Evaluation of a Method for Rapid Detection of Penicillinase-Producing \textit{Neisseria gonorrhoeae} in Urethral Exudates

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Received 20 June 1988/Accepted 19 September 1988

Penicillinase-producing \textit{Neisseria gonorrhoeae} (PPNG) strains were first isolated in 1976 (6). Since then, PPNG strains have spread to all continents, and their increase has become a dramatic problem in developing countries for two major reasons: (i) detection of PPNG necessitates costly laboratory investigations before therapy to avoid treatment failure, and (ii) an alternative therapy to penicillin is often more expensive.

This is a considerable financial burden for most sexually transmitted disease centers in Africa. Although a rapid method for detection of PPNG in urethral exudates has been developed (2, 3, 8), direct detection in clinical specimens was not recommended by experts of the Centers for Disease Control (1). In Bangui, Central African Republic, the proportion of PPNG rose from 0.8% in 1981 to 14% in 1985 (5) and 32% in 1987. If PPNG could be detected in time for the prescription of appropriate treatment, this would result in considerable savings and could delay the spread of antibiotic-resistant strains. We report here a simple and rapid method for detecting β-lactamase in urethral exudates from male patients with gonorrhea.

Men with urethral discharge were examined at the Institut Pasteur in Bangui in March and April 1988. First, a sample of urethral exudate was obtained by using a wire loop. Specimens were Gram stained, inoculated on a selective chocolate agar medium (Diagnostic Pasteur, Marnes-La-Coquette, France), and incubated overnight at 37°C in a candle jar. Then, a second sample was suspended in pyridine-2-azo-p-dimethylaniline cephalosporin (PADAC; Diagnostic Pasteur) (4, 7) which had been rehydrated with 0.2 ml of 5 mM cysteine (Sigma Chemical Co., St. Louis, Mo.) in 1 mM HCl (pH 3.5). A dense suspension was obtained, vortexed, and immediately incubated at 37°C. The test was read every 15 min as positive (orange-yellow) or negative ( violet). The Gram stain was read as positive if gram-negative diplococci were seen inside or attached to leukocytes. When detected, the presence of numerous other bacteria was noted. Further identification of \textit{N. gonorrhoeae} was performed after 24 to 48 h on isolated colonies with the oxidase test and sugar oxidation. β-Lactamase was detected on isolated colonies by the nitrocefin test (Cefinase; Biomerieux, Marcy l’Etoile, France) and the PADAC test as recommended by the manufacturers.

Gram-negative diplococci were seen in 68 of 70 Gram-stained smears and selected for direct detection of β-lactamase. \textit{N. gonorrhoeae} was isolated from all 68 urethral exudates, and colonies were tested for the presence of β-lactamase by both methods. More than 58% (38 of 68) of the isolates were found to produce penicillinase by the nitrocefin and PADAC tests, and 30 of 68 isolates were negative with both tests. As can be seen in Table 1, the results of direct β-lactamase detection with the PADAC test correlated very well with those of the test performed after 24 to 48 h on cultured bacteria. Although there were no false-negative results, one PPNG-negative urethral exudate was positive in the direct detection test. Examination of the Gram-stained smear of this false-positive specimen revealed numerous other gram-positive cocci and gram-negative rods. More PPNG-negative urethral exudates should be tested to evaluate the importance of other bacteria in influencing the results of the direct detection test. Thus, the sensitivity of the direct test in our series was 100%, the specificity was 96.6%, and the positive predictive value was 97.4%. The mean time to obtain a frank orange-yellow with the direct detection test was 2.75 h, with a positive result obtained in one case after 0.5 h; whereas in two cases it took 8 h. In 13 cases, we collected a sufficient quantity of urethral pus to perform a second direct detection test at room temperature (25°C). PPNG was recovered on culture from 8 of 13 such specimens. These eight corresponding exudates gave positive results in the direct test, whereas the others remained negative. However, room temperature delayed the mean time for a positive reaction of the direct detection test to 5.5 h, i.e., almost twice that required with incubation at 37°C.

In this small series, the proportion of PPNG (58%) demonstrated a dramatic increase compared with the percentage of PPNG in Bangui in 1987 (32%). During the first 5 months of 1988, the proportion of PPNG routinely isolated in our laboratory, not including the specimens of the present survey, was 58.6% (34 of 58). This should prompt national health authorities to advise an alternative antibiotic to penicillin.

Our results show that PPNG can be reliably detected in men by a rapid method using a commercially available test. The method does not require sophisticated laboratory equipment and is easy to read. The correlation between results obtained with in vitro-grown gonococci and those with urethral exudates showed that β-lactamase is expressed in...
TABLE 1. Detection of β-lactamase in urethral exudates containing gram-negative diplococci compared with recovery of PPNG in subculture

<table>
<thead>
<tr>
<th>Detection of β-lactamase activity</th>
<th>No. of urethral exudates (n = 68) (PADAC direct test)</th>
<th>No. of PPNG isolates (n = 68)</th>
<th>Nitrocefin test</th>
<th>PADAC test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>39</td>
<td>38</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>29</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

vivo. But this test will likely fail to detect PPNG in women. If we had used the results of the rapid test to select treatment, only one man would have been treated unnecessarily with penicillin. However, little is known about the possible role of other β-lactamase-producing bacteria in the same ecological niche. The direct method does not detect chromosomally determined resistance to penicillin, but neither do β-lactamase detection methods performed on cultured isolates. Thus, national surveys on antibiotic susceptibility of *N. gonorrhoeae* should be conducted to detect intrinsic resistance to penicillin and provide guidelines for alternative therapy. But the direct detection of β-lactamase in urethral exudates might be useful in countries with limited financial capabilities and where gonococcal urethritis is frequent and PPNG strains are not yet predominant.

LITERATURE CITED