

Antimicrobial Resistant Pattern of *Acinetobacter* spp.*

Nalinee Aswapokee, M.D.**

Surapee Tiengrim, M.Sc.**

Busaba Charoensook, Cert. Microbiol***

Kantima Sangsiriwut, B.Sc.***

Abstract

Acinetobacter spp. is an increasingly important opportunist in hospital environment and infects severely-ill or immunosuppressed patients. Most strains are multidrug resistance (MDR). We determined antimicrobial resistance and susceptibility of 85 strains of *Acinetobacter* spp. collected from patients hospitalized in Siriraj Hospital during 1996 to mid 1997, to 19 antimicrobial agents and one beta-lactamase inhibitor.

Ninety-four percent of *Acinetobacter* spp. were *A. baumannii*. Most strains were resistant to antimicrobial drugs using resistant breakpoints (NCCLS) for MIC₉₀.

For susceptibility pattern, imipenem, cefoperazone-sulbactam, trovafloxacin and cefepime exerted low MIC₉₀. Other drugs were less active or resistant.

For resistance, ≥ 50 percent of *Acinetobacter* spp. were resistant to cefoperazone, tetracycline, TMP-SMX. Twenty to 50 percent of strains were resistant to piperacillin, ciprofloxacin, ceftazidime, cefotaxime, doxycycline, amikacin and ofloxacin. Ten to 20 percent were resistant to piperacillin-tazobactam, levofloxacin, cefepime, sparfloxacin and minocycline. None of strains was resistant to imipenem nor cefoperazone-sulbactam.

Forty-nine percent of the strains were MDR, with the majority resistant to 5 drug classes. All strains produced beta-lactamases and 13 percent produced extended-spectrum beta-lactamases.

From the results of the study, the choice of agents for empirical treatment and graded susceptibility was recommended. (*J Infect Dis Antimicrob Agents* 1998;15:43-8.)

Acinetobacter spp. is an ubiquitous opportunistic bacteria which responsible for a wide spectrum of infections in patients with serious underlying diseases (1-3). There has been progressive decrease in antimicrobial susceptibilities among clinical isolates of acinetobacter since early 1980s (4-7). At the present time, acinetobacter strains exhibit multidrug resistance. In some area, these strains were susceptible only to polymyxin and sulbactam (9-10), and were resistant

to imipenem (9-12), rendering the strains virtually untreatable.

A majority of patients hospitalized at Siriraj Hospital, a tertiary care medical center, are severely and/or terminally ill. These populations are susceptible to multidrug resistant nosocomial pathogens. We determined antimicrobial resistance of *Acinetobacter* spp. obtained from these patients. The results will be used for guiding graded susceptibility testing and

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** Division of Infectious Diseases, Department of Medicine,

*** Department of Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

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Reprint request: Nalinee Aswapokee, M.D., Division of Infectious Diseases, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

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empirical use of antibiotics in these patients.

MATERIALS AND METHODS

Bacterial isolates

A total of 85 clinical isolates of *Acinetobacter* spp. collected during 1996 to mid 1997 from patients hospitalized at Siriraj Hospital were used. Speciation was performed by using API 20 NE[®] (Bio Mérieux Inc., France).

Antimicrobial agents

Nineteen antimicrobial agents and one beta-lactamase inhibitor were used. These were amikacin, piperacillin, cefotaxime, ceftazidime, cefoperazone, cefepime, ampicillin-sulbactam, cefoperazone-sulbactam, piperacillin-tazobactam, sulbactam, imipenem, ofloxacin, ciprofloxacin, levofloxacin, sparfloxacin, trovafloxacin, tetracycline, doxycycline, minocycline and trimethoprim-sulfamethoxazole (TMP-SMX). New agents were generous gifts from representative manufacturers. Commercially available agents were purchased from Sigma.

Susceptibility testing

Minimum inhibitory concentrations (MICs) were determined either by standard agar dilution (NCCLS) (13), or by E-test.

Standard agar dilution using Mueller-Hinton agar, pH 7.3 ± 0.1, BBL[®] were performed for most agents, whereas E-test (AB Biodisk[®]) were used only for cefotaxime, piperacillin-tazobactam and minocycline.

Disc diffusion technique were used only for sulbactam-ampicillin and piperacillin. The methods used were described by NCCLS (14).

Multidrug resistance was defined as resistance to 3 or more groups of agents of unrelated chemical structures.

Susceptibility pattern was reported as range, MIC₅₀ and MIC₉₀. Percent resistance indicated percentage of organisms possessing MIC above resistance breakpoints (NCCLS).

Beta-lactamase detection

Beta-lactamases were detected by using chromogenic cephalosporin. Extended-spectrum beta-lacta-

Table 1. *In vitro* susceptibilities of 85 strains of *Acinetobacter* spp.

Antimicrobial agents	MIC (mg/L)			% Susceptibility*
	Range	MIC ₅₀	MIC ₉₀	
Amikacin	1 — >128	4	>128	69.4
Cefotaxime	0.75 — >256	24	>256	17.1
Cefoperazone	8 — >128	64	>128	3.2
Ceftazidime	0.25 — >128	8	>128	60.0
Cefepime	0.05 — >128	4	32	67.0
Cefoperazone-sulbactam	0.25 — 32	2	8	98.0
Piperacillin-tazobactam	0.125 — >256	12	>256	60.7
Imipenem	0.05 — 4	0.25	1	100.0
Ofloxacin	0.25 — >16	0.25	16	70.9
Levofloxacin	0.1 — 16	0.25	8	77.5
Ciprofloxacin	0.05 — >16	0.5	>16	61.2
Sparfloxacin	0.005 — 8	0.025	8	75.3
Trovafloxacin	0.005 — 16	0.05	2	—
Tetracycline	2 — >128	16	>128	7.1
Doxycycline	0.025 — 128	4	128	51.7
Minocycline	0.047 — 24	0.38	16	75.0
TMP-SMX	0.05 — >32	8	>32	43.6
Sulbactam	0.25 — >128	4	128	—

* Based on susceptibility breakpoints defined by NCCLS as: amikacin ≤ 16 mg/L, cefotaxime ≤ 8 mg/L, cefoperazone ≤ 16 mg/L, ceftazidime ≤ 8 mg/L, cefepime ≤ 8 mg/L, cefoperazone-sulbactam (CFP) ≤ 16 mg/L, piperacillin-tazobactam (PIP) ≤ 16 mg/L, imipenem ≤ 4 mg/L, ofloxacin ≤ 2 mg/L, levofloxacin ≤ 2 mg/L, ciprofloxacin ≤ 1 mg/L, sparfloxacin ≤ 1 mg/L, tetracycline ≤ 4 mg/L, doxycycline ≤ 4 mg/L, minocycline ≤ 4 mg/L, TMP-SMX (TMP) ≤ 2 mg/L.

mases (ESBLs) were detected by double-disk synergy technique (15).

RESULTS

Among 85 isolates, *A. baumannii* comprised the majority (80 out of 85, 94.1%). The rest were *A. jejunii* (4 out of 85, 4.7%) and *A. haemolyticus* (1 out of 85, 1.2%).

The susceptibility results revealed that imipenem was the most active agent, beta-lactams or non-beta-lactams, with the MIC₉₀ of 1 mg/L. The second active beta-lactam was cefoperazone-sulbactam MIC₉₀ of 8 mg/L. Other beta-lactams were only moderately active or inactive. Amikacin was the only aminoglycoside used and was not active. Among fluoroquinolone group, trovafloxacin was an only active agent. Table 1 summarizes antimicrobial susceptibilities of 85 strains of *Acinetobacter* spp.

The resistant patterns showed that more than 50 percent of isolates were resistant to cefoperazone,

tetracycline and TMP-SMX, with the highest percentage for cefoperazone (67.2%). Thirty to 50 percent of *Acinetobacter* spp. were resistant to the following agents: amikacin, doxycycline, cefotaxime, ceftazidime, ciprofloxacin and piperacillin. Ten to 21 percent of isolates were resistant to the following agents: minocycline, sparfloxacin, cefepime, levofloxacin, piperacillin-tazobactam and ofloxacin. Only 2.5 percent were resistant to ampicillin-sulbactam and none were resistant to cefoperazone-sulbactam and imipenem.

There were discrepancy of percentage of resistance among individual agent in the same antimicrobial group. For example, *Acinetobacter* spp. were more resistant to cefoperazone and ceftazidime than cefotaxime, and the organisms showed highest percentage of susceptibility to minocycline than other agents in the tetracyclines group. Sulbactam-containing betalactams always covered larger proportion of susceptible organisms, even though sulbactam alone showed high MIC₉₀ (128 mg/L). Table 2 summarises resistant pattern of *Acinetobacter* spp.

Forty-nine out of 85 strains (57.6%) were resistant to 3 or more drugs of unrelated chemical structures. Thirty-seven strains were resistant to 5 drugs, 3 strains to 4 drugs and 9 strains to 3 drugs (Table 3).

All strains produced beta-lactamase enzymes and 13 percent of these were extended-spectrum beta-lactamases (ESBLs).

DISCUSSION

We use MIC₉₀ instead of MIC₅₀ to indicated activities or resistance to antimicrobial agents in this report on the basis that we are interested more on the acquired resistance (16). By using MIC₉₀, we believed that the actual proportions of susceptible and resistant isolates will be advocated, and this rendered clinical relevance.

The study revealed that very few antimicrobial agents were effective for *Acinetobacter* spp. Even potent agents like ceftazidime, ciprofloxacin, amikacin were not active. This may be due to multidrug resistant (MDR) nature of the organism. It is known that *Acinetobacter* spp. produced several detoxifying enzymes such as aminoglycoside-modifying enzymes and beta-lactamases. Resistance to amikacin in this genus is mainly due to production of 3'-aminoglycoside phosphotransferase type VI [APH(3')-VI] and 6'-aminoglycoside acetyltransferase type I [AAC (6')-I]

Table 2. Percentage resistance to antimicrobial agents of 85 strains of *Acinetobacter* spp.*

Antimicrobial agent	(%) Resistance
Cefoperazone	67.2
Tetracycline	67.1
TMP-SMX	51.8
Piperacillin	43.0
Ciprofloxacin	37.7
Ceftazidime	34.1
Cefotaxime	31.6
Doxycycline	31.6
Amikacin	30.6
Ofloxacin	20.9
Piperacillin-tazobactam	18.9
Levofloxacin	12.9
Cefepime	10.6
Sparfloxacin	10.6
Minocycline	10.2
Ampicillin-sulbactam	2.5
Cefoperazone-sulbactam	0
Imipenem	0

* Based on resistance breakpoint (NCCLS) as: amikacin \geq 32 mg/L, cefotaxime \geq 64 mg/L, cefoperazone \geq 64 mg/L, ceftazidime \geq 32 mg/L, cefepime \geq 32 mg/L, cefoperazone-sulbactam (CFP) \geq 64 mg/L, piperacillin-tazobactam (PIP) \geq 128 mg/L, imipenem \geq 16 mg/L, ofloxacin \geq 8 mg/L, levofloxacin \geq 8 mg/L, ciprofloxacin \geq 4 mg/L, sparfloxacin \geq 4 mg/L, tetracycline \geq 16 mg/L, doxycycline \geq 16 mg/L, minocycline \geq 16 mg/L, TMP-SMX (TMP) \geq 8 mg/L.

Table 3. Multidrug resistant patterns of 49 *Acinetobacter* spp.

Antimicrobial agents to which organisms are resistant	No of strains (%)*
Beta-lactams, aminoglycoside, fluoroquinolones, tetracycline, TMP-SMX	37 (75.5)
Beta-lactams, fluoroquinolones, tetracycline, TMP-SMX	1 (2.0)
Beta-lactams, aminoglycoside, tetracycline, TMP-SMX	1 (2.0)
Aminoglycoside, fluoroquinolone, tetracycline, TMP-SMX	1 (2.0)
Beta-lactams, tetracycline, TMP-SMX	5 (10.3)
Aminoglycoside, tetracycline, TMP-SMX	2 (4.1)
Beta-lactams, fluoroquinolones, TMP-SMX	2 (4.1)

* percentage of a total of 49 strains

(17-19). We were not able to characterize aminoglycoside-modifying enzymes, but the study of distribution of these enzymes revealed that they are ubiquitous (20). It was shown that 98 percent of *Acinetobacter* spp. produced cephalosporinases (Bush group 1 enzymes) (19,21) and this may explain why most strains were resistant to third generation cephalosporins. Although *Klebsiella* spp. was the predominant ESBLs producer (22,23), we found in this study that 13 percent of *Acinetobacter* spp. also produced ESBLs. The prevalence of ESBLs production in this genus is similar to that reported previously (19). Although the resistance to fluoroquinolones is increasing, the mechanism responsible for resistance has not been elucidated (24).

Most *Acinetobacter* spp. were still susceptible to imipenem, as reported previously (24-26) and in this study, albeit resistance occurred sporadically as mentioned earlier (9-12). The activities of sulbactam-containing beta-lactam agents against this genus is remarkable. In this study, we found that 98 percent of strains were susceptible of cefoperazone-sulbactam, 2 percent were intermediately susceptible and none was resistant. This was also true for sulbactam-ampicillin in which 97.2 percent were susceptible. Although it has been repeatedly shown that sulbactam-containing beta-lactams were effective against *Acinetobacter* spp. (26-29) and that sulbactam has intrinsic activity against this genus (30-33), we were not able to explain the discrepancy of our result when sulbactam alone seems not to work very well (MIC₉₀ of 128 mg/L), but the combination with cefoperazone or ampicillin, to which the organisms were resistant, exerted good activities

and excellent coverage. The inhibitory action of sulbactam on beta-lactamase production of this genus seems not to be a plausible explanation, since the most prevalence beta-lactamases in this genus are cephalosporinases, which are not inhibited by sulbactam. The activity of minocycline which is superior to other agents in the tetracyclines group agreed with other studies (34).

The majority of 85 strains (57.6%) were multiply resistant and most of these (37/49, 75%) were resistant to 5 drug classes. This finding is similar to previous reports from different locations of the world (2-3,9-11, 35), and might reflect the nature of this opportunist rather than the pattern of antimicrobial use. Using molecular approach, it was found that the mode of spread of *Acinetobacter* spp. in hospital environment occurred mostly via cross-transmission (36,37), although strains heterogeneity were sometimes detected (38). The control measures should therefore included an emphasis on improvement of hospital hygiene as well as the improvement of antimicrobial use.

The results of our study indicated that antimicrobial agents to be used empirically in severe acinetobacter infections in non-terminal illness should include either carbapenem or sulbactam-containing beta-lactam agents. Mildly infected cases may respond empirically to minocycline. The combination of antimicrobial agents may be a useful mean to achieve eradication as well as to slow emergence of resistance (39). Susceptibility testing for this genus should be graded in order to avoid pressure use (40). Based on the result in this study, the agents of first choice should include mino-

cycline, ofloxacin or fourth generation cephalosporins to which the organisms were less likely to be resistant. Carbapenem and sulbactam-containing beta-lactams, even though offered greater coverage and higher potency, should be alternative agents due to preservation of drugs. Choice of newer fluoroquinolones i.e. sparfloxacin or trovafloxacin which showed good *in vitro* activity, should be postponed until more studies indicated clinical efficacies and/or organisms are resistant to primary and/or secondary agents.

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