Acquired macrolide resistance genes and the 1 bp deletion in the mtrR promoter in Neisseria gonorrhoeae

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Received 20 September 2002; returned 4 October 2002; revised 8 October 2002; accepted 9 October 2002

The presence of macrolide-lincosamide-streptogramin B resistance genes erm(B), erm(C) and erm(F), the macrolide resistance mef(A) gene, and the DNA sequence of a 13 bp repeat in the promoter region of the mtrR gene, were determined in 62 Neisseria gonorrhoeae isolates collected between 1992 and 1999 in Seattle, Washington, USA. Eleven isolates with erythromycin and azithromycin MICs of \( \leq 0.06 \) mg/L, had no acquired genes or deletions in the 13 bp repeat region. Among 44 isolates with erythromycin MICs 1.0–16.0 mg/L, and azithromycin MICs 0.06–4.0 mg/L, 16 carried the 1 bp deletion in the mtrR promoter region alone, nine carried one or more of the four acquired macrolide resistance genes alone, and 14 carried both acquired macrolide resistance genes plus the 1 bp deletion in the mtrR promoter region. Three isolates with erythromycin MICs \( \geq 8 \) mg/L, and azithromycin MICs of 4.0 mg/L, carried only erm genes. Five isolates with MICs of 1–2 mg/L did not carry the 1 bp deletion, or any of the acquired resistance genes examined. Our data suggest that the 1 bp deletion in the mtrR promoter region is not found in all erythromycin-resistant (MIC \( \geq 1.0 \) mg/L) N. gonorrhoeae.

Keywords: N. gonorrhoeae, macrolide resistance, acquired resistance

Introduction

After almost two decades of decline, the incidence of gonococcal infection increased modestly during the late 1990s in the USA.1 However, large increases have been reported in some geographical areas among men who have sex with men (MSM).2 Gonococci recovered from MSM have been reported to differ from those infecting heterosexual men and women. These differences include increased resistance to hydrophobic molecules, including macrolide antibiotics.2–4 A 1 bp deletion of an adenine in the −35 to −10 region of the mtrR promoter has been shown to upregulate the mtrCDE operon. This leads to a four-fold increase in resistance to macrolides, penicillin and tetracycline.4 More recently, Neisseria gonorrhoeae, with increased resistance to erythromycin and azithromycin, have been identified that carry rRNA methylases encoded by either erm(B), erm(C) and erm(F), or the macrolide efflux gene mef(A).5,6

Erythromycin and azithromycin are not recommended for treatment of gonococcal infection; however, azithromycin is frequently used for chlamydial therapy, and azithromycin is used in some areas for gonorrhoea therapy. In the Seattle-King County area between 1992 and 1999, 1676 of 7370 (22.7%) N. gonorrhoeae isolates were erythromycin resistant (MIC \( \geq 1 \) mg/L), and the erythromycin and azithromycin susceptibilities were highly correlated (\( r = 0.78 \)). To understand better the various mechanisms of erythromycin resistance, 62 isolates with erythromycin MICs of \( \leq 0.06–16 \) mg/L, and azithromycin MICs of 0.03–4.0 mg/L, were investigated for the presence of mutations in the mtrR promoter region, and for the presence of erm(B), erm(C), erm(F) and mef(A).
Materials and methods

Gonococcal isolates

Sixty-two *N. gonorrhoeae*, recovered by public and private clinical laboratories in Seattle-King County, were sent to the University of Washington’s Neisseria Reference Laboratory (NRL). The identification of the isolates was confirmed, and erythromycin and azithromycin susceptibilities were performed using established methods. Erythromycin resistance was defined as an MIC of \( \geq 1 \) mg/L. Isolates included 21 resistant isolates from 1992–1998, 11 isolates with erythromycin MICs of \( \leq 0.06 \) mg/L from 1998–1999 and 30 isolates from a weighted (3:1 erythromycin-resistant:erythromycin-susceptible) random sample of urethral isolates collected during 1998–1999.

DNA hybridization for acquired genes

All isolates were assayed by whole cell dot blots and/or DNA dot blots, and probed as described previously using the following probes: *erm*(B) (5'-GAAAAGGTACTCAAC-CAAATA-3'), *erm*(C) (5'-TCAAAACATAATATAGATTAA-3'), *erm*(F) (5'-CGGGTCAGCACTTTACTATTG-3') and *mef*(A) (5'-GTTGCTGTGATTGCATCTATTAC-3'). Genes were confirmed by PCR assays as described previously. PCR amplification of a 380 bp fragment, bracketing the promoter region of the *mtrR* gene, was performed using primers mtrF1 (5'-GCCAATCAACAGGCATTCTTA-3') and mtr13R1 (5'-GTTGGAACAACGCGTCAAAC-3'). The reaction mix included DNA 100 ng and 2.5 units of Taq polymerase (Applied Biosystems, Foster City, CA, USA). A Perkin-Elmer Cetus thermal cycler was used, and the reaction was as follows: denaturation at 94°C for 3 min; then 30 cycles at 94°C for 1 min, annealing at 51°C for 1 min, and elongation at 72°C for 2 min; there was a final elongation step at 72°C for 5 min. The PCR products were run on a 1.5% agarose gel, as described previously. The University of Washington Department of Biochemistry DNA Sequencing Center sequenced the PCR products, which were compared with the wild-type sequence (GenBank database accession number Z25796).

Results and discussion

Characterization of isolates

Erythromycin and azithromycin susceptibilities are correlated in Gram-positive cocci. In this study, the *N. gonorrhoeae* isolates, with low erythromycin MIC of \( \leq 0.0.6 \) mg/L, had azithromycin MICs of 0.03–0.06 mg/L. The isolates with erythromycin MICs of 0.25–0.5 mg/L had azithromycin MICs of 0.03–0.125 mg/L, isolates with erythromycin MICs of 1–4 mg/L had azithromycin MICs of 0.06–0.5 mg/L, while isolates with erythromycin MICs of 8–16 mg/L had azithromycin MICs of 4 mg/L.

Eleven isolates with erythromycin and azithromycin MICs of 0.03–0.06 mg/L had no mutations detected in *mtrR*, and did not carry the acquired genes studied. In the seven isolates with erythromycin MICs 0.25–0.5 mg/L, one isolate possessed the 1 bp *mtrR* deletion plus *erm*(C), a second carried *erm*(B), *erm*(F) and *mef*(A), and a third carried *mef*(A). The remaining four isolates carried neither the 1 bp *mtrR* deletion nor the four acquired genes (Table 1). These isolates may have chromosomal mutations that counteracted the effect of the acquired macrolide resistance genes, and the 1 bp deletion, resulting in no increase in erythromycin resistance. Alternatively, it is also possible that their acquired genes were not expressed, or

Table 1. Characterization of the 62 *N. gonorrhoeae* isolates

<table>
<thead>
<tr>
<th>Erythromycin MIC (mg/L)</th>
<th>Azithromycin MIC (mg/L)</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>wild type no acquired genes</td>
</tr>
<tr>
<td>( \leq 0.06 )</td>
<td>0.03–0.06</td>
<td>11</td>
</tr>
<tr>
<td>0.25–0.5</td>
<td>0.06–0.125</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>0.06–0.125</td>
<td>4*</td>
</tr>
<tr>
<td>2–4</td>
<td>0.125–0.5</td>
<td>1</td>
</tr>
<tr>
<td>8–16</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20 (32%)</td>
</tr>
</tbody>
</table>

*One isolate has an insertion of a thymidine in the 13 bp *mtrR* promoter region. Three isolates have been described previously as containing *mef*(A) genes.
were non-functional. Their MICs of azithromycin were indistinguishable from those for four other isolates in this group.

Among the nine isolates with erythromycin MICs of 1 \text{mg/L}, one isolate had the 1 \text{bp} \text{mtrR} \text{deletion}, two additional isolates had this deletion plus either \text{erm}(B) and \text{erm}(F), or \text{erm}(F) and \text{mef}(A); two other isolates carried \text{erm}(C) or \text{erm}(F) alone. The remaining four isolates carried none of the acquired antibiotic resistance genes, or the 1 \text{bp} \text{mtrR} \text{deletion}, although one possessed an inserted thymidine in the 13 \text{bp} repeat region of the \text{mtrR} \text{promoter}. In one recently published study, Ng \textit{et al}.\textsuperscript{8} concluded that this insertion did not affect erythromycin susceptibilities in their clinical isolate or a laboratory-derived mutant.

There were 32 isolates with erythromycin MICs of 2–4 \text{mg/L}. Fifteen had the 1 \text{bp} \text{mtrR} \text{deletion} alone, 12 had the 1 \text{bp} deletion and carried acquired gene(s), and four isolates carried acquired genes alone. All four acquired genes were found in this group of isolates. One isolate lacked both acquired resistance genes and the 1 \text{bp} deletion (Table 1). In three isolates for which the erythromycin MIC was 8–16 \text{mg/L} and the azithromycin MIC was 4 \text{mg/L}, we found one or more of the three \text{erm} genes, but no 1 \text{bp} deletion (Table 1).

Nine of 44 (20.5\%) erythromycin-resistant isolates did not contain the 1 \text{bp} deletion in the \text{mtrR} \text{promoter}, and included three isolates with erythromycin MICs of 4 \text{mg/L}, indicating that the 1 \text{bp} deletion is not the sole cause of erythromycin or azithromycin resistance in \textit{N. gonorrhoeae}. Five strains contained neither a 1 \text{bp} \text{mtrR} \text{deletion} nor any of the four acquired resistance genes examined. There are currently 26 different rRNA methylase (\text{erm}) genes, and eight other types of gene conferring macrolide resistance.\textsuperscript{5} These five isolates may carry other known or uncharacterized acquired macrolide resistance genes. It is also possible that these five strains have other mutations, each providing a small increase in resistance. However, it is unlikely that they carry the recently described but rare mutations (Dr Ng, National Laboratory for Sexually Transmitted Diseases, National Microbiology Laboratory, Population and Public Health Branch, Health Canada, Winnipeg, Canada, personal communication) in the 23S rRNA,\textsuperscript{8} because the two isolates described with these mutations, representing a single strain, had significantly higher MICs of both erythromycin (32–64 \text{mg/L}) and azithromycin (4 \text{mg/L}) than the five isolates in this study with MICs of 1–2 \text{mg/L} erythromycin and 0.06–0.125 \text{mg/L} azithromycin. As azithromycin use continues, it is likely that \textit{N. gonorrhoeae}, with a variety of different mutations and acquired macrolide resistance genes, may be isolated, as currently described for \textit{Streptococcus} spp.\textsuperscript{9,10}

\section*{Acknowledgements}
Partial support was provided by the Ponicin Fellowship to S. L. Cousin, Jr and NIH grant 5U19AI-31448-10.

\section*{References}


