Mechanisms of Resistance and Antimicrobial Susceptibility Profile of Carbapenem-Resistant *Acinetobacter* spp. from P.R. China

C2-105

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ICAAC 2010
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AMENDED ABSTRACT

Background: Carbapenem-resistant *Acinetobacter baumannii* increased rapidly and has become a serious therapeutic challenge in P.R. China. We evaluated the resistance mechanisms and antimicrobial susceptibility profile of the clinical carbapenem-resistant *A. baumannii* isolates collected from P.R. China.

Methods: Ninety non-duplicated carbapenem-resistant *A. baumannii* isolates were collected from 12 teaching hospitals in P.R. China during 2007-2008. Susceptibility testing was performed by CLSI broth microdilution (M07-A8) method. OXA-51-like, OXA-23-like, OXA-58-like and OXA-24-like carbapenemases, KHM-, SIM-, VIM-, IMP-, and PER-encoding genes, and IS*Aba* insertion sequences were screened by PCR. Isolates were typed by pulsed-filed gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

Results: All isolates were susceptible to colistin and polymyxin B. Tigecycline was very active (MIC_{90}, 2 µg/mL) and isolates were 51.1 and 37.8% susceptible to minocycline and doxycycline, respectively. Strains from Wuhan and Urumqi had higher MIC values (MIC_{90}, >8 µg/mL) to minocycline. Susceptibility rates for fluoroquinolones, β-lactams (+/- β-lactamase inhibitors), trimethoprim/sulfamethoxazole and aminoglycosides ranged from 0 to 14.4%. All strains contained at least one carbapenemases (up to 4 per strain) distributed as follows (n; % of strains): $bla_{OXA-51-like}$ (89; 98.9%), $bla_{OXA-23-like}$ (71; 78.9%), $bla_{OXA-58-like}$ (29; 32.2%), $bla_{OXA-24-like}$ (3; 3.3%) and, bla_{PER} (22; 24.4%). One isolate which was $bla_{OXA-51-like}$-negative was identified as *Acinetobacter* genomospecies 3 by *rpoB* sequencing. IS*Aba1* was observed upstream of $bla_{OXA-51-like}$ and $bla_{OXA-23-like}$ in 16.9 and 97.2% of cases, respectively. IS*Aba3* was inserted upstream and downstream of all the $bla_{OXA-58-like}$. No metallo-β-lactamases (MBLs) were detected. Overall, nine PFGE types were observed among *A. baumannii*. PFGE type E contained 46 isolates collected from nine medical centers in different regions of P.R. China.

Conclusions: Carbapenem-resistant *A. baumannii* strains from P.R. China were all susceptible to polymyxin and tigecycline, while ~50% were inhibited by minocycline, with some regional variation. OXA-51-like, OXA-23-like and OXA-58-like carbapenemases associated with IS*Aba1* or IS*Aba3* were the most prevalent resistance mechanisms. PFGE pattern C and E were the main clones spreading in nine of the 12 hospitals in this study.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative organism that has been increasingly recognized as an important opportunistic pathogen causing nosocomial infections and outbreaks, especially among patients in intensive care units. Carbapenems are frequently the empiric therapy of choice; however, carbapenem-resistant *A. baumannii* strains have frequently been observed and are rapidly increasing in prevalence worldwide. Moreover, these strains are usually resistant to other antimicrobial agents including fluoroquinolones and aminoglycosides.

Carbapenem resistance in *A. baumannii* is often due to carbapenem-hydrolyzing Ambler class D β-lactamases and, although less frequent, to class B MBLs. Four groups of class D carbapenemases have been identified in *Acinetobacter* species: OXA-51-like (intrinsic to *A. baumannii*), OXA-23-like, OXA-58-like and OXA-24-like. Insertion sequences belonging to the IS*Aba* family are often detected upstream of class D carbapenemase-encoding genes, which provide promoter sequences, thus improving gene expression. Additionally, MBL-encoding genes bla_{IMP}, bla_{VIM} and bla_{SIM} have been reported in *A. baumannii,* leading to carbapenem resistance.

In People's Republic (P.R.) of China, carbapenem-resistant *A. baumannii* increased from 25% in 2007 to 35% in 2008 according to the Surveillance by Etest and Agar Dilution of Nationwide Isolate Resistance (SEANIR) program, a national antimicrobial resistance surveillance program in China's mainland. This study evaluated the antimicrobial susceptibility profiles and resistance mechanisms among carbapenem-resistant *A. baumannii* clinical isolates collected from China.

MATERIALS AND METHODS

Bacterial strain collection. A total of 90 carbapenem-resistant *A. baumannii* isolates were collected from 12 teaching hospitals in China during 2007-2008, as a part of SEANIR surveillance. Bacterial identification was confirmed by the clinical microbiology laboratory of Peking Union Medical College Hospital, according to standard operation procedures. The Vitek® 2 Compact automatic identification system (bioMérieux; Marcy l'Etoil, France) was used when needed. Species identification of isolates PCR-negative for $bla_{OXA-51-like}$ was confirmed by *rpoB* sequencing.

Antimicrobial susceptibility testing. Susceptibility testing was performed by Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (M07-A8) using commercially prepared and validated dry-form panels (TREK Diagnostic Systems, Ohio, USA). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control (QC) strains. Susceptibility criteria and QC ranges were in accordance with CLSI guidelines M100-S20-U (2010). Tigecycline MIC values were interpreted based on the breakpoints for Enterobacteriaceae approved by the US Food and Drug Administration (FDA; Tygacil Product Package Insert, 2005).

Screening for carbapenemase-encoding genes and IS*Aba*. Multiplex PCR approaches were used to detect carbapenemase genes. Primers targeting $bla_{OXA-51-like}$, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$ genes, and bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{KHM} variants were combined in two amplification reactions. PER-encoding genes were amplified by generic primers. Forward and reverse primers targeting IS*Aba1*, IS*Aba2* and IS*Aba3* were used in combination with those anchoring the OXA-encoding genes described above.

Molecular typing. Genetic relatedness was evaluated by pulsed-field gel electrophoresis (PFGE). Genomic DNA was prepared in agarose blocks and digested by Apa I (New England Biolabs, Massachusetts, USA). Electrophoresis was performed on the CHEF-DR III apparatus (BioRad, California, USA). PFGE profiles were analyzed using the GelCompar II software (Applied Math, Kortrijk, Belgium). Percent similarities were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.5% and 0.5%, respectively. Isolates showing similarity coefficient ≥80% were considered as genetically related (clonal type).

Multilocus sequence typing (MLST). Primers for amplification and sequencing were those as described on the website of PubMLST (http://pubmlst.org/). Amplicons were sequenced on both strands. Sequencing results were submitted to the PubMLST for analysis.

RESULTS

- Isolates were recovered from sputum (40.0%), blood (22.2%), urine (7.8%), drainage (5.6%), broncho-alveolar lavage (5.6%), cerebrospinal fluid (4.4%), lung (4.4%), other sterile site samples (5.6%) and other respiratory tract samples (4.4%).
- Only colistin ($MIC_{50/90}$, 0.5/1 µg/mL), polymyxin B ($MIC_{50/90}$, 0.5/0.5 µg/mL) and tigecycline ($MIC_{50/90}$, 1/2 µg/mL) were active against this collection of *Acinetobacter* spp. Other agents tested had limited coverage (41.1 – 100.0% resistant; Table 1).
- Susceptibility to minocycline varied regionally. Isolates from Wuhan and Urumqi exhibited MIC values (MIC_{90}, >8 µg/mL) to minocycline, higher than isolates from other regions.
- 81.1% of the strains carried acquired carbapenemase genes, distributed as follows: $bla_{OXA-23-like}$ (71; 78.9%), $bla_{OXA-58-like}$ (29; 32.2%), $bla_{OXA-24-like}$ (3; 3.3%) and bla_{PER} (22; 24.4%). MBLs were not detected. Isolates with different carbapenemases are shown in Table 2.
- $bla_{OXA-51-like}$ was detected in 89 of the 90 strains, confirming species identification. One isolate was PCR-negative for $bla_{OXA-51-like}$ and further identified as *Acinetobacter* genomospecies 3. This strain was collected from Xi'an and carried $bla_{OXA-23-like}$ and bla_{PER}.
- IS*Aba1* was noted upstream of $bla_{OXA-51-like}$ in 16.9% (15/89) of the strains (Table 2, Figure 1). IS*Aba1* was located upstream of $bla_{OXA-23-like}$ in all but 2 of the 71 strains harboring this carbapenemase gene. IS*Aba3* flanked both ends of $bla_{OXA-58-like}$ genes in all strains carrying this gene. IS*Aba1*, *2*, and *3* elements were not associated with $bla_{OXA-24-like}$ (Figure 1).
- Nine *A. baumannii* clones were identified by PFGE and two patterns (C and E) predominated. PFGE porfile C was identified among 29 strains from Wuhan and Urumqi, while profile E comprised 46 isolates from nine medical centers. Isolates from these two clusters displayed at least nine different enzyme/IS arrays (Table 2).
- One isolate each from Xi'an, Wuhan and Shenzhen clustered within three PFGE types (A, B and H), respectively, with unique enzyme contents (Table 2).
- Isolates belonging to PFGE patterns D and F had also distinct enzyme contents and were recovered from different hospitals (Table 2).
- Four isolates belonged to PFGE type G: three from Beijing carried $bla_{OXA-51-like}$ downstream of IS*Aba1* and bla_{PER}, and one from Xi'an carried the same genes, but no IS. Pattern I comprised three isolates from Shenzhen, which carried $bla_{OXA-51-like}$, $bla_{OXA-23-like}$ downstream of IS*Aba1* and bla_{PER}.
- MLST types were unique for each PFGE cluster, but were not defined for four *A. baumannii* groups.

Table 1. Antimicrobial susceptibilities of 90 clinical isolates of carbapenem-resistant *A. baumannii* isolated from Chinese hospitals during 2007-2008.

Antimicrobial agents	MIC (µg/mL) 50%	MIC (µg/mL) 90%	MIC range (µg/mL)	% Susceptible	% Resistance
Imipenem	>8	>8	2 ->8	2.2	88.9
Meropenem	>8	>8	8 ->8	0.0	97.8
Ampicillin/Sulbactam	>16	>16	4 - >16	3.3	92.2
Piperacillin	>128	>128	64 ->128	0.0	96.7
Piperacillin/Tazobactam	>64	>64	32 - >64	0.0	96.7
Ticarcillin/Clavulanic acid	>128	>128	128 - >128	0.0	100.0
Ceftriaxone	>32	>32	0.5 - >32	1.1	68.9
Ceftazidime	>16	>16	4 - >16	12.2	65.6
Cefepime	>16	>16	2 - >16	4.4	91.1
Ciprofloxacin	>4	>4	0.5 - >4	4.4	95.6
Amikacin	>32	>32	1 - >32	14.4	85.6
Gentamicin	>8	>8	2 - >8	4.4	95.6
Tobramycin	>16	>16	0.5 - >16	11.1	88.9
Colistin	0.5	1	0.5 - 2	100.0	0.0
Polymixin B	0.5	0.5	0.5 - 1	100.0	0.0
Doxycycline	>8	>8	0.06 - >8	37.8	62.2
Minocycline	4	>8	0.06 - >8	51.1	41.1
Tigecycline[a]	1	2	0.12 - 4	97.8	2.2
Trimethoprim/sulfamethoxazole	>2	>2	0.5 - >2	8.9	91.1

a. US FDA approved breakpoints for indicated Enterobacteriaceae species were used for comparison purposes only.

Table 2. Molecular characteristics and distribution of carbapenem-resistant *A. baumannii* from P.R. China (2007-2008).

PFGE	MLST	Enzymes (insertion sequences)	Medical Centers[a]	No.
A	NA[b]	OXA-51-like(IS*Aba1*), OXA-23-like, OXA-58-like(IS*Aba3*), PER	SX	1
B	ST109	OXA-51-like, OXA-23-like(IS*Aba1*), PER	WH	1
C	ST91	OXA-51-like, OXA-23-like(IS*Aba1*), OXA-58-like(IS*Aba3*)	WH, XJ	24
		OXA-51-like, OXA-23-like(IS*Aba1*), OXA-58-like(IS*Aba3*), PER	WH	3
		OXA-51-like, OXA-23-like, OXA-58-like(IS*Aba3*)	WH	1
		OXA-51-like, OXA-23-like(IS*Aba1*)	WH	1
D	NA[b]	OXA-51-like, OXA-23-like, OXA-24-like, PER	BJ	1
		OXA-51-like, OXA-24-like	JL	1
E	ST138	OXA-51-like, OXA-23-like(IS*Aba1*)	SY, PU, ZJ,WH, RJ, QD	29
		OXA-51-like(IS*Aba1*)	SY, PU, NJ, XJ, WH	9
		OXA-51-like (IS*Aba1*), OXA-23-like(IS*Aba1*), PER	PU, ZJ	1
		OXA-51-like, OXA-23-like(IS*Aba1*), PER	BJ, ZJ	4
		OXA-51-like(IS*Aba1*), PER	SY, WH	2
		OXA-51-like , PER	BJ	1
F	NA[b]	OXA-51-like, OXA-23-like(IS*Aba1*)	ZJ	1
		OXA-51-like(IS*Aba1*), OXA-23-like(IS*Aba1*)	NJ	1
G	NA[b]	OXA-51-like(IS*Aba1*), PER	BJ	3
		OXA-51-like, PER	SX	1
H	ST92	OXA-51-like(IS*Aba1*)	SZ	1
I	ST110	OXA-51-like, OXA-23-like(IS*Aba1*), PER	SZ	3

a. Medical Centers and their location: BJ, Beijing Hospital, Beijing; JL, The People's Hospital of Jilin Provence, Changchun; NJ, Jiangsu Province Hospital, Nanjing; PU, Peking Union Medical College Hospital, Beijing; QD, The Affiliated Hospital of Medical College Qingdao University, Qingdao; RJ, Rijin Hospital, Shanghai; SX, Shanxi Provincial People's Hospital, Xi'an; SY, Shengjing Hospital of China Medical University, Shenyang; SZ, Shenzhen People's Hospital, Shenzhen; WH, Tongji Hospital, Wuhan; XJ, The First Affiliated Hospital of Xijiang Medcal University, Urumq; ZJ, The First Affiliated Hospital of College of Medicine, Zhejiang University, Hangzhou
b. ST type not available.

Figure 1. Insertion sequences and carbapenemase genes detected in carbapenem-resistant *A. baumannii* strains. Direction of arrows indicate transcription orientation.

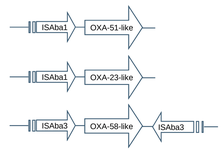
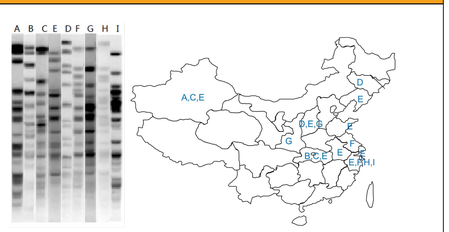


Figure 2. PFGE types and distribution observed among carbapenem-resistant *A. baumannii* isolates recovered from Chinese hospitals during 2007-2008.



CONCLUSIONS

- Carbapenem-resistant *A. baumannii* strains from P.R. China were highly resistant to most antimicrobial agents tested. Polymyxins (100.0% susceptible) and tigecycline (inhibited 97.8% of isolates at ≤2 µg/mL) were the most active agents against these strains.
- Insertion sequences that usually supply promoter regions were detected in 94.4% of the bla_{OXA}-carrying strains. These elements are remarkably important when detected upstream of bla_{OXA} as this configuration can elevate the carbapenem MICs. Additionally, these elements increase the potential for dissemination of carbapenemase genes, since they can function as a transposition system.
- Clonal spread of carbapenem-resistant *A. baumannii* was noted in China. PFGE pattern E (ST138) was the most widespread clone and detected in nine of the twelve hospitals evaluated. Different arrays of enzymes were detected within clusters.

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