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Contemporary Challenges in Human Immunodeficiency Virus Pre-exposure Prophylaxis in Belgium

PhD thesis submitted for the degree of Doctor of Medical Sciences at the University of Antwerp to be defended by

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Summary

Human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) is a very effective biomedical intervention to reduce HIV incidence and improve sexual health. PrEP has become a crucial component of HIV prevention, and its uptake is increasing globally, including in Belgium. However, PrEP coverage remains insufficient, and both PrEP care and users face numerous challenges. In this thesis, we describe some of these challenges, and potential interventions to address them.

First, we discuss the PrEP (care) discontinuation and ongoing HIV risk when discontinuing PrEP care. Factors contributing to discontinuation are multiple, include being in a monogamous relationship, reduced sexual activity, consistent condom use, and barriers to PrEP care (e.g., difficult access to the clinic). To address these barriers, we emphasize the importance of a differentiated, client-centered and low-threshold approach in PrEP delivery, as recommended by the World Health Organization. Moreover, it is crucial to consider the individual risk for HIV to assess the effectiveness of PrEP programs, beyond traditional retention in care metrics.

Subsequently, we explore two components of a syndemic affecting PrEP users: chemsex and non-consensual sex. Chemsex, the use of drugs during sexual encounters, can present risks and negative effects. We found that a substantial proportion of PrEP users in Belgium engaged in chemsex and expressed a willingness to reduce related risks through online applications and face-to-face counseling with healthcare providers. Non-consensual sex is another significant concern, with a considerable proportion of PrEP users reporting such experiences. However, seeking help after non-consensual sex incidents remains low, indicating a need for improved support services. PrEP clinics could play a vital role in addressing this syndemic, given that PrEP users are already familiar and engaged in care with these services.

Finally, we explored the emergence of antimicrobial resistance (AMR) in bacterial sexually transmitted infections (STIs), particularly *Neisseria gonorrhoeae*. AMR has been increasing in *Neisseria gonorrhoeae* following decades of antimicrobial exposure. Reducing antimicrobial consumption is crucial to slow down the emergence of AMR. Our findings suggest that reducing screening intensity for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in PrEP cohorts can significantly decrease antimicrobial consumption. We also recommend using mono-therapy with ceftriaxone instead of dual-therapy with ceftriaxone and azithromycin for the treatment of *Neisseria gonorrhoeae*. Finally, we caution against the widespread use of doxycycline prophylaxis for STIs due to the risk of inducing AMR.

In conclusion, we recognize the positive impact of PrEP on reducing HIV incidence and improving sexual health. However, challenges in PrEP care, including coverage and discontinuation, must be addressed. The syndemic of chemsex and non-consensual sex require attention and support services within PrEP clinics. Additionally, the emergence of AMR in bacterial STIs necessitates interventions to reduce antimicrobial consumption. Our research provides insights into these areas and contributes to a better understanding of optimizing PrEP programs and addressing associated challenges.

Dutch Summary

Humaan immunodeficiëntievirus (HIV) pre-expositie profylaxis (PrEP) is een zeer effectieve biomedische interventie om de incidentie van HIV te verminderen en de seksuele gezondheid te verbeteren. PrEP is een cruciaal onderdeel geworden van hiv-preventie en het gebruik ervan neemt wereldwijd toe, ook in België. De dekking van PrEP blijft echter onvoldoende en zowel PrEP zorg als PrEP gebruikers worden geconfronteerd met uitdagingen. In dit proefschrift beschrijven we enkele van deze uitdagingen en mogelijke interventies om ze aan te pakken.

Eerst bespreken we het stoppen met PrEP (zorg) en het doorlopende HIV-risico bij het stoppen met PrEP-zorg. Factoren die bijdragen aan het stoppen met PrEP (zorg) zijn onder andere het hebben van een monogame relatie, verminderde seksuele activiteit, consequent condoomgebruik en barrières voor PrEP-zorg. Om deze barrières aan te pakken, benadrukken we het belang van een gedifferentieerde, cliëntgerichte en laagdrempelige aanpak bij de levering van PrEP, zoals aanbevolen door de Wereldgezondheidsorganisatie. Bovendien is het cruciaal om rekening te houden met het individuele risico op HIV om de effectiviteit van PrEP-programma's te beoordelen, naast de traditionele retention in care-metingen.

Vervolgens onderzoeken we twee componenten van een “syndemic” bij PrEP-gebruikers: chemseks en niet-consensuele seks. Chemseks, het gebruik van drugs tijdens seksuele contacten, brengt risico's en negatieve effecten met zich mee. We toonden dat een aanzienlijk deel van de PrEP-gebruikers in België aan chemseks deed en zich bereid verklaarde om de risico's te verminderen door online applicaties en face-to-face counseling met zorgverleners. Niet-consensuele seks is een ander belangrijk problematiek, gezien een aanzienlijk deel van de PrEP-gebruikers melding maakt van dergelijke ervaringen. Het zoeken van hulp na incidenten met seks zonder consent blijft echter laag, wat duidt op een behoefte aan betere ondersteunende diensten. PrEP-klinieken zouden een vitale rol

kunnen spelen bij het aanpakken van deze syndromen, aangezien PrEP-gebruikers al bekend zijn met en betrokken zijn bij de zorg van deze diensten.

Tot slot onderzochten we de opkomst van antimicrobiële resistentie (AMR) in bacteriële seksueel overdraagbare aandoeningen (soa's), met name *Neisseria gonorrhoeae*. AMR is toegenomen bij *Neisseria gonorrhoeae* na tientallen jaren van blootstelling aan antimicrobiële middelen. Het verminderen van antimicrobieel gebruik is cruciaal om het ontstaan van AMR te vertragen. Onze bevindingen suggereren dat het verminderen van de screeningsintensiteit voor *Neisseria gonorrhoeae* en *Chlamydia trachomatis* in PrEP cohorten het gebruik van antimicrobiële middelen aanzienlijk kan verminderen. We raden ook aan om voor de behandeling van *Neisseria gonorrhoeae* mono-therapie met ceftriaxon te gebruiken in plaats van dual-therapie met ceftriaxon en azitromycine. Tot slot waarschuwen we tegen het wijdverbreide gebruik van doxycycline profylaxe voor soa's vanwege het risico op het veroorzaken van AMR.

Concluderend erkennen we de positieve invloed van PrEP op het verminderen van de hiv-incidentie en het verbeteren van de seksuele gezondheid. Er moeten echter uitdagingen in de PrEP-zorg worden aangepakt, waaronder dekking en stopzetting. De syndromen van chemsex en niet-consensuele seks vereisen aandacht en ondersteunende diensten binnen PrEP-klinieken. Daarnaast vereist de opkomst van AMR in bacteriële soa's interventies om het gebruik van antimicrobiële middelen te verminderen. Ons onderzoek biedt inzicht in deze gebieden en draagt bij aan een beter begrip van het optimaliseren van PrEP-programma's en het aanpakken van de bijbehorende uitdagingen.

Chapter 1 Introduction

1.1 PrEP: a new era in HIV prevention

November 2010 marked the beginning of a new era in human immunodeficiency virus (HIV) prevention. The results of the Pre-exposure Prophylaxis Initiative (iPrEx) trial showed a reduction of 44% in the incidence of HIV among HIV negative men who have sex with men (MSM) taking daily oral Tenofovir disoproxil and Emtricitabine (1). The efficacy of this novel biomedical intervention called pre-exposure prophylaxis (PrEP), consisting in the use of antiretroviral medication to prevent HIV acquisition, will later be confirmed by other randomized controlled trials (RCTs). The IPERGAY trial found a reduction of 86% in HIV incidence among MSM taking event-driven PrEP, a method consisting in taking PrEP before and after a sexual contact, as opposed to daily intake (Figure 1) (2). The PROUD trial showed a reduction of 86% in HIV incidence among MSM taking daily PrEP (3). This led the World Health Organization (WHO) to recommend PrEP as part of a comprehensive HIV prevention package for MSM in 2014 (4). The efficacy of PrEP has been demonstrated in other populations at risk for HIV such as sero-discordant heterosexual couples (5), heterosexual men and women in high prevalence areas (6) and injecting drug users (7) . In 2015, WHO expanded its recommendation of integrating PrEP in a comprehensive HIV prevention package to all people at substantial risk for HIV (8).

Following the WHO recommendation, PrEP has been rolled-out in several countries worldwide. The number of countries having integrated the WHO PrEP recommendation in their national guidelines increased from 26 in 2015 to 120 at the end of 2019 (9). It has been estimated that about 626.000 people had received PrEP at least once that year, with MSM, adolescent girls and young women, and sex workers as most reached priority populations (9). Later on, it has been estimated that about 4 million individuals worldwide had ever started PrEP by the end of 2022 (10).

In Belgium, Tenofovir disoproxil/Emtricitabine are available and reimbursed as PrEP through the public health insurance since 2017 (11). PrEP is reimbursed for people at substantial risk for HIV as defined by a set of criteria (Table *1*). It is dispensed through 12 HIV reference centers, at least one in each Belgian province. Both daily and event-driven PrEP are possible, although the latter is only recommended for cis-gender male and transgender female who are not taking exogenous hormones, due to pharmacokinetic reasons (12).

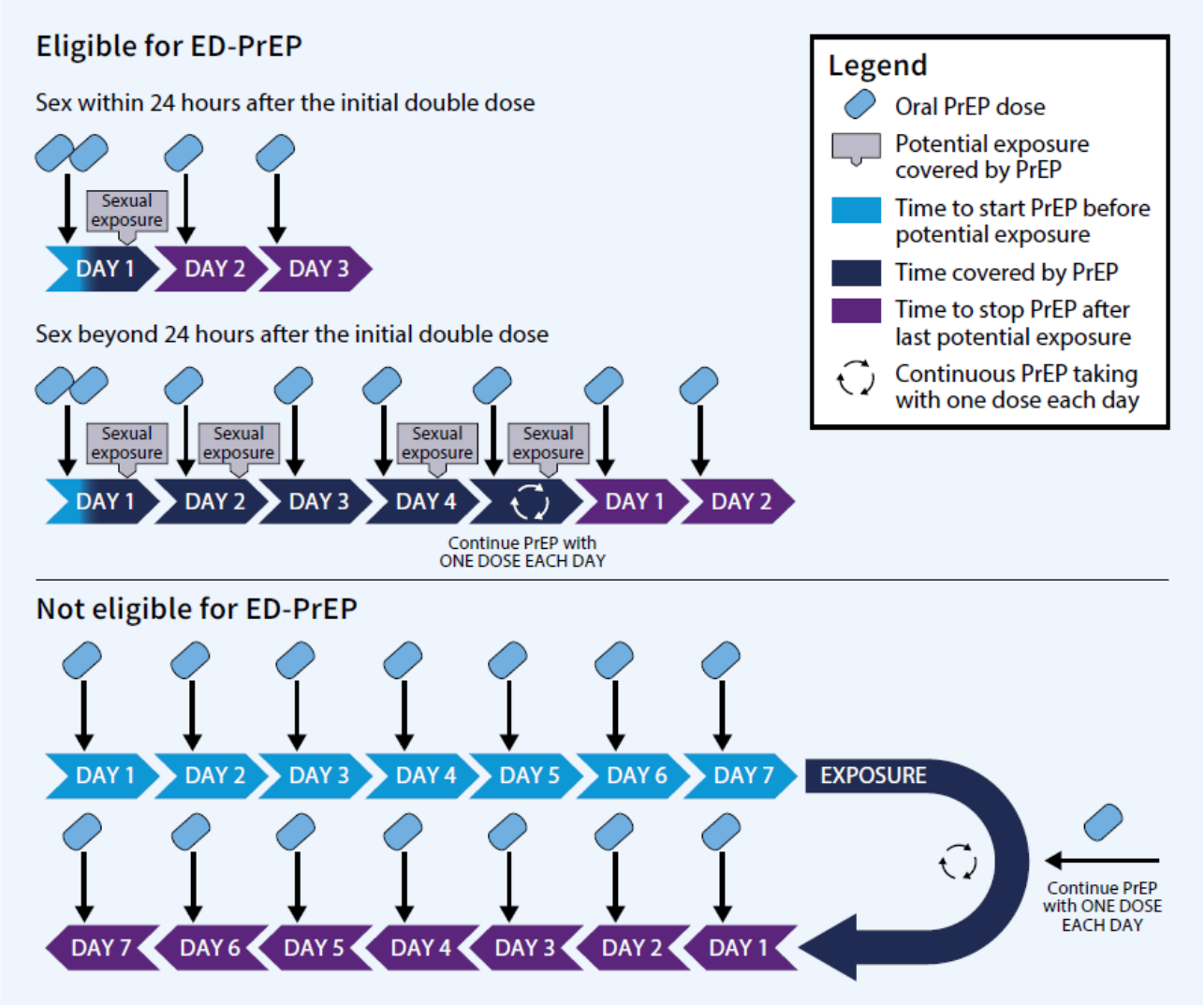


Figure 1 - Schematic representation of daily and event-driven (ED) PrEP intake *(12)*

Table 1 - Belgian PrEP reimbursement criteria

Criteria MSM at high risk of HIV infection:
(1) Condomless anal intercourse with at least two different partners in the last six months.
(2) Diagnosed with multiple sexually transmitted diseases in the last year.
(3) Taken multiple post-exposure prophylaxis treatments in the last 12 months.
(4) Used psychoactive substances while involved in sexual activities.
Criteria for other individuals at risk for HIV infection
(1) People who inject drugs.
(2) Sex workers.
(3) Individuals that are being exposed to unprotected sex and a high risk of HIV.
(4) Partners of HIV-positive patients who have a detectable viral load.

Since 2017, PrEP uptake in Belgium has been continuously increasing (13). The number of PrEP users through the public health insurance increased from 2332 in 2018 to 5227 in 2021. The vast majority of these users are men (99.2%), aged 30-49 (62.1%), and living the Dutch-speaking part of Belgium (54%, this represents 41.9 PrEP users/100 000 inhabitants in Flanders, which is lower than the 129.8 PrEP users/100 000 inhabitants found in Brussels, but higher than in Wallonia with 23.2 PrEP users/100 000 inhabitants). Among individuals having started PrEP in 2021, 98% were MSM and 71% were Belgian. While the increasing uptake of PrEP in Belgium is encouraging, more than two new HIV infections per day were still diagnosed in Belgium in 2021, pointing that there is still room for more HIV prevention. The two largest groups affected by the HIV epidemic are MSM and people with a sub-Saharan African migration background, each of these groups accounting for about half of the incident HIV cases. In contrast, MSM represent almost all PrEP users in Belgium (13), while other risk groups for HIV are largely underrepresented (14). Even though MSM seem to be well reached by PrEP in Belgium, a substantial part of this population would still be eligible for PrEP but is currently not taking it (15). This so-called “PrEP gap” leaves individuals at risk for HIV and represents a risk for onwards HIV transmission. It was estimated in 2018 that only 18% of eligible MSM in Belgium were actually taking PrEP (15, 16, 17). Luckily, this gap further closed while PrEP uptake was increasing, and it was

estimated that 45% of eligible MSM were taking PrEP in 2022 (unpublished data). The reasons for this "PrEP gap" can be explained by barriers to PrEP (care), that can be found all along the PrEP continuum of care (Figure 2) (18).

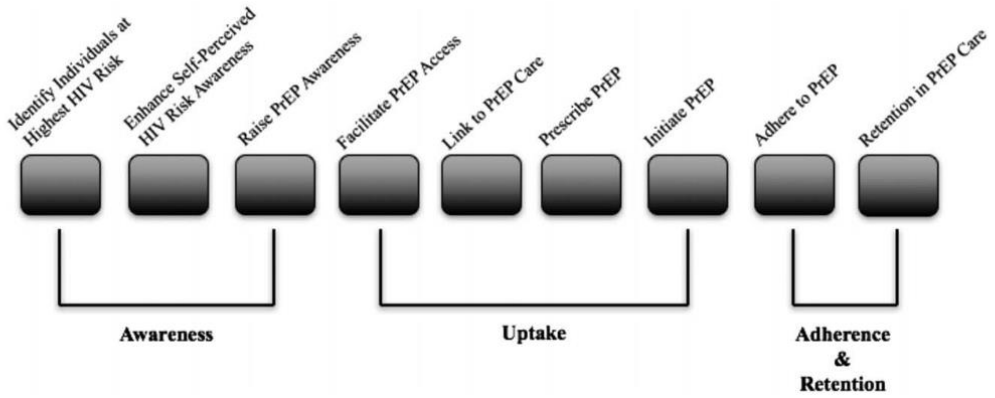


Figure 2 - PrEP continuum of care *(18)*

Firstly, awareness of PrEP, its indications and a correct self-perceived HIV risk are crucial (18). Awareness of PrEP among Belgian MSM is high as it ranges from 70-90% (15). Lack of awareness was associated with being older than 50 years old, a lower educational level, unemployment and living in a small city (15, 19). Despite a relative high awareness, willingness to take PrEP among Belgian MSM is lower and ranges from 43-70% (15). Importantly, willingness to take PrEP was higher among participants eligible for PrEP. However, still 16-33% of MSM eligible for PrEP were not willing to take it, which is worrying. These individuals were more likely to not have tested for HIV in the previous 6 months and reported a lower self-perceived risk for HIV. Discordance between objective and self-perceived HIV risk has been previously shown and poses a threat to PrEP care as these individuals might not identify themselves as PrEP candidates and, as a consequence, remain at risk for HIV (19, 20).

Following awareness and willingness to take PrEP, access to PrEP care is the next step in the continuum of care (18). PrEP care accessibility can be hampered by several structural, logistical and financial barriers (18). In Belgium, PrEP is delivered through 12 centralized, highly specialized HIV reference centers, which might limit PrEP care accessibility (11, 21).

Financial barriers might be less of an issue given that PrEP is reimbursed by the public health insurance. However, individuals who are not covered by the public health insurance are de facto excluded from PrEP care, meaning these individuals might remain at risk for HIV. For instance, it has been shown that undocumented migrants from Sub-Saharan Africa are more likely to be eligible for PrEP (and thus at high risk for HIV) than those who have access to health insurance (14). One of the main challenges in PrEP care is adherence and retention in care (18). PrEP is not a lifelong intervention and should be taken only during periods at risk for HIV, while it can be discontinued when no such risk is present (22). Therefore, assessing reasons for PrEP discontinuations as well as ongoing (or not) HIV risk when discontinuing PrEP is crucial and had rarely been done before. It will be the focus of the first part this thesis.

1.2 Syndemics as a threat to the health of PrEP users

Syndemics are defined as "the aggregation of two or more […] health conditions in a population in which there is some level of deleterious biological or behavior interface that exacerbates the negative health effects of any or all of the diseases involved." (23) Mental health disorders, sexual violence and substance use are examples of such factors that can interplay within the HIV and sexually transmitted infections (STIs) epidemics, and affect MSM and PrEP users (24). In this part of the introduction, we will describe these factors, how they share common risk factors and consequences, and can therefore reinforce each other leading to an increased burden of health consequences on PrEP users.

MSM are disproportionately affected by mental health disorders compared with heterosexual populations (25, 26, 27). A meta-analysis of mental health disorders among non-heterosexual populations showed that MSM have up to 2 times more risks of suicidal ideations and depression than their heterosexual counterparts (28). Similarly, high rates of mental health disorders have been reported among PrEP users (29). A poorer mental health state has in turn been associated with higher odds of unprotected anal intercourse, and therefore an increased risk for HIV and STIs (24, 30). But mental health issues have also

been associated with substance use and substance use disorders, both matters that have been found to be more prevalent in MSM and PrEP users compared with the general population (24, 31).

"Chemsex", or sexualized drug use, is a type of substance use of particular interest. It usually refers to the use of psychoactive drugs such as methamphetamines, gamma-hydroxybutyrate or mephedrone in a sexual context, but in theory it could relate to the use of any drug before or during sexual activities (24, 32). Engagement in chemsex is particularly prevalent among PrEP users (32). In Belgium it has been estimated that 24-48% of PrEP users engage in chemsex (13, 33). Assessing and addressing chemsex in PrEP users is important given that such practices can lead to several physical and mental health consequences. The substance use itself can lead to substance use disorders such as addiction or even overdose (34). Engagement in chemsex has been linked to high sexual risk taking such as unprotected anal intercourse and fisting, and therefore a risk for HIV and other STIs (35, 36). Moreover, unsafe injection practices present a risk for the transmission of HIV and other bloodborne viruses such as hepatitis C (32, 37). Substance use has been linked to poorer attendance of HIV clinics and poorer adherence to antiretroviral medications in HIV positive individuals, raising the fear for similar trends among PrEP users (34). Reassuringly, most studies assessing PrEP adherence among PrEP users engaging in chemsex found high levels of adherence (38, 39). Lastly, individuals engaging in chemsex had higher rates of mental health issues such as depression and anxiety and were more likely to report experiences of sexual violence (40).

Sexual violence is defined as "a sexual act that is committed or attempted by another person without freely given consent of the victim or against someone who is unable to consent or refuse" (26, 41). The exact incidence of sexual violence in MSM is hard to estimate and depends on the definition used and the recall period (42). However, all studies seem to point to a higher incidence in MSM compared with the general population. Sexual violence has been linked with several short- and long-term health consequences. Mental health disorders, including mood disorders and suicidal ideation, as well as alcohol and drug

use have been more frequently reported among victims of sexual violence (43). Higher rates of sexual risk behaviors, and higher incidences of STIs and HIV have also been described (27, 44). No data on the occurrence of sexual violence among PrEP users was available so far. We hypothesized that PrEP users might be particularly vulnerable for sexual violence due to a combination of factors such as a high number of sex partners, frequent engagement in chemsex, a high prevalence of mental health disorders and the frequent use of dating apps, which have been shown to facilitate the occurrence of sexual violence (33, 45, 46, 47).

The second part of this work will address two components of this syndemic: chemsex and sexual violence. We will particularly focus on how PrEP users affected by these issues can be supported and the role of PrEP care in providing such support.

1.3 The burden of sexually transmitted infections

Sexually transmitted infections have been on the rise worldwide since the early 1990s (48). It has been estimated that 131 million cases of chlamydia, 78 million cases of gonorrhea, and 6 million cases of syphilis occurred in the world in 2012 (49). Similar trends have been described in Europe and in Belgium (Figure 3) (50, 51, 52, 53). Several key populations, such as MSM and sex-workers, are disproportionately affected by STIs (54). In Europe in 2019, MSM accounted for 50% of all gonorrhea cases, 74% of all syphilis cases and 13% of all chlamydia cases (50, 51, 52). Higher rates of sexual risk behavior such as condomless anal sex, an important number of partners, important partner concurrency as well as a densely connected sexual networks are factors explaining the higher incidence of STIs among MSM (55).

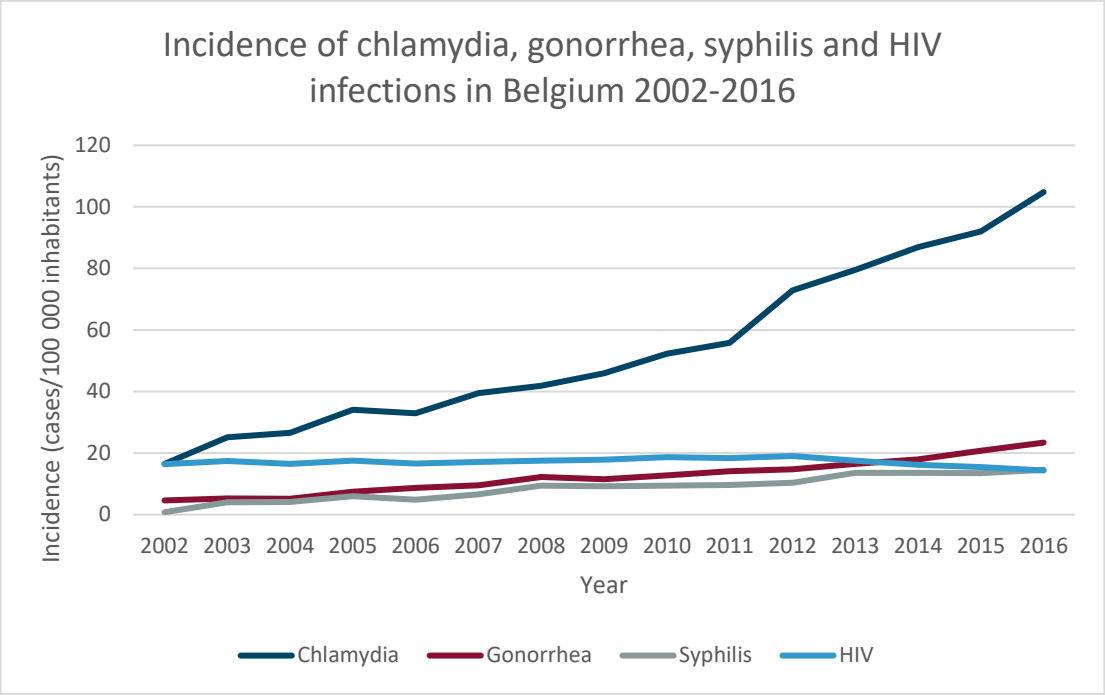


Figure 3 - Trends of chlamydia, gonorrhoeae, syphilis, and HIV infections in Belgium (2002-2016) (13, 56, 57)

Similarly, high rates of STIs have been reported among PrEP users (58). However, the link between PrEP and STIs is complex. HIV and bacterial STIs share transmission routes (e.g. unprotected anal sex). Therefore, it is logical that individuals at high risk for HIV such as PrEP users are also at high risk for STIs (58). Moreover, high rates of STIs among PrEP users might partly be explained by the intense screening protocols in place in this population. Most PrEP guidelines recommend 3-monthly screening for STIs (59, 60). It has been shown that engaging individuals in care increases their testing frequency which in turn might increase detection and thus spuriously increase the incidence of these infections (61, 62). Lastly, the roll-out of PrEP, alike all successful HIV prevention interventions, has raised concerns of "risk compensation", an increase in risky behavior due to a decrease in perceived risk (63, 64, 65). In the case of PrEP, the protection it confers against HIV would lead to an increase in sexual risk behavior such as condomless anal sex, which could in turn increase the transmission of other STIs. However, the effect of PrEP on the incidence of STIs is conflicting, with some studies having found an increase in the incidence of STIs

following PrEP initiation (64, 66), while others did not (67, 68). There is a growing body of evidence showing that a minority of PrEP users concentrate the majority of STIs (66, 69). A Belgian study found that approximately 78% of incident STIs among PrEP users were found in 36% of the users (69). This subgroup of PrEP users engaged in particularly high-risk sexual behaviors such as a high number of sex partners, a high number of condomless anal sex partners and chemsex.

How PrEP and STIs are linked is a matter of debate, but even more controversial are the interventions needed to control the STIs epidemic in this population. In the past decades, the emergence of antimicrobial resistance (AMR) in bacterial STIs, particularly *Neisseria gonorrhoeae* has led to rethink STI control strategies in key populations such as MSM on PrEP (70).

1.4 Antimicrobial resistance in *Neisseria gonorrhoeae*

Neisseria gonorrhoea (NG) has become resistant to all classes of antimicrobials used against it and there are concerns it might become untreatable in the near future (71). Several cases of extensively drug resistant NG, displaying resistance to ceftriaxone and azithromycin, the combination of antibiotics used to treat it, have been described in the United Kingdom, Australia and Austria (72, 73). It is likely that AMR in NG has emerged following decades of antimicrobial exposure (74). AMR has frequently emerged in core-groups heavily exposed to antimicrobials such as PrEP users (75). For example, the consumption of macrolides in PrEP users is higher than the average consumption of any European country and 52 times higher than the country with the lowest consumption (76). The mechanisms by which antimicrobial consumption can lead to AMR in NG are multiple and complex (71). However, one mechanism seems to play a prominent role: the uptake of genetic material from commensal *Neisseria species (spp.)* through transformation (77). Commensal *Neisseria* spp. are much more prevalent than pathogenic *Neisseria* spp. and, therefore, they are much more exposed to antimicrobials used for other indications than pathogenic *Neisseria* spp. (78). As a consequence, they face a greater selection pressure than pathogenic *Neisseria*

spp. to develop AMR. AMR determinants acquired by commensal *Neisseria* spp. can subsequently be transferred to NG under antimicrobial pressure (71). Interventions to reduce antimicrobial consumption, and thus slow down the emergence of AMR are urgently needed. The third part of this work will cover the issue of antimicrobial resistance in STIs. We will first assess how antimicrobial resistance in commensal *Neisseria* spp. has evolved in place and time in the last decades, and in relation with pathogenic *Neisseria* spp.

One of the main drivers of antimicrobial consumption in PrEP users is screening and subsequent treatment for NG and *Chlamydia trachomatis* (CT). As previously mentioned, most PrEP guidelines recommend 3-monthly screening for NG and CT, at three sites (urethra, pharynx, ano-rectal) (59, 60). The rationale for this recommendation is that the majority of these infections in MSM are extra-genital and asymptomatic (79). Early detection and treatment of these asymptomatic infections would lead to a decrease in transmission and ultimately to a reduction in the incidence and prevalence (80, 81). However, the evidence supporting such and effect of screening for NG and CT is scarce (82). International guidelines stipulate that there should be evidence from high quality RCTs on the benefits of a screening program before it should be introduced (83, 84). No such RCT had been performed so far to assess the impact of screening for NG and CT on the incidence of these infections. Furthermore, screening and subsequent treatment for NG and CT might be counterproductive.

Figure *4* shows how screening for NG and CT could lead to more AMR in NG in populations with a high network connectivity such as PrEP users: screening and treatment for NG might eradicate NG in some individuals but would also induce AMR in commensal *Neisseria* spp. (85) Given that the sexual network connectivity remains unchanged, individuals might become re-infected with NG, but this time AMR determinants acquired by commensal *Neisseria* spp. can be transferred to NG. In the end, the prevalence of NG would not decline, but NG strains with AMR determinants would also be present. In the third part of this work, we will assess several strategies to reduce antimicrobial consumption in PrEP cohorts in order to slow down the emergence of antimicrobial resistance. The first strategy we will

assess is reducing the intensity of screening for NG and CT. We will evaluate if screening for NG and CT in PrEP cohorts is efficacious in reducing the incidence of these infections, and what is the impact of different screening strategies on antimicrobial consumption.

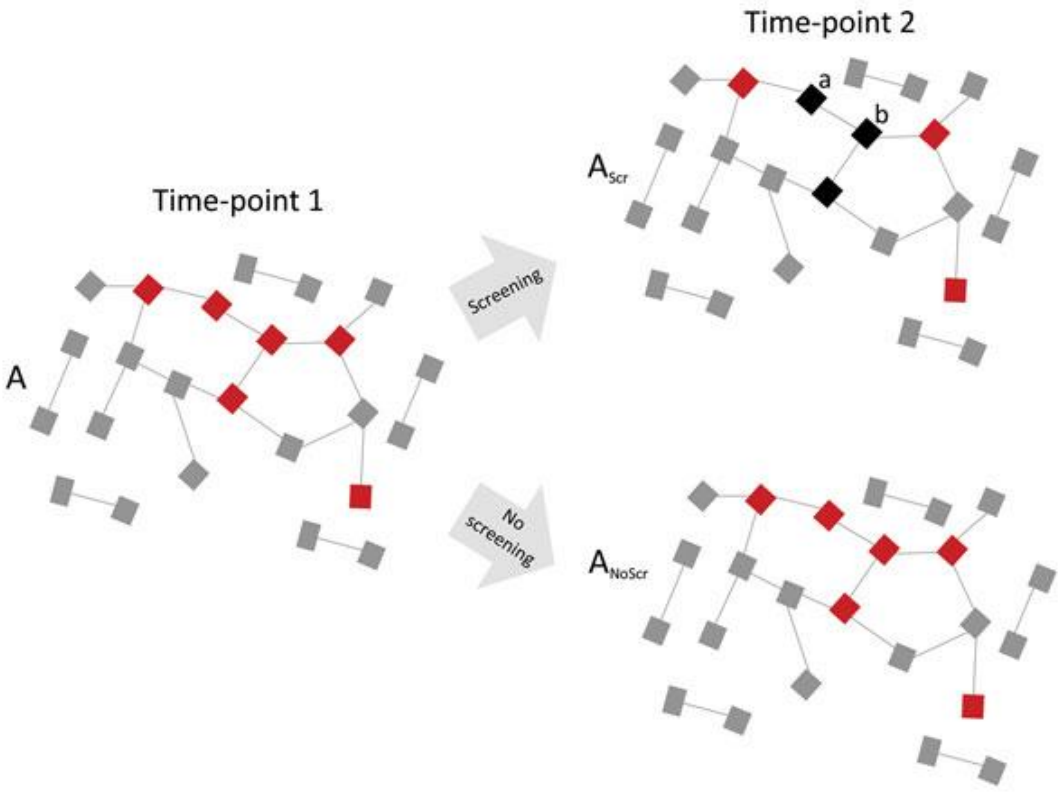
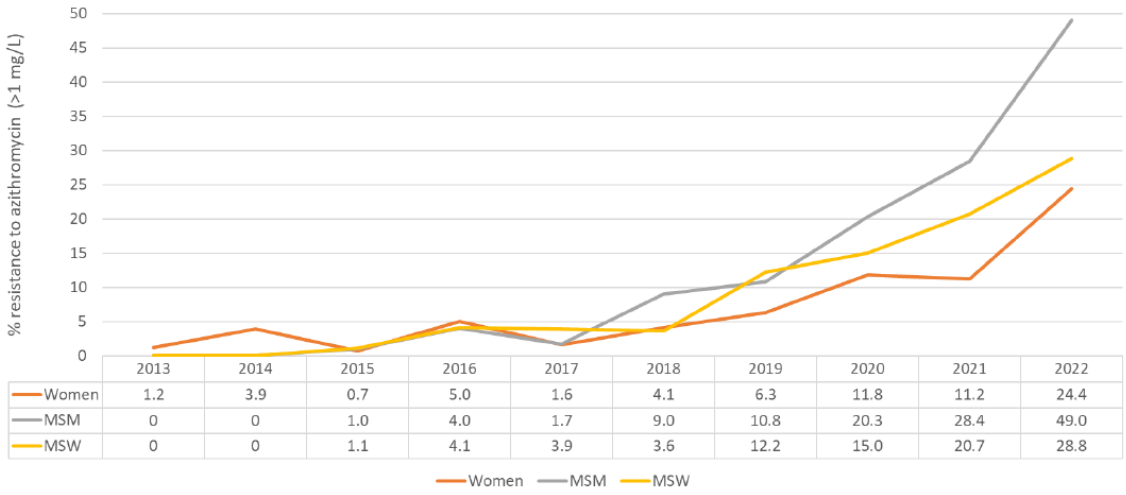


Figure 4 - Screening for NG and CT could lead to more AMR due to a dense sexual network in MSM (85)

There are currently two main options for the treatment of NG: monotherapy with ceftriaxone (CRO) or dual therapy with CRO plus azithromycin (CRO/AZM) (86, 87). Dual therapy emerged in the early 2010s and was endorsed by the United States Center for Diseases Control and Prevention (CDC) and the European International Union against Sexually Transmitted Infections (EIUSTI) (86, 87). The rationale behind dual therapy was based on the opinion of certain experts that it would delay the emergence of AMR in NG (88). Importantly, to our knowledge, no RCT has compared the efficacy of mono- with dual therapy. Two recent meta-analyses did not find a significant difference in the eradication of pharyngeal or anorectal NG between the two options (89, 90). However, the percentage of NG isolates with resistance to AZM has dramatically increased in past years. In Belgium,

for example, the proportion of clinical isolates with AZM resistance has increased from 0.2% to 33% between 2013 and 2022 (Figure 5) (91). Given that there is equipoise between both treatment options, switching from mono- to dual-therapy for the treatment of NG might be a way to reduce antimicrobial consumption in PrEP cohorts, and delay the emergence of AMR. In this work, we will assess the effect on AMR of both treatment regimens.



	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Women	1.2	3.9	0.7	5.0	1.6	4.1	6.3	11.8	11.2	24.4
MSM	0	0	1.0	4.0	1.7	9.0	10.8	20.3	28.4	49.0
MSW	0	0	1.1	4.1	3.9	3.6	12.2	15.0	20.7	28.8

Figure 5 - Resistance to azithromycin of NG stratified by gender and sexual transmission 2013-2022. MSW: Men who have sex with women (91)

1.5 Doxycycline post-exposure prophylaxis: a game changer or a threat?

A new intervention to control bacterial STIs, that is at odds with all the precited antibiotic sparing interventions, has emerged in recent years: doxycycline post-exposure prophylaxis (DoxyPEP) (92). It entails the use of doxycycline within 72 hours after a condomless sexual contact. DoxyPEP has been shown to reduce the occurrence of CT infections by 70-88%, and of syphilis infections by 73-87% (93, 94). Data on the efficacy of DoxyPEP on the occurrence of NG is conflicting. A study in the USA has shown a 55% reduction in NG cases (94), whereas a study in France did not find an effect on NG infections (93). This difference might be explained by the higher baseline tetracycline prevalence in the French study (93).

While the use of DoxyPEP seems promising for bacterial STIs control, DoxyPEP has not been included in any recommendation so far. The main concern is that it could induce AMR in NG, as discussed above, but also in other pathogens (95). Despite being not formally recommended, it has been reported that 2-23% of MSM are already using DoxyPEP (96, 97, 98, 99), and acceptability for this new intervention has been shown to be high among MSM and healthcare professionals (100, 101, 102). DoxyPEP will be the focus of the last part of this thesis. More specifically, we will assess the awareness and use of antibiotics for STI prevention among Belgian PrEP users and how Doxy-PEP might induce AMR to several antimicrobials in NG.

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Chapter 2 Objectives

The objective of the present research is to describe contemporary challenges faced by PrEP care and users in Belgium and potential interventions to address these challenges. The main challenges that will be addressed are:

A. Barriers to PrEP care and retention in PrEP care
B. The presence of syndemics and the need for risk-reduction strategies, and
C. The emergence of AMR in bacterial STIs

Each of these challenges has its own objectives, methods, and related publications that are detailed in the following table.

Challenge	Objective	Chapter
A. Barriers to PrEP care and retention in PrEP care	Objective A.1: to explore which factors are associated with PrEP care discontinuation, the reasons for discontinuation and to explore to what extent patients who discontinue PrEP care are still at risk for HIV	3
B. The presence of syndemics and need for additional risk-reduction strategies	Objective B.1: to explore the occurrence of engagement in chemsex, its perceived negative effects, the willingness to reduce chemsex and associated risks, and the preferred options or tools to reduce such risks among PrEP users in Belgium	4.1
	Objective B.2: to assess the occurrence and forms of lifetime non-consensual sex, factors associated with recent experiences of non-consensual sex and to explore help-seeking behavior after non-consensual sex experiences PrEP users in Belgium	4.2
C. The emergence of AMR in bacterial STIs	Objective C.1: to assess how antimicrobial susceptibility in commensal Neisseria has varied over place and time and in relation to the	5.1

	pathogenic *Neisseria*	
	Objective C.2: to assess the impact on macrolide consumption of switching from triple-site, 3-monthly to single-site 6-monthly screening for NG and CT in PrEP users	5.2
	Objective C.3: to assess the effect of screening MSM on PrEP for NG and CT on the incidence of these infections, the incidence of symptomatic infections, the incidence of syphilis infections and on antibiotic consumption as well as the PrEP users' perceptions towards STI screening	5.2
	Objective C.4: to assess the impact on the resistome of mono- vs dual therapy for the treatment of NG	5.2
	Objective C.5: to assess the awareness and use of STI prophylaxis among HIV PrEP users in Belgium	5.3
	Objective C.6: to assess if doxycycline post-exposure prophylaxis could induce resistance (in NG) to other classes of antimicrobials	5.3

Chapter 3

Barriers to PrEP care and retention in PrEP care

3.1 PrEP care discontinuation: reasons, associated factors and HIV risk

Vanbaelen T, Rotsaert A, Jacobs BKM, et al. Why Do HIV Pre-Exposure Prophylaxis Users Discontinue Pre-Exposure Prophylaxis Care? A Mixed Methods Survey in a Pre-Exposure Prophylaxis Clinic in Belgium. *AIDS Patient Care STDS* 2022; 36(4): 159-67.

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Why Do HIV Pre-Exposure Prophylaxis Users Discontinue Pre-Exposure Prophylaxis Care? A Mixed Methods Survey in a Pre-Exposure Prophylaxis Clinic in Belgium

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Abstract

It remains unclear why patients discontinue HIV pre-exposure prophylaxis (PrEP) care and to what extent they remain at risk for HIV when they do. We reviewed routinely collected medical records and patient questionnaires and performed an e-mail/telephone survey to assess reasons for discontinuing PrEP care, ongoing risks for HIV infection, and associated factors. Patients with more than two registered PrEP visits from a PrEP clinic in Antwerp, Belgium between June 2017 and February 2020 were included in this study. Patients who did not return for a visit after October 30, 2019 and who were not transferred out were considered as having discontinued PrEP care. A total of 143/1073 patients were considered as having discontinued PrEP care. Patients who discontinued PrEP care were more likely to be younger than those who remained in care (35 vs. 38 years old, $p < 0.01$). The most common reasons for discontinuation were having stopped using PrEP (62/101, 61.4%) and "COVID-19" ($n = 35$, 34.7%). The most common reasons for stopping PrEP use was a decreased sexual activity due to coronavirus disease 2019 (COVID-19; 21/62, 33.9%) or not COVID-19 related (10/62, 16.1%), a monogamous relationship (20/62, 32.3%) and consistent condom use (7/62, 11.3%). Among respondents who reported about current HIV risk the majority reported being at low risk either by still taking PrEP (32/91, 35.2%), consistently using condoms, or limiting number of sex acts or partners (58/91, 52.7%). No HIV seroconversion was reported.

Keywords: HIV pre-exposure prophylaxis, PrEP discontinuation, HIV, prevention, PrEP

Introduction

HIV PRE-EXPOSURE PROPHYLAXIS (PrEP) is a very effective biomedical intervention to prevent HIV acquisition, when correctly taken.[1–3] PrEP is recommended for all individuals at substantial risk for HIV infection, such as men who have sex with men (MSM).[1,4,5] It has been implemented in many countries and its uptake is continuously increasing.[6] It is estimated that >1,300,000 individuals initiated PrEP in the second quarter of 2021.[7]

PrEP uptake, adherence, and retention in care are needed for PrEP to be effective.[8] Improving PrEP awareness and uptake are important first steps for effective PrEP implementation and is already extensively studied. Various studies have demonstrated that adherence is crucial for PrEP to be efficacious.[9] PrEP persistence, the correct and sustained use of PrEP over time, is also critical, but has until now received less scientific attention.[10]

PrEP is not considered a lifelong intervention, but should be taken in periods of increased risk for HIV infection. As HIV risk can vary over time (e.g., due to changes in sexual behavior), PrEP can be discontinued or restarted depending on such risk.[11,12] On the contrary, discontinuing PrEP during periods of HIV risk is to be avoided given the risk of HIV acquisition.[13–15]

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Title – Why do HIV pre-exposure prophylaxis users discontinue PrEP care? A mixed methods survey in a PrEP clinic in Belgium

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Abstract

It remains unclear why patients discontinue HIV pre-exposure prophylaxis (PrEP) care and to what extent they remain at risk for HIV when they do. We reviewed routinely collected medical records and patient questionnaires, and performed an email/telephone survey to assess reasons for discontinuing PrEP care, ongoing risks for HIV infection and associated factors. Patients with more than two registered PrEP visits from a PrEP clinic in Antwerp, Belgium between June 2017 and February 2020 were included in this study. Patients who did not return for a visit after 30/10/2019 and who were not transferred out were considered as having discontinued PrEP care. A total of 143/1073 patients were considered as having discontinued PrEP care. Patients who discontinued PrEP care were more likely to be younger than those who remained in care (35 vs 38 years old, $p<0.01$). The most common reasons for discontinuation were having stopped using PrEP (62/101, 61.4%) and 'COVID-19' (n=35, 34.7%). The most common reasons for stopping PrEP use were a decreased sexual activity due to COVID-19 (21/62, 33.9%) or not COVID-19 related (10/62, 16.1%), a monogamous relationship (20/62, 32.3%) and consistent condom use (7/62, 11.3%). Among respondents who reported about current HIV risk the majority reported being at low risk either by still taking PrEP (32/91, 35.2%), consistently using condoms or limiting number of sex acts or partners (58/91, 52.7%). No HIV seroconversion was reported.

Keywords: HIV pre-exposure prophylaxis, PrEP discontinuation, HIV, prevention, PrEP

Introduction

HIV pre-exposure prophylaxis (PrEP) is a very effective biomedical intervention to prevent HIV acquisition, when correctly taken (1–3). PrEP is recommended for all individuals at substantial risk for HIV infection, such as men who have sex with men (MSM) (1,4,5). It has been implemented in many countries and its uptake is continuously increasing (6). It is estimated that over 1.300.000 individuals initiated PrEP in the second quarter of 2021 (7).

PrEP uptake, adherence and retention in care are needed for PrEP to be effective (8). Improving PrEP awareness and uptake are important first steps for effective PrEP implementation and is already extensively studied. Various studies have demonstrated that adherence is crucial for PrEP to be efficacious (9). PrEP persistence, the correct and sustained use of PrEP over time, is also critical, but has until now received less scientific attention (10). PrEP is not considered a lifelong intervention, but should be taken in periods of increased risk for HIV infection. As HIV risk can vary over time (e.g. due to changes in sexual behavior), PrEP can be discontinued or restarted depending on such risk (11,12). On the contrary, discontinuing PrEP during periods of HIV risk is to be avoided given the risk of HIV acquisition (13–15).

Many PrEP patients discontinuing care do so in the first months following PrEP initiation (16–18). One recent review across different countries and populations, including MSM, showed that average retention in PrEP care was 51% after 6 months and 43% after twelve months (8). Patients who discontinue PrEP care are more likely to be younger and predominantly choose event-based PrEP (18,19). Potential reasons for discontinuing PrEP care are a lower perceived risk for HIV, fear of side-effects or experiencing logistical and financial barriers (17,19).

Recently, the COVID-19 pandemic has been responsible for major changes in sexual behavior and prevention. Sexual risk-taking and PrEP use both declined during the first periods of physical distancing and restriction measures (20,21). Hence, the need for PrEP

care may have been less in this period. Furthermore, sites where PrEP is provided may have been temporarily closed, or its providers may have been temporarily predominantly occupied in periods requiring more care and attention to COVID-19. Understanding whether or how PrEP care is discontinued in such periods can be important to anticipate subsequent or similar phases of the COVID-19 pandemic.

In Belgium, PrEP is reimbursed through the public health care system since mid-2017 for people at substantial risk for HIV acquisition (22). PrEP care is centralized in 12 HIV Reference Centers (HRC). To get PrEP reimbursed, patients visit an HRC where eligibility is verified. Next, a reimbursement request is submitted to the health insurance fund, which is to be renewed yearly. A total of 4071 persons initiated PrEP before 2020, the vast majority (97.3%) of them being MSM (23). Information on PrEP discontinuation however is lacking.

The objective of this study was to explore which factors are associated with PrEP care discontinuation and the reasons. An additional objective was to explore to what extent patients who discontinue PrEP care are still at risk for HIV. These insights can complement current knowledge on PrEP care retention and effectiveness of PrEP programs, as well as assess the need for additional tools or interventions to improve retention in PrEP care.

Methods

Design

Retrospective analysis of routinely collected medical records and questionnaire data, in addition to a cross-sectional email and telephone survey.

Sample selection

The setting of this study is a PrEP clinic in Antwerp, Belgium. All patients with more than two PrEP visits between the roll-out of PrEP in Belgium (01/06/2017) and the start of the

COVID-19 period (28/2/2020) were selected. In general, to initiate PrEP, patients first come for a screening visit where information is provided, eligibility for reimbursement is controlled and medically required tests are performed. During a second visit, test results are provided, as well as further counselling and a PrEP prescription.

Data retrieval for this study was November 2020. Patients who did not return for one year prior the start of the analysis were theoretically not able to get PrEP reimbursed given the required yearly renewal of the reimbursement. Hence, patients who had not returned since 30/10/2019 were considered as *having potentially discontinued PrEP care*. Patients who interrupted and re-engaged in PrEP care were not considered as having potentially discontinued PrEP care.

Data collection

Questionnaires

Patients were asked to fill in a questionnaire at each PrEP consultation. For this analysis we used data on socio-demographic characteristics and sexual behavior collected during the first PrEP visit.

Medical records

An electronic medical record is held by the healthcare providers during the PrEP consultation and contains all medically relevant information. The medical records of patients who *potentially discontinued PrEP care* were examined for a reason for not returning. If during medical records examination, patients were found to have an appointment planned or had a consultation between the censor date and the final analysis, they were not anymore considered as *having potentially discontinued PrEP care*.

Telephone and email survey

Patients who *potentially discontinued PrEP care* and for whom no reason for not returning was found in the medical records were informed via email about the study and asked consent for an interview. They were provided the option to decline participation or to provide the information directly via email. Patients who did not decline, nor provided information about the reason for not returning, were contacted via telephone for a brief interview about the reasons for not returning for FU, PrEP use, HIV testing and preferred settings for PrEP FU. Respondents could provide multiple answers for each question. The interviews were recorded with patients' consent.

Answers were classified in pre-defined categories. If no pre-defined category fitted the answer, it was reported as free text in the category "other".

Participants were provided the option for a new PrEP appointment at the end of the interview.

Data analysis

Data concerning the reasons for having discontinued PrEP care, obtained via medical records, email or telephone were grouped into recurring categories and described using absolute numbers and proportions.

Patients who were classified as having *potentially discontinued PrEP care* (as defined above) and who did not report being transferred out in medical records or during the telephone or email survey were considered as having *discontinued PrEP care*. This category also included patients for whom no information was collected due of lack of contact details or answer. All other patients were considered as having *remained in care.* We compared patients having discontinued care with those remaining in care to find associations between sociodemographic characteristics, sexual risk factors, and PrEP care continuation.

We defined HIV risk as low if, based on medical records or telephone/email survey, participants reported either still taking PrEP, consistently using condoms or a limited number of sex acts or partners (e.g. being in a monogamous relationship with a HIV negative or HIV positive undetectable partner, having no sexual contacts at all, …)

Continuous variables were described with mean/median and standard deviation/interquartile range. Categorical variables were described using proportions. Associations between categorical variables were tested using chi-square and associations between categorical and continuous variables using Student's t-test or Mann Whitney U test.

Retention in care was analyzed using survival analysis and Kaplan-Meier curves.

Statistical analysis and graphical representation was performed in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical approval

The study received ethical approval from the Institutional Review Board of the Institute of Tropical Medicine, Antwerp (IRB 1352-20) and the ethics committee of the University hospital of Antwerp (18/33/368). Patient consent was asked before filling the questionnaires and before participating to the telephone/email survey. The data were pseudonymized before analysis.

Results

Sample selection

Among the 1073 selected PrEP patients, 169 were considered as *having potentially discontinued PrEP care* (Figure 1). For 26 of those, we found a valid reason for not returning in medical records (e.g. transferred to another clinic, Appendix C) and thus they were not

further contacted. We collected data via 49 telephone interviews and 26 email answers. Eleven patients had missing contact information, 52 patients did not respond and five declined to participate, leading to a response rate of 56.8%. We found that 26 patients were transferred to another center which brings the final number of patients considered as *having discontinued PrEP care* to 143 and the number of patients considered having *remained in care* to 930.

Retrospective analysis of PrEP questionnaires

The median age of our total sample of patients was 38 years (IQR 30-46; Table 1) at baseline. The majority was male (99.7%), highly educated (62.2%), of Belgian nationality (85.6%) and from Antwerp province (75.2%). The median number of sex partners in the three months preceding the first visit was 6 (IQR 4-12). Almost all participants (99.3%) had sex with men, 42% reported using party drugs during sex and 9.8% reported never using condoms for anal sex in the three previous months.

The survival analysis of PrEP care showed a probability of remaining in FU of 93.9% (95% CI 92.5-95.4), 91% (95% CI 89.2-92.9), 87.6% (95% CI 85.5-89.9) and 86% (95% CI 83.7-88.4) at 3,6,9 and 12 months respectively. (Figure 2)

Patients who discontinued PrEP care were more likely to be younger when compared with those in care (median age 35 vs 38, p-value < 0.01, Table 1). There were no significant differences in gender, educational level, country or province of origin and in sexual practices.

Results from telephone/email survey and medical records

<u>Reasons for PrEP care discontinuation</u>

The most common reasons for discontinuing PrEP care were having stopped using PrEP (n=62, 61.4%, Table 2), COVID-19 (n=35, 34.7%) and being followed-up elsewhere (n=26,

25.7%). COVID-19 was responsible for various reasons for PrEP care discontinuation, such as decreased sexual activity, fear to visit the PrEP clinic during the first wave of the pandemic (e.g. avoiding public places), or assuming that healthcare providers would prioritize COVID-19 over PrEP care, such as the following participant explains:

"[…] with COVID I thought [the clinic] would have other concerns than PrEP users." (male, 47 years old, 6 months of FU)

One participant passed away (0.99%) and 6 had moved abroad (5.9%). Other reasons for PrEP care discontinuation included having forgotten or missed an appointment (n=7, 6.9%), or no longer feeling the need for PrEP FU (n=2, 1.9%). Particular barriers experienced that lead to PrEP care discontinuation included difficulties accessing the clinic (e.g. distance, opening hours, …; n=7, 6.9%), finding the procedures for PrEP FU too much (n=4, 3.9%), or experiencing side-effects of PrEP (n=3, 2.9%) such as the following participant:

"I only used PrEP for a very short time. I took them periodically, usually when I went to the sauna ... When I take them, I feel the effect in my body (a kind of rush) .... Usually this feeling is limited, but a few times it was so intense that I had to vomit ! So in that respect it was not really a success." (male, 52 years old, 6 months of FU)

Among patients who stopped using PrEP, the majority did so because of a reduced sexual activity due to the COVID-19 pandemic (n=21, 33.9%,) or because of a novel monogamous relationship with a HIV negative or HIV positive partner with undetectable viral load (n=20, 32.3%). Other reasons were a reduced sexual activity not related to COVID-19 (n=10, 16.1%), consistent condom use (n=7, 11.3%), having moved abroad (n=4, 6.5%), health-related issues (n=3, 4.8%) and difficulties to make an appointment (n=2, 3.2%). The following participant explained how PrEP well fitted within a particular period of his life:

"I stopped de facto the behavior that made PrEP needed. […] when I was in the 40s [year old, …], for the first time in my life, I started experimenting with drugs to call it that way…

and what in lingo is called chemsex […], after 1.5 years or 1 year three quarters, that behavior has almost, but I can in fact say completely, disappeared." (male, 52 years old, 14 months in FU)

Estimated risk for HIV infection when having discontinued PrEP care

No HIV seroconversion was reported among the participants in the email and telephone survey. Among the participants who reported information on HIV risk in the survey or medical records, the vast majority (90/91, 98.9%) reported having a low risk for HIV infection either by still taking PrEP, consistent condom use, or a limited number of sex acts or partners (e.g. being in a monogamous relationship with a HIV negative or HIV positive undetectable partner). One participant reported sex acts which were not covered by PrEP nor condoms after his last visit:

"(…) the reason why I went into the PrEP program is because I often travel […] and I was afraid to be contaminated there […] At some point I received an email from [the clinic] and I didn't answer… Then I lost the thread and I think I was unsubscribed from the program, I can't really remember, but it came from my side. (…) I did have risky contacts [after that]." (male, 46 years old, 3.5 months of FU)

Discussion

We found that the main reason for PrEP care discontinuation was having stopped using PrEP. Among the participants who stopped using PrEP, the majority did so because of a decreased self-perceived risk for HIV. However, particular barriers such as difficulties accessing the clinic or experiencing side-effects also lead to patients stopping PrEP use.

The finding that the majority stops using PrEP due to a decreased self-perceived risk for HIV infection is in line with the results of previous studies (24–28). However, while these findings sound reassuring, some studies have found that a reduced self-perceived risk for

HIV does not always corresponds with a real decreased risk for infection (29,30). For example, Blumenthal et al found that 38% of the PrEP patients underestimated their HIV risk and this proportion went up to 90% in people who had a high-risk of HIV according to objective criteria (29). Interventions focused on improving self-estimation of HIV risk should be explored in order to allow patients to correctly and safely stop and re-start PrEP.

We found that some participants experienced barriers that have led them to discontinue PrEP care, such as (fear of) side-effects, difficulties to maintain the PrEP FU schedule, to access the clinic or finding the procedures too much, as found elsewhere (24–28). In contrast with other studies (24–26), none of the participants reported a financial burden of PrEP as reason for not returning for FU or for stopping with PrEP. This might be due to PrEP being partially reimbursed in Belgium for people at substantial risk for HIV, making it more affordable for people who have access to the public health care system (22). Additionnal interventions or alternative PrEP care delivery models should be explored in order to address the barriers experienced by PrEP care users and make the thresholds for PrEP care access and persistence as low as possible. For example, PrEP care decentralization or de-medicalization as well as new PrEP modalities (e.g.: injectable PrEP) are potential interventions to achieve such goals (31).

Some participants also reported COVID-19 as reason for not discontinuing for PrEP care or stopping PrEP use, either because of a reduced sexual activity imposed by social-distancing measures, or because of fear for public spaces or difficult access of the PrEP clinic. It has been previously described that the early stages of the COVID-19 pandemic have been responsible for major disruptions in PrEP care services as well as changes in sexual behavior and prevention practices (20,21,32,33). Further research is needed to assess how these have evolved during the different waves of the pandemic. Interventions aiming at improving retention in care when COVID-19 restrictions are in place, such as tele-care, must also be explored (34).

We found a retention rate of 90.3% at 12 months among the PrEP patients in this clinic. This is much higher than the average 43% reported by a recent review of PrEP care retention, based on studies in various countries and risk groups (8). Studies focusing on MSM in the United States also describe a drop in retention rate in the first year after PrEP initiation (16,17,35). This discordant finding may be explained by the selection criteria (> 2 visits) we applied to define PrEP care discontinuation and because we did not take into account patients who temporarily interrupt PrEP and later re-engaged in care.

Reassuringly, no HIV seroconversion was reported by patients who discontinued PrEP care in our clinic. This contrasts with other studies that found a higher HIV incidence among people who discontinued PrEP (14,15). Our finding could be due to the fact that the vast majority of those patients reported being protected against HIV either by still taking PrEP either by a reduced self-perceived risk.

Our study has several limitations. First, our selection criteria include patients who attended more than two PrEP visits which makes it prone to survival bias and could explain our high retention rate compared to other studies (8), moreover due to our sample selection criteria, our total sample is not likely to be representative of all PrEP patients. Second, the email and telephone survey was performed 1-3 years after the last PrEP visit which could induce recall bias. Third, the survey was performed by a PrEP care provider of the clinic and answers might have been subject to social desirability bias, as reasons for discontinuing PrEP care directly related to the clinic could have been underreported. Fourth, COVID-19 has been frequently cited as reason for PrEP (care) discontinuation although it doesn't fit the timeframe of our study. PrEP care discontinuation was defined as not returning after 30/10/2019, when COVID-19 was still out of the picture. Multiple explanations for this finding are possible. PrEP users do not always attend quarterly visits consistently (18) and it is not known when exactly the patients stopped PrEP after their last visit. Another explanation could be the fact that multiple answers for the same question could be provided by the patients, while COVID-19 could be a reason for not having re-started PrEP at the time of the survey it might not have been the reason for stopping PrEP initially.

Moreover, due to insufficient detail about the reasons for discontinuation it was not always clear what the main reason was, e.g. having limited access due to self-quarantine, or limited availability of services. Also, sometimes the discontinuation was multifactorial, making it impossible to distinguish between reasons, e.g. no more need for PrEP due to reduced sexual contacts, or reduced sexual contacts due to COVID-19 restrictions. Fifth, we did not perform HIV testing and HIV seroconversion data is based on self-reporting during the telephone/email survey. Finally, we could not obtain information from all patients who discontinued PrEP care in our clinic, which makes our sample not likely to be representative of all patients who discontinued PrEP care.

These limitations notwithstanding, this study showed that, while PrEP patients in our study discontinued PrEP care for various reasons, most of them thought to be at low risk for HIV infection when doing so. It is known that PrEP can be discontinued during periods at lower risk for HIV and restarted should the risks reappear (11). It is crucial that patients correctly estimate their risk for HIV infection in order to safely decide when and how to take PrEP. Alternative or novel strategies are also required to address potential barriers to PrEP care, particularly in times of COVID-19 where sexual activities and prevention services face many disruptions.

Table 1 - sociodemographic characteristics, PrEP use and behavioral factors

	Total sample*	**Discontinued PrEP care†**	**Patients remained in care‡**	**p-value**
	N=1073, n (%)	N=143, n (%)	N=930, n (%)	
Sociodemographics				
Age (years) (median; IQR)	38; 30-46	35; 27-44	38; 31-47	**<0.01**
Gender				
Man	1070 (99.7%)	143 (100)	927 (99.7)	1
Education§				
Higher Education	534 (62.2)	62 (55.9)	472 (63.2)	0.39
Country of origin‖				
Belgium	730 (85.6)	101 (91.8)	629 (84.7)	0.06
Province of origin				
Antwerp	807 (75.2)	99 (69.2)	708 (76.1)	0.09
Sexual practices				
Number of sexual partners previous 3 months (median; IQR)	6; 4-12	6;4-10	7;4-14	0.06
Gender of sexual partners¶				
Men	952 (99.3)	123 (98.4)	829 (99.4)	0.51
Condom use during anal sex in the previous 3 months**				
Never	89 (9.8)	8 (7)	105 (13.2)	0.45
Use of party drugs during sex in the previous 3 months††				
Yes	387 (42)	57 (47.5)	330 (41.1)	0.22

* total sample of patients having had > 2 visits.

† Discontinued PrEP care being defined as having had > 2 visits, not returning for FU after 30/10/2019 and not being transferred out.

‡ still in care being defined as not belonging to the "discontinued PrEP care" category

§ Missing answers total sample/discontinued PrEP care/patients remained in care: n = 215/32/183

‖ Missing answers total sample/discontinued PrEP care/patients remained in care: n = 220/33/187

¶ Missing answers total sample/discontinued PrEP care/patients remained in care: n = 114/18/96

** Missing answers total sample/discontinued PrEP care/patients remained in care: n = 165/29/136

†† Missing answers total sample/discontinued PrEP care/patients remained in care: n = 151/23/128

Table 2 – Telephone/email survey and medical records combined results

	Total N=101, n(%)
Reported HIV protection*	
yes	90(98.9)
no	1(1,1)
Reasons for PrEP care discontinuation†	
Doesn't use PrEP anymore	62(61,4)
COVID-19	35(34,7)
FU elsewhere	26(25,7)
Difficulties of access of the clinic (not COVID-19 related)	7(6,9)
Forgot or missed previous appointment	7(6,9)
Moved abroad	6(5,9)
Too many procedures for PrEP FU	4(3,9)
Side effects	3(2,9)
No need for FU	2(1,9)
Death	1(0,99)

* Defined as still taking PrEP, consistently using condom or being in a monogamous relationship with a HIV negative partner or HIV positive undetectable partner. Denominator = 91, due to lack of information for 10 participants.

† multiple answers possible

List of abbreviations: COVID-19: coronavirus disease 19, FU: follow-up, PrEP: pre-exposure prophylaxis

Figure 1 - Sample selection

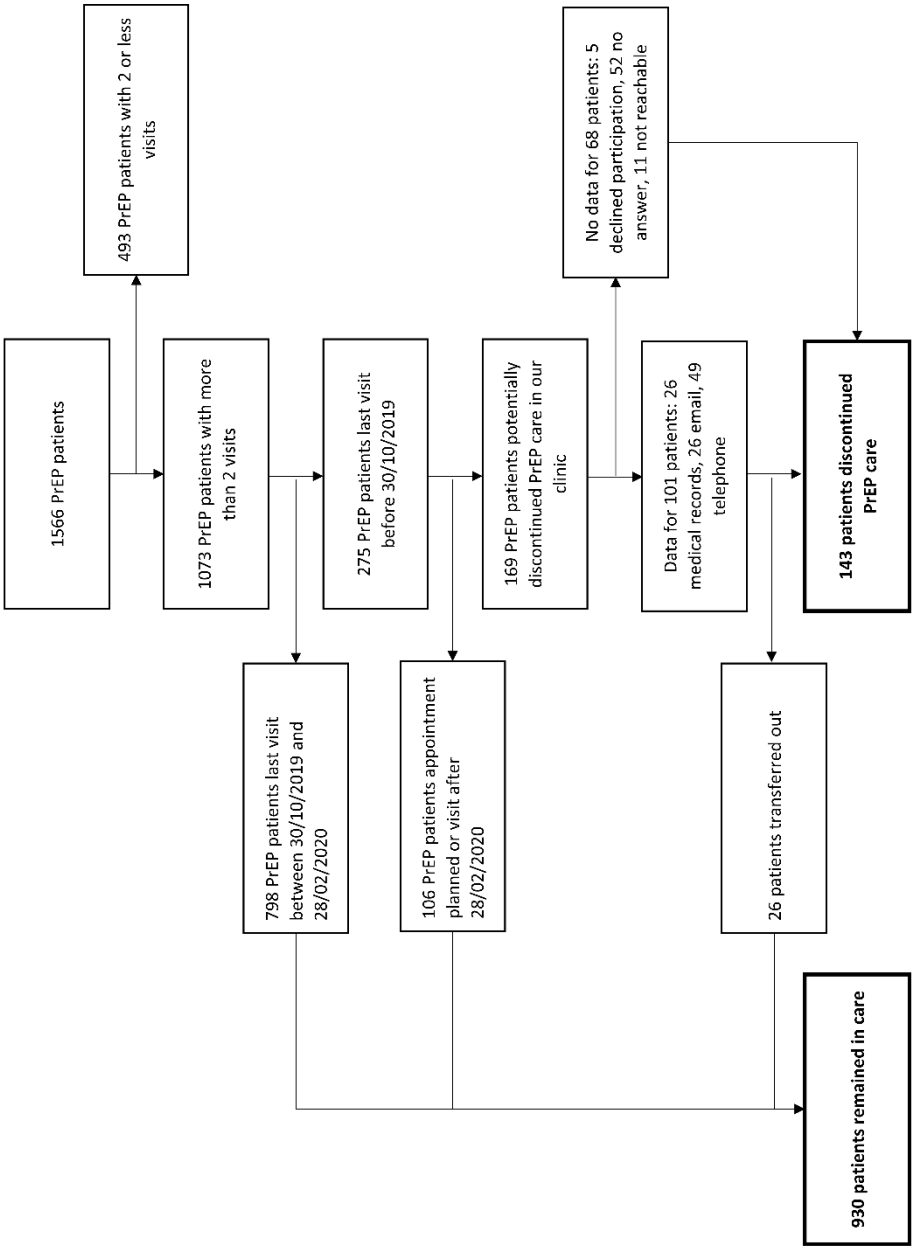


Figure 2 - Kaplan Meier curve of PrEP follow-up. FU = Follow-Up

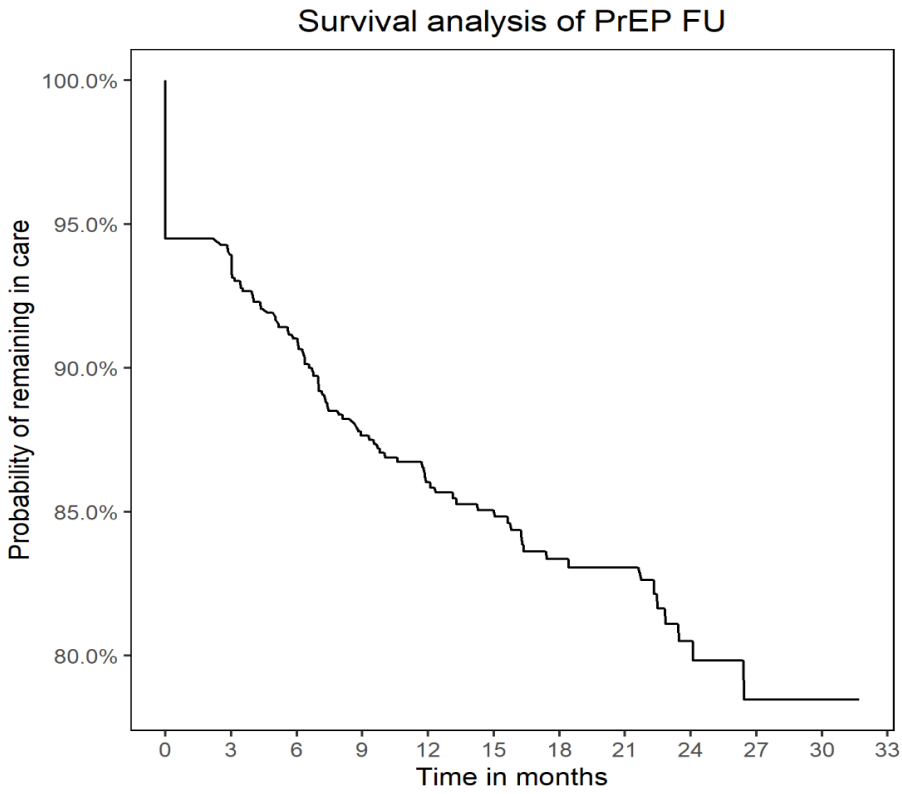
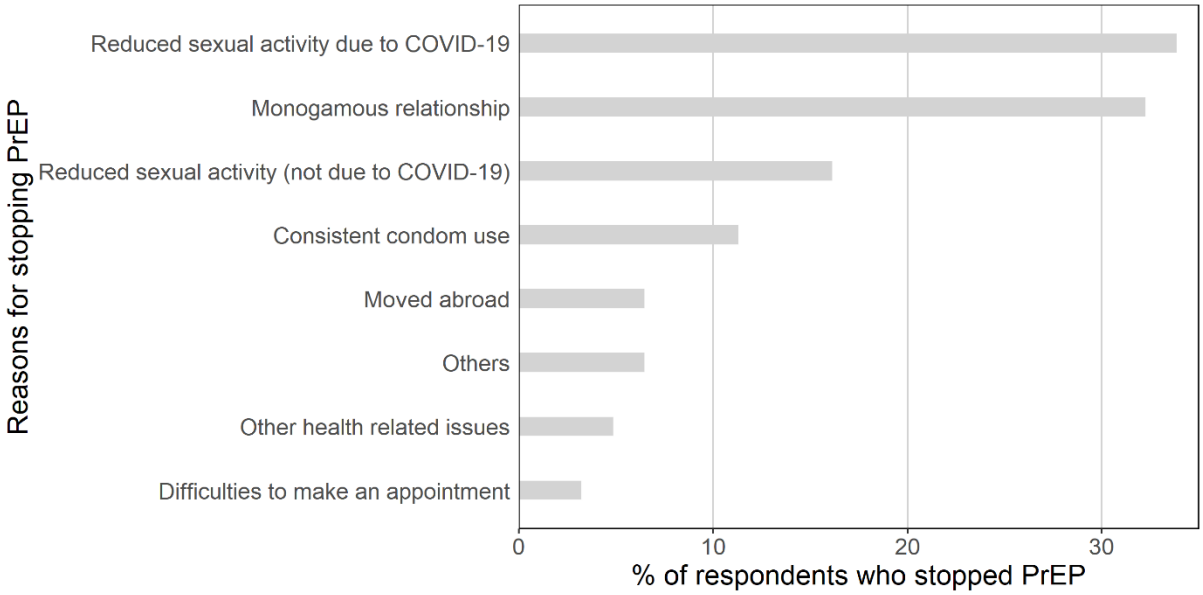


Figure 3 - Reasons for stopping PrEP – PrEP = Pre-Exposure Prophylaxis*



* multiple answers possible

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Appendix A – results LTFU telephone interviews

	Telephone interviews n (%)
HIV risk (N=49)	
Seroconversion	
yes	0 (0)
no	49 (100)
Protection*	
yes	48 (97,9)
no	1 (2)
Reasons for PrEP care discontinuation (N=49)†	
Stopped using PrEP	32 (65,3)
FU elsewhere	13 (26,5)
No need for FU	1(2)
Forgot or missed previous appointment	5(10,2)
Difficulties of access of the clinic (not COVID-19 related)	6(12,2)
Too many procedures for PrEP FU	2(4)
COVID-19	22(44,9)
Death	1(2)
Moved abroad	4(8,1)
Reasons for stopping PrEP (N=32)†	
Monogamous relationship	8(25)
Reduced sexual activity due to COVID-19	17(53,1)
Consistent condom use	4(12,5)
Others	4(12,5)
Reduced sexual activity (not due to COVID-19)	6(18,8)
Difficulties to make an appointment	2(6,3)
Moved	3(9,4)
Other health related issues	3(9,4)
Follow up of those still taking PrEP (N=17)	
Followed-up in another PrEP clinic	11(64,7)
Followed-up by GP	2(11,8)
Still had PrEP pills	4(23,5)
COVID-19 reasons for discontinuing PrEP care (N=22)†	
Reduced sexual contacts	19(86,4)
Did not wish to come to the clinic	3(13,6)
Blocked abroad	1(4,5)
Difficulty to make an appointment	1(4,5)
New appointment given	

yes	11(22,4)
no	38(77,6)

* defined as either reporting still taking PrEP, having no risks or systematically using condoms

† multiple answers possible

List of abbreviations: COVID-19: coronavirus disease 19, FU: follow-up, GP: general practitioner, HIV: human immunodeficiency virus, LTFU: lost to follow up, PrEP: pre-exposure prophylaxis

Appendix B - results LTFU email answers

	Email answers n (%)
Protection* (N=22)	
Yes	20(91)
No	0(0)
Not specified	2(9)
Reasons for PrEP care discontinuation (N=26)†	
COVID-19	12(46,1)
Doesn't use PrEP anymore	16(61,5)
FU elsewhere	6(23,1)
Missed or forgot previous appointment	2(7,7)
Difficulties of access of the clinic (not COVID-19 related)	1(3,9)
No need for FU	1(3,9)
Side effects	1(3,9)
Too many procedures for PrEP FU	2(7,7)
New appointment given	
yes	3(11,5)
no	23(88,4)

* defined as either reporting still taking PrEP, having no risks or systematically using condoms

† multiple answers possible

List of abbreviations: COVID-19: coronavirus disease 19, FU: follow-up, LTFU: lost to follow up, PrEP: pre-exposure prophylaxis

Appendix C - Reasons for PrEP care discontinuation retrieved from medical records

	Medical records N= 26, n(%)
Side effects	1(3,9)
COVID-19 (didn't wish to come to the clinic)	1(3,9)
Death	1(3,9)
Transfer HIV clinic	7(26,9)
Stopped taking PrEP	14(53,9)
Moved abroad	2(7,7)
List of abbreviations: COVID-19: coronavirus disease 19, HIV: human immunodeficiency virus, PrEP: pre-exposure prophylaxis	

Appendix D – Eligibility criteria for reimbursement of PrEP in Belgium

Criteria for the reimbursement of PrEP in Belgium are as follow:

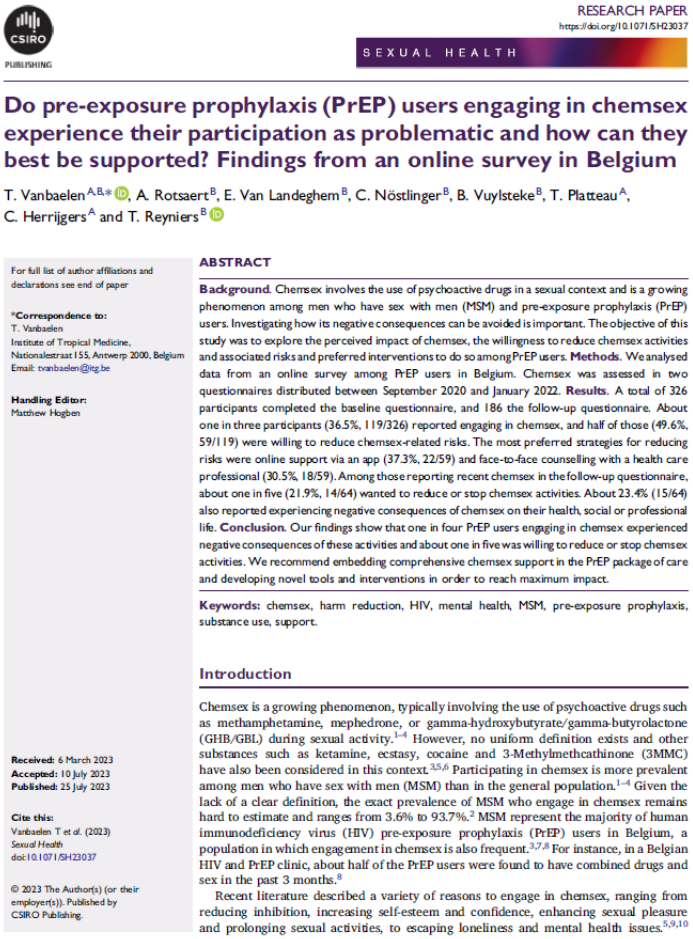
- MSM (men having sex with men) at very high risk of HIV infection :
    - People who have had unprotected anal sex with at least two partners in the past 6 months;
    - People who have had multiple STDs (syphilis, chlamydia, gonorrhea, or primary hepatitis B or C infection) in the past year;
    - People who have used PEP more than once a year;
    - People who use psychoactive substances during sexual activity
- High-risk individuals with individual risk :
    - PWID (People who inject drugs) who share needles ;
    - People in prostitution who are exposed to unprotected sex;
    - People in general exposed to unprotected sex at high risk of HIV infection ;
    - Partner of an HIV-positive patient without viral suppression (newly on treatment or no viral suppression with adequate treatment)”

Chapter 4

Syndemics and the need for additional risk reduction

4.1 Chemsex: occurrence, consequences, and preferred support strategies

Vanbaelen T, Rotsaert A, Van Landeghem E, et al. Do pre-exposure prophylaxis (PrEP) users engaging in chemsex experience their participation as problematic and how can they best be supported? Findings from an online survey in Belgium. *Sex Health* 2023; 20(5): 424-30.



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SEXUAL HEALTH

Do pre-exposure prophylaxis (PrEP) users engaging in chemsex experience their participation as problematic and how can they best be supported? Findings from an online survey in Belgium

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ABSTRACT

Background. Chemsex involves the use of psychoactive drugs in a sexual context and is a growing phenomenon among men who have sex with men (MSM) and pre-exposure prophylaxis (PrEP) users. Investigating how its negative consequences can be avoided is important. The objective of this study was to explore the perceived impact of chemsex, the willingness to reduce chemsex activities and associated risks and preferred interventions to do so among PrEP users. **Methods.** We analysed data from an online survey among PrEP users in Belgium. Chemsex was assessed in two questionnaires distributed between September 2020 and January 2022. **Results.** A total of 326 participants completed the baseline questionnaire, and 186 the follow-up questionnaire. About one in three participants (36.5%, 119/326) reported engaging in chemsex, and half of those (49.6%, 59/119) were willing to reduce chemsex-related risks. The most preferred strategies for reducing risks were online support via an app (37.3%, 22/59) and face-to-face counselling with a health care professional (30.5%, 18/59). Among those reporting recent chemsex in the follow-up questionnaire, about one in five (21.9%, 14/64) wanted to reduce or stop chemsex activities. About 23.4% (15/64) also reported experiencing negative consequences of chemsex on their health, social or professional life. **Conclusion.** Our findings show that one in four PrEP users engaging in chemsex experienced negative consequences of these activities and about one in five was willing to reduce or stop chemsex activities. We recommend embedding comprehensive chemsex support in the PrEP package of care and developing novel tools and interventions in order to reach maximum impact.

Keywords: chemsex, harm reduction, HIV, mental health, MSM, pre-exposure prophylaxis, substance use, support.

Introduction

Chemsex is a growing phenomenon, typically involving the use of psychoactive drugs such as methamphetamine, mephedrone, or gamma-hydroxybutyrate/gamma-butyrolactone (GHB/GBL) during sexual activity.[1–4] However, no uniform definition exists and other substances such as ketamine, ecstasy, cocaine and 3-Methylmethcathinone (3MMC) have also been considered in this context.[3,5,6] Participating in chemsex is more prevalent among men who have sex with men (MSM) than in the general population.[1–4] Given the lack of a clear definition, the exact prevalence of MSM who engage in chemsex remains hard to estimate and ranges from 3.6% to 93.7%.[2] MSM represent the majority of human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) users in Belgium, a population in which engagement in chemsex is also frequent.[3,7,8] For instance, in a Belgian HIV and PrEP clinic, about half of the PrEP users were found to have combined drugs and sex in the past 3 months.[8]

Recent literature described a variety of reasons to engage in chemsex, ranging from reducing inhibition, increasing self-esteem and confidence, enhancing sexual pleasure and prolonging sexual activities, to escaping loneliness and mental health issues.[5,9,10]

Title - Do PrEP users engaging in chemsex experience their participation as problematic and how can they best be supported? Findings from an online survey in Belgium

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Summary text

One third of HIV pre-exposure prophylaxis users engage in chemsex (or sexualized drug use) and about one in four experience negative consequences of it. Nearly half of them reported to be willing to reduce the chemsex-related risks with healthcare providers and online apps as preferred support options. We recommend embedding comprehensive chemsex support in the PrEP package of care and developing novel tools and interventions in order to reach maximum impact.

Abstract

Background – Chemsex involves the use of psychoactive drugs in a sexual context and is a growing phenomenon among men who have sex with men and PrEP users. Investigating how its negative consequences can be avoided is important. The objective of this study was to explore the perceived impact of chemsex, the willingness to reduce chemsex activities and associated risks and preferred interventions to do so among PrEP users.

Methods – We analyzed data from an online survey among PrEP users in Belgium. Chemsex was assessed in two questionnaires distributed between September 2020 and January 2022.

Results – A total of 326 participants completed the baseline questionnaire, and 186 the follow-up questionnaire. About one in three (36.5%, 119/326) reported engaging in chemsex, and half of those (49.6%, 59/119) were willing to reduce chemsex-related risks. The most preferred strategies for reducing risks were online support via an app (37.3%, 22/59) and face-to-face counselling with a healthcare professional (30.5%, 18/59). Among those reporting recent chemsex in the follow-up questionnaire, about one in five (21.9%, 14/64) wanted to reduce or stop chemsex activities. About 23.4% (15/64) also reported experiencing negative consequences of chemsex on their health, social or professional life.

Conclusion – Our findings show that one in four PrEP users engaging in chemsex experienced negative consequences of these activities and about one in five was willing to reduce or stop chemsex activities. We recommend embedding comprehensive chemsex support in the PrEP package of care and developing novel tools and interventions in order to reach maximum impact.

Keywords – chemsex; HIV; pre-exposure prophylaxis; substance use; support; harm reduction; MSM; mental health

Introduction

Chemsex is a growing phenomenon, typically involving the use of psychoactive drugs such as methamphetamine, mephedrone, or gamma-hydroxybutyrate/gamma-butyrolactone (GHB/GBL) during sexual activity (1–4). However, no uniform definition exists and other substances such as ketamine, ecstasy, cocaine and 3-Methylmethcathinone (3MMC) have also been considered in this context (3,5,6). Participating in chemsex is more prevalent amongst men who have sex with men (MSM) than in the general population (1–4). Given the lack of a clear definition, the exact prevalence of MSM who engage in chemsex remains hard to estimate and ranges from 3.6% to 93.7% (2). MSM represent the majority of human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) users in Belgium, a population in which engagement in chemsex is also frequent (3,7,8). For instance, in a Belgian HIV and PrEP clinic, about half of the PrEP users were found to have combined drugs and sex in the past three months (8).

Recent literature described a variety of reasons to engage in chemsex, ranging from reducing inhibition, increasing self-esteem and confidence, enhancing sexual pleasure and prolonging sexual activities, to escaping loneliness and mental health issues (5,9,10). However, it also involves risks related to substance use, including addiction and overdose (6,11). Chemsex is associated with behaviors that can increase the risk for sexually transmitted infections (STIs) such as condomless anal sex, group sex and transactional sex (2,3,12). Various studies found that MSM engaging in chemsex had higher rates of hepatitis C, HIV and bacterial STIs such as syphilis or gonorrhoeae (2,3,11–13). It has been demonstrated that mental health issues, such as depression, anxiety, and suicidal ideation are more frequently present in MSM engaging in chemsex (14,15). Chemsex can thus act as a syndemic condition with other psychosocial problems within the HIV and STIs epidemics (4,6,16). Therefore, concerns have been raised that chemsex may hamper the effectiveness of prevention interventions such as PrEP, for example by increasing HIV risk behaviors or decreasing adherence to PrEP (3,16). However, research regarding the effects of chemsex on chemsex participants' lives are limited.

In July 2022, the World Health Organization acknowledged the need for addressing this growing chemsex phenomenon among MSM (4). It recommends a patient-centered, non-judgmental approach that covers all chemsex-related harms from drug-related risks to mental and sexual health (4). It remains unclear which interventions and strategies would be most effective in reducing the risks associated with chemsex.

The main objective of this study was to explore the perceived negative effects of chemsex among PrEP users in Belgium, their willingness to reduce chemsex and associated risks, and their preferred options or tools to reduce such risks. Such insights could help develop acceptable and effective strategies to support MSM engaging in chemsex and reduce the negative consequences of chemsex.

Methods

Study design and participants

We conducted an online survey among PrEP users in Belgium to investigate sexual behavior and PrEP-related topics. The detailed methodology of this survey has been published previously (17). Briefly, between September 2020 and January 2022, we distributed three questionnaires with approximately six months in between (one baseline and two follow-up questionnaires). The baseline questionnaire assessed mainly occurrence of chemsex-related activities, willingness to reduce related risks and preferred support strategies. In the second follow-up questionnaire, we further explored interesting themes that emerged from the baseline questionnaire, such as the perceived impact of chemsex (see Appendix 1). Therefore, only data from these two questionnaires is presented in this analysis. In this study, we defined chemsex as combining stimulant drugs and sex.

Participants were recruited through social media of community organizations, HIV reference centers delivering PrEP and social or sexual networking applications such as Grindr. Eligibility criteria were being at least 16 years old; reporting an HIV negative or

unknown serostatus; living in Belgium; and having used PrEP in the six months preceding the baseline questionnaire. Participants consenting to be contacted for follow-up questionnaires were invited to complete these via a personal link sent via email. The questionnaires were available in Dutch, English and French and pilot tested by research team members and MSM community organization representatives. Participants who completed all three questionnaires could win one of three €100 vouchers

Baseline questionnaire

In the baseline questionnaire, we assessed socio-demographic factors (e.g., age, education level, gender), sexual behavior as well as engagement in chemsex in the previous three months. We assessed the latter by using the question “In the last three months, how much of the sex you've had has been under the influence of stimulant drugs?”. Participants had to choose among the following answers: “none of it”, “almost none of it", “less than half”, “about half”, "more than half”, “almost all of it” and “all of it”. Participants who answered “none of it” were categorized as not having engaged in chemsex in the previous 3 months and all other participants as having engaged in chemsex. We then used filter logics in the questionnaire so that the following questions pertaining to chemsex only needed to be answered among those indicating to have engaged in chemsex. The willingness to reduce chemsex-related risks was assessed using the question “Would you be willing to reduce the risks that accompany chemsex?”. Participants could select the following options: “certainly, yes”, “rather yes”, “rather not” and “certainly not”. This variable was recoded, and the first two options were categorized as “willing to reduce the risks that accompany chemsex” and the two last options as “not willing to reduce the risks that accompany chemsex”. The willingness to receive specific chemsex-related support was assessed among those willing to reduce the risks that accompany chemsex, using the following question: “What would help you to reduce your risks that accompany chemsex?”. Participants were presented with several options among which to choose, as well as a free text “other” option (see Appendix 1).

Follow-up questionnaire

In the second follow-up questionnaire, we assessed sexual behavior and engagement in chemsex in the previous six months. Using a similar methodology as for the baseline questionnaire, we used filter logics to additionally assess the perceived negative effects of chemsex and the willingness to reduce or stop chemsex among participants who reported engagement in chemsex. Lastly, all participants were asked whether they would like more attention to be paid to chemsex during PrEP consultations. The detailed questions can be found in Appendix 1.

Data analysis

We describe numerical variables using medians and interquartile ranges, and categorical variables using absolute numbers and proportions. To assess a potential attrition bias, we compared socio-demographic factors between participants of the baseline and follow-up questionnaires using Mann-Whitney U test for medians and chi-square test or Fisher's exact test for proportions.

Ethics approval

We obtained ethical approval from the Institutional Review Board of the Institute of Tropical Medicine (IRB 1380/20). All participants provided consent before participation in the study. We pseudonymized all data before and upon data retrieval.

Results

Sample description

In total, 326 participants completed the baseline questionnaire, among whom 256 (78.5%) provided contact details and consented to participate in the follow-up questionnaires. One hundred eighty-seven (73.0%) participants completed the second follow-up questionnaire.

At baseline, the median age was 42 years (IQR 34-50, Table 1). Most participants were male (97.2, 317/326), highly educated (81.6%, 266/326), born in Belgium (85.6%, 279/326) and had health insurance (98.2%, 320/326). In the three months prior the baseline questionnaire, about half the participants reported having had one or more steady partners (50.3%, 164/326) and 1-5 occasional partners (48.5%, 158/326). About two thirds reported having had 1-15 anonymous partners (64.4%, 210/326). Most participants reported having had sex weekly with their steady partner(s) (52.9%, 64/121) and monthly with their occasional partners (42.5%, 111/261) or anonymous partners (41.1%, 92/224). We found no significant differences in these variables between respondents of the baseline and follow-up questionnaires (Table 1).

Baseline questionnaire

In the baseline questionnaire, about one-third (36.5%, 119/326) of the participants reported to have engaged in chemsex in the past three months. Among those, 57.9% (69/119) reported that half or more of the sexual encounters they had in the past three months were under the influence of stimulant drugs (Table 2). About one in five (17.6%, 21/119) chemsex users reported having been combining sex and drugs for less than one year, 54.6% (65/119) for one to five years and 27.7% (33/119) for more than five years (Table 2).

Among those who reported chemsex activities in the past three months, about half (49.6%, 59/119) reported to be willing to reduce the risks that accompany chemsex. Online support through an app was the most preferred support strategy (37.3%, 22/59), followed by face-to-face counselling with a health professional (30.5%, 18/59).

Follow-up questionnaire

In the follow-up questionnaire, about a third (34.2%, 64/187) of the participants reported having engaged in chemsex in the past six months (Table 3). Among those, 23.4% (15/64)

also reported that chemsex sometimes had a negative impact on their health, social or professional life. Again 15 participants (23.4%) were concerned or very concerned that chemsex could lead to more negative consequences in the future. Fourteen participants engaging in chemsex activities (21.9%, 14/64) reported to be likely or extremely likely wanting to stop or reduce chemsex. A third of all participants (35.8%, 67/187) would like to see more attention given to chemsex during a PrEP consultation, while this was 40.6% (26/64) among those engaging in chemsex.

Discussion

Our study is among the first to assess the perceived impact of chemsex, the willingness to reduce chemsex activities and associated risks and preferred interventions to do so among PrEP users. We found that one in four experienced negative consequences of chemsex on their daily lives. We also found that half the PrEP users engaging in chemsex were willing to reduce the risks that accompany chemsex with support through an app or face-to-face counselling with health professionals as preferred options.

The finding that almost one in four PrEP users engaging in chemsex experienced negative impact on their health, social or professional life, resonates with a similar study among MSM in Ireland (11). However, in a Dutch study it was found that only 9% of MSM engaging in chemsex experience a negative impact on their daily live (5). Our data also show that one in five is willing to stop or to engage less in chemsex related activities, similar as in the Dutch study (19%) (5). Our data also show that the majority of PrEP users engaging in chemsex do not experience a negative impact and are not willing to reduce or stop chemsex activities. It has been shown that some persons engaging in chemsex are well aware of the risks inherent to chemsex and apply different harm-reduction strategies by themselves, such as controlling the choice of drugs or the frequency of intake and therefore mitigate these harms (18). Nevertheless, our findings corroborate that a substantial part of those engaging in chemsex activities experience a negative impact on their lives and, among them there is an undeniable willingness to reduce chemsex activities and its related harms. There

is a lack of effective behavioral interventions to address the risks that accompany chemsex (4). Therefore, finding ways to address this need will be crucial for achieving such behavioral changes.

We found that the most preferred strategies for reducing chemsex related risks were online support through an app or face-to-face counselling with a health care professional. This was also found in the Irish study where sexual health services and online tools were the preferred chemsex support options (11). Although counselling holds promise to support people who engage in chemsex, this type of support is disconnected from actual chemsex events. Smartphone applications may enable real-time support before, during and after chemsex, at times chosen by the user. Such applications have shown to be effective in digital health promotion in a wide range of health-related domains, for instance adherence to HIV medication or smoking cessation (19,20). Among MSM, online tools have been proven to be effective and acceptable for different HIV and STI prevention interventions (21). Digital tools for chemsex support have been considered as having a promising potential (22). Recently, an app for chemsex support has been developed in Belgium (23). This app, consisting in an information module and an individual support module, is evidence-based and was developed in collaboration with MSM engaging in chemsex. Results on the effectiveness of this app are still pending but acceptability was very high in a pilot study among MSM (23). Our findings confirm that there may be great potential in developing and evaluating digital tools to support chemsex and reduce associated risks.

Health care professionals in general, and sexual health service professionals in particular, are often cited as a preferred source of information or support by respondents engaging in chemsex activities (5,11,24). This is in line with our study's findings as about 41% of those engaging in chemsex would like more attention to be paid to chemsex during PrEP consultations. These results emphasize the need for a comprehensive approach during (PrEP) consultations. This may be achieved by training and involving designated sexual health professionals. PrEP consultations represent an opportunity to do prevention on chemsex by informing, raising awareness, and promoting safe drug practices. They also

represent an opportunity to assess and address the negative consequences and, if necessary, refer patients to adequate support services.

A surprising finding was that 6.8% preferred 'group counselling' to adapt their chemsex behavior. Group counselling is an approach that is often acknowledged by (community) organizations (25). Nevertheless, in our study, such an approach was only preferred by a small proportion of participants.

It is unlikely that chemsex can effectively addressed via a one-size-fits-all strategy and various harm-reduction strategies already exist (6,26). Strong et al. proposed an integrated harm-reduction scheme based on three chemsex related harms: HIV, drug, and sex related harms (6). Given this wide range of chemsex-related harms, the diversification of options, from support by health care professionals and apps to community or peer-based interventions may be crucial to reach a maximum of users in need of support and to tackle as much chemsex related harms as possible (6,22,24,27).

Potential self-selection is a limitation to our study, inherent to the study design, and cannot be fully excluded. Hence, the sample might not be representative of the entire PrEP population. Furthermore, due to the drop-out of participants between the baseline and the follow-up questionnaires, the sample size in the follow-up questionnaire is rather small, which may have introduced an information bias in our results. Secondly, as we asked about the occurrence of certain behaviors in the last three or six months, a recall bias cannot be excluded. Given the sensitive and intimate nature of this topic, participants might be prone to social desirability bias. We consider these potential biases may have led to an underestimation of chemsex, being a potentially stigmatized behavior. There are some inconsistencies in the formulation of the different questionnaires (e.g.: chemsex use in the past six months was assessed in the baseline questionnaire whereas chemsex use in the past three months was assessed in the follow-up questionnaire), making comparisons between these timepoints impossible. Finally, the survey took place over more than a year in periods of different COVID-19 restrictions. Since these restrictions impacted sexual

behaviors (28), it cannot be excluded that COVID-19 and the related restrictions may have affected our results.

Despite these limitations, our study sheds light on the magnitude of chemsex among PrEP users in Belgium, its associated perceived negative consequences, and the preferred support approaches to reduce chemsex-related harms. More research is needed on effective chemsex support approaches and their implementation in sexual health care services, with a focus on online interventions and trained health care professionals. Moreover, research is also required on how to raise awareness on the currently existing and future support options for chemsex users, and on how to address the barriers to chemsex support, in order to maximize their effectiveness.

Conclusion

In conclusion, we found that at least one in five PrEP users engaging in chemsex would like to reduce or stop engaging in such activities. Online applications and support from health care professionals were the most preferred approaches for chemsex-support. Based on our results, we recommend embedding comprehensive chemsex support in the PrEP package of care and support the development of novel strategies and tailored interventions to address the risks and potential health problems that accompany chemsex.

Table 1 - Comparison of socio-demographic factors and sexual behavior at baseline between the baseline and follow-up questionnaires

	Baseline questionnaire (N=326)	**Follow-up questionnaire (N=187)**	**p-value**
Age	42 (34-50)	46 (38-53)	0.29
Born in Belgium	279 (85.6)	161 (86.1)	0.76
Higher education completed	266 (81.6)	150 (80.2)	0.45
Public Health Insurance	320 (98.2)	183 (97.9)	0.64
Gender: man	317 (97.2)	183 (97.9)	0.22
How many steady partners do you have?			0.55
None	162 (49.7)	93 (49.7)	
1	131 (40.2)	79 (42.2)	
2	20 (6.1)	9 (4.8)	
3	5 (1.5)	3 (1.6)	
>3	8 (2.5)	3 (1.6)	
How often did you have anal sex with your steady partner(s) in the last 3 months?*			0.25
Daily	6 (4.9)	1 (1.4)	
Weekly	64 (52.9)	35 (50.7)	
Monthly	42 (34.7)	26 (37.7)	
Less than monthly	9 (7.4)	7 (10.1	
How many occasional partners do you have?			0.64
none	38 (11.7)	20 (10.7)	
1-5	158 (48.5)	89 (47.6)	
6-10	54 (16.6)	35 (18.7)	
>10	76 (23.3)	43 (23.0)	
How often did you have anal sex with your occasional partner(s) in the last 3 months?†			0.39
Daily	3 (1.1)	0 (0.0)	
Weekly	101 (38.7)	59 (38.8)	
Monthly	111 (42.5)	63 (41.4)	
Less than monthly	46 (17.6)	30 (19.7)	

In the last 3 months, with how many anonymous or new sex partner(s) did you have sex ?			0.08
none	75 (23.0)	38 (20.3)	
1-15	210 (64.4)	125 (66.8)	
16-30	29 (8.9)	18 (9.6)	
31-50	8 (2.5)	6 (3.2)	
>50	4 (1.2)	0 (0.0)	
How often did you have anal sex with your anonymous partner(s) in the last 3 months?‡			0.13
Daily	2 (0.9)	0 (0.0)	
Weekly	74 (33.0)	39 (28.9)	
Monthly	92 (41.1)	59 (47.2)	
Less than monthly	56 (25.0)	37 (29.6)	

*only among respondents who reported anal sex with a steady partner (N=121/N=69 among participants of the baseline and follow-up questionnaires respectively)

†only among respondents who reported anal sex with an occasional partner (N=261/N=152 among participants of the baseline and follow-up questionnaires respectively)

‡only among respondents who reported anal sex with an anonymous partner (N=224/N=135 among participants of the baseline and follow-up questionnaires respectively)

Table 2 – Baseline questionnaire questions regarding chemsex

	Baseline questionnaire participants (N=326, n(%))
In the last three months, how much of the sex you've had has been under the influence of stimulant drugs?	
All of it	8 (2.5)
Almost all of it	26 (8.0)
More than half	14 (4.3)
About half	21 (6.4)
Less than half	17 (5.2)
Almost none of it	33 (10.1)
None of it	207 (63.5)
For how long have you been combining stimulant drugs and sex?*	
Less than 6 months	9 (7.6)
Less than 1 year	12 (10.1)
Less than 2 years	23 (19.3)
Less than 3 years	18 (15.1)
Less than 4 years	9 (7.6)
Less than 5 years	15 (15.6)
More than 5 years	33 (27.7)
Would you be willing to reduce the risks that accompany chemsex?*	
Certainly, yes	28 (23.5)
Rather yes	31 (26.1)
Rather not	50 (42.0)
Certainly not	10 (8.4)
What would help you to reduce your risks that accompany chemsex?†§	
Face-to-face counselling with health professional	18 (30.5)
Group counselling	4 (6.8)
Peer support	16 (27.1)
Online training	17 (28.8)
Online support via an app	22 (37.3)

* Question asked to participants who reported engagement in chemsex in the previous 3 months (N=119)

† Question asked to participants who reported willing to reduce the risks that accompany chemsex ("certainly, yes" and "rather yes", N=59)

§ multiple answers possible

Table 3 – Follow-up questionnaire questions regarding chemsex

	Follow-up questionnaire participants (N=187, n(%))
How often were you under the influence of stimulant drugs during sex (=chemsex) in the past 6 months?	
Never	123 (65.8)
Almost never	20 (10.7)
Less than half	7 (3.7)
About half of it	10 (5.3)
More than half	8 (4.3)
Almost always	17 (9.1)
Always	2 (1.1)
How often does the use of chemsex negatively affects your health, your social life or your professional life?*	
Never	30 (46.9)
More not than yes	19 (29.7)
Sometimes yes, sometimes no	15 (23.4)
More yes than not	0 (0)
Every time	0 (0)
How concerned are you that chemsex could have more negative consequences for you in the future?*	
Not concerned at all	13 (20.3)
Not concerned	15 (23.4)
Neutral	21 (32.8)
Concerned	14 (21.9)
Very concerned	1 (1.6)
To what extent would you like to have less or stop chemsex?*	
Extremely unlikely	4 (6.2)
Unlikely	13 (20.3)
Neutral	33 (51.6)
Likely	12 (18.8)
Extremely likely	2 (3.1)
* Questions asked to participants having reported chemsex (N=64)	

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4.2 Non-consensual sex: experiences and help-seeking behavior

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Non-Consensual Sex and Help-Seeking Behavior Among PrEP Users in Belgium: Findings from an Online Survey

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ABSTRACT

Non-consensual sex poses a threat not only to sexual health but also to mental and physical health in general. HIV pre-exposure prophylaxis (PrEP) users might be particularly vulnerable to non-consensual sex because of interplaying factors such as mental health disorders, a high number of sex partners, engagement in chemsex, and the widespread use of dating apps. The objectives of this study were to assess the occurrence of non-consensual sex, its associated factors, and related help-seeking behavior among PrEP users. We analyzed data from an online survey among PrEP users in Belgium (09/2020-02/2022). Almost one in five participants (34/187, 18.2%) reported having ever experienced non-consensual sex. The most reported form was having sex against one's will, followed by having been given drugs against one's will, and having had sex without a condom against one's will. The vast majority of those who had experienced non-consensual sex (29/34, 85.3%) did not seek help afterward, mostly due to a lack of perceived need (21/29, 72.4%). Reported barriers to seeking help were shame (6/29, 20.7%) and lack of awareness of help services (3/29, 10.3%). Having experienced non-consensual sex in the past five years was associated with younger age and suicidal ideation in a multivariable logistic regression model. We conclude that addressing barriers to non-consensual sex help services is crucial to maximize their use and minimize the consequences of non-consensual sex experiences. PrEP consultations also represent an opportunity to offer such help given PrEP users are already familiar with these PrEP services and engaged in care.

Introduction

Human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) is a very effective biomedical intervention to prevent HIV acquisition, if taken correctly (O Murchu et al., 2022). Studies have shown that PrEP use can also improve sexual health by reducing fear of HIV and enhancing sexual satisfaction in general (Grov et al., 2021; Zimmermann et al., 2021). Moreover, it has allowed individuals engaging in sexual risk behavior to enroll in care and has improved sexual health knowledge (Grov et al., 2021). However, PrEP users are still disproportionally affected by a range of physical and psychological harms, such as higher rates of sexually transmitted infections (STIs), substance use disorders, and mental health issues (Nöstlinger et al., 2020; Strong et al., 2022; Werner et al., 2018). If we want to optimally support PrEP users in maintaining a safe and healthy sexuality, we require more insights into how such needs can be better addressed.

As in other high-income countries, most PrEP users in Belgium are men who have sex with men (MSM; Hayes et al., 2019; Sciensano, 2022). Previous studies have shown that MSM report non-consensual sex[1] experiences more frequently than heterosexual populations (Coxell et al., 1999; Finneran & Stephenson, 2013). Non-consensual sex includes all types of sexual experiences that occurred without consent or were against one's will (Drückler et al., 2021; Ratner et al., 2003). It can take many forms such as sexual intercourse against one's will, forced condom removal, or verbal sexual harassment (Basile et al., 2014). In a national representative sample of Belgian adults, 78% of non-heterosexual respondents reported some form of sexual victimization in their lifetime and they were two times more likely to have experienced non-consensual sexual victimization compared with heterosexual populations (Schapansky et al., 2021). We hypothesized that some PrEP users might be particularly at risk to experience non-consensual sex due to a combination of a high number of sex partners, frequent engagement in chemsex (the use of stimulant drugs in a sexual context), a high prevalence of mental health disorders, and frequent use of dating apps (Hoenigl et al., 2020; King et al., 2008; Phillips et al., 2014; Rotsaert, Reyniers, Jacobs et al., 2022). However, to date the association between such factors and non-consensual sex experiences among PrEP users has not been explored. Such insights are important to identify PrEP users who may be the most at risk for non-consensual sex.

Victims of non-consensual sex are more likely to report sexual risk taking, substance use, mental health disorders, STIs, and HIV infections (Buller et al., 2014; Jones et al.,

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[1]Various terms are used in the literature such as non-consensual sex, sexual violence, sexual assault, etc. We have chosen to use non-consensual sex because it reflects a broader range of experiences than, for instance, "sexual assault". Moreover, we chose this term to stay in line with recent publications on the topic among MSM (e.g., Drückler et al., 2021). However, when citing other studies, we have chosen to preserve the original study terminology.

Title – Non-consensual sex and help-seeking behavior among PrEP users in Belgium: findings from an online survey

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Abstract

Non-consensual sex poses a threat not only to sexual health but also to mental and physical health in general. HIV pre-exposure prophylaxis (PrEP) users might be particularly vulnerable to non-consensual sex because of interplaying factors such as mental health disorders, a high number of sex partners, engagement in chemsex, and the widespread use of dating apps. The objectives of this study were to assess the occurrence of non-consensual sex, its associated factors, and related help-seeking behavior among PrEP users. We analyzed data from an online survey among PrEP users in Belgium (09/2020-02/2022). Almost one in five participants (34/187, 18.2%) reported having ever experienced non-consensual sex. The most reported form was having sex against one's will, followed by having been given drugs against one's will, and having had sex without a condom against one's will. The vast majority of those who had experienced non-consensual sex (29/34, 85.3%) did not seek help afterwards, mostly due to a lack of perceived need (21/29, 72.4%). Reported barriers to seeking help were shame (6/29, 20.7%) and lack of awareness of help services (3/29, 10.3%). Having experienced non-consensual sex in the past five years was associated with younger age and suicidal ideation in a multivariable logistic regression model. We conclude that addressing barriers to non-consensual sex help services is crucial to maximize their use and minimize the consequences of non-consensual sex experiences. PrEP consultations also represent an opportunity to offer such help given PrEP users are already familiar with these PrEP services and engaged in care.

Keywords – pre-exposure prophylaxis, non-consensual sex, HIV, help services, mental health

Introduction

Human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) is a very effective biomedical intervention to prevent HIV acquisition, if taken correctly (1). Studies have shown that PrEP use can also improve sexual health by reducing fear of HIV and enhancing sexual satisfaction in general (2,3). Moreover, it has allowed individuals engaging in sexual risk behavior to enroll in care and has improved sexual health knowledge (2). However, PrEP users are still disproportionally affected by a range of physical and psychological harms, such as higher rates of sexually transmitted infections (STIs), substance use disorders, and mental health issues (4–6). If we want to optimally support PrEP users in maintaining a safe and healthy sexuality, we require more insights in how such needs can be better addressed.

As in other high-income countries, most PrEP users in Belgium are men who have sex with men (MSM) (7,8). Previous studies have shown that MSM report non-consensual sex[1] experiences more frequently than heterosexual populations (9,10). Non-consensual sex includes all types of sexual experiences that occurred without consent or were against one's will (11,12). It can take many forms such as sexual intercourse against one's will, forced condom removal, or verbal sexual harassment (13). In a national representative sample of Belgian adults, 78% of non-heterosexual respondents reported some form of sexual victimization in their lifetime and they were two times more likely to have experienced non-consensual sexual victimization compared with heterosexual populations (14). We hypothesize that some PrEP users might be particularly at risk to experience non-consensual sex due to a combination of a high number of sex partners, frequent

[1] Various terms are used in the literature such as non-consensual sex, sexual violence, sexual assault, etc. We have chosen to use non-consensual sex because it reflects a broader range of experiences than, for instance, "sexual assault". Moreover, we chose this term to stay in line with recent publications on the topic among MSM (e.g., Drückler et al., 2021). However, when citing other studies, we have chosen to preserve the original study terminology.

engagement in chemsex (the use of stimulant drugs in a sexual context), a high prevalence of mental health disorders, and frequent use of dating apps (15–18). However, the association between such factors and non-consensual sex experiences among PrEP users has not been explored yet. Such insights are important to identify PrEP users who may be the most at risk for non-consensual sex.

Victims of non-consensual sex are more likely to report sexual risk taking, substance use, mental health disorders, STIs, and HIV infections (12,19,20). Moreover, it has been shown that these factors can act as a syndemic, i.e., interact synergistically and therefore reinforce each other, leading to a higher burden of disease in PrEP users (6,21). Non-consensual sex experiences have also been associated with other short- and long-term mental and physical health harms such as smoking, obesity, suicidal ideation, or cardio-vascular diseases (12,19,22). Being able to mitigate these harms is crucial. An important first step towards achieving this, is to ensure that victims of non-consensual sex seek and receive help. Previous studies show that only a minority of those who have experienced non-consensual sex do so (23–25). However, the help seeking behavior of PrEP users who are victims of non-consensual sex is currently not known.

The objectives of this study were to assess (1) the occurrence and forms of lifetime non-consensual sex, (2) the factors associated with recent experiences of non-consensual sex, and (3) the help-seeking behavior after non-consensual sex experiences among PrEP users in Belgium. Such insights are crucial to ensure adequate support and reduce the potential harms of non-consensual sex on PrEP users' health.

Methods

Participants

We conducted an online survey among PrEP users in Belgium. We recruited participants through the social media of community organizations, HIV reference centers, and

social/sexual networking applications. Eligibility criteria were: being at least 16 years old; reporting an HIV negative or unknown serostatus; living in Belgium; and having used PrEP in the six months preceding the baseline questionnaire. Three questionnaires were distributed at intervals of approximately six months (one baseline and two follow-up questionnaires) between September 2020 and January 2022. More details on the methodology of this survey have been published elsewhere (26). For the present study we selected participants who completed the second follow-up questionnaire, in which we asked questions pertaining to non-consensual sex, sexual behavior, STIs, and mental health. Socio-demographic characteristics were retrieved from the baseline questionnaire.

Measures

The first objective of this study was to assess the occurrence and forms of lifetime non-consensual sex among PrEP users. For that purpose, we used the question "Have you ever had sex that was partly or completely against your will or without your consent (non-consensual)? Non-consensual sex is any form of sexually transgressive behavior, verbal, physical, intentional, or unintentional, where there is clearly no mutual consent and/or which is not voluntary", followed by some examples of non-consensual sex. We used filter logics in the questionnaire so that questions pertaining to non-consensual sex only needed to be answered among those reporting having experienced non-consensual sex. A list of different forms of non-consensual sex was shown (e.g.: I had sex against my will, I had sex without a condom against my will) and participants were asked to select the ones they had experienced, with a free text 'other' option. The complete list of questions regarding non-consensual sex can be found in Table 1.

The second objective of this study was to explore factors associated with non-consensual sex among PrEP users. We assessed mental health issues using the patient health questionnaire 2-item (PHQ-2) and generalized anxiety disorder 2-item (GAD-2) screening tools, both including two questions with four items ranging from 0 to 3 (27,28). As recommended, we used a cut-off of 3 to define major depression disorder and generalized

anxiety disorder. We screened for suicidal ideation via the last question of the PHQ-9: “Over the last 2 weeks, how often have you been bothered by the following problems? Thoughts that you would be better off dead or of hurting yourself in some way” (29). We dichotomized the answering options into “yes” for reporting any occurrence of such thoughts in the previous two weeks versus “no”. We assessed sexual behavior in the previous six months by inquiring about the number of occasional and anonymous partners, the frequency of condom use with such partners, and engagement in chemsex. We also asked about the occurrence of any STI and the use of recreational drugs in the previous six months. Other variables used in the present study were socio-demographic characteristics, including age, self-assigned gender, education level, country of birth, and social health insurance status.

The third objective of this study was to explore the help-seeking behavior of PrEP users having experienced non-consensual sex. For that purpose, we used the question “Have you ever sought help after experiencing non-consensual sex?”. As for the first question regarding non-consensual sex, we used filter logics in the questionnaire so that this questions only needed to be answered among those reporting having experienced non-consensual sex. Lastly, we asked “Why did you NOT seek help after experiencing non-consensual sex?” to participants having answered “No” to the previous question. A list of different reasons for not seeking help was shown and participants were asked to select the ones that applied to them, with a free text ‘other’ option.

Data Analysis

We described numerical variables using medians and interquartile ranges, and categorical variables using absolute numbers and proportions. We conducted logistic regression to identify associations between having experienced non-consensual sex in the past five years and socio-demographic factors, sexual behavior, mental health issues, and drug use. We first performed univariable logistic regression to select the variables to include in a multivariable logistic regression analysis. Variables significantly associated with non-

consensual sex in the univariable regression analysis were selected using a likelihood ratio test with a significance level set at 0.1. The multivariable model was built using stepwise selection, based on likelihood ratio test and a significance level set at 0.05.

We used R studio version 4.2.0 for these analyses ("R Core Team," 2022).

Ethical approval and consent

For this study we received ethical approval from the Institutional Review Board of the Institute of Tropical Medicine (IRB 1380/20). All participants provided consent before participation in the study. All data were pseudonymized upon retrieval.

Results

Sample description

A total of 187 participants completed the second follow-up questionnaire (Table 2). All but four participants self-identified as men (97.9%, 183/187), two participants as trans-men, and two as trans-women. Median age was 46 years old (IQR 38-53). The majority had been born in Belgium (86.1%, 161/187), had completed or were enrolled in higher education (80.2%, 150/187), and had social health insurance (97.9%, 183/187).

Occurrence and forms of non-consensual sex experiences

A total of 34 participants (34/187, 18.2%) reported having ever experienced non-consensual sex (Table 1). For almost half of them (18/34, 52.9%), the last experience was more than five years ago and for about a quarter (9/34, 26.4%) less than one year ago. The most frequently reported form of non-consensual sex was having sex against one's will (19/34, 55.9%) followed by having been given drugs against one's will (8/34, 23.5%), and having had sex without a condom against one's will (7/34, 20.6%). Other forms of non-consensual sex were reported in free text such as forced penetration (2/34, 5.9%), being in

pain and the partner refusing to stop (1/34, 2.9%), or not feeling capable of saying “no” (1/34, 2.9%). Around 40% of those having reported non-consensual sex were under the influence of alcohol or drugs when it occurred (14/34, 41.2%).

Factors associated with recent non-consensual sex experiences

In the univariable logistic regression analysis, participants having experienced non-consensual sex in the past five years were more likely to be younger [OR 0.95 (95%CI 0.89-0.99)], to have screened positive for anxiety [OR 3.23 (95%CI 0.83-10.59)] or suicidal ideation [OR 4.54 (95%CI 1.50-13.33)]. In the multivariable logistic regression model, only younger age [aOR 0.95 (95%CI 0.89-1)] and suicidal ideation [aOR 4.32 (95%CI 1.45-12.87)] remained significantly associated with non-consensual sex after controlling for other factors (Table 2).

Help-seeking behavior after non-consensual sex experiences

The vast majority did not seek help after experiencing non-consensual sex (29/34, 85.3%). The main reason for not seeking help was not feeling the need to do so (21/29, 72.4%), followed by being ashamed of what happened (6/29, 20.7%), and not knowing where to receive help (3/29, 10.3%). One trans-woman respondent reported fearing for her job if she sought help and fearing she would be treated differently than other women. She reported having sought help only at the time she developed symptoms of HIV infection. Another respondent reported being 14 years old when the non-consensual sex episode occurred and not realizing at that time that what happened was not acceptable.

Discussion

The objectives of the present study were three-fold: firstly, assess the occurrence and forms of non-consensual sex among PrEP users in Belgium. With regard to this objective, we found that one in five PrEP users had ever experienced non-consensual sex, with having

sex against one's will and being given drugs in a sexual context against one's will as most frequent forms of non-consensual sex reported. Secondly, we aimed to assess factors associated with non-consensual sex. In our sample, non-consensual sex was significantly associated with younger age and suicidal ideation. Thirdly, we aimed to explore help-seeking behavior of PrEP users who had experienced non-consensual sex. The majority had not sought help due to not having felt the need to do so. However, some respondents reported lack of awareness about where to find help and shame as barriers to seeking help after non-consensual sex.

The frequency of non-consensual sex experiences varies between studies, depending on the population studied, the definition used, and the recall period (9). We found a lower frequency of non-consensual sex than a recent Dutch study which reported a five-year incidence of 18.1% among MSM recruited through sexual networking applications (11). Several factors might explain the difference between our results and those of the Dutch study. The Dutch study recruited participants exclusively through sexual networking applications, which has been shown to facilitate some form of sexual violence (31), whereas we also recruited participants through community organizations and HIV reference centers. Furthermore, the Dutch study focused on MSM in Amsterdam, an exclusively urban setting, while our study was performed throughout Belgium. Nevertheless, the fact that one in five PrEP users reported non-consensual sex in our study is worrying, given the consequences non-consensual sex can have on health.

The second finding of our study, namely that recent non-consensual sex experiences is associated with younger age, is consistent with the findings of other studies (9,11,14). Several explanations for this finding have been proposed by Schapansky et al. Firstly, technology might have facilitated some forms of non-consensual sex, therefore exposing more threats to young adults rather than older adults. For instance, it has been shown that dating apps, mostly used by younger individuals, can facilitate sexual assault by multiple mechanisms such as facilitating meetings between victims and perpetrators (31,32). Secondly, younger individuals might have a higher awareness of consent in a sexual context

due to the attention it has received (mostly online) in recent years, following the #metoo movement. Lastly, recall bias is more likely to occur in older individuals (Schapansky et al., 2021). We also found an association between non-consensual sex and suicidal ideation. While this type of association between mental health issues and non-consensual sex experiences has been discussed extensively (10,19,20,33,34), determining causality would be impossible given the likely complexity of the relationship between these two factors. Mental health disorders have been described as both vulnerability factors and consequences of non-consensual sex (12,35,36). Moreover, non-consensual sex and mental health seem to be intertwined at multiple ecological levels, making this relationship even more complex (33). Our findings underline that particular attention to non-consensual sex experiences and related issues should be given in PrEP users who present mental health disorders and those who are younger.

Regarding our last objective, the finding that the majority of participants who experienced non-consensual sex did not seek help resonates with the results of previous studies conducted among men (24,37) and among lesbian, gay, bisexual, and queer individuals (38). While the main reason for not seeking help in our study was a lack of perceived need, some participants reported barriers to seeking help such as being ashamed of what had happened and not knowing where to do so. Barriers to non-consensual sex help services have been described at multiple levels (24,25). Individual-level barriers include shame and lack of acknowledgement of the event (24,25). At the social and societal levels, fear of negative reactions, lack of access or availability, and cultural and gender norms have been reported (24,25,38). To address these barriers, several countries, including Belgium, have developed sexual assault care centers, where victims of non-consensual sex can receive medical, psychological, and legal support at one-stop centers (39–41). It is crucial that these services are well-known, low-threshold, and offer non-judgmental, multidisciplinary care in order to address these multiple barriers. In Belgium, PrEP users are followed up via HIV reference centers (18). Given that PrEP users are already familiar with and engaged in care at these HIV centers, the PrEP follow-up consultations also represent an opportunity to prevent, address, and counsel them on the issue of non-consensual sex. It is shown that

PrEP users prefer sexual health care professionals to address other problems frequently found in this population such as problematic chemsex (42). Moreover, the World Health Organization recently recommended integrating broader health interventions in the PrEP package of care for MSM, such as mental health or substance use disorder support (43). Including support after non-consensual sex in the PrEP package of care could also be a way to improve broader health in PrEP users. However, further research is required to investigate how help services can be tailored to best address the needs of PrEP users with regard to non-consensual sex experiences.

Seeking help early after non-consensual sex can mitigate some of its short- and long-term consequences (44). For example, providing post-exposure prophylaxis for HIV can avoid HIV seroconversions, which might be needed if PrEP was not taken. It can also represent an opportunity to offer mental health support and organize a schedule of HIV and STI testing. It has also been shown that offering early interventions after sexual assault decreases the occurrence of mental health consequences (45,46). Therefore, it is important to sensitize the public to the potential consequences of non-consensual sex and raise awareness about the importance of seeking help to encourage the victims to do so.

This study has several limitations: first, our analysis is based on a relatively small number of participants, which might affect the generalizability of our results. Nevertheless, we believe that our exploratory study offers some important insights on non-consensual sex experiences in PrEP users. Second, potential self-selection inherent to the online study design cannot be fully excluded. Hence, the sample may not be representative of the entire PrEP population. Third, the results might be subject to recall bias and, given the sensitive and intimate nature of the topics explored, subjects might be prone to social desirability bias, which could have led to underreporting. Finally, The PHQ-2 and GAD-2 tools were designed as screening tools, and positive results should be complemented by further investigations. Therefore, we might have overestimated the occurrence of mental health issues.

Conclusion

This study among PrEP users in Belgium aimed to assess the occurrence and forms of non-consensual sex, factors associated with recent non-consensual sex experiences, and help-seeking behavior after having experienced non-consensual sex. We found that one in five PrEP users have experienced non-consensual sex at some point in their lives. Younger age and suicidal ideation were associated with a recent non-consensual sex experience. The majority of PrEP users having experienced non-consensual sex did not seek help due to a lack of perceived need, shame, or not knowing where to find help. Raising awareness about this issue and ensuring help is available and accessible is important to mitigate the potential consequences of non-consensual sex on physical and mental health. This can be achieved through the existing one-stop sexual assaults centers. Furthermore, we also recommend particular attention be given to topics such as non-consensual sex during PrEP clinic consultations, either preventively or to help address experiences that have already occurred.

Table 1 - results of the survey questions regarding non-consensual sex (N=187)

	N (%)
Have you ever had sex that was partly or completely against your will or without your consent (non-consensual)?	
Yes	34 (18.2)
No	153 (81.8)
When was the last time this happened?†	
More than 5 years ago	18 (52.9)
Less than 5 years ago	7 (20.6)
Less than a year ago	4 (11.8)
Less than 6 months ago	1 (2.9)
Less than 1 month ago	4 (11.8)
Which of these "sex without consent"-scenarios have happened before*	
I had sex against my will	19 (55.9)
I had sex WITHOUT a condom against my will (while I wanted to use condoms)	7 (20.6)
I had been given drugs against my will in a sexual context	8 (23.5)
I was photographed or filmed against my will	4 (11.8)
I passed out and didn't know what was happening	3 (8.8)
Other	8 (23.5)
Have you ever had non-consensual sex under the influence of alcohol or drugs?†	
Yes	14 (41.2)
No	20 (58.8)
Have you ever sought help after experiencing non-consensual sex?†	
Yes	5 (14.7)
No	29 (85.3)
Why did you NOT seek help after experiencing non-consensual sex?*§	
I didn't feel the need to do that	21 (72.4)
I didn't know where to get help	3 (10.3)
I was ashamed to report what had happened.	6 (20.7)
Other (Please specify)	2 (5.9)

* multiple answers possible

† Only for respondents who reported having ever experienced non-consensual sex: N=34

§ Only for respondents who reported not having sought help after having experienced non-consensual sex: N=29

Table 2 - Factors associated with non-consensual sex in the past 5 years, results from uni- and multi-variable logistic regression analysis (N=187)

	Total (N=187, n(%))	**Non-consensual sex past 5 years no (N=171, n(%))**	**Non-consensual sex past 5 years yes (N=16, n(%))**	**Univariable logistic regression**		**Multivariable logistic regression**	
				OR (95% CI)	**P-value**	**aOR (95% CI)**	**P-value**
Sociodemographic characteristics							
Median age (IQR)	46 (38-53)	46 (38.5-53.5)	34.5 (31-49.8)	**0.95 (0.89-0.99)**	**0.04**	**0.95 (0.89-1)**	**0.04**
Identified as male	183 (97.9)	169 (98.8)	14 (87.5)	**0.08 (0.01-0.73)**	**0.02**		
Born in Belgium	161 (86.1)	146 (85.4)	15 (93.8)	2.56 (0.47-47.46)	0.31		
Higher education completed or enrolled	150 (80.2)	137 (80.1)	13 (81.2)	1.07 (0.32-4.87)	0.91		
Social health insurance	183 (97.9)	168 (98.2)	15 (93.8)	0.26 (0.03-5.59)	0.32		
Mental Health							
GAD-2 score							
< 3	167 (89.3)	155 (90.6)	12 (75.0)	Ref.			
≥ 3	20 (10.7)	16 (9.4)	4 (25.0)	3.23 (0.83-10.59)	0.08		
PHQ-2 score							
< 3	164 (87.7)	152 (88.9)	12 (75)	Ref.			
≥ 3	23 (12.3)	19 (11.1)	4 (25)	2.66 (0.69-8.57)	0.14		
Thoughts of dying or hurt oneself (past 2 weeks)							
No	155 (82.69)	146 (85.4)	9 (56.2)	Ref.		Ref.	

Yes	32 (17.1)	25 (14.6)	7 (43.8)	**4.54 (1.50-13.33)**	**<0.01**	**4.32 (1.45-12.87)**	**0.01**
Drug use and chemsex (past 6 months)							
Drug use							
No	108 (57.8)	98 (57.3)	10 (62.5)	Ref.			
Yes	79 (42.2)	73 (42.7)	6 (37.5)	0.81 (0.26-2.27)	0.69		
Engagement in chemsex							
No	123 (65.8)	111 (64.9)	12 (75)	Ref.			
Yes	64 (34.2)	60 (35.1)	4 (25)	0.61 (0.16-1.86)	0.40		
Sexual behavior (past 6 months)							
Being paid for sex							
No	178 (95.2)	163 (95.3)	15 (93.8)	Ref.			
Yes	9 (4.8)	8 (4.7)	1 (6.2)	1.36 (0.07-8.16)	0.78		
Having paid for sex							
No	176 (94.1)	161 (94.2)	15 (93.8)	Ref.			
Yes	11 (5.9)	10 (5.8)	1 (6.2)	1.07 (0.06-6.2)	0.94		
Any STI diagnose							
No	118 (63.1)	109 (63.7)	9 (56.2)	Ref.			
Yes	69 (36.9)	62 (36.3)	7 (43.8)	1.37 (0.47-3.85)	0.56		
Group sex							
No	68 (36.4)	63 (36.8)	5(31.2)	Ref.			
Yes	119 (63.6)	108 (63.2)	11 (68.8)	1.28 (0.44-4.22)	0.65		

N anonymous partners							
<10	94 (50.3)	86 (50.3)	8 (50)	Ref.			
≥10	93 (49.7)	85 (49.7)	8 (50)	1.01 (0.35-2.87)	0.98		
N occasional partners							
<5	106 (56.7)	96 (56.1)	10 (62.5)	Ref.			
≥5	81 (43.3)	75 (43.9)	6 (37.5)	0.76 (0.25-2.16)	0.62		
Condom use for anal sex with anonymous partners*							
Always	11 (7.5)	9 (6.7)	2 (16.7)	Ref.			
Sometimes	71 (48.3)	65 (48.1)	6 (50.0)	0.44 (0.10-2.24)			
Never	65 (44.2)	61 (45.2)	4 (33.3)	0.60 (0.15-3.06)	0.53		
Condom use for anal sex with occasional partners†							
Always	3 (2.5)	3 (2.7)	0 (0.0)	Ref.			
Sometimes	46 (37.7)	42 (59.8)	4 (8.7)				
Never	73 (59.8)	67 (59.8)	6 (60.0)		0.76		

Note: Values in bold indicate statistically significant results

Abbreviations: 95% CI, 95% confidence interval; GAD, generalized anxiety disorder; IQR, interquartile range; N, number of; OR, odds ratio; aOR, adjusted odds ratio; PHQ, patient health questionnaire, STI, sexually transmitted infection

* Number of respondents reporting sex with anonymous partners in the past 6 months: N=147

† Number of respondents reporting sex with occasional partners in the past 6 months: N=122

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Chapter 5

The emergence of AMR in bacterial STIs

5.1 The role of commensal Neisseria species in the emergence of AMR in NG

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Global epidemiology of antimicrobial resistance in commensal *Neisseria* species: A systematic review

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ABSTRACT

Background: Commensal *Neisseria* species (spp). represent an important reservoir of antimicrobial resistance genes for pathogenic *Neisseria* spp. In this systematic review, we aimed to assess the antimicrobial susceptibility of commensal *Neisseria* spp. and how this has evolved over time. We also aimed to assess if commensal *Neisseria* spp. showed intrinsic resistance to four antimicrobials - penicillin, azithromycin, ceftriaxone and ciprofloxacin. *Methods:* Pubmed and Google Scholar were searched following the PRISMA guidelines. Articles reporting MICs of commensal *Neisseria* spp. were included according to inclusion/exclusion criteria, and the quality of the articles was assessed using a pre-designed tool. Individual and summary measures of penicillin, azithromycin, ceftriaxone and ciprofloxacin MICs were collected. Additional data was sought to perform a comparison between the MICs of pathogenic and commensal *Neisseria* spp.
Results: A total of 15 studies met our criteria.We found no evidence of intrinsic AMR in commensal *Neisseria* spp. We did find evidence of an increasing trend in MICs of commensal *Neisseria* spp. over time for all antimicrobials assessed. These findings were similar in various countries. Eight additional studies were included to compare pathogenic and commensal *Neisseria* spp.
Conclusion: The MICs of commensal *Neisseria* spp. appear to be increasing in multiple countries. Surveillance of MICs in commensals could be used as an early warning system for antimicrobial resistance emergence in pathogens. Our findings underline the need for antibiotic stewardship interventions, particularly in populations with high antimicrobial consumption.

1. Introduction

The genus *Neisseria* includes species that are both pathogenic (*Neisseria meningitidis* and *N. gonorrhoeae*) and commensals to humans (e.g., *N. cinerea, N. mucosa, N. subflava, N. lactamica*) (Dorey et al., 2019). The commensal *Neisseria* spp. are predominantly residents of the oropharynx and have been shown to play an important role in human health (Deasy et al., 2015; Liu et al., 2015). They come into frequent contact with pathogenic *Neisseria* spp. in the oropharynx, which provides the opportunity to exchange genetic material – predominantly via transformation (Spratt et al., 1992; Wadsworth et al., 2018; Fiore et al., 2020). Numerous studies have established that this genetic exchange is important in the genesis of resistance to antimicrobials in the pathogenic *Neisseria* spp. Wadsworth et al., (2018; Fiore et al., 2020). The most prominent genes involved in this transformation include *penA, mtrCDE, rplB, rplD, rplV* and *gyrA*. The acquisition of sections of these genes from commensal *Neisseria* spp. has played an important role in the acquisition of penicillin, cephalosporin, macrolide and fluoroquinolone resistance in *N. meningitidis/N. gonorrhoeae* (Wadsworth et al., 2018; Fiore et al., 2020; Manoharan-Basil et al., 2021).

Antimicrobial resistance (AMR) may emerge earlier and spread more extensively in commensals than in pathogenic *Neisseria* spp. (Fiore et al., 2020; Dong et al., 2020). This has led to call for surveillance of AMR in commensal *Neisseria* spp. (Fiore et al., 2020; Dong et al., 2019; Kenyon and Schwartz, 2018). Proponents of this view argue that commensals are more at risk for the emergence of AMR due to their considerably higher

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Title - Global epidemiology of antimicrobial resistance in commensal *Neisseria* species: a systematic review

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Running title - AMR in commensal Neisseria species, a systematic review

Abstract

Background – Commensal *Neisseria* species (spp.) represent an important reservoir of antimicrobial resistance genes for pathogenic *Neisseria* spp. In this systematic review, we aimed to assess the antimicrobial susceptibility of commensal *Neisseria* spp. and how this has evolved over time. We also aimed to assess if commensal *Neisseria* spp. showed intrinsic resistance to four antimicrobials - penicillin, azithromycin, ceftriaxone and ciprofloxacin.

Methods – Pubmed and Google Scholar were searched following the PRISMA guidelines. Articles reporting MICs of commensal *Neisseria* spp. were included according to inclusion/exclusion criteria, and the quality of the articles was assessed using a pre-designed tool. Individual and summary measures of penicillin, azithromycin, ceftriaxone and ciprofloxacin MICs were collected. Additional data was sought to perform a comparison between the MICs of pathogenic and commensal *Neisseria* spp.

Results – A total of 15 studies met our criteria. We found no evidence of intrinsic AMR in commensal *Neisseria* spp. We did find evidence of an increasing trend in MICs of commensal *Neisseria* spp. over time for all antimicrobials assessed. These findings were similar in various countries. Eight additional studies were included to compare pathogenic and commensal *Neisseria* spp.

Conclusion – The MICs of commensal *Neisseria* spp. appear to be increasing in multiple countries. Surveillance of MICs in commensals could be used as an early warning system for antimicrobial resistance emergence in pathogens. Our findings underline the need for antibiotic stewardship interventions, particularly in populations with high antimicrobial consumption.

Keywords: antimicrobial resistance, commensal *Neisseria*, antimicrobial susceptibility, *Neisseria* spp.

Introduction

The genus *Neisseria* includes species that are both pathogenic (*N. meningitidis* and *N. gonorrhoeae*) and commensals to humans (e.g., *N. cinerea, N. mucosa, N. subflava, N. lactamica*) (1). The commensal *Neisseria* spp. are predominantly residents of the oropharynx and have been shown to play an important role in human health (2, 3). They come into frequent contact with pathogenic *Neisseria* spp. in the oropharynx, which provides the opportunity to exchange genetic material – predominantly via transformation (4-6). Numerous studies have established that this genetic exchange is important in the genesis of resistance to antimicrobials in the pathogenic *Neisseria* spp. (5, 6). The most prominent genes involved in this transformation include penA, mtrCDE, rplB, rplD, rplV and gyrA. The acquisition of sections of these genes from commensal *Neisseria* spp. has played an important role in the acquisition of penicillin, cephalosporin, macrolide and fluoroquinolone resistance in N. *meningitidis/N. gonorrhoeae* (5-7).

Antimicrobial resistance (AMR) may emerge earlier and spread more extensively in commensals than in pathogenic *Neisseria* spp. (6, 8). This has led to call for surveillance of AMR in commensal *Neisseria* spp. (6, 9, 10). Proponents of this view argue that commensals are more at risk for the emergence of AMR due to their considerably higher prevalence (close to 100%) than that of pathogenic *Neisseria* spp. (typically 0.01 to 10%) (9, 10). This higher prevalence means that commensals are more likely to be affected by bystander selection – selection for AMR by antimicrobials used for other indications (11). It is not, however, known if relevant resistance associated mutations are more prevalent in commensal versus pathogenic *Neisseria* spp. In addition, it is unknown if commensal *Neisseria* spp. may be intrinsically resistant to certain classes of antimicrobials. If they are not, is the prevalence of AMR increasing in commensal *Neisseria* spp.?

To address these questions, we performed a systematic review of antimicrobial susceptibility in commensal *Neisseria* spp. Our overarching research question was how

antimicrobial susceptibility in commensal *Neisseria* spp. has varied over place and time and in relation to the pathogenic *Neisseria* spp.

Methods

Systematic Review of MICs in commensal Neisseria spp.

This review was performed according to the PRISMA guidelines [12]. All the steps were performed independently by two reviewers (CK and TV). PRISMA checklists are presented in appendix A.

Search strategy

PubMed and Google Scholar were searched for articles published until March 21, 2021. Reference lists of relevant articles were checked for additional titles for inclusion in the review. Key words used for the search were “Antimicrobial Resistance”, “Antimicrobial Susceptibility”, “MIC”, “Minimum inhibitory concentration”,

“Neisseria” and specific names of each species of commensal *Neisseria* that has been isolated in humans (see appendix B)

Selection process and criteria

Titles and abstracts of all the articles retrieved through the search were screened. Duplicates were removed manually. Articles in German and Japanese were translated using DeepL Translator (www.deepl.com).

Articles reporting the MICs of commensal *Neisseria* spp. were included. Studies were included or excluded according to the following predefined criteria:

Inclusion criteria:

1. Reports individual or summary measures of MICs of commensal *Neisseria* spp.
2. Abstracts and full text available
3. Drug sensitivity testing done in a laboratory setting
4. Clinic- and population-based samples, national surveillance samples and case series

Exclusion criteria

1. Case reports of single isolates
2. Studies that did not report the year or country the isolates were obtained from
3. Studies not reporting MICs of penicillin, ceftriaxone, ciprofloxacin or azithromycin.

Data extraction

Data extraction was done using a predesigned database using Microsoft Excel. Information extracted included article information (DOI, first author, year of publication, period of data collection and country), study design (population sampled, sample size), method of species ID and antimicrobial susceptibility testing methodology.

We extracted the following summary measures of MIC distribution in as far as they were reported: median, range, interquartile range (IQR), MIC 50, MIC 90 for each *Neisseria* species by study period and country. If those were not reported but individual measurements were, we calculated summary measures per species per study. Data was extracted for the following antimicrobials: penicillin, ceftriaxone, ciprofloxacin and azithromycin.

Article quality assessment

The quality and risk of bias of each article was assessed using a tool based on the review from Tadesse et al. (13). This tool was modified for the purposes of this study and contained

11 criteria to evaluate study design, period and setting, sample collection, processing, storage and type of antimicrobial susceptibility testing performed (see appendix C). This quality assessment was not used for article inclusion/exclusion.

Comparison of MICs between N. lactamica and pathogenic Neisseria spp.

In addition to the systematic analysis, we compared the MIC distributions of the pathogenic *Neisseria* spp. with those of *N. lactamica* per year and country. The rationale for this comparison was to assess if *N. lactamica* MICs (azithromycin, benzylpenicillin, ceftriaxone, ciprofloxacin) were different than those of *N. meningitidis* and *N. gonorrhoeae*. *N. lactamica* was chosen for these analyses as more data was available than for any other commensal. The main analysis was directed at the comparison between *N. lactamica* and *N. meningitidis* for three reasons: 1. There is sufficient data about those two species to perform a comparison; 2. These two species are frequently surveyed in the same programs; 3. Unlike *N. gonorrhoeae*, these species are not predominantly sexually transmitted and their prevalence and antimicrobial susceptibilities are less likely to be affected by differences in sexual behaviour and the intensity of Sexually Transmitted Infections control activities. Where studies reported relevant antimicrobial susceptibility data for both *N. lactamica* and *N. meningitidis*, this data was used. When this type of study was not available, a literature search was performed in PubMed and Google Scholar to find large well conducted surveys that assessed the corresponding MIC distributions in *N. meningitidis* and *N. gonorrhoeae*. Preference was given to studies reporting MIC distributions from the same city or country, same or similar year and that used a similar method to ascertain MIC. The only study providing antimicrobial susceptibility data for *N. lactamica* in Japan did so for cefotaxime, ampicillin, azithromycin and tosufloxacin (14). To enable comparisons, antibiotics from the same class were used as proxies for each other. Cefotaxime MICs was used as a proxy for ceftriaxone, ampicillin to represent penicillin and tosufloxacin to represent ciprofloxacin MICs. All MIC values were converted in mg/L.

Data analysis

We compared the changes in antimicrobial susceptibility per species over time in individual countries. We report all summary measures of antimicrobial susceptibility (median, IQR or range).

EUCAST (v. 11.0) breakpoints in *N. gonorrhoeae* were used to define AMR in all *Neisseria* species: ceftriaxone resistance, > 0.125 mg/L; ciprofloxacin resistance, > 0.06 mg/L; and benzylpenicillin resistance, > 1 mg/L (available at: http://www.eucast.org). The epidemiological cut-off of 1mg/L was used for azithromycin as EUCAST does not provide a breakpoint for this antibiotic.

The results of the comparisons between commensal and pathogenic *Neisseria* spp. are presented graphically with forest plots for each antibiotic separately. Median MICs and range are displayed on the plots using a log2 scale.

Meta-analysis was not conducted because of the small number of isolates available per country per time point and variations in how antimicrobial susceptibility was determined and summarized. We did not conduct tests to assess if differences in MIC distributions were statistically significant. This was related to factors such as differences in study design between samples being compared and the fact that none of the studies we reviewed provided individual sample level MIC data. Only a limited number of studies reported interquartile ranges and we thus used medians and ranges to compare MICs between groups. The graphics and calculations were produced using R version 4.0.2.

Intrinsic resistance

To evaluate if a *Neisseria* species exhibited evidence of intrinsic resistance to a particular antimicrobial, we assessed if any isolate of that species, including the older samples, had MICs below the EUCAST breakpoints for *N. gonorrhoeae*. If any isolates of a species were susceptible according to EUCAST breakpoints, then this species was classified as not having intrinsic resistance [15].

Results

A. Systematic review of MICs in commensal *Neisseria* spp.

The literature search identified 295 studies (Figure 1). Of these 4 were excluded due to duplication, 274 were excluded based on title and abstract and 15 full-text articles were reviewed. Of these 8 studies met the inclusion/exclusion criteria. Seven studies were included through other sources which brings the total number of studies included in the review to 15 (Table 1).

Evolution of MICs in commensal Neisseria spp. over time

Table 2A contains all summary measures of MICs by study and species. The most relevant findings are highlighted hereunder.

N. cinerea

The earliest study to report antimicrobial susceptibilities was that of Berger et al., who found low penicillin MICs (range 0.00015-0.0006 mg/L) in 28 clinical isolates of *N. cinerea* from Germany pre-1961 (Table 2A) (16). A different study reported a low penicillin MIC (0.04mg/L) for one isolate of *N. cinerea* obtained from Germany in 1962 (17). By the early 1980s, penicillin MICs in this organism were higher than in the previous studies, between 0.125 and 1 mg/L in the USA (18) and 0.16 to 0.64 mg/L in France (17). A larger study conducted in France between 1973 and 1997 (n=183) also showed high MICs (median MIC 0.5 mg/L [range 0.125-8]) (19).

N. subflava

A study from Belgium that used an identical protocol to compare the MICs of historical isolates from the early 1980s with isolates obtained in 2019 found an increase in MICs over time (azithromycin: median 1 to 176 mg/L; ceftriaxone: median 0.03 to 0.38 mg/L) (20).

The data from Asia shows that ceftriaxone MICs were higher in Vietnam in 2016 (median 0.064) than in Japan in 2005 (median 0.03) (9, 21). A small study from Spain in 1996 found high penicillin MICs in *N. subflava* (median 1mg/L [range 0.06-4] and *N. mucosa* (median 1mg/L [range 0.12-1] (22).

Intrinsic resistance

We found no evidence of intrinsic antimicrobial resistance to any antimicrobial considered in any of the *Neisseria* species under review (Table 2A).

B. Comparison of MICs in pathogenic *Neisseria* spp. vs *N. lactamica*

To perform a comparison between pathogenic and commensal *Neisseria* spp., eight studies were included (6 of *N. gonorrhoeae* and 2 of *N. meninigitidis* MICs). Relevant study characteristics are provided in Table 1. Studies from five countries included data that enabled us to compare MIC distributions between commensal *Neisseria* (*N. lactamica*) and *N. meningitidis/N. gonorrhoeae* (Fig. 2).

Spain

Two large surveys of antimicrobial susceptibility in Spain in the 1990s found higher penicillin MICs in *N. lactamica* (n=286, median 0.25 mg/L [range 0.12 to 1]) than *N. meningitidis* (n=700, median 0.06 mg/L [range 0.007-0.5]; Fig. 2; Table 2B) (23, 24). A national survey in 1997-1998 found a gonococcal penicillin MIC distribution (median 0.25 mg/L [range <0.007-16]) which was similar to that of *N. lactamica* (25).

These same three surveys found similar ciprofloxacin MICs in the three species. The ciprofloxacin MICs were slightly higher in *N. lactamica* (median 0.003 mg/L [range 0.0015-0.5]), than *N. gonorrhoeae* (median 0.0015 mg/L [range <0.0015-0.25]) which was in turn slightly higher than those of *N. meningitidis* (median 0.006 mg/L [range 0.0003-0.012]).

The ceftriaxone MICs reported in these surveys were highest in *N. lactamica* (median 0.0015 mg/L [range 0.0007-0.06]), followed by *N. gonorrhoeae* (median 0.0007 mg/L [range 0.0003-0.007]) and then *N. meningitidis* (median 0.0007 mg/L [range 0.00007-0.015]).

A previous study of 30 *N. lactamica* strains from the early 80s found lower MICs for both penicillin (median 0.2 mg/L [range 0.1-0.8]) and ceftriaxone (median 0.0007 mg/L [range 0.003-0.0015]) compared with the more recent studies (26).

Belgium

A study in Belgium evaluated the MICs of all oropharyngeal *Neisseria* spp. isolated from 96 individuals in 2019 (27). MICs were found to be higher in *N. lactamica* than *N. meningitidis* for both azithromycin (median 1.5 mg/L [range 1-2] and median 0.5 [range 0.19-6] mg/L, respectively) and ciprofloxacin (median 0.127 mg/L [range 0.06-0.19] and median 0.004 mg/L [range 0.002-0.125]) but not ceftriaxone (median 0.008 mg/L [range 0.008-0.008] and median 0.008 mg/L [range 0.008-1]; Fig 2, Table 2B). Ceftriaxone (median 0.016 mg/L [range 0.016-0.5]) and ciprofloxacin (median 0.5 mg/L [range 0.002-32]) MICs were higher in 642 *N. gonorrhoeae* isolates evaluated in the Belgian national surveillance report for 2019 than the corresponding MICs for *N. lactamica* or *N. meningitidis* (28). In contrast, the *N. gonorrhoeae* azithromycin MICs from this report (median 0.19 mg/L [range 0.03-256]) were lower than those for *N. lactamica* and *N. meningitidis*.

Germany

Karch et al., compared the penicillin MIC distributions of *N. lactamica* (n=123, collected during a meningococcal carriage study in 1999/2000) and *N. meningitidis* (n=129, randomly selected from invasive isolates received by the national reference laboratory in 2006) (29). The penicillin MICs were higher in *N. lactamica* (median 0.38 [range 0.064-2 mg/L]) than *N. meningitidis* (median 0.064 [range 0.016-0.25 mg/L]). These penicillin MICs in *N. lactamica*

were also higher than those reported for 150 isolates of *N. gonorrhoeae* obtained between 1988-1992 (median 0.125 [range 0.002-128]) (30).

China

A large clinical study from Shanghai between 2005 and 2018 found high ciprofloxacin MICs for *N. meningitidis* (median 0.125 mg/L [range 0.015-1]) but even higher MICs in circulating commensal *Neisseria* spp. (median 0.25 [range 0.015-16]; Table 1; Fig. 2) (31). A further survey conducted in Shanghai children between 2014 and 2016 found similarly high ciprofloxaxin MICs for *N. lactamica* (median 0.25 [range 0.06-1]) (32). Gonococcal ciprofloxacin MICs (n=159) obtained in Shanghai in 2004-2005 were higher than both those for *N. meningitidis* and *N. lactamica* (median 8 mg/L [range 0.06-64]) (33). A later study showed similarly high ciprofloxacin MICs for *N. gonorrhoeae* (n=366) obtained in Shanghai in 2017 (median 16 mg/L [range 0.004-32]) (8).

Japan

Variations in the ciprofloxacin MICs between *Neisseria* species in Japan were very similar to those found in China with very high MICs in *N. gonorrhoeae* (median 8 mg/L [range 0.06-32]), followed by *N. lactamica* (median 0.5 mg/L [range 0.015-1]) and then *N. meningitidis* (median 0.004 mg/L [range 0.004-0.125]; Table 1; Fig 2) (14, 34, 35). Penicillin MICs were also markedly elevated in *N. lactamica* (median 1 mg/L [range 0.5-4]) and *N. gonorrhoeae* (median 1 mg/L [range 0.06-64]) in comparison to *N. meningitidis* (median 0.031 mg/L [range 0.016-0.25]) (14, 34, 35). Ceftriaxone MICs were highest in *N. lactamica* (median 1 mg/L [range 1-8]) followed by *N. gonorrhoeae* (median 0.06 mg/L [range 0.06-0.125]) and *N. meningitidis* (median 0.004 mg/L [range 0.004-0.004]) (14, 34, 35).

Discussion

Although little has been published evaluating the antimicrobial susceptibility of commensal *Neisseria* spp., the data that has been published is instructive. Our findings suggest that commensal *Neisseria* spp. are not intrinsically resistant to the antimicrobials evaluated here when utilizing *N. gonorrhoeae* breakpoints. Thus *N. cinerea* was highly susceptible to penicillin in the 1960s (16). Penicillin MICs in this organism, however, appear to have increased steadily in the ensuing decades. A similar pattern has been established for *N. meningitidis* and *N. gonorrhoeae* (36, 37). Of six bacterial species tested, *N. gonorrhoeae* was the most susceptible to penicillin in the early antibiotic era (36). Following decades of antibiotic exposure, penicillin MICs of *N. gonorrhoeae* have increased considerably (36, 37). By 2018, isolates with penicillin and ceftriaxone MICs of 1 mg/L or above were being reported from Japan (38). Whilst there is considerably less data available for commensal *Neisseria* spp., the available data suggests that commensals have undergone a similar evolution. This is most evident for penicillin in *N. cinerea*, but likely also applies to *N. lactamica* and *N. subflava*. We found weak evidence that ceftriaxone, azithromycin, penicillin and ciprofloxacin MICs have been increasing in these species over the past few decades.

We also found specific populations where this increase seemed to be more pronounced. The median ciprofloxacin MICs for *N. lactamica*, *N. meningitidis* and *N. gonorrhoeae*, for example, were all higher in China than any other country included in our comparison. A systematic review of gonococcal antimicrobial resistance in China confirmed the high ciprofloxacin MICs but also documented how rapidly ciprofloxacin resistance emerged in China – ciprofloxacin resistance increased from 13% to 94% of gonococcal isolates between 1995 and 2003 (39).

A striking feature of our analysis was how much higher the ciprofloxacin MICs were in *N. lactamica* than *N. meningitidis* in China and elsewhere. In the studies we used from China, 98.5 % of *N. lactamica* versus 68.7% of *N. meningitidis* were resistant to ciprofloxacin (32).

Our comparison of MIC distributions revealed that, in general, the azithromycin, benzylpenicillin and ciprofloxacin MICs were higher in *N. lactamica* than *N. meningitidis*. As far as ceftriaxone was concerned, the same was true in one large study from Spain and one study from Japan, whereas in a smaller study from Belgium, the MIC distributions were very similar between these two species (20, 23, 24). In general, MIC distributions for ceftriaxone in *N. gonorrhoeae* were higher than those of *N. meningitidis* and not too dissimilar to those of *N. lactamica*.

This high prevalence of resistance in commensal *Neisseria* spp. is more than a theoretical risk. Phylogenetic and transformation experiments have revealed that transformation from resistant isolates of *N. cinerea* was a likely source of penicillin and cephalosporin resistance in both *N. gonorrhoeae* and *N. meningitidis* (38, 40). In the case of *N. gonorrhoeae*, epidemiological evidence pointed to Japan as one of the likely locations where this transformation occurred (40). Phylogenetic analyses from China demonstrated that over half of the fluoroquinolone resistance conferring mutations in *N. meningitidis* were acquired from *N. lactamica* (31). Similarly, increases in azithromycin resistance in *N. gonorrhoeae* have been linked to the spread of mosaic mtrCDE genes acquired from commensal *Neisseria* spp. (5). The incidence of this horizontal gene transfer (HGT) between *Neisseria* spp. is appreciable. One longitudinal study, for example, found evidence of HGT between *N. meningitidis* and *N. lactamica* in 15 loci over a 6-month period in the two individuals that were co-colonized by both bacteria at baseline (41).

What is the reason for the increasing antimicrobial resistance in commensal *Neisseria* spp. ? Commensal *Neisseria* spp. are a key constituent of a healthy oro-pharyngeal microbiome (42, 43). Broad spectrum antimicrobials have been shown to be effective at eradicating the pathogenic *Neisseria* spp., but to have little effect on the prevalence of commensal *Neisseria* spp. (44). Broad spectrum antimicrobials do however select for antimicrobial resistance in commensal *Neisseria* spp. (27, 44). Populations with high levels of antimicrobial consumption have been shown to be at high risk for the emergence of antimicrobial resistance in both *N. gonorrhoeae* (10, 45-47) and commensal *Neisseria* spp.

(27). Further research is required to better define the types and intensity of antimicrobial exposure required to select for the genesis and spread of antimicrobial resistance of antimicrobial resistance in commensal *Neisseria* (48).

There are a number of limitations to this review. Very few studies have been published on this topic. Those that have been published have numerous methodological weaknesses, including small sample sizes and non-random samples. Comparisons between studies are further hampered by differences in how species were identified, and MICs assessed. For Japan we had to use antibiotics from the same class as proxies for the antibiotics of interest as no other data was available. Moreover, species identification in older studies might be subject to misclassification bias. Lastly, our definition of intrinsic resistance is based on EUCAST breakpoints for *N. gonorrhoeae* as no such breakpoints are defined for commensal *Neisseria* spp.

These limitations notwithstanding, our findings suggest the need to better understand and arrest the further emergence of AMR in commensal *Neisseria* spp. In the case of *N. gonorrhoeae*, a number of studies (but not all studies (49)) have found a link between population level consumption of a class of antimicrobials and the prevalence of class concordant AMR (50, 51). Various lines of evidence suggest that differential intensity of antimicrobial consumption is likely to be the key driver of AMR in commensal *Neisseria* spp. (9, 20, 52).

Taken as a whole, the findings of our review support the argument that surveillance of MICs of commensal *Neisseria* spp. may be a useful early warning system of excess antimicrobial exposure and increased risk for the emergence of AMR in *N. gonorrhoeae* and other pathogens (52). In a similar vein, the findings motivate for intensified antimicrobial stewardship. Whilst this is important in general populations, special attention should be focused on core-groups with high rates of partner change such as HIV pre-exposure prophylaxis cohorts due to the frequency with which gonococcal AMR has emerged in such populations (10, 53).

Table 1 - Selected characteristics of the studies included in the systematic review and comparison of MICs between pathogenic *Neisseria* spp. and *Neisseria lactamica*

Study First Author & reference	Year of publication	Study period	Country	Sampling method	Number of isolates	MIC testing methodology	Method of species ID	Species assessed	Relevant antimicrobials assessed
Studies included from the systematic review									
Laumen (20)	2020	1979-1990	Belgium	29 isolates collected between 1979 and 1990 and kept in the Institute of Tropical Medical Medicine's historical collection of *Neisseria*.	29	Agar dilution	NS	Nm, Ns, No, Nma	Ceftriaxone, azithromycin
Laumen (20)	2020	2019	Belgium	10 men with a diagnosis of anogenital Ng had their oropharynges swabbed on 2 separate occasions and all *Neisseria* cultured and MICs assessed	27	E-test	GS, O, MALDI-TOF	Nm, Ns, No, Nma, Ng	Ceftriaxone, azithromycin
Laumen (27)	preprint	2019-2021	Belgium	A subgroup of 64 MSM using HIV pre-exposure prophylaxis of a randomized clinical trial (PreGo) and 20 employees of the Institute of Tropical Medicine.	26	E-test	GS, O, MALDI-TOF	Nm, Nl	Ceftriaxone, azithromycin, ciprofloxacin
Chen (31)	2019	2005-18	China	198 N. meningitidis and 293 commensal *Neisseria* isolates collected between 2005 and 2018 in Shanghai. The *N. meningitidis* isolates were obtained from invasive meningococcal isolates (n=46) and asymptomatic carriers (n=152). The commensal *Neisseria* isolates were all obtained from carriers, including *N. lactamica* (n=252).	491	Agar dilution	GS, O, MALDI-TOF	Nl, Np, Ns, Nc, Nmu, No, Nm	Ciprofloxacin
Shen (32)	2019	2014-16	China	Carriage survey performed in 11 kindergartens and 15 schools in Shanghai. Posterior oropharyngeal swabs collected from 2239 children younger than 15 years.	200	Agar dilution	GS, O, MALDI-TOF	Nl	Ciprofloxacin
Berger (16)	1962	1961	Germany	28 strains cultivated from the human pharynx. Further details pertaining to sample selection were not provided.	28	Agar dilution	GS, O, biochem	Nc	Penicillin

Karch (29)	2015	1999-2000	Germany	*N. lactamica* strains (n = 123) collected during the Bavarian meningococcal carriage study in winter 1999/2000.	123	E-test	GS, O, biochem	Nl	Penicillin
Karch (29)	2015	2006	Germany	*N. meningitidis* strains (n = 129) randomly selected from invasive isolates received by the German reference laboratory for meningococci in 2006.	129	E-test	GS, O, biochem	Nm	Penicillin
Furuya (21)	2007	2005-2006	Japan	45 clinical isolates of *N. subflava* collected from the oropharynx of 40 Japanese men with urethritis and 5 women who were sex workers	45	Agar dilution	BD BBLCRYSTAL N/H, and VITEK NHI	Ns	Penicillin, ceftriaxone, ciprofloxacin
Takei (14)	2020	2015	Japan	7 N. lactamica strains detected in Chiba Children's Hospital during the 2015 surveillance study for N. meningitidis were analyzed. Strains detected in specimens from 389 patients younger than 15 years who presented with respiratory symptoms.	7	Micro dilution	biochem, MALDI-TOF	Nl	Azithromycin, ampicillin, cefotaxime, tosufloxacin
Saez Nieto (22)	1998	NS	Spain	112 isolates cultured from oropharyngeal swabs from 40 randomly chosen individuals among university personnel	112	Agar dilution	GS, O, biochem	Nmu, Ns	Penicillin
Arreaza (23)	2002	1996-8	Spain	286 isolates cultured during two meningococcal carriage surveys between 1996 and 1998	286	Agar dilution	GS, O, biochem	Nl	Penicillin, ciprofloxacin, ceftriaxone
Kochi (19)	1999	1973-97	France	183 strains of *N cinerea*, isolated from various human biological specimens and sent to the National Meningococcal Reference Center between 1973 and 1997. MIC was defined for 124/183 strains.	124	Agar dilution	GS, O, biochem	Nc	Penicillin
Bowler (17)	1994	1962-82	France/Germany	Four isolates of *N. cinerea*, 3 from France and one from Germany had their penicillin MICs assessed as part of a series of transformation experiments with Nm	4	NS	NS	Nc	Penicillin

Knapp (18)	1984	1981-83	USA	Four isolates of *N. cinerea* were obtained from clinical isolates from various centres in the USA and further characterized	4	Agar dilution	GS, O, biochem	Nc	Penicillin
Dong (9)	2021	2016-17	Vietnam	207 men who have sex with men had pharyngeal swabs performed and *Neisseria* species identified. We report results of patients who didn't report any antibiotic use in the past 6 months.	265	E-Test	GS, O, MALDI-TOF	Ng, Nm, Ns, Nmu, No	Ceftriaxone
Nieto (26)	1990	1979-1983	Spain	30 N. lactamica and 30 N. polysaccharea strains isolated from nasopharynges of children. Nm not included because strains described according to resistance pattern.	60	Agar dilution, disk diffusion	GS, O, biochem	Nl, Np	Penicillin, ceftriaxone
Studies included to perform a comparison between commensal and pathogenic Neisseria									
Dong (8)	2020	2017	China	Neisseria gonorrhoeae isolates were collected from male patients with uncomplicated urogenital gonorrhea at the Shanghai Skin Disease Hospital in conjunction with the China GASP. The first 30 N. gonorrhoeae isolates of each month in 2017 (except for 36 isolates collected in July, making a total of 366 isolates)	366	Agar dilution	GS, O, biochem	Ng	Penicillin, ciprofloxacin, azithromycin, ceftriaxone
Schäfer (30)	1995	1988-1992	Germany	150 strains of Neisseria gonorrhoeae isolated between 1988 and 1992 from urethral, cervical, vaginal, anal and pharyngeal swabs in female prostitutes.	150	Agar dilution	GS, O, biochem	Ng	Penicillin, ciprofloxacin, azithromycin
Arreaza (25)	2003	1997-98	Spain	2966 gonococcal isolates received at the Spanish National Reference Laboratory from 1983 to 2001. We used the results for the isolates from the years closest to	55	Agar dilution	NS	Ng	Penicillin, ceftriaxone, ciprofloxacin

				those when the Nl isolates were obtained. (=1997-1998)					
Arreaza (24)	2000	1996-97	Spain	789 isolates obtained from a study of asymptomatic Nm carriers (between 1996 and 1997). Results were reported separately for serogroup C (n=89) and non-serogroup C (n=700). We used the results for the larger sample size.	700	Agar dilution	GS, O, biochem	Nm	Penicillin, ceftriaxone, ciprofloxacin
Watanabe (34)	2007	1990-2004	Japan	Strains isolated from meningococcal meningitis, pneumonia, and healthy carriers during a 15-year period from 1990 to 2004. 100 strains of Nmen: 33 isolated from patients with meningococcal meningitis; 24 from patients with septicemia, pneumonia, rhinosinusitis, etc. other than meningitis.; 6 strains from STD, 33 strains from healthy carriers, 3 patient-derived strains, 1 unknown.	100	Agar dilution	NS	Nm	Penicillin, ceftriaxone, ciprofloxacin
Hamasuna (35)	2013	2009-10	Japan	As part of a national surveillance project of Ng AMR, urethral swabs were obtained from male patients older than 16 years with symptoms of urethritis at 51 participating facilities including departments of urology in hospitals and private clinics that specialized in urology.	83	Agar dilution	NS and NAAT confirmed (Cobas amplicore STI-1)	Ng	Penicillin, ceftriaxone, ciprofloxacin, azithromycin

Yang Yang (33)	2006	2004-2005	China	Clinical N. gonorrhoeae isolates collected from 159 consecutive male patients with symptoms of urethritis at the Shanghai Skin Disease and STD Hospital between 2004 and 2005.	159	Agar dilution	GS, O, Biochem	Ng	Penicillin, ciprofloxacin, ceftriaxone
De Baetselier (28)	NA	2019	Belgium	642 N. gonorrhoeae clinical isolates obtained during 2019 from participating centres were sent to the Belgian national Ng reference laboratory and had their MICs assessed	642	E-test, (Agar dilution)	NS	Ng	Ceftriaxone, azithromycin, ciprofloxacin

Abbreviation list: Biochem: biochemical tests, GS: gram staining, MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization-Time Of Flight, MIC: minimum inhibitory concentration, NA: not applicable, NAAT: nuclei acid amplification tests, Nc: *Neisseria cinerea*, Ng: *Neisseria gonorrhoeae*, Nl: *Neisseria lactamica*, Nm: *Neisseria meningitidis*, Nma: *Neisseria macacae*, Nmu: *Neisseria mucosa*, No: *Neisseria oralis*, Np: *Neisseria polysaccharea*, Npe: *Neisseria perflava*, Ns: *Neisseria subflava*, Nsi*: Neisseria sicca*, NS: not specified, O: oxydase tests

Tables 2A and 2B - Summary measures of antimicrobial susceptibility by study and species

Table 2A - Summary measures of antimicrobial susceptibility by study and species – results of the systematic review

Species	Year	Author	Country	Antimicrobial	N isolates	Median MIC (mg/L)	Range min (mg/L)	Range max (mg/L)
N. lactamica	1996-1998	Arreaza (23)	Spain	penicillin	286	0,25	0,12	1
N. lactamica	1996-1998	Arreaza (23)	Spain	ceftriaxone	286	0,0015	0,0007	0,06
N. lactamica	1996-1998	Arreaza (23)	Spain	ciprofloxacin	286	0,003	0,0015	0,5
N. cinerea	1961	Berger (16)	Germany	penicillin	28		0,00015	0,0006
N. cinerea	1962-1982	Bowler (17)	France, Germany	penicillin	4	0,24	0,04	0,64
Various commensal *Neisseria* species	2005-2018	Chen (31)	China	ciprofloxacin	293	0,25	0,015	16
N. flavescens	2016-2017	Dong (9)	Vietnam	ceftriaxone	76	0,047	0,047	
N. macacae	2016-2017	Dong (9)	Vietnam	ceftriaxone	7	0,047		
N. oralis	2016-2017	Dong (9)	Vietnam	ceftriaxone	2	0,056		
N. subflava	2016-2017	Dong (9)	Vietnam	ceftriaxone	33	0,064		
N. subflava	2005-2006	Furuya (21)	Japan	penicillin G	45	0,5	0,06	2
N. subflava	2005-2006	Furuya (21)	Japan	ceftriaxone	45	0,03	0,001	0,12
N. subflava	2005-2006	Furuya (21)	Japan	ciprofloxacin	45	0,25	0,008	8
N. lactamica	1999-2000	Karch (29)	Germany	penicillin G	123	0,38	0,064	2
N. cinerea	1981-1983	Knapp (18)	USA	penicillin	4		0,125	1
N. cinerea	1973-1997	Kochi (19)	France	penicillin	124	0,5	0,125	8
N. lactamica	2019	Laumen (27)	Belgium	azithromycin	2	1,5	1	2
N. lactamica	2019	Laumen (27)	Belgium	ciproflocaxin	2	0,127	0,064	0,19
N. lactamica	2019	Laumen (27)	Belgium	ceftriaxone	2	0,008	0,008	0,008
N. subflava	2019	Laumen (20)	Belgium	azithromycin	10	176	0,047	256
N. subflava	2019	Laumen (20)	Belgium	ceftriaxone	10	0,38	0,023	2
N. macacae	2019	Laumen (20)	Belgium	azithromycin	3	8	4	256
N. macacae	2019	Laumen (20)	Belgium	ceftriaxone	3	0,094	0,032	0,125
N. oralis	2019	Laumen (20)	Belgium	azithromycin	2	3	3	3
N. oralis	2019	Laumen (20)	Belgium	ceftriaxone	2	0,273	0,047	0,5
N. subflava	1983	Laumen (20)	Belgium	azithromycin	7	1	0,025	4

N. subflava	1983	Laumen (20)	Belgium	ceftriaxone	7	0,03	0,015	0,06
N. macacae	1983	Laumen (20)	Belgium	azithromycin	5	8	4	8
N. macacae	1983	Laumen (20)	Belgium	ceftriaxone	5	0,06	0,03	0,125
N. oralis	1983	Laumen (20)	Belgium	azithromycin	2	4	4	4
N. oralis	1983	Laumen (20)	Belgium	ceftriaxone	2	0,06	0,06	0,06
Various commensal *Neisseria* species	1998	Saez Nieto (22)	Spain	penicillin			0,06	4
N. lactamica	1979-1983	Nieto (26)	Spain	ceftriaxone	30	0,0007	0,0003	0,0015
N. lactamica	1979-1983	Nieto (26)	Spain	penicillin	30	0,2	0,1	0,8
N. polysaccharea	1979-1983	Nieto (26)	Spain	ceftriaxone	30	0,0004	0,0003	0,025
N. polysaccharea	1979-1983	Nieto (26)	Spain	penicillin	30	0,25	0,05	0,8
N. lactamica	2014, 2016	Shen (32)	China	ciprofloxacin	200	0,25	0,06	1
N. lactamica	2015	Takei (14)	Japan	azithromycin	7	1	0,25	1
N. lactamica	2015	Takei (14)	Japan	ampicillin	7	1	0,5	4
N. lactamica	2015	Takei (14)	Japan	tosufloxacine	7	0,5	0,015	1
N. lactamica	2015	Takei (14)	Japan	cefotaxime	7	1	1	8

Table 2B - Summary measures of antimicrobial susceptibility by study and species – results for pathogenic Neisseria species (studies included to perform a comparison between commensal and pathogenic Neisseria)

Species	**Year**	**Author**	**Country**	**Antimicrobial**	**N isolates**	**Median MIC (mg/L)**	**Range min (mg/L)**	**Range max (mg/L)**
N. gonorrhoeae	2019	De Baetselier (28)	Belgium	azithromycin	642	0,19	0,003	256
N. gonorrhoeae	2019	De Baetselier (28)	Belgium	ceftriaxone	642	0,016	0,016	5
N. gonorrhoeae	2019	De Baetselier (28)	Belgium	ciprofloxacin	642	0,5	0,002	32
N. gonorrhoeae	2019	De Baetselier (28)	Belgium	penicillin	642	0,5	0,015	64
N. meningitidis	2019	Laumen (27)	Belgium	azithromycin	34	0,5	0,19	6
N. meningitidis	2019	Laumen (27)	Belgium	ciproflocaxin	34	0,004	0,002	0,125
N. meningitidis	2019	Laumen (27)	Belgium	ceftriaxone	34	0,008	0,008	1
N. meningitidis	2019	Laumen (20)	Belgium	azithromycin	5	0,75	0,25	0,75
N. meningitidis	2019	Laumen (20)	Belgium	ceftriaxone	5	0,016	0,016	0,016
N. meningitidis	1983	Laumen (20)	Belgium	azithromycin	15	0,5	0,25	4
N. meningitidis	1983	Laumen (20)	Belgium	ceftriaxone	15	0,002	0,001	0,06

N. gonorrhoeae	2004-2005	Yang Yang (33)	China	penicillin	159	32	0,05	64
N. gonorrhoeae	2004-2005	Yang Yang (33)	China	ciprofloxacin	159	8	0,06	64
N. gonorrhoeae	2004-2005	Yang Yang (33)	China	ceftriaxone	159	0,03	0,004	0,25
N. gonorrhoeae	2017	Dong (8)	China	ciprofloxacin	366	16	0,004	32
N. meningitidis	2005-2018	Chen (31)	China	ciprofloxacin	198	0,125	0,015	1
N. gonorrhoeae	1988-1992	Schäfer (30)	Germany	penicillin	150	0,125	0,002	128
N. meningitidis	2006	Karch (29)	Germany	penicillin G	129	0,064	0,016	0,25
N. meningitidis	1990-2004	Watanabe (34)	Japan	penicillin G	100	0,031	0,016	0,25
N. meningitidis	1990-2004	Watanabe (34)	Japan	ceftriaxone	100	0,004	0,004	0,004
N. meningitidis	1990-2004	Watanabe (34)	Japan	ciprofloxacin	100	0,004	0,004	0,125
N. gonorrhoeae	2009-2010	Hamasuna (35)	Japan	penicillin G	83	1	0,06	64
N. gonorrhoeae	2009-2010	Hamasuna (35)	Japan	ceftriaxone	83	0,06	0,06	0,125
N. gonorrhoeae	2009-2010	Hamasuna (35)	Japan	azithromycin	83	0,125	0,06	2
N. gonorrhoeae	2009-2010	Hamasuna (35)	Japan	ciprofloxacin	83	8	0,06	16
N. meningitidis	1996-1997	Arreaza (24)	Spain	penicillin	700	0,06	0,007	0,5
N. meningitidis	1996-1997	Arreaza (24)	Spain	ceftriaxone	700	0,0007	0,00007	0,015
N. meningitidis	1996-1997	Arreaza (24)	Spain	ciprofloxacin	700	0,006	0,0003	0,012
N. gonorrhoeae	1997-1998	Arreaza (25)	Spain	penicillin	55	0,25	0,007	16
N. gonorrhoeae	1997-1998	Arreaza (25)	Spain	ceftriaxone	55	0,0007	0,0003	0,007
N. gonorrhoeae	1997-1998	Arreaza (25)	Spain	ciprofloxacin	55	0,0015	0,0015	0,25
N. meningitidis	2016-2017	Dong (9)	Vietnam	ceftriaxone	10	0,002		

Figure 1 - Flowchart of study selection

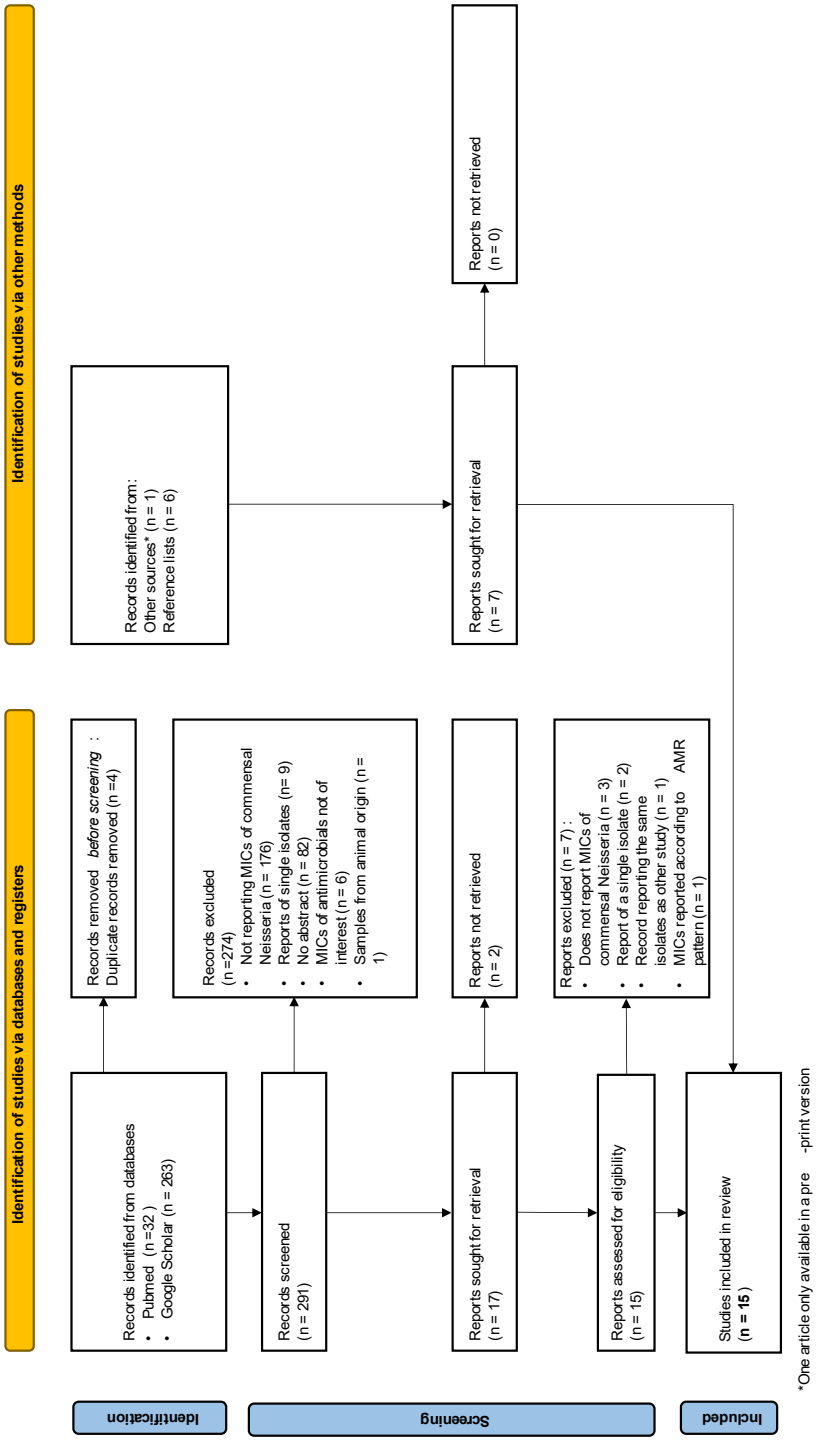
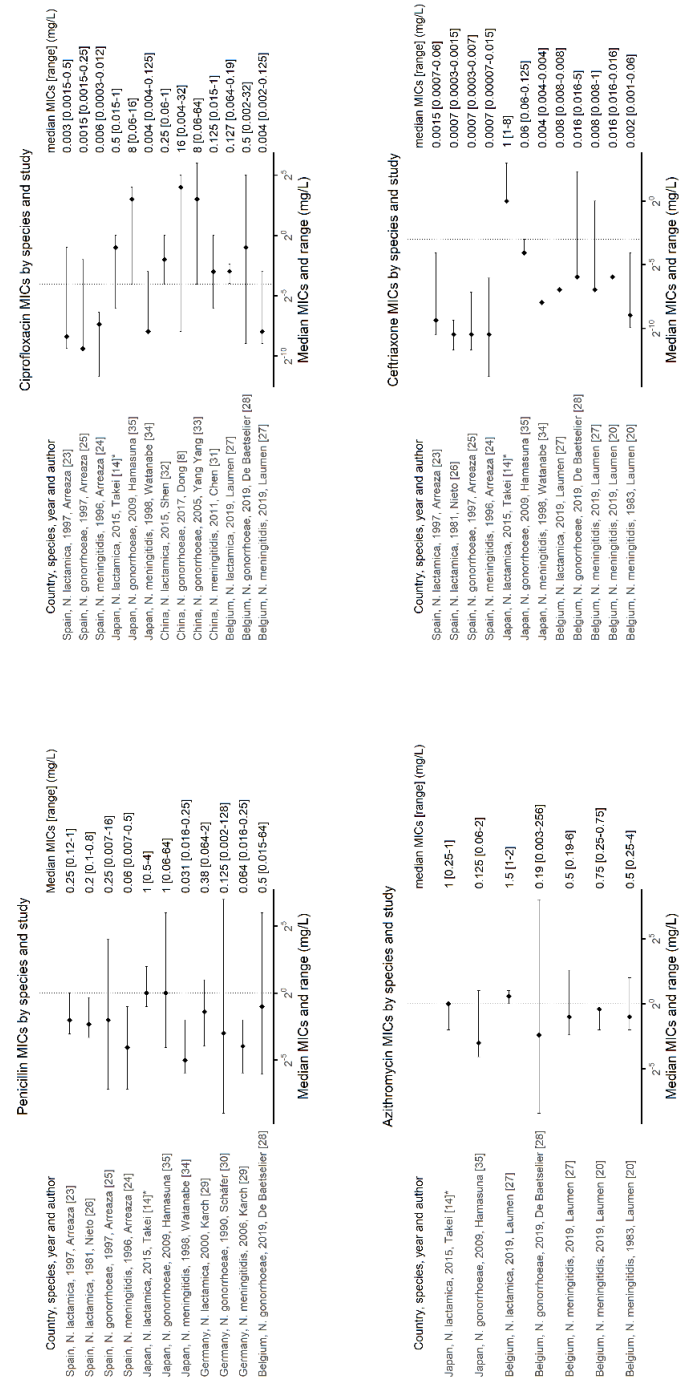


Figure 2 – Penicillin, azithromycin, ceftriaxone and ciprofloxacin minimum inhibition concentrations (MICs, mg/L) median and range for *N. gonorrhoeae, N. meningitidis* and *N. lactamica* by study. The horizontal dotted line represents the EUCAST (v. 11.0) breakpoint or epidemiological cut-off for the corresponding antimicrobials. * For this study we used an antibiotic of the same class as proxies, cefotaxime for ceftriaxone, tosufloxacin for ciprofloxacin and ampicillin for penicillin.



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Appendix A – PRISMA checklists

PRISMA 2020 Main Checklist

Topic	No.	Item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Line 59-84
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Line 86-89
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Line 109-123
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Line 97-103, appendix B
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	appendix B
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Line 93-95,106
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Line 93-95, 125-137

Topic	No.	Item	Location where item is reported
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Line 132-137
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Line 125-130
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Line 93-95, 139-145
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Line 132-134
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item 5)).	Line 132-199
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Line 134-135, 169-174
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Line 125-137, 181-183
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Line 125-137, 181-199
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NA

Topic	No.	Item	Location where item is reported
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Line 201-209, figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	figure 1
Study characteristics	17	Cite each included study and present its characteristics.	table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	appendix C
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	table 2a and b, lines 211-313
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	appendix C
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	figure 2
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Line 315-360
	23b	Discuss any limitations of the evidence included in the review.	Line 362-370
	23c	Discuss any limitations of the review processes used.	Line 3621-370
	23d	Discuss implications of the results for practice, policy, and future research.	Line 380-387
OTHER INFORMATION			

Topic	No.	Item	Location where item is reported
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Line 389-390
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Line 389-390
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Line 398-400
Competing interests	26	Declare any competing interests of review authors.	Line 402-403
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Line 395-396

PRIMSA Abstract Checklist

Topic	No.	Item	Reported?
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Partially
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes

Topic	No.	Item	Reported?
Synthesis of results	6	Specify the methods used to present and synthesize results.	Partially
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	No
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	No
Registration	12	Provide the register name and registration number.	No

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. MetaArXiv. 2020, September 14. DOI: 10.31222/osf.io/v7gm2. For more information, visit: www.prisma-statement.org

Appendix B – Search strategy

Pubmed (last consulted March 21 2021)

("antimicrobial resistance"[Title/Abstract] OR "antimicrobial susceptibility"[Title/Abstract] OR "MIC"[Title/Abstract] OR "minimum inhibitory concentration"[Title/Abstract]) AND ("Neisseria"[Title/Abstract] AND ("lactamica"[Title/Abstract] OR "bacilliformis"[Title/Abstract] OR "cinerea"[Title/Abstract] OR "elongata"[Title/Abstract] OR "flavescens"[Title/Abstract] OR "macacae"[Title/Abstract] OR "mucosa"[Title/Abstract] OR "oralis"[Title/Abstract] OR "polysaccharea"[Title/Abstract] OR "sicca"[Title/Abstract] OR "subflava"[Title/Abstract] OR "flava"[Title/Abstract])) AND 1901/01/01:2021/03/21[Date - Publication]

32 results

Google Scholar (last consulted March 21 2021)

allintitle: neisseria resistance -gonorrhoeae

259 results

Appendix C – Article quality assessment

The quality of each article was assessed using a tool based on the review from Tadesse et al.[13] This tool was modified for the purposes of this study and contains 11 criteria. This quality assessment was not used for inclusion/exclusion.

Criteria	**% of studies fulfilling the criteria**
Is the research design described?	96%
Does the study state the period of time during which samples were specifically collected?	84%
Is the setting of the study and data acquisition clearly described; for example, hospital acquired versus community acquired?	48%
Are the criteria for enrolment in the study clearly stated?	24%
Is there a clear description of the types of specimen collected?	52%
Are details of conditions of sample storage like temperature and place described?	40%
Is the duration of sample storage described?	8%
Are the media for culture described?	88%
Did the study describe the total number of isolates?	100%
Does the study describe the type of susceptibility testing used?	88%
Did the study specify the testing standard used (e.g. CLSI, EUCAST)	64%

5.2 Interventions impacting the emergence of AMR in bacterial STIs

5.2.1 Reducing NG/CT screening intensity as a tool to slow down AMR in NG

Vanbaelen T, Van Dijck C, De Baetselier I, et al. Screening for STIs is one of the main drivers of macrolide consumption in PrEP users. *International Journal of STD and AIDS* 2021; 32(12).

Letter to the Editor

INTERNATIONAL JOURNAL OF STD & AIDS

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Screening for STIs is one of the main drivers of macrolide consumption in PrEP users

Dear Editors

A recent publication by Forster et al. explored the demographic and behavioural factors associated with antimicrobial susceptibility to ceftriaxone and azithromycin of *Neisseria Gonorrhoeae* (NG). This article showed an alarming increase in NG geometric mean azithromycin minimum inhibitory concentration (MIC) between 2014/2015 and 2017/2018. Moreover they found a higher geometric mean azithromycin MIC in men who have sex with men (MSM) compared with other groups. Given the emergence of antimicrobial resistance in NG, the authors emphasize the need for interventions in order to reduce the inappropriate use of azithromycin.[1]

In our clinic, we made use of a natural experiment, where we changed from triple-site, 3-monthly to single-site 6-monthly screening to assess the impact of screening intensity on macrolide consumption.

From October 2015 until May 2018 PrEP was provided to 197 MSM and 3 transgender women via an open-label prospective cohort study that served as implementation trial for PrEP in Antwerp, Belgium (the Be-PrEP-ared study).[2] Participants underwent 3-site 3-monthly screening for *Chlamydia trachomatis* (CT) and NG for at least 18 months' follow-up. A retrospective analysis of macrolide prescriptions during this study revealed a macrolide consumption of 12.05 defined daily doses/1000 individuals/day (DID) which is 4–7 times higher than thresholds associated with inducing macrolide resistance in a range of bacterial species.[3,4]

By the end of the study, PrEP was re-imbursed in Belgium and participants of the Be-PrEP-ared study were invited to routine PrEP care after study completion, along with new PrEP patients. During routine care, screening for CT/NG was performed at a single site every 6 months, due to Belgian testing reimbursement regulations. The antimicrobial treatment of CT/NG followed the then contemporary IUSTI guidelines.[5–7] CT was typically treated with azithromycin or doxycycline and NG with ceftriaxone and azithromycin. Based on clinical records, WHO standard methodology was used to calculate screening intensity and macrolide consumption (in DID) in a period of 'routine PrEP', between October 2019 and December 2020, which was compared to the results from the Be-PrEP-ared period.

A total of 1305 patients attended the PrEP clinic during this routine PrEP period, 1297 were male and 8 were female. We performed 2060 CT/NG nucleic acid amplification tests (NAATs), representing 2.16 tests/person/year, whereas during the Be-PrEP-ared study 12 tests were performed per person per year (Table 1).

Macrolide consumption was 3.27 DID during the routine PrEP period. To assess the impact of COVID-19 restrictions we repeated the same calculation for two different periods: 10/2019-03/2020 and 04/2020-12/2020. Macrolide consumption declined slightly from 3.61 DID in the first period to 3.17 DID in the second period.

In the routine PrEP period, macrolide consumption was thus almost four-fold lower than during Be-PrEP-ared. The main driver of macrolide prescriptions in PrEP cohorts with 3-site, 3-monthly screening is the treatment of asymptomatic CT/NG infections.[3,8] Most guidelines still recommend 3-site 3-monthly screening among PrEP users although the evidence supporting this is scarce. In particular, more intense screening for CT/NG has not been shown to reduce the prevalence of these infections compared to less intense screening.[9,10] We conclude that less intensive screening of CT/NG in PrEP cohorts offers a way to reduce macrolide consumption. Alternative antimicrobial regimens, including those limiting the use of azithromycin, could also be considered.[7] The dramatic increases in macrolide resistance in *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and other bacteria in Belgium and elsewhere suggest the urgent need to

Table 1. Screening intensity for *Chlamydia trachomatis* (CT)/*Neisseria gonorrhoeae* (NG) and macrolide consumption during the 'routine PrEP' and Be-PrEP-ared periods.

	Be-PrEP-ared period	Routine PrEP period
Screening intensity CT/NG	12 tests/patient/year	2.16 tests/patient/year
Macrolide consumption	12.05 DID	3.27 DID

CT: Chlamydia trachomatis; NG: Neisseria gonorrhoeae; DID: doses/1000 individuals/day.

Title – Screening for STIs is one of the main drivers of macrolide consumption in PrEP users

Authors – Vanbaelen Thibaut[1], Van Dijck Christophe[1], De Baetselier Irith[1], Florence Eric[1], Reyniers Thijs[2], Vuylsteke Bea[2], Jacobs Bart K.M.[1], Kenyon Chris[1]

Affiliations – [1]Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; [2]Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium

Dear Editors,

A recent publication by Forster et al. explored the demographic and behavioral factors associated with antimicrobial susceptibility to ceftriaxone and azithromycin of *Neisseria gonorrhoeae* (NG). This article showed an alarming increase in NG geometric mean azithromycin minimum inhibitory concentration (MIC) between 2014/2015 and 2017/2018. Moreover, they found a higher geometric mean azithromycin MIC in men who have sex with men (MSM) compared with other groups. Given the emergence of antimicrobial resistance in NG, the authors emphasize the need for interventions in order to reduce the inappropriate use of azithromycin (1).

In our clinic, we made use of a natural experiment, where we changed from triple-site, 3-monthly to single-site 6-monthly screening to assess the impact of screening intensity on macrolide consumption.

From October 2015 until May 2018 PrEP was provided to 197 MSM and 3 transgender women via an open-label prospective cohort study that served as implementation trial for PrEP in Antwerp, Belgium (the Be-PrEP-ared study).(2) Participants underwent 3-site 3-monthly screening for *Chlamydia trachomatis* (CT) and NG for at least 18 months' follow-up. A retrospective analysis of macrolide prescriptions during this study revealed a macrolide consumption of 12.05 defined daily doses/1000 individuals/day (DID) which is 4 to 7 times higher than thresholds associated with inducing macrolide resistance in a range of bacterial species.(3,4)

By the end of the study, PrEP was re-imbursed in Belgium and participants of the Be-PrEP-ared study were invited to routine PrEP care after study completion, along with new PrEP patients. During routine care, screening for CT/NG was performed at a single site every 6 months, due to Belgian testing reimbursement regulations. The antimicrobial treatment of CT/NG followed the then contemporary IUSTI guidelines.(5–7) CT was typically treated with azithromycin or doxycycline and NG with ceftriaxone and azithromycin. Based on clinical

records, WHO standard methodology was used to calculate screening intensity and macrolide consumption (in DID) in a period of 'routine PrEP', between October 2019 and December 2020, which was compared to the results from the Be-PrEP-ared period.

A total of 1305 patients attended the PrEP clinic during this routine PrEP period, 1297 were male and 8 were female. We performed 2060 CT/NG nucleic acid amplification tests (NAAT), representing 2.16 tests/person/year, whereas during the Be-PrEP-ared study 12 tests were performed per person per year (Table 1).

Macrolide consumption was 3.27 DID during the routine PrEP period. To assess the impact of COVID-19 restrictions we repeated the same calculation for two different periods: 10/2019-03/2020 and 04/2020-12/2020. Macrolide consumption declined slightly from 3.61 DID in the first period to 3.17 DID in the second period.

In the routine PrEP period, macrolide consumption was thus almost four-fold lower than during Be-PrEP-ared. The main driver of macrolide prescriptions in PrEP cohorts with 3-site, 3-monthly screening is the treatment of asymptomatic CT/NG infections.(3,8) Most guidelines still recommend 3-site 3-monthly screening among PrEP users although the evidence supporting this is scarce. In particular, more intense screening for CT/NG has not been shown to reduce the prevalence of these infections compared to less intense screening.(9,10) We conclude that less intensive screening of CT/NG in PrEP cohorts offers a way to reduce macrolide consumption. Alternative antimicrobial regimens, including those limiting the use of azithromycin, could also be considered.(7) The dramatic increases in macrolide resistance in *N. gonorrhoeae, M. genitalium* and other bacteria in Belgium and elsewhere suggest the urgent need to incorporate this type of stewardship into plans of how intensively to screen for CT/NG in PrEP and other populations.

Table 1 – Screening intensity for *Chlamydia trachomatis* (CT)/*Neisseria gonorrhoeae* (NG) and macrolide consumption during the 'routine PrEP' and Be-PrEP-ared periods.

	Be-PrEP-ared period	**Routine PrEP period**
Screening intensity CT/NG	12 tests/patient/year	2.16 tests/patient/year
Macrolide consumption	12.05 DID	3.27 DID

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Summary

Background

Guidelines recommend three-site (urine, anal, pharynx) three-monthly (3X3 screening) screening for *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) in men who have sex with men (MSM) and transgender women (TGW) taking HIV pre-exposure prophylaxis (PrEP). We present the first randomized controlled trial to compare the effect of screening versus non-screening for NG/CT on the incidence of these infections in MSM and TGW taking PrEP.

Methods

A multicenter, randomized, controlled trial of 3X3 screening for NG/CT versus non-screening was conducted among MSM and TGW taking PrEP in five HIV reference centers in Belgium. Participants attended the PrEP clinics quarterly for 12 months. NG/CT was tested at each visit in both arms, but results were not provided to the non-screening arm, if asymptomatic. The primary outcome was the incidence rate (IR) of NG/CT infections in each arm, assessed in the per-protocol population. Non-inferiority of the non-screening arm was proven if the upper limit of the 95% confidence interval of the IR ratio (IRR) was lower than 1·25. This trial is completed and the trial protocol was registered at clinicaltrials.gov (NCT04269434).

Findings

Between September 21, 2020 and June 4, 2021, 508 subjects were randomized to the 3X3 screening arm and 506 to the non-screening arm. The overall IR of NG/CT was 0·155 cases/100 person-days (95%CI 0·128-0·186) in the 3x3 screening arm and 0·205 (95%CI 0·171-0·246) in the non-screening arm. The IR was significantly higher in the non-screening arm (IRR 1·318, 95%CI 1·068-1·627). Participants in the non-screening arm had a higher

incidence of CT infections and symptomatic CT infections. There were no significant differences in NG infections. Participants in the non-screening arm consumed significantly less antimicrobials. No serious adverse events were reported.

Interpretation

We failed to show that non-screening for NG/CT is non-inferior to 3-site 3-monthly screening in MSM and TGW taking PrEP in Belgium. However, screening was associated with higher antibiotic consumption and had no effect on the incidence of NG. Further research is needed to assess the benefits and harms of NG/CT screening in this population.

Research in context

Evidence before this study

We searched PubMed until April 06, 2023 for reports of randomized, controlled, clinical trials reporting the effect of screening for *Neisseria gonorrhoeae* or *Chlamydia trachomatis* on the prevalence or incidence of these infections. We used the search terms “chlamydia” OR “gonorrh*” AND “screening” OR “testing” AND “trial”. We found no reports of such trials for *Neisseria gonorrhoeae*. We found two randomized controlled trials assessing the effect of screening for *Chlamydia trachomatis* in the general population. A randomized, step-wedge, controlled trial explored the effect of yearly screening for *Chlamydia trachomatis* among more than 300.000 men and women aged 16-29 in the Netherlands and did not show a reduction in positivity rates (odds ratio 0·96, 95%CI 0·83-1·10, p-value=0·52) nor estimated population prevalence (3% in the control group vs 2·6% in the intervention group). An Australian cluster randomized controlled trial assessed the effect of yearly screening for *Chlamydia trachomatis* in about 4000 men and women aged 16-29 and did not show a significant reduction in the prevalence of this infection (adjusted relative difference 0·9 (95% CI 0·5 to 1·6; p=0·67).

Added value of this study

We describe the results of the first randomized controlled trial to compare screening for *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) versus non-screening among men who have sex with men (MSM) and transgender women (TGW) taking HIV pre-exposure prophylaxis. In the primary analysis, we found that non-screening was associated with an overall higher incidence of NG/CT infections (IRR 1·318, 95%CI 1·068-1·627), but this difference was driven by non LGV-CT infections alone (IRR 1·435, 95%CI 1·098-1·875) as no difference in NG infections was found (IRR 1·212, 95%CI 0·940–1·564). Given that asymptomatic participants in the non-screening arm were not aware of a positive NG/CT result and thus not treated, two consecutive NG/CT diagnosis in this arm might represent

the same, untreated infection. Therefore, we performed a sensitivity analysis, controlling for this 'untreated-infections-bias' in the non-screening arm. In this sensitivity analysis, we found no difference in terms of NG and/or CT incidence between both arms. Screening and subsequent treatment for NG/CT was associated with a 21 to 45% increase in antimicrobial consumption.

Implications of all the available evidence

Our study found that 3-site, 3-monthly NG/CT screening in MSM and TGW taking HIV-PrEP could lead to a reduction in the incidence of CT infections but not NG infections and comes at the cost of higher antimicrobial consumption. Therefore, more studies are needed to assess the benefits and harms of NG/CT screening in this population.

Introduction

International guidelines stipulate that screening programs should only be introduced once they have met a set of criteria: the benefits should outweigh the harms, screening should be cost-effective and there should be scientific evidence of screening program effectiveness (1). No RCT has ever been conducted to evaluate the efficacy of screening for *Neisseria gonorrhoeae* (NG) or *Chlamydia trachomatis* (CT) in men who have sex with men (MSM) and transgender women (TGW) (2). Two large cluster RCTs have been conducted to evaluate the effect of screening for CT in general populations (3,4). Both found no significant impact of screening on the prevalence of CT. No RCTs have been conducted to evaluate the efficacy of screening for NG (5).

Ecological analyses have found that countries where MSM are more intensively screened for NG/CT do not have a lower incidence and prevalence of asymptomatic or symptomatic NG/CT cases (6). One study that used self-reported data from two surveys in 2010 and 2017 of over 100,000 MSM from 46 European countries found that the intensity of NG/CT screening increased over time, but the intensity of screening was positively associated with the number of symptomatic NG/CT cases (6). The authors concluded that intensive screening may abrogate the development of an immune response to these infections which paradoxically increases the risk of subsequent re-infection. In the case of CT, there is experimental data from animal models, an observational clinical study and some epidemiological evidence to support this 'arrested immunity' hypothesis (7). A number of authors have argued for more frequent NG/CT screening in MSM (8). They have largely based this call on modelling studies, some of whom have found that two- to three-monthly screening reduces incidence, and the finding that more frequent screening detects more infections which, if treated, will reduce the population prevalence (8). Partly as a response to these arguments and evidence of increasing incidence of these infections in many countries, numerous guidelines have increased the recommended intensity of screening for NG/CT to 3-monthly, 3-site (anorectum, urethra and pharynx) testing in MSM taking HIV pre-exposure prophylaxis (PrEP) (9)

We have shown that screening MSM for NG/CT results in high levels of macrolide, cephalosporin and tetracycline consumption (10). For instance, three-site, three-monthly screening results in up to 12 defined daily doses of macrolides per 1000 inhabitants per year (DID) (11). This high antimicrobial consumption exceeds the approximate thresholds for the induction of antimicrobial resistance (AMR) in *Streptococcus pneumoniae, Mycoplasma genitalium* and *Treponema pallidum* by 5- to 9-fold (12). Screening MSM for NG/CT may therefore select for AMR in these and other bacteria such as *Helicobacter pylori* and NG. In a previous study, for example, we found a positive ecological association between the intensity of screening MSM for NG/CT and reduced gonococcal susceptibilities to cephalosporins (13). However, this study was prone to the ecological-inference fallacy. Increased antimicrobial consumption is of particular concern in PrEP users as gonococcal AMR has frequently emerged in such core-groups heavily exposed to antimicrobials (14). For instance, the proportion of NG isolates with azithromycin resistance in Belgium has increased from 2 to 33% in less than a decade, and this increase is more pronounced among MSM (15). A similar but more dramatic increase in macrolide- and multidrug-resistance has occurred in *Mycoplasma genitalium* in Belgium, meaning that we are regularly confronted with individuals with untreatable infections (16). Interestingly, we showed that changing NG/CT screening intensity in a PrEP cohort from three-monthly, three-site to one-site, six-monthly reduced the consumption of macrolides from 12·05 to 3·27 DID without any noticeable adverse clinical consequences (11). Such insights are important given that there is evidence that a decline in macrolide consumption can lead to a decline in the prevalence of antimicrobial resistance in bacteria such as group A streptococci (17).

Given the unclear benefits and the potential harms of screening MSM taking PrEP for NG and CT, authors have underlined the urgent need for RCTs on this topic (5). In this paper we present the results from the first RCT to compare the effect of screening on the incidence of NG/CT infections in MSM and TGW on PrEP. We also assessed the effect of screening on the incidence of symptomatic NG/CT infections, syphilis infections and antibiotic consumption as well as the PrEP users' perceptions towards STI screening.

Methods

Study design

We performed a multicenter, randomized, controlled clinical trial of three-site three-monthly screening for NG/CT versus non-screening among MSM and TGW taking HIV-PrEP in Belgium. The study took place in five HIV reference centers in Belgium (Institute of Tropical Medicine (ITM) in Antwerp, Saint-Pierre University Hospital and Erasme University Hospital in Brussels, Ghent University Hospital in Ghent and Liège University Hospital in Liège). A qualitative sub-study was embedded within the trial at ITM to explore PrEP users' perceptions towards STI screening. This study was approved by the Institutional Review Board of ITM (1360/20) and by the Ethics Committees of the University Hospital of Antwerp (20/27/377), Saint-Pierre University Hospital (20-07-05), Ghent University Hospital (BC-08167), Erasme University Hospital (P2020/321) and Liège University Hospital (2020-240). Written consent was obtained from all participants in Dutch, French, or English. The study protocol is available in the Appendix p.6.

Participants

All men followed-up for PrEP in these five centers were approached for study inclusion. Inclusion criteria were 1) being able and willing to provide informed consent, 2) being born as male, 3) being 18 years old or more, 4) having had oral sex and/or anal sex with another man in the last 12 months, 5) being enrolled in a Belgian PrEP center and 6) being willing to comply with the study procedures. Exclusion criteria were 1) being enrolled in another interventional trial, 2) testing positive for HIV at screening and 3) having symptoms of proctitis or urethritis. Participants provided written informed consent.

Randomization and masking

Subjects who met all inclusion criteria were randomized 1:1 into the non-screening (intervention) or 3x3 screening (control) arms. The randomization list was prepared by an independent statistician using SAS 9.4 (SAS Institute, Cary NC). To ensure (approximate) treatment balance within study sites, the randomization list was blocked by site using variable block sizes (block size four or six). The overview of the randomization list was not shared with the investigators until trial database lock. Study participants, doctors and nurses were not blinded. The study statistician was blinded until approval of the statistical analysis plan.

Procedures

As in routine PrEP care, participants were asked to attend 3-monthly visits at the PrEP clinic. The study duration was 12 months, hence five study visits were planned. One baseline visit took place at day 0 and four subsequent visits at months 3, 6, 9 and 12, each within a window of one week earlier and 6 weeks later.

At the baseline visit, after eligibility assessment, informed consent procedure and randomization, socio-demographic characteristics, sexual behavior, STI history in the past 12 months and antibiotic use in the past 6 months were collected. A first-void urine sample, pharyngeal swab and anorectal swab were collected. The pharyngeal swab was collected by the physician, whereas both other samples were self-collected. Samples per participant were pooled and tested for NG and CT by nucleic acid amplification techniques (NAAT). Those who tested positive were recalled for treatment according to current guidelines.(18) This generally entailed ceftriaxone 500mg or 1g intra-muscularly with or without azithromycin 2g orally for NG and doxycycline 200mg/day orally for seven days for CT and 21 days for LGV. Syphilis and HIV testing was performed on a blood sample.

At the month 3, 6 and 9 visits, symptoms compatible with an STI, STIs diagnosed, antibiotic use and sexual behavior since the last visit were recorded. A first-void urine sample, pharyngeal swab and anorectal swab were collected from all participants. For

asymptomatic participants in the 3x3 screening arm, these samples were analyzed and, if positive, participants were recalled for treatment according to current guidelines. In the non-screening arm, results were only provided when symptoms were present. Asymptomatic participants in the non-screening arms were thus not informed of the result of these samples, nor was the physician who performed the study visit. All participants who reported symptoms either during a study visit, or between study visits were tested and treated as per current guidelines.

At the month 12 visit, data were collected as for the previous visits. A first-void urine sample, pharyngeal swab and anorectal swab were collected and analyzed for NG/CT for all participants. If positive, participants from both arms were treated as per current guidelines. HIV and syphilis testing was performed on blood samples every 3 months.

Study participants were able to attend the PrEP/STI clinic at any point in between the scheduled visits for any health problems. Participants were encouraged to attend the clinic for any symptoms compatible with an STI. Participants who received a partner notification for an STI were tested and treated according to the current guidelines. Test-of-cure visits were performed according to local protocols.

For the qualitative sub-study, social scientists trained in qualitative research, conducted three focus group discussions (FGD), among randomly selected ITM study participants. Each FGD consisted of three to five participants. To maximize variation in perceptions, two in-depth interviews (IDIs) with PrEP users of the clinic who declined participation to the main study were performed. The interviewers obtained a verbal informed consent from each participant prior to the start of the FGDs and IDIs. Audio-recording took place upon agreement. FGDs and IDIs were conducted in Dutch and online via a secured platform, respecting General Data Protection Regulation.

NG and CT testing was performed at each site's laboratory. The three samples were pooled per patient and visit according to a validated pooling strategy. Positive samples for CT were

sent to the National Reference Center for STIs (ITM) for genotyping to detect LGV serovars. HIV and syphilis testing was performed according to local protocols.

Outcomes

The primary outcome was the overall incidence of NG/CT infections in each arm. Each participant could contribute one diagnosis of CT and one diagnosis of NG per scheduled or unscheduled visit. Only laboratory-confirmed diagnoses made between scheduled visits, performed inside or outside of the study clinic were included.

Secondary outcomes were ceftriaxone, azithromycin and doxycycline exposure in the two study arms (expressed in daily defined doses (DDD) per 1000 persons years according to WHO methodology), incidence rate of symptomatic NG and CT and incidence rates of syphilis and HIV.

All NG/CT diagnoses were included in the primary outcome. Hence, it was implicitly assumed that every diagnosis was a new infection. Recent studies have shown that the median durations of untreated pharyngeal and ano-rectal NG infections are 16 and 9 weeks respectively, and the duration of untreated CT infections 6 and 13 weeks, respectively (19,20). Therefore, it is possible that an NG/CT infection detected at the 3 to 12 month visit in the non-screening arm was simply a non-resolved infection that was already present at the prior visit. This could spuriously increase the measured incidence in the non-screening arm as the same infection would be counted twice. Therefore, a sensitivity analysis was performed to deal with this 'untreated-infection bias'. In this analysis, consecutive diagnoses of the same type (e.g. CT at two consecutive visits) in the non-screening arm were counted as one infection unless the prior diagnosis was a symptomatic one (and therefore treated), or if the participants reported having used antibiotics efficacious against the relevant STI between both diagnoses.

In addition, a pre-specified sub-group analysis was performed by stratifying the participants according to STI risk behavior. We hypothesized that the effects of screening for NG/CT could be different in individuals with a lower number of sexual partners given the lower sexual network connectivity in these individuals. For that purpose, participants that consistently reported 4 or less partners in all 5 study visits were categorized as lower-risk and all other participants were categorized as higher risk. Finally, a separate, non-pre-specified analysis was added using gonorrhoea and chlamydia separately as outcomes.

All FGDs and IDIs were transcribed verbatim and pseudonymized. Data were collected and analyzed iteratively using a thematic analysis approach and Nvivo. We inductively developed an initial coding scheme. Subsequently, we re-read all transcripts with the focus on describing the variation in perceptions towards testing for asymptomatic and symptomatic NG/CT infections and how the emergence of antibiotic resistance influences these perceptions.

The largest safety concern for this study was that the participants in the non-screening arm could experience a higher incidence of symptomatic NG/CT. Rather than reporting each symptomatic episode of NG/CT as an adverse event, an independent data and safety monitoring board (DSMB) evaluated if the non-screening arm had an unacceptably high incidence of symptomatic NG/CT. For this purpose, the DSMB included two independent STI experts (Infectious Disease Physicians/Epidemiologists) and the study statistician to evaluate the incidence of symptomatic NG and CT in both arms at two interim time points: once 50% and 100% of all study participants had completed their month 6 visit. It was decided that serious consideration would be given to stopping the study if the incidence of symptomatic NG and CT infections in the non-screening arm was double that of the screening arm.

Statistical analysis

For the primary outcome, estimates were based on a negative-binomial regression model with number of diagnoses as dependent variable, study arm and study site as independent variable and log(visit number) as offset. This model also provided an estimate of the log incidence rate ratio (IRR, no screening versus screening), together with 95% confidence interval. The predicted values and standard errors estimated from the regression models were used to calculate the 95% CI for the IR. The standard formula for Wald confidence intervals was then used in the log scale and exponentiated. Non-inferiority of the 'no screening' arm was concluded if the upper limit of the 95% confidence interval was lower than 1·25. The same methodology was applied for the secondary outcomes except for antimicrobial consumption for which a rate ratio was calculated, with number of DDDs as dependent variable. The number needed to screen was calculated by dividing 1 by the absolute risk reduction between both arms.

The primary analysis was performed following the per-protocol (PP) approach. Participants who had fewer than 3 visits with NG/CT results or did not follow the randomized intervention were excluded from the PP analysis. Participants were excluded from the intention to treat (ITT) analysis if they did not attend any of the follow-up visits.

Participants in each intervention arm were described with respect to baseline characteristics. The description was done in terms of median (interquartile range) and mean (standard deviation) for continuous characteristics and using counts and percentages for categorical characteristics.

Based on a previous study, we estimated an average number of diagnosis per subject of 0·72 over four visits (21). The 'no screening' arm was considered to be non-inferior if there is an increase of maximal 25% in number of diagnoses (i.e., increase of an average of 0·72 to 0·90 per 4 visits). Assuming that 95% of the participants would have data on all four follow-up visits, and 5% would have data on only three visits, the required sample size to obtain 80% power at a significance level of 5% was 912. Assuming an additional 10% drop out rate, the final sample size was estimated to be 1014 participants.

We estimated the duration of NG and CT infections in the non-screening arm by calculating the time difference in days between the estimated infection date and the estimated clearance date. The infection date was defined as the mid-point between the diagnosis date and the date of the previous negative test The clearance date was either the date where a treatment was provided, or the midpoint between the last positive test result and the first subsequent negative test.

All statistical analyses were performed using R (version 4.2).

The trial protocol was registered at clinicaltrials.gov (NCT04269434).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

A total of 2409 individuals were approached for the study between the 21st of September 2020 and the 4th of June 2021, among whom 1014 were randomized (508 in the 3X3 screening arm and 506 in the non-screening arm, Figure 1). A total of 38 participants did not attend any follow-up visit and were excluded from the analysis. We excluded 275 participants from the per protocol analysis, 206 had out of window visits, 133 had fewer than three visits with NG/CT results and eight participants in the non-screening arm did not follow the randomized intervention. The study ended on the 26th of August 2022. The baseline characteristics as well as number of sex partners were well-balanced between the two arms (Table 1).The number of sex partners and unprotected sex partners remained stable across all study visits in both arms (Appendix p.1)

A total of 196 NG cases and 224 CT cases were diagnosed in the non-screening arm after the baseline visit, and 164 NG cases and 157 CT cases were found in the 3X3 screening arm

(Table 2). In the primary analysis, the incidence of NG/CT was 0·205 cases/100 person-days (95%CI 0·171-0·246) in the non-screening arm and 0·155 (95%CI 0·128-0·186) in the 3X3 screening arm (Table 3). The incidence rate (IR) of NG/CT was higher in the non-screening arm compared with the 3X3 screening arm (IR ratio (IRR) 1·318, 95%CI 1·068-1·627; Table 3; Figure 2) and the upper-limit of the 95% confidence interval included the non-inferiority cut-off of 1·25, indicating we cannot conclude non-inferiority of non-screening compared with 3X3 screening. The incidence rate ratio of symptomatic NG/CT was 1·373 (95%CI 0·963-1·956; Table 3). Participants in the non-screening arm consumed less azithromycin, ceftriaxone and doxycycline (Table 4) compared with the 3X3 screening arm. The incidence of syphilis was not significantly higher in the non-screening arm compared with the 3X3 screening arm (Table 3)

In the PP sensitivity analysis accounting for the untreated-infection bias, there was no difference between arms in terms of the incidence rate of NG/CT (IRR 1·093, 95%CI 0·895-1·334; Figure 2, Table 3), but the 95%CI of the incidence rate ratio included the non-inferiority cut-off of 1·25.

Results were similar between the PP and ITT analysis, except for the incidence of syphilis that was higher in the non-screening arm compared to the 3X3 screening arm in the ITT analysis (Appendix p.2).

Differences in NG/CT incidence were driven by differences in CT incidence. We could not establish a difference in NG incidence in the PP analysis (Table 3; Figure 2) or in symptomatic NG incidence. The incidence of CT and symptomatic CT was higher in the non-screening arm. However, there was no difference in CT incidence in the sensitivity analysis. Based on these results, the estimated number needed to screen for symptomatic and asymptomatic CT infections was 25·55 and 10·92, respectively (Appendix p.3).

A total of 231 participants reported less than five sex partners at all study visits and where thus considered as lower-risk participants and the remaining 783 participants were

considered as higher-risk participants. Higher-risk participants had a higher incidence of NG/CT in the non-screening arm compared with the 3X3 screening arm, in the primary analysis (Table 3) but this difference disappeared in the sensitivity analysis, when accounting for the untreated-infection bias. Similar results were obtained for the incidence rates of CT cases and symptomatic CT cases. However, no difference was found in terms of the incidence of NG cases or symptomatic NG cases in these participants. The IRRs in lower-risk participants were not different.

The median (IQR) estimated duration of NG infections in the non-screening arm was 72·5 days (52·5-98·0), and of CT infections 90·5 days (53·0-132·4).

Symptomatic participants typically presented with mild symptoms and no participant reported severe outcomes or adverse events (Appendix p.4). The number of unscheduled visits and visits for partner notification can be found in Appendix p.5.

Participants of the qualitative sub-study reported mixed reactions towards non-screening for asymptomatic NG/CT. The fact that these STIs are mostly asymptomatic and self-limiting, without causing serious complications or harm to the individual, were mentioned as arguments against screening.

"Why would you try to detect something if you have no symptoms? And that is actually not very dangerous either? Even if you pass it on." (FGD 3, ID 32)

The main reported disadvantage of non-screening was the possibility of ongoing transmission to sexual partners. For some participants, not testing and treating was accompanied with feelings of guilt, risk, and irresponsibility. Some participants suggested adjusting the testing strategy according to the number of sexual contacts a person has, and whether or not condoms are used.

“Assuming that a condom is almost never used because there is PrEP. And that there are about five to six or so changing contacts per month. With that in mind, I feel safer being fully tested all the time. If I had a steady partner, and if someone were to come once a month, I would think: okay, let me get tested once every six months.” (FGD 2, ID 26)

The qualitative data showed that perceptions towards AMR varied. Some participants were concerned about the emergence of AMR and/or stated they preferred to avoid using antibiotics when possible. Others reported a lack of knowledge on the subject.

“I compare it to a scale and I find it difficult to see where that carries the most weight: is the weight in the sense of antibiotic resistance, or is the weight in the sense of I'm walking with an asymptomatic gonorrhoea infection that I could spread to many others. I, personally, find that a difficult balancing act.” (FGD 2, ID 26)

Lastly, not all participants were familiar with the natural course of NG/CT infections and the mechanisms of AMR. As knowledge increased during the sessions, participants’ attitudes sometimes shifted towards non-screening for asymptomatic NG/CT.

Discussion

This RCT did not establish that non-screening for NG/CT in MSM and TGW on PrEP is non-inferior to 3-site 3-monthly screening with respect to NG/CT incidence. The overall incidence of NG/CT was significantly higher in the non-screening arm compared to the screening arm in the primary analysis. However, in the sensitivity analysis, controlling for the untreated-infections bias, we could not show a statistically significant difference in the incidence of NG/CT between both arms. Differences in NG/CT incidence were driven by a higher incidence of CT in the non-screening arm, as the incidence of NG did not differ. The incidence of symptomatic CT was also higher in the non-screening arm. Participants in the screening arm consumed considerably more antimicrobials compared with the non-screening arm. Among higher-risk participants, the incidence of NG/CT, CT and

symptomatic CT were higher as well. These results provide the first RCT-based evidence of the benefits and harms of screening for NG/CT in MSM on PrEP.

Our finding that screening was associated with a lower incidence of CT but not NG is commensurate with the presumed longer duration of infection for CT and possible higher proportion of CT infections that are asymptomatic in MSM (20,22). For instance, a systematic review found that chlamydia had a longer duration of infection than gonorrhoea in both the oropharynx and anorectum in MSM (20). Hence, periodic screening for NG/CT might detect more CT infections as NG infections might have cleared spontaneously between screening timepoints. While the findings of our study do not provide strong support to continue screening for NG in MSM in PrEP cohorts, they do provide some evidence to support screening for CT (22). Nonetheless, it is possible that screening may exert its effect at an individual- and/or population-level. For this reason, it is critical to evaluate the benefits and harms of screening for NG/CT at both levels.

Besides the population-level effect, other elements should be taken into account when assessing the impact of screening for NG/CT. An increase in the incidence of NG/CT infections PrEP users resulting from a non-screening strategy might result in an increased transmission and subsequent morbidity in other populations. For instance, there is evidence of bridging transmission of NG between MSM and women (23). The additional NG infections in women could result in increased adverse events such as infertility. Moreover, a modelling study has suggested that screening for NG might allow for early detection and treatment of already resistant strains, and therefore limit their spread (24). Lastly, other aspects such as the impact of screening on the costs for both patients and health insurance are also important.

We have previously established that intense screening for NG/CT is a key driver of high antibiotic consumption in PrEP users (10). In a similar vein, reducing the intensity of screening for NG/CT in PrEP users has been shown to result in a large reduction in macrolide consumption (11). However, screening and subsequent treatment for CT may be less likely

to induce AMR than screening for NG. This is because treatment guidelines recommend the less-resistogenic doxycycline for CT therapy compared to NG therapy where ceftriaxone with or without azithromycin (both WHO 'reserve' antimicrobials) are advised (25). We calculated that 10·92 men would need to be screened at three sites every three months for a year to prevent one asymptomatic CT infection and 25·55 to prevent one symptomatic CT infection. This would require 2·34 courses of doxycycline therapy for each symptomatic CT infection prevented.

In our study, higher-risk participants had a higher incidence of asymptomatic NG/CT infections. Previous studies have similarly found that the majority of STIs in PrEP cohorts were diagnosed in a small subgroup with a high rate of partner turnover (26). In such individuals, the high number of partners results in a dense sexual network which generates a high equilibrium prevalence for STIs such as NG and CT (27). Intensive screening for these STIs in this group may reduce this prevalence but would place evolutionary pressures on these STIs to acquire mutations that would enable them to regain their equilibrium prevalence. This could be via evading the diagnostic tests used (as has occurred with CT (28)), or via the emergence of AMR as has transpired on multiple occasions with NG (14). Therefore, although the effect of screening for CT was greatest in those with higher STI risk behavior, screening in this group may confer the greatest risk for the emergence of AMR. Modeling studies have suggested that intensive screening may reduce the prevalence of NG/CT to such an extent that the consumption of antibiotics may be reduced in this group (29). These modeling studies are, however, at odds with the results of observational studies which have found that the screening MSM for NG/CT was not associated with reduced prevalence regardless of how intensive the screening (30).

We found an increased incidence of syphilis infections in the non-screening arm compared to the 3X3 screening arm in the ITT analysis. This finding could be explained by the higher consumption of doxycycline and ceftriaxone, two antimicrobials effective against *Treponema pallidum*, in the screening arm. Given that the incubation period of primary syphilis is typically 10-90 days and the fact that syphilis infections are frequently

asymptomatic in this population, treating NG/CT with either of these antimicrobials could have reduced the incidence of syphilis. This reduction in syphilis incidence should be taken into account when assessing the benefits and harms of screening for NG/CT in PrEP users.

Our study had several limitations. The untreated-infections-bias meant that our primary analysis overestimated the incidence of NG/CT infections in the non-screening arm. Controlling for this bias in our sensitivity analysis may, however, have underestimated NG/CT incidence in the non-screening arm. Due to the pooling of samples used for NG/CT testing, the anatomical site of infection was unknown which might have impacted our results. Moreover, the assays used for NG/CT testing do not allow to discriminate viable infections from non-viable infections. The use of such assays could lead to a better estimation of the incidence of infections and should be included in future trials. Furthermore, given the number of sex partners reported by participants, there might have been contamination between study arms. Another limitation is that the participants and physicians were not blinded. This might have resulted in altered behavior. This RCT took place in different periods of COVID-19 restrictions. It has been shown that PrEP users decreased their number of partners in the periods of COVID-19 restrictions (31). We cannot exclude that our results were impacted by changing behaviors and might thus not be representative of periods with no restrictions. Additionally to the measurement bias in our outcome, we cannot dismiss the presence of selection bias in the per-protocol estimates and in the intention to treat estimates due to the large number of excluded participants due to out-of-window visits and due to missing outcome data. Finally, the qualitative sub-study was conducted among 12 PrEP users at one study site, it is possible that this small sample size did not allow us to reach saturation in the PrEP users' perceptions regarding NG/CT screening, and we cannot exclude that there are variations in these perceptions between study sites.

The introduction of doxycycline post exposure prophylaxis (PEP) could have a profound influence on STI screening (32). By reducing the incidence of CT and NG, doxycycline PEP could reduce the benefit and need for 3X3 screening for these infections. Conversely the

combination of intensive screening and doxycycline PEP could have a large impact on the transmission of these infections (32). It is also possible that the high levels of antimicrobial consumption resulting from these interventions would do more harm than good in terms of AMR and microbiome damage (33).

The main reason to screen for NG/CT in MSM and TGW is to reduce the incidence of symptomatic infections and secondarily to reduce the incidence/prevalence of infections in the population. In our RCT, screening reduced the incidence of CT but not NG. The effect on CT incidence disappeared once we controlled for the untreated-infections bias. We found that screening resulted in a lower incidence of symptomatic CT infections but not symptomatic NG infections. Screening was however associated with a 21 to 45% increase in consumption of antimicrobials. In conclusion, our study shows that 3-site, 3-monthly NG/CT screening in MSM and TGW taking HIV-PrEP could lead to a reduction in the incidence of CT infections but not NG infections and comes at the cost of higher antimicrobial consumption. Therefore, more studies, including studies with doxycycline PEP arms, are needed to assess the benefits and harms of NG/CT screening in this population.

Figure 1 – Trial profile

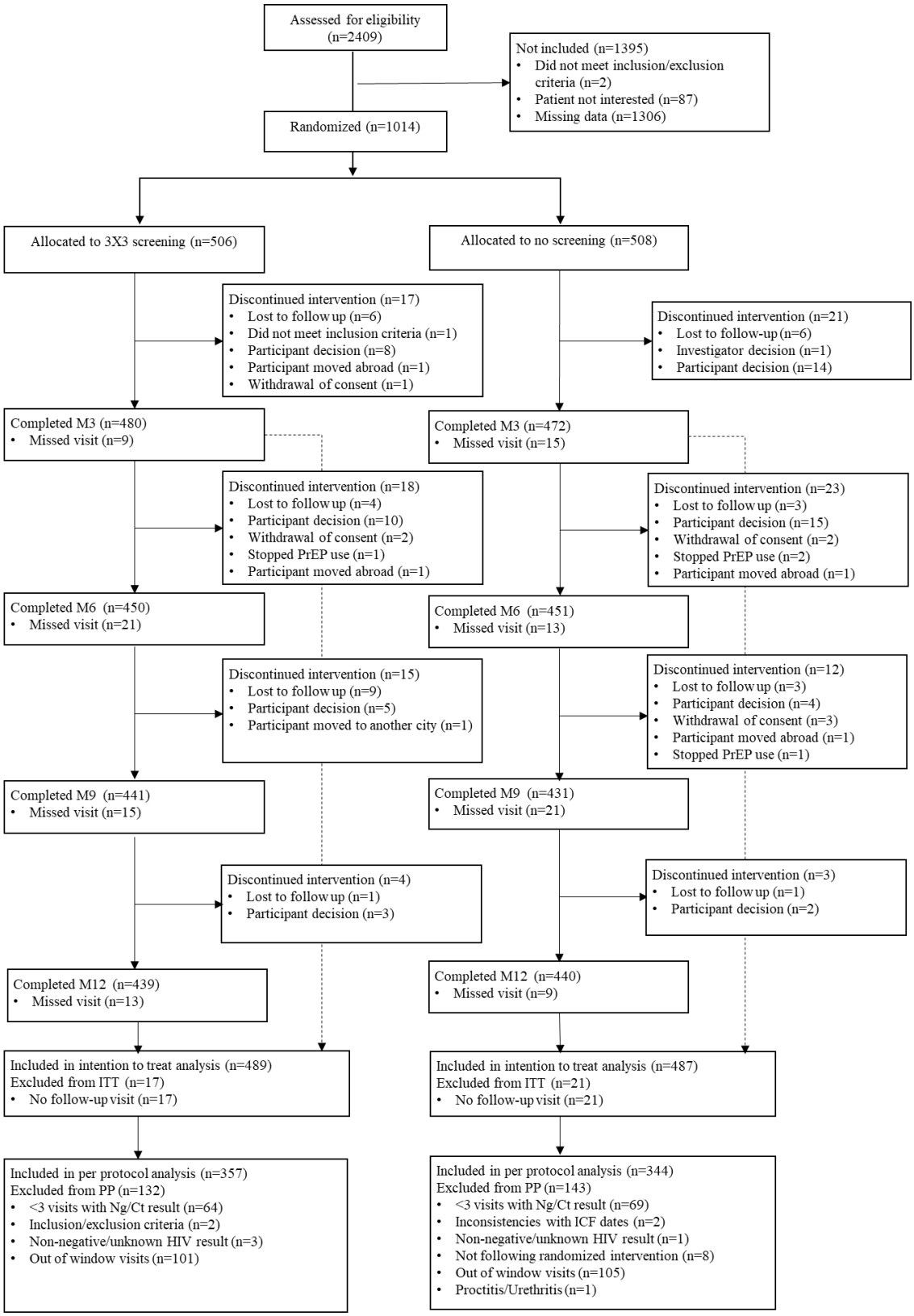

Figure 2 - Forest plot of the incidence rate ratios (IRR) of *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) infections in the primary and sensitivity analyses. The vertical dotted line represents the non-inferiority margin of 1·25

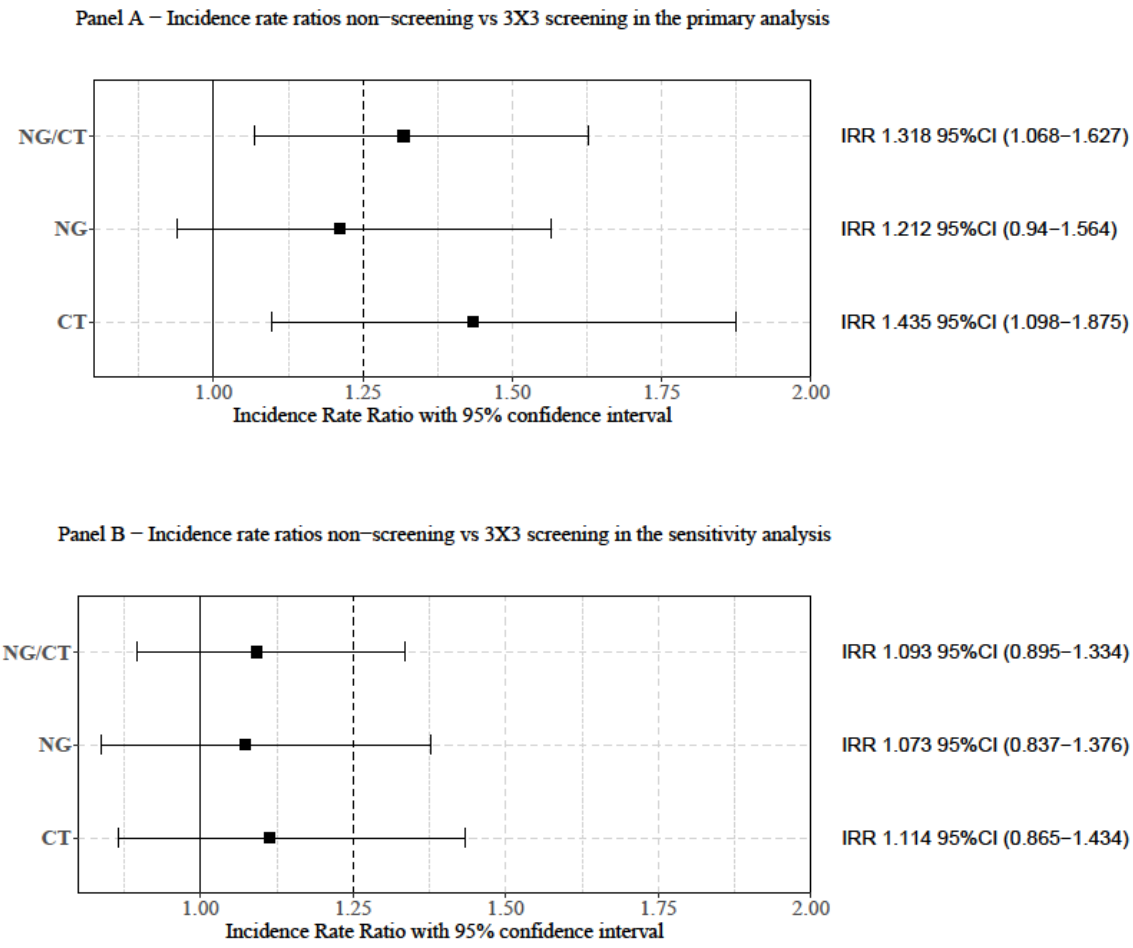

Table 1 – Baseline characteristics in both study arms

	3 x 3 Screening (N=506) n (%)/Median (IQR)	**Non-screening (N=508) n (%)/Median (IQR)**	**Total population (N=1014) n (%)/Median (IQR)**
Age (years)	39 (33 - 47)	39 (32·5 - 48)	39 (33 - 47)
Gender			
Man	506 (100%)	505 (99·4%)	1011 (99·7%)
Transgender woman	0 (0%)	3 (0·6%)	3 (0·3%)
Number of sex partners (past 3 months)	4 (2 - 8)	4 (2 - 8)	4 (2 - 8)
Number of unprotected sex partners (past 3 months)	2 (1 - 5)	2 (1 - 5)	2 (1 - 5)
Any antibiotic use (past 6 months)	192 (37·9%)	173 (34·1%)	365 (36·0%)
Cephalosporins	67 (13·2%)	77 (15·2%)	144 (14·2%)
Macrolides	81 (16·0%)	94 (18·5%)	175 (17·3%)
Penicillin	63 (12·5%)	47 (9·3%)	110 (10·8%)
Quinolones	11 (2·2%)	5 (1·0%)	16 (1·6%)
Tetracyclines	57 (11·3%)	54 (10·6%)	111 (10·9%)
List of abbreviations: IQR, interquartile range			

Table 2 - Number of NG and CT cases diagnosed during the study (baseline visit excluded)

	Neisseria gonorrhoeae n (%)	**Chlamydia trachomatis (non-LGV) n (%)**	**Chlamydia trachomatis (LGV) n (%)**
Total number of cases	360	381	24
Non-screening arm	196 (54.4)	224 (58.8)	10 (41.6)
3X3 screening arm	164 (45.5)	157 (41.2)	14 (58.3)
Symptomatic cases (n (%))	104 (28.8)	66 (18.4)	10 (41.7)
Non-screening arm†	56 (53.8)	43 (65.2)	3 (0.3)
3X3 screening arm†	48 (46.2)	23 (34.8)	7 (0.7)
† % among symptomatic infections N=104 for NG, N=66 for non-LGV CT, and N=10 for LGV CT List of abbreviations: CT, *Chlamydia trachomatis;* LGV, *Lymphogranuloma venereum*			

Table 3 - Incidence rate and incidence rate ratio of NG/CT and symptomatic NG/CT (per protocol analysis)

	Total population				**Stratified analysis ≥5 partners**				**Stratified analysis <5 partners**			
	Primary analysis		**Sensitivity analysis**		**Primary analysis**		**Sensitivity analysis**		**Primary analysis**		**Sensitivity analysis**	
	Estimate (95% CI)	p-value	**Estimate (95% CI)**	**p-value**	**Estimate (95% CI)**	**p-value**	**Estimate (95% CI)**	**p-value**	**Estimate (95% CI)**	**p-value**	**Estimate (95% CI)**	**p-value**
NG/CT cases												
*IR non screening**	0·205 (0·171 - 0·246)	··	0·169 (0·141 - 0·200)	··	0·236 (0·196 - 0·284)	··	0·194 (0·162 - 0·233)	··	0·0009 (0·0004 - 0·002)	··	0·0007 (0·0003 - 0·0016)	··
*IR 3 x 3 screening**	0·155 (0·128 - 0·186)	··	0·154 (0·128 - 0·184)	··	0·182 (0·150 - 0·220)	··	0·181 (0·151 - 0·217)	··	0·0006 (0·00003 - 0·0015)	··	0·0006 (0·0003 - 0·0014)	··
IRR												
3 x 3 screening	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··
Non screening	1·318 (1·068 - 1·627)	0·0102	1·093 (0·895 - 1·334)	0·385	1·290 (1·040 – 1·599)	0·021	1·071 (0·874 - 1·312)	0·511	1·430 (0·694 – 2·944)	0·332	1·178 (0·594 - 2·334)	0·640
NG/CT symptomatic												
*IR non screening**	0·046 (0·032 - 0·066)	··	··	··	0·055 (0·038 - 0·079)	··	··	··	0·000 (0· 000 - 0· 000) †	··	··	··
*IR 3 x 3 screening**	0·034 (0·023 - 0·049)	··	··	··	0·040 (0·027 - 0·059)	··	··	··	0·000 (0· 000 - 0· 000) †	··	··	··
IRR												
3 x 3 screening	1 (Ref)	··	··	··	1 (Ref)	··		··	1 (Ref)	··	··	··
Non screening	1·373 (0·963 - 1·956)	0·0801	··	··	1·352 (0·940 – 1·945)	0·104	··	··	1·473 (0·353 – 6·155)	0·595	··	··

NG cases												
*IR non screening**	0·099 (0·078 - 0·125)	··	0·089 (0·055 - 0·112)	··	0·116 (0·091 - 0·147)	··	0·103 (0·081 - 0·130)	··	0·000 (0·000 - 0·000) †	··	0·000 (0·000 - 0·000) †	··
*IR 3 x 3 screening**	0·081 (0·064 - 0·103)	··	0·082 (0·065 - 0·104)	··	0·095 (0·074 - 0·122)	··	0·096 (0·076 - 0·122)	··	0·000 (0·000 - 0·000) †	··	0·000 (0·000 - 0·000) †	··
IRR												
3 x 3 screening	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··
Non screening	1·212 (0·940 - 1·564)	0·138	1·073 (0·837 - 1·376)	0·579	1·213 (0·826 – 1·367)	0·637	1·062 (0·685 - 1·256)	0·626	1·041 (0·389 – 2·787)	0·936	1·041 (0·389 – 2·787)	0· 936
NG symptomatic												
*IR non screening**	0·024 (0·015 - 0·040)	··	··	··	0·029 (0·018 - 0·048)	··	··	··	0·000 (0·000 - 0·000) †	··	··	··
*IR 3 x 3 screening**	0·021 (0·013 - 0·035)	··	··	··	0·025 (0·015 - 0·042)	··	··	··	0·000 (0·000 - 0·000) †	··	··	··
IRR												
3 x 3 screening	1 (Ref)	··	··	··	1 (Ref)	··	··	··	1 (Ref)	··	··	··
Non screening	1·162 (0·757 - 1·783)	0·492	··	··	1·155 (0·742 - 1·801)	0·522	··	··	1·117 (0·225 - 5·533)	0·893	··	··
CT cases												
*IR non screening**	0·104 (0·083 - 0·130)	··	0·079 (0·063 - 0·099)	··	0·117 (0·093 - 0·148)	··	0·090 (0·071- 0·114)	··	0·0006 (0·0002 - 0·002)	··	0·0004 (0·0002 - 0·001)	··
*IR 3 x 3 screening**	0·072 (0·056 - 0·092)	··	0·071 (0·056 - 0·089)	··	0·085 (0·066 - 0·109)	··	0·083 (0·0465- 0·106)	··	0·0003 (0·0001 - 0·001)	··	0·0003 (0·0001 - 0·001)	··

IRR												
3 x 3 screening	1 (Ref)	..	1 (Ref)	..	1 (Ref)	..	1 (Ref)	..	1 (Ref)	..	1 (Ref)	..
Non screening	1·435 (1·098 - 1·875)	0·008	1·114 (0·865 - 1·434)	0·404	1·375 (1·041 - 1·815)	0·025	1·077 (0·826 - 1·403)	0·586	1·902 (0·783 - 4·620)	0·156	1·351 (0·584 - 3·128)	0·482
CT symptomatic												
*IR non screening**	0·021 (0·012 - 0·034)	..	..	..	0·024 (0·014 - 0·041)	..	..	..	0·000 (0·000 - 0·000) †	..	..	..
*IR 3 x 3 screening**	0·011 (0·006 - 0·020)	..	..	..	0·014 (0·008 - 0·025)	..	..	..	0·000 (0·000 - 0·000) †	..	..	..
IRR												
3 x 3 screening	1 (Ref)	..	..	..	1 (Ref)	..	..	..	1 (Ref)	..	..	..
Non screening	1·798 (1·038 - 3·117)	0·037	..	..	1·743 (0·990 - 3·067)	0·054	..	..	2·301 (0·209 - 25·400)	0·496	..	..

List of abbreviations: CT: *Chlamydia trachomatis*; 95% CI: 95% confidence interval; IR: incidence rate; IRR: incidence rate ratio; NG: *Neisseria gonorrhoeae*

* Incidence Rate in cases/100 person-days

† The incidences in these instances were in the magnitude of 10e-7, thus both the point estimate and the confidence intervals appear as 0 in the table

Table 4 - Rate and ratio of antibiotic consumption (per protocol analysis)

	Total population		**Stratified analysis ≥ 5 partners**		**Stratified analysis <5 partners**	
	Primary analysis				**Primary analysis**	
	Mean Estimate (95% CI)	**p-value**	**Mean Estimate (95% CI)**	**p-value**	**Mean Estimate (95% CI)**	**p-value**
Antibiotic consumption						
Azithromycin						
*IR non screening**	0·0046 (0·0043 - 0·0050)	··	0·512 (0·367 - 0·713)	··	0·139 (0·051 - 0·381)	··
*IR 3 x 3 screening**	0·0059 (0·0075 - 0·0063)	··	0·691 (0·505 - 0·945)	··	0·257 (0·096 - 0·689)	··
RR						
3 x 3 screening	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··
Non screening	0·788 (0·719 - 0·863)	<0·0001	0·741 (0·493 - 1·112)	0·148	0·543 (0·124 - 2·208)	0·393
Ceftriaxone						
*IR non screening**	0·0004 (0·0004 - 0·0006)	··	0·053 (0·041 - 0·068)	··	0·015 (0·006 - 0·038)	··
*IR 3 x 3 screening**	0·0008 (0·0007 - 0·0009)	··	0·099 (0·081 - 0·121)	··	0·017 (0·007 - 0·038)	··
RR						
3 x 3 screening	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··
Non screening	0·561 (0·426 - 0·739)	<0·0001	0·540 (0·398 – 0·733)	<0·0001	0·913 (0·312 – 2·677)	0·869
Doxycycline						
*IR non screening**	0·0044 (0·0041 - 0·0048)	··	0·595 (0·374 - 0·948)	··	0·141 (0·031 - 0·644)	··
*IR 3 x 3 screening**	0·0081 (0·0075 - 0·0086)	··	1·028 (0·636 – 1·661)	··	0·381 (0·075 – 1·924)	··
RR						

3 x 3 screening	1 (Ref)	..	1 (Ref)	..	1 (Ref)	..
Non screening	0·55 (0·515 - 0·0·588)	<0·0001	0·579 (0·319 - 1·052)	0·073	0·369 (0·034 - 3·991)	0·412
List of abbreviations: 95%CI: 95% confidence interval; RR: rate ratio * rate in DDD/100 person-days						

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Supplementary material

Appendix 1 - number of sex partners and unprotected sex partners in all study periods

	3 x 3 Screening Median (IQR)	**No-screening Median (IQR)**	**Pooled Median (IQR)**
Baseline			
Number of sex partners (3M)	4 (2 - 8)	4 (2 - 8)	4 (2 - 8)
Number of unprotected sex partners (3M)	2 (1 - 5)	2 (1 - 5)	2 (1 - 5)
Month 3			
Number of sex partners (3M)	4 (2 - 8)	4 (2 - 8)	4 (2 - 8)
Number of unprotected sex partners (3M)	2 (1 - 5)	2 (1 - 5)	2 (1 - 5)
Month 6			
Number of sex partners (3M)	5 (2 - 10)	5 (3 - 10)	5 (3 - 10)
Number of unprotected sex partners (3M)	3 (1 - 5)	3 (1 - 5)	3 (1 - 5)
Month 9			
Number of sex partners (3M)	5 (3 - 10)	5 (3 - 10)	5 (3 - 10)
Number of unprotected sex partners (3M)	3 (1 – 7)	3 (1 – 7)	3 (1 – 7)
Month 12			
Number of sex partners (3M)	5 (3 - 10)	5 (3 - 10)	5 (3 - 10)
Number of unprotected sex partners (3M)	3 (1 – 7)	3 (1 – 7)	3 (1 – 7)
List of abbreviations: 3M, past 3 months; IQR, interquartile range			

Appendix 2 – Incidence of NG/CT and symptomatic NG/CT (intention to treat analysis)

	Total population				**Stratified analysis =>5 partners**				**Stratified analysis <5 partners**			
	Primary analysis		**Sensitivity analysis**		**Primary analysis**		**Sensitivity analysis**		**Primary analysis**		**Sensitivity analysis**	
	Mean Estimate (95% CI)	**p-value**	**Mean Estimate (95% CI)**	**p-value**	**Mean Estimate (95% CI)**	**p-value**	**Mean Estimate (95% CI)**	**p-value**	**Mean Estimate (95% CI)**	**p-value**	**Mean Estimate (95% CI)**	**p-value**
NG/CT symptomatic												
*IR No screening**	0.209 (0.181 - 0.242)	··	0.169 (0.147 - 0.196)	··	0.238 (0.205 - 0.277)	··	0.193 (0.165 - 0.224)	··	0.100 (0.063 - 0.159)	··	0.082 (0.053 - 0.128)	··
*IR Screening**	0.157 (0.135 - 0.184)	··	0.156 (0.135 - 0.181)	··	0.184 (0.157 - 0.217)	··	0.181 (0.155 - 0.212)	··	0.069 (0.042 - 0.113)	··	0.071 (0.045 - 0.111)	··
IRR												
3 x 3 Screening	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··	1 (Ref)	··
Non-Screening	1.321 (1.101 - 1.585)	0.0027	1.082 (0.912 - 1.285)	0.367	1.289 (1.069 – 1.553)	0.008	1.058 (0.886 - 1.261)	0.530	1.421 (0.772 – 2.616)	0.259	1.154 (0.650 - 2.049)	0.626
NG/CT symptomatic												
*IR No screening**	0.047 (0.035 - 0.063)	··	··	··	0.053 (0.039 - 0.072)	··	··	··	0.021 (0.010 - 0.047)	··	··	··
*IR Screening**	0.035 (0.026 - 0.048)	··	··	··	0.041 (0.029 - 0.057)	··	··	··	0.015 (0.006 - 0.036)	··	··	··
IRR												
3 x 3 Screening	1 (Ref)	··	··	··	1 (Ref)	··	··	··	1 (Ref)	··	··	··
Non-Screening	1.329 (0.970 - 1.820)	0.0768	··	··	1.302 (0.937 – 1.809)	0.116	··	··	1.408 (0.524 – 3.787)	0.498	··	··

List of abbreviations: CT: Chlamydia Trachomatis; 95% CI: 95% confidence interval; IR: incidence rate; IRR: incidence rate ratio; NG: Neisseria Gonorrhoeae;
Values in bold are significant
* Incidence Rate in cases/100 person-years

Appendix 3 – detailed calculation of the number needed to screen

	Risk non-screening arm*	**Risk screening arm**	**Absolute risk difference (95%CI)**	**Number needed to screen (95%CI)†**
Chlamydia trachomatis cases	0.44	0.31	0.13 (0.07-0.19).	7.69 (5.27-13.97)
Chlamydia trachomatis symptomatic cases	0.10	0.06	0.04 (0.01-0.07)	25.55 (13.85-165.18)
Chlamydia trachomatis asymptomatic cases	0.15	0.25	0.1 (0.04-0.15)	10.92 (6.78-28.10)
* number of events/number of participants † 1/absolute risk difference List of abbreviations: 95%CI, 95% confidence interval				

Appendix 4 – Total number of NG and CT cases and proportion of symptomatic infections during the study in the primary analysis (baseline visit excluded)

	Neisseria gonorrhoeae **n (%)**	***Chlamydia trachomatis*** **(non LGV) n (%)**	***Chlamydia trachomatis*** **(LGV) n (%)**
Total number of cases	360	381	24
Non-screening arm	196 (54.4)	224 (58.8)	10 (41.6)
3X3 screening arm	164 (45.5)	157 (41.2)	14 (58.3)
Symptomatic infections (n (%))	104 (28.8)	66 (18.4)	10 (41.7)
Non-screening arm†	56 (53.8)	43 (65.2)	3 (0.3)
3X3 screening arm†	48 (46.2)	23 (34.8)	7 (0.7)
*Proctitis**	9	7	4
*Urethritis**	13	6	0

* possible underreporting of the type of symptoms present

† % among symptomatic infections N=104 for NG, N=66 for non-LGV CT, and N=10 for LGV CT

List of abbreviations: LGV, *Lymphogranuloma venereum*

Appendix 5 – Number of unscheduled visits and visits for partner notification in each study arm

	3X3 screening arm	**Non screening arm**
Number of unscheduled visits	45	80
Number of visits for partner notification	11	24

5.2.2 **Switching from dual- to monotherapy for the treatment of NG**

Vanbaelen T, Florence E, Van Dijck C, et al. Effect on the Resistome of Dual vs Monotherapy for the Treatment of Neisseria gonorrhoeae: Results From a Randomized Controlled Trial (ResistAZM Trial). *Open Forum Infect Dis* 2023; 10(10).

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MAJOR ARTICLE

Effect on the Resistome of Dual vs Monotherapy for the Treatment of *Neisseria gonorrhoeae*: Results From a Randomized Controlled Trial (ResistAZM Trial)

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Background. No randomized controlled trial (RCT) has compared the impact on the resistome of ceftriaxone (CRO) plus azithromycin (AZM) vs CRO for the treatment of *Neisseria gonorrhoea* (NG).

Methods. This was an open-label, single-center, RCT comparing the effect on the resistome of CRO plus AZM vs CRO for the treatment of NG. Men who have sex with men (MSM) with genital, anorectal, or pharyngeal NG infection were randomized into the CRO/AZM and CRO arms. Oral rinse and anorectal samples were taken for culture and resistome profiling at 2 visits (baseline and day 14). The primary outcome was the ratio of mean macrolide resistance determinants in anorectal samples from day 14 between arms.

Results. Twenty individuals were randomized into the CRO/AZM arm and 22 into the CRO arm. We found no significant difference in the mean macrolide resistance determinants in the day 14 anorectal samples between arms (ratio, 1.05; 95% CI, 0.55–1.83; $P = .102$). The prevalence of baseline macrolide resistance was high (CRO/AZM arm = 95.00%; CRO arm = 90.91%).

Conclusions. We could not demonstrate a significant effect of dual CRO/AZM therapy on the resistome compared with CRO alone, likely due to a high baseline resistance to AZM. Interventions to prevent the emergence of antimicrobial resistance in MSM are needed.

Keywords. antimicrobial resistance; macrolide; men who have sex with men; *Neisseria gonorrhoeae*; resistome.

Neisseria gonorrhoeae (NG) has developed resistance to all antimicrobials used against it, and there are concerns that it might become untreatable in the near future [1]. One important mechanism in the development of antimicrobial resistance (AMR) in NG is the uptake of genetic material through transformation [1, 2]. Several studies have shown that NG acquired cephalosporin, sulfonamide, and macrolide resistance genes from commensal *Neisseria* species (spp.) [1, 3]. Commensal *Neisseria* spp. are much more prevalent than pathogenic *Neisseria* spp. [4]. As a consequence, they face a greater selection pressure than pathogenic *Neisseria* spp. to develop AMR if exposed to high levels of antimicrobials in a population [4]. AMR determinants from commensal *Neisseria* spp. can subsequently be transferred to NG under antimicrobial pressure [1]. Worryingly, AMR in commensal *Neisseria* spp. has been increasing in multiple countries, an effect that has been most pronounced in the populations most exposed to antimicrobials, such as men who have sex with men (MSM) taking HIV preexposure prophylaxis (PrEP) [5, 6].

There are currently 2 main options for the treatment of NG: monotherapy with ceftriaxone (CRO) or dual therapy with CRO plus azithromycin (CRO/AZM) [7–9]. Dual therapy emerged in the early 2010s and has been endorsed by the United States Centers for Disease Control and Prevention (CDC) and the European International Union against Sexually Transmitted Infections (EIUSTI) [7, 9]. The rationale behind dual therapy was based on the opinion of certain experts that it would delay the emergence of AMR in NG [10]. Importantly, to our knowledge, no randomized controlled trial (RCT) has compared the efficacy of mono with dual therapy. However, 2 recent meta-analyses did not find a significant difference in the eradication of pharyngeal or anorectal NG between the 2 options [11, 12]. In recent years, several guidelines, including those from the CDC and EIUSTI, have changed their recommendations to endorse monotherapy as the preferred or alternative treatment [7–9]. The main reason

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Title – Effect on the resistome of dual- vs monotherapy for the treatment of *Neisseria gonorrhoeae*: results from a randomized controlled trial (ResistAZM Trial)

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Key points

- We couldn't demonstrate an increase in pheno- or genotypic macrolide resistance following dual-therapy with ceftriaxone plus azithromycin compared to mono-therapy with ceftriaxone.
- This lack of increase might be due to the high prevalence of macrolide resistance at baseline.

Keywords – *Neisseria gonorrhoeae*; resistome; antimicrobial resistance; macrolide; men who have sex with men

Abstract

Background

No randomized controlled trial (RCT) has compared the impact on the resistome of ceftriaxone (CRO) plus azithromycin (AZM) versus CRO for the treatment of *Neisseria gonorrhoea* (NG).

Methods

Open-label, single center, RCT comparing the effect on the resistome of CRO plus AZM versus CRO for the treatment of NG. Men who have sex with men (MSM) with genital, anorectal or pharyngeal NG infection were randomized into the CRO/AZM and CRO arms. Oral rinse and anorectal samples were taken for culture and resistome profiling at two visits (baseline and day 14). The primary outcome was the ratio of mean macrolide resistance determinants in anorectal samples from day 14 between arms.

Results

Twenty individuals were randomized in the CRO/AZM arm and 22 in the CRO arm. We found no significant difference in the mean macrolide resistance determinants in the day 14 anorectal samples between arms (ratio=1.05, 95%CI 0.55-1.83, p-value=0.102). The prevalence of baseline macrolide resistance was high (CRO/AZM arm=95.00%, CRO arm=90.91%).

Conclusion

We could not demonstrate a significant effect of dual CRO/AZM therapy on the resistome compared to CRO alone, likely due to a high baseline resistance to AZM. Interventions to prevent the emergence of antimicrobial resistance in MSM are needed.

Introduction

Neisseria gonorrhoeae (NG) has developed resistance to all antimicrobials used against it and there are concerns that it might become untreatable in the near future (1). One important mechanism in the development of antimicrobial resistance (AMR) in NG is the uptake of genetic material through transformation (1,2). Several studies have shown that NG acquired cephalosporin, sulfonamide and macrolide resistance genes from commensal *Neisseria* species (spp.) (1,3). Commensal *Neisseria* spp. are much more prevalent than pathogenic *Neisseria* spp. (4) As a consequence, they face a greater selection pressure than pathogenic *Neisseria* spp. to develop AMR if exposed to high levels of antimicrobials in a population(4). AMR determinants from commensal *Neisseria* spp. can subsequently be transferred to NG under antimicrobial pressure (1). Worryingly, AMR in commensal *Neisseria* spp. has been increasing in multiple countries, an effect that has been most pronounced in populations most exposed to antimicrobials, such as men who have sex with men (MSM) taking HIV preexposure prophylaxis (PrEP)(5,6).

There are currently two main options for the treatment of NG: monotherapy with ceftriaxone (CRO) or dual therapy with CRO plus azithromycin (CRO/AZM) (7–9). Dual therapy emerged in the early 2010s and had been endorsed by the United States Center for Diseases Control and Prevention (CDC) and the European International Union against Sexually Transmitted Infections (EIUSTI)(7,9). The rationale behind dual therapy was based on the opinion of certain experts that it would delay the emergence of AMR in NG (10). Importantly, to our knowledge, no randomized controlled trial (RCT) has compared the efficacy of mono- with dual therapy. However, two recent meta-analyses did not find a significant difference in the eradication of pharyngeal or anorectal NG between the two options (11,12). In the past years, several guidelines, including those from the CDC and EIUSTI have changed their recommendations to endorse monotherapy as the preferred or alternative treatment (7–9). The main reason for this switch is that the percentage of NG isolates with resistance to AZM has dramatically increased. In Belgium, for example, the proportion of clinical isolates with AZM resistance has increased from 0.2% to 33% between

2013 and 2022(13). Similar trends have been described in other countries (7,8). These trends have been driven primarily by the emergence and spread of gonococcal clones with mosaic sections of their MtrCDE efflux pumps acquired from commensal *Neisseria* spp. (3,14–18) Studies have found that these clones are prevalent in core groups such as HIV-PrEP cohorts(17).

This has generated the hypothesis that dual therapy was partially responsible for the recent increase in gonococcal resistance to macrolides. The high levels of macrolides used in populations such as PrEP cohorts would have directly selected for macrolide resistance in commensal *Neisseria* spp. via bystander selection (19). Because azithromycin has a long tissue half-life, its long post-therapy tail could also have provided a selective advantage for gonococcal strains to acquire macrolide resistance from commensal *Neisseria* spp. in populations with intense AZM exposure (20). Whilst the available evidence suggests there is equipoise in the efficacy of both dual and monotherapy for the treatment of NG (11), no study that we are aware of has evaluated the effect on the resistomes. In this paper, we present the results of an RCT which assessed the impact on the resistome of both therapeutic regimens. We hypothesized that the receipt of CRO/AZM results in a greater increase in macrolide resistance genes in the anorectal resistome and in macrolide resistance in oropharyngeal commensal streptococci and *Neisseria* spp. than CRO.

Methods

Study design, setting and participants

We performed an open label, single center, RCT to compare the effect on the resistome of CRO plus AZM dual therapy (CRO/AZM) versus CRO monotherapy for the treatment of NG. The study took place at the HIV/STI clinic of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. Individuals with a diagnosis of symptomatic or asymptomatic genital, anorectal or pharyngeal NG detected in routine care were approached for the study. Inclusion criteria were being able and willing to provide written informed consent, being

assigned male sex at birth, being at least 18 years old, and having a confirmed diagnosis of urethral, anorectal or pharyngeal NG by molecular detection or, for patients with urethritis, a Gram/methylene blue stain of a urethral smear showing intra-cellular diplococci and >10 white blood cells/field. Exclusion criteria were the use of any macrolide antibiotics in the previous 6 months, a known contra-indication or allergy to ceftriaxone, azithromycin or lidocaine, and the presence of any other condition, including the suspicion or diagnosis of sexually transmitted infections (STIs), that required the administration of an antibiotic other than CRO at enrollment.

Randomization

Subjects who met all the inclusion- and exclusion criteria were randomized with a 1:1 ratio into the CRO/AZM and CRO study arms. The randomization list was prepared by an independent sponsor biostatistician using SAS 9.4 (SAS Institute, Cary NC) and was not shared with the study team until the database was locked.

Study procedures

Two study visits were planned - a baseline visit and a follow-up visit at day 14 (+/- 1 day).

During the baseline visit we collected a sample from the site where NG was detected for NG culture. In addition, we collected oral rinse samples (21), using 15 ml sterile phosphate buffered saline (PBS, Oxoid^{TM}, Dulbecco A) for culturing oropharyngeal streptococci and *Neisseria* spp., and anorectal swabs (Eswab^{TM} medium, COPAN Diagnostics Inc., Brescia, Italy) for resistome profiling. Urine samples were collected by the patient. Oral rinse samples were self-collected under the supervision of the study physician after having received instructions. The oral rinse samples were stored at -80°C using skim milk with 30% glycerol. For the anorectal swabs, participants could opt for self-collection or collection by the study physician.

Data were collected on STI history, HIV status, HIV-PrEP use, number of sex partners (past 3 months), and antibiotic use (past 12 months). An oral examination was performed, and a physical examination if deemed necessary. Participants then received their allocated treatment. In the CRO/AZM group, participants received ceftriaxone 1g single dose intramuscularly (IM) and azithromycin 2g single dose orally under the supervision of the study physician. Participants in the CRO group received ceftriaxone 1g single dose IM alone.

At the day 14 visit, samples from the previously infected sites were taken for molecular detection and culture to assess the NG clearance, as part of routine care. In addition, we collected oral rinse samples for streptococci and *Neisseria* spp. culture, and anorectal swabs for resistome profiling, as described above. Data were collected on HIV status, HIV-PrEP use, and antibiotic use since the last visit.

Laboratory procedures

NG molecular testing was performed using the Abbott RealTime CT/NG assay. Positive NG samples were confirmed using in-house real-time polymerase chain reaction (PCR)(22). NG was cultured on GC selective agar (Becton Dickinson, Heidelberg, Germany) and if positive, antimicrobial susceptibility testing of ceftriaxone, ciprofloxacin and azithromycin was done using Etests (BioMérieux, France).

Culture of oral commensal *Neisseria* spp. and streptococci was performed with and without azithromycin (2ug/ml), according to Laumen et al (21) (Appendix p.1).

Shotgun metagenomic sequencing and bioinformatic analyses

The anorectal swabs were shipped on dry ice to Eurofins Genomics for DNA isolation, library preparation and metagenomic sequencing. The raw sequencing data have been deposited with links to BioProject accession number PRJNA974953 in the NCBI BioProject

database (https://www.ncbi.nlm.nih.gov/bioproject/). Bioinformatic analyses was carried out according to Van Dijck et al. (23) (Appendix p.1).

Outcomes

In this paper, we defined macrolide resistance as resistance to macrolides, lincosamides and streptogramins. The primary outcome was the ratio of mean macrolide resistance determinants in the day 14 visit anorectal samples between the two treatment arms. This ratio was calculated by dividing the mean normalized read count of macrolide resistance determinants categorized at the class level (macrolides, lincosamides and streptogramins) in the CRO/AZM group by the corresponding mean normalized read count in the CRO group. We also calculated the proportion of individuals carrying macrolide resistance genes. For that purpose, any measurement in the normalized macrolide resistance determinants above 0 was deemed as macrolide resistance.

The secondary outcomes also included the ratio of mean resistance determinants applied to each non-macrolide antibiotic class in the day 14 visit anorectal samples. Additionally, three indicators of multidrug resistance were created. The first indicator represented participants who carried resistance genes to more than one of the following non-macrolide antibiotics: aminoglycosides, betalactams, fluoroquinolones, and tetracyclines. A second indicator was created with the addition of trimethoprim and sulfonamides to the previous indicator. A third indicator represented participants who carried resistance genes to both macrolides and non-macrolides.

Based on culture results, the difference in the proportion of oropharyngeal commensal Neisseria and streptococci that are macrolide resistant between the two treatment arms at both visits was calculated by dividing the number of colonies on the plates containing azithromycin by the number of colonies on the plates without azithromycin. Lastly, an indicator representing the proportion of individuals presenting at least one resistant colony, for streptococci and commensal *Neisseria* spp. separately, at baseline was created.

The primary analysis was performed using the intention-to-treat (ITT) approach. In the ITT analysis, all randomized participants who gave at least a sample on day 14 were analyzed according to their randomized allocation, even if they received another intervention, showed protocol violations prior to or during the study or were lost to follow-up. In the per-protocol analysis, only participants who received the intervention and followed the protocol as planned were included.

Statistical analysis

Assuming a 2.5-fold increase in the ratio of macrolide resistance determinants in the CRO/AZM group compared to the CRO group, a sample size of 42 patients was estimated to detect this effect size at a significance level of 0.05 and with a power of 80%. The rationale behind this effect size estimation was based on a previous study, the MORDOR trial, that found a 7-fold increase in this ratio following repeated mass administration of AZM in Niger (24). Given the difference in the study populations between our study and the MORDOR trial, we used a much lower effect size. Our sample size calculation was corrected for a drop-out rate of 5%.

We described baseline characteristics using medians and interquartile ranges for continuous variables and absolute numbers and proportions for categorical variables.

The primary analysis of assessing the ratio of the mean normalized macrolide resistance determinants in the anorectal microbiome between the two arms was done using a permutation test with 10000 permutations. The normalized macrolide resistance read counts were calculated by dividing the number of macrolide resistance reads by the total number of bacterial reads in the sample. The resulting proportion was then multiplied by 10^6 to generate normalized resistance read counts per million reads. A 95% confidence interval (CI) for the ratio of the two arms was estimated using permutation. In cases where multiple comparisons were made, the p-values were adjusted using the Benjamini-Hochberg method for family-wise error. The secondary analysis regarding mean normalized

resistance read counts of non-macrolide antibiotic class in anorectal samples was done similarly.

Proportions are presented with Wilson's 95% CI and compared using Fisher's exact test. The latter was also used to compare the patient count with adverse events in the two arms. Means and medians were compared using Mann-Whitney U test and, for paired samples, Wilcoxon signed rank test. No subgroup- nor interim analysis were performed. All computations were made using R version 4.2.3 (25)

Ethical clearance and trial registration

All participants provided written informed consent at baseline. The Institutional Review Board of the ITM, the Ethics Committee of the University Hospital of Antwerp and the Competent Authorities of Belgium (FAMHP) approved the trial. The study was carried out in compliance with the Declaration of Helsinki and according to the most recent Good Clinical Practice guidelines, it was registered in the EudraCT public registry (EUDRACT 2021-003616-10).

Results

Between January 17 and May 9, 2022, a total of 64 individuals were approached for the study. Twenty-two were not included due to not meeting the inclusion and/or exclusion criteria or due to not being able to return for the day 14 visit (Figure 1). The remaining 42 individuals were randomized (22 to the CRO arm and 20 to the CRO/AZM arm). Two participants in the CRO arm were excluded from the ITT analysis as they did not attend the day 14 visit.

All participants were male. The median age at baseline was 40 years (IQR 29.3-44.0;Table 1). A total of nine (9/42, 21.43%) participants reported being HIV positive, and 27 (27/42, 64.29%) reported taking HIV-PrEP. Participants had a median of five sex partners (IQR 3-

8.25) in the past three months, and 18 (18/42, 42.86%) used antibiotics in the past 12 months. Socio-demographic and sexual risk-taking characteristics were well-balanced between both arms (Table 1).

In the primary analysis, the mean normalized macrolide resistance determinants count in ano-rectal samples at day 14 was 110.3 counts/million reads (95%CI 64.54-156.06) in the CRO arm and 167.53 counts/million reads (95%CI 97.86-237.19) in the CRO/AZM arms (Table 2). Their ratio was not statistically significant (ratio 1.05, 95%CI 0.55-1.83, p-value=0.102; Table 2, Figure 2). Likewise, there was no statistically significant difference in non-MLS determinants in anorectal samples on day 14 between the two arms (Table 2). The proportions of participants with macrolide resistance were 90.91% (95%CI 76.39-99.11) in the CRO arm and 95.00% (95%CI 76.39-99.11) in the CRO/AZM arm at day 0, and 100% (95%CI 83.89-100) in both arms at day 14. These differences were not statistically significant. The proportions of participants with multidrug-resistance at day 14 were not statistically significant between both arms (Appendix p.2)

Based on culture results, the mean proportion of streptococci/commensal *Neisseria* spp. that were macrolide resistant at day 0 was 66.66%/51.40% in the CRO arm and 68.61%/48.64% in the CRO/AZM arms, respectively (Appendix p.3-4). At day 0, 100% (95%CI 89..75-100) of individuals had at least one macrolide resistant streptococci colony and 92.50% (95%CI 74.4-95.20) at least one macrolide resistant commensal *Neisseria spp.* colony (Appendix p.5).

Similar results were obtained in the per-protocol analysis (Appendix p.6).

A total of six participants reported adverse events deemed as drug-related by the investigators (Appendix p.7). No serious adverse event was reported. No difference between both arms was found in terms of adverse events.

Discussion

Our study did not show a difference in the abundance of macrolide and non-macrolide resistance determinants in anorectal samples 14 days after administration of CRO or CRO plus AZM. The prevalence of macrolide resistance was high at baseline and remained high at day 14 in both arms. The prevalences of multidrug-resistance on day 14 were similar between both arms.

These findings contrast with the results of previous studies. An RCT compared phenotypic macrolide resistance in oro-pharyngeal streptococci after a course of azithromycin or clarithromycin versus placebo among more than 200 healthy volunteers in Belgium (26). This study showed a large increase in macrolide resistance from approximately 30% to 80% in both intervention arms, and no increase in the placebo arm. The increase in macrolide resistance persisted throughout the study, up to 180 days. A cluster RCT among children in Niger evaluated the effect on the resistome of 6-monthly mass azithromycin distribution vs placebo for a total study duration of four years (24). This study found that azithromycin had a pronounced effect on pheno- and genotypic resistance. The prevalence of resistance to erythromycin in oral streptococci increased to a mean 12.3% in the azithromycin arm compared to 2.9% in the placebo arm (27). Likewise, a substantial increase in the abundance of genes conferring macrolide and non-macrolide resistance in the gastro-intestinal tract was seen in participants receiving azithromycin. Participants in the intervention arm had 7.5 times more macrolide resistance determinants than the placebo arm at the end of the trial (27).

The discrepancy between our results and those of previous studies might be attributed to the different prevalence of macrolide resistance at baseline. Whilst considerable caution should be exercised in comparisons between studies using different methodologies, the proportion of individuals with phenotypic macrolide resistance in oral streptococci in our study at baseline (100% in both arms) was considerably higher than the 2.9% in the Niger study (24). The prevalence of macrolide resistance in commensal *Neisseria* spp. in our study

at baseline was also high, over 90% in both arms. In a similar vein, the mean proportion of streptococci/commensal *Neisseria* spp. that were macrolide resistant at day 0 (around 50 and 70%, respectively) was higher in our study than the 30% baseline macrolide resistance found in streptococci in the Belgian volunteer study (26).

The high prevalence of macrolide resistance in both commensal *Neisseria* and streptococcal species found in our study is alarming for a number of reasons. The prevalence of macrolide resistance has been increasing not only in NG but also in invasive streptococcal infections (28). As already noted, the rapid increase in macrolide resistance in NG has been driven by NG lineages that have acquired mosaic MtrCDE efflux pumps from various commensal *Neisseria* spp. (3,14–18) A number of authors have argued that antimicrobial resistance in commensal *Neisseria* serves as a critical early warning system of excess antimicrobial consumption and risk of AMR emerging in the pathogenic *Neisseria* species (4,20).

These findings also suggest that a saturation of macrolide resistance determinants in our study population before the intervention may explain the lack of an effect of dual therapy on macrolide resistance. The very high prevalence of resistance to macrolides at baseline we found might be explained by intensive antimicrobial consumption in our study population. About 40% of the participants reported use of antimicrobials in the 12 months prior to the baseline visit. The use of antimicrobials is correlated with antimicrobial resistance in several pathogens, such as NG and *Streptococcus pneumoniae* (29,30). We have previously shown that macrolide consumption in a Belgian PrEP cohort was 52-fold higher than the community level consumption of certain European countries. Moreover, this macrolide consumption exceeds thresholds known to be associated with high rates of AMR in *Mycoplasma genitalium, Treponema pallidum*, and *Streptococcus pneumoniae* by 5- to 9-fold (31,32). Likely as a result of this intense consumption, the prevalence of macrolide resistance in *Treponema pallidum* and *Mycoplasma genitalium* in MSM attending our STI clinics is over 90% and 33.6% in *N. gonorrhoeae* (13,33,34). Further evidence for the saturation hypothesis comes from a previous study which found very high

levels of macrolide, fluoroquinolone and cephalosporin resistance in oral commensal *Neisseria* spp. in MSM attending our STI clinic, but no difference between the groups who had, and had not, consumed antimicrobials in the preceding 6 months (6). The prevalence of resistance to these antimicrobials in both groups was, however, considerably higher than that in the general population (6). In addition, in this study, macrolide resistance associated genes in the oropharynx were over two times more abundant in the MSM than in the general population (23).

These findings suggest the need for interventions to reduce antimicrobial consumption in populations at risk for the further emergence of AMR. We have calculated that dual therapy for NG and *Chlamydia trachomatis* (CT) infections is the major driver of macrolide consumption in our PrEP cohort (35). Switching from dual- to mono- therapy for the treatment of NG might be a way to reduce macrolide consumption.

Our study has several limitations. First, we based our sample size calculation on a study performed on a different population. Although we tried to adapt to this difference by reducing the expected effect size, we cannot exclude that our study was underpowered to evaluate the effects in our population. Second, we assessed the impact of CRO/AZM vs CRO at only one timepoint, 14 days after the administration of the antimicrobials. Therefore, we cannot infer what the results would have been at other time points. Fourth, neither participants nor physicians were blinded, which might have led to altered behavior between the study visits.

Other types of evidence should be considered when choosing between mono- and dual-therapy for the treatment of NG. As noted above, two systematic reviews of observational studies found no difference in efficacy at curing NG between mono- and dual-therapy (11,12). In addition, a combined individual and ecological level analysis of determinants of gonococcal macrolide and cephalosporin minimal inhibitory concentrations (MICs) from over 20,000 isolates in 26 European countries found that dual therapy was associated with a higher azithromycin MIC than monotherapy and no difference was found in ceftriaxone

MICs (36). A key argument for the introduction of dual therapy is that the azithromycin would protect the ceftriaxone from the acquisition of resistance (10). The available evidence does not support this supposition. Moreover, a number of studies have noted that the excess consumption of antimicrobials such as macrolides may exert much of its effects at the population level (37), and other studies have found that a population-level reduction in macrolide consumption effectively leads to a reduction in macrolide resistance in streptococci(38). Together with the increasing prevalence of macrolide resistance, these findings have motivated the authors of certain guidelines to return to monotherapy as the preferred gonococcal treatment (7).

Conclusion

Our study did not find that dual-therapy resulted in an increase in pheno- or genotypic macrolide resistance compared with mono-therapy. This lack of increase might have been due to the high prevalence of macrolide resistance at baseline, which in turn was likely due to the high antimicrobial consumption in the study population. Previous studies have found that macrolide consumption leads to a substantial and prolonged increase in macrolide and non-macrolide resistance determinants and that reducing macrolide consumption can reduce the prevalence of macrolide resistance. Switching from dual- to monotherapy for NG is one way to achieve this. Despite the negative results of our study, we conclude that the evidence reviewed above in combination with the observed high baseline levels of macrolide resistance in our study supports the switch to monotherapy for NG.

Table 1 - Socio-demographic, sexual risk taking and characteristics of Neisseria gonorrhoeae (NG) infection at baseline*

	CRO (n=22) **n (%)/median (IQR)**	**CRO/AZM (n=20)** **n (%)/median (IQR)**	**Total sample (n=42)** **n (%)/median (IQR)**
Age in years	40 (28.5 - 41.75)	41.5 (29.75 - 45)	40 (29.25 - 44)
HIV status:			
Positive	5 (22.73)	4 (20)	9 (21.43)
Negative	17 (77.27)	16 (80)	33 (78.57)
Number of partners (last 3 months)	5 (3 - 6)	5 (3.75 - 10)	5 (3 - 8.25)
Use of antibiotics (last 12 months)	8 (36.36)	10 (50)	18 (42.86)
Amoxicillin/Clavulanic acid	0 (0)	2 (10)	2 (4.76)
Ceftriaxone	0 (0)	0 (0)	0 (0)
Doxycycline	0 (0)	0 (0)	0 (0)
Penicillin	0 (0)	0 (0)	0 (0)
PrEP use: yes	14 (63.64)	13 (65)	27 (64.29)
NG infection			
Symptomatic	7 (31.82)	6 (30)	13 (30.95)
Asymptomatic	15 (68.18)	14 (70)	29 (69.05)
NG infection site			
Anorectal	2 (9.09)	1 (5)	3 (7.14)
Urethral	4 (18.18)	5 (25)	9 (21.43)
Pooled (urethral, anorectal, pharyngeal)	16 (72.73)	14 (70)	30 (71.43)

*There was no statistical difference between the two arms in any of these variables

List of abbreviations: AZM, azithromycin; CRO, ceftriaxone; HIV, human immunodeficiency virus; IQR, interquartile range; NG, *Neisseria gonorrhoeae;* PrEP, pre-exposure prophylaxis.

Table 2 - Primary analysis results, comparison of mean read counts of normalized macrolide and non-macrolide resistance determinants in ano-rectal samples at day 14 (intention to treat analysis)

	CRO+AZM (95%CI)	**CRO (95%CI)**	**Ratio (CRO+AZM/CRO) (95% CI)**	**p-value**
Determinants				
MLS	167.53 (97.86 - 237.19)	110.3 (64.54 - 156.06)	1.05 (0.55 - 1.83)	0.1026
Aminoglycosides	22.22 (14.98 - 29.46)	34.41 (11.36 - 57.45)	1.12 (0.47 - 2.19)	1
Betalactams	89.82 (68.36 - 111.28)	110.46 (80.17 - 140.76)	1.01 (0.69 - 1.44)	1
Bacitracin	1.11 (0 - 2.96)	4.63 (0 - 11.21)	3.79 (0.05 - 20.15)	1
Glycopeptides	0.14 (0 - 0.4)	0.26 (0 - 0.77)	0.83 (0 - 1.91)	1
Trimethoprim	1.55 (0.4 - 2.69)	2.73 (0.7 - 4.77)	1.19 (0.32 - 3.15)	1
Cationic antimicrobial peptides	5.52 (0 - 14.65)	16.52 (0 - 38.45)	2.82 (0.07 - 14.64)	1
Mupirocin	0 (0 - 0)	0.84 (0 - 1.86)	1.49 (0 - 5.34)	1
Metronidazole	0.06 (0 - 0.19)	0.2 (0 - 0.61)	1.2 (0 - 3.22)	1
Fluoroquinolones	23.9 (0 - 68.13)	9.44 (0 - 21.97)	6.44 (0.02 - 46.02)	1
Sulfonamides	0.62 (0 - 1.76)	5.47 (0 - 11.84)	10.6 (0.01 - 148.86)	1
Tetracyclines	423.8 (361.52 - 486.09)	348.12 (286.41 - 409.82)	1.01 (0.79 - 1.27)	0.5621

List of abbreviations: AZM, azithromycin; CRO, ceftriaxone; 95% CI, 95% confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range; MLS, macrolide lincosamides streptogramines

Figure 1 - Trial profile

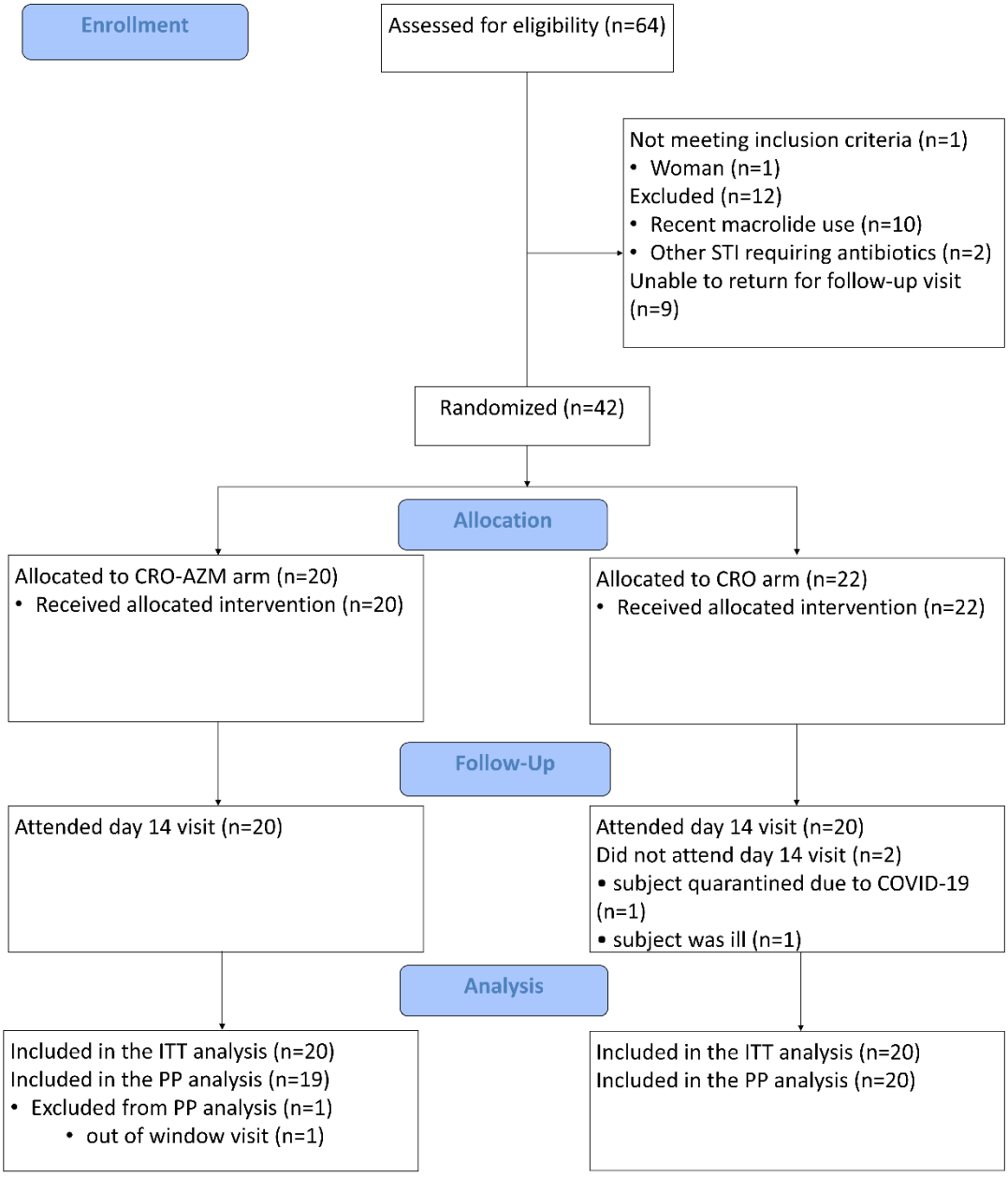
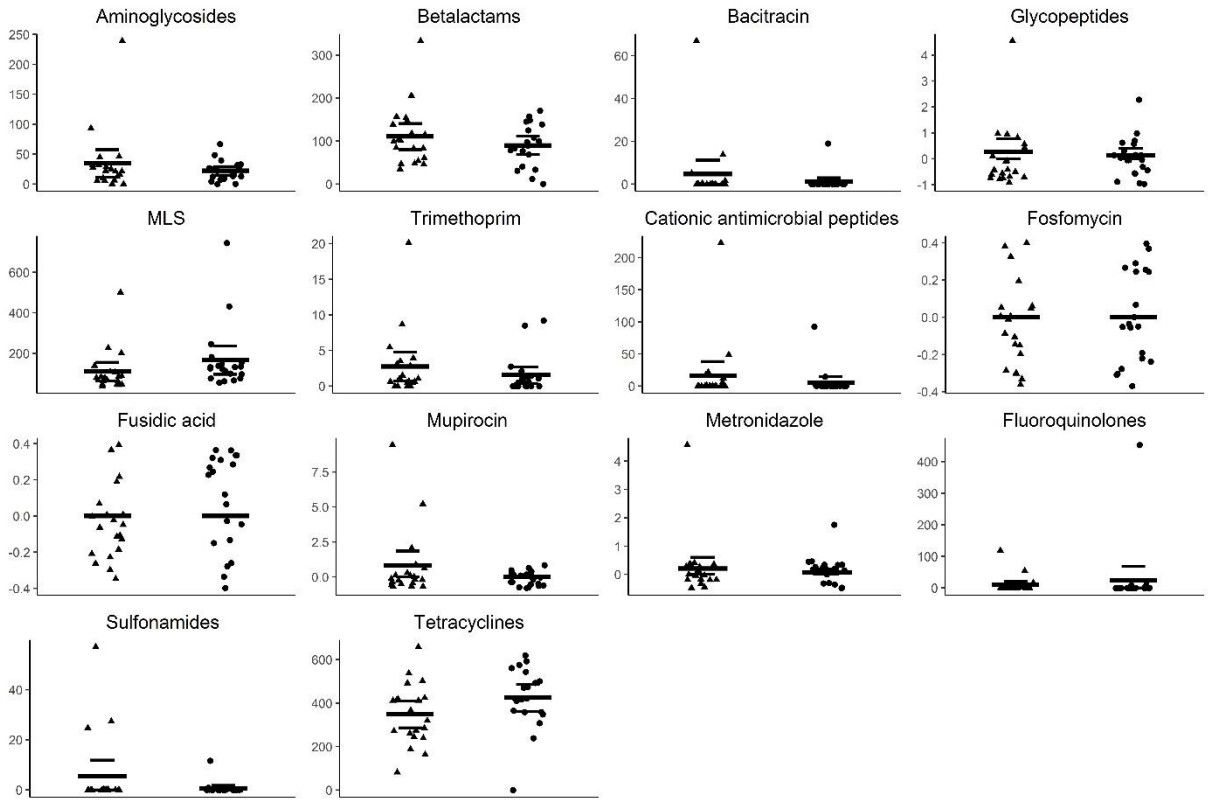


Figure 2 - Normalized abundance of resistance determinants (reads/million) at day 14, by treatment arm in reads per million (triangle: CRO, circle: CRO/AZM). Points represent actual measurements, elongated horizontal lines represent means and shorter horizontal the lower and upper bounds of the 95% CI



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Appendix 1 – Details of the laboratory procedures (*Streptococci* and commensal *Neisseria spp. culture)*, shotgun metagenomic sequencing and bioinformatic analyses

Laboratory procedures (Streptococci and commensal Neisseria spp. *culture)*

For culture of commensal *Neisseria* spp. and *Streptococcus* spp., LBVT.SNR and Columbia CNA agar, containing 5% sheep blood were used, respectively. Oral rinse samples were serially diluted in PBS and 100µl of the 10^{-2}, 10^{-3} dilutions were spread using a plate spinner, on respective plates with and without the addition of 2µg/ml azithromycin (Sigma Aldrich, Steinheim am Albuch, Germany). The concentration of 2µg/ml was chosen based on a previous study[6]. Plates were incubated up to 48 hrs at 37^0C in a 5-7% CO_2 incubator. The total number of colonies on the plates with and without 2µg/ml azithromycin were determined, counts were taken from the plate with 20-200 colonies.

Shotgun metagenomics

The anorectal swabs were shipped on dry ice to Eurofins Genomics for DNA isolation, library preparation and metagenomic sequencing. The DNA was isolated followed by library preparation using the TruSeq DNA library kit (Illumina Inc., San Diego, CA, USA). The libraries were multiplexed using Nextera DNA library preparation kit (Illumina Inc., San Diego, CA, USA) and sequenced on NextSeq6000 v2, 2×150 bp reads to generate 6Gb reads per sample.

Bioinformatic analyses

Bioinformatic analyses was carried out according to Van Dijck et al[23]. In brief, initial quality control of the raw reads was carried out using FASTQC[39]. The raw reads were trimmed for quality (Phred ≥20) and length (≥32 bases) using trimmomatic (v0.30)[40]. The host reads were removed from the raw reads by mapping the reads against the human reference genome (GRCh38, accession GCF_000001405.26) using Burrows-Wheeler Aligner (BWA-

MEM) (v0.7.17-r1188) using default parameters[41]. Abundance and diversity of the resistomes were characterized by mapping the non-human reads to the MEGARes v2.0 database using BWA-MEM with default parameters[42]. The ResistomeAnalyzer (https://github.com/cdeanj/resistomeanalyzer) was used to classify the antibiotic resistance genes with a gene fraction greater than 80% into types, classes, and gene groups. Single nucleotide polymorphisms and genes conferring resistance exclusively to non-drug compounds were not used in the downstream analyses.

Appendix 2 – Multidrug resistance proportions at day 14 in the CRO and CRO/AZM arms

	CRO **% (95% CI)**	**CRO/AZM** **% (95% CI)**	**p-value**
Indicator 1: **Aminoglycosides, Betalactams, Fluoroquinolones, Tetracyclines**	100 (83.89 - 100)	95 (76.39 - 99.11)	1
Indicator 2: **Aminoglycosides, Betalactams, Fluoroquinolones, Tetracyclines, Trimethoprim, Sulfonamides**	100 (83.89 - 100)	95 (76.39 - 99.11)	1
Indicator 3: **Aminoglycosides, Betalactams, Fluoroquinolones, Tetracyclines AND macrolides**	100 (83.89 - 100)	95 (76.39 - 99.11)	1
List of abbreviations: AZM, azithromycin; CRO, ceftriaxone; 95% CI, 95% confidence interval			

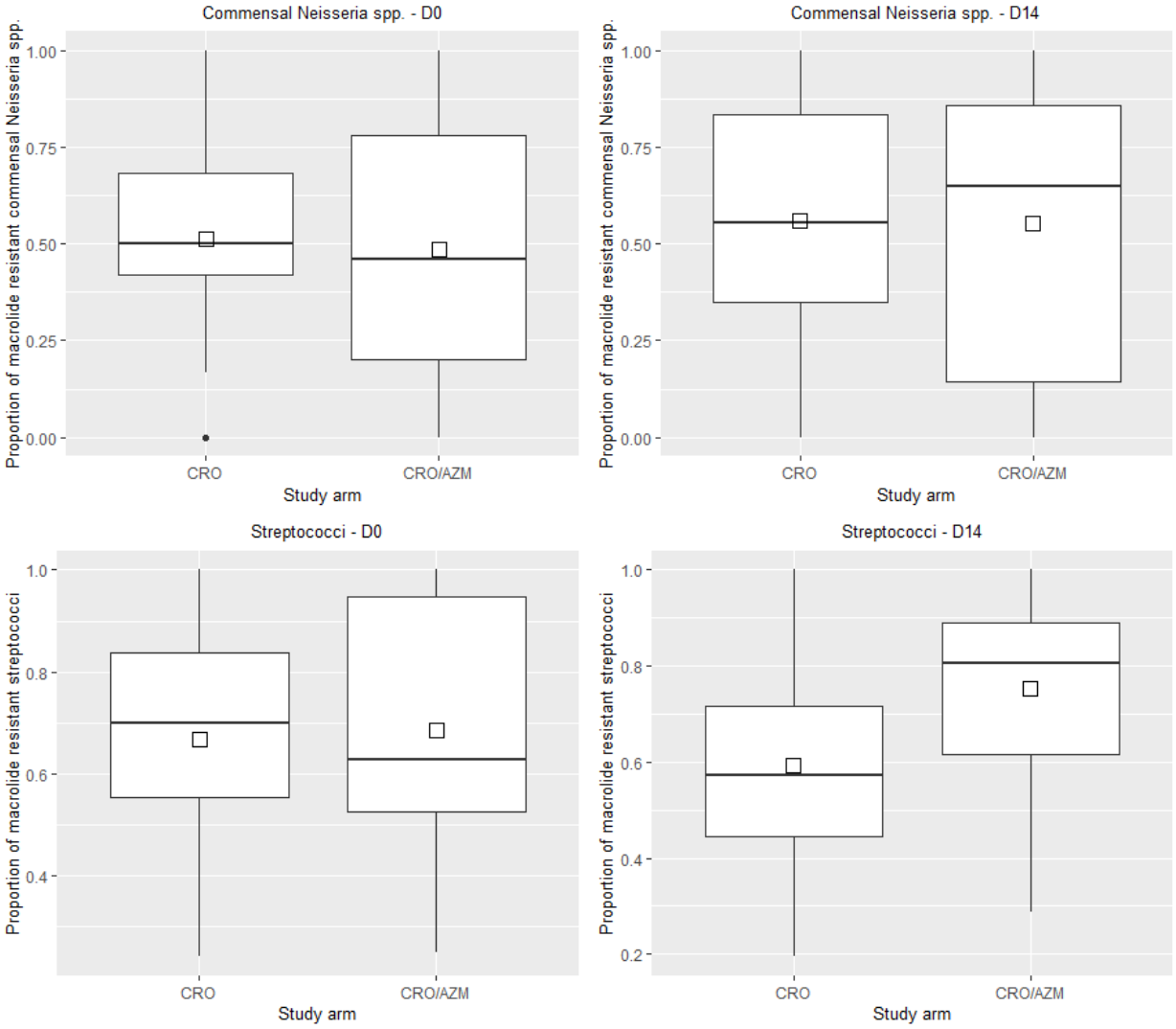
Appendix 3 – Mean and median proportions of streptococci and commensal *Neisseria spp.* that were macrolide resistant in the CRO and CRO/AZM arms at day 0 and day 14

	Streptococci mean (median)	**p-value***	**Commensal *Neisseria spp.* mean (median)**	**p-value***
CRO day 0	66.66% (69.88)		51.40% (50.00)	
CRO day 14	59.32% (57.22)	0.196	56.04% (55.43)	0.978
CRO/AZM day 0	68.61% (62.69)		48.64% (46.15)	
CRO/AZM day 14	75.21% (80.62)	0.568	55.21% (65.00)	0.679

List of abbreviations: AZM, azithromycin; CRO, ceftriaxone

*Wilcoxon sign rank test comparing the change in proportions within study arms between day 0 and day 14

Boxplot of mean macrolide resistant streptococci and commensal *Neisseria spp.* at day 0 and day 14

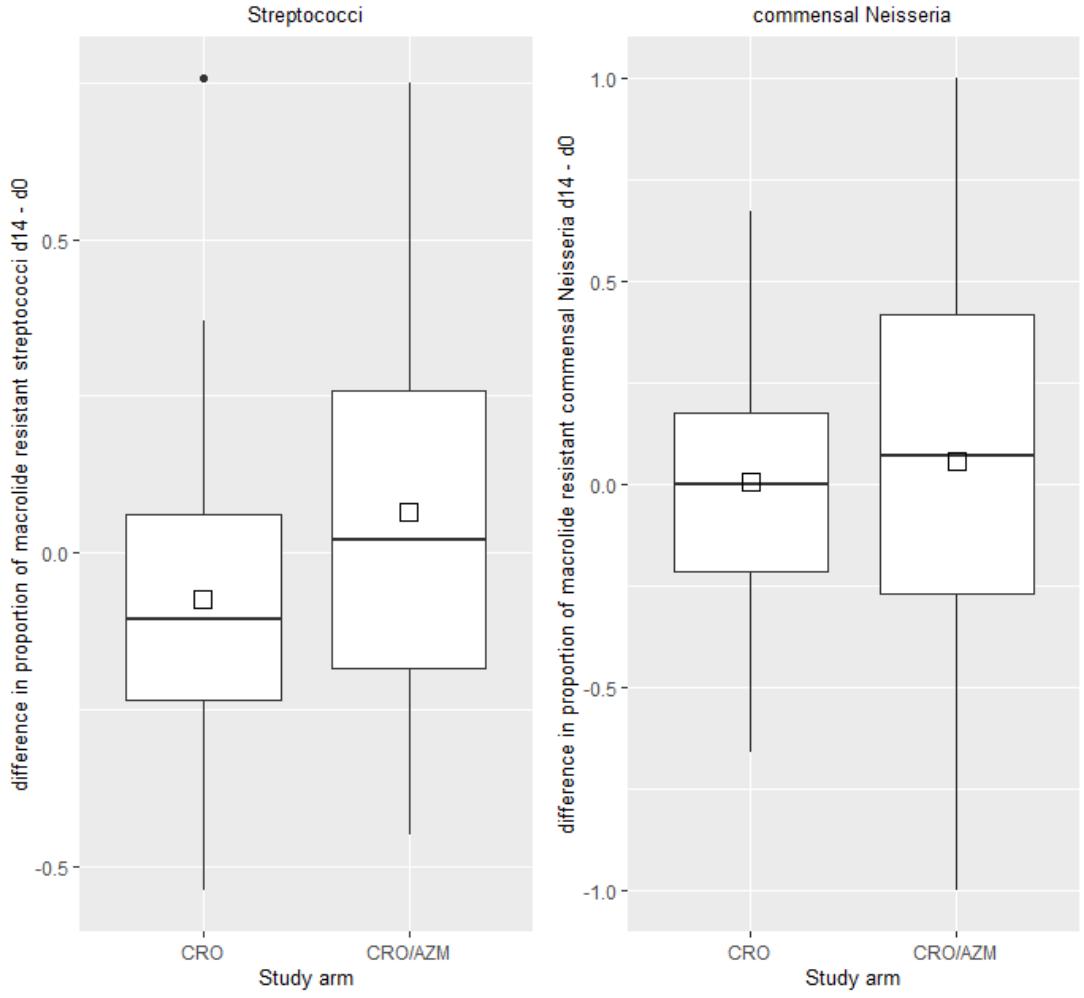


Appendix 4 – Differences between day 14 and day 0 in the proportions of streptococci and commensal *Neisseria spp*. that were macrolide resistant, in each arm.

	CRO mean (median)	**CRO/AZM mean (median)**	**p-value**
Streptococci (day 14 – day 0)	-7.3% (-10.6)	6.6% (2.2)	0.267
Commensal *Neisseria* spp. (day 14 – day 0)	0.7% (0.0)	5.6%(6.8)	0.679

List of abbreviations: AZM, azithromycin; CRO, ceftriaxone

Boxplots of individual differences (day 14 – day 0) for culture results by treatment arm.



Appendix 5 – proportions of participants presenting at least one macrolide resistant streptococci colony and commensal *Neisseria* spp. colony per arm and per study visit

	Streptococci	**Commensal *Neisseria spp.***
CRO day 0	100%	90.5%
CRO day 14	100%	83.3%
CRO/AZM day 0	100%	94.7%
CRO/AZM day 14	100%	73.7%
List of abbreviations: AZM, azithromycin; CRO, ceftriaxone		

Appendix 6 - Permutation analysis results for comparison of normalized resistance determinants, the endpoint is the ratio of CRO+AZM/CRO (per protocol analysis)

Determinants	**Ratio (CRO + AZM/CRO) (95% CI)**	**p-value**
MLS (Macrolides, lincosamides, streptogramines)	1.05 (0.54 - 1.84)	0.0971
Aminoglycosides	1.10 (0.47 - 2.12)	0.970
Betalactams	1.01 (0.70 - 1.42)	0.970
Bacitracin	3.59 (0.05 - 19.66)	0.970
Glycopeptides	0.86 (0 - 2.02)	0.970
Trimethoprim	1.19 (0.33 - 3.03)	0.970
Cationic antimicrobial peptides	2.74 (0.06 - 13.74)	0.970
Mupirocin	1.53 (0 - 5.62)	1
Metronidazole	1.24 (0 - 3.39)	0.970
Fluoroquinolones	6.68 (0.02 - 45.26)	0.970
Sulfonamides	11.02 (0 - 156.70)	1
Tetracyclines	1.01 (0.81 - 1.23)	0.0979
List of abbreviations: AZM, azithromycin; CRO, ceftriaxone; CI, confidence interval		

Appendix 7 – adverse events reported at day 14

	Pooled (N=42) n (%)	**CRO (N=22) n (%)**	**CRO/AZM (N=20) n (%)**	**p-value**
Any adverse event	6 (14.2%)	2 (4.8%)	4 (9.5%)	0.4
Abdominal pain*	1 (12.5%)	0 (0%)	1 (25.0%)	
Nausea*	3 (50.0%)	0 (0%)	3 (75.0%)	
Pain at injection site*	3 (50.0%)	2 (100%)	1 (25.0%)	
Pre-syncope*	1 (12.5%)	0 (0%)	1 (25.0%)	
Any drug-related adverse event	6 (14.2%)	2 (4.8%)	4 (9.5%)	0.4
Any serious adverse event	0 (0)	0 (0)	0 (0)	1
List of abbreviations: AZM, azithromycin; CRO, ceftriaxone * % among participants having reported adverse events, multiple answers possible				

5.3 Doxycycline prophylaxis and the risk of AMR

Vanbaelen T, Reyniers T, Rotsaert A, et al. Prophylactic use of antibiotics for sexually transmitted infections: awareness and use among HIV PrEP users in Belgium. *Sex Transm Infect* 2022; 98(8): 625.

Prophylactic use of antibiotics for sexually transmitted infections: awareness and use among HIV PrEP users in Belgium

Thibaut Vanbaelen ,[1,2] Thijs Reyniers ,[2] Anke Rotsaert,[2] Bea Vuylsteke ,[2] Eric Florence,[1] Chris Kenyon ,[1] Irith De Baetselier[1]

Doxycycline prophylaxis has been shown to reduce the incidence of chlamydia and syphilis infections in various studies, but researchers are worried about increasing antimicrobial resistance (AMR).[1] Although not currently recommended, STI prophylaxis has been reported in high-risk men who have sex with men, with proportions ranging from 2% to 10%.[2] We assessed the awareness and use of STI prophylaxis among HIV pre-exposure prophylaxis users in Belgium in a nested cross-sectional online survey in December 2021 and January 2022. Participants were recruited through social media, HIV reference centres and social/sexual networking applications. A total of 187 participants completed the survey. The median age was 46 years (IQR 38–53). The majority were born in Belgium (161/187, 86.1%) and identified themselves as male (183/187, 97.9%). Fifty-four participants (28.9%) had ever heard of STI prophylaxis, 21 (11.3%) knew someone who used it and 6 (3.2%) reported having used STI prophylaxis themselves. Three users reported taking it only after sex and three both before and after sex. Two had used doxycycline, one azithromycin, one amoxicillin and antibiotic was unknown in two cases. Two participants reported having taken STI prophylaxis in the previous month, two in the previous 1–6 months and two >12 months ago. Two participants had obtained these antibiotics through an HIV/STI clinic, one from a sex partner and three reported using leftovers. Although the use of STI prophylaxis was limited in our sample, a substantial proportion of participants were aware of STI prophylaxis or knew persons using it, suggesting that this phenomenon may be more common than initially thought. Another concern is that some antibiotics with a high propensity to induce AMR or no efficacy in reducing the occurrence of bacterial STIs were used. Sensitisation of patients and healthcare providers is needed, as well as further research on the net benefits and risks of STI prophylaxis.

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Title – Prophylactic use of antibiotics for sexually transmitted infections - awareness and use among HIV PrEP users in Belgium

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Doxycycline prophylaxis has been shown to reduce the incidence of chlamydia and syphilis infections in various studies, but researchers are worried about increasing antimicrobial resistance (AMR) (1). Although not currently recommended, STI-Prophylaxis has been reported in high-risk men who have sex with men, with proportions ranging from 2% to 10% (2). We assessed the awareness and use of STI prophylaxis among HIV PrEP users in Belgium in a nested cross-sectional online survey in December 2021 and January 2022. Participants were recruited through social media, HIV reference centers and social/sexual networking applications. A total of 187 participants completed the survey. The median age was 46 years (IQR 38-53). The majority were born in Belgium (161/187, 86.1%) and identified themselves as male (183/187, 97.9%). Fifty-four participants (28.9%) had ever heard of STI-Prophylaxis, 21 (11.3%) knew someone who used it and 6 (3.2%) reported having used STI-Prophylaxis themselves. Three users reported taking it only after sex and three both before and after sex. Two had used doxycycline, one azithromycin, one amoxicillin and antibiotic was unknown in two cases. Two participants reported having taken STI-Prophylaxis in the previous month, two in the previous 1-6 months and two more than 12 months ago. Two participants had obtained these antibiotics through an HIV/STI clinic, one from a sex partner and three reported using leftovers. Although the use of STI-Prophylaxis was limited in our sample, a substantial proportion of participants were aware of STI-Prophylaxis or knew persons using it, suggesting that this phenomenon may be more common than initially thought. Another concern is that some antibiotics with a high propensity to induce AMR or no efficacy in reducing the occurrence of bacterial STIs were used. Sensitization of patients and healthcare providers is needed, as well as further research on the net benefits and risks of STI-prophylaxis.

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Vanbaelen T, Manoharan-Basil SS, Kenyon C. Doxycycline Postexposure Prophylaxis Could Induce Cross-Resistance to Other Classes of Antimicrobials in Neisseria gonorrhoeae : An In Silico Analysis. *Sex Transm Dis* 2023; 50(8): 490-3.

Doxycycline Postexposure Prophylaxis Could Induce Cross-Resistance to Other Classes of Antimicrobials in *Neisseria gonorrhoeae*: An In Silico Analysis

Thibaut Vanbaelen, MD, Sheeba Santhini Manoharan-Basil, PhD,* and Chris Kenyon, MD, PhD, MPH*†*

Abstract: We found that tetracycline resistance–associated mutations and genes in *Neisseria gonorrhoeae* are linked to mutations causing resistance to other antimicrobials. Therefore, the use of doxycycline postexposure prophylaxis may select for resistance to other antimicrobials.

Three randomized controlled trials have now established that doxycycline postexposure prophylaxis (PEP) can reduce the incidence of chlamydia and syphilis in men who have sex with men (MSM).[1–3] The Doxycycline Postexposure Prophylaxis (Doxy PEP) study, the largest and most rigorous of these studies, found that doxycycline also reduced the incidence of *Neisseria gonorrhoeae*.[3] As a result of these findings, certain clinics in San Francisco are now offering doxycycline PEP to a proportion of MSM attending their clinics.[4]

A major concern about the widespread use of doxycycline PEP is that it will induce resistance to tetracyclines in *N. gonorrhoeae* and other bacterial species. Two doxycycline PEP studies have evaluated the effect of doxycycline on tetracycline resistance in *N. gonorrhoeae*. Both found no statistically significant effect, but the duration of follow-up was short, and the number of gonococcal isolates tested was small (n = 9 isolates[1] and n = 47 isolates[3]).

An underexplored risk of doxycycline PEP is the selection of resistance to other classes of antimicrobials. The excess use of antimicrobials has been frequently associated with the selection of cross-resistance to related and unrelated classes of antimicrobials in a number of bacterial species.[5] This effect can be direct or indirect. In the direct pathway, tetracyclines have been noted to induce mutations that confer cross-resistance to fluoroquinolones, β-lactams, and other classes of antimicrobials in *Escherichia coli* in vitro.[6,7] Tetracyclines can also act indirectly. If, for example, the genetic determinants of doxycycline resistance in *N. gonorrhoeae* are strongly linked to markers of resistance to other antimicrobials, then the use of doxycycline may indirectly select for resistance to these other antimicrobials. This has been shown for other species, such as the selection for macrolide resistance in *Streptococcus pyogenes*.[8]

To test this indirect-pathway hypothesis, we assessed the extent to which tetracycline-resistance-associated mutations (RAMs) were clonally distributed in *N. gonorrhoeae* and if these RAMS were associated with resistance-conferring mutations to other classes of antimicrobials.

We tested the 2 major determinants of reduced susceptibility to tetracyclines—*tetM* and *rpsJ* V57M. High-level tetracycline resistance (>16 mg/L) is typically due to the plasmid-mediated acquisition of the *tetM* gene.[9] The *rpsJ* V57M substitution reduces the affinity of the 30S ribosome subunit for tetracyclines and results in lower-level resistance.[9]

MATERIALS AND METHODS

N. gonorrhoeae Collection

We analyzed the 2375 gonococcal isolates from the 2018 Euro-GASP survey (https://pathogen.watch/collection/eurogasp2018). This survey collected the samples from individuals who had culture-positive gonococcal infection episodes in 26 European Union and European Economic Area countries via a validated sampling methodology.[10,11] Whole-genome sequencing was performed, and genogroups and AMR determinants were deduced from quality-checked genomic data.[10]

DATA ANALYSIS

All known RAMs were grouped per gene to construct a binary variable per gene that indicated if any RAM was present in that isolate. For *gyrA*, for example, if any of the known GyrA RAMs were present (S91F, D95A, D95G, D95N), the GyrA variable was coded as 1 and coded as 0 if no RAMs were found. The RAMs used to construct the variables are as follows: *gyrA* (S91F, D95A, D95G, D95N), *parC* (D86N, S88P, E91K), *penA* (A311V, V316T, I312M, ins346D, T483S, P551S, G542S, G545S), *ponA* (L421P), *porB1a* (G120K, G120D/A121D), mtr promoter (a57del), and *folP* (R228S).[10] To assess for clonality, we assessed the prevalence of *rpsJ* V57M and *tetM* by genogroup. This analysis was limited to the genogroups with more than 50 isolates. Statistical analyses were conducted using Stata V16 and the χ^2 test to compare groups.

RESULTS

Clonality by Genogroup

We found strong evidence of clonal spread of *rpsJ* V57M and *tetM* by genogroup (Fig. 1).

rpsJ

In 7 of the 11 genogroups with more than 50 isolates, all the isolates had the *rpsJ* V57M mutation (n = 592). For the 4

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Authors' Contributions: C.K., T.V., and S.S.M.-B. conceptualized the study. C.K. was responsible for the statistical analyses. All authors read and approved the final draft.
Ethics Statement: This analysis involved a secondary data analysis of anonymized public-access data.
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Title - Doxycycline Post Exposure Prophylaxis could induce cross-resistance to other classes of antimicrobials in Neisseria gonorrhoeae: an in-silico analysis

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Abstract

We found that tetracycline resistance associated mutations in *Neisseria gonorrhoeae* are linked to mutations causing resistance to other antimicrobials. Therefore, the use of doxycycline PEP may select for resistance to other antimicrobials.

Keywords – doxycycline PEP, AMR, cross resistance, PrEP

Introduction

Three randomized controlled trials have now established that doxycycline post exposure prophylaxis (PEP) can reduce the incidence of chlamydia and syphilis in men who have sex with men (MSM) (1-3). The Doxycycline Post Exposure Prophylaxis (Doxy PEP) study, the largest and most rigorous of these studies, found that doxycycline also reduced the incidence of *Neisseria gonorrhoeae* (3). As a result of these findings, certain clinics in San Francisco are now offering doxycycline PEP to a proportion of MSM attending their clinics (4).

A major concern about the widespread use of doxycycline PEP is that it will induce resistance to tetracyclines in *N. gonorrhoeae* and other bacterial species. Two doxycycline PEP studies have evaluated the effect of doxycycline on tetracycline resistance in *N. gonorrhoeae.* Both found no statistically significant effect, but the duration of follow-up was short, and the number of gonococcal isolates tested were small (n=9 (1) and n=47 (3)).

An underexplored risk of doxycycline PEP is the selection of resistance to other classes of antimicrobials. The excess use of antimicrobials has been frequently associated with the selection of cross resistance to related and unrelated classes of antimicrobials in a number of bacterial species (5). This effect can be direct or indirect. In the direct pathway, tetracyclines have been noted to induce mutations that confer cross resistance to fluoroquinolones, beta-lactams and other classes of antimicrobials in *Escherichia coli* in vitro (6, 7). Tetracyclines can also act indirectly. If, for example, the genetic determinants of doxycycline resistance in *N. gonorrhoeae* are strongly linked to markers of resistance to other antimicrobials, then the use of doxycycline may indirectly select for resistance to these other antimicrobials. This has been shown for other species, such as the selection for macrolide resistance in *Streptococcus pyogenes* (8).

To test this indirect-pathway hypothesis, we assessed the extent to which tetracycline-resistance-associated mutations (RAMs) were clonally distributed in *N. gonorrhoeae* and if

these RAMS were associated with resistance-conferring mutations to other classes of antimicrobials.

We tested the two major determinants of reduced susceptibility to tetracyclines – tetM and rpsJ V57M. High- level tetracycline resistance (>16mg/L) is typically due to the plasmid-mediated acquisition of the tetM gene (9). The rpsJ V57M substitution reduces the affinity of the 30S ribosome subunit for tetracyclines and results in lower-level resistance (9).

Materials and methods

N. gonorrhoeae collection

We analyzed the 2375 gonococcal isolates from the 2018 Euro-GASP survey (https://pathogen.watch/collection/eurogasp2018). This survey collected the samples from individuals who had culture-positive gonococcal infection episodes in 26 EU and EEA countries via a validated sampling methodology (10, 11). Whole genome sequencing was performed, and genogroups and AMR determinants were deduced from quality-checked genomic data (10).

Data analysis

All known RAMs were grouped per gene to construct a binary variable per gene that indicated if any RAM was present in that isolate. For gyrA, for example, if any of the known GyrA RAMs were present (S91F, D95A, D95G, D95N), the GyrA variable was coded as 1 and coded as 0 if no RAMs were found. The RAMs used to construct the variables are as follows: gyrA (S91F, D95A, D95G, D95N), parC (D86N, S88P,E91K), penA (A311V, V316T, I312M, ins346D, T483S, P551S, G542S, G545S), ponA (L421P), porB1a (G120K,G120D/A121D), mtr promoter (a57del) and folP (R228S) (10). To assess for clonality, we assessed the prevalence of rpsJ V57M and tetM by genogroup. This analysis was limited to the genogroups with

more than 50 isolates. Statistical analyses were conducted using Stata V16 and the Chi-squared test to compare groups.

Results

Clonality by genogroup

We found strong evidence of clonal spread of rpsJ V57M and tetM by genogroup (Fig. 1).

rpsJ

In 7 of the 11 genogroups with more than 50 isolates, all the isolates had the rpsJ V57M mutation (n=592). For the 4 other genogroups, only 15/336 (4.4%) isolates had this mutation (Fig. 1).

tetM

Three genogroups had a high prevalence of tetM (233/266 (87.6%)). The remaining 8 genogroups had a low prevalence of tetM (11/662 (1.7%); Fig. 1).

Cross resistance

rpsJ

The rpsJ V57M mutation was present in 1816 (76.5%) of isolates. The presence of rpsJ V57M was strongly positively associated with all RAMs assessed excluding the mtrR promoter A57 deletion, where the association was negative (Table 1). The strongest associations were for gyrA, parC, penA, porB1a and folP RAMs. In the presence of rpsJ V57M the prevalence of gyrA RAMs was 66.5% versus 1.8% in the absence of rpsJ V57M (P <0.001). The corresponding prevalence of RAMs if rpsJ V57M was present/absent for parC

was 51.2%/1.1%, for penA was 99.9%/68.3%, for porB1a was 29.7%/0.7% and for folP 98.6%/45.8%; all P<0.001).

tetM

tetM was present in a smaller proportion of isolates (520/2375 (21.9%)). gyrA, penA, and folP RAMs were also more prevalent in isolates with tetM (P <0.001; Table 1). In contrast, parC, ponA, porB1a, mtrR promoter and mtrD mosaics were less prevalent in isolates with tetM (P <0.001).

Cross resistance by genogroup

rpsJ

The four genogroups with a low prevalence of rpsJ V57M had less than 10% prevalence of gyrA, parC, porB1b and the mtrR promoter mosaic mutations (Fig. 2). In contrast, the prevalence of these mutations in the 7 genogroups with the rpsJ V57M was over 70% for gyrA (6 genogroups), parC (4 genogroups), porB1b (3 genogroups) and the mtrR promoter mosaic (1 genogroup). The prevalence of penA mutations was over 70% in all genogroups except for G387, where the prevalence of penA, rpsJ mutations and tetM were all less than 10%.

tetM

In the three high-tetM-prevalence genogroups, the prevalence of gyrA and penA RAMs was over 70% in 2 and 3 genogroups, respectively (Fig. 1). The prevalence of parC RAMs was less than 10% in each of these genogroups. The low tetM prevalence genogroups included examples with a low and high prevalence of each of the RAMs analysed.

Discussion

Our analysis revealed strong evidence of clonal spread of rpsJ, tetM and other RAMs by genogroup. We also found strong individual-isolate-level associations between the presence of the rpsJ V57M mutation and gyrA, penA, porB1a, mosaic mtrR promoter/mtrD and folP RAMs (amongst others). These mutations play a crucial role in driving resistance to beta-lactams, fluoroquinolones, folate antagonists and macrolides.

These findings can be parsimoniously explained by the linkage of these RAMs in certain genogroups. Certain genogroups have a high prevalence of RAMs to multiple classes of antimicrobials. For example, almost all the isolates from G1407 genogroup, have rpsJ penA, gyrA, parC and porB1b RAMs. Likewise, G12302 has a high prevalence of the above mutations along with the mtrR promoter mosaic. Antibiotic selection pressure has previously been shown to play a crucial role in determining the rise and fall of gonococcal genogroups (12-14). For example, the use of the less efficacious oral cefixime as the preferred treatment for gonorrhoea in Europe was linked to the rise of G1407 with its mosaic penA gene and reduced susceptibility to cefixime (10, 15). Because almost all isolates in this genogroup have gyrA and parC RAMs conferring resistance to fluoroquinolones, the use of cefixime indirectly resulted in an increased prevalence of fluoroquinolone resistance (13, 15, 16). A number of gonococcal infections that failed treatment with ceftriaxone were from this genogroup 1407 (17).

In the Doxycycline Post Exposure Prophylaxis (DoxyPEP) study, the average doxycycline consumption was 16 doses of 200mg doxycycline per month in the PEP arm (3). This consumption is 442-fold higher than the mean consumption of tetracyclines in a typical HIV PrEP cohort (18). The combination of this intense consumption of doxycycline and the high equilibrium prevalence of *N. gonorrhoeae* in PrEP cohorts (around 10% (19, 20)) would mean it would be unsurprising if the widespread use of doxycycline PEP in MSM would provide a selective advantage for genogroups with tetracycline resistance - such as G1407 and G12302. Since these genogroups are resistant to other classes of antimicrobials,

doxycycline PEP could indirectly select for resistance to these other classes of antimicrobials.

It is important to note that a number of the RAMs assessed were negatively associated with the presence of tetM. We do not have an explanation for this finding, but it may result in a more complex association between doxycycline PEP and the selection of cross resistance to other antimicrobials. Other limitations of our analysis include the fact that this study was limited to one world region for a single year. Although Euro-GASP has been shown to produce resistance prevalence estimates that are broadly representative of the participating countries (11), a major weakness is the small sample sizes provided by certain countries (10).

In summary, we found strong individual- and genogroup-level associations between the presence of the rpsJ V57M mutation and gyrA, penA, porB1a, mosaic mtrR promoter/mtrD and folP RAMs. These findings suggest that studies and programmes using doxycycline PEP would be advised to monitor for the emergence of resistance to other antimicrobials in *N. gonorrhoeae* and other bacterial species.

Figure 1 - Proportion of gonococcal isolates with resistance associated mutations (RAMs) in rpsJ, penA, gyrA, parC, porB1b, mtr promoter mosaic and tetM per genogroup (in genogroups with more than 50 isolates)

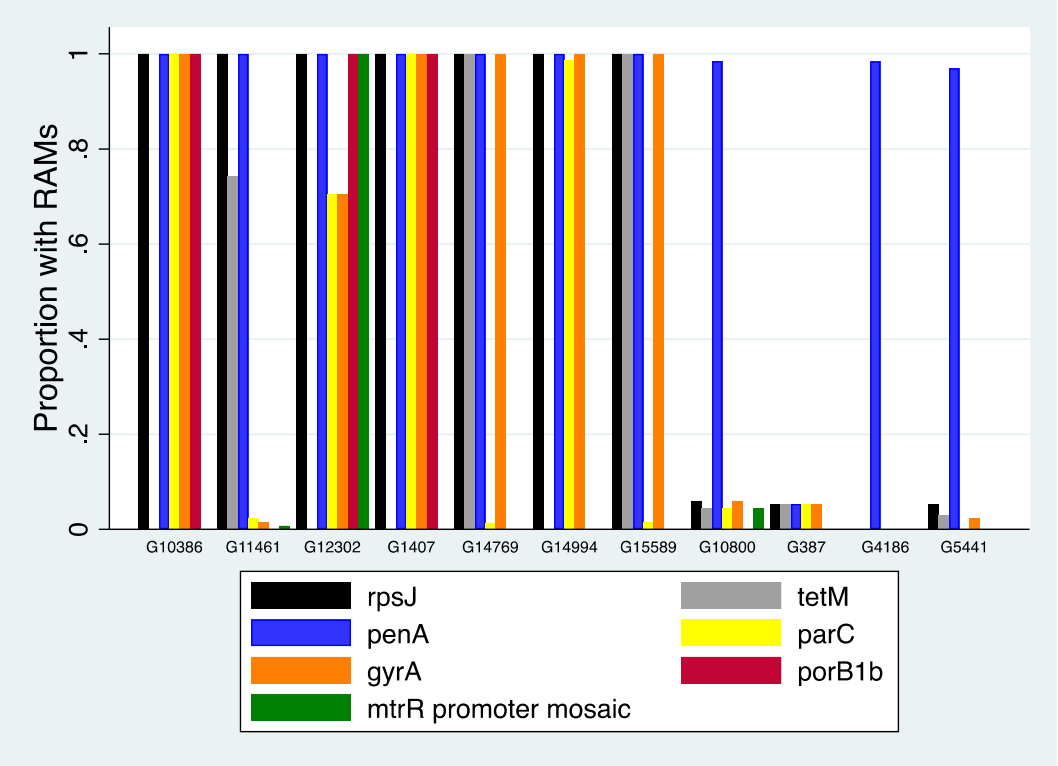

Table 1 - Prevalence of various resistance associated mutations (RAMs[#]) according to presence or absence of rpsJ V57M and tetM. Number (row percentage)

		***rpsJ* WT**	***rpsJ* V57M**	***tetM* absent**	***tetM* present**
***gyrA* RAMs[#]**	*Absent*	549 (47.5)	608 (52.6)	1001 (86.5)	156 (13.5)
	Present	10 (0.8)	1208 (99.2)**	854 (70.1)	364 (30.0)**
***parC* RAMs[#]**	*Absent*	553 (38.4)	886 (61.6)	1088 (75.6)	351 (24.4)
	Present	6 (0.6)	930 (99.4)**	767 (81.9)	169 (18.1)**
penA RAMs[#]	*Absent*	177 (99.4)	1 (0.6)	176 (98.9)	2 (1.1)
	Present	382 (17.4)	1815 (82.6)**	1679 (76.4)	518 (23.6)**
***folP* RAM[#]**	*Absent*	303 (92.1)	26 (7.9)	321 (97.6)	8 (2.4)
	Present	256 (12.5)	1790 (87.5)**	1534 (75.0)	512 (25.0)**
***mtrR* promoter a57 del**	*Absent*	359 (20.3)	1406 (79.7)	1283 (72.7)	482 (27.3)
	Present	200 (32.8)	410 (67.2)**	572 (93.8)	38 (6.2)**
***ponA*[#]**	*Absent*	373 (26.4)	1038 (73.6)	1029 (72.9)	382 (27.1)
	Present	186 (19.3)	778 (80.7)**	826 (85.7)	138 (14.3)**
***porB1a*[#]**	*Absent*	555 (30.3)	1276 (69.7)	1345 (73.5)	486 (26.5)
	Present	4 (0.7)	540 (99.3)**	510 (93.8)	34 (6.3)**
***mtrR* promoter mosaic**	*Absent*	556 (26.0)	1581 (74.0)	1620 (75.8)	517 (24.2)
	Present	3 (1.3)	235 (98.7)**	235 (98.7)	3 (1.3)**
***mtrD* mosaic**	*Absent*	559 (26.0)	1589 (74.0)	1631 (75.9)	517 (24.1)
	Present	0 (0)	227 (100)	224 (98.7)	3 (1.3)**
tetM	*Absent*	553 (29.8)	1302 (70.2)	NA	NA
	Present	6 (1.2)	514 (98.9)**	NA	NA

** P<0.001; NA – Not Applicable; WT – wild type

[#] : gyrA (S91F, D95A, D95G, D95N), parC (D86N, S88P,E91K), penA (A311V, V316T, I312M, ins346D, T483S, P551S, G542S, G545S), ponA (L421P), porB1a (G120K,G120D/A121D), mtr promoter (a57del) and folP (R228S)

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Chapter 6 Discussion

HIV prevention relies on a combination of behavioral, structural and biomedical interventions that should be tailored to a person's needs and circumstances (1). PrEP has become a cornerstone of this HIV prevention combination package. As discussed in the introduction, the roll-out of PrEP in the world and in Belgium is ongoing, and uptake is constantly increasing. However, despite these efforts, PrEP coverage remains insufficient and both PrEP care and users encounter numerous challenges. Therefore, it is essential to evaluate the impact of PrEP on the HIV epidemic and on sexual health to determine if it meets the high expectations set by early studies. This section of the thesis aims to briefly address this question before describing how our findings have filled in some of the research gaps regarding the contemporary challenges faced by PrEP care and users in Belgium.

6.1 Does PrEP meet its expectations?

HIV incidence has been declining in Belgium and worldwide for over a decade (2, 3). Various factors contribute to this decrease, such as the widespread implementation of effective antiretroviral therapy, which suppresses viral replication and prevents onward transmission, also known as treatment as prevention (TasP) (4). Assessing the exact added value of PrEP on the decline of HIV incidence is challenging. However, several lines of evidence suggest a positive effect of PrEP on HIV incidence. A modeling study conducted in Belgium examined the added value of TasP, outreach testing, and PrEP on the HIV epidemic (5). The study compared the number of new HIV infections and the budget impact of implementing different prevention strategies: outreach testing + TasP, outreach + TasP + PrEP, and no additional prevention measures. Without additional prevention, it was projected that by 2030, there would be 1 350 new HIV infections per year. In contrast, the first scenario (outreach testing + TasP) estimated 865 new infections per year, and the second scenario (outreach + TasP + PrEP) estimated 663 new infections per year. The

second scenario also resulted in the largest reduction in the total pharmaceutical budget (a decrease of 33.7M€ compared to 20.6M€ in the first scenario). Other modeling studies have suggested that achieving sufficient PrEP coverage among at-risk populations, particularly MSM, has the potential to eliminate the HIV epidemic in this population (6, 7). Recently, real-world data from a 10-year longitudinal cohort study in Australia supported these findings, by showing a stronger decline in HIV incidence among MSM following the introduction of PrEP (8). Similarly, in Belgium, the decline in new HIV infections seems sharper among MSM since the introduction of PrEP in 2017 (Figure *6*) (3).

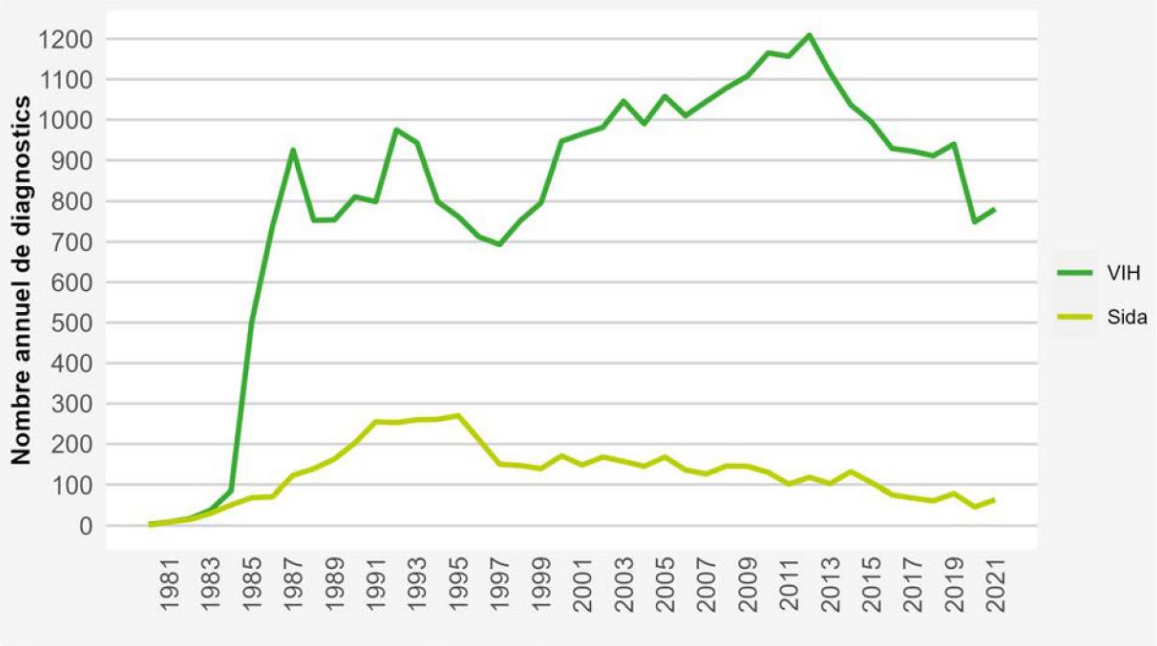

Figure 6 - Number of new HIV infections in Belgium 1981-2021 (3)

However, it is unlikely that PrEP alone will allow to achieve the WHO goals of ending the HIV epidemic by 2030 (9). Other prevention methods, such as condom use or TasP, also have their place in HIV prevention (10). Beside their role in HIV prevention, condoms provide protection against other STIs and, for that reason, they are always recommended in combination with PrEP. As mentioned in the introduction, the roll-out of PrEP has raised concerns of “risk compensation”, an increase in risk behavior, for instance a decrease in condom use, following PrEP implementation. However, evidence regarding PrEP and risk compensation is conflicting (11, 12, 13). Some studies have shown higher rates of

condomless anal intercourse among PrEP users (13), or following PrEP initiation (14), while other studies have found no differences (15, 16), and some even found a decrease in such behaviors (17).

Beside the positive impact on the HIV epidemic, PrEP has been shown to improve the (sexual) health of its users in multiple ways. Studies have indicated that PrEP has a positive effect on mental health and on the quality of users' sex lives (18, 19, 20). It reduces sexual anxiety, primarily associated with a reduced fear of HIV, while increasing self-esteem and sexual pleasure (6, 7, 8). PrEP has also been reported to improve health knowledge, particularly regarding HIV and STIs, and reduce HIV-related stigma within the MSM community (20). Moreover, PrEP enables high-risk individuals to engage in care, providing access to regular STI testing and a range of related services. PrEP consultations can also offer opportunities to address other issues faced by users, such as drug use or mental health issues (21).

However, we have seen that PrEP coverage remains insufficient and that both PrEP care and users face many challenges. In this thesis we explored some research gaps on describing and addressing some of these challenges. We will now summarize how our findings filled in these gaps.

6.2 Barriers to PrEP care and retention in PrEP care

The first objective of this thesis, related to the barriers to PrEP care, was to identify and explore factors associated with PrEP care discontinuation, reasons for discontinuation, and ongoing HIV risk among individuals who discontinued PrEP (care). We have shown that the majority of PrEP users who discontinued PrEP care did so due to having stopped using PrEP or due to the COVID-19 epidemic ongoing at the time of our study. Among those who stopped PrEP, the main reasons therefore were being in a monogamous relationship, a reduced sexual activity or consistent condom use. However, some users also reported barriers to PrEP care such as difficult access to the clinic and too many procedures for PrEP

follow-up. To ensure continued engagement in PrEP care and adequate protection against HIV, it is crucial to establish a low threshold, differentiated PrEP delivery model. A differentiated delivery model can be defined as “a client-centred approach that simplifies and adapts services, in ways that both serve the needs of affected people better and reduce unnecessary burdens on the health system” and is endorsed by WHO in its 2022 guidelines on HIV, viral hepatitis, and STI prevention, diagnosis, treatment, and care (22). It has been shown that providing PrEP care represents an important additional workload for the Belgian HRCs (23), and this has sometimes led to long waiting times for PrEP consultations. One way to tackle the waiting times, to reduce the workload in HRCs, and to tend towards a differentiated delivery model for PrEP is to involve family physicians (FPs) in PrEP care. A recent study in Belgium has shown a high willingness among FPs to provide PrEP care (24). Four potential roles of FPs in PrEP care were identified, namely being a low-threshold entry point for advice on PrEP, identifying potential PrEP candidates, initiating appropriate care for PrEP candidates and providing follow-up for PrEP. However, here as well some barriers need to be addressed before involvement of FPs can be achieved. Training FPs on PrEP and sexual health related topics, as well as setting up a collaboration between FPs and HRCs were identified as important steps for the implementation of PrEP care in primary care. Recently, the importance of a collaboration between HRCs and FPs in PrEP care has been acknowledged by the Belgian social security, as it has allowed that one in two trimestral visits for PrEP care can be done by FPs (25).

Importantly, the vast majority of PrEP users who discontinued PrEP care in our study reported not being at risk for HIV anymore. Such information is crucial to complement the classical retention in care metrics, that, in the case of PrEP, do not allow for a comprehensive assessment of PrEP care programs efficiency. Given that PrEP is not a lifelong intervention and that it can be discontinued in periods where no HIV risk is present, assessing PrEP initiation and/or discontinuation without assessing HIV risk is incomplete. PrEP discontinuation in the absence of HIV risk should not be considered a failure of PrEP programs, as opposed to discontinuing PrEP care when HIV risk is still present. This is consistent with the concept of “prevention-effective adherence” (Figure 7) (26). This

concept takes into account the variability in HIV risk and the use of other HIV prevention methods to measure whether an individual is at risk for HIV, and is particularly suitable for PrEP evaluation (26). However, despite being more accurate, collecting such individual-level data in routine PrEP monitoring is challenging, and most guidelines recommend the use of proxy's such as rates op PrEP care initiation and discontinuation to monitor PrEP programs (27).

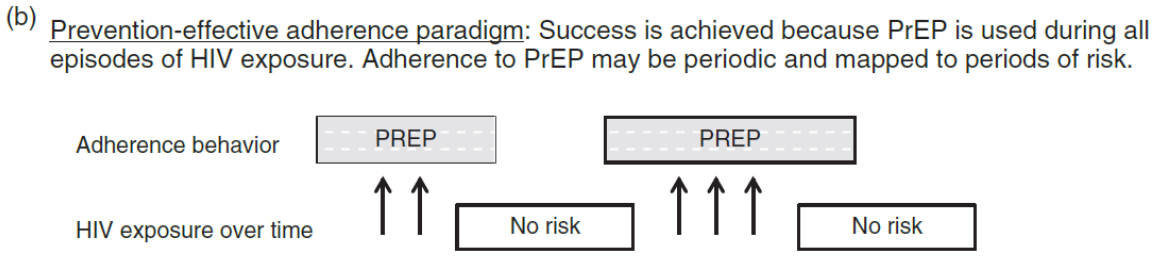

Figure 7 - Schematic representation of the concept of prevention effective adherence (26)

6.3 Syndemics and the need for additional risk reduction strategies

Afterwards, we explored two components of a syndemic that poses a threat to the health of PrEP users, namely engagement in chemsex and non-consensual sex. Regarding chemsex, our objective was to explore the occurrence of engagement in chemsex, its perceived negative effects, the willingness to reduce chemsex and associated risks, and the preferred options or tools to reduce such risks among PrEP users in Belgium. We found that more than one third of the PrEP users engaged in chemsex in the three months prior to our study. Among them, a substantial part reported experiencing negative consequences of chemsex on their health, social and professional life, and was willing to reduce the risks related to chemsex. The most preferred strategies to do so were support through an online application and face to face counselling with a healthcare provider. Online applications have been shown to be acceptable and effective among MSM for different HIV an STI prevention interventions (28). The main advantage from such apps is that they can offer support at the time chosen by the user, before, during or after a chemsex session, given

that they are constantly accessible on the users' devices. Individuals engaging chemsex have reported that access to immediate, easily accessible, and reliable information is needed to mitigate chemsex-related risks (29). For instance, these applications can be used to search for harmful drug combinations during a chemsex session. Further research is needed to assess whether such applications are effective in reducing chemsex-related risks in real-world settings.

The fact that healthcare professionals are one of the preferred support options is consistent with previous findings in the literature (30, 31). This finding underlines the potential role of PrEP clinics in providing such support. Moreover, about one third of the PrEP users reported willing more attention to be paid to chemsex during PrEP consultations. Given that PrEP users are already engaged in care and familiar with these services, PrEP clinics seem particularly suited for providing specific support on chemsex-related topics. Addressing chemsex has been endorsed by WHO as essential health intervention for MSM (28), and we recommend it to be included in the PrEP package of care.

The second component of the syndemic explored in this thesis was non-consensual sex. We aimed to describe the occurrence and forms of non-consensual sex, the factors associated with recent experiences of non-consensual sex, and to explore help-seeking behavior after non-consensual sex experiences among PrEP users in Belgium. We found that approximately one in five PrEP users ever experienced non-consensual sex, with the majority not seeking help afterward. Seeking help following non-consensual sex experiences is crucial given the association with several short- and long-term health consequences such as mental health disorders, STIs and HIV infections (32, 33). The main reason for not seeking help in our study was not feeling the need to do so. However, other respondents reported barriers to seeking help such as shame of reporting what happened or lack of knowledge about available support services. Several countries, including Belgium, developed sexual assault centers, where victims of non-consensual sex can seek psychological, medical and legal help at a one-stop center (34, 35). While such centers are very important, they are still rare. Broadening support through other channels can help

extend coverage and reduce the barriers to accessing assistance. Given the higher prevalence of non-consensual sex among MSM and the fact that MSM represent the majority of PrEP users, PrEP clinics can play a vital role in informing, preventing, and providing support to PrEP users who have experienced non-consensual sex.

As mentioned in the introduction, these two components are part of a syndemic and are thus interplaying at different levels, resulting in an increased burden on the health of PrEP users. Explaining how all these factors exactly interact is complex. Causality cannot be inferred from the available literature. For instance, mental health disorders might be a vulnerability factor leading to substance use but could also be the consequence of substance use (36, 37). Other factors such as social aspects or cultural norms might also be involved, making the relationship even more complex (38). The same might be true for the association between sexual violence and mental health disorders or substance use (39). However, on the light of all the factors described hereabove, it is clear that mental health issues, substance use, sexual violence and the HIV and STIs epidemics are linked. All these factors are highly prevalent among MSM and PrEP users, share common pathways and consequences. Therefore, they all have the potential to reinforce each other and increase their burden, which underlines the importance of offering support regarding these issues.

6.4 The emergence of AMR in bacterial STIs

In the next chapter of this thesis, we moved on to the topic of STIs, and more specifically how to prevent the emergence of AMR in bacterial STIs. We first assessed how antimicrobial susceptibility in commensal *Neisseria* spp. has varied over place and time and in relation to the pathogenic *Neisseria*. We have shown that AMR has been increasing in commensal *Neisseria* spp. following decades of antimicrobial exposure. Such insights are crucial given that commensal *Neisseria* spp. harboring resistance genes can transfer them to pathogenic *Neisseria* spp. This has led several authors to recommend using commensal *Neisseria* spp. as an early-warning system for the emergence of AMR in pathogenic *Neisseria* spp. (40) While AMR surveillance programs exist for NG, to our knowledge no

similar program has been developed for commensal *Neisseria spp.* Our findings corroborate the importance of setting-up such a system.

Subsequently, we explored several options to reduce antimicrobial consumption in PrEP cohorts. First, we assessed the effect of screening MSM on PrEP for NG and CT on antibiotic consumption and on the incidence of these infections. We found that reducing screening intensity from 3-site 3-monthly to 1-site 6-monthly led to a drastic reduction in macrolide consumption, one antimicrobial particularly prone to inducing AMR. We also found that not screening for NG and CT in PrEP cohorts leads to an increase in CT infections, but not in NG infections. However, this difference disappeared when performing a sensitivity analysis, controlling for a bias in one of the study arms (Figure 8). This finding is commensurate with the longer duration of CT infections compared with NG infections, when left untreated (41, 42). Screening for NG and CT was associated with a 28-84% increase in antimicrobial consumption. As mentioned in the introduction of this thesis, interventions to reduce antimicrobial consumption in core-groups with high consumption are urgently needed. Reducing screening intensity for NG and CT in MSM taking PrEP is one way to achieve this. Based on our findings, the benefits of screening for NG and CT, namely a potential decrease in CT infections, come at the cost of a very high antimicrobial use, and therefore, do not seem to outweigh the harms. However, careful consideration should be given to the potential increase in CT infections. Increased incidence of bacterial STIs among MSM could lead to an increased transmission, also to other populations. For instance, there is evidence of bridging transmission of NG between MSM and women (43). An increase in these infections in women could result in increased adverse events such as infertility, and such effects should thus be taken into account when assessing the benefits and harms of screening MSM on PrEP for NG/CT.

We also assessed the impact on the resistome of monotherapy with CRO vs dual therapy with CRO and AZM for the treatment of NG. We could not show that dual therapy was more likely to induce resistance compared to mono-therapy. This finding may be attributed to the high prevalence of AMR determinants in the study population before the study, indicating a saturation effect where further resistance could not be induced. This saturation

effect may be a result of the already high antimicrobial consumption in the population. These findings contrast with previous studies that showed a pronounced and prolonged effect of macrolide consumption on AMR (44, 45). However, in these studies, baseline resistance was much lower compared with ours. Our findings, taken as a whole, underline once again the importance of reducing antimicrobial consumption in PrEP cohorts. It has been shown that macrolide-sparing options for the treatment of bacterial STIs, such as switching from dual- to mono-therapy for the treatment of NG can decrease macrolide consumption up to 16-fold (46). Therefore, even if we couldn't show an effect of dual-therapy on the resistome, we recommend using mono-therapy with ceftriaxone for the treatment of NG.

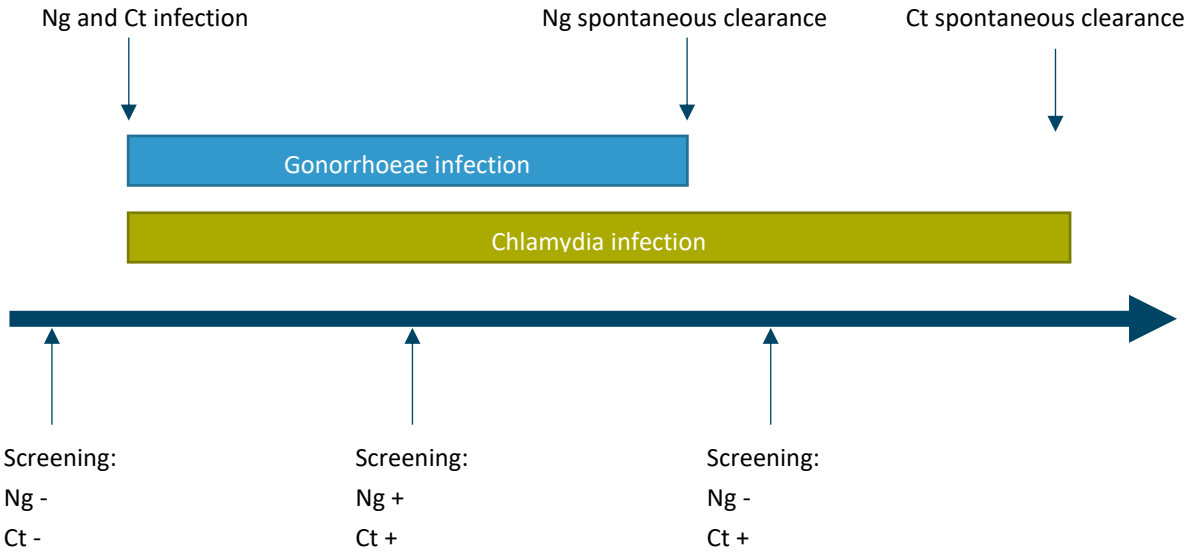

Figure 8 - Schematic representation of the untreated infections in the non-screening arm bias of the Gonoscreen study

The last part of this thesis focused on antibiotic prophylaxis for STIs and the risk of AMR linked to this new STI control intervention. We have shown that the use of antibiotics to prevent STIs was limited among PrEP users in Belgium so far. However, among the few users, some used antibiotics with a high propensity to induce AMR or with no proven efficacy in reducing the occurrence of bacterial STIs. We have also shown that the use of doxycycline PEP may select for resistance to other antimicrobials, given that tetracycline resistance associated mutations in NG are linked to mutations causing resistance to other antimicrobials. Several authors have now raised concerns about the potential detrimental

effect of widespread doxyPEP implementation, given the risk of associated AMR (47, 48). Our findings support these concerns, and we advise for caution before recommending the use of doxyPEP in MSM taking PrEP. Further research should evaluate the exact impact of doxyPEP on AMR in order to assess the potential harms linked with its widespread and prolonged use.

6.5 Conclusions

PrEP has made significant progress since its initial rollout in the mid-2010s. Its impact on the HIV epidemic is undeniable. Despite increasing coverage and uptake, more efforts are required to overcome barriers to PrEP care and reach a maximum number of users to achieve the maximum impact. Implementing a multidisciplinary, low threshold, differentiated PrEP delivery model is crucial in this regard. Such a model should address the concomitant health and psychosocial needs of PrEP users, including issues related to chemsex and non-consensual sex. Another critical aspect affecting the health of PrEP users is the emergence of AMR in bacterial STIs. Decades of antimicrobial exposure have led to situations where some bacterial STIs are becoming untreatable. Therefore, urgent interventions to reduce antimicrobial consumption are needed. Discontinuing screening for NG and CT infections within PrEP cohorts may be one approach, although careful consideration should be given to the potential increase in CT infections. Switching from dual therapy to monotherapy for the treatment of NG is another strategy to consider. On the contrary, DoxyPEP, an intervention that is at odds with the previously cited antibiotic sparing options, is making its way in STI control strategies. We have shown some of the potential harms associated with DoxyPEP, namely the selection of resistance to doxycycline and other antimicrobials in NG. Further research is needed to assess the exact effect of DoxyPEP on AMR, before its formal implementation.

When considering aspects such as AMR or novel interventions such as doxyPEP, the expertise of HIV/STI clinics is essential. Monitoring AMR, particularly in commensal *Neisseria spp.*, or assessing the impact of doxyPEP on AMR requires specific knowledge and

laboratory techniques that are not routinely available. To be able to achieve this, having PrEP users involved in PrEP care in HRCs is crucial. However, this should be balanced with the need to develop a differentiated PrEP delivery model and to decrease workload on PrEP clinics. Furthermore, in this thesis we recommend including other aspects of sexual health such as chemsex support in PrEP care. We must keep in mind that providing PrEP care already represents an additional workload on HRCs. Adding more tasks to the healthcare professionals involved in PrEP care might increase the workload even more and come at additional costs. Involving FPs in PrEP care might be a step toward a low threshold, differentiated PrEP delivery model, and a way to reduce the workload on PrEP clinics. However, the workload of FPs is also high, and FPs mentioned requiring additional training before being able to implement PrEP care in their practices and underlined the importance of collaborating with HRCs. A differentiated PrEP delivery model should take all these parameters into account and provide an efficient distribution of the tasks that is acceptable for PrEP users, FPs and HRCs. One important step toward such a model has recently been done, as the Belgian social security has formally allowed the collaboration between FPs and HRCs for PrEP care. Ultimately, improving the sexual health and reducing HIV incidence should remain the primary objectives of comprehensive PrEP programs.

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List of abbreviations

AMC:	antimicrobial consumption
AMR:	antimicrobial resistance
aOR:	adjusted odds ratio
AZM:	azithromycin
Biochem:	biochemical tests
CDC:	United States Center for Diseases Control and Prevention
CI:	confidence interval
CLSI:	Clinical and Laboratory Standards Institute
COVID:	coronavirus disease 2019
CRO:	ceftriaxone
CT:	*Chlamydia trachomatis*
DDD:	defined daily dose
DID :	defined daily dose/1000 individuals/day
DNA:	deoxyribonucleic acid
DoxyPEP:	doxycycline post-exposure prophylaxis
DSMB:	data and safety monitoring board
(E)IUSTI :	(European) International Union against Sexually Transmitted Infections
EUCAST:	European Committee on Antimicrobial Susceptibility Testing
Euro-GASP:	European Gonococcal Antimicrobial Surveillance Programme
FAMHP:	Federal Agency for Medicines and Health Products
FGD:	focus group discussion
FP:	family physician
FU :	follow-up
GAD-2:	generalized anxiety disorder 2-item
GHB/GBL:	gamma-hydroxybutyrate/gamma-butyrolactone
GP:	general practitioner
GS:	gram staining
HGT:	horizontal gene transfer
HIV:	human immunodeficiency virus
HRC:	HIV Reference Center
ICF:	informed consent form
IDI:	in-depth interview
IM:	intra-muscular
iPrEX:	pre-exposure Prophylaxis Initiative
IQR:	interquartile range
IR(R) :	incidence rate (ratio)
IRB :	institutional review board
ITM:	Institute of Tropical Medicine

ITT:	intention to treat
KCE:	Belgian Healthcare Knowledge Center
LGV:	*Lymphogranuloma Venereum*
LTFU :	lost to follow up
MALDI-TOF:	matrix-assisted laser desorption/ionization-time of flight
MIC:	minimum inhibitory concentration
MLS:	macrolide lincosamides streptogramines
MSM:	men who have sex with men
NA:	not applicable
NAAT:	nuclei acid amplification tests
Nc:	*Neisseria cinerea*
NG/Ng:	*Neisseria gonorrhoeae*
Nl*:*	*Neisseria lactamica*
Nm*:*	*Neisseria meningitidis*
Nma*:*	*Neisseria macacae*
Nmu*:*	*Neisseria mucosa*
No*:*	*Neisseria oralis*
Np*:*	*Neisseria polysaccharea*
Npe*:*	*Neisseria perflava*
Ns*:*	*Neisseria subflava*
Nsi*:*	*Neisseria sicca*
NS:	not specified
O:	oxydase tests
PEP:	post-exposure prophylaxis
PHQ-2:	patient health questionnaire 2-item
PHQ-9:	patient health questionnaire 9-item
PP:	per protocol
PrEP:	pre-exposure prophylaxis
PRISMA:	preferred reporting items for systematic reviews and meta-analyses
PWID	people who inject drugs
RAM:	resistance-associated mutation
RCT:	randomized controlled trial
RR:	rate ratio
STD:	sexually transmitted disease
STI:	sexually transmitted infection
TasP:	treatment as prevention
USA:	United States of America
WHO:	World Health Organization
3MMC:	3-Methylmethcathinone

Curriculum vitae

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CURRENT OCCUPATION:

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PREVIOUS WORK EXPERIENCES

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OTHER TRAINING

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- Spanish: near native

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Scientific conferences

Oral presentations

Does screening for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* affect the incidence of these infections in MSM taking HIV-PrEP? Results from a randomized, multicenter, controlled trial. Presented at the 2023 STI & HIV World Congress, 24-28 July 2023. Chicago, United States.

Self-sampling with oral rinse to detect oropharyngeal *Neisseria gonorrhoeae* among men who have sex with men (SSONG study): preliminary results. Presented at the 2023 Hepatitis and HIV conference, 13-15 November 2023. Madrid, Spain.

Poster presentations

Why do PrEP users not return for their follow-up? A mixed methods survey in a PrEP clinic in Antwerp, Belgium. Presented at the 18TH European AIDS Conference, 27-30 October 2021. Online.

Why do PrEP users not return for their follow-up? A mixed methods survey in a PrEP clinic in Antwerp, Belgium. Presented at the 9th Belgian Research AIDS & HIV Consortium Symposium, 30 November 2021. La Hulpe, Belgium.

STI-Prophylaxis awareness and use among HIV-PrEP users in Belgium. Presented at the 23rd International Union against Sexually Transmitted Infections, 4-7 September 2022. Victoria Falls, Zimbabwe

STI-Prophylaxis awareness and use among HIV-PrEP users in Belgium. Presented at the 10th Belgian Research AIDS & HIV Consortium Symposium, 23 November 2022. La Hulpe, Belgium.

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