Coexistence of bla_{OXA-23} with bla_{NDM-1} and armA in clinical isolates of Acinetobacter baumannii from India

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Keywords: mixed carbapenemases, ISAba1, high-level resistance to aminoglycosides

Sir,
The production of carbapenemases is the most common mechanism responsible for carbapenem resistance in Acinetobacter baumannii. They include metallo-β-lactamases (VIM, IMP and SIM types), which have been sporadically reported in some parts of the world, and acquired OXA-type carbapenemases, which have been more frequently identified worldwide and are clustered in three major subfamilies (bla\textsubscript{OXA-23}, bla\textsubscript{OXA-24} and bla\textsubscript{OXA-58}). High levels of resistance to aminoglycosides due to the production of plasmid-encoded 16S rRNA methylase in Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter spp. have been documented since 2003. The present study was undertaken to determine the mechanism responsible for the carbapenem resistance.

Three carbapenem-non-susceptible A. baumannii were isolated from patients in the intensive care unit of a tertiary care hospital in Chennai, India in April 2010. Species identification and antibiotic testing were carried out using an automated machine (VITEK-2). The MICs of carbapenems and aminoglycosides were determined using the CLSI agar dilution method, while tigecycline MICs were determined using the EUCAST broth microdilution method. All the isolates were resistant to all the β-lactams, aminoglycosides and quinolones, and were only susceptible to tigecycline and colistin.

Double disc synergy test (DDST) and modified Hodge test (MHT) were used for detection of metallo-β-lactamases and other carbapenemases, respectively. PCR screening was performed for OXA-type carbapenemases (bla\textsubscript{OXA-23}, -24, -51 and -58-like) and the known metallo-β-lactamase genes.

All three isolates showed positivity for both DDST and MHT, and PCR yielded the products with expected sizes for bla\textsubscript{OXA-51}, -23-like and bla\textsubscript{NDM-1} (Table 1). Sequencing of both bla\textsubscript{OXA-23} and bla\textsubscript{NDM-1} genes showed 100% identities with previously reported genes. In all three isolates, the bla\textsubscript{OXA-23} gene was adjacent to insertion element IS\textsubscript{Aba1}, which provides the promoter required for expression of linked resistance genes; bla\textsubscript{OXA-51-like} genes were also found in all of the isolates, but were not activated by IS\textsubscript{Aba1}. Conjugation experiments using Escherichia coli J53 as the recipient were unsuccessful. Plasmids of Enterobacteriaceae were not detected among A. baumannii by PCR-based replicon typing.

Susceptibility testing showed increased MICs of carbapenems and an unusual phenotype of broad-spectrum high-level resistance to aminoglycosides, including amikacin, gentamicin, netilmicin and tobramycin (MICs > 256 mg/L), with susceptibility to tigecycline (MIC = 0.5–1 mg/L). PCR screening was performed for 16S rRNA methylase-encoding genes (armA, rmtA, rmtB, rmtC, rmtD and npmA). All three isolates showed positivity for the armA gene (Table 1).

NDM-1 metallo-β-lactamase-mediated resistance to carbapenems in Enterobacteriaceae has been found in many parts of India. However, to our knowledge, this is the first

Table 1. Analysis of the three A. baumannii by MIC, DDST, MHT and PCR for resistance determinants

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (mg/L)</th>
<th>DDST</th>
<th>MHT</th>
<th>16S rRNA methylase</th>
<th>NDM-1</th>
<th>OXA-51</th>
<th>OXA-23</th>
<th>OXA-51+IS\textsubscript{Aba1}</th>
<th>OXA-23+IS\textsubscript{Aba1}</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A28</td>
<td>&gt;512 &gt;512 &gt;256 &gt;256 &gt;256 &gt;256 1</td>
<td>+</td>
<td>+</td>
<td>armA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>A32</td>
<td>&gt;512 &gt;512 &gt;256 &gt;256 &gt;256 &gt;256 0.5</td>
<td>+</td>
<td>+</td>
<td>armA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>A36</td>
<td>&gt;512 &gt;512 &gt;256 &gt;256 &gt;256 &gt;256 0.5</td>
<td>+</td>
<td>+</td>
<td>armA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; NET, netilmicin; TOB, tobramycin; TGC, tigecycline; DDST, double disc synergy test; MHT, modified Hodge test.

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report of the coexistence of the bla\textsubscript{OXA-23} gene with bla\textsubscript{NDM-1} and armA in clinical isolates of \textit{A. baumannii} in India.

\textit{A. baumannii} isolates have been found to carry mixed carbapenemase genes giving very broad-spectrum antibiotic resistance profiles. The emergence of these powerful coexistent resistance mechanisms described here will further seriously limit future therapeutic options.

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### Transparency declarations

None to declare.

### References

10. Karihikeyan K, Toleman M, Giske CG et al. First report of the coexistence of bla\textsubscript{OXA-48} or bla\textsubscript{OXA-48-like} with bla\textsubscript{NDM-1} in Enterobacteriaceae from India. In: Abstracts of the Twentieth European Congress of \textit{Clinical Microbiology and Infectious Diseases}, Vienna, Austria, 2010. Abstract P-742. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.
11. Toleman M, Karihikeyan K, Sharma M et al. Recent epidemic emergence of bla\textsubscript{NDM-1} metallo-\textbeta-\textit{lactamase} in enteric organisms from India is mostly linked to A/C type plasmids. In: Abstracts of the Twentieth European Congress of \textit{Clinical Microbiology and Infectious Diseases}, Vienna, Austria, 2010. Abstract O-134. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.

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### Sequence types of Portuguese carbapenem-resistant \textit{Acinetobacter baumannii} isolates collected over 10 years

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**Keywords:** \textit{A. baumannii}, MLST, IMP-5 \textbeta-\textit{lactamase}, European clone II

Sir,

\textit{Acinetobacter baumannii} is recognized as one of the most problematic nosocomial pathogens, whose infections are often associated with epidemic spread and outbreaks of multidrug-resistant strains. The identification of resistant clones with epidemic potential is crucial to understanding the epidemiology of this microorganism, and to enable the review and implementation of adequate infection control guidelines. In Portugal, a sublineage of \textit{A. baumannii} European clone II was identified by PFGE and amplified fragment length polymorphism in different Portuguese hospitals. The sublineage was responsible for the spread of the multidrug-resistant phenotype associated with this strain, including resistance to imipenem. Despite the good discrimination obtained by these typing methods, they are laborious and fingerprinting results are not always transferable between laboratories, as protocols and thresholds may differ. The development of multiplex sequence typing (MLST) using housekeeping genes for the characterization of \textit{A. baumannii} isolates allows the comparison of results between laboratories, contributing to a global epidemiological understanding of the relationship between \textit{A. baumannii} phenotypes and genotypes.

The main objective of this study was, therefore, to identify the sequence types (STs) of carbapenem-resistant isolates of \textit{A. baumannii} collected in Portugal between 1997 and 2009. Our carbapenem-resistant \textit{A. baumannii} collection (540 isolates) showed three antimicrobial susceptibility patterns: A, resistant to all \textbeta-lactams, including carbapenems, but susceptible to quinolones and aminoglycosides (collected from 1998 to 1999); B, resistant to all antibiotics, except tobramycin and/or amikacin for some isolates and colistin (collected from 1999 to 2009); and C, resistant to \textbeta-lactam antibiotics and susceptible to gentamicin, netilmicin, tobramycin, amikacin and colistin (collected from 2004 to 2009). Fifteen isolates were selected from these groups according to the antimicrobial susceptibility profile, the date of isolation, the geographical location of the hospital and the genotype profile.