Detection of Tripoli metallo-β-lactamase 2 (TMB-2), a variant of bla_{TMB-1}, in clinical isolates of Acinetobacter spp. in Japan

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Keywords: MBLs, TMB-1, carbapenemases

Sir,

Metallo-β-lactamase is an important resistance determinant among Gram-negative bacteria, and some metallo-β-lactamase genes are encoded on mobile gene elements that can spread among various clinically important bacterial species.1 TMB-1 (Tripoli metallo-β-lactamase 1) was first identified in 2012 in an Achromobacter xylosoxidans strain isolated from a hospital environment sample in Tripoli, Libya.2 Here, we report two cases in which bla_{TMB-1}-positive non-baumannii Acinetobacter spp. were isolated from patients with no history of international travel.

The first case, a carbapenem-resistant Acinetobacter sp. (MRY12-142) was isolated from a urine sample in December 2011. This case had no recent history of international travel. For the second case, Acinetobacter sp. (MRY12-226) was isolated from necrotic tissue in July 2012. This patient also had no international travel history. These two cases were identified in two hospitals that are separated by more than 1000 km, and there was no epidemiological link between the two cases.

Acinetobacter spp. were sequenced by the partial rpoB gene and the 16S–23S rRNA gene spacer region,3,4 which revealed Acinetobacter pittii in the first case and Acinetobacter genomospecies 14BJ in the second case. Both isolates were resistant to penicillins, cephalosporins, imipenem, meropenem and trimethoprim/sulfamethoxazole, but susceptible to fluoroquinolones, amikacin, and minocycline according to MICs determined by the VITEK2 system (bioMérieux, Lyon, France) and the recommended breakpoints of CLSI 2012.5 Metallo-β-lactamase production was screened using a disc containing sodium merceptoacetic acid (SMA) (Eiken, Tokyo, Japan).6 For both isolates, the growth inhibitory zone around the imipenem and ceftazidime discs expanded upon the addition of the SMA disc, which is strongly indicative of metallo-β-lactamase production.

Based on PCR analyses, both isolates were negative for bla_{OXA-1}, bla_{OXC}, bla_{IMP} bla_{VIM-1}, bla_{OXA-2}, bla_{OXA-23-like}, bla_{OXA-26-like}, bla_{OXA-51-like} and bla_{OXA-58-like}. However, PCR analyses for class 1 integron gene cassettes, in which primers targeted the 5′-conserved region (CS) and 3′-CS, revealed two bands of ~1.2 kbp and 1.8 kbp in both isolates. Sequence analysis of the 1.2 kbp PCR products of both isolates showed that the class 1 integron gene cassette contained only one gene that had 99% amino acid identity with TMB-1, and was thus designated TMB-2. The 738 bp sequence of bla_{TMB-2} was identical to that of bla_{TMB-1}, except for one substitution at nucleotide position 544, which caused an amino acid change from serine to proline at position 228 according to the class B standard numbering7 (GenBank accession numbers AB758277 and AB758278). Sequence analysis for the 1.8 kbp PCR product of MRY12-142 was conducted and showed that the class 1 integron gene cassette contained aac(6′)-Ib and a hypothetical open reading frame.

The PCR product of the class 1 integron gene cassette containing bla_{TMB-2} was ligated into pGEM-T (Promega, WI, USA) and transformed into Escherichia coli strain DH5α. In addition, pGEM-T harbouring bla_{TMB-1} was obtained by site-directed mutagenesis and transformed into E. coli DH5α to evaluate the role of this single amino acid substitution on antimicrobial susceptibility, the MICs being determined by Etest (bioMérieux). As shown in Table 1, the TMB-2-producing transformant was resistant to ceftazidime and susceptible to aztreonam, similar to the TMB-1-producing transformant. However, the TMB-2-producing transformant showed >256-fold and 16-fold lower MICs for doripenem and meropenem, respectively, compared with the TMB-1-producing transformant. The MICs of imipenem were not

Table 1. Antimicrobial susceptibility of isolates and strains determined by Etest

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>A. pittii MRY12-142</th>
<th>A. genomospecies 14BJ MRY12-226</th>
<th>E. coli DH5α (pGEM-T–TMB-2)</th>
<th>E. coli DH5α (pGEM-T–TMB-1)</th>
<th>E. coli DH5α (pGEM-T)</th>
<th>E. coli DH5α</th>
<th>A. pittii ATCC 19004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>32</td>
<td>32</td>
<td>0.064</td>
<td>0.094</td>
<td>0.047</td>
<td>0.047</td>
<td>16</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>0.25</td>
<td>0.38</td>
<td>6</td>
</tr>
<tr>
<td>Imipenem</td>
<td>16</td>
<td>&gt;32</td>
<td>2</td>
<td>1</td>
<td>0.38</td>
<td>0.38</td>
<td>0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>2</td>
<td>32</td>
<td>0.064</td>
<td>0.064</td>
<td>0.75</td>
</tr>
<tr>
<td>Doripenem</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>0.064</td>
<td>32</td>
<td>0.032</td>
<td>0.032</td>
<td>0.19</td>
</tr>
</tbody>
</table>
different for the two strains. Both transformants also showed an apparent expansion of the growth inhibitory zone around the cef-tazidime disc upon addition of the SMA disc.

It has been reported that carbapenem resistance among non-baumannii Acinetobacter spp. is usually due to the production of metallo-β-lactamase. To our knowledge, this study is the first to report an Acinetobacter spp. positive for blaTMB-2 and to identify a new variant, blaTMB-2. It is also the first report to identify blaTMB in clinical isolates. The low MICs of carbapenemes for transformant cells suggests that additional resistance mechanisms, such as the production of other classes of β-lactamase, a reduction in outer membrane protein expressions and an acceleration of efflux pump activities, may be involved in the carbapenem resistance of parental clinical isolates of Acinetobacter spp. Although the same phenomenon was reported in the IMP-type, it is notable that one amino acid substitution from serine to proline in TMB-2 has drastically decreased the MICs of meropenem and doripenem. As another patient had a history of international travel nor any epidemiological link, it is possible that blaTMB-2 had been endemic in Japan but unrecognized because of its reduced ability to hydrolyse carbapenemes. The unrecognized spread of blaTMB-2 could be a concern as it can turn to blaTMB-1 by only one nucleotide substitution. Although this report discusses only two cases, it may be important to evaluate the spread of this emerging metallo-β-lactamase gene among non-baumannii Acinetobacter spp.

Acknowledgements
We thank Kumiko Kai, Yumiko Yoshimura and Yoshie Taki for technical assistance and we thank Sunao Matsubayashi and Yuki Koura for providing clinical isolates and information.

Funding
This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H24-Shinkou-Ippan 010).

Transparency declarations
None to declare.

References