Keeping track of antimicrobial resistance for *Neisseria gonorrhoeae* in Auckland, New Zealand: past, present and future considerations

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**ABSTRACT**

**AIMS:** *Neisseria gonorrhoeae* (NG) has developed resistance to a wide range of antimicrobials. Population level data is essential to determine empiric treatment regimes. We sought to identify antimicrobial resistance patterns for NG in the Auckland region from 2008–2016, and review the utility of current methods of antimicrobial resistance testing.

**METHODS:** Antimicrobial susceptibilities and demographic data from NG isolates derived from patients attending the Auckland Regional Sexual Health Service and Auckland City Hospital were analysed to determine resistance rates and trends over time. Antimicrobial susceptibility testing was performed by agar dilution using Clinical and Laboratory Standards Institute (CLSI) interpretation criteria.

**RESULTS:** Results for 2,302 isolates from 1,941 patients were analysed. While ciprofloxacin resistance increased between 2008 and 2011, resistance rates for all antibiotics declined from 2013–2016. In 2016, 22% (53) of isolates were resistant to ciprofloxacin, 7% (17) to penicillin, 31% (76) to tetracycline and 0.8% (2) exhibited decreased susceptibility to ceftriaxone.

**CONCLUSION:** Ceftriaxone is still suitable as a component of gonorrhoea treatment in our region but resistance to other agents prohibits their use for empiric treatment regimens. Current methods of detecting antimicrobial resistance for NG needed to be updated so that they are fit for purpose.

Since the beginning of the antibiotic era, strains of *Neisseria gonorrhoeae* (NG) have developed resistance to a wide range of antimicrobials, including sulphonamides, tetracycline, penicillin, ciprofloxacin, ceftriaxone and azithromycin.1

However, widespread use of molecular detection for NG and other sexually transmitted infections has led to a reduction in samples submitted for NG culture, and has meant that patient level individual isolate susceptibility information is rarely available to guide definitive antibiotic therapy. Therefore, population level data, such as that provided from active surveillance programmes, is essential to determine empiric treatment regimes. Many countries, including New Zealand, participate in national or supranational surveillance programmes.2-4 Although the New Zealand national public health laboratory (Institute of Environmental Science and Research, ESR) periodically reports gonococcal resistance data, there have not been any longitudinal studies documenting NG antimicrobial resistance for New Zealand in recent years.5
Our laboratory submits gonococcal antimicrobial susceptibility data annually to the World Health Organization (WHO) Western Pacific region Gonococcal Antimicrobial Surveillance Programme (GASP). These data are derived from patients attending the Auckland Regional Sexual Health Service, as well as inpatients and outpatients from Auckland City Hospital. A continuous database of susceptibility results for these antibiotics has been maintained since 2008.

The aim of this study was to identify antimicrobial resistance (AMR) patterns for NG in the Auckland region over the period 2008–2016, to review the current approach to AMR surveillance, and use this knowledge to focus our AMR surveillance strategy to support both local and national decision-making for empiric treatment guidance.

Methods

Antimicrobial susceptibility results for non-duplicate isolates of NG, derived from patients attending the Auckland Regional Sexual Health Service and Auckland City Hospital between January 2008 and December 2016 were included. Referred samples from external laboratories were excluded. Samples from patients attending general practices are processed elsewhere.

Antimicrobial susceptibility testing and breakpoints

Antimicrobial susceptibility testing was performed using an agar dilution method (breakpoint technique) for penicillin, tetracycline, ciprofloxacin and ceftriaxone (Clinical and Laboratory Standards Institute, CLSI).6 Our laboratory participates in the WHO Western Pacific region GASP annual external quality assessment programme.

During the study period, antimicrobial susceptibility testing results were interpreted, and reported using CLSI criteria. Accordingly, penicillin resistance was categorised by production of penicillinase as determined by nitrocefinase disc testing (BD, Becton Dickinson, Melbourne, Australia) or in its absence, as chromosomal mediated resistance. High-level tetracycline resistance was defined as minimum inhibitory concentration (MIC) MIC ≥16mg/L.7

In addition, for ceftriaxone, decreased susceptibility was defined as isolates in the MIC range 0.06–0.12mg/L, in accordance with the Australian Gonococcal Surveillance Programme (AGSP) criteria.8

The MIC required to inhibit 50% and 90% of isolates were calculated for each antibiotic.

For comparative purposes, European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria9 are also presented in Table 1.

Statistical methods

Cochran-Armitage trend tests were used to assess trends over time; otherwise Chi square was used to compare proportions. Patient level information was analysed by sex and anatomical site.

Ethical review was not required for this audit-related activity.

Results

Over the study period, 2,302 isolates were tested for antimicrobial susceptibility, from 1,941 patients. Most (1,704, 74%) isolates were from men. The number of isolates varied between 158 and 315 per year.

Among men, the isolates were cultured from urethral samples (1,255, 74%), from rectal samples (302, 18%) and from throats (91, 5%), as well as from other sites or unspecified sites (56, 3%). Among females isolates were cultured from urogenital sites (urethra, vagina, cervix, 502, 88%), from rectal samples (17, 3%) and from throats (22, 4%), as well as from other sites or unspecified sites (56, 9%).

In 2016, 22% (53/245) of isolates were resistant to ciprofloxacin, 7% (17/245) to penicillin, 0.8% (2/245) exhibited decreased susceptibility to ceftriaxone, and 31% (76/245) were resistant to tetracycline. Overall susceptibility over the study period is presented in Table 1. Trends in susceptibility are shown in (Figure 1).
Penicillin resistance

The vast majority of isolates (n=2,256, 98%) were non-susceptible to penicillin. This was mostly attributable to reduced susceptibility (78%, n=1,793) by chromosomal mediated changes to penicillin binding proteins, in which strains have mutations which increase the risk of further mutations conferring outright resistance, with less, 20% of isolates (n=463) exhibiting resistance according to CLSI breakpoints. Resistance was chromosomally mediated in 12% (n=286), and by the production of penicillinase in 8% (n=177) of isolates. The resistance rates significantly decreased from 26% in 2008 to 7% in 2016 (p =0.004), with a corresponding increase in the proportion of isolates falling into the reduced susceptibility category (70% in 2008 to 92% in 2016, p<0.001). The proportion of susceptible isolates was low, and reduced further over the study period (4% in 2008 to 0.8% in 2016, p<0.002). Higher rates of resistance were seen in males (22%, 378/1,704 isolates) compared with females (15%, 87/597 isolates, p<0.001), and in extra-genital (31%, 135/432 isolates) compared with urogenital (18%, 363/1,763, p<0.001) isolates.

Figure 1: Trends in antibiotic resistance for Neisseria gonorrhoeae isolates, LabPlus 2008–2016.

Table 1: Antimicrobial susceptibility of N. gonorrhoeae isolated at LabPlus 2008–2016.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Isolates tested</th>
<th>Interpretive criteria</th>
<th>Number (%) Susceptible</th>
<th>Number (%) Intermediate/ decreased susceptibility</th>
<th>Number (%) Resistant</th>
<th>MIC 50*</th>
<th>MIC 90*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>2,300</td>
<td>CLSI</td>
<td>1,512 (66)</td>
<td>787 (34)</td>
<td>0.004</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EUCAST</td>
<td>1,508 (66)</td>
<td>788 (34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>2,301</td>
<td>CLSI</td>
<td>45 (2)</td>
<td>1,793 (78)</td>
<td>463 (20)</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EUCAST</td>
<td>45 (2)</td>
<td>463 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2,299</td>
<td>CLSI</td>
<td>390 (17)</td>
<td>396 (35)</td>
<td>1,113 (48)</td>
<td>1</td>
<td>&gt;16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EUCAST</td>
<td>577 (25)</td>
<td>1,113 (48)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>2,296</td>
<td>CLSI</td>
<td>2,296 (100)</td>
<td>0</td>
<td>0.008</td>
<td>0.03</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AGSP</td>
<td>109 (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EUCAST</td>
<td>2,296 (100)</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: *The minimum concentration of antibiotic required to inhibit 50% and 90% of isolates.
CLSI: Clinical and Laboratory Standards Institute.
EUCAST: European Committee on Antimicrobial Susceptibility Testing.

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Ceftriaxone decreased susceptibility

Overall, 4.6% (n=109) of isolates exhibited decreased susceptibility to ceftriaxone during the study period. Variation in this proportion was seen with time, with rates highest in 2009, 2010 and 2012, with a trend towards increasing susceptibility over the study period (p<0.001). No isolates resistant to ceftriaxone were detected through breakpoint testing over the study period.

The proportion of samples with decreased ceftriaxone susceptibility was higher (13%, 54/432) from extra-genital, compared with urogenital sites (3%, 52/1763 isolates, p<0.001), and for men (6%, 101/1,704 isolates) compared with women (1.4%, 8/568 isolates p<0.001). Accordingly, the MIC 90 of isolates from males (0.03mg/L) was higher than that for isolates from females (0.016mg/L), and the MIC 90 for extra-genital (rectal/throat) isolates (MIC 0.06mg/L) was higher than urogenital isolates (0.03mg/L).

Ciprofloxacin resistance

Overall, 34% (n=787) of isolates were resistant to ciprofloxacin, with two trends evident: resistance rates increased from 24% in 2008 to a peak of 45% in 2011 (p<0.001) and then declined to 22% in 2016 (p=0.002). Isolates from men were more likely to be resistant (35%, 598/1,704), compared with those from females (32%, 189/597, p=0.006) as were those from extra-genital (42%, 182/432 isolates) compared with urogenital sites (32%, 832/1,763, p<0.001).

Tetracycline resistance

Overall, 48% (n=1,113) of isolates were resistant to tetracycline, with 24% (n=547) exhibiting high-level resistance. Resistance rates were highest in the period 2008–2012 (48–66%), and declined to 31% in 2016 (p<0.001).

No difference in resistance rates were seen between isolates from males and females, but extra-genital isolates were more likely to be resistant (57%, 246/432 isolates) compared with urogenital isolates (47%, 832/1,763 isolates, p<0.001).

Discussion

Consistent with other countries, we report high levels of resistance to multiple antimicrobials among local NG isolates in a predominantly STI clinic-based population. Resistance rates to ciprofloxacin (22%), penicillin (21%), and tetracycline (42%), from 2008–2016 remained well above the WHO-recommended 5% threshold for the use of these agents as empiric therapy for gonorrhoea infections.10

There has been temporal changes in susceptibility patterns, reflecting worldwide trends.2,4,8 In the early study period (2008–2011), rates of ciprofloxacin resistance were increasing, and around 5% of isolates had decreased susceptibility to ceftriaxone. However, from 2013–2016, resistance rates for penicillin, ciprofloxacin and tetracycline declined, and rates of decreased ceftriaxone susceptibility reduced to less than 5%. These trends probably represent fluctuations in circulating clones.8 However, the number of strains of NG harbouring resistance with the capacity to spread is likely to be increasing, as evident by the increasing number of reports of clones associated with treatment failure, placing significant pressure on our limited antibiotic options for this organism.8

We found that isolates from men, and those from extragenital sites, were overall more likely to be resistant than those from urogenital sites. Though we do not have behavioural information to confirm this, as reported elsewhere, this finding likely reflects the fact that these sites are more likely to be sampled in men who have sex with men (MSM), a group known to have increased rates of NG resistance compared with the general population.12

In New Zealand, ciprofloxacin resistance was uncommon before the turn of the century. Rates increased exponentially from <5% to 40% between 2000 and 2011, due to the introduction of resistant gonococcal strains from overseas and their subsequent dissemination via sexual networks.5,13 This increase in resistance necessitated changes
to local treatment guidelines, including the recommendation of ceftriaxone as the standard empirical treatment for gonococcal infections, more recently given in combination with azithromycin. Consequently, resistance rates for ciprofloxacin declined after 2012, but remain well above thresholds recommended for use as empiric therapy. The role of this drug is currently limited to a minority of patients, where susceptibility results for their isolate are available at the time of clinical review. In these situations, it is recommended that it be administered with azithromycin, because resistance still occurs when this drug is used as monotherapy below the breakpoint for resistance.

Although resistance to penicillin is relatively uncommon, most strains in our region have reduced susceptibility to penicillin, again with the potential to accumulate further mutations, and become fully resistant, so this antibiotic is not appropriate for use. Tetracyclines have never been recommended for the treatment of NG, because of high resistance rates and the requirement for multiple-dose therapy, but this could change in the future, as other options are lost, if susceptibility results were available to guide treatment.

Decreased susceptibility to ceftriaxone remains low in our setting, and this agent is still suitable as a component of empiric treatment. However, worldwide there are increasing reports of ceftriaxone treatment failure. This is due to an increasing number of circulating clones of ceftriaxone susceptible gonorrhoea that are harbouring mosaic PBP mutations, which increase the likelihood of de-novo generation of resistance. Though no patients in New Zealand have had ceftriaxone resistant NG to date, we know that non-adherence to recommended treatment among healthcare providers is common in our region, and there are concerns that monotherapy with ceftriaxone or azithromycin may contribute to increasing resistance in our population. As has been demonstrated with ciprofloxacin, importation of resistant strains can rapidly lead to dissemination, and ongoing vigilance is required.

Azithromycin resistance appears to be an emerging issue in our population, likely through both de-novo resistance and spread of already resistant strains, but until now monitoring of azithromycin susceptibility has not been part of local surveillance activities. Resistance is of concern because the current empiric regime relies on azithromycin to protect ceftriaxone activity, and high rates of resistance to this agent could hasten development of ceftriaxone resistance. Additionally, for patients unable to receive ceftriaxone, some current international guidelines allow the administration of azithromycin as monotherapy; this regime appears to be in jeopardy.

In line with current therapeutic options and our antimicrobial resistance patterns, we have changed our approach to NG AMR testing to support both individual and population-based treatment guidance. We now perform real-time susceptibility testing of NG isolates so that patients infected with resistance strains can be identified earlier, and test-of-cure performed where clinically warranted. This involves determining the MIC for ceftriaxone and azithromycin using a gradient diffusion method, along with susceptibility to ciprofloxacin by disc diffusion for all NG isolates. We started performing azithromycin susceptibility testing in mid-2017, and to date the results have shown that azithromycin resistance is present in 21% of NG isolates in the Auckland region (32/153 isolates tested, unpublished data).

Whole genome sequencing has recently been applied to local gonococcal isolates and offers a powerful surveillance tool for both genotypic resistance and epidemiological surveillance. In the clinical setting, the availability of rapid, sensitive, gonorrhoea detection and genotypic resistance point of care tests would allow definitive, tailored therapy at the time high-risk patient presents for review. However, this technology is not yet widely available, and is currently limited to detection of a narrow range of mutations. In addition, in order to detect newer resistance mechanisms there would be an ongoing requirement for phenotypic testing to be performed on a proportion of isolates.

With increasing reports of treatment failure worldwide, it is essential that clinicians in New Zealand do not become complacent about the risks and consequences of widespread resistance to the
current antibiotic options for NG, which include increasing rates of pelvic inflammatory disease, tubal infertility and neonatal eye disease. Given the high azithromycin rates that we have noted, it is also essential that antimicrobial stewardship interventions occur in the community to address potential sources of inappropriate azithromycin administration, such as encouraging the use of multiple dose doxycycline regimes for symptomatic chlamydia infections, as recommended in recent updates to national guidelines, rather than the use of single-dose azithromycin.

In summary, changes in susceptibility patterns of N. gonorrhoeae have occurred in our region over the last eight years, consistent with those reported worldwide. Though ceftriaxone remains a suitable component of empiric therapy in our region, rates of resistance for other antimicrobials remain high. We have tailored our AMS for NG to reflect clinical and surveillance needs by provision of real-time results, and the introduction of susceptibility testing for azithromycin. In the future, developments in point of care testing may allow rapid, individualised therapy for patients in STI clinics.

Competing interests:
Nil.

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