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Antibiotic Susceptibility Survey of *Neisseria gonorrhoeae* in Thailand

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The antibiotic susceptibilities of *Neisseria gonorrhoeae* isolates obtained from patients attending sexually transmitted disease clinics in Cholburi and Bangkok, Thailand, were determined by agar dilution. Some 28.2% of isolates produced β-lactamase. A total of 97.9% of β-lactamase-positive and 51% of β-lactamase-negative isolates tested were resistant to penicillin (MICs, ≥2 μg/ml), 70% of isolates tested were resistant to tetracycline (MICs, ≥2 μg/ml), and 91% of isolates tested were susceptible to spectinomycin (MICs, ≤64 μg/ml). The MICs for 90% of isolates for the other drugs tested were 2 μg/ml for erythromycin, 2 μg/ml for cefoxitin, 1 μg/ml for cefuroxime, 0.125 μg/ml for cefpodoxime, 0.06 μg/ml for cefotaxime, 0.25 μg/ml for ceftazidime, 0.03 μg/ml for ceftizoxime, 0.03 μg/ml for ceftriaxone, 0.03 μg/ml for cefixime, 0.06 μg/ml for aztreonam, 0.008 μg/ml for ciprofloxacin, 0.125 μg/ml for norfloxacin, and 0.075 μg/ml for ofloxacin. Fewer than 1.5% of isolates were resistant to the extended-spectrum cephalosporins tested. Some 0.3% or fewer isolates were resistant to broad-spectrum cephalosporins, fluoroquinolones, or the monobactam aztreonam. Antibiotic resistance among *N. gonorrhoeae* isolates from Cholburi and Bangkok in May 1990 appeared to be primarily limited to penicillin and tetracycline, which are no longer used to control gonorrhea. Spectinomycin, which has been in general use against gonorrhea in Thailand since 1983, has dwindling utility, with resistance at a level of 8.9%.

Periodic monitoring of the underlying susceptibility profiles of the *Neisseria gonorrhoeae* strains prevalent in areas of frequent transmission may provide clues regarding treatment options and emerging drug resistance. The sex industry in Thailand sustains very high rates of transmission of sexually transmitted diseases; among them, gonorrhea is foremost in frequency and multiple antibiotic resistance is common. In 1983, the high prevalence of tetracycline-resistant, β-lactamase-producing *N. gonorrhoeae* strains led to the adoption of spectinomycin as the recommended therapy for gonorrhea in Thailand (15). While spectinomycin-resistant strains developed rapidly in both the Republic of Korea and the Philippines, spectinomycin has retained its effectiveness in Thailand and remains the drug of choice for the

TABLE 1. Antibiotic susceptibilities of *N. gonorrhoeae* isolates in Thailand

Antibiotic[a]	MIC (μg/ml)			No. of isolates tested
	90%	50%	Range	
Penicillin G (βL neg)	4.000	2.000	0.125–64.0	239
Penicillin G (βL pos)	>64.000	64.000	1.000–>64.0	94
Erythromycin	2.000	0.500	<0.030–4.0	332
Tetracycline	4.000	2.000	<0.060–32.0	333
Spectinomycin	64.000	64.000	8.000–>128.0	305
Cefoxitin	2.000	1.000	0.060–8.0	332
Cefuroxime	1.000	0.500	<0.030–>4.0	333
Cefpodoxime	0.125	0.030	<0.004–>4.0	331
Aztreonam	0.060	0.015	0.001–>4.0	331
Cefixime	0.030	<0.001	<0.001–8.0	328
Cefotaxime	0.060	0.030	<0.001–8.0	333
Ceftazidime	0.250	0.060	0.008–>4.0	333
Ceftizoxime	0.030	0.015	<0.001–>4.0	333
Ceftriaxone	0.030	0.008	<0.001–1.0	333
Norfloxacin	0.125	0.060	<0.015–>4.0	332
Ofloxacin	0.075	0.038	<0.005–2.5	333
Ciprofloxacin	0.008	0.004	<0.001–2.0	329

[a] βL neg, β-lactamase negative; βL pos, β-lactamase positive.

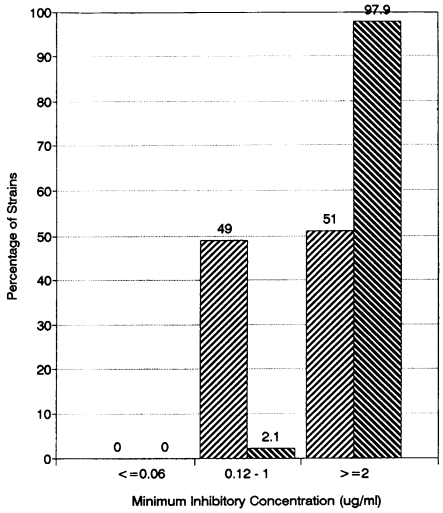

FIG. 1. Relationship of β-lactamase carriage to the in vitro penicillin susceptibilities of 239 β-lactamase-negative (▨) and 94 β-lactamase-positive (▧) isolates.

* Corresponding author.

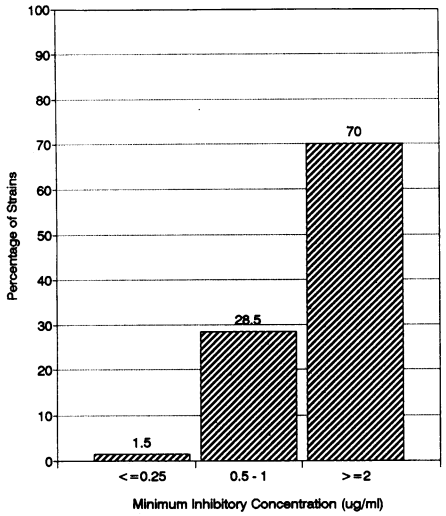

FIG. 2. Distribution of tetracycline susceptibility in 333 isolates.

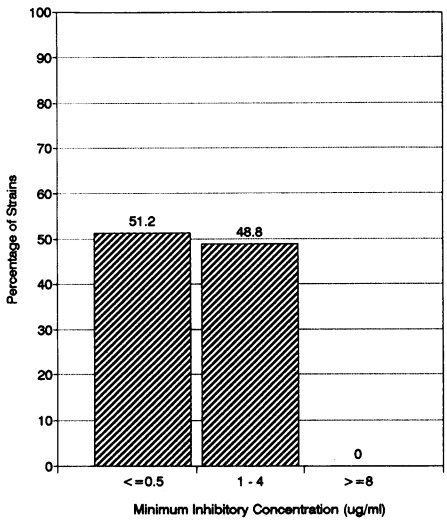

FIG. 4. Distribution of erythromycin susceptibility in 332 isolates.

treatment of gonorrhea (1, 4, 7, 8, 15). However, cases of gonorrhea arising in visitors to Thailand are frequently seen by practitioners in other countries where other treatment protocols are recommended. The goal of the present study was to examine the antibiotic susceptibility profiles of the *N. gonorrhoeae* strains prevalent in Cholburi and Bangkok, Thailand, and to screen for possible ceftriaxone-resistant strains. During May 1990, physicians at sexually transmitted disease clinics in Cholburi and Bangkok provided the clinical isolates of *N. gonorrhoeae* used in this study.

MATERIALS AND METHODS

Gonococcal isolates. Isolates of *N. gonorrhoeae* were obtained from patients with symptomatic sexually transmitted disease and men and women attending public health clinics in Cholburi and Bangkok during May 1990. There was no selection of isolates; all isolates that grew on subculture were tested. No information was available on the incidence of treatment failures or repeat isolates from the same patient. Initial isolations were made on modified Thayer-Martin agar (BBL Microbiology Systems, Cockeysville, Md.). Suspect colonies were identified by colony morphology, Gram staining, oxidase activity (SpotTest oxidase reagent; Difco Laboratories, Detroit, Mich.), and reaction in the Gonochek II monoclonal antibody test (Du Pont Co., Wilmington, Del.). Overnight subcultures were placed into a cryoprotective medium (Trypticase soy broth [BBL] with 20% glycerol [Mallinckrodt, Inc., Paris, Ky.]) and were frozen at −70°C until they were tested. All testing reported here was performed in the authors' laboratory in Hawaii. Thawed specimens were plated onto chocolate agar prepared from GC agar base (BBL), 1% bovine hemoglobin (BBL), and 1% IsoVitaleX (BBL). Pure colonies reisolated on chocolate agar were tested after 18 h of growth in second subcultures.

β-Lactamase testing. β-Lactamase production was tested

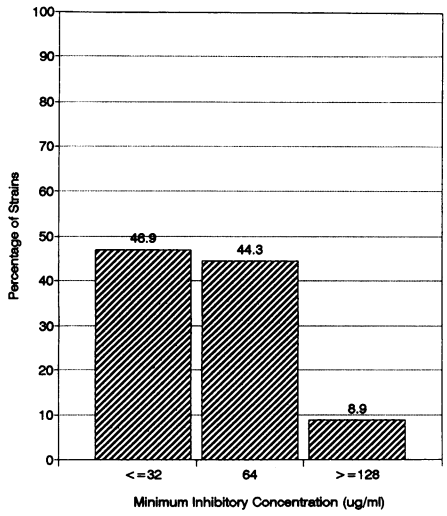

FIG. 3. Distribution of spectinomycin susceptibility in 305 isolates.

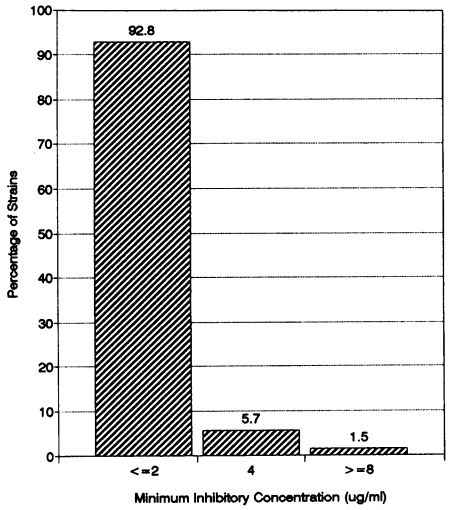

FIG. 5. Distribution of cefoxitin susceptibility in 332 isolates.

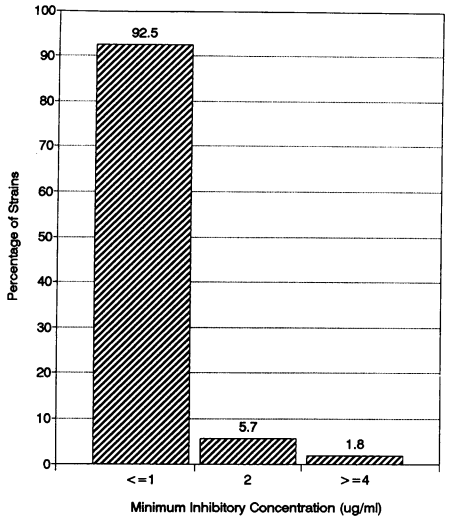

FIG. 6. Distribution of cefuroxime susceptibility in 333 isolates.

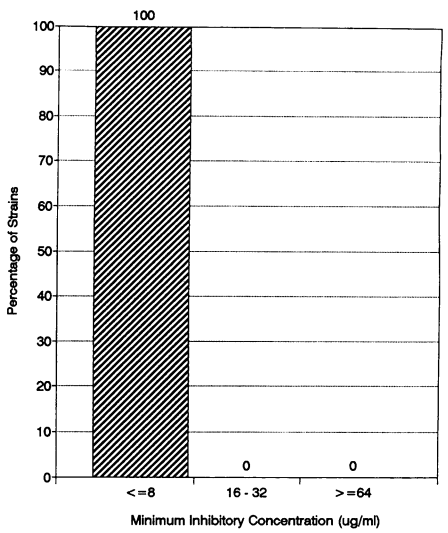

FIG. 8. Distribution of cefotaxime susceptibility in 333 isolates.

by using nitrocefin disks (Cefinase; BBL), with *Haemophilus influenzae* ATCC 10211 used as a negative control.

Antimicrobial agents. The following standard antimicrobial reference powders were provided by the indicated manufacturers: penicillin G, Marsham Pharmaceuticals, Cherry Hill, N.J.; tetracycline hydrochloride and cefixime, Lederle Laboratories, Wayne, N.J.; spectinomycin hydrochloride and cefpodoxime proxetil, The Upjohn Co., Kalamazoo, Mich.; ciprofloxacin hydrochloride, Miles Pharmaceuticals, West Haven, Conn.; cefoxitin sodium and norfloxacin sodium, Merck Sharp & Dohme, West Point, Pa.; cefuroxime sodium and ceftazidime, Glaxo, Inc., Research Triangle Park, N.C.; cefotaxime sodium, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; ceftizoxime sodium, Smith, Kline & French, Philadelphia, Pa.; ceftriaxone, Roche Laboratories, Nutley, N.J.; erythromycin base, Abbott Pharmaceuticals, Inc., North Chicago, Ill.; Ofloxacin, Imperial Pharmaceuticals, Manila, Philippines; and aztreonam, E. R. Squibb and Sons, Inc., Princeton, N.J.

Antibiotic stock solutions were prepared in reagent-grade water and were frozen at −70°C. Serial twofold dilutions of the antibiotics were prepared in reagent-grade water on the day of use.

Antimicrobial susceptibility testing. Antibiotic susceptibility testing was conducted as described previously (12). The quality control organisms inoculated onto each plate were *Staphylococcus aureus* ATCC 29213 and ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922. Subcultures were incubated in a humidified atmosphere of 5% CO_2 for 24 h at 35°C. MICs were read as the lowest concentration of antibiotic that inhibited growth (9). Antimicrobial susceptibility was judged according to breakpoints previously defined in the literature (5, 6, 10).

Statistical analysis. The effect of β-lactamase production on the susceptibility of an organism to each drug was

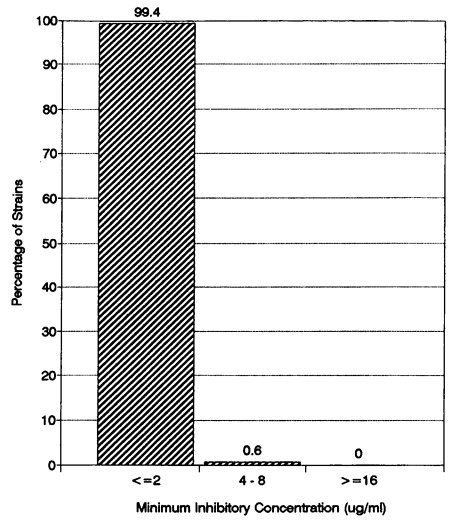

FIG. 7. Distribution of cefpodoxime susceptibility in 331 isolates.

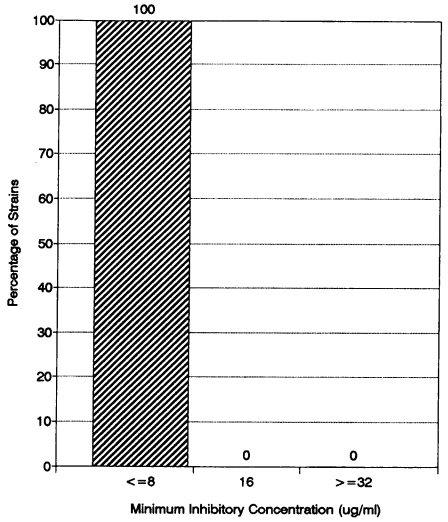

FIG. 9. Distribution of ceftazidime susceptibility in 333 isolates.

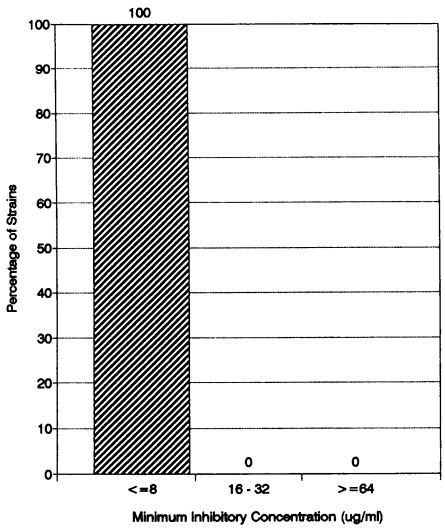

FIG. 10. Distribution of ceftizoxime susceptibility in 333 isolates.

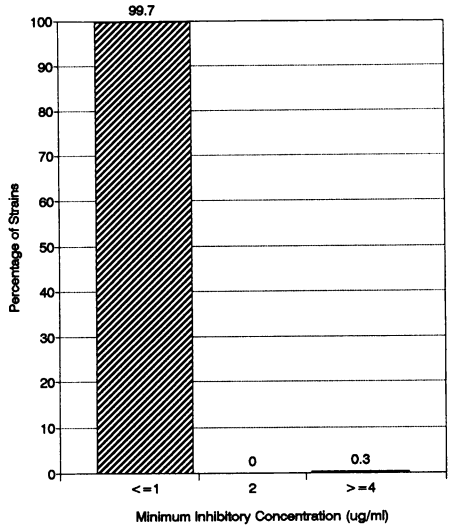

FIG. 12. Distribution of cefixime susceptibility in 328 isolates.

examined by chi-square analysis with EpiInfo Version 5.00 (Centers for Disease Control Epidemiology Program Office, Atlanta, Ga.). Significance was defined as $P \leq 0.05$. Data are presented separately for penicillin, because β-lactamase production was found to have a significant effect on the susceptibilities of the organisms to penicillin.

RESULTS

Three hundred thirty-three isolates of *N. gonorrhoeae* were examined to determine their antibiotic susceptibilities. The MICs for 50% (MIC_{50}s) and 90% (MIC_{90}s) of isolates tested and the range of MICs are given in Table 1 for each antibiotic tested. Where significant, differences between β-lactamase-positive and β-lactamase-negative strains are presented separately. β-Lactamase was produced by 94 of 333 (28.2%) of the isolates. Susceptibility to penicillin was significantly reduced in strains that produced β-lactamase; 117 of 239 (49%) β-lactamase-negative strains were moderately susceptible, whereas 92 of 94 (97.9%) β-lactamase-positive strains were resistant ($P < 0.001$) (Fig. 1). Chromosomal resistance to penicillin was detected in 122 of 239 (51%) β-lactamase-negative strains. The MIC_{50} (2 μg/ml) and the MIC_{90} (4 μg/ml) of tetracycline were above the accepted criteria for susceptibility (Fig. 2) (10). Of 305 specimens examined, 278 (91.2%) were spectinomycin susceptible (Fig. 3), with the MIC_{50} and MIC_{90} both equal to 64 μg/ml. The MIC_{90}s for the other drugs tested were 2 μg/ml for erythromycin, 2 μg/ml for cefoxitin, 1 μg/ml for cefuroxime, 0.125 μg/ml for cefpodoxime, 0.06 μg/ml for cefotaxime, 0.25 μg/ml for ceftazidime, 0.03 μg/ml for ceftizoxime, 0.03 μg/ml for ceftriaxone, 0.03 μg/ml for cefixime, 0.06 μg/ml for aztreonam, 0.008 μg/ml for ciprofloxacin, 0.125 μg/ml for norfloxacin, and 0.075 μg/ml for ofloxacin. More than 98% of the isolates were categorized as susceptible or moderately susceptible to each of this final group of antibiotics (5, 6, 10).

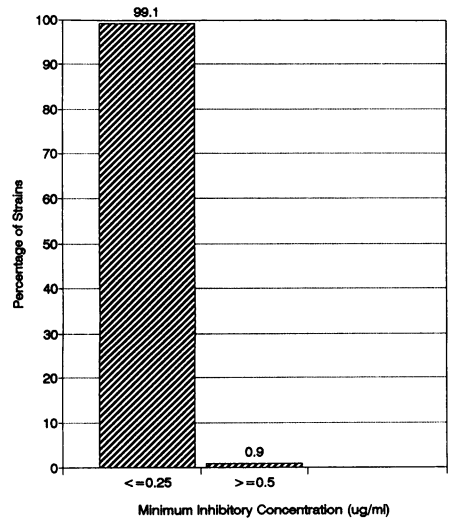

FIG. 11. Distribution of ceftriaxone susceptibility in 333 isolates.

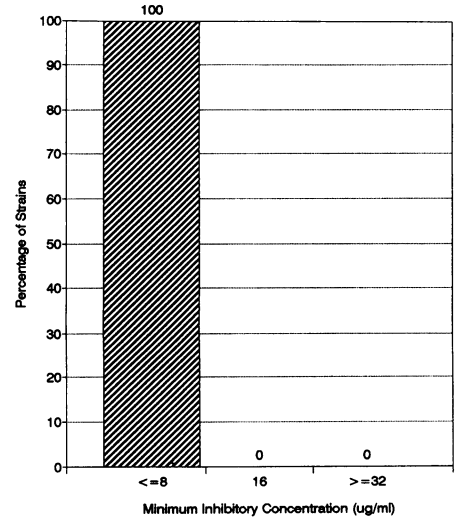

FIG. 13. Distribution of aztreonam susceptibility in 331 isolates.

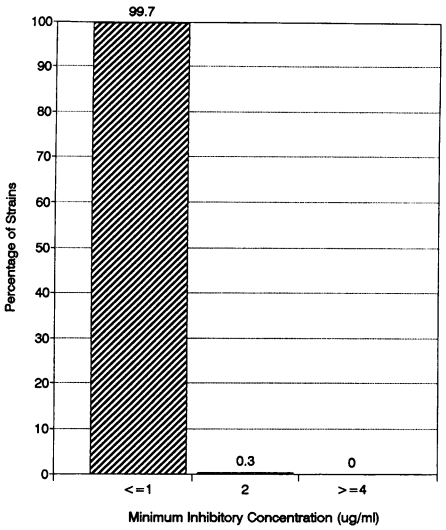

FIG. 14. Distribution of ciprofloxacin susceptibility in 329 isolates.

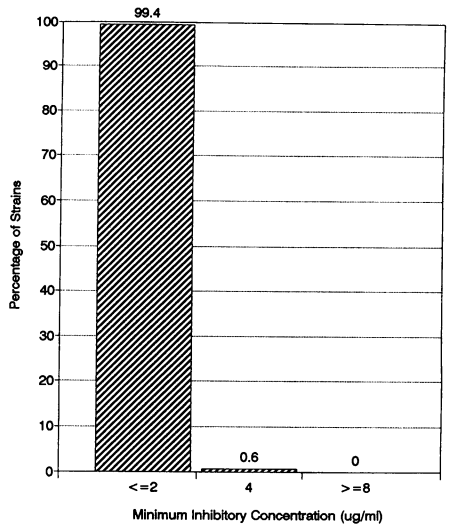

FIG. 16. Distribution of ofloxacin susceptibility in 333 isolates.

The distributions of susceptibilities to each of these antimicrobial agents are shown in Fig. 4 through 16, respectively.

DISCUSSION

Previous researchers have documented the resistance of *N. gonorrhoeae* to both penicillin and tetracycline in Thailand (2). While the prevalence of β-lactamase-producing strains appears to have declined from the 45% (reported in 1983) to 50% (reported in 1988), in the present study, 94 of 333 (28.2%) isolates produced β-lactamase (14, 15). The apparent reduction in the prevalence of β-lactamase-producing strains of *N. gonorrhoeae* is anomalous and probably reflects a temporal sampling error. Susceptibility to penicillin did not increase with a decline in the rate of β-lactamase production; however, 92 of 94 (97.9%) β-lactamase-producing isolates and 122 of 239 (51%) β-lactamase-negative isolates were categorized as penicillin resistant (Fig. 1), possibly as a consequence of penicillin therapy for unrelated illnesses or self-medication by sex industry workers with penicillin or penicillin congeners (13). Self-medication among sex industry employees in the Philippines has been cited as a significant factor in fostering antibiotic resistance, and the likelihood that the same practice occurs in Thailand is high (3). Tetracycline resistance in 233 of 333 (70%) (Fig. 2) isolates indicates that significant selective pressure resulting from its continued use for sexually transmitted diseases and other illnesses exists as well.

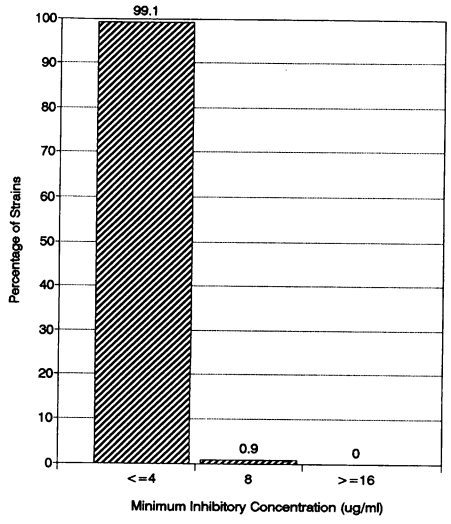

FIG. 15. Distribution of norfloxacin susceptibility in 332 isolates.

Spectinomycin was first used as the primary drug of choice against gonorrhea on a wide scale in Korea and the Philippines in 1981 (1, 8, 11). Spectinomycin-resistant isolates were reported in Korea in 1981 and the Philippines in 1981, and spectinomycin resistance reached 22.2% in the Philippines by 1988 (7). Spectinomycin became the primary therapeutic drug for the treatment of gonorrhea in Thailand in 1983 following a series of studies which documented penicillin-producing *N. gonorrhoeae* rates of up to 71% (14). Five years later (1987 and 1988), in a survey of patients with sexually transmitted diseases in Bangkok, Cholburi, Chiengmai, and Songkhla, Thailand, none of 3,200 *N. gonorrhoeae* isolates was spectinomycin resistant (15). We now document a spectinomycin resistance rate of 8.9% in these isolates from Bangkok and Cholburi from 1990.

The isolates tested demonstrated consistently high rates of susceptibility to extended-spectrum and broad-spectrum cephalosporins. Three extended-spectrum cephalosporins, cefoxitin, cefuroxime and cefpodoxime, each showed good activity against these isolates, with susceptibility rates of 98.5, 98.5, and 100%, respectively. The broad-spectrum cephalosporins cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and cefixime and the monobactam aztreonam demonstrated very high activities against these isolates with susceptibility rates of 100, 100, 100, 99.1, 100, and 100%, respectively. The susceptibility of *N. gonorrhoeae* to the fluoroquinolones ciprofloxacin, norfloxacin, and ofloxacin was 100% for each drug. It is possible however, that the in vivo response to the fluoroquinolones may not be adequately predicted by the results of MIC testing, and surrogate testing with nalidixic acid may be a more reliable predictor of the efficacy of ciprofloxacin in vivo than is direct testing (16).

We documented the continuing widespread resistance of *N. gonorrhoeae* isolates from Thailand to penicillin and tetracycline. More than 98% of isolates were susceptible to each of the expanded- and broad-spectrum cephalosporins tested. Susceptibility to the fluoroquinolones tested was universal. Spectinomycin may be approaching the end of its utility as a first-line drug for the treatment of gonorrhea in Thailand, with 8.9% of isolates now resistant.

ACKNOWLEDGMENTS

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