian demonstration project PRELUDE found that 20% of MSM would prefer event-driven PrEP (i.e., dosing around specific risk events) and 14% periodic PrEP (i.e., daily dosing during periods of increased risk). However, in PRELUDE, the choice was hypothetical, i.e., participants had to take PrEP daily.[18] In the United Kingdom, 1 study found that among 293 MSM who purchased PrEP on the internet, 16% was following the event-driven regimen.[19] In France, where both daily PrEP and event-driven PrEP are provided since 2015, about 6 out of 10 PrEP users have been prescribed event- driven PrEP.[20] However, it could be questioned whether this high proportion in France is due to the IPERGAY study, in the sense that MSM in France would have been more familiar with this dosing regimen. Taking into account these findings, there is now evidence that at least 1 in 4 of MSM at high risk of HIV acquisition would prefer event-driven PrEP in high income countries, such as Belgium.

Our study results made it possible to outline a profile of MSM who prefer event-driven PrEP: they had less frequently anal sex, had fewer sex partners, and were less likely to engage in specific sexual risk-taking activities such as group sex in the 3 months preceding study participation. Although MSM preferring event-driven PrEP are at sufficient risk of HIV acquisition, considering the PrEP eligibility criteria, they report relatively less risk behaviours than those opting for daily PrEP. They also consider themselves to be able to anticipate when they will be at risk, thus preferring event-driven PrEP. Hence, it is clear that daily PrEP may not be suitable for all MSM at high risk of HIV, and that event-driven regimens could better suit the prevention needs of a specific group of MSM with less frequent sexual risk-taking.

We ensured that participants were well informed about different regimens before choosing. However, it cannot be excluded that the information and counselling provided has influenced participants' preferences in either way. Another limitation is that the enrollment procedure was slow, which may mean that participants enrolled at the beginning of the study (October 2015) may not be entirely comparable with those enrolled later (December 2016). An additional analysis to control for this potential bias did not show any association between time of enrollment and preferred PrEP regimen (not shown in results). The slow enrollment may also have led to the relative large number of persons (n = 81) who had become unreachable, unable, or unwilling to participate. Given the lack of data of people who preregistered but were not screened, we were unable to detect a selection bias in this regard. Different eligibility criteria

could have resulted in a different study population. Informing potential participants about the eligibility criteria before preregistration may have reduced the number of those not meeting inclusion criteria. Preventive counselling at the screening visit may have influenced sexual behaviour in the week before enrollment.

Since June 2017, PrEP is reimbursed in Belgium for persons at increased risk of HIV and can be obtained through AIDS Reference Clinics (i.e., specialized public HIV treatment centres). In case of eligibility, physicians fill out a reimbursement request, to be submitted to the social health insurance of the future user.[21] The approval is attributed for 1 year, renewable and ensures that users pay € 11,9 maximum as co-payment per bottle (i.e., 30 pills). After 9 months, approximately 1350 requests were approved, almost exclusively MSM.[21] This number is high when compared with the early uptake in other countries such as France (since 2015) and the United States (since 2012), relative to the number of inhabitants.[22,23] It confirms the high demand and acceptance of this prevention method within this at-risk population. The eligibility criteria for PrEP among MSM and screening procedures used in our study, which are now mostly used in Belgium, have been effective in selecting a subgroup of MSM at substantial risk of HIV. This is corroborated by the self- reported sexual risk behaviours that correspond well to known HIV sexual risk factors (e.g., high number of sexual partners),[10] and by the high prevalence of STIs at screening (e.g., 39.5% had at least 1 bacterial STI). These results also provide further evidence that routine screening for STIs among MSM when initiating PrEP is important.[11] Preferably, this includes testing in 3 different sites (i.e., rectal, urethral, and pharynx), and testing for hepatitis C virus.[17,24]

The World Health Organization currently recommends a daily regimen only.[11] However, the World Health Organization also acknowledges that good practices for implementing PrEP should be people-centred, organized around users' needs and preferences.[11] The efficacy of event-driven PrEP has been demonstrated in the IPERGAY trial,[8] and in the open-label phase efficacy increased to 97% compared with the placebo group.[25] It could be argued that the high efficacy found in IPERGAY may be due to the high number of tablets used. However, in a sub analysis focusing on IPERGAY participants with infrequent sexual intercourse and, hence, nondaily pill intake (i.e., less than 15 tablets per month), efficacy increased to 100%.[26] Two European demonstration projects, i.e., the Be-PrEP-ared and AmPrEP now also show that there is a real demand for event-driven PrEP.[16,17] Therefore, we strongly recommend that event-driven PrEP be considered as a valuable alternative regimen in guidelines for the provision of PrEP among MSM. Integrating event-driven PrEP into clinical practice could lead to reduced numbers of pills used and reduced public healthcare expenditure.[27]

Concerns have been raised that event-driven PrEP is less forgiving of missed doses, which may compromise adherence, thus leading to higher chances of seroconverting

and developing resistance.[13,28,29] In HIV Prevention Trials Network (HPTN) 067, participants were randomized to take either daily or nondaily regimens (i.e., time-driven and event- driven).[30–32] Although adherence levels (i.e., coverage of sex acts) for daily PrEP were significantly better among young women in Cape Town and MSM in Harlem than for nondaily regimens, they were comparable among MSM in Bangkok. Whether adherence to event-driven PrEP would be better when MSM can self-select their preferred regimen under real-life conditions remains to be studied. It could be hypothesized that tailoring PrEP use to users' preferences and prevention needs leads to better adherence through improved motivation.

In our study, MSM coming forward for PrEP seemed to be well aware of their risk of HIV, reflected by their PrEP regimen choice. However, it should be noted that almost all participants considered it likely that they would change their dosing regimen the following year. It could mean that participants are aware that their risk of HIV may vary over time, and that PrEP use may be adapted accordingly.[33] The prospective data from Be-PrEP-ared will be important to shed more light on the dynamics of how, when, and why MSM at high risk of HIV switch regimen, maintain adherence, or discontinue PrEP use. It was surprising that employment status and average net income were associated with PrEP regimen choice, given that PrEP in Be-PrEP-ared was provided for free. Potential explanations are that participants choosing event-driven PrEP anticipated they would have to pay for the high costs of the medication after the study because PrEP was not yet reimbursed at the time of data collection. Alternatively, it may reflect socioeconomic disparities in health behaviour.[34] This second explanation may be particularly plausible when taken into account that PrEP knowledge and acceptability have also been shown to differ along the traditional lines of health inequality.[35,36] Further studies are needed to better understand intraindividual and interindividual variation in PrEP use and how this may relate to different dynamics in sexual risk behaviours or potential disparities in PrEP use?

9.2.6 Conclusion

Event-driven PrEP was preferred by about 1 of 4 PrEP users at high risk of HIV infection in our study, which may better suit their prevention needs. Implementing and including both regimens in PrEP provision for MSM could lead to better tailored HIV prevention approaches.

9.2.7 Acknowledgements

The authors thank all members of the community advisory board for their continuous support in the Be- PrEP-ared study. They thank Be-PrEP-ared participants for their participation.

9.2.8 References

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9.2.9 Appendix 1. Be-PrEP-ared Study Team

Maureen Aerts; Laura Albers; Jozefien Buyze; Tania Crucitti; Irith De Baetselier; Vicky Cuylaerts Marjan Van Esbroeck; Eric Florence; Nikki Foque; Katrien Fransen; Chris Kenyon; Marie Laga; Hanne Landuyt; Christiana Nöstlinger; Thijs Reyniers; Bart Smekens; Yven Van Herrewege; Kurt Van Lent; Harry Van Loen; Jef Verellen; Bea Vuylsteke; and Kristien Wouters

9.3 ANNEX 3 – LGV SPECIFIC REQUEST FORM

REFERENTIECENTRUM VOOR SEKSUEEL OVERDRAAGBARE AANDOENINGEN
Aanvraagformulier voor CONFIRMATIE van *CHLAMYDIA TRACHOMATIS - LGV*

***Labocode**

GELIEVE DIT FORMULIER MET HET STAAL OP TE STUREN NAAR HET REFERENTIELABORATORIUM
Apr. D. VAN DEN BOSSSCHE
Instituut Tropische Geneeskunde - Klinisch Referentielaboratorium (KRL)
Kronenburgstraat 43/3 - 2000 Antwerpen (België)
Tel : 03/247.65.52 - 03/247.64.45 / Fax : 03/247.07.89 / E-mail : dvandenbossche@itg.be

***Gegevens over het laboratorium dat het staal opstuurt**

Naam klinisch bioloog : .....
Naam laboratorium : .....
Adres : .....
Postcode/Woonplaats : .....
Tel. : ..... Fax : .....
E-mail: .....
Naam + RIZIV nr aanvragende arts: .....
.....

Gegevens over de patiënt

*Naam (initialen/andere code) : .....
*Geslacht : ☐ M ☐ V ☐ andere ☐ onbekend
*Geboortedatum (of leeftijd) : .....
*Postcode/Woonplaats : .....
*Geboorteland : .....
Beroep : .....
Nationaliteit : .....
Recent verblijf buitenland : ☐ ja ☐ neen
Zo ja, land of streek : .....

Gegevens over het staal

Vermoedelijke identificatie : .....
*Identificatienummer : .....
*Gebaseerd op :

❑ Isolatie
❑ PCR
❑ Antigen detectie (ELISA)
❑ Andere : .....

*Oorsprong :

❑ Anaal secreet
❑ Genitale ulcus
❑ Genitaal secreet
❑ Inguinaal aspiraat

❑ Biopt: .....
❑ Andere: .....
❑ Onbekend

Datum staalafname: .....

Voorbehouden voor het referentielaboratorium

***Klinische en epidemiologische gegevens**

Symptomen

❑ Asymptomatisch
❑ Urethritis
❑ Epididymitis
❑ Cervicitis
❑ Proctitis
❑ Genitale ulcer
❑ Inguinale Lymfadenopathie
❑ Andere: .....
❑ Onbekend

Vermoedelijke transmissie

❑ Heteroseksueel
❑ Homoseksueel
❑ Biseksueel
❑ Werkzaam in prostitutie
❑ Contact met sexwerker
❑ Onbekend

HIV Status

❑ Negatief
❑ Negatief op PrEP
❑ Positief
❑ Nieuwe HIV diagnose
❑ Onbekend

Co-infecties

❑ Geen
❑ Gonorrhoeae
❑ Chlamydia non-LGV
❑ Hepatitis B
❑ Herpes genitalis
❑ Syfilis
❑ Andere: .....
❑ Onbekend
❑ Trichomonas vaginalis
❑ Mycoplasma genitalium
❑ Hepatitis C

*** ABSOLUUT IN TE VULLEN**

Aanvraagformulier_Chlamydia_20210610

CURRICULUM VITAE

Personal Information

Name: De Baetselier Irith

Date of birth: 24th December 1980

Nationality: Belgian

Home address: August van Putlei 131, 2150 Borsbeek, Belgium

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Current function: Research expert – Coordinator of STI reference activities at the clinical reference laboratory of ITM

Education and Degrees:

University studies:

- 2019-2021: PhD - Improving Sexually Transmitted Infection diagnostics and control among Men who have sex with Men in Belgium, University Antwerp, Belgium and Institute of Tropical Medicine, Antwerp, Belgium
- 2006: Master in Biomedical Science, University Antwerp, Belgium

Languages:

- Dutch: mother tongue
- English: fluent in writing and speaking
- French: good in writing and speaking

Professional experience:

Employment History	
Current Post:	Expert Scientist
Institution:	Institute of Tropical Medicine (ITM)
Start date:	06/2018
Description of current post:	Coordinator and focal point of the National Sexually Transmitted Infections (STI) reference centre at ITM Research fellow with a large interest in HIV/STI prevention, diagnostics and antimicrobial resistance. Extensive expertise with the design and follow-up of multi-centric prospective- or cross-sectional studies concerning HIV/STI prevention in high- and low-resource countries with a focus onto the necessary laboratory activities. Supervision and training of on-site laboratory activities, implementation of a Quality Control system and Good Clinical Laboratory Practices in Belgium, Asia and African countries.

Previous Posts		
Dates	**Institution**	**Job Title**
2010-2018	Institute of Tropical Medicine, Antwerp	Junior Scientist
2008-2010	Clinitude, Leuven	Clinical Research Associate
2007-2008	MSource, Brussels	Clinical Research Associate

Participation at (inter)national conferences (accepted poster and oral presentations):

- World HIV & STI Congress (ISSTDR) 2021. Online conference – accepted presentations:
    - The impact of the Covid-19 pandemic on the trends of Sexually transmitted infections in Belgium. Results of an STI clinic (poster)
    - Heterogeneity of Mycoplasma genitalium resistance to macrolides and fluoroquinolones among men who have sex with men initiating PrEP in West-Africa (oral)
- International Union against STIs (IUSTI) 2020. Online conference – no poster presentations
- International Union against STIs (IUSTI) 2019, Tallinn, Estonia – accepted oral poster presentation:
    - Detection of macrolide resistance in Mycoplasma genitalium still represents some challenges for commercial kits. Baetselier Irith, Smet Hilde, Crucitti Tania
- World HIV & STI Congress (ISSTDR) 2019, Vancouver, Canada – accepted poster presentations:
    - Population structure of Lymphogranuloma venereum in Belgium: Surveillance data from 2010 until 2017. **De Baetselier I**, Cuylaerts V, Smet H, De Deken B, Thys W, Abdellati S, Meehan C, Crucitti T
    - To pool or not to pool STI samples in MSM using PrEP? Results of the CohMSM-PrEP Study (ANRS 12369 - Expertise France**. I. De Baetselier**, B. Vuylsteke, I. Yaya, A. Dagnra, A. Yeo, H. Faye Ketté, S. Diandé, J. Yaka, G. Kadanga, I. Traoré, V. Cuylaerts, H. Smet, E. Dah, E. Mensah, B. Dembele, A. Koné, C. Laurent, T. Crucitti for the CohMSM PrEP study group
    - Prevalence of STIs among MSM initiating PrEP in West-Africa (CohMSM-PrEP ANRS 12369 - Expertise France**) I. De Baetselier**, T. Crucitti, I. Yaya, B. Dembele, E. Mensah, E. Dah, A. Koné, H. Fayé Ketté, A. Dagnra, A. Yeo, S. Diandé, C. Laurent, B. Vuylsteke for the CohMSM PrEP study group
- Research for Prevention (R4P) 2018, Madrid, Spain – accepted poster presentations:
    - Lymphogranuloma venereum among MSM using PrEP in Belgium. Time to test! **De Baetselier I**, Wouters K, Vuylsteke B, Reyniers T, Buyze J, Laga M, Crucitti T.
    - High level of macrolide resistance of *Mycoplasma genitalium* found among MSM at high risk for HIV in a Belgian PrEP demonstration

project. **De Baetselier I,** Smet H, Wouters K, Vuylsteke B, Reyniers T, Laga M, Crucitti T

- Scientific seminar on infectious diseases Sciensano, 17 May 2018, Brussels, Belgium – accepted poster presentation
    - Surveillance of Sexually Transmitted Infections in Belgium: Data from 2017. **De Baetselier I**, Smet H, Cuylaerts V, De Deken B, Abdellati S, Thys W, Crucitti T

- World HIV & STI Congress (ISSTDR) 2017, Rio de Janeiro, Brazil – accepted poster presentations:
    - *Mycoplasma genitalium* and *Trichomonas vaginalis* detection a cohort of men who have sex with men in Belgium: Evaluation of the Diagenode S-DIagMGTV Multiplex kit. **De Baetselier I**, Smet H, Vuylsteke B, Crucitti T
    - Colli-Pee: A new device to collect first-void urine at home for molecular detection of STIs. Evaluation & acceptability by MSM in a PrEP study in Belgium. **De Baetselier I**, Vankerckhoven V, Manon de Koeijer, Reyniers T, Smet H, Vuylsteke B, Crucitti T
- African Society of Laboratory Medicine (ASLM) 2014, Cape-Town, South-Africa – accepted poster presentations
    - Evaluation of a Chlamydia trachomatis rapid test in Rwanda: The BioChekSwab Rapid Test. **De Baetselier I**, Mwambarange L, Cuylaerts V, Musengamana V, Agaba S, Kestelyn E, van de Wijgert J, Crucitti T
    - Implementation and evaluation of the Presto combined qualitative real time CT/NG assay in Rwanda. **De Baetselier I**, Mwambarange L, Cuylaerts V, Musengamana V, Rusine J, Muvunyi C, Mukarurangwa A, van de Wijgert J, Kestelyn E, Crucitti T
    - Building effective onsite laboratories in developing countries: A case study of Rinda Ubuzima. Lambert Mwambarangwe, Vicky Cuylaerts, Viateur Musengamana, Stephen Agaba, Evelyne Kestelyn, Tania Crucitti, **Irith De Baetselier**, Jennifer Van Nuil, Janneke van de Wijgert, Jean Claude Ndagijimana
- BREACH meeting 28/09/2012, Brussels, Belgium – no poster presentations
- African Society of Laboratory Medicin (ASLM) 2012, Cape-Town, South-Africa – accepted poster presentations
    - Introducing an External Quality Assessment Scheme for CD4 Absolute Count Analysis in a Phase III HIV Prevention Trial in Sub-Saharan Africa. **Irith De Baetselier**, Tine Vermoesen, Katrien Fransen, Lut Van Damme, Tania Crucitti

- Implementing Good Clinical Laboratory Practices in different laboratories in sub-Saharan Africa for a phase III clinical trial. **Irith De Baetselier**, Katrien Fransen, Gustav Venter, Elizabeth Rammutla, Walter Agingu, Theonest Ndyetabura, Lut Van Damme ,Tania Crucitti

- CROI 2012, Seattle, USA – no poster presentations

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