Gentamicin susceptibility among a sample of multi-drug resistant *Neisseria gonorrhoeae* isolates in India

Manju Bala\(^a\), Vikram Singh\(^{a,b}\), Aradhana Bhargava\(^a\), Monika Kakran\(^a\), Naveen Chandra Joshi\(^a\) and Ravi Bhatnagar\(^b\)

**Running Title:** Gentamicin efficacy against MDR *N. gonorrhoeae*

Apex Regional STD Teaching, Training & Research Centre, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India\(^a\); Department of Life Sciences, SunRise University, Alwar, Rajasthan, India\(^b\)

**Correspondence:**
Dr. Manju Bala, Consultant and Professor (Microbiology), Apex Regional STD Teaching, Training & Research Centre, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India. Telephone number: 91-11-26196740, Fax number: 91-11-26163072, Email: manjubala_2@hotmail.com
ABSTRACT

Antimicrobial susceptibility testing of 258 *N. gonorrhoeae* isolates by Etest determined that 60.1% were MDR while 5% strains had decreased susceptibility to currently recommended extended-spectrum cephalosporins (ESCs). Among these, 84.5% MDR and 76.9% strains having decreased susceptibility to ESCs were susceptible to gentamicin. No MDR isolate was resistant to gentamicin. These *in vitro* results suggest that gentamicin might be an effective treatment option for the MDR strains and in dual therapy for gonorrhea. However, further research regarding the clinical treatment outcomes is essential.

**Key words:** *Neisseria gonorrhoeae;* Gentamicin; MDR; AMR surveillance
Neisseria gonorrhoeae (N. gonorrhoeae) clinical isolates resistant to extended-spectrum cephalosporins (ESCs), the last remaining options for empiric therapy, and most other antimicrobials have been reported in recent years throughout the world (1-4). Organizations such as the World Health Organization (WHO), the US Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) are considering infections caused by multidrug-resistant (MDR) and extensively-drug resistant (XDR) N. gonorrhoeae as a major public health problem (5-7). With the increasing prevalence of drug resistance, there is an urgent need for new and effective treatment options. Gentamicin has been used for the past two decades for management of gonorrhea in Malawi (8,9). Gentamicin has been considered as an option for the treatment of infections due to the MDR and XDR strains and also for future treatment of gonorrhea particularly, in dual therapy in other geographic regions (10-12).

Earlier studies have focused on the in vitro susceptibility of N. gonorrhoeae to gentamicin (8,13-17). Some of these studies were on a limited number of isolates (13,17). To the best of our knowledge, no study has analysed the in vitro susceptibility of gentamicin against MDR strains. Therefore, this study was aimed at determining the (1) trend of in vitro susceptibility of N. gonorrhoeae isolates to gentamicin during 2010-2015 (2) status of gentamicin susceptibility for MDR strains and strains with decreased susceptibility to ESCs and (3) profile of gentamicin less susceptible strains for eight other antimicrobials recommended at present and in past for treatment of gonorrhea.

A total of 258 N. gonorrhoeae isolates obtained from consecutive patients attending STD clinic from August 2010 to December 2015 were identified on the basis of colony morphology, Gram staining, oxidase, superoxol and carbohydrate utilization test (18). Susceptibility testing for gentamicin and other antimicrobials such as ESCs (ceftriaxone,
cefopodoxime, cefixime), penicillin, tetracycline, ciprofloxacin, spectinomycin, and azithromycin was performed using Etest strip as per manufacturer instructions (BioMerieux SA, France) on GC agar (Becton, Dickinson and Company (BD), Sparks, MD, USA) containing 1% vitamin growth supplement (HiMedia, India) or isovitalex (BD). Susceptibility testing for above antibiotics (except cefixime) was also performed by Calibrated Dichotomous Sensitivity (CDS) disc diffusion technique (2,19,20).

The strains were interpreted as susceptible, less susceptible and resistant (2,19,21,22). MDR isolates were defined as quinolone resistant *N. gonorrhoeae* (QRNG)+penicillinase producing *N. gonorrhoeae* (PPNG); QRNG+tetracycline resistant *N. gonorrhoeae* (TRNG); QRNG+PPNG+TRNG; QRNG+azithromycin resistant (AzR) and QRNG+PPNG+TRNG+AzR (7).

*N. gonorrhoeae* isolates were tested for β-lactamase production by the chromogenic cephalosporin method (18). Quality control was performed using the WHO reference strains (WHO C and F-P) and ATCC 49226. This centre regularly participates in WHO external quality assurance scheme and its results have been described previously (2). The chi-squared test was applied for comparison of trend data and *p* value was determined.

Table 1 shows the profile of MDR *N. gonorrhoeae* strains and their susceptibility pattern to gentamicin. Of 258 isolates examined in the study, 155 were MDR. No MDR strain was resistant to gentamicin and 84.5% were susceptible to it. All the four MDR strains in 2010 were susceptible to gentamicin and gentamicin susceptibility for MDR strains decreased to 73.9% in 2013 (*p*=0.69). However, susceptibility increased to 83.3% in 2015 (*p*=0.77).

During the study period, 13 (5%) isolates had decreased susceptibility to ESCs i.e. ceftriaxone, cefpodoxime and cefixime. No strain was resistant to all the three ESCs. Out of these 13 isolates, 10 (76.9%) were susceptible and 3 (23.1%) were less susceptible to
gentamicin. MICs for gentamicin susceptible strains ranged from 0.5-4 mg/L and for all less susceptible strains it was 8 mg/L.

The trend of gentamicin susceptibility and susceptibility profile of gentamicin less susceptible strains to other antimicrobials is shown in Table 2. During the study period, 90.7% (234/258) isolates were susceptible to gentamicin. The proportion of gentamicin less susceptible strains increased ($p=0.27$) from 0% in 2010 to 15.4% in 2013 and subsequently decreased ($p=0.37$) to 8.8%. Out of 24 gentamicin LS strains, all (100%) were susceptible to spectinomycin and azithromycin and 87.5% were susceptible to ESCs. None of the gentamicin LS strains were susceptible to penicillin and ciprofloxacin. Plasmid mediated resistance to tetracycline was observed in 5 out of 24 gentamicin LS strains.

The current study was undertaken in the WHO Regional Reference Laboratory (RRL) for the Gonococcal Antimicrobial Surveillance Program (GASP) for South-East Asia Region (SEAR) countries. This RRL regularly carries out surveillance of gonococcal AMR (2,4,) and assists in implementing the WHO ‘Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in N. gonorrhoeae’ (23,24). One of the core components of this global action plan is to identify alternative effective treatment strategies. This WHO RRL has included gentamicin in routine AMR surveillance since August 2010 to evaluate its efficacy.

No resistance to gentamicin was reported in the present study and in the study from Mongolia, Europe, and Canada (13,14,16). In our study, only 9.3% of the gonococcal isolates were less susceptible to gentamicin, in contrast to a European study, where in majority (83%) of the isolates were less susceptible. This discrepancy might have been due to the agar dilution method used in the European study and geographical differences (14,22).

Our findings are more similar to studies from Mongolia and Brazil, where 100% isolates were susceptible [13,17]. Observations of the European study (14) are similar to that from Malawi (9) and Canada (16) where the majority of isolates were less susceptible.
In the present study, majority (76.9%) of isolates were within a wide MIC range of 0.5-4 mg/L, while 95%, 99.7% and 100% of European, Argentinian and Kenyan isolates were within a more narrow MIC range of 4-8 mg/L, 4-8 mg/L and 4-16 mg/L, respectively (14,15,25). In comparison, only 9.1% isolates in our study had MIC value of 8 mg/L. Our findings are similar to Mongolia, where MICs of 82.2% isolates were between 0.5-2 mg/L (13). In previous studies, gentamicin MICs ranging from 1-32 mg/L in Indonesia (26,27), 2-16 mg/L in the USA (28), ≤2-16 in Canada (16) and 0.5-32 mg/L in Malawi (8) were reported.

The correlation between in vitro susceptibility and clinical outcomes has not yet been established for gentamicin. Only one study determined the clinical efficacy of gentamicin in the treatment of *N. gonorrhoeae* two years after its use in Malawi (9). Preliminary findings from a recent American study examining the effectiveness of combination of gentamicin with azithromycin suggested high efficacy of this regimen (11). However, this study did not determine the efficacy of the individual antibiotics, or efficacy of gentamicin for extra-genital infections. The latest CDC STD treatment guidelines recommend dual treatment with single doses of gentamicin plus azithromycin in suspected treatment failures cases with the recommended regimen (ceftriaxone plus azithromycin) (29). However, all these potential treatment regimens require comprehensive *in vitro* and *in vivo* evaluations and recommendations to be made based on clinical trials, not only *in vitro* results.

To conclude, there is a clear need to identify and evaluate possible future treatment options in view of concerns about emergence of resistance to ESCs. Gentamicin is an effective, inexpensive and a relatively safe antibiotic which has shown high *in vivo* efficacy in Malawi and USA (9,11). ESC resistant gonococci are unlikely to exhibit cross-resistance to gentamicin as cephalosporins act on bacterial cell wall synthesis whereas gentamicin disrupts protein synthesis. A single intramuscular injection of antibiotic paves the way for
outpatient management and reduces the risk of vestibular and renal toxicity seen with high drug concentrations for longer periods for other infections (10). No resistance to gentamicin was observed in the present study and its high in vitro susceptibility against MDR strains and strains with decreased susceptibility to currently recommended ESCs suggests its potential for future use as a treatment of gonococcal infections. However, because of limitation of the present study being at a single site although over multiple years, more studies worldwide are required to inform gentamicin efficacy at local, national, regional level.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs. Leelamma Peter for her excellent technical assistance. We thank the Medical Superintendent, VMMC & Safdarjung Hospital for permitting us to carry out this study. The authors are thankful to department of life sciences, SunRise University for granting permission to Mr. Vikram Singh to carry out his PhD thesis work at the RRL. The authors are indebted to Prof. John W. Tapsall, Dr. Monica M Lahra and Ms. Athena Limnios, WHO collaborating centre for STD, Department of Microbiology, The Prince of Wales Hospital, Sydney, Australia for supplying WHO Reference strains and low concentration antibiotic discs (Oxoid).

Funding This study was funded partially by the National AIDS Control Organization, India (F.9(58)DSACS/STI(AD)/NACP-III/2009-10) and Indian Council of Medical Research, India (ID No. 5/3/3/4/2013-ECD-I dated 13.3.15, RFC NO. ECD/ADHOC/41/2014-15).

Conflict of interest None declared.

REFERENCES


Table 1 Profile of MDR Neisseria gonorrhoeae isolates during 2010-2015 and their susceptibility pattern to gentamicin

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of isolates tested</th>
<th>QRNG+ PPNG n (%)</th>
<th>QRNG+ TRNG n (%)</th>
<th>QRNG+ AzR n (%)</th>
<th>QRNG+ TRNG+ PPNG+ AzR n (%)</th>
<th>Total MDR Strains n (%)</th>
<th>Gentamicin S n (%)</th>
<th>Gentamicin LS n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August to December 2010</td>
<td>8</td>
<td>0 (12.5)</td>
<td>3 (37.5)</td>
<td>0</td>
<td>4 (50)</td>
<td>4 (100)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>42</td>
<td>6 (14.3)</td>
<td>2 (4.8)</td>
<td>16 (38.1)</td>
<td>0</td>
<td>26 (62)</td>
<td>24 (92.3)</td>
<td>2 (7.7)</td>
</tr>
<tr>
<td>2012</td>
<td>71</td>
<td>24 (33.8)</td>
<td>0</td>
<td>20 (47.8)</td>
<td>0</td>
<td>2 (2.8)</td>
<td>46 (64.8)</td>
<td>40 (86.9)</td>
</tr>
<tr>
<td>2013</td>
<td>39</td>
<td>5 (12.8)</td>
<td>3 (7.7)</td>
<td>15 (38.5)</td>
<td>0</td>
<td>0</td>
<td>23 (59)</td>
<td>17 (73.9)</td>
</tr>
<tr>
<td>2014</td>
<td>41</td>
<td>10 (24.4)</td>
<td>5 (12.2)</td>
<td>11 (26.8)</td>
<td>0</td>
<td>0</td>
<td>26 (63.4)</td>
<td>21 (80.8)</td>
</tr>
<tr>
<td>2015</td>
<td>57</td>
<td>10 (17.5)</td>
<td>0</td>
<td>17 (30.6)</td>
<td>3 (11.5)</td>
<td>0</td>
<td>30 (52.6)</td>
<td>25 (83.3)</td>
</tr>
<tr>
<td>Total</td>
<td>258</td>
<td>55 (21.3)</td>
<td>11 (4.3)</td>
<td>82 (31.7)</td>
<td>5 (1.9)</td>
<td>2 (0.8)</td>
<td>155 (60.1)</td>
<td>131 (84.5)</td>
</tr>
</tbody>
</table>

QRNG, quinolone resistant *N. gonorrhoeae*; PPNG, penicillinase producing *N. gonorrhoeae*; TRNG, tetracycline resistant *N. gonorrhoeae*; AzR, azithromycin resistant; MDR, multi-drug resistant; S, susceptible; LS, Less susceptible.
Table 2 Trend of gentamicin susceptibility and antimicrobial susceptibility profile of gentamicin less susceptible *Neisseria gonorrhoeae* isolates to other antimicrobials

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of isolates tested</th>
<th>Gentamicin LS n (%)</th>
<th>Ceftriaxone, Cefpodoxime and Cefixime n (%)</th>
<th>Penicillin n (%)</th>
<th>Tetracycline n (%)</th>
<th>Ciprofloxacin n (%)</th>
<th>Azithromycin n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>DS</td>
<td>PPNG</td>
<td>CMR</td>
<td>LS</td>
<td>TRNG non-TRNG</td>
</tr>
<tr>
<td>Aug. to Dec. 2010</td>
<td>8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>42</td>
<td>2 (4.8)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2012</td>
<td>71</td>
<td>6 (8.4)</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2013</td>
<td>39</td>
<td>6 (15.4)</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2014</td>
<td>41</td>
<td>5 (12.2)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2015</td>
<td>57</td>
<td>5 (8.8)</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>258</td>
<td>24 (9.3)</td>
<td>21 (87.5)</td>
<td>9 (37.5)</td>
<td>5 (20.8)</td>
<td>10 (41.7)</td>
<td>5</td>
</tr>
</tbody>
</table>

LS, less susceptible; n, number; S, susceptible; DS, decreased susceptibility; PPNG, penicillinase producing *N. gonorrhoeae*; CMR, chromosomally mediated resistance; TRNG, tetracycline resistant *N. gonorrhoeae*; R, resistant; HLR, high level resistance.