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In Vitro Activities of Aztreonam, Imipenem, and Amoxycillin-Clavulanate against Ampicillin-Resistant *Haemophilus influenzae*

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Two hundred and fifty-seven ampicillin-resistant clinical isolates of *Haemophilus influenzae* were tested by disk diffusion and MIC determination for susceptibility to aztreonam, imipenem, and amoxycillin combined with clavulanate. The modal MICs and MICs for 50 and 90% of isolates of all three antimicrobial agents for the 157 β-lactamase-positive strains did not differ significantly from figures obtained with 2,201 ampicillin-susceptible *H. influenzae* by the same methods. Aztreonam and amoxycillin-clavulanate were less active, as reflected by an increase in these parameters, against 38 β-lactamase-negative isolates requiring ≥4 μg of ampicillin per ml for inhibition and 62 strains considered to have an intermediate degree of nonenzymic (intrinsic) resistance to ampicillin (zone diameters of <20 mm with 2-μg ampicillin disks and MICs of 1 or 2 μg/ml). There was no detectable difference in imipenem activity against these 100 strains compared with that observed against the ampicillin-susceptible group. Of the 24 strains requiring at least 4 μg of imipenem per ml for inhibition, 13 also showed reduced susceptibility to ampicillin (5 β-lactamase-positive and 8 β-lactamase-negative isolates). A lack of correlation between reduced susceptibility to imipenem and the other β-lactams was observed.

The prevalence of β-lactamase-positive *Haemophilus influenzae* in the United Kingdom increased significantly between 1977 and 1981 (7, 15) but has changed little in the last 5 years (16). Studies in several other countries have reported that a very high prevalence of such strains is found in some areas, with a greater incidence among type b strains (2, 4, 8). The chemical configurations of the monobactam aztreonam and the carbapenem imipenem (*N*-formimidoyl thienamycin) confer marked stability in the presence of a wide variety of β-lactamases (11, 20), while the combination of amoxycillin with the β-lactamase inhibitor clavulanic acid protects the aminopenicillin from attack by many such enzymes (18).

Studies with all three compounds against β-lactamase-producing *H. influenzae* have suggested that activity is retained in the presence of enzyme production (predominantly TEM-1), and several have found that activity is reduced to a variable extent against the small numbers of β-lactamase-negative strains examined that show non-enzyme-mediated (intrinsic) resistance to ampicillin (6, 14).

A 1986 national survey of resistance among clinical isolates of *H. influenzae* (16) provided 100 β-lactamase-negative strains that show reduced zone diameters (<20 mm) with 2-μg ampicillin disks and that are inhibited by concentrations of ampicillin ranging from 1 to 64 μg/ml. The in vitro activities of the β-lactamase-stable compounds against these intrinsically resistant isolates were compared with results obtained with 157 β-lactamase-positive and 2,201 ampicillin-susceptible strains.

MATERIALS AND METHODS

Antibiotics. Standard laboratory powders were obtained from Beecham Pharmaceuticals (ampicillin), Merck Sharp & Dohme (imipenem), and E. R. Squibb & Sons (aztreonam). Augmentin was supplied as Adatabs (Mast Laboratories) containing amoxycillin and clavulanic acid in the ratio 2:1.

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Source and identification of isolates. The 2,458 strains were collected from 23 United Kingdom clinical laboratories during the first 3 months of 1986. Organisms were identified as *H. influenzae* if they were XV dependent by disk testing on nutrient agar (Southern Group Laboratories), CO_2 nonrequiring, and porphyrin production test negative (9). Strains showing irridescence on Fildes agar were serotyped by slide agglutination using antisera from Wellcome Reagents Ltd.

Susceptibility testing. Strains were grown for 5 h at 37°C in nutrient broth containing 5% inactivated Fildes extract (Oxoid Ltd.). Dilutions (1/100) were made in peptone water and were used (i) for inoculation by swabbing of DST (Oxoid) agar plates, supplemented with 0.25% (vol/vol) lysed horse blood and 10 μg of NAD per ml, to which 6-mm disks containing 2 μg of ampicillin, 30 μg of aztreonam, 10 μg of imipenem, and 2 μg of amoxycillin and 1 μg of clavulanic acid were applied; and (ii) for inoculation by a Denley multipoint inoculator (giving an approximate inoculum size of 10^4 CFU) of DST agar plates supplemented as described above to which doubling dilutions of antibiotics had been added to produce final plate concentrations ranging from 256 to 0.008 μg of ampicillin per ml, 64 to 0.008 μg of aztreonam per ml, 64 to 0.12 μg of imipenem per ml, and 256 to 0.12 μg of amoxycillin per ml combined with clavulanic acid in a 2:1 concentration ratio. Organisms showing ampicillin disk zone diameters of <20 mm were tested against the full range of antibiotic concentrations, while those showing zones of ≥20 mm were tested only against the following ranges: 16 to 0.008 μg of aztreonam per ml, 8 to 0.12 μg of imipenem per ml, and 16 to 0.12 μg of amoxycillin per ml combined with clavulanate as described above; and on 2-μg/ml ampicillin plates. All plates were incubated for 18 h at 37°C in an atmosphere of 95% air and 5% CO_2.

β-Lactamase detection. Strains showing zones of <20 mm and/or for which ampicillin MICs were ≥2 μg/ml were tested

TABLE 1. Modal and calculated MICs of three β-lactamase-stable β-lactams for four groups demarcated by susceptibility to ampicillin

Organisms (no. of isolates)	MIC (μg/ml)[a]								
	Aztreonam			Amoxycillin-clavulanate[b]			Imipenem		
	50%	Mode	90%	50%	Mode	90%	50%	Mode	90%
Ampicillin susceptible (2,201)	0.06	0.06	0.12	0.50	0.50	1.00	1.00	1.00	2.00
β-Lactamase positive (157)	0.06	0.06	0.12	0.50	0.50	1.00	1.00	1.00	2.00
β-Lactamase negative; zone diam, <20 mm; MIC, 1 or 2 μg/ml (62)	0.12	0.12	0.50	1.00	1.00	4.00	1.00	1.00	2.00
β-Lactamase negative; MIC, ≥4 μg/ml (38)	0.25	0.50	1.00	4.00	4.00	8.00	1.00	1.00	2.00

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b] MICs of amoxycillin.

for β-lactamase production by iodometric (3) and acidometric (Oxoid β-lactamase strips) methods.

RESULTS

The modal MICs and MICs for 50 and 90% of isolates of the three β-lactamase-stable compounds were clearly very similar for the 157 β-lactamase-positive and 2,201 ampicillin-susceptible strains (Table 1). (The latter group comprised 2,169 isolates showing ampicillin zones of ≥20 mm and MICs of <2 μg/ml and 32 showing zones of 18 or 19 mm and MICs of 0.25 or 0.5 μg/ml.) These MIC parameters were much higher for the amoxycillin-clavulanate combination (expressed throughout as the concentration of amoxycillin present) and aztreonam but not imipenem against the two groups showing intrinsic resistance to ampicillin. Table 2 shows the distribution of the 257 strains considered to have some degree of resistance to ampicillin according to MICs of the four agents tested and includes a comparison of the β-lactamase-positive and -negative groups.

Isolates with non-enzyme-mediated ampicillin resistance accounted for 100% of strains requiring at least 4 μg of amoxycillin-clavulanate per ml, 90% of those requiring ≥0.5 μg of aztreonam per ml, and three of the four strains requiring concentrations of ≥8 μg of imipenem per ml for inhibition. However, 13 (54%) of the 24 strains inhibited by ≥4 μg of imipenem per ml showed ampicillin zone diameters of <20 mm, of which 5 were β-lactamase positive and 8 showed intrinsic resistance.

Of these 24 isolates, 2 (imipenem MICs, 4 and 16 μg/ml) required at least 0.5 μg of aztreonam per ml for inhibition; both were β-lactamase negative and inhibited by ≥4 μg of ampicillin and amoxycillin-clavulanate per ml. The remaining 22 isolates in this group were all inhibited by ≤0.25 μg of aztreonam per ml, and only those with non-β-lactamase-mediated resistance to ampicillin showed reduced susceptibility to amoxycillin-clavulanate.

Of the 66 type b strains, 12 produced β-lactamase and 6 were nonproducers of enzyme requiring ≥4 μg of ampicillin per ml for inhibition. All type b strains were inhibited by <4 μg of imipenem per ml. Four of the six strains showing intrinsic resistance to ampicillin were inhibited by ≥0.5 μg of aztreonam per ml, and all six were inhibited by ≥4 μg of amoxycillin-clavulanate per ml.

The coefficient of correlation between zone sizes and MICs was very poor for amoxycillin-clavulanate ($r = -0.43$). There was no correlation for aztreonam ($r = -0.13$) and imipenem ($r = -0.17$).

DISCUSSION

As predicted based on the known β-lactamase stability of these three β-lactams and in agreement with previous studies, no difference in activity was observed against the ampicillin-susceptible and β-lactamase-producing ampicillin-resistant groups.

Although clavulanic acid has weak broad-spectrum antibacterial activity, its combination with amoxycillin produced no enhancement of activity against strains showing intrinsic resistance to ampicillin compared with the activity of ampicillin alone; 75% of isolates in this group were inhibited by amoxycillin concentrations (in combination with clavulanate) equal to or one dilution step from the respective MICs of ampicillin. This is in agreement with studies which compared in vitro activities of ampicillin and amoxycillin alone against β-lactamase-negative *H. influenzae* (14). The coefficient of correlation between zone size and MIC was poor—a problem noted by others (21; P. J. Turner, Ph.D. thesis, University of Aston, Birmingham, England, 1985)—but strains requiring ≥4 μg of amoxycillin-clavulanate per ml for

TABLE 2. Distribution of 157 β-lactamase-positive and 100 β-lactamase-negative isolates showing reduced zones (<20 mm) with 2-μg ampicillin disks according to MICs of the four β-lactams tested

Drug	β-Lactamase	No. of isolates for which MIC (μg/ml) was as follows:											
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
Ampicillin	+							1	1	5	15	18	117
	−								38	24	23	6	9
Amoxycillin-clavulanate[a]	+						33	73	48	3			
	−							16	28	15	30	9	2
Aztreonam	+		8	22	88	35	3	1					
	−	1	2	2	16	26	23	19	7	3	1		
Imipenem	+					6	11	31	61	43	5		
	−					4	2	27	47	12	5	2	1

[a] MICs of amoxycillin.

inhibition all showed zones of <20 mm with the combination disk.

Aztreonam is highly active in vitro against *H. influenzae*, but concentrations higher than the mode were needed to inhibit a proportion of the intrinsically resistant group. Even so, for all 2,458 strains tested, MICs were lower than the 8-μg/ml susceptibility breakpoint previously adopted for members of the family *Enterobacteriaceae* (1). While the ampicillin susceptibility breakpoint for members of the *Enterobacteriaceae* is also 8 μg/ml, that for *Haemophilus* spp. is 2 μg/ml (13). Extrapolation of this value to aztreonam might not be unreasonable but would still exclude only four isolates from the susceptible range. On the basis of in vitro susceptibility testing results and what is known about levels achieved in blood and tissues (19), aztreonam appears likely to be a suitable agent for the treatment of most *H. influenzae* infections for which parenteral therapy is required.

There was a lack of correlation between reduced susceptibility to imipenem and any of the other agents tested. In contrast with findings for aztreonam and amoxycillin-clavulanate, strains with intrinsic resistance to ampicillin accounted for only one third of the 24 isolates requiring ≥4 μg of imipenem per ml for inhibition. Reduced susceptibility to imipenem without cross-resistance to other β-lactams has been described in other species, including *Pseudomonas aeruginosa*, in which it has been suggested that this selectivity may be due to the presence of an imipenem-specific porin (17). Findings of this study support a hypothesis that changes occur at sites of penetration through the outer membrane of *H. influenzae* which are unique to this molecule and confer a degree of resistance (although all but four isolates were inhibited by less than the susceptibility breakpoint MIC adopted for clinical trials [10]).

The predictive values of the 10-μg imipenem and 30-μg aztreonam disks in relation to the observed MICs of these antibiotics are poor. The 2-μg ampicillin disk has been found by others to provide more reliable results than larger-value disks for *H. influenzae* with reduced susceptibility, whether or not the resistance was enzyme mediated (12, 22). In addition, recently published results comparing discrimination of different disk concentrations of a new cephalosporin suggest that 5 or 10 μg is superior to 30 μg for many species, including *H. influenzae* (5). Hence, smaller-value disks may prove to be more suitable for this species.

Although the prevalence of *H. influenzae* isolates possessing a degree of intrinsic resistance to ampicillin has increased in the United Kingdom in the past 5 years, the actual numbers remain small (16). The increasing use of β-lactams resistant to enzymic hydrolysis may lead to a greater prevalence of those organisms among clinical isolates. A proportion will exhibit reduced susceptibility to other β-lactams and may pose considerable therapeutic problems, particularly in areas where *H. influenzae* isolates are frequently resistant to the non-β-lactam agents commonly used in treatment.

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