Journal of Antimicrobial Chemotherapy (2007) **60**, 1163–1167
doi:10.1093/jac/dkm305
Advance Access publication 29 August 2007

JAC

High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea

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Received 25 April 2007; returned 21 June 2007; revised 9 July 2007; accepted 19 July 2007

***Objectives*: To investigate antimicrobial resistance in clinical isolates of *Acinetobacter* spp. from two Korean hospitals.**

***Methods*: Two hundred and sixty-five isolates of *Acinetobacter* spp. from two Korean hospitals were collected and were identified to species level using partial *rpoB* gene sequences. Antimicrobial susceptibility testing was performed using a broth microdilution method.**

***Results*: *rpoB* gene sequences indicated that 214 isolates (80.8%) were *Acinetobacter baumannii*, and allowed these to be classified into three subgroups (I, II and III); 142 isolates (53.6%) belonged to subgroup I, 54 (20.4%) to subgroup II and 18 (6.8%) to subgroup III. Forty-eight isolates (18.1%) and 74 isolates (27.9%) were resistant to polymyxin B and colistin, respectively. However, antimicrobial resistance rates varied markedly between subgroups. While *A. baumannii* subgroup I showed low resistance rates to polymyxin B and colistin (2.1% and 7.0%, respectively), subgroups II and III showed high resistance rates to these antibiotics (38.9% and 64.8% in subgroup II and 72.2% and 88.9%, in subgroup III, respectively). Multidrug resistance was also significantly more frequent in subgroup I (45.1%) than in subgroups II and III (13.0% and 16.7%, respectively).**

***Conclusions*: Our data indicate that subgroup identification of *A. baumannii* may aid selection of appropriate antimicrobial agents for the treatment of *Acinetobacter* infections.**

Keywords: *rpoB*, antimicrobial resistance, subgrouping

Introduction

Acinetobacter species have increasingly been recognized as hospital-acquired pathogens mainly in immunocompromised patients and patients in intensive care units (ICUs).[1] In ICUs, the prevalence of infections by *Acinetobacter* spp. currently account for 2% to 10% of all nosocomial Gram-negative bacterial infections in the United States and Europe.[2] To date, 10 nomenspecies and 4 genomospecies have been isolated from humans; *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Acinetobacter parvus*, *Acinetobacter radioresistens*, *Acinetobacter schindleri*, *Acinetobacter ursingii* and genomospecies 3, 13TU, 10 and 11. Of these, *A. baumannii*, *A. calcoaceticus* and genomospecies 3 and 13TU are the most frequently isolated and clinically relevant.[3] They are commonly referred to as the *A. baumannii–A. calcoaceticus* complex because they are genetically closely related and cannot be easily differentiated by phenotypic methods in the clinical microbiology laboratory.

Emergence of pandrug-resistant or multidrug-resistant (MDR) *A. baumannii* strains has become a serious clinical problem in

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many parts of the world, especially in some Asian countries.[4] Antimicrobial options for the treatment of MDR *A. baumannii* are limited, including polymyxins or sulbactam.[1] In this study, we identified *Acinetobacter* isolates from two Korean hospitals using partial *rpoB* gene sequencing, which has been used for the identification of several bacterial species,[5] and tested their antimicrobial susceptibilities *in vitro*.

Materials and methods

Bacterial isolates

A total of 265 non-duplicate clinical isolates of *Acinetobacter* spp., which were collected from two tertiary-care hospitals in Korea, were tested in the study. One hundred and six isolates were from blood of patients in the Chonnam National University Hospital (CNUH; Gwangju) during the period from March 2002 to May 2006. The other 159 isolates from various specimens were collected at the Samsung Medical Center (SMC; Seoul) between January and May 2006; 76 isolates from sputum, 31 from tracheal aspirate, 7 from bile, 6 isolates from blood, pus and urine, respectively, and other various specimens. We also included three strains representing European clones I (RUH875), II (RUH134) and III (RUH5875).

rpoB gene analysis

To identify *Acinetobacter* species and to analyse the intraspecific variation of *A. baumannii*, we determined the partial *rpoB* gene sequence of 265 isolates of *Acinetobacter* spp. using primers Ac1055F (GTGATAARATGGCBGGTCGT) and Ac1598R (CGBG CRTGCATYTTGTCRT).[5] We obtained unambiguous 468 bp sequences from all isolates, which included one of the variable regions of the *rpoB* gene, zone 2.[5]

Antimicrobial susceptibility testing

In vitro susceptibility testing was performed with all isolates of *Acinetobacter* spp. using the broth microdilution method according to CLSI guidelines.[6] Fourteen antimicrobial agents were tested: imipenem, meropenem, polymyxin B, colistin, tetracycline, ciprofloxacin, rifampicin, amikacin, cefepime, ceftriaxone, cefoperazone/sulbactam, ceftazidime, piperacillin/tazobactam and ampicillin/sulbactam. The interpretive criteria used were those established in CLSI standard M100-S16.[6] *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. MDR was defined in accordance with Paterson.[7]

Results

Based on partial (468 bp) *rpoB* gene sequences, we identified 12 *Acinetobacter* species (8 nomenspecies and 4 genomospecies) among the 265 isolates. Of these, 214 isolates (80.8%) were classified as *A. baumannii*. Genomospecies 3 was the second most common species (24 isolates, 9.1%). Other species were represented by one to five isolates, and six isolates could not be assigned to species level although they clustered within the genus *Acinetobacter* in the *rpoB* gene tree. *A. calcoaceticus* and *A. parvus* were found only in the CNUH, and *Acinetobacter baylyi*, *A. haemolyticus*, *A. junii*, *Acinetobacter tjernbergiae*, *A. ursingii* and genomospecies 11 were isolated only in the SMC. Isolates within the same species or subgroup in *A. baumannii* showed *rpoB* sequence divergence of $<2\%$, equivalent to no more than nine nucleotide changes.

The 214 *A. baumannii* isolates were further divided into three subgroups, subgroups I, II or III, based on phylogenetic clustering (Figure 1): 142 (66.4%) isolates belonged to subgroup I; 54 isolates (20.4%) to subgroup II; and 18 isolates (6.8%) to subgroup III. *A. baumannii* CIP 70.34 (=ATCC 19606), which is the type strain of *A. baumannii*, and three representative strains of European clones I, II and III also belonged to subgroup I. Subgroup I included 47 isolates (44.3%) from the CNUH and 95 isolates (59.7%) from the SMC. While 17 isolates were grouped into subgroup III in the CNUH, only one isolate from SMC belonged to this subgroup.

For all isolates of *Acinetobacter* spp., resistance rates to polymyxin B and colistin were 18.1% and 30.6%, respectively (Table 1). MIC_{90}s of polymyxin B and colistin were 8 and 32 mg/L, respectively. Resistance rates to carbapenems were 8.3% (imipenem) and 11.7% (meropenem). Resistance rates and MIC_{90}s of the other antimicrobials are shown in Table 1.

Antimicrobial resistance rates varied by subgroups of *A. baumannii* (Table 1). Resistance rates to polymyxin B and colistin in *A. baumannii* subgroup I were only 2.1% and 7.0%, respectively. They were also low in genomospecies 3 (4.2% and 8.3%, respectively). However, they were very high in *A. baumannii* subgroups II and III. Polymyxin B resistance rates of *A. baumannii* subgroups II and III were 38.9% and 72.2%, respectively. In addition, most isolates of *A. baumannii* subgroups II and III were resistant to colistin (resistance rates, 64.8% and 88.9%, respectively). MIC_{90}s of polymyxin B and colistin were also markedly different among *A. baumannii* subgroups and genomospecies 3 (Table 1).

We identified 88 MDR *Acinetobacter* isolates (33.2%) (Table 2); most (64 isolates, 72.7%) belonged to subgroup I. The MDR rate was significantly higher in *A. baumannii* subgroup I (45.1%) followed by *A. baumannii* subgroups II (13%) and III (16.7%) ($P < 0.001$) (Table 2). Polymyxin B and colistin showed good activity against MDR *A. baumannii* subgroup I isolates but they showed poorer activity against MDR *A. baumannii* isolates belonging to subgroups II and III.

Discussion

Acinetobacter spp. have become important nosocomial pathogen due to the emergence and rapid spread of MDR strains.[1] Very few antimicrobial agents can be reliably used for effective therapy of MDR *Acinetobacter* infections.

In this study, we identified *Acinetobacter* species based on *rpoB* gene analysis. Although we used only partial *rpoB* gene sequences, we could identify most isolates of *Acinetobacter* species.[5] Based on the identification using the *rpoB* gene, we have identified the first isolates of *A. baylyi* and *A. tjernbergia* from patient specimens: these species had previously been identified from activated sludge plants.[8] Our results may indicate that more diverse *Acinetobacter* species have the potential to infect humans.

Figure 1. Phylogenetic grouping of 265 isolates of *Acinetobacter* spp. inferred from partial *rpoB* gene sequences. This tree was constructed by the neighbour-joining method. Only reference strains of La Scola *et al.*[5] are indicated. Three *A. baumannii* subgroups are represented.

Table 1. Antimicrobial resistance among *A. baumannii* isolates

Antimicrobial	Total		*A. baumannii* subgroup I ($n = 142$)		*A. baumannii* subgroup II ($n = 54$)		*A. baumannii* subgroup III ($n = 18$)		*P*
	R (%)	MIC_{90} (mg/L)	*R* (%)	MIC_{90} (mg/L)	*R* (%)	MIC_{90} (mg/L)	*R* (%)	MIC_{90} (mg/L)	
Polymyxin B	18.1	8	2.1	2	38.9	8	72.2	32	<0.001[a]
Colistin	30.6	32	7.0	2	64.8	64	88.9	>64	<0.001[a]
Tetracycline	26.4	>64	39.4	>64	9.3	8	11.1	64	<0.001[a]
Ciprofloxacin	28.7	>64	45.1	>64	1.9	1	16.7	>64	<0.001[b]
Rifampicin	2.3	8	1.4	8	3.7	4	0.0	4	0.436[a]
Amikacin	30.2	>128	37.3	>128	18.5	128	11.1	>128	0.011[b]
Meropenem	11.7	16	14.1	16	3.7	1	5.6	1	0.113[a]
Imipenem	8.3	8	8.5	8	0.0	1	5.6	1	0.021[a]
Cefepime	28.7	>64	38.7	>64	16.7	>64	11.1	32	0.005[b]
Ceftriaxone	32.8	>128	44.4	>128	14.8	>128	16.7	>128	<0.001[b]
Cefoperazone/sulbactam[c]	—	>64/32	—	>64/32	—	8/4	—	64/32	—
Ceftazidime	35.1	>64	45.8	>64	13.0	>64	16.7	>64	<0.001[b]
Piperacillin/tazobactam	25.3	>256/4	43.0	>256/64	1.9	16/4	11.1	256/4	<0.001[a]
Ampicillin/sulbactam	23.4	>64/32	40.1	>64/32	0.0	4/2	11.1	64/32	<0.001[a]

[a]Fisher's exact test.
[b]χ^2 test.
[c]Breakpoint for cefoperazone/sulbactam was not available.[6]

In this study, three subgroups were identified within *A. baumannii* based on *rpoB* gene sequences. These subgroups were phylogenetically distinct from one another, although they clustered into a single clade robustly (Figure 1). More interestingly, subgrouping of *A. baumannii* isolates based on partial *rpoB* gene sequences correlated with antimicrobial resistance profiles. While most isolates belonging to subgroup I were susceptible to polymyxin B and colistin, many isolates of subgroups II and III were resistant to these agents (Table 1). Emergence and spread of polymyxin-resistant *Acinetobacter* spp. poses a serious therapeutic concern, because no antimicrobial agents except tigecycline are available for treatment of MDR *Acinetobacter* infections.[9] Colistin or polymyxin B resistance in *Acinetobacter* spp. is rare worldwide.[10] Due to many colistin- or polymyxin B-resistant isolates belonging to the *A. baumannii* subgroups II and III, colistin or polymyxin B resistance rates among *Acinetobacter* spp. were 30.6% and 18.1% in this study. This means that accurate identification of *Acinetobacter* spp. is needed to select appropriate antimicrobial agents, although most colistin-resistant isolates of subgroups II and III were susceptible to other antimicrobials. In addition, higher rates of resistance to colistin may also be relevant in clinical settings, because it is less toxic than polymyxin B.

Table 2. Antimicrobial resistance rates among multidrug-resistant *Acinetobacter* species

	Total ($n = 88$) (%)	*A. baumannii* subgroup I ($n = 64$) (%)	*A. baumannii* subgroup II ($n = 7$)	*A. baumannii* subgroup III ($n = 3$)
MDR (%)	33.2	45.1	13.0	16.7
Antimicrobial				
polymyxin B	12 (13.6)	1 (1.6)	4	1
colistin	16 (18.2)	1 (1.6)	6	2
tetracycline	61 (69.3)	56 (87.5)	—	1
ciprofloxacin	73 (83.0)	64 (100)	—	2
rifampicin	6 (6.8)	2 (3.1)	2	—
amikacin	75 (85.2)	53 (82.8)	7	2
meropenem	31 (35.2)	20 (31.3)	2	1
imipenem	22 (25.0)	12 (18.8)	—	1
cefepime	74 (84.1)	55 (85.9)	—	2
ceftriaxone	82 (93.2)	62 (96.9)	5	3
ceftazidime	82 (93.2)	62 (96.9)	5	3
piperacillin/tazobactam	65 (73.9)	61 (95.3)	—	2
ampicillin/sulbactam	61 (69.3)	56 (87.5)	—	2

Carbapenems also showed different resistance profiles among *A. baumannii* subgroups. Unlike polymyxins, imipenem and meropenem showed good *in vitro* activities against isolates of *A. baumannii* subgroup II. Out of 83 polymyxin-resistant isolates, only 5 and 7 isolates were resistant to imipenem or meropenem (6.0% and 8.4%, respectively).

Reports of a number of outbreaks of nosocomial infections caused by *Acinetobacter* isolates might indicate a propensity to dissemination within hospitals.[1] Thus, polymyxin resistance in *Acinetobacter* spp. could increase within a short time, although in our collection it was restricted to particular *A. baumannii* subgroups. The increasing trend of resistance to colistin or polymyxin B, which is now considered often to be the last choice for treatment of *Acinetobacter* infections, warrants continuous surveillance.

Acknowledgements

Drs Je Chul Lee (Kyungpook National University, Korea) and Lenie Dijkshoorn (Leiden University Medical Centre, The Netherlands) provided isolates of European clones.

Funding

This study was done with the support of the Asian-Pacific Research Foundation of Infectious Diseases (ARFID; Seoul, Korea).

Transparency declarations

None to declare.

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