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RESEARCH NOTE

Carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* isolates in the United Arab Emirates

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ABSTRACT

Five carbapenem-resistant *Acinetobacter baumannii* isolates, collected from the United Arab Emirates in 2006, were investigated to identify the mechanism(s) responsible for carbapenem resistance. Genotyping was performed by pulsed-field gel electrophoresis, and the location of the bla_{OXA-23} gene was determined by using the endonuclease I *Ceu*I technique and mating-out assays. The four isolates in which the bla_{OXA-23} gene was located on the chromosome within a Tn*2006* composite transposon were clonally related. The single non-clonally related isolate harboured the bla_{OXA-23} gene on a 70-kb transferable plasmid. This study reports on the dissemination of OXA-23-producing *A. baumannii* isolates in the Middle East.

Keywords *Acinetobacter baumannii*, carbapenem resistance, OXA-23, transposon, United Arab Emirates

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Carbapenem resistance in *Acinetobacter baumannii* is increasingly being observed worldwide. Several studies have reported metallo-β-lactamases (MBLs) [1], but the most common mechanism is related to carbapenem-hydrolyzing class D β-lactamases (CHDLs) [2]. Three main acquired CHDL gene clusters have been described in *A. baumannii*, containing bla_{OXA-23}-like, bla_{OXA-40}-like or bla_{OXA-58}-like genes. The bla_{OXA-23} gene is either plasmid-borne or chromosome-borne [3,4], and *A. radioresistens* was recently identified as the progenitor of bla_{OXA-23}-like genes [5].

From July 2006 to November 2006, five carbapenem-resistant *A. baumannii* isolates causing fatal infections in five patients were collected from the adult intensive-care unit of Sheikh Khalifa Medical City. Whereas two isolates were considered to be colonizing agents, three were involved in infections, two of which caused septicaemia. The age of the patients ranged from 30 to 60 years, and all of them had been treated with colistin and tobramycin, except patient 3, who was treated with colistin and tigecycline (Table 1).

The isolates were identified after 16S rRNA gene sequencing [6]. MICs of imipenem and meropenem were determined using antibiotic disks from Sanofi Diagnostics Pasteur (Marne-La-Coquette, France) [7] and were found to be 32 mg/L (Table 2). The isolates were resistant to almost all antibiotics, including aminoglycosides, fluoroquinolones and β-lactams (Table 2).

The production of MBLs was evaluated using the MBL Etest, combining imipenem and EDTA as recommended by the manufacturer (AB Biodisk,

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Table 1. Clinical features related to *Acinetobacter baumannii* isolates

Isolate number	Imipenem susceptibility	Date of first isolation (day/month/year)	Date of hospitalization (day/month/year)	Source of isolation	Infected/ colonized	Treatment for *A. baumannii* infection	Underlying disease	Outcome
1	Resistant	24/7/2006	2/6/2006	Blood	Colonized	Colistin and tobramycin	Duodenal ulcer, peritonitis, septic shock	Died
2	Resistant	13/8/2006	14/7/2006	Blood	Colonized	Colistin and tobramycin	Pneumonia	Died
3	Resistant	2/10/2006	1/10/2006	Blood	Infected	Colistin and tigecycline	Pancreatitis	Died
4	Resistant	6/10/2006	4/4/2006	Blood	Infected	Colistin and tobramycin	Peritonitis	Died
5	Resistant	3/11/2006	15/10/2006	CSF	Infected	Colistin and tobramycin	Polytrauma	Died

CSF, cerebrospinal fluid.

Table 2. MICs of β-lactams for the five OXA-23-positive *Acinetobacter baumannii* isolates, *Escherichia coli* TOP10 (pAS1), reference strain *E. coli* TOP10, *A. baumannii* transconjugant expressing OXA-23, and reference strain *A. baumannii* BM 4547

β-Lactam(s)	*A. baumannii* isolates 1, 2, 4 and 5	*A. baumannii* isolate 3	*E. coli* TOP10 (pAS1)	*E. coli* TOP10	*A. baumannii* BM 4547 (bla_{OXA-23})	*A. baumannii* BM 4547
Amoxycillin	>512	>512	>512	2	>512	16
Amoxycllin + CLA	512	>512	16	2	64	16
Ticarcillin	>512	>512	>512	1	>512	8
Ticarcillin + CLA	>512	>512	256	1	>512	8
Piperacillin	256	256	4	1	>512	4
Piperacillin + TZB	256	256	4	1	>512	4
Cephalotin	256	256	1	4	256	256
Cefuroxime	256	256	1	1	16	16
Ceftazidime	256	256	<0.06	<0.06	4	4
Cefotaxime	256	256	<0.06	<0.06	32	32
Cefepime	256	256	<0.06	<0.06	256	32
Cefpirome	256	256	<0.06	<0.06	>512	64
Aztreonam	128	>512	<0.06	0.06	128	128
Imipenem	32	32	0.25	<0.06	16	0.25
Meropenem	32	32	0.32	<0.06	>32	1

CLA, clavulanic acid (4 μg/mL); TZB, tazobactam (4 μg/mL); *E. coli* TOP10 (pAS1) expressing the chromosomally located bla_{OXA-23} gene from plasmid pBK-CMV cloning vector; *A. baumannii* BM 4547 bla_{OXA-23} expressing the bla_{OXA-23} plasmid-borne gene.

Solna, Sweden), but all isolates gave negative results. However, PCR experiments followed by sequence analysis led to the identification of bla_{OXA-23}, in addition to the natural bla_{OXA-69} β-lactamase gene, in all isolates. The insertion sequence IS*Aba1* was detected upstream of the bla_{OXA-23} gene in all isolates; however, no colinearity was found between IS*Aba1* and bla_{OXA-69}, thus making any IS*Aba1*-mediated overexpression of this naturally occurring carbapenemase gene unlikely [8].

Genotypic comparison was performed by pulsed-field gel electrophoresis according to the manufacturer's recommendations (Bio-Rad, Marnes-la-Coquette, France). Whole cell DNA of the *A. baumannii* isolates was digested with *Apa*I overnight at 37°C (New England Biolabs, St Quentin-en-Yvelines, France). Electrophoresis was performed with a CHEF DRII apparatus (Bio-Rad) through a 1% agarose gel in 0.5× Tris–borate–EDTA buffer. Migration conditions were as follows: temperature, 14°C; voltage, 6 V/cm; and switch angle with one linear switch ramp of 3–8 s for 10.5 h, and then 12–20 s for 10.5 h. The analysis showed that four of the five carbapenem-resistant isolates belonged to a single clone according to the Tenover criteria. This result is in accordance with the results of bla_{ampC} gene sequencing as previously described [9], indicating perfect identity among isolates 1, 2, 4 and 5, whereas a 10-bp divergence was found in isolate 3 (data not shown). Interestingly, all four patients infected with the same clonal strain had received the same antimicrobial regimen, and identical MICs were determined for their isolates (Table 2). Thus, the dissemination of an OXA-23-producing clone within this hospital was assessed, but, interestingly, another OXA-23-producing strain had also caused infection of a patient.

To search for a chromosomal location of the β-lactamase gene, the endonuclease I *Ceu*I (New England Biolabs) was used, which digests a 26-bp sequence in the *rrn* genes for the 23S rRNA [10], and the fragments were separated by pulsed-field gel electrophoresis. DNA–DNA hybridization of the fragments was then performed, using the Southern technique as previ-

ously described [11], with two probes: an 884-bp PCR-generated probe specific for the 16S and 23S rRNA genes, and a 589-bp probe specific for the bla_{OXA-23} gene. Labelling of the probes and signal detection were carried out using a non-radioactive labelling and detection kit, according to the manufacturer's instructions (GE Healthcare, Saclay, France). The endonuclease I *Ceu*I technique revealed a chromosomal location of the bla_{OXA-23} gene in the four clonally related isolates (isolates 1, 2, 4 and 5) with a hybridization signal obtained for a *c*. 40-kb band, whereas the bla_{OXA-23} gene was located on a 70-kb plasmid in isolate 3 (data not shown). To assess the plasmid location of bla_{OXA-23} in isolate 3 more precisely, mating-out experiments were performed with isolate 3 as donor and *A. baumannii* BM 4547 as recipient strain, with selection on ticarcillin (50 mg/L) and rifampicin (50 mg/L), as previously described [12]. The bla_{OXA-23}-containing plasmid was transferred by conjugation from *A. baumannii* 3 into *A. baumannii* BM 4547. MICs for the bla_{OXA-23} transconjugants are indicated in Table 2.

In order to investigate the genetic structures surrounding the bla_{OXA-23} gene, cloning experiments were performed. Total DNA of *A. baumannii* isolates was digested with the *Sac*I restriction enzyme, ligated into the *Sac*I site of plasmid pBK-CMV, and transferred by electrotransformation into *Escherichia coli* TOP10, as previously described [13]. Recombinant plasmids were selected on Trypticase Soy agar plates containing ticarcillin (50 mg/L) and kanamycin (30 mg/L). Recombinant plasmid pAS1, obtained from *A. baumannii* isolate 1, was sequenced on both strands using an Applied Biosystems sequencer (ABI 3100, Foster City, CA), which allowed identification of the bla_{OXA-23} gene as part of transposon Tn*2006*, as observed previously [14]. Analysis of nucleotide sequences at the 3′-extremity of Tn*2006* showed that its insertion had occurred in the chromosome close to *guaD*, a gene encoding a putative guanine deaminase sharing 88% amino acid identity with that of *A. baumannii* ATCC 17198 [15]. The 5′-extremity of Tn*2006* was not available from recombinant plasmid pAS1. Thus, primer 5′-guanine (5′-ATCGTACGGCATAAGTCTGC-3′) was designed, on the basis of the genome sequence of *A. baumannii* ATCC 17198, and the remaining sequence of *guaD* was successfully amplified. Sequencing of the obtained PCR product revealed a duplication of the target site composed of a 9-bp sequence (AAGTTTTTG) at both Tn*2006* ends, indicating that the Tn*2006* acquisition was likely to have occurred through a transposition mechanism. This structure was identified in *A. baumannii* isolates 1, 2, 4 and 5. In isolate 3, IS*Aba1* was detected upstream of the bla_{OXA-23} gene, but the 3′-extremity of Tn*2006* was not identified by PCR mapping. Unfortunately, attempts to clone the bla_{OXA-23} gene from isolate 3 remained unsuccessful. Thus, it is likely that a different structure was associated with the bla_{OXA-23} gene in the plasmid in isolate 3.

This study provides the first description of the dissemination of carbapenem-resistant *A. baumannii* isolates carrying Tn*2006* on their chromosome. It is also the first report of OXA-23-producing *A. baumannii* isolates in the Middle East. It further emphasizes the major role of CHDLs, and in particular of OXA-23, in the emergence of carbapenem resistance in *Acinetobacter* spp.

TRANSPARENCY DECLARATION

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RESEARCH NOTE

In vitro activity of tigecycline and occurrence of tetracycline resistance determinants in isolates from patients enrolled in phase 3 clinical trials for community-acquired pneumonia

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ABSTRACT

The *in vitro* activity of tigecycline was evaluated against baseline pathogens isolated from patients enrolled in phase 3 clinical trials for community-acquired pneumonia conducted in 29 countries worldwide. Tigecycline was active against the most prevalent pathogens, including *Streptococcus pneumoniae* (MIC_{90} 0.06 mg/L), *Staphylococcus aureus* (MIC_{90} 0.25 mg/L), *Haemophilus influenzae* (MIC_{90} 0.5 mg/L) and *Klebsiella pneumoniae* (MIC_{90} 1 mg/L). Twelve isolates of *S. pneumoniae* expressing *tet*(M) and two isolates of *K. pneumoniae* producing extended-spectrum β-lactamases isolated during the study were susceptible to tigecycline. The excellent *in vitro* activity of tigecycline against these clinical isolates confirmed its potential utility against pathogens associated with community-acquired pneumonia.

Keywords Community-acquired pneumonia, *in vitro* susceptibility, respiratory isolates, tigecycline

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Tigecycline, the 9-t-butylglycylamido derivative of minocycline, was developed by Wyeth in

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