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Antibiotic Susceptibility of *Neisseria gonorrhoeae* Strains from Europe and Africa

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The in vitro activities of 16 antimicrobial agents were tested by a plate dilution method against 268 unselected isolates of *Neisseria gonorrhoeae* from Belgium, Rwanda, Swaziland, and Zaire. Fifteen β-lactamase-producing strains isolated in Europe from various origins were also tested. There were significant regional variations in antimicrobial agent susceptibility, even among the African isolates, with the Rwandan and Zairean strains being most resistant. Benzylpenicillin and ampicillin were equally active in all but the β-lactamase-producing strains. Among the cephalosporins, cefotaxime was by far the most active, followed by cefuroxime, cefamandole, cefoxitin, and cefaclor, in that order. All strains were susceptible to spectinomycin, thiamphenicol, kanamycin, and rifampin, with the exception of one highly rifampin-resistant isolate and a moderately thiamphenicol-resistant strain. Twenty-six percent of the isolates were highly resistant to streptomycin. Six percent of the gonococci had a minimal inhibitory concentration for tetracycline greater than 2 μg/ml. Clavulanic acid inhibited the β-lactamase activity of the gonococci tested and improved markedly the activities of ampicillin and amoxicillin against β-lactamase-producing strains.

The recent emergence of β-lactamase-producing strains of *Neisseria gonorrhoeae* (2) and the high prevalence of isolates with low susceptibilities to penicillin in many developing countries (7, 11, 13-15) have made the selection of alternative drugs for the treatment of gonorrhea imperative. An essential feature of any alternative drug is that it must be active in vitro. We investigated the in vitro activities of 16 antimicrobial agents against isolates of *N. gonorrhoeae* from Belgium and three African countries. The strains were tested as part of a continuous surveillance program on the antibiotic susceptibility of gonococci in these countries. Fifteen β-lactamase-producing strains were also included.

MATERIALS AND METHODS

Strains. A total of 283 unselected isolates of *N. gonorrhoeae* were studied. The strains were isolated in 1977 and 1978 in Belgium (168 isolates), Rwanda (41 isolates), Swaziland (54 isolates), and Kinshasa, Zaire (5 isolates). Among the β-lactamase-producing strains, 1 was isolated in our laboratory in Antwerp, Belgium, and 14 were received from various laboratories. All the strains were preserved by lyophilization after testing for the presence of β-lactamase by the chromogenic cephalosporin test (6).

N. gonorrhoeae WHO reference strains III, V, and VII and *Staphylococcus aureus* ATCC 25923 were included in each experiment.

Antibiotics. National standards of benzylpenicillin, ampicillin, tetracycline hydrochloride, erythromycin, rifampin, kanamycin, and streptomycin were provided by the Laboratory of Pharmaceutical Standards, Ministry of Health, Brussels, Belgium. Sulfamethoxazole and trimethoprim were supplied by Roche Products, Basel, Switzerland; cefuroxime was supplied by Glaxo Laboratories, Ltd., Greenford, Middlesex, England; cefamandole and cefaclor were supplied by Eli Lilly & Co., Indianapolis, Ind.; cefoxitin was supplied by Merck Sharp & Dohme International, Rahway, N.J.; cefotaxime (a new semisynthetic cephalosporin) was supplied by Roussel-UCLAF, Paris, France; amoxicillin was supplied by Beecham Research Laboratories, Ltd., Brentford, Middlesex, England; thiamphenicol was supplied by Zambon s.p.a., Bresso (Milan), Italy; and spectinomycin was supplied by the Upjohn Co., Kalamazoo, Mich. The combination sulfamethoxazole-trimethoprim was tested in the ratio 19:1. For the β-lactamase-producing strains, combinations of ampicillin and amoxicillin with the β-lactamase inhibitor clavulanic acid (ratio, 2:1) were also tested.

Susceptibility tests. Minimal inhibitory concentrations (MICs) were determined by an agar dilution method. With a multipoint replicator, suspensions from overnight cultures in 5 ml of blood-saponin broth (tryptic soy broth [Difco Laboratories, Detroit, Mich.] with 1% saponin [Merck] and 5% horse blood) were inoculated onto plates containing doubling dilutions of the antibiotics. The test medium was GC (gonococcus) agar (Difco) supplemented with 1% hemoglobin (Difco) and 1% IsoVitaleX (Baltimore Biological Lab-

oratory, Cockeysville, Md.), except for sulfamethoxazole-trimethoprim, which was tested on Diagnostic Sensitivity Test agar (Oxoid Ltd., London, England) with 1% saponin (Merck) and 5% horse blood. The inoculum was approximately 10^4 colony-forming units. After incubation for 24 h at 37°C in a 10% CO_2 atmosphere, the MIC was determined as the lowest concentration of drug yielding no growth or a barely visible haze.

RESULTS

The distributions and median values of minimal inhibitory concentrations from the non-β-lactamase-producing strains are given in Table 1. A bimodal distribution was found for the penicillin MICs. Forty-four percent of 268 isolates were less susceptible to penicillin (MIC ≥ 0.06 μg/ml), with 25% having an MIC greater than 0.25 μg/ml.

Ampicillin and amoxicillin were very similar in their activities on the gonococci tested. They gave a narrower range of MICs than that of penicillin and were more active against strains relatively resistant to penicillin. On a weight basis, cefotaxime was by far the most active of all the antibiotics tested, with MICs as low as 0.0005 μg/ml. The other cephalosporin analogs tested showed somewhat lower activities than that of penicillin. Cefuroxime was the most active, and cefamandole and cefaclor gave MICs as high as 8 μg/ml. The bimodal distribution of the results for cephalosporins was not as clear as it was for penicillin.

Tetracycline MICs showed a normal distribution and peaked at 0.5 μg/ml. Twenty-three percent had MICs greater than 1.0 μg/ml. One isolate had an MIC of 2 μg/ml for rifampin, and another was highly resistant (MIC > 64 μg/ml). Both strains were isolated in Antwerp. An isolate from Swaziland had an MIC of 16 μg/ml for thiamphenicol, which inhibited 82% of the strains at 1.0 μg/ml.

Seven percent of the strains were relatively resistant to erythromycin (MIC > 2.0 μg/ml). Spectinomycin inhibited all gonococci within a narrow range, as did kanamycin. All strains were fully susceptible. Twenty-six percent of all gonococci displayed high-level resistance to streptomycin (MIC ≥ 128 μg/ml). The combination sulfamethoxazole-trimethoprim showed a unimodal distribution, with the modus at 4 μg/ml.

The results of the tests with 15 β-lactamase-producing gonococci are given in Table 2. None of the strains was isolated in Africa. All the cephalosporin analogs showed higher MICs than they had with the non-β-lactamase-producing strains. The order of activity as evaluated by median value was cefotaxime > cefuroxime > cefoxitin > cefamandole > cefaclor. Cefotaxime, cefuroxime, and cefoxitin were most stable against β-lactamase activity, with only two- to threefold increases of the median MICs as compared with the values for the non-β-lactamase-producing strains, whereas the median values for cefaclor and cefamandole multiplied by 25 and 50, respectively. Ampicillin and amoxicillin showed very similar results, and both were much more active when used in combination with clavulanic acid.

The distribution of MICs for penicillin according to the geographical origins of the isolates is shown in Table 3. The patterns of the susceptibilities of gonococci from Belgium and Swaziland were very similar, with a first modus at 0.03 μg/ml and with 47 and 46%, respectively, being less susceptible. Isolates from Rwanda were more resistant and gave a multimodal distribution. Eighty-five percent were less susceptible (MIC > 0.03 μg/ml), and 24% had MICs between 1 and 4 μg/ml, none producing β-lactamase. The Belgian and Swazi isolates showed very similar susceptibilities for all the other antibiotics, whereas the Rwandan strains were always less susceptible, with the exception of rifampin.

DISCUSSION

Our results confirm previous observations that regional variations in antibiotic susceptibilities do exist. Whereas 47% of the Belgian strains and 46% of the Swazi strains are less susceptible to penicillin (MIC ≥ 0.06 μg/ml), as much as 85% of the Rwandan gonococci have MICs greater than 0.03 μg/ml. The figures for Belgium are comparable to those found in other European countries (12), but the prevalence of decreased susceptibility is lower than that in the United States (4). There has been no significant increase in relative resistance to penicillin in Belgium since 1974 (5).

Gonococcal isolates from an unselected urban population in Zaire were more resistant to penicillin than those from a rural population in Rwanda; however, interpretation of these data must be cautious due to the small number of strains tested, and this high resistance rate needs further investigation. There is no obvious explanation for the significant difference in penicillin susceptibility of *N. gonorrhoeae* strains in African countries. It has been suggested that widespread, indiscriminate use of oral penicillin and its congeners in doses inadequate for cure may select and facilitate the dissemination of gonococci relatively resistant to these drugs. Self-medication is a widespread practice in most African countries, and antibiotics are freely available at local markets. Although most studies conclude that there is higher penicillin resist-

TABLE 1. *Susceptibility of non-β-lactamase-producing N. gonorrhoeae strains to 16 antimicrobial agents*

Antimicrobial agent	No. of strains	Median MIC (μg/ml)	No. of strains with MIC (μg/ml) of:																	
			0.0005	0.001	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Penicillin	268	0.042					9	50	66	24	25	28	27	22	16	1					
Ampicillin	268	0.12					4	1	36	47	51	53	55	21							
Amoxicillin	227	0.10						2	32	38	57	30	52	16							
Cefuroxime	268	0.04				2	30	38	48	59	27	18	25	18	3						
Cefamandole	260	0.08					1	46	34	41	26	24	31	36	8	12	1				
Cefaclor	249	0.21					7	23	30	15	25	39	18	30	35	21	6				
Cefoxitin	250	0.25						2		20	20	85	50	28	45						
Cefotaxime	243	0.003	39	24	34	54	21	25	22	23	1										
Tetracycline	260	0.45										57	89	54	45	14	1				
Rifampin	241	0.18							17	14	41	121	46		1						1
Thiamphenicol	241	0.23								19	34	80	43	22	36	6		1			
Erythromycin	235	0.25					9	17	4	10	19	61	45	42	12	16					
Spectinomycin	244	13.26														7	44	105	88		
Kanamycin	255	5.68												5	23	48	123	56			
Streptomycin	256	15.32												1	1	15	37	82	53		67
Sulfamethoxazole-trimethoprim[a]	252	3.30												8	45	111	67	10	11		

[a] MIC is expressed as micrograms of the mixture sulfamethoxazole-trimethoprim (19:1) per milliliter.

TABLE 2. *In vitro susceptibilities of β-lactamase-producing N. gonorrhoeae strains*

Antimicrobial agent	No. of strains with MIC (μg/ml) of:																
	Tested	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Penicillin	15															3	12
Ampicillin	15															3	12
Amoxicillin	15															4	11
Ampicillin + clavulanic acid[a]	15								2	3	6	4					
Amoxicillin + clavulanic acid	15								3	4	5	3					
Cefuroxime	15					4	1	4	3	1	2						
Cefamandole	12									1	1		4	6		•	
Cefaclor	12											1	2	9			
Cefoxitin	15									4	8	1	2				
Cefotaxime	15	1	6	3	1	2	2										
Tetracycline	14								1	8	2	1	2				
Rifampin	15					5	1		9								
Thiamphenicol	15									4	1	10					
Erythromycin	15					1	1	4		8		1					
Spectinomycin	15												6	2	7		
Kanamycin	12											3	8	1			
Streptomycin	12											1	1				10
Sulfamethoxazole-trimethoprim[b]	14											4	4	6			

[a] Combination of ampicillin–clavulanic acid at a ratio of 2:1; MIC is expressed as micrograms of ampicillin per milliliter.

[b] MIC is expressed as micrograms of the mixture sulfamethoxazole–trimethoprim (19:1) per milliliter.

TABLE 3. *Distribution of MICs for penicillin according to geographical origin*

Origin	No. of strains	Median MIC (μg/ml)	No. of strains with MIC (μg/ml) of:									
			0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4
Belgium	168	0.03	3	38	49	20	12	16	14	15	1	
Swaziland	54	0.03	6	10	13	1	4	5	6	5	4	
Rwanda	41	0.15		2	4	3	9	6	7	1	8	1
Zaire	5							1		1	3	

ance of gonococci in Africa as compared with Europe (1, 7, 9, 13), our data suggest that important regional variations also occur in Africa. This clearly indicates the need for continued regional monitoring of gonococcal antibiotic susceptibility in this part of the world. β-Lactamase-producing gonococci were not found during this survey.

Among the cephalosporin analogs tested, cefotaxime was by far the most active on a weight basis. The cephalosporins differed in stability against β-lactamase activity, with cefaclor and cefamandole being least stable. All the cephalosporins tested were more active than the first-generation cephalosporins (3, 8). Most of the other antimicrobial agents tested inhibited all the strains at levels achievable in blood after systemic administration of moderate doses, with the exception of streptomycin, for which high-level resistance (MIC ≥ 128 μg/ml) was found in 26% of all strains. A highly rifampin-resistant strain (MIC ≥ 128 μg/ml) was isolated in Belgium, where this antibiotic is sporadically used in the treatment of gonorrhea. Most interesting was the inhibition of β-lactamase activity by clavulanic acid (10), which improved markedly the activities of ampicillin and amoxicillin against the β-lactamase-producing gonococci tested. The combination of β-lactamase-labile penicillins S and clavulanic acid deserves further clinical investigation in infections by β-lactamase-producing gonococci.

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