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Antimicrobial Susceptibility of *Acinetobacter* Species

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The in vitro activities of 16 antimicrobial agents against 180 *Acinetobacter* strains isolated from blood cultures ($n = 162$), central venous catheters ($n = 11$), and cerebrospinal fluids ($n = 7$) were studied. MICs were determined by a microtiter broth dilution method. Considerable differences in antimicrobial drug susceptibility against strains belonging to different species could be demonstrated. *Acinetobacter baumannii* isolates ($n = 108$) were generally more resistant than isolates identified as species other than *A. baumannii*. Multidrug resistance was common among *A. baumannii* isolates. Of the antimicrobial agents tested, imipenem was highly active against all *A. baumannii* isolates, and the other agents tested were only moderately active or inactive. Good activity against *Acinetobacter* species strain 3 was demonstrated for imipenem, amikacin, and ciprofloxacin. Most of the strains belonging to other species were susceptible to imipenem, ciprofloxacin, expanded-spectrum cephalosporins, amoxicillin-clavulanate, and the aminoglycosides but were resistant to ampicillin and older cephalosporins.

Acinetobacter species are opportunistic pathogens of low virulence. Their contribution to nosocomial infection, however, has been increasing over the past 30 years (3, 5, 17). Though widely prevalent in nature (2) and generally regarded as commensals of human skin and respiratory and genitourinary tracts (1, 9), they have been implicated as the cause of serious infectious diseases such as meningitis, pneumonia, tracheobronchitis, endocarditis, wound infections, and septicemia, mostly involving patients with impaired host defenses (5). Several outbreaks of hospital infection have been described, many of them due to contamination of hospital equipment and the hands of personnel. Treatment of serious infections due to *Acinetobacter* spp. is complicated by the widespread multidrug resistance of the organism (4, 10, 13, 21).

Until recently, the genus *Acinetobacter* contained the single species *Acinetobacter calcoaceticus* subdivided into the two subspecies or biovars *A. calcoaceticus* subsp. *anitratus* and *A. calcoaceticus* subsp. *lwoffii*. *A. calcoaceticus* subsp. *anitratus* was frequently reported to be much more resistant to antibiotics than *A. calcoaceticus* subsp. *lwoffii* (4, 5, 11, 18).

In 1986, the taxonomy of the genus *Acinetobacter* was changed extensively by Bouvet and Grimont, who outlined 12 different species by DNA-DNA-hybridization, including the named species *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii*, and *A. lwoffii* and six unnamed genomic species (6). Most *A. baumannii* strains and all *Acinetobacter* species strains 3 and 10 strains represent organisms that were formerly classified as *A. anitratus*, whereas all *A. junii*, *A. lwoffii*, and *Acinetobacter* species strain 11 strains were formerly classified as *A. lwoffii*.

Most studies reporting data on antimicrobial drug susceptibilities of *Acinetobacter* spp. were not based on this new taxonomy (4, 11, 18, 21). We therefore decided to study the in vitro activities of several antimicrobial agents against 180 *Acinetobacter* strains belonging to different species.

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MATERIALS AND METHODS

Bacterial strains. All strains were collected at our institution over a period of 18 months from different patients in 11 hospitals, with more than 30 different departments being involved. Isolates were stored in glycerol broth at −70°C. Only strains from relevant clinical sources were included in this work. A total of 162 strains were isolated from blood cultures, 11 were isolated from central venous catheters, and 7 were isolated from cerebrospinal fluid. All strains were identified by carbon source utilization tests according to the simplified identification scheme of Bouvet and Grimont (7). Of these strains, 108 were identified as *A. baumannii*, and 72 could be assigned to other species. Seventeen strains represented *Acinetobacter* species strain 3, 16 were *A. johnsonii*, 15 were *A. lwoffii*, 6 were *A. junii*, 5 were *Acinetobacter* species strain 10, 3 were *Acinetobacter* species strain 12, 3 were *A. haemolyticus*, 2 were *Acinetobacter* species strain 6, and 5 could not be classified. Biotyping was performed on all strains identified as *A. baumannii* as described by Bouvet and Grimont (7). The origins of strains and distributions of species are shown in Table 1.

TABLE 1. Origins of *Acinetobacter* isolates and distributions of species

Species	No. of isolates			
	Blood culture	Central venous catheter	Cerebrospinal fluid	Total
A. baumannii	95	10	3	108
A. haemolyticus	3			3
A. johnsonii	16			16
A. junii	6			6
A. lwoffii	14		1	15
Acinetobacter species strain 3	15		2	17
Acinetobacter species strain 6	2			2
Acinetobacter species strain 10	5			5
Acinetobacter species strain 12	3			3
Acinetobacter strains, ungrouped	3	1	1	5
Total	162	11	7	180

TABLE 2. In vitro activities of 16 antimicrobial agents against 180 isolates of different *Acinetobacter* species

Species	*n*	MIC[a]	Ampicillin	Mezlocillin	Piperacillin	Augmentin	Cefazolin	Cefoxitin	Cefuroxime	Cefotaxime	Ceftazidime	Ceftriaxone	Aztreonam	Imipenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin
A. baumannii biotype 9	95	Range	8–>64	≤4–>256	≤4–>256	2–32	>64–>64	32–>64	8–>64	2–64	≤1–>64	2–64	4–>64	≤0.5–2	≤4–>128	≤1–>32	≤1–>32	≤0.25–>8
		MIC_{50}	16	16	8	4	>64	>64	32	8	8	8	16	≤0.5	32	>32	16	>8
		MIC_{90}	>64	>256	>256	8	>64	>64	>64	16	32	32	>64	≤0.5	64	>32	>32	>8
A. baumannii biotype 6	13	Range	8–>64	16–>256	8–>256	4–16	>64–>64	32–>64	16–>64	4–>128	2–16	4–>128	8–32	≤0.5–1	≤4–>128	≤1–>32	≤1–>32	≤0.25–>8
		MIC_{50}	16	16	16	8	>64	>64	16	4	2	4	16	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	>64	>256	>256	8	>64	>64	>64	>128	16	64	16	≤0.5	>128	>32	2	1
A. haemolyticus	3	Range	2–32	16–32	8–16	≤1–2	>64–>64	16–16	8–16	4–4	2–4	≤1–4	2–8	≤0.5–≤0.5	≤4–32	≤1–2	≤1–16	≤0.25–0.5
		MIC_{50}	8	32	16	≤1	>64	16	8	4	2	2	4	≤0.5	16	2	8	≤0.25
		MIC_{90}	32	32	16	2	>64	16	16	4	4	4	8	≤0.5	32	2	16	0.5
A. johnsonii	16	Range	≤1–>64	≤4–>256	≤4–>256	≤1–8	16–>64	4–>64	2–>64	≤1–>128	≤1–>64	≤1–>128	≤1–32	≤0.5–1	≤4–≤4	≤1–>32	≤1–>32	≤0.25–4
		MIC_{50}	≤1	32	16	≤1	>64	>64	16	4	4	≤1	4	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	4	64	128	4	>64	>64	>64	32	16	16	32	≤0.5	≤4	4	2	0.5
A. junii	6	Range	≤1–4	≤4–16	≤4–8	≤1–2	16–>64	2–8	≤1–8	≤1–4	≤1–2	≤1–4	≤1–4	≤0.5–≤0.5	≤4–>128	≤1–≤1	≤1–≤1	≤0.25–≤0.25
		MIC_{50}	2	16	≤4	≤1	>64	4	4	2	2	2	2	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	4	16	8	2	>64	8	8	4	2	4	4	≤0.5	>128	≤1	≤1	≤0.25
A. lwoffii	15	Range	≤1–4	≤4–128	≤4–128	≤1–2	≤1–>64	≤1–>64	2–>64	≤1–>128	≤1–>64	≤1–>128	≤1–>64	≤0.5–≤0.5	≤4–8	≤1–4	≤1–2	≤0.25–≤0.25
		MIC_{50}	≤1	8	8	≤1	>64	16	4	2	2	≤1	8	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	2	64	64	2	>64	>64	>64	8	8	8	32	≤0.5	≤4	≤1	≤1	≤0.25
Acinetobacter species strain 3	17	Range	4–32	16–64	8–32	2–8	>64–>64	>64–>64	16–>64	8–16	2–8	4–16	8–32	≤0.5–≤0.5	≤4–32	≤1–>32	≤1–8	≤0.25–>8
		MIC_{50}	16	32	16	4	>64	>64	32	8	4	8	16	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	32	64	32	4	>64	>64	>64	16	8	16	32	≤0.5	≤4	>32	8	1
Acinetobacter species strain 6	2	Range	≤1–4	≤4–8	≤4–≤4	≤1–≤1	16–>64	2–8	2–8	≤1–4	≤1–2	≤1–4	2–4	≤0.5–≤0.5	≤4–≤4	≤1–≤1	≤1–≤1	≤0.25–≤0.25
		MIC_{50}	≤1	≤4	≤4	≤1	16	2	2	≤1	≤1	≤1	2	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	4	8	≤4	≤1	>64	8	8	4	2	4	4	≤0.5	≤4	≤1	≤1	≤0.25
Acinetobacter species strain 10	5	Range	16–>64	16–128	8–128	4–16	>64–>64	32–>64	16–32	4–8	4–8	4–4	16–32	≤0.5–4	≤4–32	4–>32	≤1–16	≤0.25–≤0.25
		MIC_{50}	32	32	16	4	>64	>64	16	4	8	4	32	≤0.5	16	16	8	≤0.25
		MIC_{90}	>64	128	128	16	>64	>64	32	8	8	4	32	4	32	>32	16	≤0.25
Acinetobacter species strain 12	3	Range	≤1–2	8–16	8–8	≤1–≤1	>64–>64	8–16	4–8	≤1–4	≤1–2	≤1–4	8–16	≤0.5–≤0.5	≤4–≤4	≤1–≤1	≤1–≤1	≤0.25–0.5
		MIC_{50}	2	16	8	≤1	>64	8	4	2	≤1	≤1	8	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	2	16	8	≤1	>64	16	8	4	2	4	16	≤0.5	≤4	≤1	≤1	0.5
Acinetobacter strains, ungrouped	5	Range	≤1–>64	≤4–>256	≤4–>256	≤1–16	16–>64	4–32	2–16	≤1–4	≤1–4	≤1–4	2–16	≤0.5–8	≤4–32	≤1–16	≤1–16	≤0.25–≤0.25
		MIC_{50}	≤1	8	≤4	≤1	>64	16	4	2	2	2	4	≤0.5	≤4	≤1	≤1	≤0.25
		MIC_{90}	>64	>256	>256	16	>64	32	16	4	4	4	16	8	32	16	16	≤0.25

[a] MIC_{50} and MIC_{90}, MICs for 50 and 90% of isolates tested, respectively.

TABLE 3. Percentage of isolates fully susceptible at NCCLS breakpoints[a]

Species	n	% of isolates fully susceptible to:															
		AMP	MZ	PIP	AUG	CFZ	FOX	CRM	CFT	CAZ	CAX	AZT	IPM	AMK	GM	TOB	CP
A. baumannii biotype 9	95	9	63	64	91	0	0	1	68	68	68	46	100	36	2	2	6
A. baumannii biotype 6	13	8	69	77	92	0	0	0	85	85	85	46	100	85	77	92	92
A. haemolyticus	3	67	33	100	100	0	0	67	100	100	100	100	100	67	100	33	100
A. johnsonii	16	94	38	63	100	0	13	44	69	81	81	81	100	100	94	94	94
A. junii	6	100	100	100	100	0	100	100	100	100	100	100	100	83	100	100	100
A. lwoffii	15	100	80	87	100	7	40	67	93	93	93	60	100	100	100	100	100
Acinetobacter species strain 3	17	18	29	71	100	0	0	0	59	100	88	12	100	94	88	88	94
Acinetobacter species strain 6	2	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	100
Acinetobacter species strain 10	5	0	40	60	60	0	0	0	100	100	100	0	100	60	20	40	100
Acinetobacter species strain 12	3	100	100	100	100	0	67	100	100	100	100	67	100	100	100	100	100
Acinetobacter strains, ungrouped	5	80	80	80	80	0	40	80	100	100	100	80	80	80	80	80	100

[a] AMP, ampicillin; MZ, mezlocillin; PIP, piperacillin; AUG, augmentin; CFZ, cefazolin; FOX, cefoxitin; CRM, cefuroxime; CFT, cefotaxime; CAZ, ceftazidime; CAX, ceftriaxone; AZT, aztreonam; IPM, imipenem; AMK, amikacin; GM, gentamicin; TOB, tobramycin; CP, ciprofloxacin.

Susceptibility testing. MICs were determined by a microtiter broth dilution method. Four or five colonies were suspended in 0.5 ml of brain heart infusion broth and incubated for 6 h at 35°C. A 0.01-ml portion of the suspension was transferred into 25 ml of autoclaved distilled water. Microtiter plates (MicroScan MIC Plus Type MK Dried Panels; Baxter-Travenol, West Sacramento, Calif.) were inoculated with a Renok inoculator according to the manufacturer's recommendations to achieve a final well concentration of 1×10^5 to 4×10^5 CFU/ml. Each plate contained 16 different lyophilized antimicrobial agents serially diluted in Mueller-Hinton broth, and the concentrations of most agents tested ranged in twofold steps from 1/8 through 4 times the breakpoint for fully susceptible isolates adopted by the National Committee for Clinical Laboratory Standards (NCCLS). Plates were incubated for 18 h at 35°C. The MIC was defined as the lowest concentration of drug that prevented visible growth. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 served as controls.

RESULTS AND DISCUSSION

This study presents the susceptibilities of nine different species of the genus *Acinetobacter* to 16 antimicrobial agents. The activities of the various agents against different *Acinetobacter* species are shown in Table 2. Table 3 shows the percentages of isolates fully susceptible at NCCLS breakpoints.

A. baumannii strains were generally more resistant than strains identified as species other than *A. baumannii*. This is consistent with three recently published reports (8, 19, 20). Within *A. baumannii* strains, isolates belonging to biotype 9 were more resistant than other biotypes, confirming previous data (10). Among species other than *A. baumannii*, strains of *Acinetobacter* species strains 3 and 10 and *A. johnsonii* showed the highest resistance. The most susceptible strains were found in the species *A. junii* and *A. lwoffii*; similar data were published recently (19).

In terms of MICs for 90% of the isolates, the most active agent against *A. baumannii* strains was imipenem. No strain resistant to imipenem was found, whereas others have reported 5% of *Acinetobacter* strains being resistant to imipenem (13). Amoxicillin-clavulanate showed moderate activity, whereas ampicillin, broad-spectrum penicillins, cephalosporins, aminoglycosides, and ciprofloxacin were less active.

The trend towards resistance to expanded-spectrum cephalosporins was also demonstrated by Joly-Guillou et al. (13) and seemed to be related to the presence of cephalosporinases (14, 16). Recently, the presence of an extended broad-spectrum β-lactamase was reported (12). Others have also found increasing resistance of *A. baumannii* strains to modern quinolones (10, 20) as well as to amikacin and tobramycin (13). Resistance to amikacin was shown to be due to the presence of an aminoglycoside phosphotransferase (15).

Excellent activity against *Acinetobacter* species strain 3 strains was shown for imipenem, amikacin, ciprofloxacin, and amoxicillin-clavulanate. Of the other beta-lactams tested, only ceftazidime showed moderate in vitro activity. Isolates constituting *Acinetobacter* species strain 3 differed from *A. baumannii* strains in their greater susceptibility to ciprofloxacin and the aminoglycosides.

Against isolates identified as species other than *A. baumannii* and *Acinetobacter* species strain 3, imipenem, amikacin, ceftazidime, ceftriaxone, amoxicillin-clavulanate, and ciprofloxacin exhibited good activity. Some strains were even susceptible to ampicillin and older cephalosporins. We found only one unclassified proteolytic *Acinetobacter* strain which was resistant to imipenem.

Treatment of nosocomial infections due to *A. baumannii* species has become more and more complicated by the rapid increase in the resistance of these species to most antimicrobial agents (4, 10, 13, 21). Often, imipenem remains the only effective treatment. The overuse of new drugs may have contributed to an escalation of resistance. Treatment of infections due to species other than *A. baumannii* does not seem to pose serious problems. It should be noted that the percentage of strains identified as species other than *A. baumannii* was greater than previously reported (7, 8).

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