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Variability of antimicrobial susceptibility of commensal Neisseria species supports its use as a marker of excessive antimicrobial consumption – reflections from the results of a four-country study

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Abstract

This perspective explores the utility of commensal *Neisseria* species as an early warning sign of excessive antimicrobial consumption. Little is known as to how the prevalence and antimicrobial susceptibility of various commensal *Neisseria* species varies between populations around the world. We compared the prevalence and antimicrobial susceptibilities of oral commensal *Neisseria*

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Authors' contributions

CK conceptualized the study. CK conducted the statistical analyses and wrote the first draft of the paper. All authors read, contributed to, and approved the final draft.

Declarations of competing interest

The authors have no competing interests to declare.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work no AI tools were used.

Ethical approval

Ethics approval was obtained for each study as detailed in the study reports.

Supplementary materials

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species in the general population and cohorts of men who have sex with men (MSM) in four countries with available data – Belgium, Italy, Japan and Vietnam. In individuals where *Neisseria* spp. were detected, N. *subflava* was present in 70–100% of individuals in the different studies. The N. *subflava* azithromycin and ciprofloxacin minimum inhibitory concentrations (MICs) were higher in the MSM than in the general population. The MICs of all *Neisseria* spp. were very similar in the general populations of Belgium and Italy. For all *Neisseria* spp., azithromycin and ceftriaxone MICs were higher, whereas ciprofloxacin MICs were lower in Belgium and Italy than in Japan. The higher azithromycin and ciprofloxacin MICs observed in the cohorts of MSM compared to the general population and the higher ciprofloxacin MICs in Japan compared to Belgium and Italy are commensurate with the most commonly antimicrobial prescribed in these populations. Our results support using commensal *Neisseria* species as an early warning system of excessive antimicrobial consumption.

Keywords

Neisseria ; Commensals; Oropharynx; Antimicrobial resistance; Belgium; Italy; Japan; Vietnam

Introduction

It is increasingly appreciated that commensal *Neisseria* spp. play an important role in *Neisseria gonorrhoeae* antimicrobial resistance (AMR). Commensal *Neisseria* spp. colonize the oral cavities of most humans [1]. This means they could be exposed to AMR selection pressure every time someone takes an antimicrobial and could be used as an early warning system of excessive antimicrobial consumption in key populations [1]. Gonococcal AMR has typically emerged in populations with dense sexual networks and high antimicrobial consumption, such as sex workers and men who have sex with men (MSM), including those taking HIV pre-exposure prophylaxis (PrEP) [2]. This gonococcal AMR has frequently emerged via horizontal gene transfer of resistance from commensal *Neisseria* spp [1,6].

We and others have developed a relatively simple method to conduct surveillance of antimicrobial susceptibility in oral *Neisseria* spp. using an oral rinse with sterile water [3–5]. We have proposed that an annual survey of the antimicrobial susceptibilities of oral *Neisseria* spp. in key populations such as MSM and sex workers could provide a strategy to see if the population had been exposed to excessive antimicrobials in the preceding period [6]. In our first survey of our HIV PrEP cohort in Antwerp, Belgium, we found that the azithromycin and ciprofloxacin minimum inhibitory concentrations (MICs) of commensal *Neisseria* species were considerably higher than those of the Antwerp general population [3]. In subsequent studies we have found similar findings in settings as diverse as Italy, Japan and Vietnam [7–9].

In this perspective piece, we summarize this research and compare the *Neisseria* spp. MIC distributions in general populations and core-groups of MSM in these four different countries.

The sampling and processing methods used for each study sample are described in detail elsewhere and are summarized in SBox 1 and STable 1 [3,7–9]. Antimicrobial use data

for Belgium, Italy, Japan, the Netherlands and Vietnam in 2000 and 2015 were taken from IQVIA's MIDAS information services [10]. Antimicrobial usage is reported as defined daily doses (DDD) per 1,000 individuals. The Netherlands was included as a country with low antimicrobial consumption [10]. Multivariate linear regression was used to assess if MICs were independently associated with particular populations controlling for *Neisseria* spp. composition. The MICs were log transformed for these analyses to create a more normal distribution. STATA MP v.16 was used for all the analyses (SBox 2).

Variations in Neisseria *spp. prevalence and diversity between studies*

A lower proportion of participants in Japan (25.3%) and the cohort of MSM in Belgium (67.2%) had *Neisseria* spp. detected than those from the other studies, where these bacteria were detected from all participants ($P < 0.05$; STable 1). In all studies, *Neisseria subflava* was the most frequently detected species. Among individuals harbouring *Neisseria* spp., N. *subflava* colonization ranged from 70% to 100% across the different studies (STable 1). The number of *Neisseria* spp. detected per individual was higher in the general population (median 2, IQR 1–2), than the cohort of MSM (median 1, IQR 0–2) in Belgium ($P < 0.05$; STable 1).

Comparisons of Neisseria *spp. antimicrobial susceptibilities and diversity between general populations and MSM*

In both Belgium and Italy, N. *subflava* azithromycin and ciprofloxacin (but not ceftriaxone) MICs were higher in MSM than in the general population (azithromycin: median 4 mg/L, IQR 3–256 mg/L and median 3 mg/L, IQR 2–4 mg/L, respectively; ciprofloxacin: median 0.25 mg/L IQR 0.023–0.5 and median 0.023 mg/L, IQR 0.012–0.38 mg/L, respectively; all $P < 0.05$; Table 1; STable 2). When these analyses were done for all *Neisseria* spp., no differences were evident (STable 3). A multivariate model controlling for *Neisseria* species confirmed that in both countries, azithromycin and ciprofloxacin MICs were higher in MSM than in the general population (STable 4). In the case of Belgium, ceftriaxone MICs were also higher among MSM in this multivariate model.

Comparisons of Neisseria *spp. antimicrobial susceptibilities between general populations*

The *Neisseria* spp. MICs were very similar in the general populations of Belgium and Italy (Table 1). However, for all *Neisseria* spp. and N. *subflava*, azithromycin and ceftriaxone MICs were higher and ciprofloxacin MICs lower in Belgium and Italy than in Japan (All $P < 0.001$; Table 1). These same associations were found in multivariate testing (STable 3).

Comparisons of Neisseria *spp. antimicrobial susceptibilities between MSM populations*

There was no difference in ceftriaxone MICs for both N. *subflava* and all commensal *Neisseria* spp. between the cohorts of MSM in Belgium and Italy (Table 1). Azithromycin MICs for N. *subflava* were, however, higher in Belgium, an effect also seen in multivariate testing (STable 3). In the case of ciprofloxacin, MICs were lower in Belgium in all *Neisseria* species, but not in the analysis restricted to N. *subflava* or the multivariate analysis (Table 1; STable 3). The ceftriaxone and ciprofloxacin MICs were higher in Vietnam than Belgium and Italy. This was also true for ciprofloxacin MICs of N. *subflava* (Table 1).

Comparisons of Neisseria *spp. antimicrobial susceptibilities between N.* gonorrhoeae *and N.* subflava

Ceftriaxone and azithromycin MICs were higher in N. *subflava* than N. *gonorrhoeae* in all three countries, where this could be assessed (STable 4). In contrast, the ciprofloxacin MICs of N. *subflava* were lower than those of N. *gonorrhoeae* in two countries (Italy and Japan).

Antimicrobial consumption

In 2015, the consumption of cephalosporins, fluoroquinolones and macrolides was between 2.2 and 46.6-fold higher in each of the four countries than in the Netherlands (STable 5). This effect was most pronounced for cephalosporins (11.8 to 46.6-fold higher). In 2015, the consumption of cephalosporins was higher in Japan (1587 DDD) and Vietnam (3867 DDD) than in Belgium (981 DDD) and Italy (1041 DDD). The consumption of fluoroquinolones in 2000 was higher in Japan (1949 DDD) than in other countries (368–913 DDD). However, by 2015, fluoroquinolone use was lower in Japan (954 DDD) and Vietnam (1162 DDD) than in Belgium (1246 DDD) and Italy (1486 DDD).

We found that the antimicrobial susceptibilities of commensal *Neisseria* spp. were very similar in the general populations of Belgium and Italy. The same was true for the cohorts of MSM from these countries. The higher azithromycin MICs in MSM in Belgium compared to Italy may be due to the selection criteria for this sample, which included having had a bacterial STI diagnosed in the past 2 years and, consequently, they may have been more exposed to antimicrobials than the Italian cohort. In previous studies, we noted a particularly high consumption of macrolides in this population [11]. In keeping with previous analyses, we found that in both these countries, the azithromycin and ciprofloxacin MICs were higher in the MSM than in the general populations [3,7]. These findings can be parsimoniously explained by the higher consumption of macrolides and fluoroquinolones in MSM with high rates of partner change and multiple STIs [12]. These findings are supportive of the concept that AMR surveillance of commensal *Neisseria* spp. can be used as a marker of antimicrobial consumption [1,8].

In a similar vein, the higher ceftriaxone and azithromycin MICs of N. *subflava* than N. *gonorrhoeae* lend support to the proposal to use commensal *Neisseria* spp. as canaries in the antimicrobial coalmines [1,8]. It is important to note that horizontal gene transfer from *Neisseria* commensals in general and N. *subflava,* in particular, have played a key role in the genesis of gonococcal cephalosporin and macrolide resistance [13].

The median ciprofloxacin MICs for commensal *Neisseria* spp. and N. *gonorrhoeae* were approximately 10-fold higher in Japan than in Belgium and Italy. This finding is compatible with the higher consumption of fluoroquinolones in Japan in 2000. It is, however, at odds with the lower consumption of fluoroquinolones in Japan by 2015. We also found that ciprofloxacin MICs of commensal *Neisseria* spp. were higher in MSM in Vietnam than in Belgium and Italy despite lower fluoroquinolone consumption in Vietnam, according to the MIDAS estimates we used. These MIDAS estimates may be underestimates of consumption [14]. In addition, consumption of fluoroquinolones in core-groups with a high rate of partner turnover may be more important than fluoroquinolone use in the general population. In

our MSM cohort from Vietnam, the ciprofloxacin MICs of commensal *Neisseria* spp. were higher in individuals reporting antimicrobial use in the preceding 6 months [8]. A number of analyses in Japan have also found that high exposure to fluoroquinolones in core-groups played an important role in the emergence of gonococcal fluoroquinolone resistance in core-groups and more generally [15,16]. Excessive exposure in coregroups could also explain the higher ciprofloxacin MICs of N. *gonorrhoeae* than N. *subflava* in Italy and Japan. It could not, however, explain the high ciprofloxacin MICs of commensal *Neisseria* spp. in the Japanese general population. We hypothesize that this may be a part of the syndemic of fluoroquinolone resistance noted in a wide range of bacterial species in East Asia. This includes N. *lactamica* in China (99% ciprofloxacin resistance) [17], N. *gonorrhoeae* in parts of China/Vietnam (over 90% ciprofloxacin resistance) and a variety of other bacterial species [18]. Plausible causes of this syndemic are high consumption of fluoroquinolones by humans and food animals [18,19]. Our results provide further evidence for the need for reducing fluoroquinolone consumption from these sources. Whilst phylogenetic analyses have shown that the transformation of AMR has played an important role in the emergence of fluoroquinolone resistance in N. *meningitidis* in Asia [17], this has not been the case for N. *gonorrhoeae* [20]. Future studies are required that include more accurate antimicrobial consumption data.

The other major limitations of our analysis were differences in methodology, as noted above and outlined in STable 1. In particular the studies used different methods to obtain oropharyngeal samples which may have resulted differences in Neisseria spp. detected. In addition, we only had data from four countries and in the case of Japan and Vietnam, only data from the general population and MSM, respectively. We also only had gonococcal MIC data for a limited number of populations. Some of the sample sizes were small, and some of the collection took place during the COVID-19 pandemic, which may have affected the results. We did not have more recent antimicrobial consumption data or data pertaining to core-groups. We only conducted basic statistical analyses between studies. Our analysis was limited to a single genus. It would be useful to compare the utility of *Neisseria* spp. with other genera, such as rectal *Escherichia* and oral streptococci, as early warning systems of excessive antimicrobial consumption. Finally, our analysis was weakened by the fact that we did not include a population with low antimicrobial consumption.

Despite these limitations, our analysis confirms the utility of using the antimicrobial susceptibility of commensal *Neisseria* as an early warning system of excessive antimicrobial use. In the case of azithromycin and ceftriaxone, we found that N. *subflava* MICs were significantly higher than those of N. *gonorrhoeae* in all three countries with available data. This finding is compatible with the higher prevalence of N. *subflava* (close to 100%) than N. *gonorrhoeae* (between 0.1% and 10%), ensuring that N. *subflava* is more affected by bystander selection [21]. Further work is required to ascertain 1) the N. *subflava* MIC distributions in populations with lower use of antimicrobials; 2) if increases in MIC distributions are reversible; 3) if there are MIC thresholds that populations can aim to stay below to reduce the probability of AMR emerging in commensals and being transferred to pathogenic *Neisseria* spp. In addition, we found that populations of MSM in Belgium and Italy with high consumption of antimicrobials had higher MICs than the general population. Ciprofloxacin MICs in Japan and Vietnam were markedly higher than those

in Europe. These findings provide additional motivations for stewardship interventions to reduce excessive antimicrobial consumption.

Data sharing

Further data are available as a Supplement to this manuscript. Additional related documents, such as the study protocols, laboratory analysis plans, and informed consent forms, can be obtained from the corresponding author.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Country-level comparisons of azithromycin, ceftriaxone and ciprofloxacin MICs in all commensal *Neisseria* spp, N. *subflava* and N. *gonorrhoeae* (For example, '↑' in the 'Belgium v. Japan' column indicates that for this antimicrobial, the MICs were significantly higher in Belgium than in Japan).

	Antimicrobial	Belgium v. Japan	Belgium v. Italy	Italy v. Japan	Belgium v. Vietnam	Italy v. Vietnam
General population						
All commensal *Neisseria* spp.	Azithromycin	↑***	-	↑***	NA	NA
	Ceftriaxone	↑***	-	↑***	NA	NA
	Ciprofloxacin	↓***	-	↓***	NA	NA
N. *subflava*	Azithromycin	↑***	-	↑***	NA	NA
	Ceftriaxone	↑***	-	↑***	NA	NA
	Ciprofloxacin	↓***	-	↓***	NA	NA
Multivariate controlling for Neisseria species composition	Azithromycin	↑**	-	↑**	NA	NA
	Ceftriaxone	↑**	-	↑**	NA	NA
	Ciprofloxacin	↓**	-	↓**	NA	NA
N. *gonorrhoeae*	Azithromycin	↓***	↓***	↑**	NA	NA
	Ceftriaxone	↓***	-	↓***	NA	NA
	Ciprofloxacin	↓***	↓***	↓**	NA	NA
MSM						
All commensal *Neisseria* spp.	Azithromycin	NA	-	NA	NA	NA
	Ceftriaxone	NA	-	NA	↓***	↓***
	Ciprofloxacin	NA	↓**	NA	↓***	↓***
N. *subflava*	Azithromycin	NA	↑*	NA	NA	NA
	Ceftriaxone	NA	-	NA	-	↓***
	Ciprofloxacin	NA	-	NA	↓***	↓***
Multivariate controlling for Neisseria species composition	Azithromycin	NA	↑**	NA	NA	NA
	Ceftriaxone	NA	-	NA	-	↑**
	Ciprofloxacin	NA	-	NA	↑**	↑**

N commensal Neisseria isolates per study: Belgium General Population (132), Belgium MSM (101), Italy General Population (116), Italy MSM (148), Japan (424), Vietnam (259).

* $P < 0.05$

** $P < 0.005$

*** $P < 0.0005$.

'NA' - not available; '-' no statistically significant difference.