

## Susceptibility to Cephalosporins of Penicillin-Susceptible and Penicillin-Resistant Strains of *Neisseria gonorrhoeae* from Philadelphia

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Using agar dilution, we determined MICs of penicillin, cefoxitin, ceftriaxone, cefmetazole, tetracycline, and spectinomycin for 129 strains of *Neisseria gonorrhoeae*. All strains were susceptible to ceftriaxone (MIC range,  $\leq 0.008$  to  $0.06 \mu\text{g/ml}$ ) and spectinomycin ( $16$  to  $32 \mu\text{g/ml}$ ). The MICs for 50, 90, and 100% of strains tested were  $1.0$ ,  $2.0$ , and  $>8.0 \mu\text{g/ml}$ ;  $0.12$ ,  $1.0$ , and  $>8.0 \mu\text{g/ml}$ ;  $0.5$ ,  $1.0$ , and  $2.0 \mu\text{g/ml}$ ; and  $1.0$ ,  $2.0$ , and  $>8.0 \mu\text{g/ml}$  for cefmetazole, penicillin, cefoxitin, and tetracycline, respectively. Seven strains were  $\beta$ -lactamase producers; eight were chromosomally resistant to penicillin. There was a log-linear relation for non- $\beta$ -lactamase-producing strains between the MICs of cefmetazole, cefoxitin, and tetracycline and the MIC of penicillin (Pearson  $r = 0.787$ ,  $0.544$ , and  $0.358$ , respectively).

The treatment strategy for presumed or microbiologically documented gonorrheal infection has changed in the light of new information regarding the susceptibility of *Neisseria gonorrhoeae* to penicillin and tetracycline (6). Most laboratories do not test each strain for antimicrobial susceptibilities; thus clinicians rely on epidemiologic data from regional or national authorities to guide therapy. Cephalosporins such as cefoxitin and ceftriaxone have been widely used as primary therapy for gonorrhea. The purpose of our study was to determine the susceptibility patterns of *N. gonorrhoeae*, collected from men seen at clinics in two urban Philadelphia neighborhoods, to five drugs commonly used in the treatment of gonorrhea: penicillin, tetracycline, spectinomycin, cefoxitin, and ceftriaxone. We also tested the activity of cefmetazole, a novel cephalosporin being used in clinical trials, as chemotherapy for gonorrhea. We determined the incidence of penicillin resistance due to  $\beta$ -lactamase production, and we examined the relation between susceptibility to penicillin and susceptibility to other antibiotics among non- $\beta$ -lactamase-producing strains.

### MATERIALS AND METHODS

Between March and October 1987, isolates of *N. gonorrhoeae* were collected from men seen at Episcopal Hospital and women seen at Temple University Hospital, both in north Philadelphia. The isolates were identified in the clinical microbiology laboratories by growth on Thayer-Martin agar, Gram stain appearance, oxidase activity, and coagglutination with monoclonal antibodies (Phadebact Monoclonal GC Omni Test; Pharmacia Diagnostics, Piscataway, N.J.) or by carbohydrate utilization (Fastidious Fermenters Duo Tubes and Trio Tubes; Carr-Scarborough Microbiologicals, Inc., Decatur, Ga.). Isolates were frozen at  $-70^\circ\text{C}$  in 1-ml samples of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 20% glycerol until they were thawed for batch susceptibility testing. The control strain was a laboratory isolate whose susceptibilities to penicillin and tetracycline were determined by agar dilution in a previous experiment (data not published).  $\beta$ -Lactamase activity was

determined by the chromogenic cephalosporin assay (Cefinase; BBL).

Antimicrobial susceptibility testing was performed by the agar dilution method in accordance with guidelines of the National Committee on Clinical Laboratory Standards (10). The medium used was Proteose no. 3 agar (Difco Laboratories, Detroit, Mich.) supplemented with hemoglobin (BBL) and IsoVitaleX (BBL) enrichment to achieve a final concentration of 1% for each supplement. The frozen isolates were thawed at room temperature and subcultured twice onto chocolate agar before susceptibility testing was done. The inoculum was prepared in Trypticase soy broth from a 24-h culture on chocolate agar. The final inoculum was  $10^4$  CFU, and the inoculated plates were incubated in 5%  $\text{CO}_2$  for 24 h at  $35^\circ\text{C}$ . The MIC was defined as that concentration of antibiotic which permitted no bacterial growth. A single colony or a fine haze was considered no growth.

Antibiotic reference powders of penicillin and tetracycline were purchased from Sigma Chemical Co., St. Louis, Mo. The other reference powders were provided by their manufacturers: cefoxitin (Merck Sharp & Dohme, West Point, Pa.), ceftriaxone (Hoffmann-LaRoche Inc., Nutley, N.J.), and cefmetazole and spectinomycin (both from The Upjohn Co., Kalamazoo, Mich.).

The influence of culture site and gender on antibiotic susceptibility was assessed by one-factor analysis of variance using  $\log_2$  MICs. Log MICs of cefmetazole, cefoxitin, and tetracycline were compared with those of penicillin by using the Pearson correlation coefficient, linear regression, and analysis of variance. Parametric regression assumptions and the propriety of linear modeling were examined by using plots of standardized residuals (7). The logarithmic scale was chosen to avoid giving undue statistical weight to strains with high MICs.

### RESULTS

We collected 129 unique *N. gonorrhoeae* strains from 128 patients; 100 were from men who attended a clinic for sexually transmitted diseases in a poor, urban neighborhood in Philadelphia, and 29 were from women who attended a family planning and gynecology clinic in another poor neigh-

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TABLE 1. MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of test antibiotics for all strains of *N. gonorrhoeae*

Antibiotic	MIC (µg/ml) <sup>a</sup>		
	Range	50%	90%
Penicillin	0.015-16.0	0.12	1.0
Cefmetazole	0.06-16.0	1.0	2.0
Cefoxitin	0.12-2.0	0.5	1.0
Ceftriaxone	≤0.008-0.06	≤0.008	≤0.008
Spectinomycin	16.0-32.0	16.0	32.0
Tetracycline	0.12-16.0	1.0	2.0

<sup>a</sup> 50% and 90%, MIC for 50 and 90% of strains, respectively.

borhood nearby. Among the men, 91 strains were cultured from the urethra, 5 from the urethra and pharynx, 1 from the pharynx, and 3 from the rectum. Among the women, 11 strains were cultured from the cervix alone, 12 from the cervix and the rectum, 4 from the rectum alone, and 2 from the cervix and pharynx. When multiple sites were infected or colonized in the same patient, the MICs for all the strains were determined. With the exception of a man in whom different strains were found in the urethra and pharynx, the MICs for all strains from multiple sites were identical, and only one isolate from each patient was used for further analysis. There were no significant differences among isolates obtained from men or women or from different body sites.

The MIC range and the MICs for 50 and 90% of the strains (MIC<sub>50</sub> and MIC<sub>90</sub>) for each antimicrobial agent are shown in Table 1. Ceftriaxone was the most active of the antibiotics tested for all strains, with an MIC<sub>90</sub> of ≤0.008 µg/ml. Spectinomycin was essentially equally active for all strains, and no strains showed resistance to it. Cefoxitin was more active than cefmetazole, though both showed good activity. Cefoxitin and cefmetazole had similar MIC<sub>50</sub>s (<1.0 µg/ml) for β-lactamase-producing and penicillin-susceptible strains. Penicillin had the widest range of activity, with MICs of 0.015 to >8 µg/ml. The geometric mean MICs for the 15 penicillin-resistant strains are listed in Table 2. The strains with a penicillin MIC of 1 or 2 µg/ml were defined as chromosomally resistant. The MICs of cefoxitin, cefmetazole, and tetracycline were higher for these strains than for those with a penicillin MIC of <1 or >8 µg/ml. There was substantial low-level resistance to tetracycline, and a few strains had tetracycline MICs of >8 µg/ml, suggesting a plasmid-mediated mechanism of resistance.

The logarithmic relation of penicillin MICs to cefmetazole, cefoxitin, and tetracycline MICs for all non-β-lactamase

TABLE 2. Geometric means MICs for penicillin-susceptible and penicillin-resistant strains of *N. gonorrhoeae*

Antibiotic	MIC (µg/ml) for:		
	Penicillin-susceptible strains (n = 114)	CMR strains (n = 8)	Penicillinase-producing strains (n = 7)
Penicillin	0.14	1.2	16.0
Cefmetazole	0.76	7.3	0.67
Cefoxitin	0.41	1.1	0.45
Ceftriaxone	0.008	0.009	0.008
Spectinomycin	24.0	23.0	29.0
Tetracycline	0.65	3.4	1.2

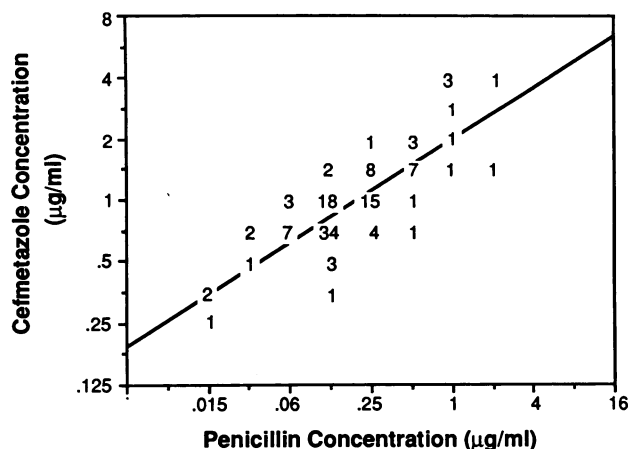


FIG. 1. Relation between penicillin MICs and cefmetazole MICs for 122 non-β-lactamase strains of *N. gonorrhoeae*. Each numeral along the regression line shows the number of strains at that position. The line is computed from the Pearson linear regression equation.

producers is shown in Fig. 1 to 3. There was a significant log-linear association between cefmetazole susceptibility and penicillin susceptibility (Pearson  $r = 0.787$ ,  $P < 0.001$ ). There was a log-linear association of lesser magnitude between cefoxitin and penicillin ( $r = 0.544$ ,  $P < 0.001$ ). For tetracycline and penicillin the correlation coefficient was 0.358 ( $P < 0.001$ ), suggesting a real, but weak, association. To confirm the propriety of linear parametric analysis, we constructed plots of standardized residuals (data not shown). These plots showed that a line was, in fact, an appropriate representation for the correlation of the MICs of various antibiotics to those of penicillin.

DISCUSSION

Public health authorities recommend treatments for gonorrhoea which give a greater than 95% chance of cure (2). Until 1976, increasing doses of short-acting penicillin compensated for decreasing susceptibility to penicillin (5). But the emergence of a plasmid-mediated β-lactamase enzyme in *N. gonorrhoeae* required the use of regimens which did not

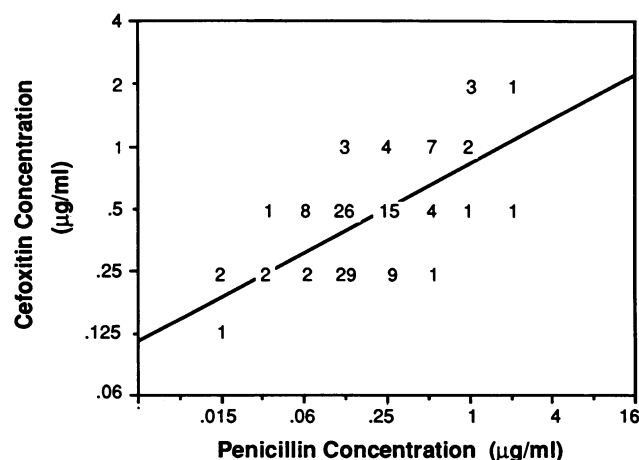


FIG. 2. Relation between penicillin MICs and cefoxitin MICs. Details about the regression line and datum points are presented in the legend to Fig. 1.

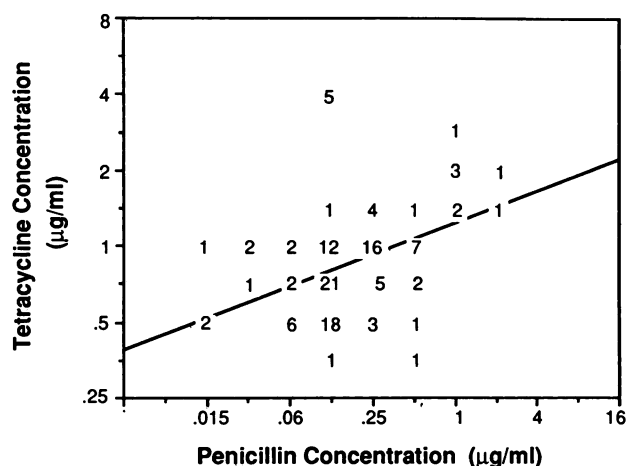


FIG. 3. Relation between penicillin MICs and tetracycline MICs. Details about the regression line and datum points are presented in the legend to Fig. 1.

rely on penicillin (2). Increased resistance to tetracycline (8) and spectinomycin (13) has made these regimens less attractive.  $\beta$ -Lactamase-resistant cephalosporins such as cefoxitin (1) and ceftriaxone (11) were found to be effective in penicillinase-producing *N. gonorrhoeae* infections.

Because of the fastidious growth requirements of *N. gonorrhoeae*, the National Committee on Clinical Laboratory Standards has recommended either of two supplemented test media for MIC determination (10). However, there are discrepancies between these media in MIC results for *N. gonorrhoeae*. There is a medium-dependent shift of 1 or 2 dilutions in the MICs of tetracycline (3). Since the breakpoint for intermediate tetracycline resistance is near the median MIC, such a shift may have a large impact on the frequency of resistance.

In addition to resistance of *N. gonorrhoeae* to penicillin due to  $\beta$ -lactamase production, there is chromosomally mediated penicillin resistance (CMR) of *N. gonorrhoeae*, defined by a penicillin MIC of  $\geq 1$   $\mu\text{g/ml}$  (12). This resistance arises from a series of mutations in the penicillin-binding proteins of *N. gonorrhoeae* which results in a decreased affinity for penicillin as a substrate (4). Although our CMR strains were susceptible to spectinomycin and ceftriaxone, some  $\beta$ -lactamase-stable cephalosporin or cephamycin antibiotics were less potent against these strains than against penicillinase-producing or penicillin-susceptible strains.

It has been shown that for strains of *N. gonorrhoeae* which were less penicillin susceptible, MICs of some antibiotics (e.g., doxycycline and chloramphenicol) and drugs (e.g., acridine orange and ethidium bromide) were higher than for more penicillin-susceptible strains (9). These relations were log linear. We also found that a log-linear increase in resistance to penicillin in our isolates correlated with increased resistance to cefmetazole, whether the MICs were above or below any breakpoint used to define resistance. This correlation may reflect penicillin-binding protein or outer membrane differences among strains which affect penicillin and cefmetazole susceptibilities in a parallel manner. In our 1987 study population, CMR strains and penicillinase-producing strains each made up about 6% of all

isolates. CMR strains of *N. gonorrhoeae*—also the most cefoxitin and tetracycline resistant—were not distinguished from penicillin-susceptible strains by the clinical laboratories which isolated them. Since it is not known whether the pattern of mutations which led to CMR could lead to increased resistance to very potent  $\beta$ -lactams (such as ceftriaxone),  $\beta$ -lactam resistance among *N. gonorrhoeae* bears close scrutiny.

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