Survey of Neisseria gonorrhoeae antimicrobial susceptibility in Ontario

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ABSTRACT: The minimal inhibitory concentrations (MICs) of penicillin, tetracycline, erythromycin, cefoxitin, ceftriaxone and spectinomycin were determined for 300 consecutive strains of Neisseria gonorrhoeae collected from physicians' offices in Ontario. Only four isolates were found to produce beta-lactamase. Of the remaining 296 isolates, five (1.7%) had penicillin MICs greater than or equal to 1 mg/L, 78 (26.3%) had tetracycline MICs greater than or equal to 1 mg/L, 13 (4.4%) had cefoxitin MICs greater than or equal to 1 mg/L and 43 (14.5%) had erythromycin MICs greater than or equal to 1 mg/L. Two isolates (0.7%) had high level tetracycline resistance with MICs greater than or equal to 16 mg/L. All N gonorrhoeae isolates were susceptible to ceftriaxone and to spectinomycin. Can J Infect Dis 1990;1(4):136-138

Key Words: Neisseria gonorrhoeae, Resistance

A N IMPORTANT CLINICAL PROBLEM CONCERNING THE treatment of Neisseria gonorrhoeae infections is the increase in antimicrobial resistance, which can be plasmid-mediated, chromosomally mediated, or both (1). Penicillinase-producing N gonorrhoeae was first isolated in Ontario in 1978 (2); the first penicillinase-producing N gonorrhoeae in Canada was isolated in 1976. In Ontario, the Ministry of Health reported that penicillinase-producing N gonorrhoeae accounted for 2.7% of all gonococcal strains isolated in 1986 (2). In Quebec, Rousseau (3) reported that penicillinase-producing N gonorrhoeae accounted for 3.4% of N gonorrhoeae strains isolated between July 1987 and July 1988.

These N gonorrhoeae isolates may have been referred because of special characteristics such as antimicrobial resistance. Therefore, the prevalence of antimicrobial resistance may be overestimated. In order to obtain a more representative account of the type and prevalence of antimicrobial resistance in N gonorrhoeae, consecutive organisms isolated from specimens obtained from physicians' offices located in each major region of Ontario were studied.

MATERIALS AND METHODS

N gonorrhoeae isolates were obtained from the 22 MDS Laboratories in Ontario between October 1987 and May 1988. (MDS Laboratories are privately operated medical laboratories which provide services to approximately 3000 physicians throughout Ontario.) A data collection sheet was submitted with the cultures of N gonorrhoeae. Each isolate represented one clinical case. The isolates were identified by Gram stain, oxidase, carbohydrate consumption and the Gonogen coagglutination test (Bio-Mega Diagnostic Inc) (4).
TABLE 1
Susceptibility of 296 beta-lactamase negative isolates of Neisseria gonorrhoeae

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number of isolates with indicated minimal inhibitory concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.001</td>
</tr>
<tr>
<td>Penicillin</td>
<td>39</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>29</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>113</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>4</td>
</tr>
</tbody>
</table>

*Breakpoint between susceptible and resistant susceptibility categories

Isolates were stored at -70°C in glycerol until they were thawed for susceptibility testing. Three reference strains (WHO III, WHO V, WHO VII) were used as recommended by the World Health Organization (5). The isolates were tested for beta-lactamase activity by the chromogenic cephalosporin test (Cefinase; BBL Microbiology Systems, Maryland).

Antimicrobial susceptibility testing was performed in a single run by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (6) with the appropriate controls. Agar dilution testing was done using a GC medium base (Difco Laboratories, Michigan) supplemented with 1% Vitox (Oxoid, Basingstoke, United Kingdom) and 1% powdered hemoglobin (GIBCO, Wisconsin). With penicillin-, erythromycin-, ceftriaxone- and spectinomycin-containing media, it has been shown that addition of hemoglobin does not produce significantly different minimal inhibitory concentrations (MICs) compared to media without hemoglobin (7,8). The thawed isolates were passed twice on chocolate agar. The inoculum was suspended in trypticase soy broth (BBL Microbiology Systems). A final inoculum of 10^4 colony forming units was delivered to the antibiotic-containing agar plates. The inoculated plates were incubated at 35°C for 24 h in 5% carbon dioxide. MIC was defined as the lowest concentration of antibiotic which inhibited bacterial growth. A single colony or faint haze was regarded as no growth. The antimicrobial reference powders used in susceptibility testing were kindly provided by the manufacturers.

RESULTS

Three hundred and sixty-eight consecutive isolates of N gonorrhoeae were collected from October 1987 to May 1988. Seventy-two isolates failed to grow on subculture. These lost isolates were evenly distributed over the time of the study and were not lost from any particular region. Four isolates (1.3%) were penicillinase-producing N gonorrhoeae. These were not included in the MIC determinations. Of the remaining 296 isolates, five (1.7%) had penicillin MICs greater than or equal to 1 mg/L, 78 (26.3%) had tetracycline MICs greater than or equal to 1 mg/L, 13 (4.4%) had cefoxitin MICs greater than or equal to 1 mg/L and 43 (14.5%) had erythromycin MICs greater than or equal to 1 mg/L. Two isolates (0.7%) had high level tetracycline resistance with MICs greater than or equal to 16 mg/L. These two isolates originated from St Catharines, Ontario. All N gonorrhoeae isolates were susceptible to ceftriaxone and spectinomycin (Table 1).

DISCUSSION

This study indicates that the majority of N gonorrhoeae isolates collected in Ontario are susceptible to antimicrobial agents using the breakpoints published in the M7-A standards of the National Committee for Laboratory Standards (6). The more current M7-A2 standards (9) were not available at the time of this study. One isolate had a MIC to penicillin of 8 mg/L. This isolate had MICs to tetracycline, erythromycin and ceftriaxone of 1.0, 0.50 and 0.001 mg/L, respectively. However, two isolates demonstrated high level tetracycline resistance, with MICs greater than or equal to 16 mg/L. This level of resistance suggests that the use of tetracycline would be associated with a high treatment failure rate. The activity of spectinomycin has decreased since the report of Dillon et al (10), although all isolates remained susceptible (MICs less than or equal to 32 mg/L, range 8 to 32).

The Centre for Disease Control provides guidelines for the detection, management and control of N gonorrhoeae. Three levels of activity are characterized based on the endemicity of penicillinase-producing N gonorrhoeae. Nonendemic, endemic and hyperendemic areas are defined as locales in which the proportions of penicillinase-producing N gonorrhoeae are less than 1%, 1 to 3% and
greater than 3%, respectively (1). Thus, Ontario would be categorized as an endemic area based on the current study. In terms of surveillance, if the prevalence of penicillinase-producing *N gonorrhoeae* is less than 1%, then testing for beta-lactamase activity on all gonococcal isolates is sufficient (1). In endemic areas, all gonococcal isolates should be examined for beta-lactamase production, and only isolates from children and from patients with pelvic inflammatory disease would undergo other antimicrobial susceptibility testing (1). In hyperendemic areas, all gonococcal strains should undergo both beta-lactamase testing and antimicrobial agar dilution susceptibility testing (1).

In 1987, Toma et al (11) described four high level tetracycline-resistant *N gonorrhoeae* isolates from 286 cultures tested in a one month period at the Laboratory Services Branch of the Ontario Ministry of Health. As a result of this finding they recommended that laboratories in the province should routinely screen *N gonorrhoeae* isolates for tetracycline resistance. The present study found that antimicrobial resistance in *N gonorrhoeae* isolates from physicians' offices in Ontario is not as prevalent as that described in studies with *N gonorrhoeae* isolates from reference laboratories. However, MICs in the present study were higher than those obtained in a Canadian study done in 1973-74 by Dillon et al (10). Thus there is a need for continued surveillance of *N gonorrhoeae* susceptibility patterns by reference laboratories. Moreover, routine diagnostic laboratories in Ontario should continue to screen for penicillinase-producing *N gonorrhoeae*, but it is not currently necessary to screen routinely for chromosomally mediated resistance or for tetracycline resistance in *N gonorrhoeae*.

REFERENCES
1. Centres for Disease Control. Antibiotic-resistant strains of *Neisseria gonorrhoeae*. Policy guidelines for detection, management and control. MMWR 1987;36:5S.
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