Detection of NDM-1-producing Enterobacteriaceae in Romania: report of the SENTRY Antimicrobial Surveillance Program

Gram-negative organisms producing NDM metallo-ß-lactamases (MßLs) were detected in India as early as 2006 (Castanheira et al., 2011) and the Indian subcontinent has been considered the primary source of these MßL-producing organisms (Poirel et al., 2011). The Balkan region that includes South-East European countries, such as Albania, Bulgaria, Greece and portions of Croatia, Italy, Macedonia, Romania and Serbia among other nations, has also been considered as a possible secondary source of NDM-producing isolates (Jovcic et al., 2011; Poirel et al., 2011). Although reports have demonstrated the presence of NDM-encoding genes in a few isolates from some of these countries or from patients that originated from the Balkan region (Poirel et al., 2011), there are scarce data on the prevalence of these organisms from Romania.

One hundred Enterobacteriaceae isolates were collected in three Romanian hospitals during 2011 and were submitted to the SENTRY Antimicrobial Surveillance Program for processing. Isolates were identified by standard bacterial identification methodologies or using matrix-assisted laser desorption/ionization-time of flight MS (MALDI Biotyper; Bruker Daltonics), where necessary. Isolates were susceptibility tested by reference broth microdilution methods and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were concurrently tested for quality assurance with all results within the expected ranges (CLSI, 2013).

Among the isolates tested, only three (3.0 %) displayed elevated MIC values (≥ 2 mg l⁻¹) when tested against

Table 1. Demographic information, antimicrobial susceptibility and molecular characterization of carbapenemase-producing Enterobacteriaceae from Romania

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Carbapenemase-producing isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial species</td>
<td>E. cloacae</td>
</tr>
<tr>
<td>Carabepenemase</td>
<td>NDM-1</td>
</tr>
<tr>
<td>City</td>
<td>Bucharest</td>
</tr>
<tr>
<td>Patient</td>
<td>59-year-old male</td>
</tr>
<tr>
<td>Source</td>
<td>UTI</td>
</tr>
<tr>
<td>City</td>
<td>Cluj-Napoca</td>
</tr>
<tr>
<td>Patient</td>
<td>68-year-old male</td>
</tr>
<tr>
<td>Source</td>
<td>LRT</td>
</tr>
<tr>
<td>City</td>
<td>Bucharest</td>
</tr>
<tr>
<td>Patient</td>
<td>71-year-old male</td>
</tr>
<tr>
<td>Source</td>
<td>BSI</td>
</tr>
<tr>
<td>MIC (mg l⁻¹)*</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>8 (R)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8 (R)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>&gt;32 (R)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;8 (R)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16 (R)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;16 (R)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;64 (R)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2 (I)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;8 (R)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;16 (R)</td>
</tr>
<tr>
<td>Tetracycline†</td>
<td>1 (S)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>&gt;4 (R)</td>
</tr>
<tr>
<td>Colistin</td>
<td>&gt;4 (NA)</td>
</tr>
<tr>
<td>PFGE profile</td>
<td>A</td>
</tr>
<tr>
<td>Plasmid size</td>
<td>97 kb</td>
</tr>
<tr>
<td>Plasmid incompatibility type</td>
<td>HI2</td>
</tr>
<tr>
<td>Additional resistance genes</td>
<td>qnrA, rmtC</td>
</tr>
<tr>
<td></td>
<td>blaOXA-1/30</td>
</tr>
<tr>
<td></td>
<td>qnrB, rmtC</td>
</tr>
<tr>
<td></td>
<td>blaCTX-M-15, blaCTX-M-15</td>
</tr>
</tbody>
</table>

BSI, Bloodstream infection; LRT, lower respiratory tract infection; UTI, urinary tract infection; ND, not determined.

*Interpretations: R, resistant; I, intermediate; S, susceptible; NA, not available per CLSI criteria except for tigecycline.
†USA–FDA breakpoints were applied when available (Tygacil 2012 package insert; Wyeth Pharmaceuticals; available at http://labeling.pfizer.com/showlabeling.aspx?id=491).

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imipenem and meropenem. These isolates were evaluated for the presence of carbapenemase-encoding genes using multiplex reactions targeting blaKPC,
blaNDM, blaGES, blaNMRC-A, blagMD, blaMP,
blaVIM, blaOXA-PM, blaIM, blaSIM, 1, blaSIM-1, blaSIM-1,
blaKIM-1, blaNDM, blaNDM-1 and blaABC-1.
Two carbapenem-resistant Enterobacter
cloacae yielded positive amplification for
blaNDM-1, and sequencing confirmed that
the isolates carried blaNDM-1. One Klebsiella
pneumoniae was positive for blaOXA-AP.
These isolates were recovered in Bucharest
(one NDM-1-producing E. cloacae and the
OXA-48-producing K. pneumoniae) and
Cluj-Napoca (one NDM-1-producing
E. cloacae). All patients were male and the
isolates were considered the cause of the
reported clinical infection (urinary, lower
respiratory or bloodstream; Table 1).
The carbapenemase-producing organisms
were resistant to several antimicrobial
agents tested (Table 1). The NDM-1
producing E. cloacae from Cluj-Napoca
displayed aztreonam MIC results of a
susceptible level (0.5 mg l\(^{-2}\))
and the isolate from Cluj-Napoca carried
blaOXA-1/30, qnrB and rmtC. Qnr and 16SrRNA
methyrase proteins were likely to
encode or contribute to elevated MIC
values for fluoroquinolone and
aminoglycoside in both isolates. The OXA-
48-producing isolates harboured only
genes encoding extended-spectrum \(\beta\)-
lactamases (CTX-M-15 and DHA-1).
More evidence is needed to corroborate
the hypotheses that the Balkan region is a
source of NDM-producing isolates;
however, this report confirms the presence
of isolates producing this M\(\beta\)L in another
country that is geographically associated
with the Balkans. IMP and VIM types
have been described from Romania
(Mereuță et al., 2007), and in this study
we describe isolates carrying the genes
encoding NDM-1 and OXA-48.
Furthermore, plasmids from both NDM-
1-producing strains, collected in hospitals
located in different Romanian cities, had
the same size and incompatibility factor,
suggesting that this genetic structure
could be disseminated in this region or
country. These findings highlight a
possible endemic carbapenem-resistant
enteric population problem in Romania,
and focused infection control practices
and surveillance of emergent strains seem
warranted in this country.

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