Gonorrhea among Men Who Have Sex with Men: Outbreak Caused by a Single Genotype of Erythromycin-Resistant Neisseria gonorrhoeae with a Single–Base Pair Deletion in the mtrR Promoter Region

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During 1995–1997, an outbreak of 66 cases of gonorrhea caused by an erythromycin-resistant (Eryr; MIC >1.0 µg/mL) prototrophic (proto) auxotype IB-1 serovar of Neisseria gonorrhoeae occurred in King County, Washington; 65 cases involved men who have sex with men (MSM), which accounted for ~37% of infections among MSM during this period. Isolates from 19 of these 65 cases of infection were analyzed by DNA sequencing of the polymerase chain reaction–amplified promoter region of the mtrR gene and by pulsed-field gel electrophoresis (PFGE) analysis of genomic DNA after NheI and SpeI digestion. Eighteen of the 19 isolates had a 1-bp A/T deletion in a 13-bp inverted repeat of the mtrR promoter region and shared a single PFGE type. Among MSM who provided data about sexual behavior, 37 (64%) of 58 MSM infected by the proto/IB-1 Eryr strain reported having >2 sex partners during the past 60 days, compared with 32 (30%) of 106 MSM infected by other strains (P < .001). This clonal outbreak of gonorrhea illustrates the ongoing need for behavioral preventive interventions among MSM.

Although the incidence of gonorrhea has declined during the AIDS era, an outbreak of infection caused by erythromycin-resistant (Eryr) Neisseria gonorrhoeae among men who have sex with men (MSM) occurred in Seattle–King County, Washington, during 1995 [1]. Previous studies [2, 3] indicated that isolates of N. gonorrhoeae from MSM were more likely than isolates from heterosexual patients to resist inhibition by a variety of structurally different hydrophobic compounds, including fecal lipids, bile salts, detergents (e.g., Triton X-100), and hydrophobic antibiotics (e.g., erythromycin). The genetic basis of resistance of N. gonorrhoeae to these compounds has been attributed to mutations in the multiple transferable resistance (mtr) efflux system [2–4], which is encoded by the mtrCDE operon [5] and regulated by a transcriptional repressor, mtrR [6]. Resistance to erythromycin and Triton X-100 can serve as a phenotypic marker for Mtr-mediated resistance in N. gonorrhoeae [2, 3, 7].

Our study of the Seattle–King County outbreak focused on characterizing the promoter region of the mtrR gene, because a 1-bp deletion in the −35 to −10 region of the mtrR promoter leads to significantly increased resistance to hydrophobic agents [2, 4, 8]. Genetic relatedness of these outbreak-causing isolates was examined by auxotyping and serotyping, as well as by pulsed-field gel electrophoresis (PFGE) [9–11]. Finally, the sexual behaviors of MSM infected with gonorrhea during the outbreak were examined to assess the possible association between increased risk taking and the spread of particular strains.

Methods

Selection of gonococcal isolates. All Eryr gonococci that were prototrophic (proto) and porin serovar IB-1 and that were recovered from MSM seen in the Public Health Seattle–King County Sexually Transmitted Disease (STD) Clinic were available for study. These comprised 73 isolates from 65 MSM. One proto/IB-1 erythromycin-susceptible isolate from the same period was selected as a control.

For analysis of mutations in the promoter region of the mtrR gene and for PFGE typing analysis, isolates from 19 of the 65 men were selected by random lot, weighted by distribution of cases over the outbreak period. The control strain was included for both assays.

Antimicrobial susceptibilities. Penicillin and erythromycin MICs were determined by the agar dilution method of the National Committee for Clinical Laboratory Standards (Villanova, PA). An Eryr strain was defined by a MIC >1.0 µg/mL, which is consistent with a previous report [12] and with levels observed in strains of

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the Mtr phenotype [3]. To confirm the Mtr phenotypic trait, Triton X-100 (Sigma, St. Louis) MICs were determined for the 73 isolates and for the control strain, by established methods [2, 3].

Auxotyping, serotyping, and PFGE. Auxotyping, serotyping, and DNA preparation for PFGE typing were done as described elsewhere [9–11]. For PFGE analysis, chromosomal DNA was digested overnight with NheI and SpeI (Promega, Madison) separately. The digested DNA was analyzed by use of the contour-clamped homogeneous electric field (CHEF) DR III apparatus with cooling module (Bio-Rad Laboratories, Richmond, CA) set at 14°C through each run. The CHEF DR III was controlled by 2 program blocks. Block 1 was run for 4 h, with pulse time ranging from 10 s initially to 30 s at the end; block 2 ran for 20 h, with pulse time again increasing from 10 s initially to 30 s at the end. The criterion for classifying PFGE types was a difference of ≥3 DNA fragments for either enzyme [13]. PFGE type variants were defined by PFGE patterns showing a 1–2-band difference from a predominant prototype pattern.

Analyses of mtrR. There is a 250-bp gap separating the mtrR gene and the mtrCDE gene complex, which is transcribed divergently from the mtrR, and the mtrR promoter is situated between mtrR and mtrCDE [8]. The mtrR promoter region was examined by polymerase chain reaction (PCR) with primers CELI (5'-GA-CATGTTCA GCGATGATAGG-3'), which anneals 120 bp downstream from the mtrR translational stop codon, and KH9#3 (5'-GACGACAGT GCAATGCAACG-3'), which anneals 24 bp downstream from the mtrC [2, 4] translational start codon, amplifying a 1087-bp fragment. This DNA fragment covered mtrR, the mtrR-mtrC intervening region, including the mtrR promoter sequence, and part of mtrC. Gonococcal genomic DNA was used as the template. PCR was done by 30 cycles of amplification; each cycle consisted of 1 min of denaturation at 95°C, 1 min of annealing at 60°C, and 1 min of elongation at 72°C. PCR products were purified by use of QIAquick PCR purification kits (Qiagen, Chatsworth, CA) according to the manufacturer’s instructions. The primer for sequencing was KH9#2 (5'-CGTTCCGGT CGGT T GACG-3') [8], which annealed 11 bp downstream from the translational mtrR start codon. DNA sequencing was done with the AmpliTaq cycle kit (Perkin Elmer, Norwalk, CT) with α-[33P]dATP incorporation. Examination of completeness of the 13-bp inverted repeat (5'-AAAAAGACTTTTTT-3') within the −35 to −10 region of the mtrR promoter [4, 8] was emphasized, because a 1-bp A/T deletion within the inverted repeat would lead to loss of mtrR expression and consequently would increase expression of mtrCDE, thus causing resistance to hydrophobic agents in N. gonorrhoeae [2, 4, 8].

Clinical and behavioral data. Information from a standardized clinic record form was entered into a computer database and subsequently analyzed by use of Epinfo, version 6 (Centers for Disease Control and Prevention, Atlanta, GA). Patient-specific information was considered for the visit during which specimens positive for N. gonorrhoeae were collected.

Results

Although gonorrhea incidence continued to decline among heterosexual men, cases of gonorrhea detected among MSM attending the STD clinic increased steadily each quarter, from 6 during April–June 1995 to 44 during January–March 1996, and then declined steadily to 9 during April–June 1997 (figure 1). Among 197 MSM with gonorrhea during this period, gonococcal isolates were available from 175 for susceptibility testing and phenotype. Of these, 94 isolates (54%) had erythromycin MICs >1.0 µg/mL; 65 of these were proto/IB-1 isolates, whereas 29 belonged to 12 other auxotype-serovar combinations. During the same time period, 57 (15%) of 376 strains recovered from heterosexual men had erythromycin MICs ≥1.0 µg/mL (P < .001). Only 1 of these 57 Ery+ isolates from heterosexual men belonged to the proto/IB-1 phenotype.

Seventy-three proto/IB-1 Ery+ isolates of N. gonorrhoeae were recovered from the 65 MSM. The Triton X-100 MICs were >8200 µg/mL for 70 of these isolates, 2048 µg/mL for 1, and 256 µg/mL for 2. The Triton X-100 MIC for the erythromycin-susceptible control strain was 256 µg/mL. For the subset of 19 isolates selected randomly for DNA sequencing and PFGE typing, the erythromycin MICs were 1–4 µg/mL, and the penicillin MICs were 1–8 µg/mL, whereas the control strains’ MICs were 0.5 and 0.03 µg/mL, respectively. The relationships between PFGE type, DNA sequencing results, and MICs are shown in table 1.

Table 1. Pulsed-field gel electrophoresis (PFGE) typing and DNA sequencing of multiple transferable resistance (Mtr) phenotypes of 19 prototrophic/IB-1 isolates of Neisseria gonorrhoeae from an outbreak among men who have sex with men in King County, Washington.

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>A/T deletiona</th>
<th>PFGE type</th>
<th>Erythromycin (µg/mL)</th>
<th>Triton X-100 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Yes</td>
<td>Type 1</td>
<td>2–4</td>
<td>&gt;8200</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Type 1 variant</td>
<td>1–4</td>
<td>&gt;8200</td>
</tr>
<tr>
<td>1</td>
<td>No</td>
<td>Other</td>
<td>2</td>
<td>2048</td>
</tr>
</tbody>
</table>

NOTE. NheI and SpeI were used for PFGE analysis. PFGE type variant was defined as PFGE pattern showing a ≤2-band difference from the predominant prototype pattern. The mtrR promoter and its flanking region were amplified by polymerase chain reaction; products were sequenced to examine mutation events in the mtrR promoter.

a A/T deletion refers to mutation detected in the 13-bp inverted repeat (5'-AAAAAGACTTTTTT-3') within the −35 to −10 positions of the mtrR promoter.
DNA sequencing of the mtrR promoter region revealed that 18 of the 19 isolates had a 1-bp A/T deletion within the 13-bp inverted repeat. The 1 remaining isolate, which had a Triton X-100 MIC of 2048 µg/mL, carried an intact 13-bp inverted repeat in the mtrR promoter region. PFGE analysis with NheI and SpeI showed that these 18 isolates having the A/T deletion shared the same PFGE type or a variant that differed by only 2 DNA restriction fragments with both enzymes (table 1). All 18 isolates had Triton X-100 MICs >2000 µg/mL. The 1 isolate that had no A/T deletion in the 13-bp inverted repeat had a PFGE type different from that of the 18 isolates with an A/T deletion. The control isolate also had a complete 13-bp inverted repeat and a different PFGE type.

Among MSM who provided sexual exposure data on standardized clinic interview forms, 37 (64%) of 58 infected with the proto/IB-1 Eryr phenotype and 32 (30%) of 106 infected with other phenotypes (P < .001) reported having >2 sex partners during the past 60 days. Seventeen (28%) of 60 MSM infected with the outbreak strain and 24 (22%) of 109 MSM infected with other gonococcal phenotypes were human immunodeficiency virus (HIV)–seropositive.

Discussion

This study described an outbreak caused by a unique genotype of Eryr N. gonorrhoeae that is characterized by a deletion in the 13-bp inverted repeat in the mtrR promoter region and that elsewhere has been shown to be responsible for resistance to erythromycin and Triton X-100 [2, 4]. Although erythromycin is not useful for treatment of gonorrhea, erythromycin resistance is a marker for resistance to a variety of hydrophobic compounds. Epidemiologic data suggest that this genotype, which carries a mutation in the mtr efflux system, had a survival advantage in the MSM population, perhaps because of resistance to killing by fecal lipids [3]. Strains of N. gonorrhoeae with the mutation may be indigenous in Seattle–King County, where 2 of 6 rectal isolates obtained from MSM during 1978–1980 had the same mutation [2, 3]. This outbreak apparently represented a sentinel event that indicated increased sexual risk taking among some MSM or the entry of the strain into an existing extended sexual network of MSM who already were engaged in high-risk sexual behaviors. Disappearance of this phenotype, after a 2-year outbreak, occurred during a period when total cases of gonorrhea among MSM seen in the STD clinic declined somewhat and was not related to specific interventions targeting the epidemic phenotype. Subsequent reports have documented an increased prevalence of gonorrhea among MSM in the western United States [1, 14], and an outbreak of syphilis is now occurring among MSM in Seattle–King County [15]. Efforts to reduce the transmission of sexually transmitted pathogens, including HIV, among MSM must be redoubled.

Acknowledgments

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