Markedly Reduced Azithromycin and Ceftriaxone Susceptibility in Commensal *Neisseria* Species in Clinical Samples From Belgian Men Who Have Sex With Men

To the Editor—Dong et al found high levels of antimicrobial resistance (AMR) in commensal oropharyngeal *Neisseria* species in Vietnamese men who have sex with men (MSM) and linked this to recent antibiotic consumption [1]. They proposed that these resistant commensals constitute a reservoir of resistance genes for incoming *Neisseria gonorrhoeae*. Studies have confirmed that horizontal gene transfer contributes to gonococcal resistance to macrolides and cephalosporins [2, 3]. Therefore, monitoring the antimicrobial susceptibilities of commensal *Neisseria* in key populations for the emergence of AMR such as MSM [4] may provide an early warning system for gonococcal AMR. This provided the motivation to assess the susceptibility of commensal *Neisseria* to these antimicrobials in MSM attending the sexually transmitted infection (STI) clinic of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. In addition, we compared these results to susceptibilities of *Neisseria* isolates archived at ITM and susceptibilities reported in previous studies.

Between January and May 2019, 10 MSM attending the STI clinic with a diagnosis of anogenital gonorrhea were enrolled. After informed consent, oropharyngeal and anal swabs were taken, before immediate treatment with ceftriaxone 500 mg intramuscularly and azithromycin 2 g orally. Sampling was repeated 14 days later. All swabs were inoculated onto blood and modified Thayer-Martin agar and incubated in 5% carbon dioxide at 36.5°C for 24 hours. Colonies with a *Neisseria*-compatible morphology were subcultured, Gram staining and oxidase test were performed, and *Neisseria* species were identified by matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS). Minimum inhibitory concentrations (MICs) of ceftriaxone and azithromycin were determined by E-test. Ethical approval was obtained from the University of Antwerp Ethics Committee. Additionally, *Neisseria* species from ITM's historical collection (clinical and reference isolates) were evaluated for ceftriaxone and azithromycin susceptibility using agar dilution.

From 40 swabs, 27 *Neisseria* isolates were grown. MALDI-TOF MS classified them into 5 different *Neisseria* species (10 strains of *N. subflava*, 3 *N. macacae*, 2 *N. oralis*, 5 *N. meningitidis*, and 7 *N. gonorrhoeae*). The MICs of isolates from day 14 were similar to those from day 0 (data not shown). Ceftriaxone and azithromycin MICs were high. For example, the ceftriaxone MICs for the most prevalent commensal in our study, *N. subflava* (median, 0.38 mg/L [range, 0.023–2.0 mg/L]) were considerably higher than those from the only study we could find reporting MICs for this organism (median, 0.03 mg/L [range, 0.001–0.12 mg/L]) [5] and those from ITM's collection (median, 0.03 mg/L [range, 0.015–0.06 mg/L]). Likewise, the azithromycin MICs of currently circulating *N. subflava* were considerably higher than those from the historical collection (Table 1). As a limitation, it should be noted that MALDI-TOF MS can accurately identify *N. gonorrhoeae*, whereas species identification of commensal *Neisseria* may show overlap [6].

Given the ease with which *N. gonorrhoeae* can acquire AMR from commensal *Neisseria*, our finding of high MICs of commensal *Neisseria* is concerning and suggests the need for larger studies to better characterize the problem. Studies are also required to evaluate if enhanced antibiotic stewardship activities in this population could reduce the MICs of commensal *Neisseria*.

Table 1. Azithromycin and Ceftriaxone Minimum Inhibitory Concentrations of *Neisseria* Isolates in the Current Study, the Japanese Study, and the Institute of Tropical Medicine's Collection

	Neisseria subflava			*Neisseria macacae*		*Neisseria oralis*		*Neisseria meningitidis*	
Parameter	Belgium, 2019 (n = 10)	Japan, 2005 (n = 45)	ITM, Historical (n = 7)	Belgium, 2019 (n = 3)	ITM, Historical (n = 5)	Belgium, 2019 (n = 2)	ITM, Historical (n = 2)	Belgium, 2019 (n = 5)	ITM, Historical (n = 15)
Azithromycin	176 (0.047 to >256)	…	1 (0.25–4)	8 (4 to >256)	8 (4–8)	3 (3–3)	4 (4–4)	0.75 (0.25–0.75)	0.5 (0.25–4)
Ceftriaxone	0.38 (0.023–2.0)	0.03 (0.001–0.12)	0.03 (0.015–0.06)	0.094 (0.032–1)	0.06 (0.03–0.125)	0.273 (0.047–0.5)	0.06 (0.06–0.06)	0.016 (0.016–0.016)	0.002 (0.001–0.060)
Method	Etest	Agar dilution	Agar dilution	Etest	Agar dilution	Etest	Agar dilution	Etest	Agar dilution
Reference	Current study	Furuya et al, 2007 [5]	Current study	Current study	Current study	Current study	Current study	Current study	Current study

Data are presented as the median (range) minimum inhibitory concentration, mg/L.
Abbreviation: ITM, Institute of Tropical Medicine (Antwerp, Belgium).

Note

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Reply to Laumen et al

To the Editor—In their letter, Laumen et al report decreased susceptibility to azithromycin and ceftriaxone among commensal *Neisseria* species obtained from men who have sex with men (MSM) at a sexually transmitted infection clinic in Belgium [1]. The findings of decreased susceptibility to ceftriaxone among commensal *Neisseria* species are similar to our report from MSM in Hanoi, Vietnam [2]. Those findings are important, as commensal *Neisseria* in the oropharynx are an important reservoir for antimicrobial resistance in *Neisseria gonorrhoeae* [3]. Genomic analysis indicates that antimicrobial use drives gonococcal evolution to specific drug-resistant lineages [4]; however, data on the impact of antibiotics on the resistome of commensal *Neisseria* species remain sparse.

There is a paucity of data on the prevalence and distribution of commensal *Neisseria* species among different populations. Approximately half of the *Neisseria* sp. identified in our report were *N. flavescens* (47%; 125/207), whereas these were not identified by Laumen et al. In that report, the most commonly identified species was *N. subflava* (37%; 10/27), which was the second most common species in our report (21.2%; 57/207). We both identified *N. meningitidis* in approximately 5% of isolates. Those variations highlight different distributions between 2 populations, although interpretation of differences is limited by low numbers of participants.

Laumen et al reported high median minimum inhibitory concentrations (MICs) to azithromycin and ceftriaxone for *N. subflava* and *N. macacae*. While not reported in our previous study, we now share median MIC data for ceftriaxone, stratified by self-reported antibiotic use within the past 6 months, for each of the 4 *Neisseria* species identified in their letter and *N. flavescens* (Table 1). Participants with antibiotic use had higher median MICs, which we used to compare with Laumen's report. For *N. subflava*, we found a median MIC to ceftriaxone of 0.094 (interquartile range [IQR], 0.047–0.110), which is lower than that reported by Laumen et al (0.38), although higher than Furuya et al (0.03) [5]. For *N. meningitidis*, median MIC = 0.064 (IQR, 0.002–0.094), higher than that of Laumen et al (0.016). The MICs for both *N. macacae* and *N. oralis* in our report were lower than their reported MICs. *N. flavescens*, the most commonly isolated strain in our study, had a median MIC of 0.047 (IQR, 0.032–0.094). Given the variations in MICs, it is likely that particular strains are harboring genetic determinants of antibiotic resistance, which might increase the risk of transferring antibiotic resistance to *N. gonorrhoeae*.

We concur that using matrix-assisted laser desorption/ionization time-of-flight mass-spectrometer represents a limitation to fully characterize the prevalence of different commensal *Neisseria* species,

Table 1. Minimum Inhibitory Concentrations to Ceftriaxone for 5 Commensal Oropharyngeal *Neisseria* Species From Men Who Have Sex With Men in Hanoi, Vietnam, Stratified by Antibiotic Exposure in the Past 6 Months

	Ceftriaxone MIC		
Species and Antibiotic Use[a]	n	Median	IQR
N. flavescens			
No	76	0.047	0.032–0.094
Yes	49	0.064	0.032–0.125
N. macacae			
No	7	0.047	0.023–0.064
Yes	5	0.064	0.047–0.064
N. meningitidis			
No	10	0.002	0.002–0.094
Yes	3	0.064	0.002–0.094
N. oralis			
No	2	0.056	0.047–0.064
Yes	2	0.034	0.004–0.064
N. subflava			
No	33	0.064	0.032–0.094
Yes	24	0.094	0.047–0.110

Abbreviations: IQR, interquartile range; MIC, minimum inhibitory concentration determined by Etest.
[a]Self-reported use antibiotics (of any class) within the past 6 months.