Jpn. J. Infect. Dis., 62, 152-154, 2009

Short Communication

Carbapenem-Resistant *Acinetobacter baumannii* Producing OXA-23 in Thailand

Pannika Ritvirool Niumsup*, Nitsara Boonkerd, Uttapoln Tansawai[1], and Mujarin Tiloklurs[2]

Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok;
[1]Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna Phitsanulok Campus, Phitsanulok; and [2]Clinical Pathology Department, Buddhachinnaraj Hospital, Phitsanulok, Thailand

(Received September 17, 2008. Accepted January 19, 2009)

SUMMARY: We investigated the resistance determinant of 13 clinical isolates of carbapenem-resistant *Acinetobacter baumannii* collected from a regional hospital in the north of Thailand. All isolates were multidrug resistant and produced the OXA-23 carbapenemase. The bla_{OXA-23} gene was found adjacent to IS*Aba1*. Furthermore, two isolates carried the metallo β-lactamase gene, bla_{IMP}. The bla_{OXA-23} and bla_{IMP} genes were plasmid-mediated according to the transformation assays. This is the first description of OXA-23-producing *A. baumannii* from Thailand.

Acinetobacter baumannii is an important cause of nosocomial infections, such as pneumonia, urinary tract infections, wound infections, and septicemia. Carbapenems are potent antimicrobial agents and commonly employed for the treatment of multidrug resistant Gram-negative bacilli infections including *A. baumannii* (1). Carbapenem-resistant *A. baumannii* have now been reported worldwide. A previous study in Thailand in the year 1998 showed that 4% of *Acinetobacter* spp. isolates were resistant to imipenem (2). According to a report by the National Antimicrobial Resistance Surveillance Center of Thailand in the year 2007, the incidence of imipenem-resistant *Acinetobacter* sp. in Thailand has dramatically increased to over 50% (http://narst.dmsc.moph.go.th). Recently, it has been reported that the mortality rate in patients with imipenem-resistant *A. baumannii* at Songklanagarind Hospital, south of Thailand, was 33.8% (3). One of the mechanisms for carbapenem resistance is the production of carbapenem-hydrolyzing β-lactamases belonging to molecular class B, including various IMP- and VIM-type metallo β-lactamases and the zinc-independent class D enzymes (oxacillinases) (4). In 2004, the chromosome-encoded OXA-72 was identified and characterized in imipenem-resistant *A. baumannii* in Thailand (GenBank accession no. AY739646); however, no data on resistance patterns was available. Aside from this single report there is little information on the prevalence or type of carbapenemase genes in *A. baumannii* in Thailand.

Buddhachinnaraj Hospital is a 1,000-bed tertiary care hospital in Phitsanulok, a city located 350 km north of Bangkok, Thailand. Our preliminary studies in the year 2005 revealed a high frequency of imipenem-resistant *A. baumannii* (57%, unpublished results). Thus we carried out this study to investigate the resistance determinant among carbapenem-resistant *A. baumannii* collected from a regional hospital in Thailand.

During the period of March - April 2006, 13 *A. baumannii* isolates were obtained from hospitalized patients. They were selected on the basis of their high-level resistance to imipenem. The isolates were identified by API-20NE (bioMerieux, Marcy-l'Etoile, France). β-Lactam MICs were determined by the agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (5). *Escherichia coli* ATCC25922 was used as the MIC reference strain. Crude cell extracts were prepared by periplasmic extractions (6) and β-lactamase activities were examined by measuring the hydrolysis of β-lactams (Spectronic® Genesys™ 5; Milton Roy, Rochester, N.Y., USA). All assays were run in triplicate. Specific activities were calculated in μmoles of β-lactam hydrolyzed per min per mg of protein. Pulsed field gel electrophoresis (PFGE) was performed with *Apa*I-digested whole-cell DNAs embedded in a 1%-agarose plug and separated in a 1%-pulsed field certified agarose gel using a CHEF Mapper® XA System (Bio-Rad, Hercules, Calif., USA) as described previously (7).

Genes encoding for Ambler class B and D carbapenemases were detected by PCR using Platinum *Taq* polymerase (Invitrogen, Carlsbad, Calif., USA) with primers and conditions specific for bla_{IMP} (8), bla_{VIM} (9), and bla_{OXA-23}-like genes (10). Plasmid DNA was prepared using the alkaline lysis method and used as a DNA template for PCR analysis. PCR products were purified using a QIAquick PCR purification kit (Qiagen, Valencia, Calif., USA) and sequenced on both strands (Applied Biosystem, Foster City, Calif., USA). The nucleotide and deduced amino acid sequences were analyzed using available software (http://www.ncbi.nlm.nih.gov). A plasmid suspension was used for the transformation experiment in *E. coli* TOP10F′ with a Gene Pulser II electroporator (Bio-Rad), and the transformants were selected on agar plates containing imipenem (1 μg/ml). For cloning of the bla_{OXA-23} gene, the PCR product was cloned into pCR2.1 TOPO (Invitrogen) and the recombinant plasmid was transformed into chemically competent cells of *E. coli* TOP10F′ by heat shock as detailed in the supplier's instructions. Transformants were selected on nutrient agar plates containing 25 μg/ml of kanamycin and 1 μg/ml of imipenem.

In the present study, 13 *A. baumannii* isolates possessed imipenem and meropenem MICs of ≥32 μg/ml. The isolate 1157 obtained at the same time from another patient was susceptible to carbapenems (Table 1).With the exception of isolate 1018, all isolates were also resistant to extended-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, and

*Corresponding author: Mailing address: Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand. Tel: +66-55-964612, Fax: +66-55-964770, E-mail: npannika@hotmail.com

Table 1. β-Lactam MICs, PFGE profiles and carbapenemase genes of *A. baumannii* isolates

Bacterial isolate	MIC (μg/ml)						PFGE type	Presence of	
	CAZ	CTX	CRO	FEP	IPM	MEM		bla_{OXA-23}	bla_{IMP}
E. coli ATCC 25922	0.25	0.125	0.031	0.062	0.25	0.031	–	NE	NE
A. baumannii 1157	16	32	128	4	4	4	–	–	–
A. baumannii 1018	8	32	32	64	64	64	I	+	–
A. baumannii 1081	>256	256	256	>256	64	128	I	+	–
A. baumannii 1263	>256	>256	>256	64	64	128	II	+	–
A. baumannii 1295	>256	>256	>256	256	128	128	III	+	–
A. baumannii 1324	>256	>256	>256	>256	128	32	IV	+	+
A. baumannii 1325	>256	>256	>256	>256	64	32	V	+	–
A. baumannii 1386	256	>256	>256	>256	128	64	VI	+	+
A. baumannii 1389	64	256	128	64	128	64	VI	+	–
A. baumannii 1414	>256	256	128	256	64	64	VII	+	–
A. baumannii 1455	>256	>256	>256	64	64	64	VIII	+	–
A. baumannii 1511	128	256	256	>256	32	32	VIII	+	–
A. baumannii 1521	>256	>256	>256	128	64	32	VIII	+	–
A. baumannii 1522	>256	>256	>256	128	32	64	VIII	+	–

CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; IPM, imipenem; MEM, meropenem; NE, not examined.

Table 2. β-Lactam MICs and β-lactamase activity of *E. coli* and *E. coli* carrying bla_{OXA-23}

Isolate	MIC (μg/ml)							β-lactamase activities[1]			
	AMP	CEF	CRO	CTX	CAZ	IPM	MEM	OXA	CTX	CAZ	IPM
E. coli TOP10F′	4	4	0.125	0.125	0.5	0.25	<0.125	ND	ND	ND	ND
E. coli TOP10F′(pOXA-23)	64	128	4	0.25	2	0.5	<0.125	3979.6 ± 22.3	8.6 ± 0.5	ND	5.2 ± 0.7

[1]: μmole substrate hydrolyzed per min per mg of protein. Results are shown as mean ± SD of from three experiments.
AMP, ampicillin; CEF, cefalothin; CRO, ceftriaxone; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; OXA, oxacillin; ND, no detectable activity.

cefepime), for which the MICs were clustered at around 64 to >256 μg/ml. The majority of *A. baumannii* isolates demonstrated weak hydrolytic activity against imipenem, a feature previously noted for the *A. baumannii* oxacillinase (4). PFGE profiles of all carbapenem-resistant *A. baumannii* isolates demonstrated eight pulsotypes (I-VIII). This result suggested that they were genetically diverse, while some isolates appeared to be closely related (Table 1).

Thirteen carbapenem-resistant *A. baumannii* isolates gave bla_{OXA-23}-like PCR products, and sequencing of seven representative PCR products confirmed the presence of bla_{OXA-23} without any point mutations (11), which was not detected in the carbapenem-susceptible isolate 1157 (Table 1). Analysis of the nucleotide sequence showed the presence of IS*Aba1* upstream of the bla_{OXA-23} gene, a genetic context that was similar to that of the prototype bla_{OXA-23} gene (12). IS*Aba1* is thought to provide a strong promoter for the high-level expression of bla_{OXA-23} (13). The bla_{OXA-23} gene was shown to be plasmid mediated by electroporation of the plasmid into *E. coli* TOP10F′, and was confirmed by PCR. Plasmid extractions from the *E. coli* TOP10F′ electrotransformant followed by restriction analysis with *Hind*III revealed a single small plasmid at ca. 30 kb. These findings are consistent with the fact that bla_{OXA-23} are mostly located on plasmids (4). The imipenem MIC for the *E. coli* TOP10F′ electrotransformant carrying bla_{OXA-23} (0.5 μg/ml) was slightly higher than that of the wild-type TOP10F′ (0.25 μg/ml).The bla_{OXA-23} PCR product was cloned into *E. coli* TOP10F′ and the recombinant plasmid was verified by PCR and sequencing. *E. coli* carrying bla_{OXA-23} displayed higher MIC values for ampicillin, cephalothin, ceftriaxone, cefotaxime, and ceftazidime than *E. coli* without plasmid. A 2-fold increase in imipenem MIC was detected in the TOP10F′ (pOXA-23) transformant but the meropenem MIC remained unchanged (Table 2). The hydrolysis of oxacillin and imipenem (albeit at a low level) confirmed that the β-lactamase acquired by this transformant corresponds to the carbapenem-hydrolyzing oxacillinases (Table 2).

The *A. baumannii* isolates producing OXA-23 carbapenemases have been repeatedly found in many countries, suggesting a worldwide distribution of these resistance determinants. In Asia, OXA-23 has frequently been identified in outbreaks of carbapenem-resistant *A. baumannii*, such as in China (14,15), Korea (10,16), and Singapore (17). This is the first description of OXA-23-producing *A. baumannii* in Thailand. The findings of OXA-23 in genetically unrelated *A. baumannii* infer that the occurrence of resistant isolates results from the transfer of the plasmid among the resistant isolates rather than the spread of one bacteria clone. However, the finding that several isolates produced identical restriction patterns on PFGE suggests that the dissemination of OXA-23 may due to the clonal spread of resistant isolates.

Carbapenem-resistant *A. baumannii* isolates have been reported which possess metallo β-lactamases instead of the plasmid-mediated class D enzymes. In the present study, amplifications for bla_{VIM}-type metallo β-lactamases were negative but we detected the bla_{IMP} allele in two isolates of carbapenem-resistant *A. baumannii* (isolate 1324 and 1386) (Table 1). Sequence analysis of the amplified fragments confirmed the presence of bla_{IMP}. The bla_{IMP} gene was located on a plasmid as verified by PCR amplification on electrotransformants. Because the bla_{IMP} has been found on the class 1 integron, and the gene cassettes in the class 1 integron are common in *Acinetobacter* spp., the IMP-positive isolates from

this study were tested for this feature. Preliminary analysis by PCR using primers targeting class 1 integron conserved sequences (CS) (5′CS and 3′CS) (18) did not detect the presence of class 1 integron, and thus the genetic context of bla_{IMP} in *A. baumannii* in this study remains unknown.

In conclusion, we demonstrated the presence of OXA-23 in carbapenem-resistant *A. baumannii* isolates, suggesting the further global spread of this enzyme. However, the possession of bla_{OXA-23} may not be sufficient to explain the carbapenem resistance. Other mechanisms, including the loss of porins, reduced affinity of penicillin-binding proteins and increase in efflux activity, may also be associated with the carbapenem resistance in these isolates. Further surveillance in Thai hospitals is essential to evaluate the prevalence of the carbapenemase gene and prevent the spread of carbapenem-resistant organisms.

ACKNOWLEDGMENTS

This work was funded by Thailand Research Fund.

We are grateful to the staff members at the Buddhachinnaraj Hospital for collecting *A. baumannii* isolates used in this study.

REFERENCES

1. Bergogne-Bérézin, E. and Towner, K.J. (1996): *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev., 9, 148-165.
2. Biedenbach, D.J., Johnson, D.M. and Jones, R.N. (1999): In vitro evaluation of cefepime and other broad-spectrum β-lactams in eight medical centers in Thailand. The Thailand Antimicrobial Resistance Study Group. Diagn. Microbiol. Infect. Dis., 35, 325-331.
3. Jamulitrat, S., Thongpiyapoom, S. and Suwalak, N. (2007): An outbreak of imipenem-resistant *Acinetobacter baumannii* at Songklanagarind Hospital: the risk factors and patient prognosis. J. Med. Assoc. Thai., 90, 2181-2191.
4. Walther-Rasmussen, J. and Hoiby, N. (2006): OXA-type carbapenemases. J. Antimicrob. Chemother., 57, 373-383.
5. Clinical and Laboratory Standards Institute (2007): Performance Standards for Antimicrobial Susceptibility Testing: 17th informational supplement. CLSI document M100-S17. Clinical and Laboratory Standards Institute, Wayne, Pa.
6. Lindström, B.E., Boeman, H.G. and Steele, B.B. (1970): Resistance of *Escherichia coli* to penicillins. J. Bacteriol., 101, 218-231.
7. Seifert, H., Dolzani, L., Bressan, R., et al. (2005): Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. J. Clin. Microbiol., 43, 4328-4335.
8. Da Silva, G.J., Correia, M., Vital, C., et al. (2002): Molecular characterization of bla_{IMP-5}, a new integron-borne metallo-β-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. FEMS Microbiol. Lett., 215, 33-39.
9. Poirel, L., Naas, T., Nicolas, D., et al. (2000): Characterisation of VIM-2, a carbapenem-hydrolysing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother., 44, 891-897.
10. Jeon, B.C., Jeong, S.H., Bae, I.K., et al. (2005): Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 β-lactamase in Korea. J. Clin. Microbiol., 43, 2241-2245.
11. Donald, H.M., Scaife, W., Amyes, S.G., et al. (2000): Sequence analysis of ARI-1, a novel OXA β-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. Antimicrob. Agents Chemother., 44, 196-199.
12. Segal, H., Garny, S. and Elisha, B.G. (2005): Is IS_{ABA-1} customized for *Acinetobacter*? FEMS Microbiol. Lett., 243, 425-429.
13. Turton, J.F., Ward, M.E., Woodford, N., et al. (2006): The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol. Lett., 258, 72-77.
14. Wang, H., Guo, P., Sun, H., et al. (2007): Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. Antimicrob. Agents Chemother., 51, 4022-4028.
15. Zhou, H., Yang, Q., Yu, Y.S., et al. (2007): Clonal spread of imipenem-resistant *Acinetobacter baumannii* among different cities of China. J. Clin. Microbiol., 45, 4054-4057.
16. Lee, J.H., Choi, C.H., Kang, H.Y., et al. (2007): Differences in phenotypic and genotypic traits against antimicrobial agents between *Acinetobacter baumannii* and *Acinetobacter genomic* species 13TU. J. Antimicrob. Chemother., 59, 633-639.
17. Koh, T.H., Sng, L.H., Wang, G.C., et al. (2007): IMP-4 and OXA β-lactamases in *Acinetobacter baumannii* from Singapore. J. Antimicrob. Chemother., 59, 627-632.
18. Lévesque, C., Piché, L., Larose, C., et al. (1995): PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother., 39, 185-191.