Author's response to reviews

Title: High co-occurrence of anorectal chlamydia with urogenital chlamydia in women visiting an STI clinic revealed by routine universal testing in an observational study; a recommendation towards a better anorectal chlamydia control in women.

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Author's response to reviews: see over
Dear editor,

Thank you for giving us the opportunity to revise our manuscript.

We also want to thank the reviewers for their very useful and helpful comments, which we addressed below. We have revised the manuscript according to the reviewers’ and editors’ comments, all comments were processed in the manuscript.

Sincerely,

On behalf of coauthors,

Geneviève van Liere.
Editor's Comments:

1. How do they know that the NAAT positive results from the anal swabs represent anorectal infection versus contamination from genital infection? Could the authors please specifically address this issue further in the discussion which is currently only brief.

Thank you for this comment. We added sentences in the discussion; ‘The large percentage of concurrent urogenital and anorectal chlamydia infections in women was therefore notable: 95% of women with anorectal chlamydia also had urogenital chlamydia, and 71% of women with urogenital chlamydia also had anorectal chlamydia. Previous studies without routine universal testing in women also reported large shares of concurrent infections (36-90%) [2, 4-10, 26]. It is not clear what causes these concurrent infections, although possible explanations could be underreporting of anal sex, autoinoculation with vaginal secretions [4, 5, 8, 9, 26] or concurrent transmission during sex. Majority (71%) of anorectal chlamydia positives did not report anal sex or symptoms. Autoinoculation from the vagina to the rectum therefore seems possible. We hypothesize that autoinoculation could also occur from the rectum to the vagina. Such (repeat) urogenital infections could lead to reproductive tract morbidity [6]. Further study on this subject is needed, for example by including anorectal chlamydia in mathematical models and by bacterial load studies, the clinical and public health impact of anorectal chlamydia in women could be explored further. Nevertheless, state of the art practice in chlamydia control entails the use of highly sensitive NAATs to test for chlamydia. Although NAATs are not yet FDA proved for anorectal testing, their use is highly recommended, accepted, and part of standard operating procedures in many care settings [14]. A positive NAAT, i.e., diagnosed anorectal chlamydia, is in practice followed by antibiotic treatment. In MSM, an anorectal swab positive for chlamydia is considered an infection, and is treated with antibiotics to prevent transmission to the population and complications in individuals. To overcome current insufficient case management of anorectal infections in women, testing and treatment strategies need to be improved, to better identify and treat infections.’.

2. The authors raise the possibility of routine anorectal testing but this will increase costs substantially. This should be discussed.

Thank you for pointing this out. We added sentences in the discussion regarding cost implications: ‘Study participation was high (93%), suggesting a high feasibility and acceptability of anorectal testing in women who do not have an indication. Therefore, routine universal anorectal screening could be an option, although this will increase costs substantially. No studies have evaluated cost effectiveness of anorectal screening for chlamydia/gonorrhoea in women. However, in MSM, anorectal screening (when prevalence > 2.69% (IQR, 1.68-3.71%)) can be a cost-effective intervention to reduce HIV infection [28-30].’.
Response to the reviewers

Reviewer #1
Marjan Javanbakht

Reviewer’s report:

General comment
This manuscript describes anatomic site distribution of chlamydial infections by routine anorectal testing among women attending an STI clinic. Data on rectal STIs in women are limited and findings from this report would add to the small number of studies reporting on this topic. However, some of the methodological issues limit enthusiasm for this manuscript and the paper would benefit from clarification, especially in the methods and results section. Specific comments are described below.

Major compulsory revisions
1. Abstract, results section: I would suggest restating the sentence starting with "Its prevalence was similar for women with indication...” to highlight the fact that there was no statistically meaningful difference by indication group, rather than stating that the prevalence was similar. In fact, the reported prevalence by indication group of 7.9%, 4.2%, and 9.2% is different.

Thank you for this comment. We rephrased the sentence in the abstract as suggested by the reviewer, also incorporating comment 8; 'Prevalence of anorectal chlamydia was 7.9% (16/203) for women with indication and 8.6% (39/451) for all other women (P=0.74).’

2. Background section, first sentence: this sentence needs referencing/citation(s).

We followed the reviewer’s suggestion. The sentence has been rephrased; ‘Chlamydia trachomatis (Ct) and Neisseria gonorrhoeae (Ng) are the most prevalent bacterial sexually transmitted infections (STIs) in women in high income countries and have major public health consequences [1-3].’ Three references were added to the sentence.


3. Background section, fourth paragraph: the first sentence is unclear. Presumably you mean that data on 'universal' screening in women is limited, not because of lack of data collection but rather because of lack of screening. Please clarify the language.
Thank you for this suggestion. We have rephrased the sentence; ‘It is unknown whether selective testing on indication misses infections in the general female population, due to lack of studies in this population.’.

4. Methods section, study population: The percent of clinic population eligible for inclusion in this study seems unusually low; Of the 6,000 annual consultations, only 663 were eligible for inclusion in this study? Please clarify.

Thank you for this comment. We have rephrased the sentence in the methods section to clarify about the study procedures; ‘Between May 2012 and July 2013, three consultation nurses (out of 13) offered all their female patients aged 18 years and older (n=663) routine testing for urogenital and anorectal chlamydia and gonorrhoea. This yielded a total of 654 consultations by 611 women for analysis (participation 92.2%).’.
Also a sentence regarding possible bias due to this inclusion procedure was added in the discussion; ‘As women attending the STI clinic were randomly assigned to a consultation nurse, possible selection bias is likely minimal.’

5.1 Methods section, study procedure and definition: The definition of sexual high risk seems very narrow. After all, there are many behaviors besides transactional sex and swinger status that would fall in this category. Was information collected on other high risk sexual behaviors such as number of partners, new or concurrent partnerships, substance use, condom use, etc?

Unfortunately, data on other high risk sexual behaviors were not available. We have added a sentence in the discussion; ‘Data on other high risk sexual behaviors (i.e., number of partners, new or concurrent partnerships, substance use, condom use) were not available, and their association with anorectal chlamydia in women could not be assessed.’.

5.2 I would suggest renaming your high risk category as prostitution/swingers to more accurately reflect the data being considered.

Thank you for this suggestion. We have renamed ‘high risk’ category as ‘prostitutes/swingers’ category in the entire manuscript. For example; ‘Determinants tested were indication (with indication versus the two other categories combined), age categories, prostitutes/swingers (prostitutes and swingers versus other women), and use of fingers/toys (versus no use of fingers/toys).’.

6a.1 Methods section, statistical analysis:
a. Per the description it sounds like you created mutually exclusive ‘indication’ categories, though it seem that there may be some misclassification relating to this particular exposure category. Were there really no women who reported symptoms/anal sex AND fingers/toy use? If so, which category were they placed in.

Thank you for this comment. We have rephrased the sentences in the methods section describing the categories; ‘Women were assigned to one of three non-overlapping hierarchically constructed indication categories based on reported behaviour and symptoms. Women in the “indication” category reported at least anal symptoms and/or anal sex, whether or not in combination with anal use of fingers and/or toys. Women who were assigned to the “fingers/toys” category only reported the anal use of fingers and/or
toys and reported no anal symptoms and no anal sex. Women who reported no anal symptoms, no anal sex, and no anal use of fingers or toys were assigned to the "without indication" category.

6a.2 Furthermore, please clarify the time frame for these behaviors. Was this in the past 6 months?

We rephrased a sentence in the methods section for more clarity; 'Each consult also included a standardised medical and sexual history taken by trained study nurses. It asked about self-reported symptoms and sexual behaviour in the past six months, i.e., 'Did you practise anal sex in the past six months?'

6a.3 Relating to the indication categories, additional information on anal symptoms would be useful.

We follow the reviewers suggestion and added information on anal symptoms in the results section; 'Anal symptoms reported were itching (n=7), ulceration (n=3), redness (n=2), discharge (n=1), pain/burning sensation (n=5), bleeding (n=3) and unspecified (n=2). Only 3 women reported a combination of (two) symptoms.'

6b. Differences between indication categories were tested using chi-square methods, though you have some cell sizes that are small enough to warrant the use of an exact method such as Fisher's exact test

Thank you for this suggestion. We now use Fisher's exact test to compare the anatomic site distribution of chlamydia with indication categories, age and prostitutes/swingers. This was changed in the methods and results section, for example; 'Finally, to assess the anatomic site distributions of urogenital and anorectal chlamydia, all women who tested positive for chlamydia were assigned to a non-overlapping distribution category: (1) urogenital only, (2) urogenital and anorectal, or (3) anorectal only. Restricting to chlamydia positives, this variable was compared over indication categories (with indication versus the two other categories combined), age categories, and prostitutes/swingers using Fisher's exact test.'

6c. Sentence starting with "Determinants tested were...", I’m not sure what is meant by “distribution categories.” Please clarify.

Thank you for this comment. By distribution categories we mean the anatomic site distribution of chlamydia; urogenital only, anorectal only or combined urogenital/anorectal. We changed the order of analyses in the methods section, analyses on distribution categories were moved to the end; 'Finally, to assess the anatomic site distributions of urogenital and anorectal chlamydia, all women who tested positive for chlamydia were assigned to a non-overlapping distribution category: (1) urogenital only, (2) urogenital and anorectal, or (3) anorectal only. Restricting to chlamydia positives, this variable was compared over indication categories (with indication versus the two other categories combined), age categories, and prostitutes/swingers using Fisher’s exact test.'

6d. Reference category for age is listed as both #25 years and #29 years. Please clarify.
Thank you for noticing this discrepancy. Reference category #25 years was a typo. The sentence was rephrased; ‘The share of infections missed was compared between indication categories, age categories (reference ≥29 years) and prostitutes/swingers using univariate and multivariate logistic regression.’.

6e. You specify two regression analyses, though it’s unclear what is being modeled. In the first, you are examining factors associated with ‘missed infections’ and the second relates to anatomic site infection. Is this correct?

This is not correct, thank you for pointing this out. Previously we examined factors associated with ‘missed infections’ with regression analyses and we examined the anatomic site distribution using Chi2, since the outcome variable (distribution category) was categorical. We followed your advice (comment 8) and collapsed ‘without indication’ and ‘fingers/toys’ into one category to describe anorectal chlamydia prevalence. Since the indication category variable then becomes dichotomous for this analysis, we now use univariate and multivariate logistic regression examine factors associated with anorectal chlamydia. Determinants tested were indication (with indication versus the two other categories combined), age categories, prostitutes/swingers (prostitutes and swingers versus other women), and use of fingers/toys (versus no use of fingers/toys). Anorectal infections in the categories "without indication" and “fingers/toys” were defined as infections missed by selective testing on indication as in current care. The share of infections missed was compared between indication categories, age categories (reference ≥29 years) and prostitutes/swingers using univariate and multivariate logistic regression. Interactions terms were added between indication categories, age categories and prostitutes/swingers in the multivariate models, but none were statistically significant and were removed from in the final models.

Finally, to assess the anatomic site distributions of urogenital and anorectal chlamydia, all women who tested positive for chlamydia were assigned to a non-overlapping distribution category: (1) urogenital only, (2) urogenital and anorectal, or (3) anorectal only. Restricting to chlamydia positives, this variable was compared over indication categories (with indication versus the two other categories combined), age categories, and prostitutes/swingers using Fisher’s exact test.’.

7. Results section, first paragraph: prevalence of anal sex seems relatively high; again please clarify how this question was asked and/or how anal sex was defined and the time frame under consideration, lifetime, past 6-months, past 3-months, etc.

We rephrased a sentence in the methods section for more clarity; ‘Each consult also included a standardised medical and sexual history taken by trained study nurses. It asked about self-reported symptoms and sexual behaviour in the past six months, i.e., ‘Did you practise anal sex in the past six months?’.’

8. Results section, indication categories and chlamydia prevalence: This goes back to a comment from above relating to the abstract section. It seems that the prevalence of anorectal chlamydia is different by indication group, though you were not able to detect a statistically meaningful difference. Describing the prevalence as ‘similar’ is inappropriate/misleading given that there may not be enough of a sample size/power to
detect this difference. You report on a total of 48 people who indicated rectal finger/toys of whom two had rectal chlamydia. I would suggest collapsing your two indication categories into one group and doing a 2X2 comparison, rather than a 3X2 comparison.

Thank you for this suggestion. We follow your advice and collapsed ‘without indication’ and ‘fingers/toys’ into one category to describe anorectal chlamydia prevalence. Since the indication category variable then becomes dichotomous for this analysis, we now use univariate and multivariate logistic regression examine factors associated with anorectal chlamydia. We rephrased the sentences in the methods section; ‘Univariate and multivariate logistic regression were used to identify determinants independently associated with anorectal chlamydia. Determinants tested were indication (with indication versus the two other categories combined), age categories, prostitutes/swingers (prostitutes and swingers versus other women), and use of fingers/toys (versus no use of fingers/toys).’

We created a separate heading 'Chlamydia prevalence and associated determinants’ and added sentences in the results section and abstract; ‘Prevalence of anorectal chlamydia was 7.9% (16/203) for women with indication and 8.6% (39/451) for the other women (categories without indication and fingers/toys ) (P=0.74). Prevalence in the three indication categories is displayed in table 1.’. This also clarifies comment 6.

9.1 Results section, missed infections by selective testing indication: it seems that the differences noted in this section were not statistically meaningful (which again may relate to sample size), and it seems unnecessary to present Odds ratios/logistic regression results to reiterate this.

Thank you for this suggestion. We rephrased sentences in the results section; ‘No determinants were found to be associated with missed anorectal infections. For example the proportion missed was 60.0% in prostitutes/swingers versus 72.0% in other women (P=0.93). The proportion missed was 70.0% in age ≤ 21 years, 81.3% in age 22-28 years and 55.6% in age ≥ 29 years (P=0.47).’.

9.2 Also, it’s unclear if these results were from multivariable analysis or unadjusted models.

We rephrased sentences in the methods section; ‘The share of infections missed was compared between indication categories, age categories (reference ≥29 years) and prostitutes/swingers using univariate and multivariate logistic regression.’

10. Results section, anatomic site distribution: it's a bit difficult to follow this data. I would suggest using a figure to highlight your findings.

We follow your suggestion and added a figure to highlight our findings.
Figure 1. Anatomic site distribution of chlamydia in women attending the STI clinic routinely screened for urogenital and anorectal chlamydia.

Minor essential revisions
1. Abstract, results section: it would be useful to state the overall prevalence of chlamydia per anatomic site.

Thank you for this suggestion. We added the overall prevalence in the abstract; 'The overall prevalence was 11.2% (73/654) for urogenital chlamydia and 8.4% (55/654) for anorectal chlamydia.'

2. Background, second paragraph: define acronyms on first use – CT and NG in first line

We rephrased the first sentence in the background section; 'Chlamydia trachomatis (Ct) and Neisseria gonorrhoeae (Ng) are the most prevalent bacterial sexually transmitted infections (STIs) in women in high income countries and have major public health consequences [1-3].'

3. Methods section, study procedures and definition: The first sentence is only partially correct and needs to be revised. There have been no validation/verification studies of self-collected rectal samples among women. The references cited are for self-collected vaginal swabs and rectal swabs in MSM.
3.1 You may also need to address this potential limitation in your discussion.

We included three references in the first sentence of the study procedures; 'Women provided self-collected vaginal swabs and self-collected anorectal swabs, which studies have proven to be a generally acceptable, valid and feasible approach [3, 24, 25].'

One study compared a provider-collected rectal swab (PRS) with a self-collected rectal swab in MSM and women. The study conclusion is; 'SRS is a feasible, valid, and acceptable alternative for MSM and women attending STI clinics, and hence should be considered for other settings as well.'
However, self-collected rectal samples are not FDA cleared. We added a sentence in the discussion to address this issue; ‘Although NAATs are not yet FDA proved for anorectal testing, their use is highly recommended, accepted, and part of standard operating procedures in many care settings [14].’.

References.
Schachter J, Philip SS: Testing men who have sex with men for urethral infection with Chlamydia trachomatis and Neisseria gonorrhoeae is only half the job, and we need the right tools. Sex Transm Dis 2011, 38(10):925-927.

Level of interest: An article of importance in its field  
Quality of written English: Acceptable  
Statistical review: Yes, and I have assessed the statistics in my report.  
Declaration of competing interests:  
I declare that I have no competing interests
Reviewer #2.

Fabian Kong.

This study add important information on an understudied areas of co-infection of chlamydia at multiple sites and importantly among women.

**Major Compulsory Revisions**

- In the BACKGROUND ...
  1. first paragraph - the authors state “In addition to infection of the urogenital tract, chlamydia and gonorrhoea can also cause anorectal infections. These might play a major role in the persistence of urogenital STI infection and the transmission of STIs to others”. The paragraph is not clear but suggests that anorectal infections may play a major role in persisting STI but the word ‘persistence’ is best avoided and maybe replaced with ‘repeat positive’ infections. Persistence has specific meanings ie latent or ‘sleeping’ chlamydia trachomatis (CT) due to exposure to interferon, penicillins etc. where once selective pressures are removed eg removal of antibiotics, the latent CT can become viable and cause repeat positive.

*Thank you for this suggestion. The term ‘persisting’ is removed from the manuscript and replaced by ‘repeat positive urogenital infections’. We rephrased the sentences in the introduction; ‘The impact of anorectal infections in women on population (public health) and individual (clinical) level are yet unknown. However, it is suggested that treatment of anorectal infections in women can help limit the spread of STI in the population [4-6] and can reduce complications in infected individuals, such as anal cancer, anal squamous intraepithelial lesions [15, 16] and reduce HIV risk [5, 6]. Moreover, the rectum might act as a reservoir and thereby play a major role in repeat positive urogenital infections [4].’.*

References were added to the statement including two new references.


2. Second paragraph – the authors state “Treatment of anorectal infections in women can help limit the spread of infection in the population and can reduce complications in infected individuals, such as transmission to other body locations and possible reproductive tract morbidity”. I am not aware of large studies that have shown that the treatment of anorectal infections are the key drivers to cross infections at other sites and therefore anorectal treatment reduces transmission to other sites ie treating rectal infections will prevent endocervical infections for example. Does the authors know if this is the case? If so, this should be referenced.

*These references were added. We also rephrased the sentence in the introduction; ‘The impact of anorectal infections in women on population (public health) and individual*
(clinical) level are yet unknown. However, it is suggested that treatment of anorectal infections in women can help limit the spread of STI in the population [4-6] and can reduce complications in infected individuals, such as anal cancer, anal squamous intraepithelial lesions [15, 16] and reduce HIV risk [5, 6]. Moreover, the rectum might act as a reservoir and thereby play a major role in repeat positive urogenital infections [4].’ We elaborated on possible autoinoculation from anorectal infections in the discussion. See also the response to comment 3.

3. In this regard can the authors also confidently say that anorectal infections are the key drivers of CT incidence among women? Perhaps maybe in MSM populations but not sure about women. Please clarify.

No, we can’t yet confidently say that anorectal infections are key drivers. In the discussion we rephrased sentences to elaborate on the issues raised by the reviewer; ‘The large percentage of concurrent urogenital and anorectal chlamydia infections in women was therefore notable: 95% of women with anorectal chlamydia also had urogenital chlamydia, and 71% of women with urogenital chlamydia also had anorectal chlamydia. Previous studies without routine universal testing in women also reported large shares of concurrent infections (36-90%) [2, 4-10, 26]. It is not clear what causes these concurrent infections, although possible explanations could be underreporting of anal sex, autoinoculation with vaginal secretions [4, 5, 8, 9, 26] or concurrent transmission during sex. Majority (71%) of anorectal chlamydia positives did not report anal sex or symptoms. Autoinoculation from the vagina to the rectum therefore seems possible. We hypothesize that autoinoculation could also occur from the rectum to the vagina. Such (repeat) urogenital infections could lead to reproductive tract morbidity [6].

Further study on this subject is needed, for example by including anorectal chlamydia in mathematical models and by bacterial load studies, the clinical and public health impact of anorectal chlamydia in women could be explored further. Nevertheless, state of the art practice in chlamydia control entails the use of highly sensitive NAATs to test for chlamydia. Although NAATs are not yet FDA proved for anorectal testing, their use is highly recommended, accepted, and part of standard operating procedures in many care settings [14]. A positive NAAT, i.e., diagnosed anorectal chlamydia, is in practice followed by antibiotic treatment. In MSM, an anorectal swab positive for chlamydia is considered an infection, and is treated with antibiotics to prevent transmission to the population and complications in individuals. To overcome current insufficient case management of anorectal infections in women, testing and treatment strategies need to be improved, to better identify and treat infections.’.

4. They might like to be more prudent in their use of the term persistent eg in the discussion.

We follow the reviewers suggestion by deleting the term ‘persistent’ throughout the manuscript and replace it by ‘repeat positive urogenital infections’. See also the response to comment 3.

5. In the RESULTS there was two sentence with “data not shown” – one regarding fingers/toys not associated with anorectal chlamydia and secondly, prevalence of anorectal chlamydia was substantial (8%) and did not differ between women who do or
do not exhibit high risk behaviour”. Although the results do not need to be tabulated, some summary statistics should be provided in the text.

We follow the reviewers’ suggestion. We added summary statistics in the results section; ‘Being prostitute/swinger was not associated with anorectal chlamydia; prevalence was 3.0% (5/168) for prostitutes/swingers versus 10.3% (50/486) for other women (P=0.13). In total, 136 women reported to have used fingers or toys, whether or not in combination with anal sex. Prevalence in those women was 5.1% (7/136) versus 9.3% (48/518) in women who did not report to have used fingers or toys (P=0.82).’.

We rephrased the methods section; ‘Univariate and multivariate logistic regression were used to identify determinants independently associated with anorectal chlamydia. Determinants tested were indication (with indication versus the two other categories combined), age categories, prostitutes/swingers (prostitutes and swingers versus other women), and use of fingers/toys (versus no use of fingers/toys).’.

· DISCUSSION and CONCLUSION
6. In the ‘study limitations’ (last paragraph of the discussion) the authors may like to discuss the issue related to potential biases in their sampling § “non-participants were slightly younger than participants” and “Gonorrhoea infections were not observed in non-participants” (methods section). In the study by Sethupathi [1] the groups most at risk of positive rectal infections included those aged <20 years and those with proven gonorhoea.

Thank you for this comment. Sentences were rephrased in the methods section; ‘Non-participants were slightly younger than participants (median 21 years versus 23 years, P<0.001). Urogenital chlamydia prevalence was similar for non-participants and participants (13.5%; (7/52) versus 11.2% (73/654), P=0.60). Gonorrhoea infections were not observed in both groups.’.

We added sentences in the discussion; In our study, the prevalence or the proportion of infections missed by current selective testing in the non-participants is unknown. Eligible non-participants were slightly younger than participants. A study by Sethupathi et al. found women most at risk for anorectal infections included women aged <20 years as was also found in current study. Therefore, the prevalence of anorectal infections may be underestimated in current study, yet due to the high response (93%), bias is expected to be minimal.’.

7. Can the authors comment/discuss the implications of their methods and results of the one women who was in the ‘without indication’ category but was positive for rectal chlamydia?

Thank you for this comment. Of the 55 anorectal chlamydia infections, 52 were combined urogenital/anorectal and only 3 were (isolated) anorectal only infections. Of the 3 anorectal only infections, 2 were in women with indication and 1 was in women without indication. Of the 55 women with anorectal chlamydia, 37 were in the ‘without indication’ category. As I understand correctly, your comment is about the anorectal only infection in the woman without indication. We added sentences in the discussion to elaborate on this case; 'In contrast to MSM, anorectal chlamydia in women was rarely isolated. In current study, one woman had an isolated anorectal infection but did not report anal sex
or symptoms. Possible explanations for this could be underreporting, a false negative urogenital test [4, 8], or autoinoculation from a spontaneously cleared urethral/vaginal infection [4, 5].

8. The authors state this “this is the first study with routine universal anorectal testing in a general group of women who visited an STI clinic and who took different sexual risks, including anal use of fingers and toys”. Two other studies have been undertaken looking at coinfection among women – one old study in 1989 (using tests less sensitive than NAATS) [2] and later one in 2010 among women attending GUM clinic (recruitment did not include all eligible women)[1] and another in 2013[3]. The authors may like to revisit this comment and compare results with these studies – esp the latter ones.

Thank you for the references. Sethupathi et al. was already in the manuscript, we added the reference by Ding et al. The latter references, which also used highly sensitive NAATs, but did not routinely test women anorectally and applied used selective testing by indication. The recruitment by Sethupathi et al. did not include all eligible women, as you mentioned. The study by Ding et al. recruited women who tested positive for urogenital chlamydia. In this study we recruited all women, with or without indication, and with or without a urogenital chlamydia. To clarify routine universal anorectal testing, we rephrased the sentence in the discussion; ‘To our knowledge, this is the first study with routine universal anorectal testing, i.e. independent of reported behaviour, symptoms or urogenital positivity, in a general group of women who visited an STI clinic and who took different sexual risks, including anal use of fingers and toys.”.

Of major concern...

9. The author states “anorectal testing in all women with urogenital infection or direct treatment effective for both urogenital and non-urogenital chlamydia (e.g. doxycycline) would detect and treat 95% of anorectal infections”. While I agree doxycycline remains the current choice for anorectal chlamydia can the authors discuss Where the 95% values comes from?

We rephrased the sentence in the discussion to clarify on where the 95% comes from; ‘When a more restricted policy is preferred, anorectal testing only in women with urogenital infection or direct treatment effective for both urogenital and non-urogenital chlamydia would detect and treat 95% of anorectal infections, since 52 of 55 anorectal infections had co-occurrence of urogenital chlamydia.”.

10. Where is the evidence for doxycycline efficacy for rectal infections? I do not believe the recommendations in the conclusion on using doxycycline is currently based on population level evidence.

This is an important comment. Indeed population level evidence is still scarce, although standard operating procedures already commonly use doxycycline and the Netherlands even included it in the guidelines. We added sentences in the discussion to address this issue; ‘The substantial anorectal chlamydia prevalence and high co-occurrence with urogenital chlamydia fuels the need for debate on what is adequate treatment for anorectal chlamydia [18-21, 23]. The currently used treatment regimes for uncomplicated anorectal chlamydia both have drawbacks; higher treatment failure rates are reported for azithromycin [19-21, 23] and compliance for doxycycline could possibly be an issue in practice [27]. More research, for example a randomised controlled trial of
azithromycin versus doxycycline, including compliance, is needed to formulate treatment recommendations.’.

Furthermore, we rephrased sentences in the abstract and discussion referring to doxycycline as the appropriate treatment. For example in the conclusion; ‘When more restricted control measures are preferred, possible alternatives include (1) anorectal testing only in women with urogenital chlamydia (problem: treatment delay or loss to follow up), and (2) direct treatment for urogenital chlamydia that is effective for anorectal chlamydia as well.’.


Thank you for the reference, we added it in the discussion. We added a sentence in the discussion regarding compliance; ‘The currently used treatment regimes for uncomplicated anorectal chlamydia both have drawbacks; higher treatment failure rates are reported for azithromycin [19-21, 23] and compliance for doxycycline could possibly be an issue in practice [27]. More research, for example a randomised controlled trial of azithromycin versus doxycycline, including compliance, is needed to formulate treatment recommendations.’.

12. what are the options for pregnant women who cannot use doxycycline after 18 weeks gestation?

We rephrased sentences in the introduction; ‘Guidelines in the UK and US recommend both single-dose azithromycin and a 7-day course of doxycycline as equal treatments for uncomplicated anorectal chlamydia in non pregnant women [12].’.
For pregnant women, azithromycin or amoxicillin (500 mg orally three times a day for 7 days) are recommended. Since this is not the scope of the article, we did not add this guideline to the manuscript.

13. Again, in the conclusion the author recommends “doing anorectal testing for all women who test positive for urogenital chlamydia”. What evidence is there for this recommendation?

Thank you for this comment. We rephrased the sentence in the discussion; ‘When a more restricted policy is preferred, anorectal testing only in women with urogenital infection or direct treatment effective for both urogenital and non-urogenital chlamydia would detect and treat 95% of anorectal infections, since 52 of 55 anorectal infections had co-occurrence of urogenital chlamydia. However, for the former option delay between urogenital and anorectal tests and subsequent treatments could be a problem in practice.’.

We added sentences in the discussion; ‘It is not clear what causes these concurrent infections, although possible explanations could be underreporting of anal sex, autoinoculation with vaginal secretions [4, 5, 8, 9, 26] or concurrent transmission during sex. Majority (71%) of anorectal chlamydia positives did not report anal sex or symptoms. Autoinoculation from the vagina to the rectum therefore seems possible. We hypothesize that autoinoculation could also occur from the rectum to the vagina. Such (repeat) urogenital infections could lead to reproductive tract morbidity [6].’.
14. What are the cost implications? Perhaps routine testing among HIV positive women only with positive chlamydia might be better than a generalised testing regime as the rectal testing data in HIV positive MSM has shown benefit [5] and cost-effectiveness among this group possibly limited to patients who seek little or no screening outside of one clinic [6].

We added sentences in the discussion regarding cost implications, including the provided references; 'Study participation was high (93%), suggesting a high feasibility and acceptability of anorectal testing in women who do not have an indication. Therefore, routine universal anorectal screening could be an option, although this will increase costs substantially. No studies have evaluated cost effectiveness of anorectal screening for chlamydia/gonorrhoea in women. However, in MSM, anorectal screening (when prevalence > 2.69% (IQR, 1.68-3.71%)) can be a cost-effective intervention to reduce HIV infection [28-30].'

We do not believe in restricting anorectal screening to HIV positive women, since HIV prevalence is low in women in the Netherlands, especially in the general female population (non-high risk). In two previous studies HIV prevalence was 0% in high risk women (swingers and prostitutes).


15. See other comments regarding of their methods with possible cross contamination of self-collected samples, uncertainty regarding if the co-infection in women are auto-innoculations or new infections and how they reached this recommendation from this study design? The conclusion and recommendations needs to be discussed in detail regarding above and changed if there is no strong evidence to support them.

Thank you for this comment. The conclusion is rephrased and the recommendations are discussed in detail in the discussion; 'The large percentage of concurrent urogenital and anorectal chlamydia infections in women was therefore notable: 95% of women with anorectal chlamydia also had urogenital chlamydia, and 71% of women with urogenital chlamydia also had anorectal chlamydia. Previous studies without routine universal testing in women also reported large shares of concurrent infections (36-90%) [2, 4-10, 26]. It is not clear what causes these concurrent infections, although possible explanations could be underreporting of anal sex, autoinoculation with vaginal secretions [4, 5, 8, 9, 26] or concurrent transmission during sex. Majority (71%) of anorectal chlamydia positives did not report anal sex or symptoms. Autoinoculation from the vagina to the rectum therefore seems possible. We hypothesize that autoinoculation could also occur from the rectum to the vagina. Such (repeat) urogenital infections could lead to reproductive tract morbidity [6]. Further study on this subject is needed, for example by including anorectal chlamydia in mathematical models and by bacterial load
studies, the clinical and public health impact of anorectal chlamydia in women could be explored further. Nevertheless, state of the art practice in chlamydia control entails the use of highly sensitive NAATs to test for chlamydia. Although NAATs are not yet FDA proved for anorectal testing, their use is highly recommended, accepted, and part of standard operating procedures in many care settings [14]. A positive NAAT, i.e., diagnosed anorectal chlamydia, is in practice followed by antibiotic treatment. In MSM, an anorectal swab positive for chlamydia is considered an infection, and is treated with antibiotics to prevent transmission to the population and complications in individuals. To overcome current insufficient case management of anorectal infections in women, testing and treatment strategies need to be improved, to better identify and treat infections. Study participation was high (93%), suggesting a high feasibility and acceptability of anorectal testing in women who do not have an indication. Therefore, routine universal anorectal screening could be an option, although this will increase costs substantially. No studies have evaluated cost effectiveness of anorectal screening for chlamydia/gonorrhoea in women. However, in MSM, anorectal screening (when prevalence > 2.69% (IQR, 1.68-3.71%)) can be a cost-effective intervention to reduce HIV infection [28-30]. When a more restricted policy is preferred, anorectal testing only in women with urogenital infection or direct treatment effective for both urogenital and non-urogenital chlamydia would detect and treat 95% of anorectal infections, since 52 of 55 anorectal infections had co-occurrence of urogenital chlamydia. However, for the former option delay between urogenital and anorectal tests and subsequent treatments could be a problem in practice. The substantial anorectal chlamydia prevalence and high co-occurrence with urogenital chlamydia fuels the need for debate on what is adequate treatment for anorectal chlamydia [18-21, 23]. The currently used treatment regimes for uncomplicated anorectal chlamydia both have drawbacks; higher treatment failure rates are reported for azithromycin [19-21, 23] and compliance for doxycycline could possibly be an issue in practice [27]. More research, for example a randomised controlled trial of azithromycin versus doxycycline, including compliance, is needed to formulate treatment recommendations.’.

‘In conclusion, prevalence of anorectal chlamydia in women was high and current selective testing on indication is not an appropriate control strategy to identify and treat anorectal chlamydia infections. Almost all women with anorectal chlamydia had concurrent urogenital chlamydia. More research is needed on the public health and clinical implications of anorectal chlamydia in women.’.

· OTHERS
16. Unsure why patient symptoms were included in the analysis and group category since most rectal infections are asymptomatic (their own sample only 3.1% of sample had symptoms) and secondarily symptoms (and/or sexual history) remain a poor predictor of rectal chlamydia infection.[7-9]. Author may like to discuss this and perhaps run a sensitivity analysis removing those with symptoms and updating table 1? Analysis by symptoms as a variable seem improbable due to small sample size?

Patient symptoms were included in the analyses together with reported anal sex because this is in many settings used as the indication for anorectal testing among women. Because this selective testing on indication misses over half of anorectal infections in MSM and high risk women (swingers), we routinely tested all women for anorectal chlamydia and gonorrhoea in this study, in addition to standard urogenital testing. One main conclusion in this study is that selective testing on indication misses 71% of
anorectal chlamydia in women visiting the STI clinic; high risk women such as swingers and prostitutes as well as the general (non high risk) female population.

17. The authors talked about possible specimen contamination by the women as they self-collected samples in their study limitations. Can the authors discuss the issues about the lack of use of serovar testing to determine that coinfections among women were either the same CT genotype (auto inoculation/re infection) and what are the possibility that rectal and cervical infections possibly might be different infections by different partners, given the sample included high risk patients. Of note, a study looking at the possible transmission of HPV by fingers (ie testing from finger tips and finger nails)[10] might be considered by the authors in future studies to use more intense laboratory tests to confirm possible transmission by this route.

Thank you for this suggestion. We added a sentence in the discussion to make recommendations for future research, including more intense laboratory tests; 'Further study on this subject is needed, for example by including anorectal chlamydia in mathematical models and by bacterial load studies, the clinical and public health impact of anorectal chlamydia in women could be explored further.’.

Serovar testing was not carried out, but could be useful to determine whether concurrent infections have the same serovar. In case the serovars are not equal, different transmission events are likely. However, no conclusions can be drawn in case the serovars are equal. In that case the infection could be an auto inoculation or (re) infection from a sex partner. Moreover, a previous study among women found low prevalence of multiple serovar infections in women with urogenital and anorectal chlamydia [ref].’.


Discretionary Revisions
Table 1: the # footnote is linked to ‘indication’ (defined as anal sex and/or symptoms) but the text also describes use of toys/fingers. Is the data/comment better separated so that “indication” only includes anal sex and/or Sx and ‘fingers/toys’ includes data related to ‘fingers/toys’?

Selective testing on indication (category indication) is based on the self report of anal sex and/or symptoms. The categories were based on these current guidelines. A category was added for women who used fingers/toys only. The women in the indication category all reported anal sex and/or symptoms, and a proportion of them also reported the use of fingers and/toys. This proportion is added in the footnote. For better clarity we added the word ‘only’ to the fingers/toys category to emphasize that these women did not report anal sex or symptoms and would not be tested if selective testing on indication was applied. See also our response to comment 16.
The term “restriction chlamydia positive” is slightly confusing and unclear. Can this be better labelled for example “positive anorectal samples only” or the like?

*Thank you for this suggestion. We replaced the term by ‘Anatomic site distribution chlamydia positives’.*

Conflict of interest: none

Fabian Kong