Isolation of Beta-Lactamase-Producing

*Neisseria gonorrhoeae*

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It is known that penicillinase-producing *Neisseria gonorrhoeae* (PPNG) are frequently found in South-East Asia and the infection does not respond to the recommended dose of penicillin. *N. gonorrhoeae* cultures isolated during June 1977 to June 1979 from Yonsei Medical Center patients were tested for β-lactamase production.

Among the 127 isolates tested one was positive by the rapid iodometric method. The culture also gave a positive result by the method of Hodge et al. No zone of inhibition was observed when its susceptibility was tested with a 10 unit penicillin disk.

The patient who yielded the organism was a 51-year-old housewife living in Seoul. Neither she nor her husband had ever traveled abroad. This result documented the presence of PPNG in this community. In the future laboratories may require careful watch to detect further spreading of PPNG.

*N. gonorrhoeae*, one of the most susceptible bacteria to penicillin previously, has been showing gradual increase of resistance to this drug *in vitro*. Penicillin frequently fails to cure the infection. Although resistant isolates are found all over the world, they are particularly prevalent in South-East Asia (Reyn, 1969b).

Despite of the gradual decrease of susceptibility, PPNG was not known until 1976 when 12 isolates were identified by the Center for Disease Control, USA (Sparling et al., 1973; CDC, 1976). Most of the isolates were from individuals who had had recent travel to South-East Asia. Since then, PPNG has been reported from many countries including England (Philips, 1976) and Japan (Onoda, 1979).

In Korea isolation of PPNG has been found in US armed forces personnel. Again they had a history of contact with the infection not in Korea but in South-East Asia. Besides the epidemiological reason, the finding of PPNG by clinical laboratories is considered very important as the infection does not respond to penicillin therapy. This study was made to see if there is any PPNG among the isolates from a general hospital's patients, most of whom were generally considered to be in the higher socioeconomic class.

*Received November 5, 1979*
MATERIALS AND METHODS

Materials received for the isolation of gonococci during the June 1977 to June 1979 period were mostly cervical swabs from patients of Yonsei Medical Center. Most of the specimens were received in Stuart transport medium tubes and Thayer-Martin medium plates were used for the isolation of gonococci (Thayer and Martin, 1966).

Identification was based on gram staining reaction and morphology, oxidase reaction and nongrowth carbohydrate degradation test (Kellogg et al., 1976).

\( \beta \)-lactamase production was tested by the rapid iodometric method (Thornsberry et al., 1977).

The method of Hodge et al. (1978) was added after January 1979. To aid the detection, most of the cultures were also tested for their inhibition zone produced by 10 unit penicillin disks (Thornsberry et al., 1977).

RESULTS

During the 2-year period, a total of 127 isolates were tested by the rapid iodometric method to detect \( \beta \)-lactamase producing strains. Among these, one isolate was found to be positive for the enzyme (Table 1). The isolate also gave a positive result by the method of Hodge et al. (Fig. 1). The isolate was sent to Statens Serum Institut, Copenhagen and \( \beta \)-lactamase production was confirmed by the chromogenic cephalosporin method. They also determined the susceptibility of the isolate and it was found resistant to both penicillin (MIC \( \geq \) 4.8 \( \mu \)g/ml) and tetracycline (MIC \( \geq \) 6.4 \( \mu \)g/ml) but susceptible to spectinomycin (MIC \( \leq \) 25 \( \mu \)g/ml).

Table 1. Result of \( \beta \)-lactamase test of the N. gonorrhoeae cultures by the rapid iodometric method

<table>
<thead>
<tr>
<th>No. of cultures tested</th>
<th>No. of cultures positive</th>
</tr>
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<tbody>
<tr>
<td>127</td>
<td>1 (0.78%)</td>
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</table>

Table 2. Susceptibility of N. gonorrhoeae cultures to 10 unit penicillin disk

<table>
<thead>
<tr>
<th>Zone diameter (mm)</th>
<th>No. of culture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No zone</td>
<td>1*</td>
</tr>
<tr>
<td>10–14</td>
<td>5</td>
</tr>
<tr>
<td>15–19</td>
<td>8</td>
</tr>
<tr>
<td>20–24</td>
<td>34</td>
</tr>
<tr>
<td>25–29</td>
<td>29</td>
</tr>
<tr>
<td>30–34</td>
<td>16</td>
</tr>
<tr>
<td>≥ 35</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>102 (100%)</td>
</tr>
</tbody>
</table>

* This isolate was PPNG.

Fig. 1. Positive test for \( \beta \)-lactamase production of the N. gonorrhoeae isolate by the method of Hodge et al. (T: test strain; C: Positive control).

A total of 102 isolates were also tested with 10 unit penicillin disks. Twenty-five cultures showed inhibition zone diameters of 30 mm and over, 63 cultures 20 to 29 mm and 14 cultures 19 mm and less. The PPNG show-
ed no inhibition zone at all (Table 2).

The patient who yielded the PPNG from her cervical swab was a 51-year-old housewife living in Seoul. The patient's chief complaints when first seen on April 4, 1979 were lower abdominal pain with dullness and vaginal discharge. Neither she nor her husband had ever traveled abroad.

**DISCUSSION**

Gonococcus was one of the most susceptible species of bacteria to penicillin when the drug was first available for clinical use (Brown and Lucas, 1967). However, in the 1960s, gradual decrease of the susceptibility together with frequent treatment failure were reported from many parts of the world (Reyn, 1969a; Willcox, 1970).

Reyn (1969b) reported the susceptibility of gonococci isolated in South-East Asia during the 1961–68 period and concluded that the incidence of strains with decreased susceptibility to penicillin was increasing.

One of the authors reported that 80.8% of gonococci isolated in 1966–67 from prostitutes in Chinhoe were relatively resistant to penicillin (Cho, et al., 1967). At least 81.2% of the cultures tested by Kim, et al. (1976) were shown to be resistant to 0.1 u/ml of penicillin. The susceptibility of the strains from Yonsei Medical Center during the 1970–75 showed 71.4% of them were relatively resistant to penicillin (Chong et al., 1976).

Despite of the fact that relatively penicillin resistant strains were frequently found, the highest minimum inhibitory concentration (MIC) remained at most around 2 u/ml (Reyn, 1969b; Chong et al., 1976; Kim et al., 1976). Therefore it was possible to cure most of gonorrhea with the USPHS recommended dosage of penicillin (Kaufman et al., 1976).

Until recently penicillinase production by gonococci were not known (Sparling, 1975). Then suddenly in 1976, PPNG appeared in South-East Asia first and then in many parts of the world (CDC, 1976; CDC, 1979). \( \beta \)-lactamase production of gonococci was shown to be mediated by R plasmid (Elwell et al., 1977). The significance of the gonorrhea due to PPNG lies in the fact that they do not respond to the recommended 4,800,000 units of penicillin. The MIC of penicillin for PPNG was reported as high as 50 \( \mu \)g/ml (Erg et al., 1979), which was much higher than that of the relatively resistant ones.

Increased incidence of the strains relatively resistant to penicillin was considered to be one of the factors which resulted in the increased number of cases of gonorrhea in the 1960s. Therefore it is quite natural to see concern over the emergence of PPNG. It may happen that gonorrhea becomes a more prevalent disease and penicillin becomes an ineffective drug for the infection. In the Philippines, penicillin resistant gonorrhea was known to be very common among prostitutes. It was learned that over half of 300 gonococcus cultures isolated during September 1978 to April 1979 from 358 prostitutes who did not respond to standard doses of penicillin were PPNG (Mayo, 1979).

Isolation of PPNG from US armed forces personnel in Korea raised a great worry of the possible spread of the strains into our community. This study with limited numbers of isolates showed that gonorrhea due to PPNG seemed to be rare at the moment at least among general hospital patients. However, the importance is the presence of and not necessarily the prevalence of such infection. Moreover, the patient who yielded the
organism was a housewife who along with her husband had never traveled abroad. In the future we may require a careful watch to detect the spread of such infection.

The patient was treated with ampicillin as the infection was not known to be caused by PPNG at the time of her visit. It was learned later that she did not improve and sought treatment at another clinic. There she was treated and cured with unknown antibiotics. The in vitro resistance to penicillin (IC$_{50} \geq 4.8\ \mu g/ml$) and the treatment failure with ampicillin clearly demonstrated the problem with PPNG. The results in USA showed that spectinomycin was the only drug that produced acceptable cure rates against penicillin resistant gonococci (Kaufman et al., 1976; MacCormack, 1977). Lately cefoxitin was shown to be an effective alternative to spectinomycin for single-session therapy of urethritis caused by PPNG (Berg et al., 1970).

For the treatment of gonorrhea, it was not necessary to test penicillin susceptibility of the isolate when PPNG was not known. However, the test is now considered necessary to select antibiotics in PPNG prevalent regions (Berg et al., 1979). β-lactamase testing by either the rapid iodometric or the acidimetric method is not very simple because they require large amounts of pure culture. Screening by the 10 unit penicillin disk (Thornsberry et al., 1977) and testing by the method of Hodge et al. (1978) were considered simpler for a busy clinical laboratory. The paper strip method of Jorgensen et al. (1977) seems also convenient for the purpose.

ACKNOWLEDGMENT

We thank Dr. Inga Lind, WHO Collaborating Center for Reference and Research in Gonococci; and Neisseria Department, Statens Seruminstitut, Copenhagen, Denmark, for the confirmation of β-lactamase production of our isolate.

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