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In Vitro Antimicrobial Production of β-Lactamases, Aminoglycoside-Modifying Enzymes, and Chloramphenicol Acetyltransferase by and Susceptibility of Clinical Isolates of *Acinetobacter baumannii*

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Antimicrobial susceptibility testing was performed on 54 epidemiologically unrelated clinical isolates of *Acinetobacter baumannii* by using a standard agar dilution technique. On the basis of the in vitro activities, imipenem and doxycycline were the most active agents, whereas amikacin, isepamicin, and the new fluorquinolones ciprofloxacin and ofloxacin presented moderate activity. Cephalosporinase activity was found in 98% of the strains, whereas lactamases of TEM type 1 and one with a pI of 7 to 7.5 were present in 16 and 11% of the strains, respectively. Resistance to aminoglycosides was explained by the production of the three classes of aminoglycoside-modifying enzymes, with predominance of aminoglycoside-3′-phosphotransferase VI in 28% of the strains.

In recent years, *Acinetobacter baumannii* has emerged as an important nosocomial pathogen in intensive care units (7, 16, 17, 34, 36, 38). This organism is capable of causing life-threating infections such as bacteremia, pneumonia, and meningitis (2, 3, 6, 32), and it is often multidrug resistant and difficult to erradicate. A progressive increase in the resistance of *A. baumannii* has been reported since 1980 (1, 11). However, information on the in vitro antibiotic susceptibilities of *A. baumannii* is quite limited. Only a few studies have examined large numbers of isolates of this organism by quantitative dilution methods. The purpose of this study was to evaluate the activity of 25 antimicrobial agents against *A. baumannii*, and since a high level of resistance to β-lactams, aminoglycosides, and chloramphenicol has been found, we also studied whether the mechanism of resistance to these antibiotics involved production of β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferase.

Susceptibility testing was performed with 54 isolates obtained from different clinical specimens in three Spanish hospitals. Identification of *A. baumannii* strains was accomplished with various biochemical tests by the API NE System (bioMerieux, Marcy l'Etoile, France) and complementary tests following the biochemical criteria of Bouvet and Grimont (4). All antimicrobial agents were obtained from their respective manufacturers in the form of standard laboratory powders. Susceptibility testing was performed by using a previously described agar dilution method and in accordance with the guidelines established by the National Committee for Clinical Laboratory Standards (28). Approximately 10^4 CFU of each isolate was inoculated onto freshly prepared media containing serial dilutions of different antimicrobial agents with a multipoint replicator. Antibiotic powders were kindly supplied by the following manufacturers: enoxacin (Laboratorios Almirall, S.A. Barcelona, Spain), ampicillin (Antibioticos, S.A., Leon, Spain), ciprofloxacin (Bayer, Leverkusen, Germany), amoxicillin, amoxicillin plus clavulanic acid, ticarcillin, and ticarcillin plus clavulanic acid (Beecham Laboratories, Brentford, United Kingdom), amikacin (Bristol-Myers Laboratories), tobramycin (Eli Lilly and Co.), trimethoprim-sulfametoxazole (Gayoso Wellcome, London, United Kingdom), ceftazidime (Glaxo Pharmaceuticals), cefotaxime and ofloxacin (Hoechst, Frankfurt, Germany), chloramphenicol (Ifesa, Barcelona, Spain); piperacillin (Lederle Laboratories, Pearl River, N.Y.), imipenem and norfloxacin (Merck Research Laboratories, Rahway, N.J.), ampicillin plus sulbactam and doxycycline (Pfizer, Inc., New York, N.Y.), ceftriaxone (Roche), gentamicin, netilmicin and isepamicin (Schering Corp., Bloomfield, N.J.), ceftizoxime (Smith Kline & French, Philadelphia, Pa.), and aztreonam (Squibb), Amoxicillin plus clavulanic acid and ampicillin plus sulbactam were tested in a ratio of 2:1, whereas ticarcillin plus clavulanic acid was tested at 2 μg of clavulanic acid per ml. Trimethoprim-sulfamethoxazole was tested in a ratio of 1:19.

The aminoglycoside-modifying enzymes were assayed by a phosphocellulose paper binding assay (15) from crude bacterial extracts of strains prepared by sonication. β-Lactamase activity was quantified by the spectrophotometric assay of nitrocefin hydrolysis (30) in 0.1 M phosphate buffer (pH 7.0), whereas β-lactamases were analyzed by isoelectric focusing as described by Matthew et al. (24). Their isoelectric points were determined by comparison with enzymes of known pIs. The chloramphenicol acetyltransferase assay was performed following the method described by Robinson et al. (33).

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TABLE 1. MICs of 25 antimicrobial drugs against 54 *A. baumannii* clinical isolates

Antibiotic	MIC (μg/ml)[a]				% Susceptible[b]
	Breakpoint	Range	50%	90%	
Amikacin	16	0.5–>256	4	128	72
Amoxicillin	8	4–>256	64	>256	2
Amoxicillin-clavulanic acid	8	4–32	16	32	16
Ampicillin	8	8–>256	64	>256	2
Ampicillin-sulbactam	8	2–64	4	16	52
Aztreonam	8	4–>256	32	128	2
Cefotaxime	8	0.5–128	16	64	31
Ceftazidime	8	0.5–32	8	32	55
Ceftizoxime	8	0.5–128	16	128	33
Ceftriaxone	8	1–128	16	64	24
Ciprofloxacin	1	0.06–64	0.5	16	70
Chloramphenicol	8	16–>256	128	256	0
Doxycycline	4	0.06–16	0.25	4	98
Enoxacin	2	0.5–>256	4	32	24
Gentamicin	4	0.5–>256	2	>256	33
Imipenem	4	0.125–1	0.5	1	100
Isepamicin	16	0.5–128	4	64	72
Netilmicin	8	0.125–>256	4	64	66
Norfloxacin	2	2–>256	8	64	18
Ofloxacin	4	0.125–32	0.5	4	72
Piperacillin	16	1–>256	32	>256	33
Ticarcillin	16	2–>256	64	256	30
Ticarcillin-clavulanic acid	16	<1–>256	32	256	31
Tobramycin	4	0.125–>256	1	256	50
Trimethoprim-sulfamethoxazole	2	0.25–32	2	32	63

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.
[b] Percentage of strains susceptible to the breakpoint concentration (28).

The results of testing the 54 *A. baumannii* strains against the 25 antibiotics and antibiotic–β-lactamase inhibitor combinations are shown in Table 1. These strains were isolated from unrelated patients and hospitals, and the majority were epidemiologically different. Almost all isolates were resistant to both ampicillin and amoxicillin. The addition of the β-lactamase inhibitor sulbactam increased the percentage of strains susceptible to ampicillin from 0 to 52%, whereas the addition of clavulanic acid to amoxicillin or ticarcillin did not significantly change the percentage of susceptible strains. In our study, more than 50% of the strains showed resistance to piperacillin, cefotaxime, ticarcillin, and ceftazidime. This result was in agreement with that presented by Mueller-Serieys et al. (26), who found that the majority of *Acinetobacter* strains tested were resistant to these antibiotics.

Acinetobacter strains are naturally resistant to cephalosporins due to the production of cephalosporinase (25), and we have found cephalosporinase activity in 98% of the strains. The cephalosporinase activity was based on the presence of bands of β-lactamase activity above pI 8. A TEM-1 enzyme (pI 5.4) was identified in 16% of the strains. The resistance to ampicillin and to carboxy- and ureidopenicillins has been attributed to the presence of TEM-1 (13, 31) or TEM-2 β-lactamases (9). Recently, Joly-Guillou et al. (20) studying 100 *Acinetobacter* strains identified a TEM-1 type enzyme in 34% of the strains. β-Lactamase activity at pI 7 to 7.5 was found in 11% of the strains, and this β-lactamase may be one of the novel broad-spectrum β-lactamases described previously (35) and designated as ceftazidimases, which have been previously described for *A. baumannii* (19).

The aminoglycosides were not uniformly active; 50% of the isolates were susceptible to tobramycin, 33% were susceptible to gentamicin, 66% were susceptible to netilmicin, and 72% were susceptible to amikacin and isepamicin. The study of susceptibility to gentamicin, tobramycin, amikacin, and netilmicin allowed us to define eight phenotypic resistance patterns. Among those, Gen^s Tob^s Amk^s Net^s (33%), Gen^r Tob^r Amk^s Net^r (19%), and Gen^r Tob^r Amk^r Net^s (20%) were the most frequently isolated. This result is in contrast to that of Muller-Serieys et al. (26), who found the phenotype of resistance to the four aminoglycosides as the most frequently encountered.

Many studies have shown the presence of aminoglycoside-modifying enzymes in clinical strains of *A. baumannii* (1, 14, 27). The most frequently occurring aminoglycoside-modifying enzyme found in our study was aminoglycoside-3′-phosphotransferase VI [APH (3′)-VI] (28%), a new type of 3′-*o*-phosphotransferase which inactivates amikacin (22, 23). Recently, Buisson et al. (5) have found a significant correlation between amikacin consumption and the emergence of amikacin resistance mediated by APH (3′)-VI in *Acinetobacter* species. The nucleotidylating enzyme 3″-adenylytransferase (9), which modifies streptomycin and spectinomycin, was detected in 15% of the strains; this enzyme has been found in other studies (13, 27). The enzyme 3-*N*-acetyltransferase I was detected in 4% of the strains and always together with APH (3′)-VI. Thus, the presence of modifying enzymes belonging to the three classes is responsible for the resistance of *Acinetobacter* strains to a great number of aminoglycosides. However, 19% of the strains that were resistant to several aminoglycosides (netilmicin, tobramycin, and gentamicin) did not contain detectable inactivating enzymatic activities and could possess other mechanisms of aminoglycoside resistance, such as diminished permeability or alteration of the binding sites.

Among quinolones, ciprofloxacin (70%) and ofloxacin (72%) were more active in vitro than norfloxacin (18%). The frequency of selecting quinolone resistant mutants seems to

be higher in nonfermenting organisms than in enterobacteria (10, 12, 21). In contrast to the results of other studies (18), all tested isolates were resistant to chloramphenicol, although we have not found chloramphenicol acetyltransferase activity in any of them.

This study suggests that β-lactam agents and aminoglycosides may not be ideal empiric agents for the treatment of *A. baumannii* infections, except for amikacin and isepamicin which together with the new fluorquinolones ciprofloxacin and ofloxacin possess moderate activity. Imipenem and doxycycline demonstrated the best in vitro activity. Doxycycline was also cited as the most active of 21 antibiotics tested by Obana et al. (29), and imipenem is normally found to be the most active antibiotic against *Acinetobacter* strains (8, 20, 37, 38).

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