Penicillin and Tetracycline Susceptibility of *Neisseria gonorrhoeae* Strains isolated during 1966 to 1975

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**ABSTRACT**

Although the decreased susceptibility of gonococci to penicillin and tetracycline is a worldwide problem in the treatment of gonorrhea, the gonococci in the West Pacific region are particularly notorious in their resistance. Using a plate dilution method, susceptibility of the gonococci isolated at this institution during 1970 to 1975 was tested to penicillin and tetracycline, which are the most widely used antibiotics for the treatment of gonorrhea. The data of this susceptibility, together with that of the strains isolated during 1966 to 1969 from prostitutes, were analyzed and herewith reported.

The range of minimum inhibitory concentration (MIC) of penicillin was 0.01 to 2.0U/ml. Among the 191 strains, 87.9% required MIC of 0.1U/ml and over, and 29.3% required 1.0U/ml and over. The range of MIC of tetracycline was from 0.125 to over 2μg/ml. Among the 120 strains, 60% required MIC of 1μg/ml and over. This in vitro evidence indicates wide prevalence of less susceptible strains which are difficult to cure with conventional doses of penicillin or with tetracycline. Comparison of the degree and the frequency of less susceptible strains by the year of isolation showed some variation, which may however have been induced by the difference of sources, rather than by the difference of time of isolation.

**INTRODUCTION**

Gonococci were one of the most susceptible organisms to penicillin in vitro. Hence the gonococcal infection was easily cured with a low dosage of penicillin when this was first available for clinical use (Brown and Lucas, 1967). However, with the increasing use of penicillin, many relatively resistant strains emerged and it became difficult to cure the infections with the conventional doses (Cradock-Watson et al., 1958; Fairbrother et al., 1961; Kjellander and Finland, 1963). Decreasing susceptibility of gonococci to penicillin and tetracycline have been observed in many parts of the world (Lucas, 1966; Reyn, 1969a) and in South-East Asia in particular (Reyn, 1969b). Despite the decreased susceptibility and the availability of newer antibiotics, penicillin and tetracycline currently remain the drugs of choice (Praiser, 1974).

It is customary not to run antibiotic susceptibility tests routinely for every isolate of gonococci, partly because of an empirical treatment based on the minimum inhibitory concentration (MIC) of gonococci eliminates...
such a necessity and partly because the disk diffusion method is not applicable for gonococci (Matson, 1974). Monitoring of the susceptibility, however, provides valuable information. Since the level of MIC is directly related to the frequency of treatment failure, it has been used as the basis of antibiotic dosage determination (Brown and Lucas, 1967).

Susceptibility of gonococci, isolated from prostitutes during 1966 to 1969, to penicillin and to tetracycline were reported by one of the authors (Cho et al., 1967; Chong and Chang, 1969). After that, some gonococci strains isolated from clinical patients in this institution each year, were tested for penicillin and tetracycline susceptibility. This result is compared with the previous one, and herewith presented.

MATERIALS AND METHODS

GC medium base (Difco), human bank blood, and Antimicrobial CNV (Difco) were used to prepare Thayer-Martin medium (Thayer and Martin, 1966). The specimens were from patients at Yonsei University Medical Center. Gonococci were isolated by inoculating swab specimens onto Thayer-Martin medium. Gram-negative diplococci that were oxidase positive and glucose fermentation positive were identified as gonococci.

Antibiotic susceptibility testing was done by an agar dilution method on antibiotic incorporated chocolate agar. Proteose peptone agar No. 3 (Difco) and human blood in 5% concentration were used to prepare chocolate agar. Solutions of sodium crystalline penicillin G (Kunwha Co.), or tetracycline hydrochloride (Chong Kun Dang Corp. or Yuhan Corp.) were incorporated into sterilized chocolate agar when it was cooled to 45°C. These chocolate plates were used on the day of preparation.

Test strains, subcultured on chocolate agar for 24 hours, were suspended in saline to make approximately the turbidity of No. 1 tube of a MacFarland nephometer. A loop of 1 mm diameter was used to inoculate the suspension. Plates were incubated in a candle jar at 36°C and the results were read after 24 hours. The MIC was defined as the lowest concentration of antibiotic inhibiting development of visible growth. The strains isolated in 1967 from prostitutes were sent to CDC, USPHS and a susceptibility test was done by them. Their data are also included in the comparison.

RESULTS

The range of MIC of penicillin of the 191 strains isolated during the period of 1966 to 1975 was 0.01 to 2.0 U/ml (Table 1, Fig. 1). The range by the year of isolation revealed 0.025 to 2.0 U in 1966-1967, 0.01 to 1.0 U in 1967, 0.1 to 2.0 U in 1968-1969, 0.1 to 1.0 U in 1970, 0.025 to 2.0 U in 1973, 0.01 to 0.5 U in 1974 and 0.025 to 1.0 U in 1975. Among these 191 strains 168 (87.9%) required 0.1 U and over of MIC, thus showing relative resistance. The proportions of relatively resistant strains were 92.3% in 1966-1967, 83.3% in 1967, 100% in 1968-1969, 100% in 1970, 80% in 1973, 73.3% in 1974 and 74.2% in 1975 (because of the difference of antibiotic concentration used to test 1966-1967 strains, adjustment of the results was made to tabulate it).

For tetracycline, 120 strains were tested during 1967 to 1975 (Table 2, Fig. 2). The lowest MIC was 0.125 and the highest over 2 µg/ml (for the 1968-1969 strains, the highest concentration used to test was 2 µg/ml). The range by year revealed 0.25 to 1.0 µg in 1967, 0.125 to over 2 µg in 1968-1969, 0.5 to 2.0 µg in 1973, 0.25 to 2.0 µg in 1974, and 0.25 to 2.0 µg in 1975. Among the 120 strains, there were
Table 1. Penicillin Susceptibility of Gonococcus Strains isolated during 1966-1975 (cumulative percent inhibited)

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>No. strains tested</th>
<th>Minimum Inhibitory Concentration (µg/ml)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>1966-1967</td>
<td>52</td>
<td>1.9</td>
</tr>
<tr>
<td>1967*</td>
<td>24</td>
<td>4.2</td>
</tr>
<tr>
<td>1968-1969</td>
<td>38</td>
<td>7.8</td>
</tr>
<tr>
<td>1970</td>
<td>16</td>
<td>23.0</td>
</tr>
<tr>
<td>1973</td>
<td>15</td>
<td>6.7</td>
</tr>
<tr>
<td>1974</td>
<td>15</td>
<td>13.3</td>
</tr>
<tr>
<td>1975</td>
<td>31</td>
<td>3.2</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* The susceptibility test was done by Dr. J.D. Thayer.

Table 2. Tetracycline Susceptibility of Gonococcus Strains isolated during 1967-1975 (cumulative percent inhibited)

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>No. strains tested</th>
<th>Minimum Inhibitory Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td>1967*</td>
<td>24</td>
<td>20.8</td>
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<tr>
<td>1968-1969</td>
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<td>1975</td>
<td>31</td>
<td>16.8</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* The susceptibility test was done by Dr. J.D. Thayer.

Fig. 1. Penicillin susceptibility of gonococcus strains.

Fig. 2. Tetracycline susceptibility of gonococcus strains.
72(60%) which were not inhibited by 0.5 μg/ml thus indicating their reduction of susceptibility. The proportion by year of the strains not inhibited by 0.5 μg/ml were 58.3% in 1967, 68.6% in 1967-1969, 60% in 1973, 66.7% in 1974, and 48.4% in 1975.

DISCUSSION

Venereal diseases are different from other infectious diseases in that they can involve any races of mankind, any age group and any social class (Brown, 1970). Among venereal diseases, gonorrhea outnumbers the others and it is known to be the first ranking reportable bacterial infection in the United States (Sencer, 1971). Marked increase of gonorrhea is reported in many parts of the world (Godden, 1973). Holmes et al. (1971) reported an increasing tendency to systemic complications of gonococcal infection in recent years.

Considering the fact that even in America, only about 10% of the cases were estimated as being reported to health authorities (Curtis, 1973), the incidences of gonorrhea in Korea would be even harder to estimate, but, its wide prevalence can be easily presumed by the great number of gonococcal isolations at general hospitals and the high rate of isolation from prostitutes (Chong and Hansen, 1968; Ministry of Health and Social Affairs, 1974).

When penicillin was first introduced, its effect was so miraculous that gonorrhea was considered to be easily controlled (King, 1958). This concept was held until a deterioration of the effect of penicillin and an increase of gonococcal infection became apparent. In 1959, Epstein reported cases of treatment failure of gonorrhea with penicillin among American military personnel in Korea. Although a treatment failure with penicillin can be attributed to several factors, the most important single one is a decreased susceptibility of gonococci (Willcox, 1970). Although the degree of susceptibility-reduction of gonococci is meager compared to that of other bacteria, it is true that those infections caused by less susceptible strains are more prone to become treatment failures (Schroeter and Pazin, 1970).

An agar dilution method of susceptibility test is reliable but too complicated for routine clinical laboratory use. It is not necessary to test every isolate of gonococci, but it is imperative to know the susceptibility of circulating strains since it may differ by the time and the area of isolation (Lucas, 1966; Stolz et al, 1974). Because of the lack of standardization of methods, there is an inherent difficulty in comparing susceptibility test results from different laboratories (Reyn et al., 1963; Willcox, 1970). Since our results are based on a limited number of strains, it may be risky to draw any conclusion out of these results alone. Moreover some strains were from prostitutes and others from patients. Therefore resistant strains may have been selected and may fail to represent all the circulating strains.

The range of MIC of penicillin of our strains isolated during 1966 to 1975 was 0.01 to 2.0 U/ml. The majority of them (87.9%) were relatively resistant, i.e., required 0.1 U/ml and over of penicillin (Table 1). Although an increasing proportion of less susceptible strains and an increasing degree of resistance have been noted for more than a decade in many parts of the world, a particular prevalence of resistant strains was noted in South-East Asia and in the Western Pacific region (Reyn, 1969b). It was shown that the range of MIC was approximately 0.06 to 5.6 U/ml in these areas. None of our strains were found to require more than 2 U/ml. Recently, decreased incidences of
resistant strains were reported in Canada and in Scandinavian countries (Reyn, 1969a; Givan and Keyl, 1974). Depending on the year of isolation, our results showed a variation in the degree of susceptibility and the proportion of resistant strains (Table 1, Fig. 1). However, it is not clear whether this indicates an actual decrease of resistant strains or merely reflects a variation induced by the difference of the source of isolation.

MIC of tetracycline of our strains isolated during 1967 to 1975 showed the lowest MIC of 0.125 and the highest of over 2.0 μg/ml, and 60% were resistant to 0.5 μg/ml (Table 2). Our strains could be considered to be similar in the degree of susceptibility to the other South-East Asian strains which were shown by Reyn (1969b) as being approximately MIC of 0.56 to 5.2 μg/ml. This resistance to tetracycline, as to penicillin, is worldwide. Particularly in the Philippines, MIC of 70% of the strains was reported to be over 1 μg/ml (Reyn, 1969b). Among our strains 60% were resistant to 0.5 μg/ml (Table 2). The proportion of strains resistant to this concentration showed minor variation during 1967 to 1974 and then some decrease in 1975. Further observation may reveal whether these figures were induced by an actual decrease of resistant strains.

The problem of treatment failure caused by the gradual increase of resistance of gonococci has been solved by increasing the antibiotic dosage. Givan and Keyl (1974) reported all of the infections due to strains with MIC of less than 1.0 U/ml, and 70% of those with MIC of 1.0 U/ml and over were cured with a single dose of 5,000,000 U aqueous procaine penicillin G. They also showed the relation of the MIC of tetracycline and the cure rate: 0.5 μg/ml, 96.5%; 1.0 μg/ml, 74%; 2.0 μg/ml and over, 52.6%. If our strains of 1966 to 1975 (Table 1) were categorized according to their method there were 29.3% of penicillin resistant strains (MIC of 1 U/ml and over), it could be speculated that a single 5,000,000 U dose of penicillin would have failed to cure 1/3 of these infections. Thin (1974) reported satisfactory results in the treatment of infections due to resistant strains when 1 g of probenecid was used with 5,000,000 U of penicillin. According to the category of Givan and Keyl, 1/3 of our strains were moderately sensitive to tetracycline (MIC of 1.0 μg/ml) and 26.7% were resistant (MIC of 2.0 μg/ml and over) (Table 2), and hence 1/3 or 1/2 of them respectively would not have been cured when 8 g of tetracycline was given in 6 days. This in vitro study clearly showed wide prevalence of gonococci strains less susceptible to penicillin and to tetracycline and gave a good reason for the frequently encountered treatment failures.

REFERENCES


Cho, S.D., Cho, S.Y. and Chong, Y.: Penicillin sensitivity of the N. gonorrhoeae cultures


