RESEARCH NOTE

REVISED Association between intensity of STI screening and development of antimicrobial resistance in *N. gonorrhoeae* in 12 cities in the USA: An ecological study [version 4; referees: 2 approved]

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Abstract
In this study, we assessed if there was a city-level association between sexually transmitted infection (STI) screening intensity in men who have sex with men and antimicrobial sensitivity in *Neisseria gonorrhoeae* in the United States, 2007 to 2013. We found positive associations between STI screening intensity and increases in minimum inhibitory concentrations for certain antimicrobials. Not all positive associations were statistically significant and the associations found to be statistically significant varied between the different analyses. Further studies are therefore required to assess if there is a causal relationship between the intensity of STI screening in MSM and gonococcal resistance.

Keywords
N. gonorrhoeae; STI screening; antimicrobial resistance; MSM

Open Peer Review

Referee Status: ✓ ✓

	Invited Referees 1	2
REVISED version 4 published 31 Oct 2018	✓ report	
REVISED version 3 published 16 Oct 2018	✗ report	
REVISED version 2 published 27 Sep 2018	✗ report	✓ report
version 1 published 10 Aug 2018	? report	? report

1 **Edward Goldstein**, Harvard T.H. Chan School of Public Health, USA

2 **Ellen Stobberingh**, Maastricht University Medical Centre (MUMC) , The Netherlands

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Comments (0)

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Author roles: Kenyon CR: Conceptualization, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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First published: 10 Aug 2018, **7**:1237 (https://doi.org/10.12688/f1000research.15569.1)

REVISED **Amendments from Version 3**

The abstract has been rewritten to more accurately reflect the findings

See referee reports

Introduction

In the United States (USA) the prevalence of antimicrobial resistance in *Neisseria gonorrhoeae* has typically been higher in men who have sex with men (MSM) than men who have sex with women (MSW) and women[1,2]. It has also frequently been noted to be highest in the West and lowest in the South[1–3]. Resistance has characteristically emerged in the West Coast and Hawaii and then spread eastward[1–3]. This patterning of spread has led to the view that a primary driver of resistance is the import of resistant gonococci from eastern Asia and other world regions[3]. In support of this theory, a number of studies have documented travel as a means of import of resistance in the USA[4,5]. A systematic review of risk factors associated with resistance in *N. gonorrhoeae,* however, found that a history of sex with partners abroad was associated with resistance in 6 studies and was not associated with resistance in 7 studies[6]. Furthermore, the evidence that travel plays a seminal role in the emergence of resistance in MSM is not that compelling. An analysis of data from the Gonococcal Isolate Surveillance Project (GISP) 2002 to 2007, for example, found a pronounced increase in ciprofloxacin-resistance in MSM and a smaller and later increase in MSW; the association with recent travel was negative in MSM and borderline positive in MSW[7].

Antimicrobial resistance results largely from exposure to antimicrobials[8,9]. This has been extensively documented *in vitro* and *in vivo* but for various reasons antimicrobial pressure at a population level may be more important than at an individual level in determining risk of development of antimicrobial resistance[8,10,11]. In the case of *N. gonorrhoeae,* extensive antimicrobial exposure in a population would be predicted to result in a high prevalence of resistance genes in the pharyngeal microbiomes that could then be taken up (via transformation) by *N. gonorrhoeae* and thereby provide it with a fitness conferring resistant phenotype in the setting of ongoing high antimicrobial consumption[12]. These insights have provided the rationale for ecological level studies that have generally found strong associations between the intensity of antimicrobial use and the prevalence of resistance to that antimicrobial[8,13]. A recent study from the USA however found no association between an increase in *N. gonorrhoeae* minimum inhibitory concentration (MIC) for azithromycin, ceftriaxone, cefixime and ciprofloxacin in the 23 GISP sites and the consumption of antimicrobials in the surrounding county[3]. A weakness of this study design was the use of total-consumption-of-antimicrobials by the entire county population as the explanatory variable. Since resistance has repeatedly emerged in certain MSM populations, it would be prudent to assess if this emergence is correlated with antimicrobial consumption in this group rather than the entire population. One major driver of antimicrobial consumptions in MSM is sexually transmitted infection (STI) screening. Because most *N. gonorrhoeae* and *Chlamydia trachomatis* in MSM are carried asymptomatically in the anorectum and oropharynx, screening for these STIs may result in a large increase in antimicrobial exposure. A modeling study for example found that increasing annual gonorrhea/chlamydia screening in an MSM population from 3 to 50% would result in a 11-fold increase in antimicrobial exposure[14]. In this exploratory paper we hypothesized that the intensity of STI testing plays a role in the genesis of resistance in MSM via the associated increase in antibiotic exposure.

Methods

We assessed if there was an ecological-city-level-association between the intensity of STI testing in MSM in the USA and the development of antimicrobial resistance in *N. gonorrhoeae*.

Data for STI screening was taken from the 2005, 2008 and 2011 National HIV Behavioral Surveillance MSM (NHBS-MSM) studies. These cross-sectional surveys done in 21 cities asked respondents about STI testing in the preceding 12 months. The 2005 survey (n=10,030) asked if respondents had been tested for syphilis/gonorrhea/another-STI during the preceding 12 months (single question), the 2008 survey (n=8,175) if they had been tested for syphilis in the preceding 12 months, and the 2011 survey (n=8,012) if they had been tested for gonorrhea, chlamydia or syphilis in the previous 12 months (3 questions).

Data for the change in city geometric mean *N. gonorrhoeae* MIC between 2005 and 2013 was taken from GISP data[3]. The geometric mean MIC was calculated as the *n*th root of the product of *n* MIC values. Spearman's correlation was used to assess if there was an association between (1) the prevalence of STI testing in each survey and the increase in geometric mean MIC of cefixime, ceftriaxone and azithromycin in *N. gonorrhoeae* between 2005 and 2013 and (2) the percent reporting screening for any STI in the 2011 survey and geometric mean MIC for the three antimicrobials in the following year. These three antibiotics were chosen since these were the recommended antibiotics for *N. gonorrhoeae* therapy since 2007[1]. All analyses were conducted in STATA 13.

Results

Twelve cities participated in both the NHBS-MSM and GISP surveys (n=9 for 2005, n=12 for 2008, n=12 for 2011). The intensity of self-reported STI testing in 2005 varied between 27% and 56% (median 43%, IQR 39–49). There was little change in the relative positions of the cities in terms of testing intensity between 2005 and 2008 (rho=0.87, p=0.002) and 2005 to 2011 (rho=0.81, p=0.008). Cities in the West tended to have higher STI testing rates than cities in the South (Figure 1). In 2011, the percent reporting testing for gonorrhea was strongly correlated with the percent reporting testing for chlamydia (rho=0.99, p<0.001) and syphilis (rho=0.98, p<0.001). In general, the *N. gonorrhoeae* geometric mean MIC for cefixime and azithromycin increased more rapidly than ceftriaxone in all cities (data not shown).

In 2005, significant positive associations were found between STI screening and the increase in MIC (2005 to 2013) of cefixime (rho=0.88, p=0.002), azithromycin (rho=0.93, p<0.001)

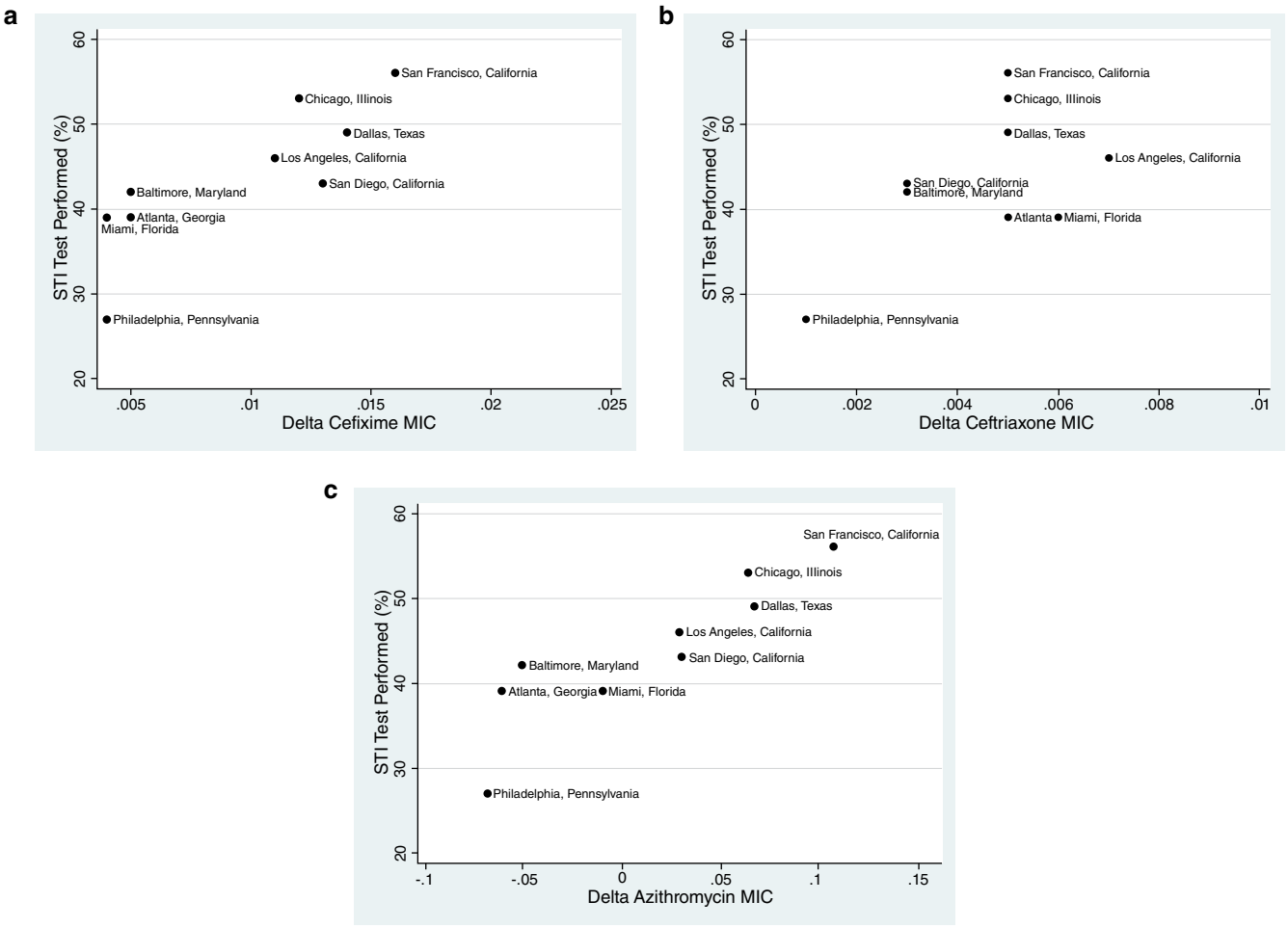

Figure 1. Scatter plots of change in the minimum inhibitory concentrations (MICs) between 2005 and 2013 and the percent of respondents reporting in 2005 that they had a bacterial STI test in the prior 12 months for (**a**) cefixime, (**b**) ceftriaxone and (**c**) azithromycin by USA city (data sources detailed in *Methods*).

but not ceftriaxone (rho=0.27, p=0.491; Figure 1). Likewise in 2008, there was a positive correlation between the percent reporting testing for syphilis in the prior 12 months and increase in MIC of cefixime (rho=0.71, p=0.010), azithromycin (rho=0.791, p=0.002) but not ceftriaxone (rho=0.36, p=0.247). A positive association was also found for the percent reporting testing for gonorrhea in 2011 and an increase in MIC for cefixime (rho=0.63, p=0.026) and azithromycin (rho=0.64, p=0.024) but not ceftriaxone (rho=0.56, p=0.062). The results for chlamydia and syphilis testing were similar (data not shown).

Spearman's correlation between percent reporting screening for any STI in 2011 and geometric mean MIC for the three antimicrobials in the following year revealed a positive association for ceftriaxone (rho=0.64, p=0.026) but not for azithromycin (rho=0.45, p=0.141) or cefixime (rho=0.31, p=0.325).

Discussion

There was a roughly two-fold variation in the proportion of MSM in different cities reporting testing for bacterial STIs. The proportion testing for bacterial STIs was associated with an increase of MIC for cefixime and azithromycin but not ceftriaxone over the time period 2005 to 2013. The correlations between percent screening in 2011 and MIC in the following year were different in that the only significant association was for ceftriaxone. Of note all six correlations between percent screening and MIC were positive. The difference between the two types of analyses related to the strengths of the associations.

A plausible reason for the lack of association between ceftriaxone and MIC change (2005–2013) is that ceftriaxone has been used almost exclusively in combination with azithromycin[3,15] and even on its own may be less susceptible to the develop of resistance than cefixime and azithromycin[16]. These findings are compatible with the theory that screening intensity plays a role in the selection of antimicrobial resistance in *N. gonorrhoeae* in MSM. Alternatively they could reflect more intense screening in sites where there is more concern about antimicrobial resistance.

The findings should however be regarded as tentative due to a number of methodological weaknesses: the sample size

was small the outcome variable (increase in geometric MIC) referred to all men sampled in GISP and not just MSM, the explanatory variable was only evaluated at three time points, the explanatory variable measured 'STI testing' and not 'STI screening' and possible confounders were not controlled for. Increased testing could, for example, be associated with other factors that may be associated with antimicrobial resistance such as greater risk behavior, more frequent travel, HIV-infection and access to medical care. Likewise we did not control for changes over time in the percent of GISP samples derived from MSM which may have influenced the geometric mean MICs. Finally, the decline in the absolute number of participants in the NHBS surveys over time and variations in participation rates by city could introduce biases.

Future studies that wish to evaluate the screening-resistance hypothesis could assess if there is an association between bacterial STI screening intensity and resistance in *N. gonorrhoeae* in bigger samples in the USA or elsewhere. Testing this hypothesis in Europe would be instructive since the proportion of MSM reporting anal screening for bacterial STIs in the prior 12 months in 38 different European countries ranges from 9.1% in Romania to 79.6% in Malta (median 18.5%, IQR 13.5–28.4)[17]. Furthermore whilst two of these countries that report high STI screening rates in MSM (the United Kingdom[17] and the Netherlands[17]) have found an association between resistance in *N. gonorrhoeae* and MSM[18,19], other countries in Europe have not found this association[15].

The recent emergence of combined azithromycin/ceftriaxone resistant *N. gonorrhoeae* provides additional motivation to better characterize the underlying determinants of the differential emergence of resistance in MSM and other populations[20]. If screening intensity is found to play a role then this could be taken into account in development of an optimal STI screening strategy.

Data availability

Data for STI screening: National HIV Behavioral Surveillance MSM (NHBS-MSM) studies

GISP data: Technical appendix of 3

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

1. Kirkcaldy RD, Harvey A, Papp JR, *et al.*: ***Neisseria gonorrhoeae*** **Antimicrobial Susceptibility Surveillance - The Gonococcal Isolate Surveillance Project, 27 Sites, United States, 2014.** *MMWR Surveill Summ.* 2016; **65**(7): 1–19.
PubMed Abstract | Publisher Full Text

2. Kirkcaldy RD, Zaidi A, Hook EW 3rd, *et al.*: ***Neisseria gonorrhoeae*** **antimicrobial resistance among men who have sex with men and men who have sex exclusively with women: the Gonococcal Isolate Surveillance Project, 2005-2010.** *Ann Intern Med.* 2013; **158**(5 Pt 1): 321–8.
PubMed Abstract | Publisher Full Text

3. Kirkcaldy RD, Bartoces MG, Soge OO, *et al.*: **Antimicrobial Drug Prescription and *Neisseria gonorrhoeae* Susceptibility, United States, 2005-2013.** *Emerg Infect Dis.* 2017; **23**(10): 1657–1663.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Iverson CJ, Wang SA, Lee MV, *et al.*: **Fluoroquinolone resistance among *Neisseria gonorrhoeae* isolates in Hawaii, 1990-2000: role of foreign importation and increasing endemic spread.** *Sex Transm Dis.* 2004; **31**(12): 702–708.
PubMed Abstract | Publisher Full Text

5. Bauer HM, Mark KE, Samuel M, *et al.*: **Prevalence of and associated risk factors for fluoroquinolone-resistant *Neisseria gonorrhoeae* in California, 2000-2003.** *Clin Infect Dis.* 2005; **41**(6): 795–803.
PubMed Abstract | Publisher Full Text

6. Abraha M, Egli-Gany D, Low N: **Epidemiological, behavioural, and clinical factors associated with antimicrobial-resistant gonorrhoea: a review [version 1; referees: 2 approved].** *F1000Res.* 2018; **7**: 400.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Goldstein E, Kirkcaldy RD, Reshef D, *et al.*: **Factors related to increasing prevalence of resistance to ciprofloxacin and other antimicrobial drugs in *Neisseria gonorrhoeae*, United States.** *Emerg Infect Dis.* 2012; **18**(8): 1290–1297.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Goossens H, Ferech M, Vander Stichele R, *et al.*: **Outpatient antibiotic use in Europe and association with resistance: a cross-national database study.** *Lancet.* 2005; **365**(9459): 579–587.
PubMed Abstract

9. Jakobsson HE, Jernberg C, Andersson AF, *et al.*: **Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome.** *PLoS One.* 2010; **5**(3): e9836.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Lipsitch M, Samore MH: **Antimicrobial use and antimicrobial resistance: a population perspective.** *Emerg Infect Dis.* 2002; **8**(4): 347–354.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Bell BG, Schellevis F, Stobberingh E, *et al.*: **A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance.** *BMC Infect Dis.* 2014; **14**(1): 13.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Kenyon C, Schwartz IS: **A combination of high sexual network connectivity and excess antimicrobial usage induce the emergence of antimicrobial resistance in Neisseria gonorrhoeae.** *Emerg Infect Dis.* 2018; **24**(7).

13. Bruyndonckx R, Hens N, Aerts M, *et al.*: **Exploring the association between resistance and outpatient antibiotic use expressed as DDDs or packages.** *J Antimicrob Chemother.* 2015; **70**(4): 1241–1244.
PubMed Abstract | Publisher Full Text

14. Buyze J, Vanden Berghe W, Hens N, *et al.*: **Current levels of gonorrhoea screening in MSM in Belgium may have little effect on prevalence: a modelling study.** *Epidemiol Infect.* 2018; **146**(3): 333–338.
PubMed Abstract | Publisher Full Text

15. Cole MJ, Spiteri G, Jacobsson S, *et al.*: **Overall Low Extended-Spectrum Cephalosporin Resistance but high Azithromycin Resistance in *Neisseria gonorrhoeae* in 24 European Countries, 2015.** *BMC Infect Dis.* 2017; **17**(1): 617.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Chisholm SA, Mouton JW, Lewis DA, *et al.*: **Cephalosporin MIC creep among gonococci: time for a pharmacodynamic rethink?** *J Antimicrob Chemother.* 2010; **65**(10): 2141–2148.
PubMed Abstract | Publisher Full Text

17. The EMIS Network: **EMIS 2010: The European Men-Who-Have-Sex-With-Men Internet Survey. Findings from 38 countries.** Stockholm: European Centre for Disease Prevention and Control; 2011.
Reference Source

18. Ison CA, Town K, Obi C, *et al.*: **Decreased susceptibility to cephalosporins among gonococci: data from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) in England and Wales, 2007-2011.** *Lancet Infect Dis.* 2013; **13**(9): 762–768.
PubMed Abstract | Publisher Full Text

19. de Vries HJ, Van der Helm JJ, Schim van der Loeff MF, *et al.*: **Multidrug-resistant Neisseria gonorrhoeae with reduced cefotaxime susceptibility is increasingly common in men who have sex with men, Amsterdam, the Netherlands.** *Euro Surveill.* 2009; **14**(37): pii: 19330.
PubMed Abstract | Publisher Full Text

20. Yasuda M, Hatazaki K, Ito S, *et al.*: **Antimicrobial Susceptibility of Neisseria gonorrhoeae in Japan from 2000 to 2015.** *Sex Transm Dis.* 2017; **44**(3): 149–153.
PubMed Abstract

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Current Referee Status: ✔ ✔

Version 4

Referee Report 01 November 2018

https://doi.org/10.5256/f1000research.18434.r40078

✔ **Edward Goldstein**
Center for Communicable Disease Dynamics (CCDD), Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

The reviewer wishes to thank the author for the edits introduced in the most recent revision. It might be helpful if the names of antimicrobials considered in the analysis were listed in the Abstract. Also, the sentence "Not all positive associations were statistically significant" probably needs editing as the term "positive associations" usually implies that they are significant. Perhaps the penultimate sentence in the Abstract could be replaced by something like "Several types of analyses relating STI screening intensity and changes in minimal inhibitory concentrations were performed, and the associations found to be statistically significant varied between the different analyses."

Competing Interests: No competing interests were disclosed.

Referee Expertise: Infectious Disease Epidemiology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Referee Report 22 October 2018

https://doi.org/10.5256/f1000research.18232.r39543

✘ **Edward Goldstein**
Center for Communicable Disease Dynamics (CCDD), Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

The reviewer wishes to thank the author for revising the submission.

In the most recent submission, the author chose to mostly report those findings in the Results section that show the effect of screening for azithromycin and cefixime MICs, but not ceftriaxone MICs. In the earlier response to the reviewer queries, the author wrote:

“we have assessed Spearman's correlation between percent reporting screening for any STI in the previous 12 months and MIC for the three antimicrobials in the following year (2012):

Azithromycin: Rho 0.45; P=0.141
Cefixime: Rho 0.31; P=0.325
Ceftriaxone: Rho=0.64; P=0.026”

“We also assessed Spearman's correlation between percent reporting screening for any STI in the previous 12 months (2011 survey) and the fold change in geometric mean MIC for the three antimicrobials between

a) 2005 and 2013:
Azithromycin: Rho 0.77; P=0.003
Cefixime: Rho 0.82; P=0.001
Ceftriaxone: Rho=0.61; P=0.034”

"b) 2009 and 2013:
Azithromycin: Rho 0.33; P=0.299
Cefixime: Rho -0.50; P=0.100
Ceftriaxone: Rho=0.58; P=0.047"

The reviewer thinks that choosing to focus on those results that stress the effect for azithromycin and cefixime MICs, but not ceftriaxone MICs could be viewed as tendentious, trying to exhibit the effect of screening on MICs for the antimicrobial that is currently used in the treatment of gonorrhea. The reviewer would suggest to give a more balanced account of the results in light of all the findings.

Competing Interests: No competing interests were disclosed.

Referee Expertise: Infectious Disease Epidemiology

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 26 Oct 2018

Chris Kenyon, Institute of Tropical Medicine, Antwerp, Belgium

Thank you for pointing this out. I agree that the abstract could be construed to exaggerate the link between screening intensity and antimicrobial resistance. I have therefore changed the abstract to the following:

We found positive associations between STI screening intensity and increases in minimum inhibitory concentrations for certain antimicrobials. Not all positive associations were statistically significant and the associations found to be statistically significant varied between the different analyses. Further studies are therefore required to assess if there is a causal relationship between the intensity of STI screening in MSM and gonococcal resistance.

Competing Interests: No competing interests were disclosed.

Version 2

Referee Report 12 October 2018

https://doi.org/10.5256/f1000research.17953.r38823

Edward Goldstein
Center for Communicable Disease Dynamics (CCDD), Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

The reviewer wishes to thank the author for the revisions and the additional analyses performed. It appears to the reviewer that the results stated in the the Abstract and the results of the additional analyses in the response to the earlier reviewer comments are not fully compatible.

The main finding of the paper, stated in the Abstract is: We found positive associations between STI screening intensity and increases in minimum inhibitory concentrations [between 2007-2013] for cefixime and azithromycin, but not ceftriaxone."

At the same time, according to the author's response:

1. "we have assessed Spearman's correlation between percent reporting screening for any STI in the previous 12 months and MIC for the three antimicrobials in the following year (2012):

Azithromycin: Rho 0.45; P=0.141
Cefixime: Rho 0.31; P=0.325
Ceftriaxone: Rho=0.64; P=0.026"

Thus the only significant relation between screening levels during the previous year and MIC is for ceftriaxone, unlike the relation between screening and increases in MIC reported in the Abstract.

2. We also assessed Spearman's correlation between percent reporting screening for any STI in the previous 12 months (2011 survey) and the fold change in geometric mean MIC for the three antimicrobials between

a) 2005 and 2013:
Azithromycin: Rho 0.77; P=0.003
Cefixime: Rho 0.82; P=0.001
Ceftriaxone: Rho=0.61; P=0.034

Thus the relation between screening and increases in MIC between 2005-2013 is significant for all the three antibiotic classes, unlike the case of the increases in MIC between 2007-2013 reported in the Abstract.

b) 2009 and 2013:
Azithromycin: Rho 0.33; P=0.299
Cefixime: Rho -0.50; P=0.100
Ceftriaxone: Rho=0.58; P=0.047

Thus the relation between screening and increases in MIC between 2009-2013 is significant only for ceftriaxone, which is exactly the opposite of the case of the increases in MIC between 2007-2013 reported in the Abstract.

Overall, the findings stated in the Abstract reflect the results of some of the analyses, while other analyses yield different results. The author needs to think of a way for framing the results to accommodate all the findings.

Competing Interests: No competing interests were disclosed.

Referee Expertise: Infectious Disease Epidemiology

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 12 Oct 2018

Chris Kenyon, Institute of Tropical Medicine, Antwerp, Belgium

Reply:

Thank you for pointing this inconsistency out. To optimally deal with this I have added the results comparing the 2011 STI screening prevalence with the 2012 MICs to the methods, results and discussion sections.

The sentence referring to the results in the abstract has been reworded to the following:
We found positive associations between STI screening intensity and increases in minimum inhibitory concentrations for cefixime and azithromycin, but not ceftriaxone when using change in city geometric mean *N. gonorrhoeae* MIC between 2005 and 2013.

The following text has been added to the methods section:
The geometric mean MIC was calculated as the *n*th root of the product of *n* MIC values. Spearman's correlation was used to assess if there was an association between (1) the prevalence of STI testing in each survey and the increase in geometric mean MIC of cefixime, ceftriaxone and azithromycin in *N. gonorrhoeae* between 2005 and 2013 and (2) the percent reporting screening for any STI in the 2011 survey and geometric mean MIC for the three antimicrobials in the following year.

The following text has been added to the results section:
Spearman's correlation between percent reporting screening for any STI in 2011 and geometric mean MIC for the three antimicrobials in the following year revealed a positive association for ceftriaxone (rho=0.64, p=0.026) but not for azithromycin (rho=0.45, p=0.141) or cefixime (rho=0.31, p=0.325).

The new results are then discussed in the discussion as follows:
The proportion testing for bacterial STIs was associated with an increase of MIC for cefixime and azithromycin but not ceftriaxone over the time period 2005 to 2013. The correlations between percent screening in 2011 and MIC in the following year were different in that the only significant association was for ceftriaxone. Of note all six correlations between percent screening and MIC

were positive. The difference between the two types of analyses related to the strengths of the associations.

Competing Interests: No competing interests to declare

Referee Report 01 October 2018

https://doi.org/10.5256/f1000research.17953.r38824

Ellen Stobberingh
Care And Public Health Research Institute (CAPHRI), Maastricht University Medical Centre (MUMC) , Maastricht, The Netherlands

No further comments

Competing Interests: No competing interests were disclosed.

Referee Expertise: My expertise includes microbiology, bacteriology, and antimicrobial susceptibility

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 24 September 2018

https://doi.org/10.5256/f1000research.16982.r38243

Ellen Stobberingh
Care And Public Health Research Institute (CAPHRI), Maastricht University Medical Centre (MUMC) , Maastricht, The Netherlands

The manuscript describes the association between STI screening and antimicrobial resistance development of N. gonorrhoeae. Although of interest there are several questions which need to be answered:

Material and Methods:

- The number of participants reduced over time: 10,030 in 2005 and 8,012 in 2011. Also there was a difference in participation rate in the different cities.
  What is the influence of the decreased participation rate over time and the variation in the participation in the different cities on the interpretation of the data?

- In 2008 (only syphilis) and in the two other years in addition N. gonorrhoeae, other STI or Chlamydia were tested. What was the reason for this difference?

- How many N. gonorrhoeae isolates were included in the different years?
- Was the microbiological method to isolate, identify and antibiotic susceptibility testing over testing similar over time? Which method was used for susceptibility testing?
- Please provide range and GM MIC values of the three antibiotica testing in the different years,

Figure 1:
- The horizontal axis mentioned an increase in MIC. What was the reference MIC?

Dicussion:
- Was there a change in therapy over time among the participants. All three antibiotica are mentioned in the guidelines, but is there information concerning the therapy prescribed over time in the different cities / participants?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: My expertise includes microbiology, bacteriology, and antimicrobial susceptibility

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Sep 2018

Chris Kenyon, Institute of Tropical Medicine, Antwerp, Belgium

The manuscript describes the association between STI screening and antimicrobial resistance development of N. gonorrhoeae. Although of interest there are several questions which need to be answered:

Material and Methods:

- The number of participants reduced over time: 10,030 in 2005 and 8,012 in 2011. Also there was a difference in participation rate in the different cities. What is the influence of the decreased participation rate over time and the variation in the participation in the different cities on the interpretation of the data?

Reply:
Indeed both the decline in the absolute number of participants and the variation by city could introduce biases. This additional limitation has been added to the discussion.

- In 2008 (only syphilis) and in the two other years in addition N. gonorrhoeae, other STI or Chlamydia were tested. What was the reason for this difference?

Reply:
These differences reflect differences in the questions asked in the various surveys. In 2008, for example, respondents were asked if they had been tested for syphilis in the preceding 12 months, whereas in the 2011 survey they were asked if they had been tested for gonorrhea, chlamydia or syphilis in the previous 12 months. This is made clear in the methods section.

- How many N. gonorrhoeae isolates were included in the different years?

Reply:
Between 2005 and 2013, 44,144 isolates were tested. The report does not detail the breakdown of number tested by year.

- Was the microbiological method to isolate, identify and antibiotic susceptibility testing over testing similar over time? Which method was used for susceptibility testing?

Reply:
There were small changes in the laboratory protocol used. The protocol used and these changes are detailed in the Kirkcaldy et al report as follows [1]:
"Gonococcal isolates collected at each sentinel clinic are subcultured at the clinic's local public health laboratory on supplemented chocolate medium and frozen in Trypticase soy broth containing 20% glycerol.

Isolates are shipped monthly to one of the regional reference laboratories, where they are tested for Beta-lactamase production and susceptibility to azithromycin, penicillin, tetracycline, ciprofloxacin, spectinomycin, cefixime, and ceftriaxone using the agar dilution method.

Isolates were inoculated on Difco GC medium base supplemented with 1% IsoVitaleX enrichment (Becton-Dickinson Diagnostic Systems, Sparks, MD). During 2005 to 2007, the lowest azithromycin concentration tested was 0.008 ug/ml; this increased to 0.03 ug/ml in 2008.

The routine testing range for azithromycin extended to 16.0 ug/ml during 2005 to 2013. Laboratories were asked to conduct agar dilution testing to identify an endpoint for isolates with an MIC of 16.0ug/ml on the initial testing run. Testing to an endpoint was not conducted on three isolates collected during 2005 to 2013. In the absence of Clinical and Laboratory Standards Institute (CLSI) breakpoints for gonococcal azithromycin susceptibility or resistance, we defined reduced azithromycin susceptibility for this analysis as an MIC of 2.0 ug/ml. Quality assurance processes are described in detail in the GISP protocol. To ensure accuracy and reproducibility of antimicrobial susceptibility results from the regional reference laboratories, a set of seven control N. gonorrhoeae strains with known MICs of various antimicrobials are included with each susceptibility

run. In addition, reference laboratories test a CDC-provided panel of 15 unidentified strains twice yearly to compare results and ensure consistency among laboratories. The results obtained from the testing of control strains and CDC-provided panels are used for internal quality assurance."

- Please provide range and GM MIC values of the three antibiotica testing in the different years,

Reply:
This data is not provided in the paper that reports the GISP results [1]. In the online Technical Appendix file of this paper the following data is however reported:

https://doi.org/10.3201/eid2310.170488

The geometric MIC of cefixime (Table 1), ceftriaxone (Table 2) and azithromycin (Table 3) by site and year. Table 8 presents the median and interdecile range of the 3 antimicrobials by site.

Figure 1:
- The horizontal axis mentioned an increase in MIC. What was the reference MIC?

Reply:
The horizontal axis is the change in MIC between 2007 and 2013.

Discussion:
- Was there a change in therapy over time among the participants. All three antibiotica are mentioned in the guidelines, but is there information concerning the therapy prescribed over time in the different cities / participants?

Reply:
This information is not provided in the report.

References:

1. Kirkcaldy RD, Bartoces MG, Soge OO, Riedel S, Kubin G, Del Rio C, et al. Antimicrobial Drug Prescription and Neisseria gonorrhoeae Susceptibility, United States, 2005-2013. Emerg Infect Dis. 2017;23(10):1657-63. Epub 2017/09/21. doi: 10.3201/eid2310.170488. PubMed PMID: 28930001; PubMed Central PMCID: PMCPMC5621530

Competing Interests: I have no competing interests

Referee Report 20 August 2018

https://doi.org/10.5256/f1000research.16982.r37099

? **Edward Goldstein**
Center for Communicable Disease Dynamics (CCDD), Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

The paper under review studies the association between the frequency of STI screening in MSM and changes in geometric mean MICs for certain antibiotics in *N. gonorrhoeae* samples in select US cities. An important potential source of bias in such analysis could be reverse causality, namely places with higher prevalence of drug resistance for gonorrhea, as well as syphilis in MSM may initiate more STI screening efforts. Below are some questions/suggestions to the author.

1. Please define geometric mean MIC. Also, since one refers to geometric mean, it might be more reasonable to examine fold changes (increase or decreases) in geometric mean MIC (or logarithm of thereof) in the correlation analysis.

2. Nine cities reported STI screening information in 2005 (Figure 1), and 12 cities reported STI screening information in 2013. The reviewer would suggest to correlate the frequency of STI screening in 2013 with

- Geometric mean MICs in 2013
- (Fold) change in geometric mean MICs between 2009 and 2013
- (Fold) change in geometric mean MICs between 2005 and 2013

For the 2005 screening frequencies, it would be interesting to correlate them with geometric mean MICs (in 2013, and possibly 2005), and not only changes in thereof.

3. In ref. 3, no association was found between rates of antibiotic prescribing and geometric mean MICs. Perhaps the results of the paper under review are affected by reverse causality, namely places with higher prevalence of drug resistance for gonorrhea, as well as syphilis in MSM may initiate more STI screening efforts. For example, the California sites had both high geometric mean MIC for ciprofloxacin in 2005 (ref. 3), and high frequency of screening (see also[1]). Currently, this possibility is not mentioned in the Discussion. The reviewer was also wondering if there is a correlation between the fold change in screening frequency and the fold change in geometric mean MICs between 2005 and 2013.

4. Are other variables in NHBS-MSM surveys (possibly rate of change of sexual partners, or a number of recent sexual partners) correlated with geometric mean MICs/changes in thereof?

5. In the Discussion, it is mentioned that “the sample size was small (9 or 12)”. Currently, only 9 cities are used in the correlation analysis, as far as the reviewer could see.

References

1. Centers for Disease Control and Prevention CDC: Increases in fluoroquinolone-resistant Neisseria gonorrhoeae among men who have sex with men--United States, 2003, and revised recommendations for gonorrhea treatment, 2004.*MMWR Morb Mortal Wkly Rep*. 2004; **53** (16): 335-8 PubMed Abstract

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Infectious Disease Epidemiology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Sep 2018

Chris Kenyon, Institute of Tropical Medicine, Antwerp, Belgium

The paper under review studies the association between the frequency of STI screening in MSM and changes in geometric mean MICs for certain antibiotics in *N. gonorrhoeae* samples in select US cities. An important potential source of bias in such analysis could be reverse causality, namely places with higher prevalence of drug resistance for gonorrhea, as well as syphilis in MSM may initiate more STI screening efforts. Below are some questions/suggestions to the author.

1. Please define geometric mean MIC. Also, since one refers to geometric mean, it might be more reasonable to examine fold changes (increase or decreases) in geometric mean MIC (or logarithm of thereof) in the correlation analysis.

Reply:
The geometric mean MIC has now been defined in the methods section.

2. Nine cities reported STI screening information in 2005 (Figure 1), and 12 cities reported STI screening information in 2013. The reviewer would suggest to correlate the frequency of STI screening in 2013 with

- Geometric mean MICs in 2013
- (Fold) change in geometric mean MICs between 2009 and 2013
- (Fold) change in geometric mean MICs between 2005 and 2013

For the 2005 screening frequencies, it would be interesting to correlate them with geometric mean MICs (in 2013, and possibly 2005), and not only changes in thereof.

Reply:
The third NHBS survey was in 2011 (not 2013) and thus we have assessed Spearman's correlation between percent reporting screening for any STI in the previous 12 months and MIC for the three antimicrobials in the following year (2012):

Azithromycin: Rho 0.45; P=0.141

Cefixime: Rho 0.31; P=0.325
Ceftriaxone: Rho=0.64; P=0.026

We also assessed Spearman's correlation between percent reporting screening for any STI in the previous 12 months (2011 survey) and the fold change in geometric mean MIC for the three antimicrobials between

a) 2005 and 2013:
Azithromycin: Rho 0.77; P=0.003
Cefixime: Rho 0.82; P=0.001
Ceftriaxone: Rho=0.61; P=0.034

b) 2009 and 2013:
Azithromycin: Rho 0.33; P=0.299
Cefixime: Rho -0.50; P=0.100
Ceftriaxone: Rho=0.58; P=0.047

3. In ref. 3, no association was found between rates of antibiotic prescribing and geometric mean MICs. Perhaps the results of the paper under review are affected by reverse causality, namely places with higher prevalence of drug resistance for gonorrhea, as well as syphilis in MSM may initiate more STI screening efforts. For example, the California sites had both high geometric mean MIC for ciprofloxacin in 2005 (ref. 3), and high frequency of screening (see also[1]). Currently, this possibility is not mentioned in the Discussion. The reviewer was also wondering if there is a correlation between the fold change in screening frequency and the fold change in geometric mean MICs between 2005 and 2013.

Reply:
Thank you for this interesting suggestion which has been added to the discussion.

4. Are other variables in NHBS-MSM surveys (possibly rate of change of sexual partners, or a number of recent sexual partners) correlated with geometric mean MICs/changes in thereof?

Reply:
Whilst we agree this would be interesting to investigate, we did not assess these correlations in this small study.

5. In the Discussion, it is mentioned that “the sample size was small (9 or 12)”. Currently, only 9 cities are used in the correlation analysis, as far as the reviewer could see.

Reply:
The sample size for 2005 was 9 and therefore in Figure 1, where one of the variables is from 2005, only 9 data points can be used. There were however 12 data points for the other 2 surveys and these were used in the analyses using these surveys.

References:

1. Kirkcaldy RD, Bartoces MG, Soge OO, Riedel S, Kubin G, Del Rio C, et al. Antimicrobial Drug Prescription and Neisseria gonorrhoeae Susceptibility, United States, 2005-2013. Emerg

Infect Dis. 2017;23(10):1657-63. Epub 2017/09/21. doi: 10.3201/eid2310.170488. PubMed PMID: 28930001; PubMed Central PMCID: PMCPMC5621530.

Competing Interests: I have no competing interests