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Gonococcal Antibiotic Resistance in Los Angeles

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One hundred and thirty-five gonococcal isolates collected from Los Angeles in 1972 were studied for antibiotic susceptibility to penicillin, ampicillin, carbenicillin, tetracycline, minocycline, doxycycline and spectinomycin. Only 12 percent of the isolates were sensitive to 0.05 μg per ml of penicillin while 35 percent required at least 0.5 μg per ml for inhibition of growth. The results were slightly better with ampicillin and nearly the same with carbenicillin. Nineteen percent of the isolates required at least 1.0 μg per ml of tetracycline for inhibition of growth and the results were similar with either minocycline or doxycycline. Forty-nine percent were sensitive to 2.0 μg per ml spectinomycin, but 37 percent required at least 8.0 μg per ml for inhibition of growth.

In this study nine of eleven isolates resistant to 1.0 μg per ml of tetracycline were also resistant to both penicillin and spectinomycin. Six came from endocervical sites of female patients who contributed only 37 percent of the total number of isolates studied.

Correlation between the agar dilution and disc diffusion methods was satisfactory with penicillin, but not with ether tetracycline or spectinomycin.

THE ALARMING TREND of antibiotic resistance to Neisseria gonorrhoeae during the past quarter century has focused even more attention on a disease that has reached epidemic proportions in the United States. Since 1970 at least two million cases of gonorrhea have occurred annually. Public health authorities are now recommending 4.8 million units of penicillin plus probenecid for uncomplicated gonococcal infection. In the 1940's, a dose of 160,000 units was considered excessive. Gonorrhea largely occurs in otherwise healthy young adults and single dose parenteral penicillin or a short course of oral tetracycline has been previously successful. As gonococcal antibiotic resistance continues, conventional therapy will no longer be effective. A knowledge of the current antibiotic susceptibility patterns and the pharmacokinetics of the antimicrobials selected are important factors in controlling this disease.

It is impractical for the usual clinical laboratory to perform critical studies on every isolate presumptively identified as N. gonorrhoeae by Gram-stain and oxidase test. Fermentation and antibiotic susceptibility studies are tedious and time-consuming. Moreover, they are not quickly available to the clinician who is anxious to prescribe appropriate chemotherapy at the time the diagnosis is first made.

For this reason we are reporting the antibiotic susceptibility patterns of gonococcal isolates surveyed from the Los Angeles area in 1972.

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Materials and Methods

One hundred and thirty-five gonococcal isolates were collected from urethral and endocervical sites of patients seen in the Los Angeles County Health Clinics in the summer of 1972. Eighty-six were from males and 49 from females. The mean age of the patients was 24.5 with a range of 12 to 56 years. All isolates were confirmed as N. gonor-

rhoeae by Gram-stain, oxidase test and carbohydrate fermentation patterns using cysteine trypticase agar (CTA, Baltimore Biologic Laboratories).

The cultures were preserved for antibiotic susceptibility testing by freezing suspensions in trypticase soy broth (TSB, Baltimore Biologic Laboratories) containing 30 percent agamma horse serum. The isolates were revived by planting thawed suspensions on dextrose starch agar slants (DSA, Baltimore Biologic Laboratories). After incubation for 18 to 24 hours in a 6 percent CO_2 atmosphere at 35°C (95°F), the isolates were resuspended in TSB to give an inoculum of 10^8 colony forming units per ml broth. Barium sulfate standards and absolute colony counts were done to insure accuracy of the inoculum size.

For the agar dilution studies, a 10^{-3} dilution of each isolate was pipetted into the wells of Steers' replicating device. Aliquots were planted on the surface of a DSA pour plate containing the antibiotic to be tested. All antibiotic standards and plates were prepared daily for each experiment. For the disc diffusion studies a 10^{-1} dilution of the isolate was streaked, using a cotton swab on the surface of a Mueller Hinton plate (Baltimore Biologic Laboratories).

Antibiotic standard powders were provided by Bristol Laboratories (penicillin G and sodium ampicillin), Roerig Laboratories (sodium carbenicillin), Lederle Laboratories (tetracycline hydrochloride and minocycline), Pfizer Laboratories (doxycycline) and Upjohn Laboratories (spectinomycin hydrochloride). The dilutions for the penicillins were 3.0, 1.5, 1.0, 0.75, 0.5, 0.25, 0.1, and 0.05 μg per ml. The dilutions for the tetracyclines were 6.0, 4.0, 2.0, 1.0, 0.5 and 0.25 μg per ml. For spectinomycin the dilutions were 16.0, 12.0, 8.0, 6.0, 4.0 and 2.0 μg per ml.

All plates were incubated in a 6 percent CO_2 atmosphere at 35°C (95°F) and read at 24 and 48 hours. No significant changes in the readings were noted. The lowest concentration of antibiotic inhibiting all visible growth constituted the minimal inhibitory concentration (MIC) for the isolate. Control isolates of known susceptibility to penicillin and tetracycline (provided by Center for Disease Control, Venereal Disease Branch, Atlanta, Georgia) were tested with each experiment and always correlated within one dilution of the known value.

For the disc diffusion study, penicillin 2 unit (Baltimore Biologic Laboratories), tetracycline 5 μg (Baltimore Biologic Laboratories) and spectinomycin 30 μg (Upjohn Laboratories) dics were used. All three discs were planted on MH plates containing each isolate to be tested. After incubation in a 6 percent CO_2 atmosphere at 35°C (95°F) the plates were examined at 24 and 48 hours. A steel caliper was used to measure the diameter of zone inhibition. No changes between the readings at 24 and 48 hours were noted.

TABLE 1.—*Comparative Susceptibility of 135 Gonococcal Isolates to Penicillins, Tetracyclines and Spectinomycin*

Antibiotic	*Minimal Inhibitory Concentrations (μg/ml)*						
	0.05	0.1	0.25	0.5	0.75	1.0	>1.0
Penicillin G ...	16	22	50	25	21	1	0
Ampicillin	13	16	97	7	2	0	0
Carbenicillin ...	9	4	14	30	63	8	7
	0.25	0.5	1.0	2.0	4.0	6.0	>6.0
Tetracycline	91	19	14	8	1	0	2
Minocycline	98	19	14	2	1	0	1
Doxycycline	78	22	19	9	5	2	0
	2.0	4.0	6.0	8.0	12.0	16.0	>16.0
Spectinomycin ..	66	11	8	7	35	5	3

Results

Overall Antibiotic Susceptibility Patterns

The susceptibility of the 135 gonococcal isolates to the antibiotic agents tested is illustrated in Table 1. For purpose of discussion, isolates with MICs of 0.5 μg per ml or greater to penicillin, 1.0 μg per ml or greater to tetracycline, and 8.0 μg per ml or greater to spectinomycin were considered insensitive or relatively resistant to the respective antibiotics.

Only 12 percent of the isolates were sensitive to 0.05 μg per ml of penicillin, while 3.5 percent were relatively resistant since their MICs were 0.5 μg per ml or greater of penicillin. Sixty-five percent were susceptible to 0.25 μg per ml or less of

TABLE 2.—*Susceptibility of Eleven Tetracycline-Resistant Isolates to Penicillin and Spectinomycin*

Id. No.	*Sex*	*Site*	*Minimal Inhibitory Concentrations (μg/ml)*		
			Tetracycline	*Penicillin*	*Spectinomycin*
1	♀	Cervix	2.0	0.5	*4.0*
2	♀	Cervix	2.0	0.5	8.0
3	♀	Cervix	2.0	0.5	8.0
4	♂	Urethra	2.0	0.5	12.0
5	♀	Cervix	2.0	0.5	12.0
6	♂	Urethra	2.0	0.5	20.0
7	♀	Cervix	2.0	0.75	12.0
8	♀	Cervix	2.0	0.75	12.0
9	♀	Cervix	4.0	0.75	12.0
10	♂	Urethra	>6.0	*0.25*	>20.0
11	♂	Urethra	>6.0	0.5	>20.0

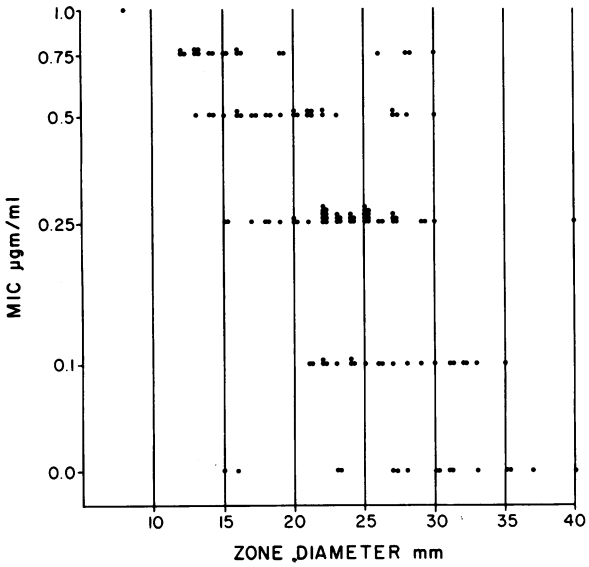

Chart 1.—Comparison of penicillin agar dilution with disc diffusion for 135 isolates of Neisseria gonorrhoeae.

penicillin, 93 percent to the same concentration of ampicillin and 42 percent to carbenicillin. Sixty-seven percent of the isolates were sensitive to 0.25 µg per ml or less of tetracycline and 19 percent required 1.0 µg per ml of tetracycline for inhibition of growth. The results were nearly identical with minocycline and doxycycline. While 49 percent of the isolates were sensitive to 2.0 µg per ml of spectinomycin, 37 percent required at least 8.0 µg per ml for inhibition of growth.

Multiple-drug Resistant Isolates

Seventy-two percent of the isolates with MICs to tetracycline of 1.0 µg per ml or greater had MICs of 0.5 µ per ml or higher to penicillin and to at least 8 µg per ml of spectinomycin. Only four of the fourteen isolates with MICs to tetracycline of 1.0 µg per ml were resistant to both penicillin and spectinomycin. However, of the 11 isolates with MICs to tetracycline of 2.0 µg per ml or greater, nine showed resistance to both (Table 2). Six of these multiple antibiotic-resistant isolates were obtained from endocervical sites of female patients who provided only 36 percent of the total number of isolates collected.

Comparison of Agar Dilution with Disc Diffusion Studies

A regression curve comparing penicillin agar-dilution MIC to the penicillin 2 unit disc-diffusion zone diameter for the isolates is shown in Chart 1. Of 48 isolates with MICs equal to or greater than 0.5 µg per ml, 32 (66 percent) had zones measuring 20 mm or less. Of 87 isolates with MICs equal to or less than 0.25 µg per ml, 76 (87 percent) had zone diameters greater than 20 mm. Of the total 43 isolates with zones 20 mm or less, 32 (74 percent) had MICs to penicillin of 0.5 µg per ml or greater. Of the total 92 isolates with zones greater than 20 mm, 76 (87 percent) had MICs equal to or less than 0.25 µg per ml of penicillin.

Satisfactory correlation did not occur between agar dilution and disc-diffusion results for tetracycline and spectinomycin. Isolates resistant by agar dilution had variable zones of inhibition by disc diffusion testing. For example, only 56 percent of the tetracycline-resistant isolates had low zones of inhibition. The results were even less impressive with spectinomycin.

Discussion

The MICs to penicillin for the gonococcal isolates surveyed in this study were higher than Martin[1] reported in his study of isolates from other metropolitan centers in 1968-69. In that study 65 percent of the isolates had MICs greater than 0.05 units per ml and 15 percent were higher than 0.5 units per ml. In our study 88 percent had MICs higher than 0.08 units per ml (0.05 µg per ml) and 35 percent had MICs higher than 0.4 units per ml (0.25 µg per ml).

The trend toward increasing resistance of gonococci to tetracycline, noted in other studies,[2-4] was also observed with our isolates. Nineteen percent had MICs to tetracycline of 1.0 µg per ml or higher. There were no significant differences among the tetracycline analogues minocycline and doxycycline. The range of susceptibility of the isolates to spectinomycin was similar to that noted by Duncan[5] and Pederson.[6] Thirty-seven percent required at least 8.0 µg per ml for inhibition of growth. How this pattern will be affected after more widespread use of spectinomycin is uncertain. Gonococcal isolates became rapidly resistant to streptomycin following its early effectiveness in treating gonorrhea in the 1940's.

We were favorably impressed with the usefulness of penicillin discs for predicting penicillin-resistant isolates. This has also been noted by other investigators.[7,8] Problems arose, however, in interpreting the tetracycline and spectinomycin disc diffusion data. It is not apparent to us why this was so. Reyn[7] has also reported discrepancies between agar dilution and disc diffusion with tet-

racycline and streptomycin. While penicillin disc testing may be a worthwhile procedure for laboratories supporting venereal disease clinics, we would hesitate to recommend it for laboratories rarely encountering gonococci. Simultaneous agar dilution studies must be done initially and checks from time to time are necessary to insure continued accuracy of the disc method.

Antibiotic resistance is a relative term for the gonococcal isolates reported in this survey, since all were inhibited by penicillin and nearly all by tetracycline and spectinomycin in the concentrations tested. However, it has clearly been shown that isolates susceptible only at the higher ranges tested are associated with more frequent therapy failures.[1,3,5,6,9] Ideally, an antigonorrheal agent should provide a serum concentration that is at least 5 to 10 times the MIC for the isolate.[9,10] Considering the variability of absorption and clearance of antimicrobials, this is often not achieved. However, since intramuscular injection of comparable doses of ampicillin[11] and carbenicillin[12] provide higher serum levels than procaine penicillin, they may be more effective agents since their *in vitro* activity against gonococci is similar to penicillin. Excellent results have been reported in field trials with either of these parenteral preparations.[11,13] Furthermore, the use of 3.5 grams of ampicillin, given in a single oral dose, preceded by one hour with 1.0 gram oral probenecid, has proven highly effective for the treatment of gonococcal urethritis in the Philippines where there is a high prevalence of penicillin-resistant gonococci.[14] This treatment was generally well tolerated by the patients. Less than 2 percent of them had significant gastrointestinal distress and no cases of rash were observed. These semi-synthetic penicillins deserve further clinical evaluation since the administration of greater than 4.8 megaunits procaine penicillin intramuscularly is likely to be painful and may not achieve reliable blood levels.

Despite the fact that tetracyclines are widely used for the treatment of gonorrhea, particularly by private physicians, there are significant disadvantages. First of all, single dose oral therapy can no longer be considered effective.[4,15] Success with multiple dose treatment depends on the reliability of patients to continue to take their medicine despite symptomatic improvement. Secondly, viable gonococci persist in secretions for longer periods increasing the risk of transmission while the patient is on treatment.[16] Lastly, there is a significant cost factor, since the newer tetracyclines, doxycycline and minocycline, are currently more expensive than the recommended doses of tetracycline hydrochloride or procaine penicillin with probenecid.

The multiple drug-resistant patterns of gonococci seen in this study have been noted elsewhere.[7,8,17,18] Why cross-resistance occurs among biologically and chemically unrelated antimicrobials is unknown. Conditioning of heterogeneous clones of gonococci by widespread use of antimicrobials appears to be the most significant factor in stimulating resistance. Sparling[4] has proposed that certain agents provoke a resistance mechanism that blocks their entry into the gonococcal cell. High level resistance may be transmitted to susceptible cells by genetic factors. It is disturbing to consider what the alternatives for therapy will be if the trend of multiple-drug resistance continues. Perhaps in the future we will understand the mechanisms more fully. Meanwhile, it remains important for the clinician to be accurately informed on the trends of antibiotic resistance so that he can prescribe the most effective therapy currently available.

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