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RESEARCH ARTICLE

REVISED Does gonorrhoea screening intensity play a role in the early selection of antimicrobial resistance in men who have sex with men (MSM)? A comparative study of Belgium and the United Kingdom [version 2; peer review: 2 approved, 1 not approved]

Chris R. Kenyon [1,2], Irith De Baetselier[3], Tania Crucitti [3]

[1]HIV/STI Unit, Institute of Tropical Medicine, Antwerp, Belgium
[2]Division of Infectious Diseases and HIV Medicine, University of Cape Town, Cape Town, South Africa
[3]HIV/STI Reference Laboratory, Institute of Tropical Medicine, Antwerp, Belgium

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Abstract

Background: It is unclear why antimicrobial resistance in *Neisseria gonorrhoeae* in the United Kingdom (UK) and the United States has tended to first appear in men who have sex with men (MSM). We hypothesize that increased exposure to antimicrobials from intensive STI screening programmes plays a role.
Methods: We assess if there is a difference in the distribution of azithromycin, cefixime and ceftriaxone minimum inhibitory concentrations (MICs) between MSM and women in the United Kingdom (UK) where 70% of MSM report STI screening in the past year vs. Belgium where 9% report STI screening in the past year. Our hypothesis is that MICs of the MSM should be higher than those of the women in the UK but not Belgium. Data for the MICs were taken from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) in the UK in 2010/2011 and 2014 and a similar national surveillance programme in Belgium in 2013/2014 (the first most complete available data). We used the Mann–Whitney test to compare the MIC distributions between MSM and women within each country
Results: In the UK the MICs for all three antimicrobials were significantly higher in MSM than women at both time points (P all <0.0005). In Belgium only the MIC distribution for azithromycin was higher in MSM (P<0.0005).
Conclusion: The findings for cefixime and ceftriaxone, but not azithromycin are compatible with our hypothesis that screening-intensity could contribute to the emergence of AMR. Numerous other interpretations of our results are discussed.

Open Peer Review

Reviewer Status ✓ ✗ ✓

	Invited Reviewers		
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1. **Henry J C de Vries**, Amsterdam Institute for Infection and Immunity (AI&II), Meibergdreef 9, Amsterdam, The Netherlands
Public Health Service (GGD) , Amsterdam, The Netherlands

2. **Michelle J Cole**, Public Health England, London, UK
Hamish Mohammed, Public Health England, London, UK

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This article is included in the Antimicrobial Resistance collection.

Jane Hallinan, Public Health England, London, UK

3. **Xiang-Sheng Chen**, Chinese Center for Disease Control and Prevention, Nanjing, China

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Chris R. Kenyon (chriskenyon0@gmail.com)

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REVISED **Amendments from Version 1**

We have made numerous changes to the second version of this article. The exact changes made are detailed in the response to reviewers sections. In summary, we have expanded the Methods section to provide more information as to the ethical approval process and the Discussion section has been considerably expanded to include points requested by the reviewers. We have also included a number of new references as requested by the reviewers.

See referee reports

Introduction

A striking feature of the patterning of antimicrobial resistance (AMR) is how it has repeatedly emerged in core-groups, either sex workers or men who have sex with men (MSM) with high rates of partner change[1]. In the last two decades AMR in the United Kingdom (UK) and the United States (USA) has tended to first appear in MSM[2–5]. In the UK for example, the prevalence of cefixime resistance (following the switch to cefixime therapy for *Neisseria gonorrhoeae* (NG) in 2005) increased from 0% in 2005 to 33.1% in 2010 in MSM, whilst remaining under 7% in heterosexual men and women (Figure 1)[2]. In the USA, UK and the Netherlands, the prevalence of AMR to at least one of ciprofloxacin/cefixime/cefotaxime/azithromycin has been noted to be higher in MSM[3,4,6]. This association has not, however, been found in other countries. An analysis of gonococcal AMR in the 24 countries participating in European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) in 2015, for example, found that cefixime and ciprofloxacin resistance rates were not higher in MSM compared to heterosexual men[7]. Azithromycin (AZM) resistance prevalence was however higher in men (both MSM and heterosexuals) than women.

We hypothesize that these differences in the emergence of AMR may be in part explained by differences in the intensity of NG/CT (*Chlamydia trachomatis*) screening for MSM. The percent of MSM who report being screened for NG/CT varies considerably between countries. In the 38 countries in the European MSM Internet Survey, for example the proportion of MSM who reported anal screening for sexually transmitted infections (STIs) ranged from 4.4% in Serbia to 70.6% in Malta (median 16.0, IQR 13.5-28.4)[8]. A higher screening intensity would be expected to translate into greater antimicrobial exposure. A study that modelled the sexual network of a population of Belgian MSM, for example, found that increasing screening intensity from 3.5% to 50% of MSM annually would reduce NG prevalence marginally but at the expense of a 12-fold increase in antimicrobial exposure[8,9].

In this preliminary study to test the hypothesis that screening intensity played a role in the selection of AMR in NG we contrast the difference in azithromycin, cefixime and ceftriaxone minimum inhibitory concentration (MIC) distributions between MSM and women in the UK (an intensive-screening country; 70% of MSM report annual STI screening[8]) with those in Belgium (a low-screening country; 9% of MSM report annual STI screening[8]) in the years 2010 to 2015. The overall consumption of these antimicrobials in these two countries was not too dissimilar. Between 2010 and 2015, Belgians consumed slightly more cephalosporins but fewer macrolides than the inhabitants

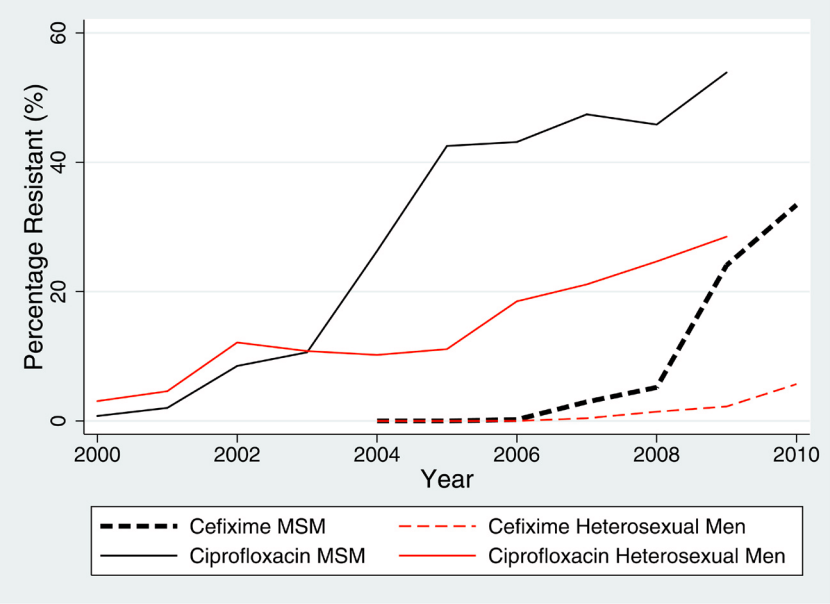

Figure 1. Percent of NG isolates in the United Kingdom showing decreased susceptibility to cefixime and ciprofloxacin in men by sexual orientation 2000–2010 (Based on data from 2,5).

of the UK (cephalosporins: 966 vs. 905 standard units per 1000/population/year; macrolides 1960 vs. 3063 standard units per 1000/population/year, respectively[10]).

National treatment guidelines for NG in the UK and Belgium

In Belgium, guidelines changed from ciprofloxacin to ceftriaxone 125mg IM or spectinomycin 2g IM in 2008[11,12]. In 2012 azithromycin was added for treatment of NG and ceftriaxone dosage was increased: ceftriaxone 500mg IM plus azithromycin 2g PO[13,14]. In the UK, cefixime 400mg PO took over from ciprofloxacin in 2005 as preferred therapy[3]. In 2011, this was switched to ceftriaxone 500mg IM plus azithromycin 1g PO[3,15]. Thus between 2008 and 2012 therapy in Belgium/the UK was mostly ceftriaxone/cefixime whereas from 2012 dual therapy was recommended in both countries.

Methods

Because the sampling and susceptibility testing methodologies vary slightly between Belgium and the UK, we do not directly compare the MICs between the two countries. Rather we assess if there is a difference in the distribution of MICs between MSM and women in each country. The rationale we use is as follows. If intensive screening in MSM plays a role in generating AMR in MSM then in the intensive-screening country we would expect to find a shift in distribution towards higher MICs in MSM compared to women for the antimicrobials used as treatment in the screening programme. In the low-screening country there should be no difference in distribution between MSM and women. We compare MSM with women rather than heterosexual men to avoid the problem of misclassification of men who occasionally have sex with men but regard themselves as heterosexual[16].

AMR surveillance in Belgium: All laboratories in Belgium are requested to send NG isolates to the National Reference Centre for STIs (NRC-STI) at the Institute of Tropical Medicine. The agar dilution method was used to determine MICs according to the CLSI guidelines[17].

AMR surveillance in the United Kingdom: The Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) is a sentinel surveillance programme for AMR in NG in the UK. It incorporates a network of genitourinary medicine (GUM) clinics chosen to give regional representation across England and Wales. Isolates from approximately 10% of patients with gonorrhoea, collected over a 3-month period (July–September) each year, undergo susceptibility testing via MIC determination using the agar dilution method at the Public Health England's sexually transmitted bacteria reference unit (PHE)[18]. Demographic and behavioural data are gathered retrospectively and then linked to laboratory data[3].

Data sets

The data for Belgium was taken directly from NRC-STI. The details regarding sexual orientation started to be reported in sufficient numbers from 2013 onwards. Because the absolute number of isolates from Belgium are low we present analyses from the combined data from 2013 and 2014.

The data for the UK was extracted from the GRASP annual reports[2,5,18]. This included digitalization of the percent distribution of MIC by sexual orientation/gender graphs using GetData Graph Digitizer 2.26. We analyze the data of 2010 for ceftriaxone and of 2011 for azithromycin and cefixime, as well as the data of 2014 for the three antimicrobials.

For the UK data ethics approval for GRASP was obtained from local regional research committees and from the northwest multicentre research ethics committee[3]. We used extracted data from publically available reports and thus no additional ethical approval was necessary. In Belgium no additional ethical approval was necessary because only fully anonymized routine surveillance data were used.

Statistical analyses

We used the Mann–Whitney test to assess if there was a difference in the MIC distributions between MSM and women within each country. Stata 13 was used for all analyses.

Results

Belgium

The STI reference laboratory received 1224 NG isolates from 78 laboratories in 2013/2014. Of these, 1150 were successfully cultured and tested. 941 (81.8%) were men, 190 (16.5%) women and 19 unknown gender. 183 (19.5%) of the men reported being heterosexual, 201 (21.4%) MSM and data was missing in 557 (59.2%) men.

The distribution of the azithromycin MICs was significantly higher in MSM compared to women (Median MIC 0.25, [IQR 0.25-0.50] vs. 0.25 [0.125-0.25]; P<0.0005) but there were no differences in the MIC distributions for cefixime or ceftriaxone (Table 1; Figure 2). The MIC distribution for azithromycin was slightly right-shifted in MSM compared to women (Figure 2). The distribution of the MICs for cefixime in women appeared bimodal, as was the MIC distribution for ceftriaxone in women and to a lesser extent in men.

United Kingdom

The number of isolates provided by the GRASP surveys was as follows: 2010: MSM 600, women 306; 2011: MSM 665, women 312; 2014: MSM 1073, women 192. For further details please refer to the individual annual reports[2,5,18].

2010–2011: The MIC distributions for all three antimicrobials were statistically significantly higher in MSM than women (Azithromycin: 0.25, [IQR 0.125-0.50] vs. 0.06 [0.03-0.125], cefixime: 0.008, [IQR 0.004-0.03] vs. 0.002 [0.002-0.004] ceftriaxone: 0.008, [IQR 0.004-0.03] vs. 0.008 [0.004-0.008]; All P<0.0005). For all three antimicrobials the distribution was right-shifted in MSM compared to women (Figure 2). The distributions of the MICs for cefixime and ceftriaxone in MSM appeared bimodal.

2014: The MIC distributions for all three antimicrobials were statistically significantly higher in MSM compared to women

Table 1. MIC distributions for MSM and women in Belgium and the United Kingdom based on data from national reporting systems.

	UK (2010/2011)		UK (2014)		Belgium (2013/2014)	
	MSM (2010: n=600, 2011: n=665)	Women (2010: n=399, 2011: n=387)	MSM (n=1073)	Women (n=192)	MSM (n=200)	Women (n=189)
Azithromycin [Median (IQR)]	0.25 (0.125-0.25)	0.06 (0.03-0.125)***	0.125 (0.125-0.25)	0.06 (0.03-0.125)***	0.25 (0.25-0.50)	0.25 (0.125-0.25)***
Cefixime [Median (IQR)]	0.008 (0.004-0.03)	0.008 (0.004-0.008)***	0.015 (0.008-0.03)	0.015 (0.008-0.015)***	0.015 (0.008-0.03)	0.015 (0.008-0.06)
Ceftriaxone [Median (IQR)]	0.008 (0.004-0.03)	0.002 (0.002-0.004)***	0.004 (0.004-0.008)	0.004 (0.002-0.004)***	0.008 (0.004-0.015)	0.008 (0.004-0.03)

*** *P*<0.0005 (P-values are from Man-Whitney tests comparing MICs distributions between MSM and women in each country); IQR – Interquartile range

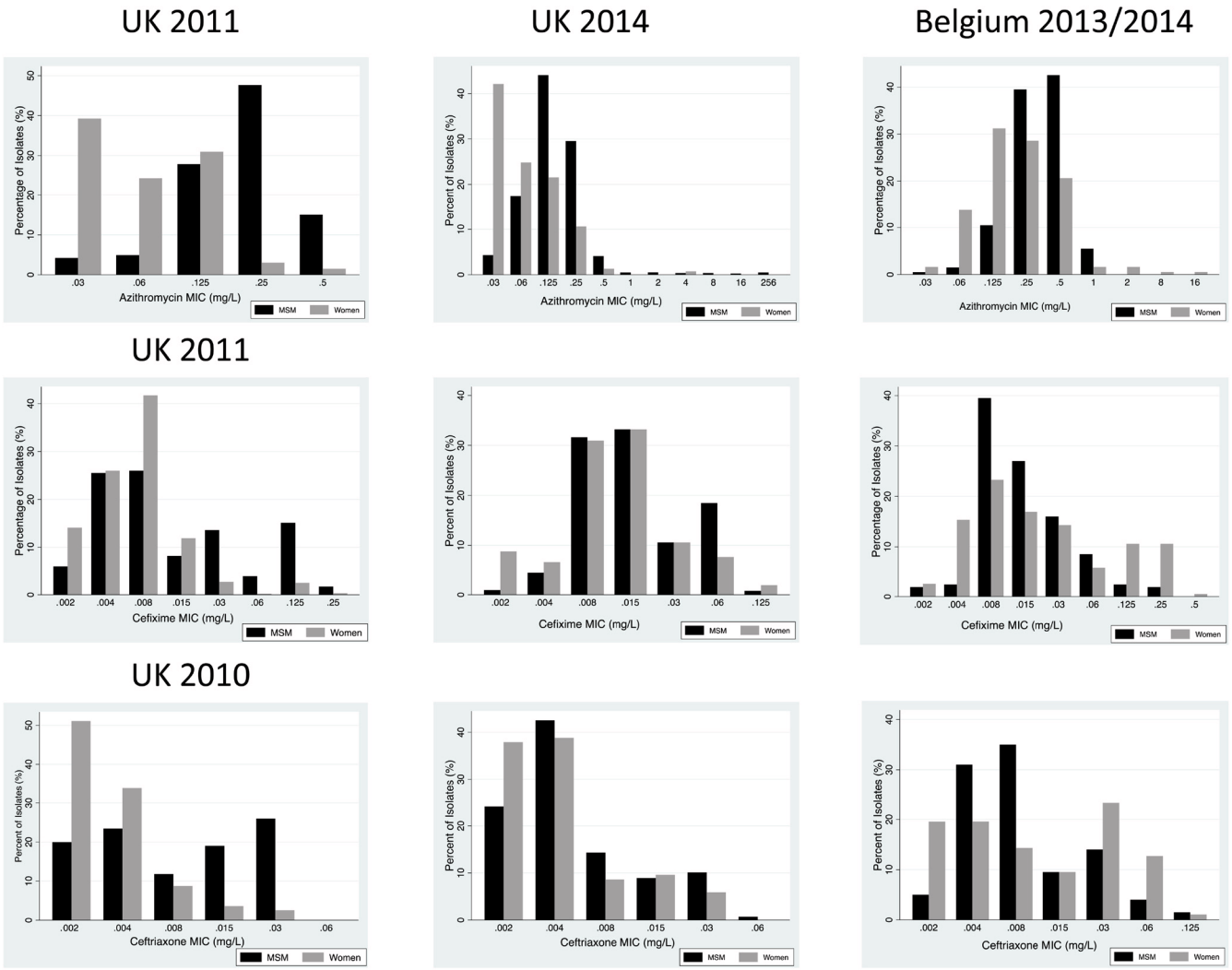

Figure 2. The percent distribution MICs of *Neisseria gonorrhoeae* isolates by gender/sexual orientation in Belgium and the United Kingdom 2010 to 2014.

and were shifted to the right but less so than in 2010 or 2011 (Figure 2, Table 1).

The distributions of the MICs for cefixime in MSM appeared bimodal, but with a shift to the left of the second mode compared to 2011. The bimodal appearance of the MIC distribution for ceftriaxone in 2014 is less pronounced compared to 2010.

Dataset 1. Minimum inhibitory concentrations distributions for Neisseria gonorrhoeae isolates analyzed

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Discussion

A better understanding of the factors underpinning the genesis of AMR in NG could assist with efforts to prevent the further development of AMR. In this study we find that the MIC distribution for azithromycin, ceftriaxone and cefixime (particularly in 2010) is right shifted in MSM compared to women in the UK. In Belgium only the distribution of azithromycin is right-shifted in this way. In addition, we find that the magnitude of this right-shift decreased in the UK between 2010/2011 and 2014. As a result, the proportion of MSM in the UK with higher ceftriaxone MICs and cefixime MICs has declined between 2010 and 2014. These findings are commensurate with UK and European surveillance data showing a decline in the proportion of third generation cephalosporin AMR[2,7,15]. A plausible reason for this decline has been the introduction of high dose ceftriaxone which has more favourable pharmacokinetic parameters than cefixime[2,3,19]. Dual therapy with azithromycin may also have played a role[7,20].

What explains the right-shifting of cefixime and ceftriaxone in MSM versus women in the UK but not Belgium? An important difference in the pharmacoecology experienced by NG in the two countries was the use of cefixime in the UK (until 2011) compared to ceftriaxone monotherapy in Belgium (until 2012). Ceftriaxone's longer half-life than cefixime may have played a role in preventing MIC drift in Belgium[19]. For a number of bug-drug combinations cefixime has been found to be more prone to AMR than other third generation cephalosporins. One of the most convincing studies of this was an *in vitro* differential selection study by Negri *et al.*, who found that cefixime was the best selector of penicillin resistance in *Streptococcus pneumoniae* (compared to amoxicillin, cefuroxime and cefotaxime[21]. The mechanism underpinning this effect has not been clearly elucidated but a number of authors have speculated that it may be related at least in part to cefixime's shorter half life. Both women and men were treated with cefixime in the UK and this would thus not explain why the right-shifting occurred predominantly/only in MSM. The higher NG screening (and therefore antibiotic exposure rates) in MSM in the UK compared to Belgium is one of many possible explanations. This explanation stems from the insight that the intensity of exposure to antimicrobials plays a crucial role in the genesis of AMR[22–26]. A range of studies have found close correlations at ecological levels between the intensity of exposure to a particular antimicrobial and AMR to that antimicrobial[22–24,27,28]. These findings have led us and others to propose the pharmacoecological theory of AMR (connectivity AMR theory) which posits that it is the combination of dense sexual networks plus excess antimicrobial consumption (such as from intense screening) that plays an important role in AMR genesis in NG[29]. The dense sex network generates the high prevalence of NG and the antimicrobial exposure then initially lowers prevalence but in the process generates a fitness advantage for resistant NG.

Arguing against the screening-intensity explanation is the fact that the right shifting of AZM occurred in MSM in both countries. This finding suggests either that some other factor is responsible for the right shifting in MSM (such as total macrolide use for all indications) or that the MSM sexual pharmacoecology is more susceptible to the development of AMR for azithromycin than other antibiotics[30]. The higher proportion of time NG spends in the rectum in MSM compared to heterosexual sexual networks, for example, could lead to an enhanced selection pressure for/availability of *mtrR*-related and *erm* mutations[30]. Macrolides have also been shown to have a particularly long adverse effect on the resistome, with changes noted for up to 4 years post therapy[31,32]. These considerations may mean that relatively low azithromycin exposure may be sufficient to generate a right shift in MIC. Gonorrhoea screening guidelines for MSM attending specialist sexual health services in the UK were updated in 2010, and this was followed by an increase in NG tests since then. Since cephalosporin resistance rates in the UK declined post 2010, this evidence is not supportive of the screening-intensity hypothesis.

We also observed changes in the bimodal distribution of cetriaxone and cefixime in 2014 versus 2010–2011 in the UK. The shift to the left of the second mode and almost disappearance of the bimodal distribution is reassuring as it may indicate that the previous emergence of a less susceptible population is temporarily under control and regaining susceptibility towards cefixime and ceftriaxone.

There are a number of alternative explanations for why AMR may arise sooner in MSM than women. MSM may be more likely to travel abroad and acquire more resistant NG in this way[4,33]. At least one study has however found that heterosexuals with gonorrhoea are more likely to report sex abroad than MSM[33]. MSM are more likely to be HIV-infected and may as a result use more antimicrobials[4]. Some studies have found that even after stratifying for HIV-infection status, MSM still report consuming more antimicrobials[4]. Both treatment of symptomatic and asymptomatic STIs may play a role here. Finally, the fact that NG spends proportionately more time in the oropharynx and rectum in MSM (compared to heterosexuals) may offer it more opportunities for acquisition of resistance genes and mutations[4,30]. It is however unlikely that these explanations can explain the differences between NG AMR in MSM vs. women in the UK compared to Belgium.

The numerous weaknesses of our study design preclude firm conclusions. These limitations include the fact that we only include two countries, and we have limited data on the full range of potential explanatory variables (such as general antimicrobial

consumption, NG/CT screening rates in women). There were also important methodological differences in how the surveillance was conducted in the two countries (such as sampling methodology, sensitivity testing). Whereas the GRASP sentinel methodology has been shown to yield fairly representative samples for the UK[34], an equivalent study has not been conducted in Belgium.

Although we cannot, on the basis of this study, conclude that the intensity of NG screening plays a role in the genesis of AMR in NG we also cannot reject this hypothesis. Further studies that could test this hypothesis include: 1) assessing the correlation between NG screening intensity in MSM and the prevalence of AMR in MSM in a greater number of countries; 2) community level randomized controlled trials assessing the impact of NG/CT screening on AMR and NG prevalence and 3) more detailed longitudinal assessments of the effects of repeated antibiotic exposure on the resistome and microbiome of MSM cohorts with higher risk behaviour[35].

Data availability

Dataset 1: Minimum inhibitory concentrations distributions for *Neisseria gonorrhoeae* isolates analyzed 10.5256/f1000research.14869.d203173[36]

Competing interests

No competing interests were disclosed.

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http://www.doi.org/10.5256/f1000research.14869.d203173

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Version 2

Reviewer Report 15 October 2018

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✘ **Hamish Mohammed**
HIV & STI Department, National Infection Service, Public Health England, London, UK
Michelle J Cole
Antimicrobial Resistanceand Healthcare Associated Infections Reference Unit (AMRHAI), National Infection Service, Public Health England, London, UK

We thank the authors for their revised version. While the proposed hypothesis is of merit, we believe the analyses are not appropriate to assess it due to the inherent biases of an ecologic analysis.
We would suggest reframing the analysis to focus on data from Belgium, as the authors have access to the raw data and could possibly compare between MSM and heterosexuals (assuming differential intensity of GC screening) with these data.
We also believe some of our previous comments were not adequately addressed:

- Comment 2 (comparing MSM and heterosexual men)
  - We disagree; there are marked differences between heterosexual men and women in the GRASP reports in respect to azithromycin resistance. In recent years, azithromycin resistance has been very similar in MSM and heterosexual men, which does not support your hypothesis.
- Comment 6 (UK screening guideline update in 2010 leading to an upturn in screening in MSM with a decline in cephalosporin resistance)
  - As mentioned previously*, the upturn in GC screening coincided with a rapid decline in cephalosporin resistance – this also does not support your hypothesis.

**In the UK, gonorrhoea screening guidelines for MSM attending specialist sexual health services were updated in 2010, and this has led to an increase in gonorrhoea tests since then. This testing trend should be considered when interpreting these findings, as the level of testing prior to 2010 would be much lower than those of more recent years. Furthermore, the upturn in screening coincided with a rapid decline in cephalosporin resistance.*

- EMIS is an excellent source of survey data from a community sample but, as you acknowledge, there may be some sampling bias between countries. Are there STI surveillance data from Belgium to indicate how many MSM are tested for GC and, if not, can we confidently conclude that MSM are more likely to be screened for GC in the UK vs.

Belgium?
- Also, in reference to your response: we would still argue that 6% and 8.3% from the Belgium national data is still a lot higher than the 1.7% and 0.4% cefixime resistance data from GRASP.
- In the UK, AMR is often imported from Asia and has spread and developed in MSM, but has not necessarily first emerged in MSM.
- The analyses of GRASP data are based on numbers extrapolated from figures using an online tool; has this tool been validated?
- Another limitation is the lack of data from Belgium in 2009/10 as we feel a similar shift in MICs in men would have been observed due to the international spread of ST1407.
- While sexual orientation data isn't available, could you examine gender differences instead?
- The GRASP ethics statement is still incorrect.

Competing Interests: Both reviewers are members of the GRASP team at PHE

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

Reviewer Report 15 October 2018

https://doi.org/10.5256/f1000research.17211.r39025

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Xiang-Sheng Chen
National Center for STD Control, Chinese Center for Disease Control and Prevention, Nanjing, China

This is an interesting and well-organized article to compare GC AMR (MIC) distributions among men who have sex with men (MSM) and women between the UK and Belgium and conclude the difference in the distribution patterns is due to intensity of screening for sexually transmitted infections. On the current version of the manuscript, I made a few additional comments as follows for authors' considerations.

1. The title refers to "gonorrhoea screening" but I could not find any information/data on gonorrhoea screening but the screening for STI in the manuscript. It may be better to change to "screening intensity for sexually transmitted infections" or "Differences in population-specific gonococcal antimicrobial resistance distributions between the United Kingdom and Belgium" and then use the screening intensity to explain the differences in text.
2. From Belgium part in the Figure 2, we can find a higher MIC distributions for cefixime and ceftriaxone among women than MSM - in contrast to what observed in the UK. Does it mean an increased intensity of screening for STI among women in Belgium? This should be

explained in the Discussion.
3. Although screening intensity can be used as proxy of intensity of antimicrobial use/exposure, the further studies indicated in the last paragraph of Discussion should include the assessment of corrections between intensity of the specific antibiotic use and the prevalence of antimicrobial resistance of NG.

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

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

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

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

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.

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

Reviewer Report 10 August 2018

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Henry J C de Vries
[1] University of Amsterdam, department of dermatology, Amsterdam Institute for Infection and Immunity (AI&II), Meibergdreef 9, Amsterdam, The Netherlands
[2] Public Health Service (GGD) , Amsterdam, The Netherlands

The manuscript has sufficiently improved. A last comment to increase clarity in figure 2, please add a subheading indicating the time and year of the data depicted above each graph, instead above the top row and left column only.

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?
Partly

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.

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

Version 1

Reviewer Report 20 July 2018

https://doi.org/10.5256/f1000research.16184.r35298

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Michelle J Cole
Antimicrobial Resistanceand Healthcare Associated Infections Reference Unit (AMRHAI), National Infection Service, Public Health England, London, UK

Hamish Mohammed
HIV & STI Department, National Infection Service, Public Health England, London, UK

Jane Hallinan
Public Health England, London, UK

This is an interesting article that tests the hypothesis that the emergence of gonococcal AMR in MSM is due to increased intensity of screening for gonorrhoea (GC).
The authors' findings are based primarily on an ecological analysis and, if these data are available

in Belgium, we believe an analysis considering the GC testing history of each person whose MIC data are included would yield useful results.
We would be keen to discuss the proposed hypothesis and interpretation of GRASP data with you, and have included comments for the authors' consideration:

1. The evidence for different levels of screening intensity in MSM is available from EMIS; is there a comparable source of these data for heterosexual women? Also, did the recruitment strategy to participate in EMIS vary between the UK and Belgium? If so, this would meant that response to the question on the history of STI screening may be less comparable between these two countries.
2. We believe a comparison of MSM with heterosexual men may be more useful, even with the limitations of underreporting of same-sex contact in heterosexually-identifying men. This is because the numbers of isolates are more comparable, women sometimes have different antimicrobial susceptibility profiles from heterosexual men and the cultures available from women are not representative of the circulating isolates in the community due to the difficulty in culturing from women.
3. In England, most gonorrhoea and chlamydia are diagnosed in people under the age of 25 years, with over one million chlamydia tests conducted annually though the National Chlamydia Screening Programme (NCSP) for 15 to 24 year olds. NCSP testing coverage is more than twice as high in women (28%) than men (11%) of that age-group (data here). Additionally, dual (CT/GC) NAAT platforms are commonly used for the NCSP and, while the positive predictive value of a gonorrhoea test in a community sample is very low, people with false positive results may be incorrectly prescribed antibiotics to treat gonorrhoea. These two aspects may work against the screening intensity hypothesis.
4. There was no evidence of an association between azithromycin resistant NG and being diagnosed previously with chlamydia or gonorrhoea (as discussed in this paper, which the authors also cite: Clifton et al[1]); ths suggests that those who get tested for STIs more frequently do not have higher azithromycin MICs.
5. The authors only considered GRASP data from 2010, 2011 and 2014, but this analysis could be strengthened by including data from more years, including more recently published data: https://www.gov.uk/government/publications/gonococcal-resistance-to-antimicrobials-surveillance-programme-grasp-report. Alternatively, could the authors please specify these three years were selected, or why data from different countries aren't compared within the same years? Also, could you please clarify why the MIC distributions for all three antibiotics were not analysed for the three time points? For example, the ceftriaxone data from the UK in 2011 could have been considered. In addition, cefixime resistance was widespread across Europe in 2010 due to the ST1407 clone. Cefixime resistance in Belgium was similar than the UK in 2010 according to the Euro-GASP data ([2]) and it would not be surprising if the burden of this resistance was in MSM in Belgium also. This analysis would be strengthened by including Belgium data from 2010/2011.
6. Interestingly, Euro-GASP 2015 and 2016 data show much higher cefixime resistance in Belgium than the UK. According to the proposed hypothesis, should the opposite pattern have been observed? It would also be interesting to speculate the level of cefixime resistance in MSM in the UK if asymptomatic, multiple-site screening was not in place.
7. In the UK, gonorrhoea screening guidelines for MSM attending specialist sexual health services were updated in 2010, and this has led to an increase in gonorrhoea tests since then. This testing trend should be considered when interpreting these findings, as the level of testing prior to 2010 would be much lower than those of more recent years. Furthermore, the upturn in screening coincided with a rapid decline in cephalosporin

resistance.

8. Between 2010 (gonorrhoea screening guideline) and 2012 (change to dual therapy), the increased screening at sexual health services would have detected more cases and these were treated with cefixime which has been shown to be less effective, particularly at the pharynx (Barbee 2013). This would suggest that the increased prevalence of cefixime resistance in MSM may have been due to the usage of an antibiotic which is less effective than ceftriaxone, rather than the result of intensive screening.
9. Figure 1 shows ciprofloxacin resistance was higher initially in heterosexual men, rather than MSM; therefore, resistance does not always emerge in MSM first.
10. The labelling of the graphs in figure 2 is unclear – could this be clarified?
11. In the discussion and in reference to the link between MSM and travel – this may be the case, but we have found no data to support this: Town et al[3]. In addition one of the references cited mentions that heterosexual men reported more sex abroad than MSM - Matteelli et al[4].
12. As you have used publicly available GRASP data collected for routine surveillance purposes, the ethics statement should be amended accordingly. Similarly, references to the 'STBRU' should be to 'PHE'.
13. As sexual orientation data was missing for 59% of the Belgium isolates, what are your views on how representative the sample of MSM whose data are considered in this analysis is?
14. As many of the MIC medians were the same and some of MIC distributions that are declared as significantly different do not appear very different on inspection, particularly for 2014 UK cefixime and ceftriaxone, it would be useful if additional analysis were performed, such as linear regression with the geometric MIC means.

References

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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?

Partly

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: All 3 reviewers work on the GRASP team at PHE

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

Author Response 26 Jul 2018

Chris Kenyon, Institute of Tropical Medicine, Antwerp, Belgium

We would be keen to discuss the proposed hypothesis and interpretation of GRASP data with you, and have included comments for the authors' consideration:

Reply:
Thank you for your interest and most useful comments. We would be most interested in collaborating on further studies along these lines.

1. The evidence for different levels of screening intensity in MSM is available from EMIS; is there a comparable source of these data for heterosexual women? Also, did the recruitment strategy to participate in EMIS vary between the UK and Belgium? If so, this would meant that response to the question on the history of STI screening may be less comparable between these two countries.

Reply:
We looked and unfortunately could not find a comparable source for women. The recruitment strategies for EMIS were similar in the two countries. Because EMIS did not collect nationally representative samples we cannot exclude the possibility of a sampling bias that differed between the UK and Belgium. The large numbers recruited in EMIS in both countries and the fact that the results for the screening and other questions are commensurate with other data sources however argues against such a bias.

1. We believe a comparison of MSM with heterosexual men may be more useful, even with the limitations of underreporting of same-sex contact in heterosexually-identifying men. This is because the numbers of isolates are more comparable, women sometimes have different antimicrobial susceptibility profiles from heterosexual men and the cultures available from women are not representative of the circulating isolates in the community due to the difficulty in culturing from women.

Reply:
Repeating the analyses with heterosexual men instead of women makes very little difference to the results. As we note above, visual comparisons of the MIC frequency distributions for azithromycin, cefixime and ceftriaxone between heterosexual men and women in the annual GRASP reports reveal little difference. We would be interested to repeat this analysis in using a larger number of countries/subpopulations as outlined in the conclusion.

1. In England, most gonorrhoea and chlamydia are diagnosed in people under the age of 25 years, with over one million chlamydia tests conducted annually though the National Chlamydia Screening Programme (NCSP) for 15 to 24 year olds. NCSP testing coverage is more than twice as high in women (28%) than men (11%) of that age-group (data here). Additionally, dual (CT/GC) NAAT platforms are commonly used for the NCSP and, while the positive predictive value of a gonorrhoea test in a community sample is very low, people with false positive results may be incorrectly prescribed antibiotics to treat gonorrhoea. These two aspects may work against the screening intensity hypothesis.

Reply:
This is a useful observation. We have thought at some length about how screening intensity may produce antimicrobial resistance (AMR) in N. gonorrhoeae (Ng). This has lead to the pharmacoecological theory of AMR (connectivity AMR theory) which posits that it is the combination of dense sexual networks plus excess antimicrobial consumption (such as from intense screening) which is responsible. The dense sex network generates the high prevalence of Ng and the antimicrobial exposure then initially lowers prevalence but in the process generates a fitness advantage for resistant Ng [4]. If this theory is correct then intensive screening in the general heterosexual population in the UK (with its low connectivity network) would not have the same effect on selecting for resistance because the prevalence of Ng is low. Populations of sex workers would also be predicted to be at risk for the genesis of AMR, which has been observed. We have added a section in the discussion to make this clearer. These considerations are best understood by means of the following diagram (Figure 1) which is taken from our recent paper on the topic [4]:

URL of Figure 1: https://wwwnc.cdc.gov/eid/article/24/7/17-2104-f2

Figure 1. High network connectivity combined with excess antimicrobial drug exposure from *N. gonorrhoeae* pre-exposure prophylaxis could produce antimicrobial resistance. A dense sexual network translates into a high equilibrium prevalence of *N. gonorrhoeae* (red squares) at time-point 1. Active *N. gonorrhoeae* screening of 50% of this population every 3 months results in 50% lower *N. gonorrhoeae* prevalence at time-point 2 (3 months later) but at the expense of an altered resistome (A_{Scr}; black squares represent 3 patients with *N. gonorrhoeae* cleared by screening/treatment. The unchanged underlying network connectivity results in a force that pushes *N. gonorrhoeae* back toward its equilibrium prevalence, placing recently cured patients at high risk for reinfection at a time when their

resistomes are enriched with resistance genes. Early reinfecting *N. gonorrhoeae* take up these resistance genes by transformation. In the absence of screening and excess antimicrobial drug use (A_{NoScr}) *N. gonorrhoeae* prevalence would not decline but there would be no pressure to select for antimicrobial resistance. Gray squares indicate uninfected persons; lines represent sexual relationships.

1. There was no evidence of an association between azithromycin resistant NG and being diagnosed previously with chlamydia or gonorrhoea (as discussed in this paper, which the authors also cite: Clifton et al1); ths suggests that those who get tested for STIs more frequently do not have higher azithromycin MICs.

Reply:
We found the paper by Clifton et al. most interesting but are also aware of the paper by Wind et al., which found that receipt of azithromycin in the previous 30 days was associated with an increased MIC [5]. More important however is the ecological perspective. It is plausible that excess AMR is exerting its effect at the population level. Thus if population A has 30 fold higher macrolide consumption than population B we know from a range of studies that macrolide resistance in *S. pneumonia* and numerous other pathobionts is much more likely to emerge in population A than B. This has been clearly shown in ecological level studies [6-8]. The association may be harder to establish at an individual level but if one thinks from a pharmacoecologic perspective (and includes considerations of how Ng can acquire AMR from Neisseriaceae and other commensals as it transits through a population) it is easy to see why this is the case [4]. The deleterious effect of macrolides on the resistome at an individual level have been long established [7]. These considerations are of considerable importance given the threat of untreatable Ng. We have recently calculated the macrolide and cephalosporin exposure that 3 site, 3 monthly screening for gonorrhoea and chlamydia places on PrEP cohort (Unpublished results). We did this via conducting a literature review of the incidence of gonorrhoea and chlamydia in PrEP studies that conducted 3 site, 3 monthly screening. We found that screening results in macrolide consumption rates that considerably exceed those in high macrolide consumption populations where consumption has been strongly associated with macrolide resistance.

1. The authors only considered GRASP data from 2010, 2011 and 2014, but this analysis could be strengthened by including data from more years, including more recently published data: https://www.gov.uk/government/publications/gonococcal-resistance-to-antimicrobials-surveillance-programme-grasp-report. Alternatively, could the authors please specify these three years were selected, or why data from different countries aren't compared within the same years? Also, could you please clarify why the MIC distributions for all three antibiotics were not analysed for the three time points? For example, the ceftriaxone data from the UK in 2011 could have been considered. In addition, cefixime resistance was widespread across Europe in 2010 due to the ST1407 clone. Cefixime resistance in Belgium was similar than the UK in 2010 according to the Euro-GASP data (2) and it would not be surprising if the burden of this resistance was in MSM in Belgium also. This analysis would be strengthened by

including Belgian data from 2010/2011.

Reply:
We agree it is frustrating not to have data from Belgium in 2010/11. As we point out in the methods section we could not use this data as we do not have data as to sexual orientation in sufficient number from this period. In the methods we state:
The details regarding sexual orientation started to be reported in sufficient numbers from 2013 onwards

For the UK data we agree it would be interesting to look at the data from all the available years. We chose the first year at or after 2010 when antimicrobial MIC frequency distributions were reported by gender/sexual orientation. This was 2010 for ceftriaxone and 2011 for azithromycin and cefixime. We then looked at all 3 antimicrobials in 2014 so as to use the same time period for the comparison with Belgium. We considered that this analysis was sufficient for our purposes but acknowledge the reviewers point that there would be a multiplicity of other ways of doing this analysis. Looking at the MIC distributions from other years we consider it likely that this would not substantially change the results. Our analysis involved comparing MIC distributions between MSM and women in the two countries and not comparing MSM or women between the two countries.

1. Interestingly, Euro-GASP 2015 and 2016 data show much higher cefixime resistance in Belgium than the UK. According to the proposed hypothesis, should the opposite pattern have been observed? It would also be interesting to speculate the level of cefixime resistance in MSM in the UK if asymptomatic, multiple-site screening was not in place.

Reply:
This is an excellent point. The cefixime resistance figures in Belgium 2015/2016 are remarkably high according to the Euro GRASP figures but it must be remembered that these figures are based on a small sample of all national samples for this time period. The full results for 2016 are 597 isolates tested of which 36 (6%°/1(0,1%) had decreased sensitivity to cefixime according to EUCAST/CLSI breakpoints. The results in 2015 were similar: 630 isolates tested with 52 (8,3%)/1(0,1%) classified as decreased sensitivity. These lower rates of resistance are a more accurate representation than the Euro GRASP figures [3].

< >In the UK, gonorrhoea screening guidelines for MSM attending specialist sexual health services were updated in 2010, and this has led to an increase in gonorrhoea tests since then. This testing trend should be considered when interpreting these findings, as the level of testing prior to 2010 would be much lower than those of more recent years. Furthermore, the upturn in screening coincided with a rapid decline in cephalosporin resistance. Between 2010 (gonorrhoea screening guideline) and 2012 (change to dual therapy), the increased screening at sexual health services would have detected more cases and these were treated with cefixime which has been shown to be less effective, particularly at the pharynx (Barbee 2013). This would suggest that the increased prevalence of cefixime resistance in MSM may have been due to the usage of an antibiotic which is less effective

than ceftriaxone, rather than the result of intensive screening. Figure 1 shows ciprofloxacin resistance was higher initially in heterosexual men, rather than MSM; therefore, resistance does not always emerge in MSM first. The labelling of the graphs in figure 2 is unclear – could this be clarified? In the discussion and in reference to the link between MSM and travel – this may be the case, but we have found no data to support this: Town et al3. In addition one of the references cited mentions that heterosexual men reported more sex abroad than MSM - Matteelli et al4. As you have used publicly available GRASP data collected for routine surveillance purposes, the ethics statement should be amended accordingly. Similarly, references to the 'STBRU' should be to 'PHE'. As sexual orientation data was missing for 59% of the Belgium isolates, what are your views on how representative the sample of MSM whose data are considered in this analysis is? As many of the MIC medians were the same and some of MIC distributions that are declared as significantly different do not appear very different on inspection, particularly for 2014 UK cefixime and ceftriaxone, it would be useful if additional analysis were performed, such as linear regression with the geometric MIC means.https://doi.org/10.3201/eid2407.172104.

Reply:
Thanks for this information which is indeed relevant and has been included as a major caveat in the discussion.

1. Between 2010 (gonorrhoea screening guideline) and 2012 (change to dual therapy), the increased screening at sexual health services would have detected more cases and these were treated with cefixime which has been shown to be less effective, particularly at the pharynx (Barbee 2013). This would suggest that the increased prevalence of cefixime resistance in MSM may have been due to the usage of an antibiotic which is less effective than ceftriaxone, rather than the result of intensive screening.

Reply:
We agree this is a possibility and have expanded the discussion section to reflect his point.

1. Figure 1 shows ciprofloxacin resistance was higher initially in heterosexual men, rather than MSM; therefore, resistance does not always emerge in MSM first.

Reply:
This is true as is the repeated emergence of AMR in sex workers and their contacts.

1. The labelling of the graphs in figure 2 is unclear – could this be clarified?

Reply:
We have asked the publisher to increase the size of the figure to a single page so as to make it easier to read the labeling.

1. In the discussion and in reference to the link between MSM and travel – this may be

the case, but we have found no data to support this: Town et al3. In addition one of the references cited mentions that heterosexual men reported more sex abroad than MSM - Matteelli et al4.

Reply:
Thanks. We have added a sentence pointing this out in the discussion.

1. As you have used publicly available GRASP data collected for routine surveillance purposes, the ethics statement should be amended accordingly. Similarly, references to the 'STBRU' should be to 'PHE'.

Reply:
Both these changes have been made.

1. As sexual orientation data was missing for 59% of the Belgium isolates, what are your views on how representative the sample of MSM whose data are considered in this analysis is?

Reply:
This is a major limitation and as we note in the limitations section means we need to be cautious in any conclusions we draw from this study.

1. As many of the MIC medians were the same and some of MIC distributions that are declared as significantly different do not appear very different on inspection, particularly for 2014 UK cefixime and ceftriaxone, it would be useful if additional analysis were performed, such as linear regression with the geometric MIC means.

Reply:
Numerous other analyses could be done but this is a very simple and limited analysis. We believe that given the major limitations of the study described above and more fully in the paper, it would be inappropriate to conduct further complicated statistical analyses. The data suggest some differences in the relationship between MIC distributions between the 2 countries. The next step should be to find better and more extensive datasets to evaluate further test the hypothesis. We conclude the paper with a description of what we think these analyses could be.

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Low Extended-Spectrum Cephalosporin Resistance but high Azithromycin Resistance in Neisseria gonorrhoeae in 24 European Countries, 2015. BMC Infect Dis. 2017;17(1):617. Epub 2017/09/13. doi: 10.1186/s12879-017-2707-z. PubMed PMID: 28893203; PubMed Central PMCID: PMCPMC5594611.
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Competing Interests: We have no competing interests

Reviewer Report 14 June 2018

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Henry J C de Vries
[1] University of Amsterdam, department of dermatology, Amsterdam Institute for Infection and Immunity (AI&II), Meibergdreef 9, Amsterdam, The Netherlands
[2] Public Health Service (GGD) , Amsterdam, The Netherlands

This is a nicely written paper proposing a new and refreshing hypothesis that gonorrhoea screening in a larger proportion of a certain population and subsequent treatment could induce AMR. Although the authors admit that they cannot confirm their hypothesis, they do claim to see an association in support of their claim. There is a multitude of other explanations for the association found which aren't properly discussed and more sound evidence is needed to confirm their statement. It is of interest though to report on their findings.

I have some comments to consider though.

1. In the method section it is stated that MSM were compared with women rather than heterosexual men to avoid the problem of misclassification of men who occasionally have sex with men but regard themselves as heterosexual. In the same fashion it is possible that heterosexual women might have sex with bisexual males and thus be exposed to the MSM pharmacoecology described here. Please consider the effect of this option in the light of choosing the control group.

2. The MIC right shift for all 3 antibiotics has decreased from 2010/11 to 2014 in the UK this is attributed to higher dosages of cephalosporins given and the addition of azi to the recommended therapy. This finding can be interpreted as an argument against the hypothesis of the authors; correct treatment of a confirmed infection does not lead to the induction of AMR since the strain is eradicated and cannot develop AMR.

3. It is stated also that ceftriaxone's longer half-life than cefixime may have played a role in preventing MIC drift in Belgium. This is counter intuitive. A longer antimicrobial half life is associated with the induction of AMR due to the prolonged exposure of bacteria to sub therapeutic concentrations of antibiotic during re-exposure. See also: Decreased Azithromycin Susceptibility of Neisseria gonorrhoeae Isolates in Patients Recently Treated with Azithromycin. Wind CM, de Vries E, Schim van der Loeff MF, van Rooijen MS, van Dam AP, Demczuk WHB, Martin I, de Vries HJC. Clin Infect Dis. 2017 Jul 1;65(1):37-45. [Ref-1]

4. In the second paragraph of the discussion it isn't mentioned that: “This explanation stems from the insight that the intensity of exposure to antimicrobials plays a crucial role in the genesis of AMR [ref 21]. Here, the reference is misquoted, Cantas et al specifically address the non-therapeutic and low-level dosage use of antimicrobials that lead to AMR induction. This is not the case in the UK setting where MSM are treated with therapeutic dosages, and only after infection has been confirmed.

References

1. Wind C, de Vries E, Schim van der Loeff M, van Rooijen M, et al.: Decreased Azithromycin Susceptibility of Neisseria gonorrhoeae Isolates in Patients Recently Treated with Azithromycin. *Clinical Infectious Diseases*. 2017; **65** (1): 37-45 Publisher Full Text

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

Yes

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?

I cannot comment. A qualified statistician is required.

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.

I confirm that I have read this submission and 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 19 Jun 2018

Chris Kenyon, Institute of Tropical Medicine, Antwerp, Belgium

Dear Prof. de Vries

Thank you for your useful suggestions which we respond to below:

1. This is a valid concern. Repeating the analyses using heterosexual men instead of women as the control group makes little difference to the MSM vs. control MIC frequency distribution curves. This is evident if one looks at the individual GRASP reports. We can share the figures for Belgium if there is interest in this.

2. and 3. It is true that we do not know why the right shift has declined. It is also true that a long half of an antibiotic is frequently associated with the induction of resistance. Azithromycin is a good example of this as noted in the reference you refer to. We have argued elsewhere that a N. gonorrhoeae (Ng) infection during the long declining half life of azithromycin is a plausible risk factor for inducing AMR (ref 27). This is however quite different to what is being argued here. If an antibiotic does not attain a Ng requires a free time above MIC for cephalosporins for at least 10-20 hours. If this is not attained this will place a selection pressure to develop AMR. Because the half life of cefixime is shorter than that of ceftriaxone (3.4 hours vs. 8.45 hours) there is a higher risk of not attaining the required free time above MIC and thereby selecting for AMR (doi:10.1093/jac/dkq289). Other factors such as the ratio of the Mutant Prevention Concentration to MIC ratio may also play a role but ultimately we do not know with certainty the reasons why cefixime is more selective for resistance.

What we do have good experimental evidence for however is that for a number of bug-drug combinations cefixime is more prone to AMR than other third generation cephalosporins. One of the most convincing studies of this was an in a in vitro differential selection study by Negri et al., who found that cefixime was the best selector of penicillin resistance in *Streptococcus pneumoniae* (compared to amoxicillin, cefuroxime and cefotaxime (PMID: 8141563). The mechanism underpinning this effect has not been clearly elucidated but a number of authors have speculated that it may be related at least in part to cefixime's shorter half life.

We will add this discussion to the next version of the paper.

4. In the next version of the paper we will include the references listed below to better back up this claim. The Cantas reference should however remain as it is a useful overview of the importance of considering total antimicrobial consumption in an ecosystem perspective. The main pathway from high antimicrobial consumption to AMR is not via subtherapeutic dosing but rather factors such as antimicrobial induced changes to the resistome and microbiome which can then be taken up by Ng via mechanisms such as transformation, plasmids. This and other mechanisms are outlined in the references below as well as refs 27-29 and 32 above.

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Competing Interests: No competing interests

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