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Comparative Susceptibility of Penicillinase-Positive and -Negative *Neisseria gonorrhoeae* to 30 Antibiotics

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The minimal inhibitory concentrations of 30 antibiotics were determined by the agar dilution method for 17 penicillinase-positive and 50 penicillinase-negative strains of *Neisseria gonorrhoeae*. The latter included 42 strains that were penicillin susceptible (pen S) (minimal inhibitory concentration, <0.125 μg/ml) and 8 strains with intermediate resistance to penicillin (pen I; minimal inhibitory concentration, 0.125 to 0.5 μg/ml). Two penicillinase-resistant penicillins (methicillin and nafcillin) were inhibitory for penicillin-resistant (pen R) strains. Three new cephalosporins (cefuroxime, cefamandole, cefaclor) and a cephamycin (cefoxitin) were bacteriostatic (minimal inhibitory concentration ≤0.8 μg/ml) for 90% of pen S, pen I, and pen R strains. Pen I strains were more resistant than pen R strains to 6 of 13 cephalosporins. Rifampin, erythromycin, spectinomycin, chloramphenicol, and the tetracyclines were inhibitory for both pen S and pen R strains. The minimal bactericidal concentrations of cefuroxime, cefamandole, cefaclor, and cefoxitin were measured for 17 pen R strains and eight pen I strains by serial dilution of the antibiotics in Trypticase soy broth supplemented with 1% IsoVitaleX and 1% hemoglobin. All tubes were subcultured after overnight incubation at 37°C. Cefuroxime and cefoxitin were bactericidal at low concentrations (minimal bactericidal concentration, ≤1.0 μg/ml) for 16 of 17 pen R strains and 6 of 8 pen I strains.

In the United States before 1973 as many as 10 to 20% of clinical isolates from cases of anogenital gonorrhea were relatively resistant to benzylpenicillin (minimal inhibitory concentration [MIC], ≤0.125 μg/ml), but they lacked penicillinase activity (13). Beginning in 1976 penicillinase-positive strains of *Neisseria gonorrhoeae* highly resistant to benzylpenicillin were found in Southeast Asia and Africa and, subsequently, in England, continental Europe, and the United States (13). If there is an increase in the as-yet-infrequent occurrence of these strains, some serious problems may arise in the treatment of gonococcal infections. From September 1976 through June 1977 the Center for Disease Control recorded 191 cases of infection caused by penicillinase-producing *N. gonorrhoeae* in the United States (13). In vitro antibiotic susceptibility tests by the agar plate dilution method were done on more than 130 of these strains with five antibiotics (benzylpenicillin, ampicillin, tetracycline, erythromycin, spectinomycin). Penicillinase-producing *N. gonorrhoeae* strains were significantly more resistant to all of these antibiotics, except for spectinomycin, than were penicillinase-negative strains of *N. gonorrhoeae* collected in the Unites States in 1974 and 1975.

The present study compares the antibiotic susceptibility patterns of 17 penicillinase-positive (penicillin-resistant [pen R]) strains of *N. gonorrhoeae* from various laboratories in the United States and England with 50 penicillinase-negative (penicillin-susceptible [pen S]) strains, the latter including 8 strains with intermediate resistance to penicillin (pen I) (MIC, 0.125 to 0.5 μg/ml). We compared the MICs of 30 antibiotics, including 10 penicillins, 13 cephalosporins, 3 tetracyclines, spectinomycin, erythromycin, chloramphenicol, and rifampin. We also determined the minimal bactericidal concentrations (MBC) of four new cephalosporins for 17 pen R and 8 pen I strains of *N. gonorrhoeae* by using a new bactericidal test in a fortified Trypticase soy broth which supported rapid growth of gonococci without autolysis.

MATERIALS AND METHODS

Organisms. A total of 67 strains of *N. gonorrhoeae* were tested. Fifty penicillinase-negative strains were collected in August and September 1977 at the Minnesota Department of Health, Minneapolis. Seventeen

penicillinase-producing strains were obtained from the following sources: L. Damsky, Minnesota Department of Health, Minneapolis; B. I. Eisenstein, School of Medicine, University of North Carolina, Chapel Hill; S. Falkow, School of Medicine, University of Washington, Seattle; W. A. Ashford, Travis Air Force Base, Calif.; A. Percival, Department of Medical Microbiology, New Medical School, Liverpool, England; J. Swanson, College of Medicine, University of Utah, Salt Lake City; and A. E. Wilkinson, Venereal Diseases Reference Laboratory, The London Hospital, London, England. The identity of the isolates was confirmed by growth on Thayer-Martin agar, Gram stain, positive oxidase reaction, and acidification of glucose but not maltose, lactose, or sucrose. The organisms were frozen in Trypticase soy broth (Difco) containing 20% (vol/vol) glycerol and stored at −90°C.

Antibiotics. Reference standard antibiotic powders were provided by the manufacturers. The powders were weighed, dissolved in the appropriate solvents (1), and diluted in sterile distilled water to a final concentration of 2,560 IU/ml (benzylpenicillin, potassium salt, and phenoxymethyl penicillin, potassium salt) or 2,560 μg/ml (all other antibiotics). The antibiotic solutions were stored at −20°C for no longer than 1 month.

MIC. Agar dilution susceptibility tests were performed by using Mueller-Hinton agar (Difco) supplemented with 1% (wt/vol) hemoglobin (Difco) and 1% (vol/vol) IsoVitaleX (Baltimore Biological Laboratory) (1, 14, 16, 18). Serial twofold dilutions of freshly thawed antibiotic solutions were made in sterile distilled water, and molten (56°C) agar was added. After mixing, the antibiotic media (20 ml) were poured into plastic petri dishes (15 by 100 mm) and allowed to harden. The assay plates were stored at 4°C for no longer than 1 week, except for amoxicillin-, ampicillin-, cefaclor-, and methicillin-containing plates, which were used within 24 h. Before use the refrigerated plates were warmed to room temperature (1 h) and dried in a 35°C incubator (1 h) with the lids ajar. A total of 17 antibiotic concentrations were tested. Expressed in international units per milliliter (benzylpenicillin, potassium salt, and phenoxymethyl penicillin, potassium salt) or in micrograms per milliliter (all others), these concentrations ranged from 0.002 through 128. In addition, an antibiotic-free control was included for each antibiotic tested.

The frozen gonococcal isolates were thawed at 37°C and inoculated onto plates of freshly prepared chocolate agar. The plates were then incubated for 18 to 24 h at 35°C in a candle extinction jar. Penicillin resistance of penicillinase-producing strains was assured by selecting colonies which grew near a 10-IU penicillin disk (Difco) placed on the plate. Several colonies were suspended in Trypticase soy broth until the turbidity matched that of a 0.5 McFarland standard. This suspension was diluted 1:20 in Trypticase soy broth and spot inoculated onto the assay plates with a Steers replicator (15). Each spot received approximately 10^3 viable units, as determined by plate counts on the adjusted inoculum (1). The spots were allowed to dry, and the plates were incubated for 18 to 24 h. The MIC was the lowest concentration of antibiotic that inhibited growth. Presence of a single colony was ignored. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included with each set of plates to control technical variables.

Disk diffusion tests. Disk diffusion susceptibility tests were performed on most of the isolates with benzylpenicillin by using a modification of the Bauer-Kirby technique (2). Assay plates (Diagnostics, Inc., Minneapolis, Minn.) contained Mueller-Hinton agar supplemented with 1% (wt/vol) hemoglobin and 1% (vol/vol) IsoVitaleX and met the conditions for quality assurance as outlined by the National Committee for Clinical Laboratory Standards (9). Several colonies of *N. gonorrhoeae* were picked from overnight growth on chocolate agar plates and suspended in Trypticase soy broth until the turbidity matched a 0.5 McFarland standard. This suspension was then streaked on assay plates. A 10-IU penicillin disk (Difco) was applied, and the plates were incubated at 35°C in a candle extinction jar for 18 to 24 h. The diameters of growth inhibition zones were measured to the nearest millimeter with calipers.

MBC. MBC determinations for cefaclor, cefamandole, cefoxitin, and cefuroxime were performed by a twofold broth dilution method (1), using Trypticase soy broth supplemented with 1% (wt/vol) hemoglobin and 1% (vol/vol) IsoVitaleX. The doubling time of *N. gonorrhoeae* in this medium was approximately 2 h, as determined by plate counts. The tubes of serially diluted antibiotics and the control tubes were inoculated with an equal volume of cell suspension adjusted to 10^5 viable units per ml and confirmed by plate counts. The tubes were incubated for 18 to 24 h in a candle extinction jar; 0.01 ml from each tube was then subcultured with a calibrated loop onto freshly prepared chocolate agar plates. The MBC was the lowest concentration of antibiotic which yielded no more than one colony on subculture after 18 to 24 h of incubation.

Beta-lactamase activity. Rapid tests for β-lactamase were performed by an acidimetric method with phenol red indicator (3), by an iodometric method (6), and by using a chromogenic cephalosporin substrate (10). A penicillinase-producing strain of *S. aureus* was used as a positive control for β-lactamase activity. Pen S strains of *N. gonorrhoeae* served as negative controls. We included only those pen R strains which were positive by all three tests for β-lactamase activity. The chromogenic cephalosporin gave the most clear-cut and quickest results.

RESULTS

MIC. The agar dilution MIC range concentrations required to inhibit 50 and 90% of the strains for 30 antibiotics and 50 pen S and 17 pen R strains of *N. gonorrhoeae* are shown in Table 1. The concentrations required to inhibit 50 and 90% of the strains for each antibiotic were calculated by probit analysis using log-probability paper. The ratio of the concentration required to inhibit 90% of the pen R strains to the concentration required to inhibit 90% of the pen S strains is also presented. The attainable peak serum level after the usual dose given orally or

TABLE 1. *Comparative susceptibilities of penicillinase-negative and positive strains of N. gonorrhoeae to each of 30 antimicrobial drugs*

Antibiotic	Penicillinase-negative strains (n = 50)[a]			Penicillinase-positive strains (n = 17)[a]			Ratio of MIC_{90} for penicillinase-positive strains to MIC_{90} for penicillinase-negative strains	Attainable serum level[b] (μg/ml)
	MIC range (μg/ml)	MIC_{50} (μg/ml)[c]	MIC_{90} (μg/ml)[c]	MIC range (μg/ml)	MIC_{50} (μg/ml)[c]	MIC_{90} (μg/ml)[c]		
Benzylpenicillin, potassium salt	0.004–0.5	0.03	0.15	0.25–64.0	8.0	46.0	306	12.0
Phenoxymethyl penicillin, potassium salt	0.015–8.0	0.23	2.1	4.0–128.0	16.0	51.2	24	4.0
Ampicillin, sodium salt	0.008–0.25	0.04	0.1	4.0–128.0	12.0	69.0	690	3.9
Amoxicillin trihydrate	0.015–0.5	0.06	0.35	4.0–128.0	20.8	128.0	366	20.6
Carbenicillin, disodium salt	0.008–1.0	0.08	1.0	4.0–16.0	5.8	9.6	9.6	160.0
Methicillin, sodium salt	0.015–4.0	0.3	3.4	1.0–2.0	1.3	1.6	0.5	32.5
Oxacillin, sodium salt	0.125–32.0	1.2	8.3	8.0–32.0	12.8	19.2	2.3	9.7
Cloxacillin	0.125–16.0	1.0	12.8	1.0–8.0	2.4	4.8	0.4	11.6
Dicloxacillin	0.06–32.0	2.0	20.0	0.5–8.0	2.0	4.2	0.2	9.5
Nafcillin	0.06–16.0	1.25	10.4	2.0–8.0	2.5	3.4	0.3	9.0
Cephalothin, sodium salt	0.03–8.0	0.3	1.4	0.25–1.0	0.35	0.7	0.5	17.6
Cephaloridine	1.0–16.0	2.1	5.8	4.0–>128.0	12.8	41.6	7.2	17.5
Cefaclor	0.015–2.0	0.09	0.43	0.125–0.5	0.19	0.3	0.7	13.0
Cephalexin	0.25–32.0	1.25	8.0	1.0–4.0	1.3	2.0	0.25	25.0
Cefatrizine	0.015–4.0	0.21	1.4	0.25–2.0	0.6	1.0	0.7	6.2
Cefuroxime	0.002–2.0	0.02	0.5	0.004–0.06	0.02	0.06	0.1	32.0
Cefoxitin	0.06–1.0	0.14	0.5	0.25–0.5	0.29	0.33	0.7	72.3
Cefamandole	0.002–2.0	0.06	0.8	0.06–1.0	0.18	0.4	0.5	25.0
Cefazolin, sodium salt	0.06–4.0	0.4	1.8	1.0–4.0	1.3	2.0	1.1	43.4
Cefadroxil	0.25–64.0	2.0	16.0	2.0–8.0	2.9	4.2	0.3	15.0
Cephapirin	0.06–16.0	0.65	5.6	1.0–8.0	2.3	4.1	0.7	15.1
Cephradine	0.25–32.0	1.5	11.2	1.0–8.0	1.8	2.8	0.25	24.2
Cephacetrile	0.5–64.0	3.0	24.0	4.0–16.0	5.3	9.1	0.4	22.0
Tetracycline	0.06–4.0	0.5	1.4	1.0–2.0	1.0	1.2	0.9	3.25
Minocycline	0.06–2.0	0.18	0.9	0.25–2.0	0.5	1.0	1.1	2.5
Doxycycline	0.06–4.0	0.3	1.3	0.25–2.0	0.5	1.0	0.8	4.0
Rifampin	0.004–0.5	0.08	0.33	0.004–0.125	0.02	0.04	0.1	7.23
Erythromycin	0.03–1.0	0.16	0.65	0.015–0.5	0.04	0.3	0.5	4.47
Chloramphenicol	0.125–1.0	0.23	0.4	1.0–2.0	1.0	1.3	3.3	12.1
Spectinomycin	1.0–32.0	8.0	32.0	8.0–16.0	8.0	10.4	0.3	100.0

[a] Values for benzylpenicillin and phenoxymethyl penicillin are given in international units per milliliter.

[b] Values given are with usual dose. Adapted in part from Hewitt and McHenry (5).

[c] MIC_{50}, Concentration required for inhibition of 50% of strains; MIC_{90}, concentration required for inhibition of 90% of strains.

intramuscularly for anogenital gonorrhea is also compared (5).

For pen S strains the most effective penicillins appeared to be (in order) ampicillin, benzylpenicillin, amoxicillin, carbenicillin, phenoxymethyl penicillin, and methicillin. Dicloxacillin, cloxacillin, nafcillin, and oxacillin were relatively ineffective. With pen R strains the most effective penicillins were (in order) methicillin, nafcillin, dicloxacillin, cloxacillin, and carbenicillin.

Amoxicillin, ampicillin, phenoxymethyl penicillin, benzylpenicillin, and oxacillin were ineffective.

For the pen S strains the most active cephalosporins were (in order) cefuroxime, cefaclor, cefoxitin (a cephamycin), cefamandole, cefatrizine, cephalothin, cefazolin, cephapirin, cephaloridine, cephalexin, and cephradine. Cefadroxil and cephacetrile were relatively inactive. For the pen R strains the most active cephalosporins were (in order) cefuroxime, cefaclor, cefoxitin, cefamandole, cephalothin, cefatrizine, cefazolin, cephalexin, cephradine, cephapirin, and cefadroxil. Cephaloridine and cephacetrile were relatively ineffective.

Among the non-beta-lactam antibiotics, the most active (in relation to attainable blood levels) against pen S strains were (in order) rifampin, chloramphenicol, erythromycin, minocycline, doxycycline, tetracycline, and spectinomycin. For the pen R strains the most active non-beta-lactam antibiotics (in comparison to attainable blood levels) were (in order) rifampin, erythromycin, spectinomycin, chloramphenicol, minocycline, doxycycline, and tetracycline.

Comparison of the ratio of the concentration required to inhibit 90% of the pen R strains to the concentration required to inhibit 90% of the pen S strains shows the following antibiotics to be relatively more active (in order) against pen R strains: cefuroxime, rifampin, dicloxacillin, cefaclor, cephradine, cephalexin, spectinomycin, cefadroxil, nafcillin, cloxacillin, cephacetrile, erythromycin, cefamandole, cephalothin, methicillin, cefoxitin, cefaclor, cephapirin, cefatrizine, doxycycline, and tetracycline. With each of these antibiotics the ratio of the concentration required to inhibit 90% of the pen R strains to the concentration required to inhibit 90% of the pen S strains was less than one.

Disk diffusion versus agar dilution penicillin MIC. A comparison of the zones of inhibition around a 10-IU disk of benzylpenicillin by diffusion on agar plates (Bauer-Kirby method) was made with the agar dilution MIC for 16 pen R strains and 26 unselected pen S strains (Fig. 1). There was a highly significant negative correlation (correlation coefficient, −0.94), as calculated by linear regression with a Hewlett-Packard HP-65 calculator. A zone of inhibition of 23 mm or less was highly predictive of penicillinase production. The more susceptible penicillinase-negative strains had inhibition zones 34 to 56 mm in diameter. There was considerable overlap in inhibition zone diameters of pen I strains (MIC, ≥0.125 μg/ml) and the more susceptible pen S strains (MIC, ≤0.064 μg/ml).

Inoculum effect. The influence of a 20-fold increase in the size of the bacterial inoculum (from 1.5×10^3 to 3×10^4 viable cells) upon the agar dilution MIC of 29 antibiotics was compared for four pen I and eight pen R strains of *N. gonorrhoeae*. For many penicillins the ratio of the MIC for a large inoculum to the MIC for a small inoculum was greater for the pen R strains, especially for carbenicillin, penicillins G and V, and ampicillin, but it was not significantly greater with penicillinase-resistant penicillins, most cephalosporins (except cefamandole and cephacetrile) and the non-beta-lactam antibiotics. With pen I strains, the size of the inoculum

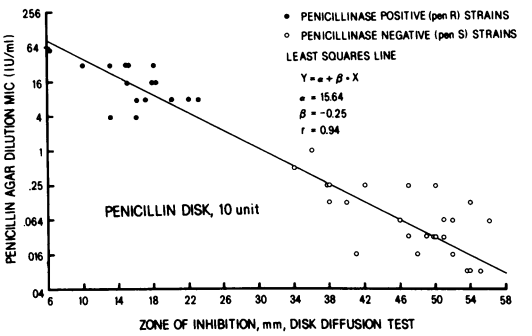

FIG. 1. *Correlation between inhibition zones with a 10-IU penicillin disk and the penicillin MIC by agar dilution method. r, Correlation coefficient.*

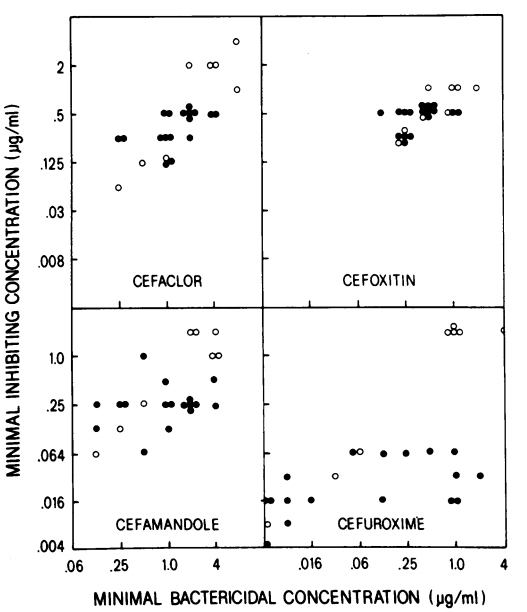

FIG. 2. *MICs and MBCs of the three most active cephalosporins and cefoxitin for pen I (○) and pen R (●) (penicillinase-positive) strains of N. gonorrhoeae.*

had less effect on the MIC; the MIC increased fourfold with the larger inoculum only for nafcillin. With all other antibiotics the ratio of the MIC for a large inoculum to the MIC for a small inoculum was no more than 1:2 for pen I strains.

MBC. Figure 2 compares the MICs and the MBCs of the three most active cephalosporins and cefoxitin against eight pen I strains and 17 pen R gonococcal strains. With the exception of cefuroxime, the four antibiotics were all bactericidal as well as bacteriostatic. There was a fairly close correlation between the MIC and MBC. Cefuroxime had the lowest MIC for the two gonococcal groups that were relatively or highly resistant to penicillin. Both the MIC and MBC of cefuroxime for some pen I strains, however, were 1 to 4 μg/ml, a level as great as for the other three antibiotics. Also, with cefuroxime the MBC was somewhat greater than the MIC for half of the pen R strains.

DISCUSSION

Pen R strains of *N. gonorrhoeae* are resistant to most oral penicillins, but they are susceptible to methicillin and, to a lesser degree, to nafcillin (4, 19). Pen I strains are more resistant than pen R strains to several penicillinase-resistant penicillins (dicloxacillin, nafcillin, cloxacillin, methicillin). As expected, pen R strains are more resistant than pen I strains to penicillinase-sensitive penicillins (benzylpenicillin, ampicillin, amoxicillin, carbenicillin) (4, 19). Pen R and pen I strains are both relatively resistant to some of the well-established cephalosporins, but they are susceptible to cephalothin, cefazolin, and three newer cephalosporins (cefamandole, cefaclor, cefuroxime) as well as cefoxitin (a cephamycin) (4, 11). Pen R strains were found to be susceptible to rifampin, erythromycin, the tetracyclines, chloramphenicol, and spectinomycin, confirming the observations of Siegel et al. (13).

Penicillinase-producing strains can be detected simply with a standard 10-IU penicillin disk on a supplemented Mueller-Hinton agar plate (20). Our data confirm and extend, by including pen R strains, earlier observations showing that the inhibition zone diameter by the disk method correlates fairly closely with the penicillin MIC determined by the agar dilution method (7, 12). This close correlation for penicillin susceptibility is true when pen R and pen S strains are compared. There is, however, a considerable overlap in the diameter of zones of inhibition observed for pen I and pen S strains.

With pen R strains the MICs for penicillin, some congeners of penicillin (especially carbenicillin), and some cephalosporins (cephacetrile, cefamandole) are notably affected by the size of the bacterial inoculum. These antibiotics may not be suitable for therapy of disseminated pen R gonococcal infections and septic complications (endocarditis, meningitis) where bacterial populations may be very large.

The penicillinase enzyme in gonococci is an important determinant for resistance to penicillin and several other penicillinase-sensitive β-lactam antibiotics. There are, however, many beta-lactam antibiotics sufficiently active against pen R strains of gonococci that they may prove useful in therapy of anogenital gonorrhea caused by pen R strains. These antibiotics include four penicillinase-resistant penicillins (methicillin, nafcillin, dicloxacillin, cloxacillin), as well as eleven cephalosporins (cefuroxime, cefaclor, cefoxitin, cefamandole, cephalothin, cefatrizine, cefazolin, cephalexin, cephradine, cephapirin, cefadroxil). Non-beta-lactams which may be effective against pen R gonococcal infections include rifampin, erythromycin, the tetracyclines, chloramphenicol, and spectinomycin. The usefulness of rifampin may be limited by the rapid acquisition of bacterial resistance.

Gonococci are autolytic in water, saline, and Mueller-Hinton broth but not in supplemented Trypticase soy broth. In the latter medium antibiotic MBCs should accurately predict antibiotic bactericidal effect (1). As yet there is little published information on the bactericidal effect in vitro of antibiotics on *N. gonorrhoeae*. It has been suggested that spectinomycin is bactericidal for gonococci, but sufficient controls to exclude autolysis were lacking (8, 17).

The high correlation between the MBCs and MICs at attainable serum levels of two of the most active new cephalosporins (cefamandole and cefaclor), as well as cefoxitin, for both pen R and pen I strains of *N. gonorrhoeae* suggests that these antibiotics can kill pen R and pen I strains. Cefuroxime may be an exception. For pen R strains the MBC of cefuroxime was often considerably greater than the MIC. The clinical usefulness of cefuroxime might be limited in gonococcal infections (pharyngitis, meningitis, endocarditis) caused by pen R strains and requiring bacterial kill for cure.

In summary, pen R strains of *N. gonorrhoeae* are highly resistant to penicillin and some of its close congeners, but they are susceptible to some penicillinase-resistant penicillins (i.e., methicillin and nafcillin). Pen R strains are also susceptible to three new cephalosporins (cefuroxime, cefamandole, cefaclor) and cefoxitin (a cephamycin), as well as to some of the older cephalosporins. Pen R strains are resistant to cephaloridine and cephacetrile. They are susceptible to

rifampin, erythromycin, spectinomycin, chloramphenicol, and the tetracyclines. Several of the new active cephalosporins (cefamandole, cefaclor, cefoxitin) are bactericidal as well as bacteriostatic for both pen R and pen I strains; cefuroxime is more bacteriostatic than bactericidal for some pen R strains. Thus, there are several antibiotics available which might be effective against gonorrhea caused by pen R strains. Of these, only spectinomycin has been recommended by the Center for Disease Control for clinical use (13).

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Evelyn C. Glatt prepared the manuscript.

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