1988-02-01

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1988, p. 259–261
0066-4804/88/020259-03$02.00/0

Vol. 32, No. 2

In Vitro Activities of 39 Antimicrobial Agents for *Branhamella catarrhalis* and Comparison of Results with Different Quantitative Susceptibility Test Methods

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Received 21 September 1987/Accepted 17 November 1987

The in vitro activities of 39 antimicrobial agents were assessed versus 74 clinical isolates of *Branhamella catarrhalis*. Resistance was observed only with penicillin and ampicillin and then only with β-lactamase-producing strains. The results of in vitro susceptibility tests with agar dilution and broth microdilution procedures were found to be comparable. The results of broth tube macrodilution tests were, in general, one twofold-concentration increment higher.

Branhamella catarrhalis is recognized as an etiologic agent of a variety of infectious diseases in humans (3). In terms of incidence and perhaps morbidity, the most important of these are acute otitis media, maxillary sinusitis, and bronchopulmonary infections. Numerous different oral and parenteral antimicrobial agents may be used to treat these diseases. The intent of this investigation was to compare the in vitro activities of selected antimicrobial agents that are of potential value in the treatment of *Branhamella* infections. In addition, the effect of susceptibility test format on MICs obtained with *B. catarrhalis* was assessed.

A total of 39 agents (Table 1) were examined versus 74 recent clinical isolates of *B. catarrhalis*. Among these 74 strains, 58 (78.4%) produced β-lactamase when tested with a conventional tube nitrocefin β-lactamase assay (7, 10). MICs for each organism-antimicrobial combination were determined by use of an agar dilution procedure which employed unsupplemented Mueller-Hinton agar (pH 7.2) with an inoculum size of 10^4 CFU per spot. Antimicrobial agents, obtained from manufacturers as laboratory-grade powders, were tested over a range of twofold-concentration increments from 0.004 to 128 μg/ml. A growth control plate containing no antimicrobial agent was included with each test. Plates were incubated at 35°C in ambient air for 20 to 24 h and examined for the presence of growth. The MIC was defined as the lowest concentration of antimicrobial agent tested which yielded no macroscopic evidence of growth of the test organism. Each determination was done twice on separate days, and the results were averaged to obtain an estimate of the MIC for each organism-antimicrobial combination. In 2,848 of the 2,885 paired determinations (98.7%), replicate MICs were the same. In no case did the results of replicate MICs vary by more than fourfold. In cases in which replicate MICs varied by one twofold-concentration increment, the higher value was assigned as the MIC. Depending on the antimicrobial agent being tested, *Staphylococcus aureus* ATCC 29213 or *Escherichia coli* ATCC 25922 was used as the daily test control.

A total of 33 strains (20 of them β-lactamase positive) were selected from among the original 74 clinical isolates of *B. catarrhalis* for additional testing with both a broth tube macrodilution and a microdilution procedure. Eight antimicrobial agents were tested (Tables 2 and 3). Cation-supplemented Mueller-Hinton broth and a final inoculum concentration of 1×10^5 to 2×10^5 CFU/ml were used with both procedures. The final broth volume in tube macrodilution tests was 2.0 ml; in microdilution tests the final broth volume was 100 μl. With these exceptions, both broth dilution procedures were performed as described above with agar dilution tests.

The results of agar dilution MIC determinations are shown in Table 1. Among the 21 beta-lactam antimicrobial agents examined in this investigation, resistance as defined by the National Committee for Clinical Laboratory Standards (9) was observed only with penicillin and ampicillin, and then only with β-lactamase-producing strains. The β-lactamase of *B. catarrhalis* appeared to abrogate, at least to some extent, the activity of 13 of these 21 beta-lactam antimicrobial agents (i.e., penicillin, ampicillin, carbenicillin, ticarcillin, cephalothin, cefaclor, cefamandole, cefuroxime, cefonicid, cefotaxime, ceftizoxime, ceftriaxone, and cefixime). However, as indicated above, resistance was observed only in the cases of penicillin and ampicillin. With the remaining eight beta-lactam antimicrobial agents (i.e., piperacillin, azlocillin, mezlocillin, cephalexin, cefoxitin, moxalactam, cefoperazone, and ceftazidime), MICs obtained with β-lactamase-producing strains were essentially equivalent to those obtained with strains that lacked β-lactamase.

When the activities of beta-lactam antimicrobial agents were compared on a weight basis, the following observations were made. Among the seven penicillins tested, carbenicillin was the least active; piperacillin, azlocillin, and mezlocillin were the most active. Cefonicid was the least active of seven first- or second-generation cephalosporins tested; cefoxitin was the most active. Finally, among the seven broad-spectrum cephalosporins examined in this investigation, cefoperazone was the least active; moxalactam was conspicuously the most active.

The combinations of amoxicillin-clavulanate, ampicillin-sulbactam, and ticarcillin-clavulanate were uniformly inhibitory at low concentrations for both β-lactamase-positive and -negative strains of *B. catarrhalis*. This is consistent with previous observations that the *B. catarrhalis* β-lactamases are inhibited by clavulanate and sulbactam (4, 8).

Resistance was not observed with any of the other 15 antimicrobial agents examined in this investigation. Among

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TABLE 1. In vitro activities of 39 antimicrobial agents for 74 clinical isolates of *B. catarrhalis*

Antimicrobial agent[a]	MIC (μg/ml)[b] for strains that were:							
	β-Lactamase positive (n = 58)				β-Lactamase negative (n = 16)			
	50%	90%	Range	Geometric mean	50%	90%	Range	Geometric mean
Penicillin	2.0	2.0	0.015–4.0	0.74	0.007	0.03	0.004–0.06	0.008
Ampicillin	2.0	2.0	0.03–4.0	0.89	0.007	0.06	0.004–0.125	0.01
Carbenicillin	4.0	8.0	0.25–16	2.0	0.25	0.5	0.25–0.5	0.38
Ticarcillin	2.0	4.0	0.5–4.0	1.4	0.125	0.5	0.125–0.5	0.20
Piperacillin	0.25	0.5	0.125–1.0	0.25	0.125	0.5	0.125–0.5	0.23
Azlocillin	0.25	0.5	0.125–0.5	0.22	0.125	0.25	0.125–0.5	0.22
Mezlocillin	0.25	0.5	0.125–0.5	0.23	0.25	0.5	0.125–0.5	0.24
Cephalothin	4.0	8.0	1.0–8.0	4.1	1.0	1.0	0.5–1.0	0.78
Cephalexin	4.0	4.0	1.0–4.0	3.1	2.0	4.0	2.0–4.0	2.75
Cefaclor	1.0	2.0	0.125–2.0	1.0	0.125	0.5	0.125–0.5	0.25
Cefoxitin	0.25	0.5	0.125–1.0	0.23	0.125	0.25	0.125–0.25	0.18
Cefamandole	4.0	8.0	0.5–8.0	4.0	1.0	1.0	0.5–2.0	0.91
Cefuroxime	2.0	2.0	0.5–4.0	1.5	0.5	1.0	0.25–2.0	0.69
Cefonicid	4.0	8.0	0.5–16	5.4	0.5	1.0	0.5–1.0	0.66
Cefotaxime	0.5	1.0	0.06–2.0	0.38	0.06	1.0	0.03–1.0	0.16
Moxalactam	0.007	0.007	0.004–0.015	0.006	0.004	0.007	0.004–0.007	0.006
Ceftizoxime	0.25	0.5	0.007–0.5	0.14	0.03	0.25	0.004–0.25	0.05
Ceftriaxone	0.5	1.0	0.004–1.0	0.17	0.004	0.25	0.004–0.25	0.02
Cefixime	0.25	0.5	0.03–1.0	0.22	0.06	0.5	0.03–0.5	0.11
Cefoperazone	0.5	2.0	0.125–2.0	0.55	0.125	1.0	0.125–1.0	0.51
Ceftazidime	0.06	0.25	0.007–0.5	0.07	0.03	0.25	0.004–0.25	0.06
Amox-clav	0.06	0.25	0.007–0.25	0.06	0.015	0.06	0.004–0.125	0.02
Amp-sulb	0.03	0.25	0.007–0.25	0.05	0.015	0.06	0.004–0.125	0.03
Ticar-Clav	0.25	0.5	0.125–1.0	0.27	0.125	0.5	0.125–0.5	0.22
Chloramphenicol	0.5	0.5	0.125–0.5	0.48	0.5	0.5	0.25–1.0	0.50
Tetracycline	0.25	0.5	0.25–0.5	0.33	0.25	0.5	0.125–1.0	0.36
Erythromycin	0.125	0.125	0.03–0.25	0.10	0.06	0.25	0.015–1.0	0.10
Erythro-sulfa	0.06	0.125	0.015–0.125	0.05	0.06	0.125	0.015–0.125	0.05
Sulfisoxazole	2.0	4.0	1.0–8.0	2.5	2.0	4.0	0.25–4.0	2.1
TMP-SMX	0.125	0.25	0.03–0.25	0.12	0.125	0.25	0.015–0.25	0.18
Rifampin	0.03	0.03	0.007–0.06	0.02	0.03	0.03	0.007–0.25	0.03
Ciprofloxacin	0.007	0.015	0.004–0.015	0.007	0.007	0.015	0.004–0.015	0.007
Pefloxacin	0.125	0.25	0.125–0.25	0.16	0.125	0.25	0.06–0.25	0.16
Aztreonam	1.0	2.0	0.125–16	1.0	0.25	2.0	0.125–4.0	0.77
Imipenem	0.125	0.25	0.03–1.0	0.11	0.125	0.25	0.03–1.0	0.10
Gentamicin	0.125	0.25	0.06–0.25	0.17	0.125	0.25	0.06–0.5	0.17
Tobramycin	0.125	0.25	0.06–0.25	0.19	0.125	0.25	0.125–0.5	0.19
Amikacin	0.5	1.0	0.125–1.0	0.47	0.5	1.0	0.125–2.0	0.63
Netilmicin	0.5	0.5	0.25–0.5	0.49	0.5	0.5	0.5	0.5

[a] Amox-clav, Amoxicillin-clavulanate (2:1); Amp-sulb, ampicillin-sulbactam (2:1); Ticar-clav, ticarcillin-clavulanate tested with a 2.0-μg/ml fixed concentration of clavulanate with various concentrations of ticarcillin; Erythro-sulfa, erythromycin-sulfisoxazole (1:64); TMP-SMX, trimethoprim-sulfamethoxazole (1:19). In all cases, the concentrations listed in the table are the concentrations of the first antimicrobial agent of the combination.

[b] 50% and 90%, MIC for 50 and 90% of strains tested, respectively.

these 15 agents, however, several patterns of activity were noted. The combination of erythromycin and sulfisoxazole was essentially comparable to either agent when tested alone. Ciprofloxacin demonstrated considerably greater activity than did pefloxacin. Similarly, imipenem was more active than aztreonam. Finally, among the four aminoglycosides tested, gentamicin and tobramycin were significantly more active than amikacin and netilmicin.

Tables 2 and 3 show comparisons of the results of agar dilution MIC determinations versus the results of broth microdilution and tube macrodilution procedures, respectively. In general, there was little difference between MICs obtained with eight antimicrobial agents when they were tested in the broth microdilution and agar dilution formats (Table 2). In contrast, MICs obtained with the broth tube macrodilution procedure were, in general, approximately one twofold-concentration increment higher than those obtained by using the agar dilution method (Table 3).

The eight antimicrobial agents selected for these compar-

TABLE 2. Comparison between results of broth microdilution and agar dilution MIC determinations with 33 strains of *B. catarrhalis*

Antimicrobial agent[a]	No. of strains with indicated ratio of broth microdilution MIC ($\log_2$) to agar dilution MIC ($\log_2$):						
	−3	−2	−1	0	+1	+2	+3
Ampicillin	1		3	24	3	1	1
Chloramphenicol		2	5	20	2	3	1
Cephalothin	2	1	1	26	1	1	1
Cefaclor		1	4	25	2		1
Tetracycline		4		22	2	4	1
Erythromycin	2	1	3	25		2	
TMP-SMX		4	6	19	3		1
Amox-clav		1	1	30		1	

[a] TMP-SMX, Trimethoprim-sulfamethoxazole (1:19); Amox-clav, amoxicillin-clavulanate (2:1).

TABLE 3. Comparison between results of broth tube macrodilution and agar dilution MIC determinations with 33 strains of *B. catarrhalis*

Antimicrobial agent[a]	No. of strains with indicated ratio of broth tube macrodilution MIC (log_2) to agar dilution MIC (log_2):						
	−2	−1	0	+1	+2	+3	+4
Ampicillin	1	1	4	19	6	1	1
Chloramphenicol	1	1	3	26	2		
Cephalothin	1	1	7	21	2	1	
Cefaclor	1	1	9	17	5		
Tetracycline	1		4	14	13	1	
Erythromycin	1	1	4	23	4		
TMP-SMX	1	2	3	13	9	3	2
Amox-clav	1		6	22	3		1

[a] TMP-SMX, Trimethoprim-sulfamethoxazole (1:19); Amox-clav, amoxicillin-clavulanate (2:1).

isons were the same as those used in a previous study aimed at defining zone diameter interpretive criteria for disk diffusion susceptibility tests with *B. catarrhalis* (6). The results of broth tube macrodilution MIC determinations in that study were essentially identical to those obtained with this method in the present investigation.

The results of this study emphasize several important points concerning the activities of selected antimicrobial agents versus *B. catarrhalis*. The in vitro activities of beta-lactam compounds for this organism vary greatly, depending on the agent. In addition, the inhibitory effect of the β-lactamases of *B. catarrhalis* on the activity of beta-lactam antimicrobial agents is variable. However, it remains that, with the exceptions of penicillin and ampicillin, resistance was not noted with any of the beta-lactam antimicrobial agents examined in this investigation with either β-lactamase-positive or -negative strains. The results of this investigation also corroborate and extend previous observations that numerous other antimicrobial agents have extensive in vitro activity for *B. catarrhalis* (1, 2, 4, 11). These include a variety of oral and parenteral agents.

Finally, it should be noted that the results of quantitative susceptibility tests with *B. catarrhalis* are influenced by test format. In general, MICs obtained using agar dilution tests with an inoculum of ca. 10^4 CFU per spot are equivalent to MICs determined using broth microdilution procedures with an inoculum of ca. 10^5 CFU/ml. In contrast, MICs obtained with the broth tube macrodilution technique using an inoculum size of ca. 10^5 CFU/ml are one twofold-concentration increment higher. This is perhaps explained by the 10-fold-higher number of organisms exposed to antimicrobial agent in the tube macrodilution format, since the inoculum effect is known to influence the results of quantitative in vitro susceptibility tests with *B. catarrhalis* (5).

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