Detection of *tet*(M) in high-level tetracycline resistant *Neisseria gonorrhoeae*

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Sir,

Antimicrobial resistance in *Neisseria gonorrhoeae* is of significant public health concern and treatment options beyond current first-line therapies are limited.[1] Effective surveillance of antimicrobial resistance in this organism is imperative to retain *N. gonorrhoeae* as a treatable disease.[2]

PHE's Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) is a sentinel surveillance programme, which tests consecutive *N. gonorrhoeae* isolates from 26 sexual health clinics across England and Wales, collected over a two- to three-month period. Isolates are tested against seven different antimicrobials, specifically penicillin, tetracycline, ciprofloxacin, cefixime, ceftriaxone, azithromycin and spectinomycin.

High-level tetracycline resistance (MICs ≥16 mg/L) [3] emerged in the species in the mid-1980s with the insertion of the *tet*(M) resistance gene into two *N. gonorrhoeae* conjugative plasmids,[4] a 42.8 Kb 'American' plasmid and a 40.5 Kb 'Dutch' plasmid.[5] The *tet*(M) product confers resistance to tetracycline by binding to the 30S bacterial ribosomal sub-unit, resulting in release of tetracycline and the continuation of protein synthesis.[6] Lower level tetracycline resistance (>1 mg/L but ≤8 mg/L) in *N. gonorrhoeae* is considered to be chromosomally mediated.[6] We sought to investigate the validity of these criteria for segregating isolates with chromosomal and plasmid-mediated tetracycline resistance and, specifically, whether some *tet*(M)-containing strains might not express high-level resistance leading to under-recognition.

One hundred and ninety-one *N. gonorrhoeae* isolates with known tetracycline susceptibility profiles (MICs ranging from ≤0.5 mg/L to ≥16 mg/L) were selected according to tetracycline MIC from those submitted to PHE as part of GRASP between 2015 and 2017. Susceptibilities had been determined by agar dilution using breakpoint plates with 1 mg/L and 8 mg/L of tetracycline in DST agar [HiMedia, India] plus 1% Vitox [Oxoid, SR0090H} and 5% lysed horse blood (TCS Biosciences, HB038] and/or by gradient strip testing (Etest® [bioMerieux, France] on GC agar base [Difco, 228950] plus 1% Vitox). Boilates were used as template for PCR targeting the *tet*(M) genes of the American and Dutch plasmids specifically.[7] Isolates were scored on the presence/absence and size of any amplicon (American plasmid: 778 bp, Dutch plasmid: 443 bp).

All isolates (n=105) with tetracycline MICs ≥16 mg/L were positive for either an American or Dutch *tet*(M) conjugative plasmid. Furthermore, no isolates (n=86) with MICs ≤8 mg/L harboured either plasmid. As expected the American plasmid predominated in the isolate panel selected, accounting for 76.2% of the high-level tetracycline resistance plasmids detected.

Subsequent analysis found that whilst all tetracycline resistance (>1 mg/L) was more likely to be found in isolates from MSM compared with heterosexual men (adjusted odds ratio (AOR) 1.39 [95% confidence interval (CI) 1.18-1.64], P<0.001), isolates with high-level resistance (≥16 mg/L) were more likely to be from heterosexual men compared with MSM (AOR: 1.45 [95% CI: 1.11-1.88], *p*=0.006) (Table 1).

Between 2015 and 2017, GRASP data revealed a year-on-year increase in the proportion of *N. gonorrhoeae* isolates that were resistant to tetracycline, from 39.4% in 2015 to 48.5% in 2017.[2] Using the confirmed correlation between MIC and presence of *tet*(M), we can infer a simultaneous

increase in the proportion of isolates with plasmid-mediated tetracycline resistance (30.1% in 2015, 41.7% in 2016 and 52.7% in 2017), which may reflect increased use of doxycycline to treat other sexually transmitted infections (STIs), such as *Chlamydia trachomatis* during this period.[8] Furthermore, the potential use of doxycycline as post-exposure prophylaxis for STIs in MSM in the era of HIV pre-exposure prophylaxis (PrEP) is of public health concern.[9] Taken together, these developments highlight the perilous situation of antimicrobial resistance in *N. gonorrhoeae*.

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Transparency declaration

None to declare

Reference List

1. Unemo M, Bradshaw CS, Hocking JS, *et al*. Sexually transmitted infections: challenges ahead. *Lancet Infect Dis* 2017; **17**: e235-e279.

2. Public Health England. Surveillance of antimicrobial resistance in *Neisseria gonorrhoeae* in England and Wales; Key findings for the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP), Data to May 2018. In: 2018; 1-25.

3. Morse SA, Johnson SR, Biddle JW, *et al*. High-level tetracycline resistance in Neisseria gonorrhoeae is result of acquisition of streptococcal tetM determinant. *Antimicrob Agents Chemother* 1986; **30**: 664-70.

4. Johnson SR, Morse SA. Antibiotic resistance in Neisseria gonorrhoeae: genetics and mechanisms of resistance. *Sex Transm Dis* 1988; **15**: 217-24.

5. Gascoyne-Binzi DM, Heritage J, Hawkey PM. Nucleotide sequences of the *tet*(M) genes from the American and Dutch type tetracycline resistance plasmids of Neisseria gonorrhoeae. *J Antimicrob Chemother* 1993; **32**: 667-76.

6. Unemo M, Del RC, Shafer WM. Antimicrobial Resistance Expressed by Neisseria gonorrhoeae: A Major Global Public Health Problem in the 21st Century. *Microbiol Spectr* 2016; **4**(3).

7. Turner A, Gough KR, Leeming JP. Molecular epidemiology of tetM genes in Neisseria gonorrhoeae. *Sex Transm Infect* 1999; **75**: 60-6.

8. Dragovic B, Nwokolo N. BASHH Clinical Effectiveness Group; Update on the treatment of Chlamydia trachomatis (CT) infection. In: 2018.

9. Williams A, Fifer H, Hughes G. Position statement on doxycycline as post-exposure prohylaxis (PEP) for STIs. In: 2017.

Table. Analysis of likelihood of infection with tetracycline resistant (>1 mg/L) or high-level tetracycline resistant (≥16 mg/L) *N. gonorrhoeae* by gender and sexual orientation (cells in bold indicate statistical significance)

				Outcome: tetracycline resistance (>1mg/L) compared to tetracycline sensitive isolates							
	Total isolates tested for tetracycline resistance in GRASP (2015-2017)	**Tetracycline resistance (MIC >1 mg/L)**	**Tetracycline resistance (MIC >1 mg/L) (row %)**	**COR**	**LCI**	**UCI**	**P value**	**AOR**	**LCI**	**UCI**	**P value**
Total	**4,251**	**1,810**	42.6%								
Year											
2015	1,699	670	39.4%	1.00	-	-	-	1.00	-	-	-
2016	1,284	525	40.9%	1.06	0.92	1.23	0.423	1.08	0.93	1.26	0.299
2017	1,268	615	48.5%	**1.45**	**1.25**	**1.68**	**<0.001**	**1.50**	**1.29**	**1.74**	**<0.001**
Sexual orientation and gender											
MSM	2,933	1,356	46.2%	**1.37**	**1.16**	**1.62**	**<0.001**	**1.39**	**1.18**	**1.64**	**<0.001**
Heterosexual men	730	282	38.6%	1.00	-	-	-	1.00	-	-	-
Women	493	128	26.0%	**0.56**	**0.43**	**0.72**	**<0.001**	**0.55**	**0.43**	**0.71**	**<0.001**
Unknown	95	44	46.3%								
				Outcome: high-level tetracycline resistance (MIC ≥16 mg/L) compared to low-level resistant							
	Total resistant (MIC >1 mg/L)	**High-level tetracycline resistance (MIC ≥16 mg/L)**	**High-level tetracycline resistance (MIC ≥16 mg/L)(row %)**	**COR**	**LCI**	**UCI**	**P value**	**AOR**	**LCI**	**UCI**	**P value**
Total	**1,810**	**745**	**41.2%**								
Year											
2015	670	202	30.1%	1.00	-	-	-	1.00	-	-	-
2016	525	219	41.7%	**1.66**	**1.30**	**2.11**	**<0.001**	**1.65**	**1.30**	**2.11**	**<0.001**
2017	615	324	52.7%	**2.58**	**2.04**	**3.26**	**<0.001**	**2.57**	**2.03**	**3.24**	**<0.001**
Sexual orientation and gender											

MSM	1,356	529	39.0%	1.00	-	-	-	1.00	-	-	-
Heterosexual men	282	136	48.2%	**1.46**	**0.12**	**1.89**	**0.004**	**1.45**	**1.11**	**1.88**	**0.006**
Women	128	55	43.0%	1.18	0.82	1.70	0.381	1.18	0.81	1.72	0.384
Unknown	44	25	56.8%								

COR - crude odds ratio

AOR - adjusted odds ratio

L/U CI - lower/upper 95% confidence interval