ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2000, p. 2543–2544 Vol. 44, No. 9
0066-4804/00/$04.00+0

In Vitro Susceptibilities of 400 Spanish Isolates of *Neisseria gonorrhoeae* to Gemifloxacin and 11 Other Antimicrobial Agents

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Received 6 March 2000/Returned for modification 11 May 2000/Accepted 3 June 2000

The in vitro activity of gemifloxacin versus those of 11 other antimicrobial agents against 400 strains of *Neisseria gonorrhoeae* was determined by microdilution with supplemented GC agar. A total of 37.5% of the strains were β-lactamase positive. A total of 70 and 6.4% of the β-lactamase-negative strains exhibited intermediate and high-level penicillin resistance, respectively. Ceftriaxone and gemifloxacin were the most active drugs (MICs at which 90% of isolates are inhibited, 0.01 versus 0.007 μg/ml, respectively), with 100% of strains inhibited by 0.12 μg/ml.

Neisseria gonorrhoeae is adept at developing mechanisms of resistance to new antimicrobial agents (6), and there is a continuing need for information on antimicrobial susceptibility patterns to help with the design of treatment regimens (2, 6).

Chromosomally mediated low-level resistance to penicillin and tetracycline was described 25 years ago (9), and high-level penicillin resistance is mediated by a TEM-1-type β-lactamase (1). Spectinomycin resistance is also due to chromosomal mutations (5). This has not affected the usefulness of this antibiotic (6), in contrast to what occurs with the sporadic resistance to newer cephalosporins. New quinolones are promising agents for the treatment of *N. gonorrhoeae* infections, but their use may carry a risk of the development of high-level resistance, as was seen following the widespread use of fluoroquinolones for the treatment of other infections (3). In addition, the development of in vivo resistance after the administration of a single dose of a quinolone as treatment for gonococcal urethritis, with the development of cross-resistance to other quinolones and concomitant resistance to tetracycline, has been described (10).

The aim of the study described here was to study the susceptibilities of 400 *N. gonorrhoeae* isolates to gemifloxacin and 11 other antimicrobial drugs, including 6 other fluoroquinolones.

The 400 clinical isolates of *Neisseria gonorrhoeae* (103 serogroup IA isolates and 297 serogroup IB isolates) were collected from 1992 to 1999 in 12 Spanish autonomous regions. The antimicrobial drugs tested are those included in Table 1. Reference standards were reconstituted according to the manufacturer's instructions, and appropriate dilutions (0.0007 to 64 μg/ml) of each drug were used in an agar dilution method with supplemented GC agar (4), similar to the methodology described by the National Committee for Clinical Laboratory Standards (NCCLS) (7). In brief, inocula were prepared by growing isolates on supplemented GC agar plates and then suspending the growth in Mueller-Hinton broth until an optical density equivalent to that of a no. 1 McFarland standard (10^8 CFU/ml) was obtained. Inoculation was performed with an automatic multi-inoculator device that dispensed a final inoculum of 10^5 CFU/spot. Incubation was performed at 37°C in a 5% CO_2 atmosphere for 18 to 20 h. *N. gonorrhoeae* 6395 (β-lactamase positive) and *N. gonorrhoeae* 3303 (penicillin resistant, β-lactamase negative) were used as controls, and interexperiment variations with these strains were no more than ±1 dilution.

The breakpoints considered are those noted in Table 1 and obtained from NCCLS document M100-S9 (7).

No differences in susceptibility or β-lactamase production were found with respect to the year of isolation, region of isolation, sample origin, or serogroup; and 37.5% of the strains were β-lactamase positive. Table 1 shows the susceptibilities of the strains to all antimicrobial drugs tested. No differences in susceptibility were found between β-lactamase-positive and -negative strains for any drug except penicillin, for which the MICs at which 50% of isolates are inhibited (MIC_{50}s), MIC_{90}s, and range of MICs were 32, 256, and 0.06 to 256 μg/ml, respectively, for β-lactamase-positive strains and 0.25, 1, and 0.003 to 4 μg/ml, respectively, for β-lactamase-negative strains. Only 23.6% of the β-lactamase-negative strains were susceptible to penicillin, with 70% of the strains being intermediate and 6.4% being resistant. The rate of susceptibility to ceftriaxone and spectinomycin was maintained at 100%, while only 13.5% strains were susceptible to tetracycline. Gemifloxacin was the most active quinolone tested, with all strains inhibited by concentrations of ≤0.12 μg/ml, followed by trovafloxacin, grepafloxacin and levofloxacin. Strains with intermediate resistance to grepafloxacin (1.5% of strains), ofloxacin (2.2%), and ciprofloxacin (6.5%) were found.

Concerns over the increase in chromosomal or plasmid-mediated penicillin resistance have led the World Health Organization (WHO) to change recommendations for first-line therapy for gonorrhoea from penicillin to spectinomycin, ceftriaxone, or ciprofloxacin (6). In this study, Spanish isolates maintained 100% susceptibility to ceftriaxone and spectinomycin, despite the different intrinsic activities of these drugs (MIC_{90}, of 0.01 versus 16 μg/ml). This is not the case for other cephalosporins such as cefoxitin, to which approximately 5% of isolates were not susceptible.

A course of tetracycline commonly follows single-dose treatments for gonorrhoea to eradicate concomitant *Chlamydia trachomatis* infection (2). In addition to eradicating coexisting chlamydial infection, sequential therapy may reduce the potential for the selection of resistant gonococci (2). This may not

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TABLE 1. Susceptibilities to gemifloxacin and 11 other antimicrobial agents of 400 isolates of *N. gonorrhoeae*[a]

Antimicrobial agent	MIC (μg/ml)			% Susceptible	% Intermediate	% Resistant
	50%	90%	Range			
Gemifloxacin	0.003	0.007	0.0005–0.12	NA[b]		
Trovafloxacin	0.007	0.03	0.002–0.25	100		
Grepafloxacin	0.003	0.03	0.002–0.25	98.5	1.5	
Levofloxacin	0.007	0.003	0.002–0.5	NA		
Ofloxacin	0.03	0.03	0.002–1	97.8	2.2	
Ciprofloxacin	0.003	0.01	0.001–0.25	93.5	6.5	
Norfloxacin	0.003	0.006	0.001–4	NA		
Tetracycline	1.0	4.0	0.03–64	13.5	54.2	32.3
Spectinomycin	16.0	16.0	4–32	100		
Ceftriaxone	0.003	0.01	0.0005–0.12	100		
Cefoxitin	1.0	2.0	0.03–4	94.8	5.2	
Penicillin	1.0	64.0	0.003–256	15.0	44.8	40.2

[a] The breakpoints used were those described in NCCLS document M100-S9 (7). The respective concentrations for the susceptible/intermediate/resistant breakpoints were ≤0.25/NA/NA μg/ml for trovafloxacin and ceftriaxone; ≤0.06/0.12 to 0.5/≥1 μg/ml for grepafloxacin and ciprofloxacin, ≤0.25/0.5 to 1/≥2 μg/ml for ofloxacin and tetracycline, ≤32/64/≥128 for spectinomycin, ≤2/4/≥8 μg/ml for cephoxitin, and ≤0.06/0.12 to 1/≥2 μg/ml for penicillin.

[b] NA, the NCCLS breakpoint is not available.

be the case in Spain, where tetracycline was poorly active against these Spanish isolates of *N. gonorrhoeae*, with approximately 85% being nonsusceptible. With respect to quinolones, the WHO recommendation for the use of ciprofloxacin as first-line therapy should be taken cautiously, since the risk of development of high-level resistance may be proportional to the prevalence of low-level resistance (3), and in this study, a 6.5% prevalence of intermediate resistance to ciprofloxacin and a 1.5% prevalence of intermediate resistance to grepafloxacin were found.

It is accepted that the levels of quinolones in serum should be 10 times greater than the MIC to predict clinical efficacy and prevent the development of resistance (8). By using as susceptibility breakpoints values 10 times lower than the peak levels in serum for the newer quinolones (i.e. ≤0.12, ≤0.25, and ≤0.5 μg/ml for gemifloxacin, trovafloxacin, and levofloxacin, respectively), 100% susceptibility to these drugs is obtained, with the MIC_{50} and MIC_{90} of gemifloxacin being lower. These drugs offer an alternative to the older quinolones (ciprofloxacin and ofloxacin) to which *N. gonorrhoeae* already shows a significant level of nonsusceptibility in a setting in which the widespread use of these drugs for the treatment of other types of infections creates selection pressure (3), in which the in vivo development of quinolone resistance has been described (10), and in which the prevalent intermediate resistance to older quinolones may increase the risk of high-level resistance (3).

This study was supported by a grant from SmithKline Beecham Pharmaceuticals, Harlow, United Kingdom.

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