First detection of bla<sub>IMI-2</sub> gene in a clinical *Escherichia coli* strain

The dissemination of carbapenem-resistant *Enterobacteriaceae* is increasing worldwide in the last decade, mainly due to the acquisition of beta-lactamase genes encoding carbapenemases. Among class A carbapenemases, KPC is widespread (14), however IMI enzymes have only been described so far in *Enterobacter* genus (1, 10, 15). The detected bla<sub>IMI</sub> genes are linked to a gene encoding a LysR-type transcriptional regulator, and as previously reported, the bla<sub>IMI-1</sub> gene is located in the chromosome of *E. cloacae* (10), whereas bla<sub>IMI-2</sub> is related to plasmids (1, 15). We report here the first description of a bla<sub>IMI-2</sub>-positive *Escherichia coli* strain.

A carbapenem-resistant *E. coli* strain W635 was recovered from a blood sample of an elderly oncologic patient (without any history of travel) who was admitted with sepsis and treated with piperacillin-tazobactam at a Spanish hospital in 2010. *E. coli* W635 was ascribed to a new sequence type registered as ST1998 (http://mlst.ucc.ie/mlst/dbs/Ecoli), and showed resistance to imipenem (IPM), meropenem (MEM), ertapenem (ETP), doripenem, ampicillin, ticarcillin, amoxicillin-clavulanic acid (AMC), cephalothin, streptomycin, nalidixic acid, ciprofloxacin, norfloxacin, sulfonamides, trimethoprim, and intermediate resistance to aztreonam and chloramphenicol by the CLSI disk diffusion method (3). A class A carbapenemase phenotype was demonstrated in this strain by double-disk synergy test (5, 9).

After multiplex PCR (8) and subsequent sequencing, a partial sequence of bla<sub>IMI-2</sub> gene was detected in *E. coli* W635. To gain insight into the bla<sub>IMI-2</sub> gene and its surrounding structure, the flanking regions were amplified by PCR using specific primers designed in this work (according to GenBank accession number AY780889). The LysR-type regulator gene (bla<sub>IMI-2R</sub>) was found upstream of bla<sub>IMI-2</sub> gene, and their genetic environment was studied by inverse PCR using PvuII and BglII restriction enzymes.
Sequence analysis revealed a total fragment of 6184 bp that was deposited in GenBank database with the accession number JN412066. A new insertion sequence of 1321 bp, designed IS\textsubscript{Ec36} by ISFinder (http://www-is.biotoul.fr/), was detected upstream of \textit{bla}\textsubscript{IMI-2R} gene. This IS belongs to IS3 family and IS2 group, and exhibits an identity of 92\% with respect to IS\textsubscript{Ec27} sequence (GenBank accession number AY857617).

The presence of genes implicated in other antimicrobial resistances (beta-lactams, aminoglycosides, quinolones, trimethoprim and sulfonamides), the study of mutations in \textit{gyrA} and \textit{parC} genes as well as the characterization of integrons and \textit{sul2} gene environment, were performed by PCR and sequencing (4, 11, 13). Table 1 shows the MICs and genotypic results.

Two different transconjugants from W635 were obtained by mating experiments, using \textit{E. coli} CSH26 as recipient and plates supplemented with MEM (8 μg/ml) and rifampicin (100 μg/ml). Detection and typing of plasmids of \textit{E. coli} W635 and transconjugant strains were carried out by PCR-based replicon typing (2, 6). Plasmids of W635 strain and its transconjugants belonging to incompatibility group I\textsubscript{I} (IncI\textsubscript{I}) and F (IncF) were subtyped by Plasmid Multilocus Sequence Typing (7, 12). \textit{E. coli} W635 contained the following typable plasmids: IncI\textsubscript{I} (ST26, CC-26), IncF (F43:A\textsubscript{-}:B\textsubscript{-}; Y2variant:A\textsubscript{-}:B\textsubscript{-}), and ColE\textsubscript{TP}. The location of \textit{bla}\textsubscript{IMI-2} in W635 strain and its transconjugants was studied by PFGE-S1 nuclease and PFGE-\textit{XbaI} southern blotting and hybridization (4) with \textit{bla}\textsubscript{IMI-2}, IncI\textsubscript{I}, IncF, and ColE\textsubscript{TP} probes. The \textit{bla}\textsubscript{IMI-2} gene in \textit{E. coli} W635 was detected in an IncF plasmid of approximately 48.5 kb. This size of plasmid is smaller than previously reported results that described the \textit{bla}\textsubscript{IMI-2} gene located on transferable plasmids with sizes of 66 kb or 80 kb (1, 15).

This is the first report of a carbapenem-resistant \textit{E. coli} strain carrying the class A carbapenemase IMI-2. The \textit{bla}\textsubscript{IMI-2} gene located in a conjugative plasmid and linked to
mobile elements might significantly spread between different *Enterobacteriaceae*, being an emerging resistance mechanism that should be tracked in the future.

The nucleotide sequence of the novel genetic environment of *bla*IMI-2 gene determined in this study was included in the GenBank database with the accession number JN412066.

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**REFERENCES**


Table 1. Resistance phenotype, genotype and genetic elements of *E. coli* strain W635, transconjugants (TC) and recipient strain.

<table>
<thead>
<tr>
<th>Strains</th>
<th>MBC of (μg/ml)</th>
<th>Resistance genes</th>
<th>Amino acid changes detected in</th>
<th>Class 1 integron</th>
<th>Incompatibility group detected (size of plasmid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP</td>
<td>ATM</td>
<td>IPM</td>
<td>MEM</td>
<td>STR</td>
</tr>
<tr>
<td>W635 donor</td>
<td>&gt;128</td>
<td>8</td>
<td>128</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>TC1</td>
<td>&gt;128</td>
<td>8</td>
<td>128</td>
<td>16</td>
<td>&gt;128</td>
</tr>
<tr>
<td>TC15</td>
<td>&gt;128</td>
<td>0.125</td>
<td>0.25</td>
<td>0.032</td>
<td>4</td>
</tr>
<tr>
<td>CSH26 recipient</td>
<td>16</td>
<td>0.125</td>
<td>0.25</td>
<td>0.032</td>
<td>4</td>
</tr>
</tbody>
</table>

- strA-strB genes were linked to sul2 gene (*repC*+*sul2*+*strA+strB+ISCR2*).
- The IncI1 and IncF were detected by hybridization in a plasmid of approximately 160 kb in the *bla*IMI-2-positive TC1 transconjugant.


ND: not determined; -: not detected.
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