An evaluation of gentamicin susceptibility of Neisseria gonorrhoeae isolates in Europe

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Objectives: The emergence of decreased susceptibility to third-generation, extended-spectrum cephalosporins in Neisseria gonorrhoeae and associated treatment failures highlights the need to consider alternatives for future therapeutic use, such as gentamicin.

Methods: The three laboratories surveying gonacoccal antimicrobial susceptibility as part of the European Network for Sexually Transmitted Infections Surveillance compared agar dilution and Etest to determine gentamicin MICs and performed the first survey of gentamicin susceptibility on 1366 gonococcal isolates from 17 European Union/European Economic Area (EU/EAA) countries in 2009.

Results: Sentinel surveillance of gentamicin susceptibility showed that 95% of European isolates were within a narrow MIC range (4–8 mg/L), with 79% showing an MIC of 8 mg/L. Most countries showed little variation, but wider MIC ranges were observed in Greece (1–16 mg/L) and France, Norway and Sweden (2–16 mg/L). While MICs for both methods generally differed by just one doubling dilution, they were lower by Etest.

Conclusions: This is the first reported evidence that the European gonococcal population susceptibility to gentamicin is similar to that reported in other world regions. Clinical trials to evaluate the therapeutic efficacy of gentamicin may be warranted.

Keywords: antimicrobial resistance, agar dilution, Etest, sentinel surveillance

Introduction

Effective treatment of Neisseria gonorrhoeae infection is critical to individual patient management and is essential in controlling gonorrhoea, but is hampered by the global dissemination of antimicrobial resistance in gonococci.1

Gonorrhoea continues to present a significant public health problem across Europe, with rates ≥5 per 100 000 population in many countries and ≥15 per 100 000 reported in Latvia, Lithuania and the UK in 2009 [European Centre for Disease Prevention and Control (ECDC), Sexually Transmitted Infections (STIs) in Europe, 1990–2009; unpublished data]. Emergence of decreased susceptibility to ceftriaxone and in particular to cefixime,1 currently recommended as first-line therapies in Europe, is a concern as treatment failures begin to be documented, at least to cefixime.1 Accordingly, third-generation, extended-spectrum cephalosporins may soon become unsuitable as first-line therapy and alternative strategies for management of gonorrhoea should be sought.

The aminoglycoside gentamicin was chosen as an alternative treatment following the emergence of penicillinase-producing N. gonorrhoeae in Africa, as it was inexpensive, can be administered in a single 240 mg dose and was shown in early trials to achieve cure rates of ≥95%.3–6 Hence, gentamicin has since been used successfully to treat gonococcal urethritis in Africa, notably in Malawi, for several years, although treatment failure has been reported.7 European surveillance of antimicrobial-resistant gonorrhoea was established in 2004 and is now undertaken by the European Network for STIs Surveillance led by the ECDC, and has identified high levels of resistance to many agents.8 If gentamicin is to be considered as part of any alternative strategy for treatment, then knowledge of the susceptibility of European gonococci to gentamicin will be essential. This study
Materials and methods

**Bacterial isolates for agar dilution and Etest comparison**

Twenty-four gonococcal isolates, evaluated as a quality assurance panel, were tested by agar dilution and Etest in the three laboratories (London, UK; Örebro, Sweden; and Copenhagen, Denmark) currently conducting sentinel surveillance. The panel comprised strains from the WHO (n=14; A, C–G and I–P), the CDC, USA (n=6), the Sexually Transmitted Bacteria Reference Laboratory (STBRL), London (n=3), and reference strain ATCC 49226.

To further compare agar dilution and Etest, the UK laboratory tested 52 isolates from the UK reference collection (n=11) and the national surveillance programme (n=41) and the Swedish laboratory examined 389 isolates from the 2009 sentinel surveillance (see below).

**Bacterial isolates for European surveillance of gonococcal antimicrobial resistance**

As part of the 2009 sentinel surveillance programme, 1366 gonococcal isolates, collected consecutively over a 3 month period from 17 European Union/European Economic Area (EU/EEA) countries, were tested. Ten countries referred >100 isolates, while the remainder referred all available isolates (ranging from 9 to 79 isolates). Susceptibility to gentamicin was determined by agar dilution in all laboratories, while Sweden additionally performed Etests on 389/1366 isolates. All isolates exhibiting an MIC of ≥8 mg/L were also tested by Etest. To control the quality of agar dilution and Etest, WHO strains (A, G, J, K, M, O and P) were tested. If MICs were within one doubling dilution of expected values, agar dilution and Etest, WHO strains (A, G, J, K, M, O and P) were additionally performed Etests on 389/1366 isolates. All isolates exhibiting an MIC of ≥8 mg/L were also tested by Etest. To control the quality of agar dilution and Etest, WHO strains (A, G, J, K, M, O and P) were tested. If MICs were within one doubling dilution of expected values, test results were included in the final dataset.

**Antimicrobial susceptibility testing**

Gonococcal cultures aged 18–24 h were suspended in saline to a turbidity equivalent to that of a 0.5 McFarland standard and, for agar dilution, 1 μL was inoculated onto the surface of GC agar (Becton Dickinson, Oxford, UK) containing 1% Vitox (Oxoid, Basingstoke, UK) (GC-VIT) and gentamicin (Sigma–Aldrich, Gillingham, UK) at doubling concentrations (1–16 mg/L). MICs were determined following 18–24 h of incubation at 36°C in 5% CO₂.

Etest strips containing gentamicin (0.016–256 mg/L) (AB bioMérieux, Solna, Sweden) were applied to the surface of a GC-VIT plate, inoculated with the same suspensions and incubated for 18–24 h at 36°C in 5% CO₂. To compare Etest and agar dilution results, Etest MICs outside the agar dilution scale were rounded up to the nearest doubling dilution.

**Results**

**Comparison of gentamicin MICs determined by agar dilution and by Etest**

The agar dilution and Etest MICs for the panel of 24 isolates circulated to assess inter-laboratory variation were all within one doubling dilution between each testing centre for each method. A consensus MIC was determined for each isolate to compare relative performances of both methods (Table 1). While the consensus MICs varied by just one doubling dilution between methods, the MIC ranges were higher for agar dilution (4–8 mg/L) than for Etest (2–4 mg/L) (Table 1).

**Table 1. Comparison of gentamicin MICs determined in three laboratories by agar dilution method and Etest for 24 quality assurance strains of N. gonorrhoeae**

<table>
<thead>
<tr>
<th>Method</th>
<th>Consensus MIC (mg/L), no. of isolates</th>
<th>Total no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etest</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*MICs of 1.5 mg/L for Etest were rounded up to 2 for comparison with agar dilution.
MICs of 3 mg/L for Etest were rounded up to 4 for comparison with agar dilution.

Additional testing of 441 clinical isolates in the UK (n=52) and in Sweden (n=389) showed that 90% of MICs (397/441) by both methods were within one dilution and the remainder within two dilutions. Again, the modal MIC was higher for agar dilution (8 mg/L, ranging from 2 to 16 mg/L) than for Etest (4 mg/L, ranging from 2 to 8 mg/L).

**Surveillance of gentamicin MICs in 17 European countries determined by agar dilution**

Gentamicin MICs ranged from 1 to 16 mg/L. The modal MIC was 8 mg/L in 79% (1081/1366) of isolates tested, while 16% (222/1366) showed an MIC of 4 mg/L. While the gonococci from most countries had an MIC of 8 mg/L, slightly lower MICs of 4 mg/L were more frequent in England and Wales, Germany, Norway and Portugal (Figure 1). Isolates from Greece showed the widest MIC range (1–16 mg/L), while MICs ranged from 2 to 16 mg/L in France, Norway and Sweden (Figure 1).

Agar dilution MICs of 16 mg/L were observed in 49 isolates from Belgium (n=13), Italy (n=9), Norway (n=8), Slovenia, Spain and Sweden (n=4), Denmark (n=3), the Netherlands (n=2) and France and Greece (n=1). Etest MICs for these were again lower than for agar dilution, at 8 mg/L for 63% (31/49) of isolates and 4 mg/L for the remaining 18 isolates (37%).

The remaining 14 isolates had MICs tested by agar dilution of 2 mg/L (13 isolates) and 1 mg/L (1 isolate).

The majority of isolates (84%, 1148/1366) were from genital sites, with 138 isolated from the rectum and 34 from the pharynx. The remainder were isolated from other sites (25) or the site of isolation was unknown (21). There was no significant association between site of infection and MIC; for genital isolates 79% (905/1148) had MICs of 8 mg/L and 3% (35/1148) had MICs of 16 mg/L, and for rectal isolates 85% (117/138) had an MIC of 8 mg/L and 4% (6/138) had an MIC of 16 mg/L (χ² test, P>0.1).

**Discussion**

This is the first survey of gonococcal gentamicin susceptibility in Europe. Among 1366 isolates tested, 95% of MICs were distributed within a narrow range of 4–8 mg/L. Isolates with higher MICs of 16 mg/L were observed by agar dilution but were lower by supplementary Etest. In previous smaller
studies, mainly conducted in the 1990s using agar dilution, gentamicin MICs of 1–32 mg/L in Indonesia,11–13 2–16 mg/L in the USA14 and 0.5–32 mg/L in Malawi7 were reported, but, similarly, MICs of 4–8 mg/L were observed most frequently. Some European countries showed lower MICs than the modal 8 mg/L or a wider range of MICs, possibly indicating greater heterogeneity in gentamicin susceptibility, although the significance of this will only be determined by longitudinal surveillance within the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP).

Agar dilution was selected as the preferred method for European sentinel surveillance as it is economical, has a high throughput and is considered the gold standard. An earlier comparison of agar dilution and Etest showed that the latter generated lower gentamicin MICs.7 The low rate of agreement between methods was partially attributed to difficulties in attaining consistency of technique in a field setting in Malawi. The current study showed also that while 90% of MICs determined by the two methods were within just one dilution, Etest typically generated lower MICs. These differences highlight that while results are broadly comparable, gentamicin MICs reported in studies using different methods should be compared with caution, particularly in the context of understanding the relationship between MIC and treatment failure. The current study demonstrated that the gentamicin MICs obtained for the quality control strains tested from the WHO reference strain panel were within one dilution of those reported for the original panel characterization.9 Use of such controls in future studies will facilitate data comparison between laboratories.

The relationship between gentamicin MIC and treatment outcome is poorly understood due to limited data and difficulties in differentiating from re-infection. Consequently, there are no interpretative criteria for gentamicin MICs, although a recent survey applied criteria based on previous clinical cure and MIC comparisons6 to define MICs of ≤4 mg/L as susceptible, 8–16 mg/L as intermediate susceptibility and ≥32 mg/L as resistant. By these criteria, all European isolates were susceptible (17%) or exhibited intermediate susceptibility (83%), with no examples of resistance observed. While presumed treatment failure is documented for infections by strains with MICs ranging from 4 to ≥16 mg/L,7 the gonococcal population in Malawi has remained relatively susceptible despite 15 years of using gentamicin to treat gonorrhoea.6 Isolates that exhibit intermediate susceptibility to a number of antimicrobial agents such as penicillin and ciprofloxacin remain responsive to treatment. While it may not be ideal to use any agents where significant numbers of isolates are showing a drift towards resistance, future choices may be limited and make this necessary. It will also need to be considered that the gonococcal population in Europe exhibits higher levels of resistance to other antimicrobial agents, particularly ciprofloxacin, than in the African studies and whether this will affect clinical outcome.

Therapeutic options for gonorrhoea are likely to be limited in the coming years and it may be necessary to have a battery of different options; gentamicin has been successfully used for gonococcal urethritis and warrants consideration. There are obvious difficulties, including the lack of data on extra-genital
infections, the true meaning of intermediate susceptibility and the appropriate dosage of this agent, which can have significant side effects. This current study suggests that gentamicin should be considered within Europe, although clinical trials to examine the efficacy of any alternative therapies for gonorrhoea and surveillance studies to define appropriate resistance breakpoint criteria and determine the true relationship to therapeutic failure will be required.

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Transparency declarations
None to declare.

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