Effect of screening for Neisseria gonorrhoeae and Chlamydia trachomatis on incidence of these infections in men who have sex with men and transgender women taking HIV pre-exposure prophylaxis (the Gonoscreen study) : results from a randomised, multicentre, controlled trial

Title - The effect of screening for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* on the incidence of these infections in men who have sex with men and transgender women taking HIV pre-exposure prophylaxis (PrEP): results from a randomized, multicentre controlled trial (the Gonoscreen study)

Authors –Thibaut Vanbaelen, Achilleas Tsoumanis, Eric Florence, Christophe Van Dijck, Diana Huis in 't Veld, Anne-Sophie Sauvage, Natacha Herssens, Irith De Baetselier, Anke Rotsaert, Veronique Verhoeven, Sophie Henrard, Yven Van Herrewege, Dorien Van den Bossche, Jean-Christophe Goffard, Elizaveta Padalko, Thijs Reyniers, Bea Vuylsteke, Marie-Pierre Hayette, Agnes Libois*, Chris Kenyon*

*These authors contributed equally

Affiliations –

Department of clinical sciences, Institute of Tropical Medicine, Antwerp, Belgium: T. Vanbaelen MD, Prof. C. Kenyon PhD, N. Herssens, I. De Baetselier PhD, A. Tsoumanis PhD, Yven Van Herrewege PhD, E. Florence PhD, C. Van Dijck PhD, D. Van den Bossche PhD

Department of General Internal Medicine, Infectious Diseases and Tropical Medicine, University Hospital of Antwerp, Belgium: E. Florence PhD

Department of Infectious Diseases, Ghent University Hospital, Gent, Belgium: D. Huis in 't Veld PhD

Department of Infectious Diseases, Liège University Hospital, Liège, Belgium: A-S. Sauvage

Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium: A. Rotsaert, T. Reyniers PhD, B. Vuylsteke PhD

Department of Family Medicine and Population Health, University of Antwerp, Antwerp, Belgium: Prof. V. Verhoeven PhD

Department of Infectious Diseases, Saint Pierre University Hospital, Université Libre de Bruxelles, Brussels, Belgium.: A. Libois

Department of Infectious Diseases, Erasme University Hospital, Université Libre de Bruxelles, Brussels: Belgium: S. Henrard, Prof. J-C. Goffard PhD

Department of Medical Microbiology, Ghent University Hospital, Ghent, Belgium: E. Padalko PhD

Department of Clinical Microbiology, Liège University Hospital, Belgium: M-P. Hayette

Correspondence to – Thibaut Vanbaelen; Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium; tvanbaelen@itg.be; +32 (0) 3 345 58 14.

Funding – Belgian Healthcare Knowledge Center (KCE - INV18-1133)

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.

Funding

Belgian Healthcare Knowledge Center

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* and *Chlamydia trachomatis* 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 *N. gonorrhoeae*. 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

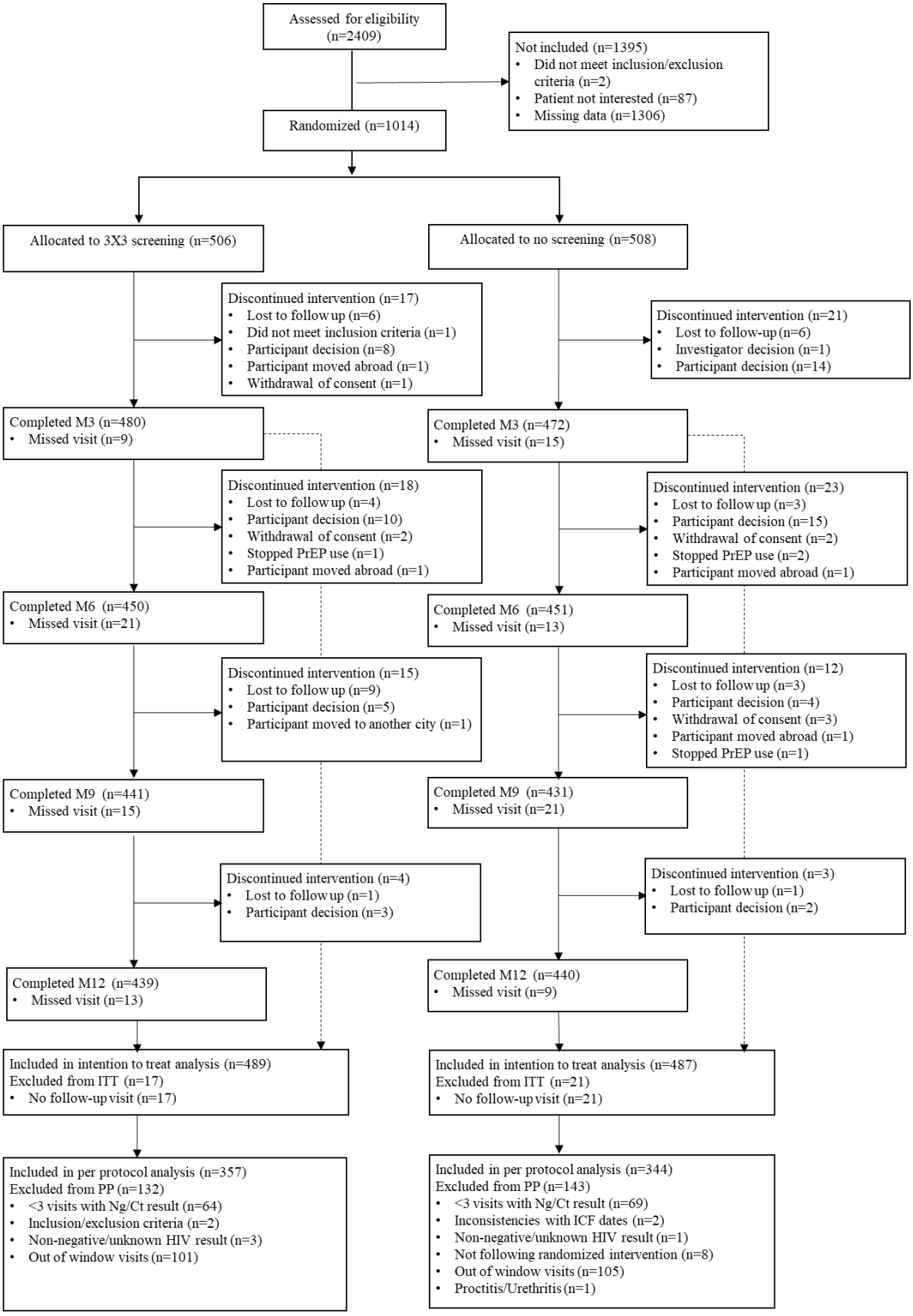

Figure 2 - Forest plot of the incidence rate ratios (IRR) of *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) infections in the primary and sensitivity analyses. The vertical dotted line represents the non-inferiority margin of 1·25

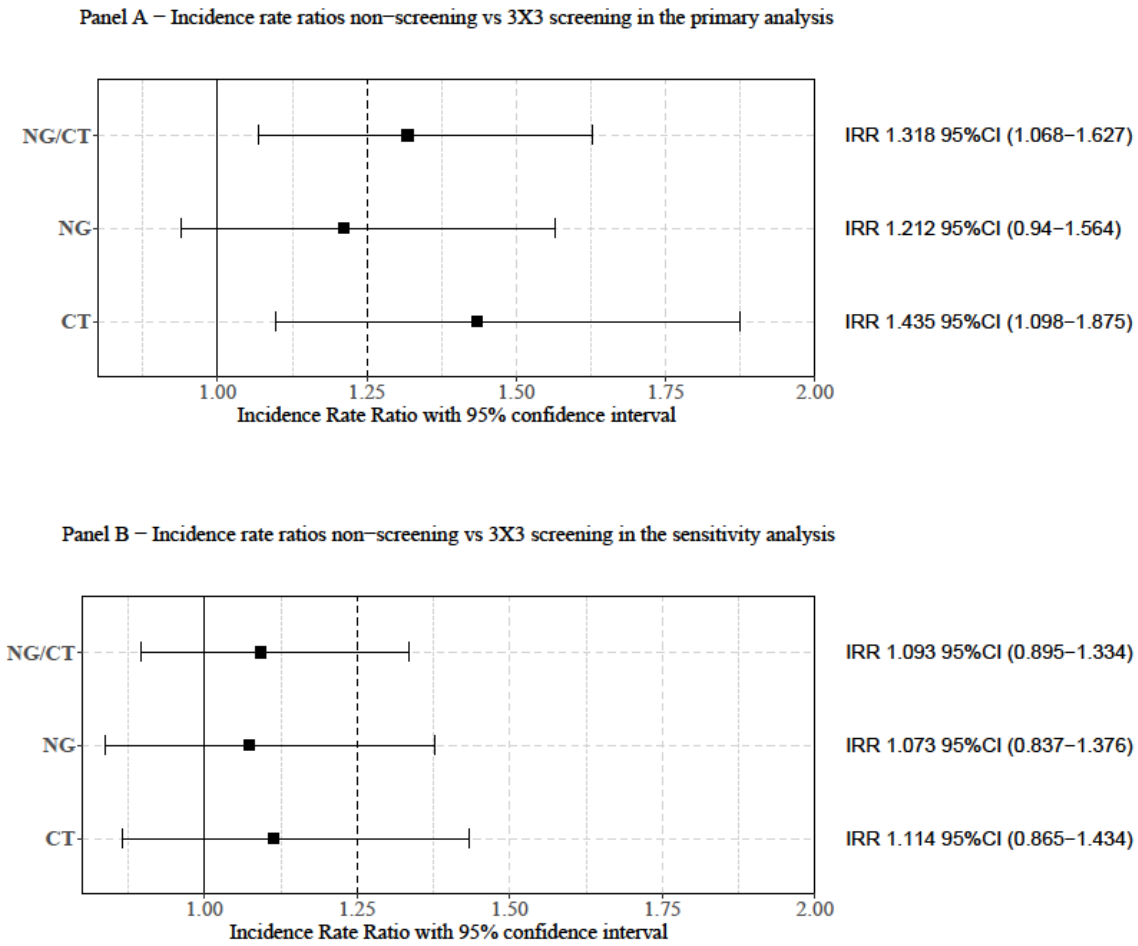

Table 1 – Baseline characteristics in both study arms

	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%)

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

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**

References

1. Andermann A, Blancquaert I, Beauchamp S, Déry V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ [Internet]. 2008 Apr [cited 2023 Mar 10];86(4):317. Available from: /pmc/articles/PMC2647421/

2. Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, et al. Screening for Chlamydia and Gonorrhea: US Preventive Services Task Force Recommendation Statement. JAMA [Internet]. 2021 Sep 14 [cited 2023 Mar 23];326(10):949–56. Available from: https://pubmed.ncbi.nlm.nih.gov/34519796/

3. Hocking JS, Temple-Smith M, Guy R, Donovan B, Braat S, Law M, et al. Population effectiveness of opportunistic chlamydia testing in primary care in Australia: a cluster-randomised controlled trial. The Lancet [Internet]. 2018 Oct 20 [cited 2021 Apr 28];392(10156):1413–22. Available from: https://pubmed.ncbi.nlm.nih.gov/30343857/

4. Van Den Broek IVF, Van Bergen JEAM, Brouwers EEHG, Fennema JSA, Götz HM, Hoebe CJPA, et al. Effectiveness of yearly, register based screening for chlamydia in the Netherlands: Controlled trial with randomised stepped wedge implementation. BMJ (Online) [Internet]. 2012 Aug 11 [cited 2021 Apr 28];345(7869). Available from: http://www.bmj.com/permissionsSubscribe:http://www.bmj.com/subscribeBMJ2012;345:e4316doi:10.1136/bmj.e4316

5. Williams E, Williamson DA, Hocking JS. Frequent screening for asymptomatic chlamydia and gonorrhoea infections in men who have sex with men: time to re-evaluate? Lancet Infect Dis [Internet]. 2023 [cited 2023 Oct 3]; Available from: https://pubmed.ncbi.nlm.nih.gov/37516129/

6. Marcus U, Mirandola M, Schink SB, Gios L, Schmidt AJ. Changes in the prevalence of self-reported sexually transmitted bacterial infections from 2010 and 2017 in two large European samples of men having sex with men – is it time to re- evaluate STI-screening as a control strategy ? PLoS One [Internet]. 2021;1–24. Available from: http://dx.doi.org/10.1371/journal.pone.0248582

7. Vickers DM, Osgood ND. The arrested immunity hypothesis in an immunoepidemiological model of Chlamydia transmission. Theor Popul Biol [Internet]. 2014 [cited 2023 Mar 9];93:52–62. Available from: https://pubmed.ncbi.nlm.nih.gov/24513099/

8. Tang EC, Vittinghoff E, Philip SS, Doblecki-Lewis S, Bacon O, Chege W, et al. Quarterly screening optimizes detection of sexually transmitted infections when prescribing HIV preexposure prophylaxis. AIDS [Internet]. 2020 Jul 1 [cited 2023 Mar 23];34(8):1181–6. Available from: https://journals.lww.com/aidsonline/Fulltext/2020/07010/Quarterly_screening_optimizes_detection_of.9.aspx

9. Center for Disease Contol and Prevention. Preexposure prophylaxis for the prevention of hiv infection in the united states - 2021 update. Atlanta; 2021.

10. Kenyon C, De Baetselier I, Wouters K. Screening for STIs in PrEP cohorts results in high levels of antimicrobial consumption. Int J STD AIDS [Internet]. 2020 Oct 1 [cited 2020 Nov

25];31(12):1215–8. Available from: http://journals.sagepub.com/doi/10.1177/0956462420957519

11. Vanbaelen T, Van Dijck C, De Baetselier I, Florence E, Reyniers T, Vuylsteke B, et al. Screening for STIs is one of the main drivers of macrolide consumption in PrEP users [Internet]. International Journal of STD and AIDS London: SAGE Publications; Jun 17, 2021 p. 1183–4. Available from: https://journals.sagepub.com/doi/full/10.1177/09564624211025940

12. Kenyon C, Manoharan-Basil SS, Van Dijck C. Is There a Resistance Threshold for Macrolide Consumption? Positive Evidence from an Ecological Analysis of Resistance Data from Streptococcus pneumoniae , Treponema pallidum, and Mycoplasma genitalium. Microbial Drug Resistance [Internet]. 2021 [cited 2021 Mar 11];1079–86. Available from: www.liebertpub.com

13. Van Dijck C, Laumen J, Zlotorzynska M, Manoharan-Basil SS, Kenyon C. Association between STI screening intensity in men who have sex with men and gonococcal susceptibility in 21 States in the USA: An ecological study. Sex Transm Infect [Internet]. 2020 Nov 1 [cited 2021 Mar 17];96(7):537–40. Available from: https://pubmed.ncbi.nlm.nih.gov/32066589/

14. Lewis DA. The role of core groups in the emergence and dissemination of antimicrobial-resistant N gonorrhoeae. Sex Transm Infect [Internet]. 2013 [cited 2021 Apr 28];89:iv47–51. Available from: http://sti.bmj.com/

15. De Baetselier I, Cuylaerts V, Smet H, Abdellati S, De Caluwe Y, Taïbi Amina, et al. Neisseria gonorrhoeae antimicrobial resistance surveillance report of Belgium –2022. Antwerp; 2022.

16. De Baetselier I, Vuylsteke B, Reyniers T, Smet H, Van den Bossche D, Kenyon C, et al. Worryingly high prevalence of resistance-associated mutations to macrolides and fluoroquinolones in Mycoplasma genitalium among men who have sex with men with recurrent sexually transmitted infections. Int J STD AIDS. 2019;2022(4):385–90.

17. Elena H, Eppälä S, Imo T, Laukka K, Aana J, Uopio -V V, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A Streptococci in Finland. Vol. 337, The New England Journal of Medicine © Copyr ight. 1997.

18. Jespers V, Stordeur S, Desomer A, Carville S, Jones C, Lewis S, et al. Diagnosis and management of gonorrhoea and syphilis. Good Clinical Practice (GCP). Brussels; 2019.

19. Barbee LA, Khosropour CM, Soge OO, Hughes JP, Haglund M, Yeung W, et al. The Natural History of Rectal Gonococcal and Chlamydial Infections: The ExGen Study. Rectal Gonorrhea and Chlamydia Natural History • cid [Internet]. 2022:1549. Available from: https://doi.org/10.1093/cid/ciab680

20. Chow EPF, Camilleri S, Ward C, Huffam S, Chen MY, Bradshaw CS, et al. Duration of gonorrhoea and chlamydia infection at the pharynx and rectum among men who have sex with men: A systematic review. Sex Health. 2016;13(3):199–204.

21. Vuylsteke B, Reyniers T, De Baetselier I, Nöstlinger C, Crucitti T, Buyze J, et al. Daily and event-driven pre-exposure prophylaxis for men who have sex with men in Belgium: results of a prospective cohort measuring adherence, sexual behaviour and STI incidence. J Int AIDS Soc [Internet]. 2019 Oct 30 [cited 2020 Nov 10];22(10). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jia2.25407

22. Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. Clinical Infectious Diseases [Internet]. 2005 [cited 2021 May 10];41(1):67–74. Available from: https://academic.oup.com/cid/article/41/1/67/325287

23. Williamson DA, Chow EPF, Gorrie CL, Seemann T, Ingle DJ, Higgins N, et al. Bridging of Neisseria gonorrhoeae lineages across sexual networks in the HIV pre-exposure prophylaxis era. Nature Communications 2019 10:1 [Internet]. 2019 Sep 5 [cited 2023 Oct 3];10(1):1–10. Available from: https://www.nature.com/articles/s41467-019-12053-4

24. Duan QI, Carmody CI, Donovan BI, Guy ID RJ, HuiID BB, Kaldor JM, et al. Modelling response strategies for controlling gonorrhoea outbreaks in men who have sex with men in Australia. New South Wales Health Pathology [Internet]. 2021; Available from: https://doi.org/10.1371/journal.pcbi.1009385

25. Unemo M, Ross J, Serwin AB, Gomberg M, Cusini M, Jensen JS. 2020 European guideline for the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS [Internet]. 2020 [cited 2021 May 14];1–17. Available from: https://www.who.int/reproductivehealth/

26. Traeger MW, Cornelisse VJ, Asselin J, Price B, Roth NJ, Willcox J, et al. Association of HIV Preexposure Prophylaxis With Incidence of Sexually Transmitted Infections Among Individuals at High Risk of HIV Infection. JAMA [Internet]. 2019;321(14):1380–90. Available from: https://jamanetwork.com/

27. Kenyon CR, Schwartz IS. Effects of sexual network connectivity and antimicrobial drug use on antimicrobial resistance in neisseria gonorrhoeae. Emerg Infect Dis [Internet]. 2018 Jul 1 [cited 2021 Apr 28];24(7):1195–203. Available from: https://doi.org/10.3201/eid2407.172104

28. Brunham RC, Rekart ML. The arrested immunity hypothesis and the epidemiology of chlamydia control. Sex Transm Dis. 2008;35(1):53–4.

29. Jenness SM, Weiss KM, Goodreau SM, Gift T, Chesson H, Hoover KW, et al. Incidence of Gonorrhea and Chlamydia Following Human Immunodeficiency Virus Preexposure Prophylaxis Among Men Who Have Sex With Men: A Modeling Study. Clin Infect Dis [Internet]. 2017 Sep 1 [cited 2023 Mar 10];65(5):712–8. Available from: https://pubmed.ncbi.nlm.nih.gov/28505240/

30. Tsoumanis A, Hens N, Kenyon CR. Is Screening for Chlamydia and Gonorrhea in Men Who Have Sex with Men Associated with Reduction of the Prevalence of these Infections? A Systematic Review of Observational Studies. Sex Transm Dis. 2018;45(9):615–22.

31. Reyniers T, Rotsaert A, Thunissen E, Buffel V, Masquillier C, Van Landeghem E, et al. Reduced sexual contacts with non-steady partners and less PrEP use among MSM in Belgium during the first weeks of the COVID-19 lockdown: Results of an online survey. Sex Transm Infect [Internet]. 2020 [cited 2021 May 18]; Available from: https://pubmed.ncbi.nlm.nih.gov/33172917/

32. Traeger MW, Mayer KH, Krakower DS, Gitin S, Jenness SM, Marcus JL. Potential impact of doxycycline post-exposure prophylaxis prescribing strategies on incidence of bacterial sexually transmitted infections. Clinical Infectious Diseases [Internet]. 2023 Aug 18;ciad488. Available from: https://doi.org/10.1093/cid/ciad488

33. Vanbaelen T, Tsoumanis A, Kenyon C. Total antimicrobial consumption in doxyPEP cohorts depends on the intensity of screening for bacterial sexually transmitted infections. Clinical Infectious Diseases [Internet]. 2023 Sep 18 [cited 2023 Oct 5]; Available from: https://dx.doi.org/10.1093/cid/ciad553

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)

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	.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				

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

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