Diversity of plasmid-mediated carbapenem-hydrolysing oxacillinases among carbapenem-resistant Acinetobacter baumannii isolates from Kingdom of Bahrain

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Sir,

Carbapenem resistance in Acinetobacter baumannii is now increasingly reported. The most prevalent mechanism of carbapenem resistance in A. baumannii corresponds to the production of acquired carbapenem-hydrolysing class D β-lactamases (CHDLs)1 encoded by blaOXA-23-like, blaOXA-40-like or blaOXA-58-like genes. Whereas the blaOXA-40 gene has been detected in Portugal, Spain and the USA, blaOXA-23 and blaOXA-58 genes have disseminated worldwide.1

The aim of our study was to identify the molecular mechanisms leading to carbapenem resistance among a panel of A. baumannii isolates exhibiting resistance or intermediate susceptibility to imipenem and collected from September 2007 to March 2008 in the Salmaniya Medical Complex, Kingdom of Bahrain, which is a 1000 bed tertiary care centre.

A total of 454 A. baumannii isolates had been recovered during the studied period, among which 262 (58%) were resistant or intermediate susceptible to imipenem. Among the latter, eight randomly selected A. baumannii isolates collected from eight patients hospitalized in different units were further analysed. They were identified using the API20 NE system and 16S rRNA gene sequencing.2 All isolates had been recovered from deep tracheal aspirates. Although two isolates were considered as colonizers, six were involved in infections and, among those, four were responsible for septicemia that led to the death of all these patients.

Use of Etest strips (AB Biodisk, Solna, Sweden) combining imipenem and EDTA did not identify metallo-β-lactamase in the isolates. PCR experiments followed by sequencing as described previously3 led to the identification of CHDL genes. A blaOXA-23 gene was found in two isolates (1 and 2), preceded by insertion sequence ISAbal as observed in transposon Tn2006;3 but no ISAbal copy was identified downstream of blaOXA-23. Isolate 3 harboured a blaOXA-58 gene that was bracketed by two copies of ISAbal3, as described previously.1 Five isolates possessed a blaOXA-40-like gene, and further sequencing revealed that this gene corresponded to blaOXA-72. The OXA-72 β-lactamase belongs to the OXA-40 subgroup together with OXA-25 and OXA-26 β-lactamases. The OXA-40-type β-lactamases differ by no more than five amino acids and possess weak carbapenemase properties. OXA-72 had been previously reported in a single Acinetobacter genomospecies 3 isolate in China,4 in a single Acinetobacter baylyi isolate in Korea5 and in several carbapenem-resistant A. baumannii isolates in Taiwan.6

Genotypic comparison was performed by PFGE, as described previously.7 The two OXA-23 producers (isolates 1 and 2) were clonally related (pulsotype I). The single blaOXA-58-positive isolate belonged to pulsotype II. The remaining blaOXA-72-positive isolates belonged to pulsotype III (isolates 4 and 5), IV (isolates 6 and 7) or V (isolate 8). All isolates were resistant to ticarcillin and piperacillin, and isolates carrying genes encoding CHDLs OXA-23 and OXA-72 were highly resistant to imipenem and meropenem, although the OXA-58 producer displayed lower MICs of imipenem and cefazidime (Table 1).

Mating-out experiments were performed as described previously,7 with OXA-23-, OXA-58- and OXA-72-positive isolates as donors and rifampicin-resistant A. baumannii BM4547 as the recipient strain. The transconjugants were selected on agar plates containing ticarcillin (50 mg/L) and rifampicin (50 mg/L). Transconjugants were obtained only with blaOXA-23-positive donors, and the transconjugants showed resistance to ticarcillin and decreased susceptibility to carbapenems (Table 1). The transconjugants harboured a 130 kb plasmid additionally conferring resistance to kanamycin and amikacin (data not shown).

Plasmid extracts, obtained as described previously,7 with OXA-58- and OXA-72-positive isolates were used for transformation experiments with A. baumannii BM4547 as the recipient strain. Electrottransformation products were selected on plates containing ticarcillin (50 mg/L). Electroporation of plasmid extracts gave rise to A. baumannii BM4547 (pOXA-72) transformants, whereas no transformant was obtained with the OXA-58-positive donor strain. The blaOXA-72 gene was located on a 10 kb plasmid in all OXA-72-positive isolates. The blaOXA-72-positive A. baumannii transformants showed resistance to ticarcillin and decreased susceptibility to carbapenems (Table 1), without any additional antibiotic resistance marker.

In order to investigate the genetic structures surrounding the blaOXA-72 gene, cloning experiments were also performed. Total DNA of A. baumannii isolate 4 was digested with the SacI or HindIII restriction enzymes, ligated into corresponding sites of plasmid pBK-CMV and transferred by electroporation into Escherichia coli TOP10 as described previously.7 E. coli strains harbouring recombinant plasmid pBK-OXA-72 were selected on agar plates containing ticarcillin (50 mg/L) and kanamycin (30 mg/L). Detailed sequence analysis of pBK-OXA-72 showed that a gene encoding a putative inner membrane protein was present 300 bp upstream of blaOXA-72, whereas downstream a gene
Table 1. MICs (mg/L) of β-lactams for representative *A. baumannii* clinical isolates of the different pulsotypes, *A. baumannii* BM4547 harbouring pOXA-72, *A. baumannii* BM4547 RifR harbouring pOXA-23, *A. baumannii* BM4547 and BM4547 RifR reference strains, *E. coli* TOP10 harbouring recombinant plasmid pBK-OXA-72 and *E. coli* TOP10 reference strain

<table>
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<th>β-Lactam(s)</th>
<th>A. baumannii BM4547 (OXA-23)</th>
<th>A. baumannii BM4547 (OXA-58)</th>
<th>A. baumannii BM4547 (OXA-72)</th>
<th>A. baumannii BM4547 (pOXA-72)</th>
<th>A. baumannii BM4547 RifR (pOXA-23)</th>
<th>A. baumannii BM4547 RifR (pOXA-72)</th>
<th>E. coli TOP10</th>
<th>E. coli TOP10</th>
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CLA, clavulanic acid (4 mg/L); TZB, tazobactam (4 mg/L).
encoding a putative replication protein was identified. No typical genetic element (such as an insertion sequence) that could explain the acquisition of \( \text{bla}_{\text{OXA-72}} \) was identified at each extremity.

In the Middle East, clonal outbreaks of infections caused by carbapenem-resistant \( A. \text{baumannii} \) strains have been reported, having occurred in the United Arab Emirates, Qatar, Iran and Iraq.\(^4-9\) In the United Arab Emirates, resistance to carbapenems was associated with the production of OXA-23;\(^9\) the carbapenem resistance mechanisms were not investigated in our study. Here we identified heterogeneity of CHDL-encoding genes as the source of carbapenem resistance among \( A. \text{baumannii} \) isolates from the same hospital. This is, to the best of our knowledge, the first report of such diversity of carbapenem resistance determinants in a restricted geographical area. This diversity of \( A. \text{baumannii} \) isolates and of CHDL genes in Bahrain may be related to the fact that this region of the Middle East has a very mixed population originating from various parts of the world.

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None to declare.

**References**


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**Emergence of extended-spectrum-AmpC-expressing \( \text{Escherichia coli} \) isolates in Belgian hospitals**

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Sir,

The overproduction of AmpC chromosomal cephalosporinases with broadened substrate activity has recently been reported in clinical isolates of \( \text{Escherichia coli} \).\(^1\) These so-called extended-spectrum AmpC (ESAC) \( \beta \)-lactamases confer reduced susceptibility to all cephalosporins, including the fourth-generation agents cefepime and cefpirome. We aimed to investigate the possible occurrence of ESAC-expressing \( E. \text{coli} \) isolates in Belgium.

Among the 6850 non-duplicate \( E. \text{coli} \) clinical isolates (one isolate/patient) that were recovered from two Belgian hospitals (Erasme Hospital, Brussels and UCL de Mont-Godinne Hospital, Yvoir) between 2004 and 2006, 83 were found to display a \( \beta \)-lactam resistance pattern consistent with AmpC overproduction on the basis of resistance to amoxicillin, amoxycillin/clavulanic acid, cefazolin and cefoxitin by the disc diffusion method using the CLSI interpretative guidelines.\(^2\) The presence of AmpC was further confirmed by analytical isoelectric focusing demonstrating a band at a pl of \( \sim 8.5–9.0 \), disappearing in the presence of oxacillin.

Fourteen out of the 83 AmpC hyper-producing isolates (Table 1) were resistant to cefazidime and cefotaxime by current EUCAST breakpoints\(^3\) and intermediate to cefepime (MIC above 1 mg/L; 1.5–8 mg/L). Sequence analysis of their chromosomal \( \text{ampC} \) genes revealed that eight of the isolates harboured an S287N mutation, while two harboured an H296P mutation, one harboured an S287N mutation, one harboured a V298L mutation and one harboured an E362K mutation. All these mutations, except the L293P mutation that is reported here for the first time, have already been reported in \( E. \text{coli} \) and were shown to be involved in the ESAC phenotype.\(^4,5\)

No mutation was found in the chromosomal AmpC of the two remaining isolates. Instead, \( IS\text{ecp1-}\text{bla}_{\text{CMY-2}} \) (plasmid-mediated \( \text{ampC} \) gene) was detected in those \( E. \text{coli} \). In one of the isolates (MIC of cefepime=4 mg/L), the reduced susceptibility to cefepime could be ascribed to the presence of a \( \text{bla}_{\text{OXA-30}} \)