NDM-1-producing Acinetobacter baumannii ST85 now in Turkey, including one isolate from a Syrian refugee

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New Delhi metallo-β-lactamase-1 (NDM-1), an acquired class B carbapenemase, is a significant clinical threat owing to the extended hydrolysis of β-lactams including carbapenems. Here, to the best of our knowledge we describe for the first time in Turkey the spread of NDM-1-producing Acinetobacter baumannii isolates recovered from intensive care unit patients. The presence of blaNDM-1 was detected by PCR and confirmed by sequencing. The clonal relationship was assessed by PFGE and multilocus sequence typing. Both isolates were positive for blaNDM-1 and were attributed with the sequence type 85. One isolate was from a Syrian refugee, whereas the second was from a patient who had never travelled outside Turkey. Our findings confirmed that the rapid spread of NDM-1-producing Gram-negative organisms could become a major challenge for the treatment and control of healthcare-associated infections in our geographical area. They suggest also that NDM-1-producing strains and/or their genetic determinants are probably being imported from Syria to neighbouring countries.

INTRODUCTION

During the past decade, carbapenemases have emerged and spread worldwide (Queennan & Bush, 2007). The carbapenemase New Delhi metallo-β-lactamase-1 (NDM-1) was first identified in 2008 in a Klebsiella pneumoniae strain from a Swedish patient transferred from India (Yong et al., 2009). Since then, the spread of NDM-1 producers has been identified in many bacterial species and isolates from patients in India, Pakistan, Oman, Kenya, Canada, USA, Australia and many European countries (UK, Austria, Belgium, Denmark, France, Germany, The Netherlands, Norway) (Kumarasamy et al., 2010; Poirel et al., 2010; Nordmann et al., 2011b). NDM-1 producers have also been reported in community-acquired infections (Kumarasamy et al., 2010; Poirel et al., 2010; Nordmann et al., 2011a).

We here report the first identification, to the best of our knowledge, of two blaNDM-1-producing isolates of carbapenem-resistant Acinetobacter baumannii in Turkey in patients hospitalized in two intensive care units (ICUs) of the University Hospital of Adana. One patient was a Syrian refugee. The molecular epidemiological characteristics of the two isolates are described.

Abbreviations: ICU, intensive care unit; NDM-1, New Delhi metallo-β-lactamase-1; ST, sequence type.

METHODS

This study was conducted in the Department of Microbiology of the Teaching Hospital, School of Medicine, Cukurova University, Adana, Turkey, in the period December 2013 to December 2014. Carbapenem-resistant A. baumannii isolates identified in the study period were collected and tested for the production of metallo-β-lactamases and their genetic determinants. Biochemical identification and drug susceptibility testing were performed by using Vitek 2 Compact (bioMérieux). The MICs of β-lactams were confirmed by Etest (bioMérieux) as previously described (Bonnin et al., 2011). A phenotypic confirmation of the carbapenemase production was performed by the modified Hodge test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Escherichia coli ATCC 25922 was used as the reference carbapenem-susceptible strain (CLSI, 2014). An imipenem–EDTA double–disk synergy test was carried out for screening the metallo-β-lactamase production (Yong et al., 2002). Species identification was confirmed by PCR of the blaOXA-51 gene (Huang et al., 2010). Multi-plex PCRs were used to screen for ESBL and carbapenemase gene sequences (Dallenne et al., 2010). The gene blaNDM-1 was searched for by using the target specific primer set NDM-Fm (5’-GGTTTGCGGATCGTGGTTTTCC-3’) and NDM-Rm (5’-CGGAAATGGCTC-ATGACGATC-3’) (Nordmann et al., 2011a). Sequencing was performed by using an ABI 310 DNA analyser (Applied Biosystems). The resulting sequences were compared with those available in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST). The clonal relationship was assessed by PFGE (Apal-PFGE) (Seifert et al., 2005). Multi-locus sequence typing was performed in accordance with the Pasteur Institute site instructions (http://pubmlst.org/abaumannii/).
RESULTS

In the period of the study, 68 samples of A. baumannii non-susceptible to imipenem and meropenem were collected. Two isolates from patients hospitalized in two different ICUs proved to carry metallo-β-lactamases and, in particular, a bla_{NDM-1} genetic sequence. Case 1 was a 49-year-old Syrian female hospitalized in September 2014 with acute renal failure and chronic gastritis. NDM-1-producing A. baumannii was isolated on 6 October 2014 from a sample of tracheal aspirate. Case 2 was a 57-year-old female living in Adana, Turkey, who had been hospitalized in the neurological ICU of the same hospital in August 2014 owing to motor neuron disease. She had no history of travel outside Turkey. In this second case also, NDM-1-producing A. baumannii was detected in a respiratory sample on 21 October 2014. The two patients had had no contact with each other, but reportedly patient care was shared between medical, nursing and support staff of the two ICUs. The antimicrobial drug-resistance patterns of the two A. baumannii isolates are summarized in Table 1. Modified Hodge test and double-disk synergy test were positive for both isolates. Both isolates were susceptible to colistin and tigecycline. The first isolate was non-susceptible and the second was susceptible to amikacin. The two isolates were positive for bla_{NDM-1} and negative for other β-lactamase genes. The two isolates shared an identical PFGE pattern. Multilocus sequence typing attributed both isolates with the sequence type (ST) 85.

DISCUSSION

NDM-1-producing bacteria are now a major global health threat (Poirel et al., 2010). A restricted number of therapeutic options are available for NDM-1 producers, including colistin and tigecycline (Yong et al., 2009; Kim et al., 2011). However, these antibiotics frequently show unfavourable pharmacokinetics and adverse effects, mainly in patients with underlying conditions.

NDM-1-producing Enterobacteriaceae, in particular K. pneumoniae and Enterobacter cloacae, have been previously reported in Istanbul, Turkey (Poirel et al., 2012, 2014). NDM-1-producing K. pneumoniae was probably imported into Turkey from Iraq (Poirel et al., 2012). NDM-1 has also recently been reported in a strain of Acinetobacter pittii isolated in 2006 in Ankara (Ignasi et al., 2014). To the best of our knowledge, our study presents the first report of NDM-1-producing A. baumannii in Turkey. NDM-1-producing A. baumannii have been previously identified in countries such as France, Algeria, Kenya, Syria and China (Chen et al., 2011; Boulanger et al., 2012; Bonnin et al., 2013; Decousser et al., 2013; Revathi et al., 2013; Rafei et al., 2014).

Of interest, our bla_{NDM-1}-carrying isolates belonged to ST85, an infrequent ST worldwide, which has recently been detected in A. baumannii carrying the bla_{NDM-1} gene in Lebanon and in France (Bonnin et al., 2013; Decousser et al., 2013; Rafei et al., 2014). In France, in particular, the first outbreak of NDM-1-producing A. baumannii, whose index case was a patient transferred from Algeria, has been described (Decousser et al., 2013). More recently, the identification in France of eight additional NDM-1-carrying ST85 isolates from patients previously hospitalized in Algeria, Tunisia and Egypt has further focused attention on North Africa as a probable reservoir for NDM-1 (Bonnin et al., 2013). In Lebanon also, A. baumannii ST85 carrying the bla_{NDM-1} gene has been isolated from Syrian patients wounded during the civil war (Rafei et al., 2014).

This study has some limitations. Epidemiological and clinical data about patients were only partially available, as well as information about policy of screening for multidrug-resistant organisms. Moreover, the primer set we used could be inadequate to detect allelic variants different from NDM-1.

Our report describes a further isolation of bla_{NDM-1}-carrying A. baumannii ST85 isolates from Adana, a city located in the south of Turkey, very close to the Syrian border, which is currently hosting many Syrian refugees. Our findings corroborate the hypothesis that NDM-1-producing A. baumannii ST85 may be highly prevalent in the Middle East and could be imported into Europe from Syria. Indeed, owing to the geopolitical conditions of this geographical area and the civil war, NDM-1-producing strains are likely being transferred from Syria to its neighbouring countries and their healthcare facilities. Our results emphasize also the crucial role of international infection control measures to contain the threatening spread of such multi-resistant organisms.

Table 1. Antimicrobial susceptibility patterns of the two A. baumannii isolated in Adana, Turkey, 2014

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>MIC (μg ml⁻¹)</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≥ 64</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>≥ 32</td>
<td>≥ 32</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>≥ 64</td>
<td>≥ 64</td>
<td></td>
</tr>
<tr>
<td>Cefoperazone/sulbactam</td>
<td>≥ 64</td>
<td>≥ 64</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥ 64</td>
<td>≥ 64</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥ 4</td>
<td>≥ 4</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>≤ 0.5</td>
<td>≤ 0.5</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≥ 8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td></td>
</tr>
<tr>
<td>Netilmicin</td>
<td>≥ 32</td>
<td>≥ 32</td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>≥ 128</td>
<td>≥ 128</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≥ 2</td>
<td>≥ 2</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>≥ 320</td>
<td>≥ 320</td>
<td></td>
</tr>
</tbody>
</table>

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REFERENCES


