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Activities of β-Lactams against *Acinetobacter* Genospecies as Determined by Agar Dilution and E-Test MIC Methods

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The agar dilution MIC method was used to test activities of ticarcillin, ticarcillin-clavulanate, amoxicillin, amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, inhibitors alone, ceftazidime, and imipenem against 237 *Acinetobacter* genospecies. A total of 93.2% of strains were β-lactamase positive by the chromogenic cephalosporin method. Overall, ampicillin-sulbactam was the most active combination against all strains (MIC at which 50% of the isolates are inhibited [MIC_{50}] and MIC_{90}, 4.0 and 32.0 μg/ml; 86.9% susceptible at ≤16 μg/ml), followed by ticarcillin-clavulanate (16.0 and 128.0 μg/ml; 85.7% susceptible at ≤64 μg/ml), piperacillin-tazobactam (16.0 and 128.0 μg/ml; 84.8% susceptible at ≤64 μg/ml), and amoxicillin-clavulanate (16.0 and 64.0 μg/ml; 54.4% susceptible at ≤16 μg/ml). Ceftazidime and imipenem yielded MIC_{50}s and MIC_{90}s of 8.0 and 64.0 μg/ml (ceftazidime) and 0.5 and 1.0 μg/ml (imipenem), respectively; 71.3% of strains were susceptible to ceftazidime at ≤16 μg/ml, and 99.2% were susceptible to imipenem at ≤8 μg/ml. Sulbactam was the most active β-lactamase inhibitor alone (MIC_{50} and MIC_{90}, 2.0 and 16.0 μg/ml); clavulanate and tazobactam were less active (16.0 and 32.0 μg/ml for both compounds). Enhancement of β-lactams by β-lactamase inhibitors was not always seen in β-lactamase-positive strains, and activity of combinations such as ampicillin-sulbactam was due to the inhibitor alone. *Acinetobacter baumannii* was the most resistant genospecies. By contrast, *Acinetobacter haemolyticus*, *Acinetobacter calcoaceticus*, *Acinetobacter johnsonii*, *Acinetobacter junii*, *Acinetobacter radioresistens*, and other non-*Acinetobacter baumannii* strains were more susceptible to all compounds tested. E-test MICs were within 1 dilution of agar dilution MICs in 38.4 to 89.6% of cases and within 2 dilutions in 61.6 to 98.6% of cases.

Gram-negative nonfermentative rods are increasingly implicated as causative agents in human disease, acquired through contact with the environmental strains as well as by nosocomial transmission (1–4, 7, 9, 29). Although *Pseudomonas aeruginosa* is the nonfermenter most commonly encountered clinically, other gram-negative nonfermentative rods are being recovered with increasing frequency from debilitated or immunosuppressed patients (1–4, 7, 9, 24, 25, 28, 29). Antimicrobial susceptibility patterns of the nonfermenters differ from those of *Enterobacteriaceae* in many respects, and among the nonfermenters many groups have susceptibility patterns which differ from each other as well as from that of *P. aeruginosa* (1–4, 7, 9, 28, 29). The unpredictability and breadth of drug resistance of many nonfermenters, and the development of new antimicrobial agents with wider spectra of activity against these organisms, make in vitro susceptibility testing an essential part of patient management.

Members of the genus *Acinetobacter* are commonly encountered in human infections in the patient groups mentioned above (5, 13, 24, 25, 28). These organisms are inherently resistant to many antimicrobials in common use, including β-lactams, macrolides, and some quinolones (1–14, 16–29). Recent changes in taxonomy of these organisms, now comprising 19 genospecies, have complicated the ability to monitor susceptibility data (28). This study has examined the susceptibility of 237 *Acinetobacter* strains to ticarcillin, ticarcillin-clavulanate, amoxicillin, amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, each β-lactamase inhibitor alone, ceftazidime, and imipenem by agar dilution and E-test methods.

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MATERIALS AND METHODS

Bacteria and antimicrobials. Organisms were all clinical isolates from Hershey Medical Center, Hershey, Pa., Case Western Reserve University and the Cleveland Clinic Foundation, Cleveland, Ohio, the California State Department of Health, Berkeley, the University of Zürich Medical School, Zürich, Switzerland, and the University of Leiden Medical School, Leiden, The Netherlands. Preliminary identification to genus level was by the NF Plus method (Innovative Diagnostic Systems, Inc., Norcross, Ga.). Screening by beta-hemolysis and by assessment of growth at 37, 41, and 44°C was also performed (28). Assignment to *Acinetobacter baumannii* or non-*A. baumannii* was by gas chromatography of cellular fatty acids (MIDI, Newark, Del.), except for five American Type Culture Collection strains (two *A. johnsonii*, two *A. haemolyticus*, and one *A. junii* strain) and twenty strains from The Netherlands (five *A. junii*, eight *A. johnsonii*, three *A. calcoaceticus*, one *A. haemolyticus*, and three *A. radioresistens* strains), for which extensive conventional and DNA homology testing was carried out. Antibiotic powders were obtained from their respective manufacturers.

Agar dilution MIC determination. Agar dilution MICs were determined for 237 strains according to standard methods with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) (15). Clavulanate was added to ticarcillin at a fixed concentration of 2.0 μg/ml, and tazobactam was added to piperacillin at a fixed concentration of 4.0 μg/ml. Clavulanate and sulbactam were added to amoxicillin and ampicillin, respectively, at a 1:2 ratio. All inhibitors were also tested alone. Mueller-Hinton plates containing antibiotic dilutions were inoculated with 10^4 CFU of organisms per spot, and plates were incubated overnight at 35°C before interpretation. Standard quality control strains were included in each run. β-Lactamase production was tested by the chromogenic cephalosporin disk method (Cefinase; BBL).

E-test MICs. MICs for 211 strains (192 *A. baumannii* and 19 non-*A. baumannii*) were determined by E-test methods according to standard practice. Amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin-clavulanate, piperacillin-tazobactam, ceftazidime, and imipenem E-test strips (AB Biodisk, Solna, Sweden) were placed radially on Mueller-Hinton plates inoculated with a 0.5 McFarland

standard. After overnight incubation at 35°C, MICs were read at the intersection of growth inhibition with the strip. All E tests were performed in duplicate, and the mean was determined.

RESULTS

The NF Plus system identified all strains to genus level only, with differentiation into glucose oxidizers and glucose nonoxidizers. Further testing by beta-hemolysis and growth at differential temperatures (28) differentiated strains as *A. baumannii* and non-*A. baumannii*. Too few non-*A. baumannii* strains were tested for valid conclusions on identification to be made by the latter methods.

Results of MIC testing are presented in Table 1. As can be seen, 93.2% of strains were β-lactamase positive. *A. baumannii* strains were the most resistant strains tested. Non-*A. baumannii* strains were more susceptible to all antimicrobials tested. Sulbactam was the most active of all the β-lactamase inhibitors tested. Enhancement of β-lactams by β-lactamase inhibitors was not always seen in β-lactamase-producing strains. Ampicillin-sulbactam was the most active β-lactam–β-lactamase inhibitor combination tested, mainly because of the antimicrobial activity of sulbactam alone. At ticarcillin-clavulanate and piperacillin-tazobactam breakpoints of ≤64.0 μg/ml (susceptibility breakpoints for *P. aeruginosa* [15]), 85.7% of strains were susceptible to ticarcillin-clavulanate and 84.8% of strains were susceptible to piperacillin-tazobactam. At breakpoints of ≤16.0 μg/ml (intermediate breakpoints for *Enterobacteriaceae* and organisms other than staphylococci [15]), 86.9% of strains were susceptible to ampicillin-sulbactam and 54.4% were susceptible to amoxicillin-clavulanate. At an intermediate breakpoint of ≤16.0 μg/ml (15), 71.3% of strains were susceptible to ceftazidime, while at an intermediate breakpoint of ≤8 μg/ml (15), 99.2% of strains (235 of 237) were susceptible to imipenem. Ceftazidime was active mainly against non-*A. baumannii* strains.

Table 2 compares the E test with the agar dilution methods for β-lactam–β-lactamase inhibitor combinations, ceftazidime, and imipenem. Duplicate E-test readings were all within 1 dilution. E-test MICs were within 1 dilution of agar dilution MICs in 38.4 to 89.6% of cases. Agreement within 2 dilutions of agar dilution MICs occurred in 61.6 to 98.6% of cases. The lowest agreement rates occurred with piperacillin-tazobactam.

TABLE 1. Antimicrobial activities against *Acinetobacter* genospecies as determined by agar dilution

Organism and drug	MIC (μg/ml)[a]		
	Range	50%	90%
A. baumannii (195/204)[b]			
Ticarcillin	1.0–256.0	32.0	256.0
Ticarcillin-clavulanate	0.015–256.0	32.0	128.0
Amoxicillin	0.25–256.0	64.0	256.0
Amoxicillin-clavulanate	0.015–128	32.0	64.0
Ampicillin	0.125–256	128.0	256.0
Ampicillin-sulbactam	0.06–256.0	4.0	32.0
Piperacillin	0.5–256.0	32.0	256.0
Piperacillin-tazobactam	0.015–256.0	16.0	128.0
Clavulanate	0.5–32.0	16.0	32.0
Sulbactam	0.5–32.0	2.0	16.0
Tazobactam	0.5–32.0	16.0	32.0
Ceftazidime	0.5–128.0	8.0	64.0
Imipenem	0.06–128.0	0.5	1.0
Non-*A. baumannii* (26/33)[c]			
Ticarcillin	0.5–256.0	8.0	64.0
Ticarcillin-clavulanate	0.015–256.0	0.06	16.0
Amoxicillin	2.0–128.0	16.0	64.0
Amoxicillin-clavulanate	1.0–64.0	8.0	16.0
Ampicillin	2.0–256.0	16.0	64.0
Ampicillin-sulbactam	0.5–64.0	2.0	4.0
Piperacillin	0.5–256.0	16.0	32.0
Piperacillin-tazobactam	0.015–256.0	0.015	16.0
Clavulanate	0.5–32.0	4.0	16.0
Sulbactam	0.5–32.0	0.5	4.0
Tazobactam	0.5–32.0	4.0	16.0
Ceftazidime	1.0–128.0	4.0	16.0
Imipenem	0.06–1.0	0.25	0.5
All strains (221/237)			
Ticarcillin	0.5–256.0	32.0	256.0
Ticarcillin-clavulanate	0.015–256.0	16.0	128.0
Amoxicillin	0.25–256.0	64.0	256.0
Amoxicillin-clavulanate	0.015–128.0	16.0	64.0
Ampicillin	0.125–256.0	64.0	256.0
Ampicillin-sulbactam	0.06–256.0	4.0	32.0
Piperacillin	0.5–256.0	32.0	256.0
Piperacillin-tazobactam	0.015–256.0	16.0	128.0
Clavulanate	0.5–32.0	16.0	32.0
Sulbactam	0.5–32.0	2.0	16.0
Tazobactam	0.5–32.0	16.0	32.0
Ceftazidime	0.5–128.0	8.0	64.0
Imipenem	0.06–128.0	0.5	1.0

[a] 50% and 90%, MICs at which 50 and 90% of the isolates are inhibited, respectively.
[b] Number of Cefinase-positive strains/number tested.
[c] *A. johnsonii* (10 strains), *A. junii* (6 strains), *A. haemolyticus* (3 strains), *A. calcoaceticus* (3 strains), *A. radioresistens* (3 strains), non-*A. baumannii* (8 strains).

DISCUSSION

The NF Plus method differentiated only between glucose-oxidizing and non-glucose-oxidizing *Acinetobacter* strains. Hemolysis and growth at differential temperatures helped in preliminary genospecies classification, and cell wall fatty acid analysis (in a few cases, as above, with more extensive testing) classified strains into *A. baumannii* and non-*A. baumannii* genospecies. Extensive biochemical testing and DNA homology analysis were performed on only a few strains, and the possibility that genospecies identification by cell wall fatty acid analysis was not always correct exists.

Resistance of *A. baumannii* strains to β-lactam and non-β-lactam antibiotics has been described (12, 20, 22). Unfortunately, this is the most commonly encountered genospecies in human infections (12, 20, 22, 28), complicating therapy especially in debilitated patients. Other *Acinetobacter* genospecies are much less commonly encountered clinically (28). The gas-liquid chromatography method employed in differentiating *A. baumannii* from non-*A. baumannii* strains is not the recognized "gold standard" method for this purpose. However, genospecies identification results for *A. baumannii* corresponded well in every case with screening by beta-hemolysis and growth at 37, 41, and 44°C. All strains identified as *A. baumannii* by gas-liquid chromatography in this study were beta-hemolysis negative and grew at all three incubation temperatures (28).

Imipenem was active, at MICs of ≤8 μg/ml, against 235 of 237 strains (99.2%). Although imipenem is usually active against acinetobacters, imipenem-resistant strains have been described (24). The superior activity of ampicillin-sulbactam to those of other β-lactam–β-lactamase inhibitor combinations, due to activity of the inhibitor alone, has been described (11, 16, 23, 26). The activity of ticarcillin-clavulanate was equivalent to that of piperacillin-tazobactam. It is interesting that en-

TABLE 2. Agreement between agar dilution and E test

Antimicrobial	No. of E-test MICs within no. of dilutions of agar dilution MIC							% of MICs within:	
	≥+3	+2	+1	0	−1	−2	≥−3	1 dilution	2 dilutions
Ticarcillin-clavulanate	27	25	54	62	19	16	8	64.0	83.4
Amoxicillin-clavulanate	32	33	28	52	36	16	14	55.0	78.2
Ampicillin-sulbactam	1	2	27	78	66	26	11	81.0	94.3
Piperacillin-tazobactam	75	45	42	33	6	4	6	38.4	61.6
Ceftazidime	6	22	68	65	33	11	6	78.7	94.3
Imipenem	3	15	85	86	18	4	0	89.6	98.6

hancement of the β-lactam by inhibitors was not universally seen in β-lactamase-producing strains. Several mechanisms of resistance to β-lactam and non-β-lactam compounds are operative in *Acinetobacter* genospecies, and production of more than one β-lactamase is not the only mechanism for β-lactam resistance in these strains (10, 17, 19). It should be noted that testing this genus with nitrocefin is not always meaningful, since multiple β-lactamases may be present, contributing to the resistance phenotype, and a negative test may not correspond in this species to β-lactam susceptibility.

Results of this study show that the E test yielded low agreement, within 1 $\log_2$ dilution, with agar dilution, only for imipenem. Duplicate testing showed results to be reproducible. The reasons for these low agreement rates are not clear but may include production of multiple β-lactamases.

Breakpoints approved by the National Committee for Clinical Laboratory Standards (15) may not apply to *Acinetobacter* genospecies, so definitive conclusions as to the relative activities of agents tested cannot be made. Because systemic *Acinetobacter* infections (usually caused by *A. baumannii*) usually occur in immunosuppressed or otherwise debilitated patients, maximal therapeutic doses are indicated. For this reason, we elected to use higher breakpoints in data analysis.

In summary, imipenem was the most active agent against all *Acinetobacter* strains tested. Ampicillin-sulbactam yielded the lowest MICs of other β-lactams tested; ticarcillin-clavulanate and piperacillin-tazobactam yielded equivalent activity, while amoxicillin-clavulanate and ceftazidime were less active.

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