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LETTER TO THE EDITOR

# VIM-1, VIM-34, and IMP-8 Carbapenemase-Producing *Escherichia coli* Strains Recovered from a Portuguese River

**Nicolas Kieffer,[a] Laurent Poirel,[a] Lucinda J. Bessa,[b] Ana Barbosa-Vasconcelos,[b] Paulo Martins da Costa,[b,c] Patrice Nordmann[a,d]**

"Emerging Antibiotic Resistance" Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland[a]; ICBAS, Abel Salazar Institute for the Biomedical Sciences, University of Porto, Porto, Portugal[b]; CIIMAR, Interdisciplinary Center for Marine and Environmental Research, University of Porto, Porto, Portugal[c]; HFR-Hôpital Cantonal, Fribourg, Switzerland[d]

The emergence of acquired carbapenemases is currently one of the most serious public health threats worldwide. These enzymes confer resistance to almost all β-lactams, including carbapenems leading to very few therapeutic options for treating patients infected by multidrug-resistant bacteria.

Here we report the identification of four environmental carbapenem-resistant *Escherichia coli* strains, E61, E201, E202, and E203. These strains were recovered in February 2015, from the Ave river, in the north of Portugal. Water samples of 100 ml were filtered through 0.45-μm-pore-size membrane filters (Millipore Corporation, USA), which were then placed on tryptone bile X-glucuronide agar (TBX) (BioKar Diagnostics, Beauvais, France) plates supplemented with imipenem (2 mg/liter). Strains growing on those selective plates were checked for carbapenemase activity by using the Carba NP test (1). Antimicrobial susceptibility testing was performed according to the standard disk diffusion method following CLSI recommendations (2) and using cation-adjusted Muller-Hinton plates (Bio-Rad, Cressier, Switzerland). The four isolates were resistant to all β-lactams, fluoroquinolones, and aminoglycosides (except amikacin), being susceptible only to tigecycline and colistin. Two out of the four isolates (E61 and E202) also remained susceptible to fosfomycin. MICs of carbapenems (imipenem, ertapenem, and meropenem) were performed using Etest strips (bioMérieux, La Balme-les-Grottes, France) (Table 1). Molecular investigations were then performed by PCR using specific primers for carbapenemase genes (3) followed by sequencing (Microsynth, Balgach, Switzerland). The phylogenetic group was determined as previously described (4) and showed that isolates E201 and E203 belonged to group E, strain E61 belonged to group A, and strain E202 belonged to group F. Multilocus sequence typing performed as described previously (5) confirmed that isolates E201 and E203 belonged to the same sequence type, whereas the two other strains were clonally unrelated. PCR and sequencing analysis revealed that strains E201 and E203 harbored the $bla_{\text{IMP-8}}$ gene and strains E61 and E202 harbored the $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-34}}$ genes, respectively.

Conjugation assays were performed in liquid medium using the azide-resistant *E. coli* J53 as the recipient strain. Transconjugants were selected onto Luria-Bertani agar plates supplemented with ertapenem (2 mg/liter) and azide (100 mg/liter) to detect the transfer of the carbapenemase genes. Transconjugants were obtained for three out of the four isolates, but not for strain E202 despite repeated attempts. Plasmid extractions were performed using the Kieser extraction method (6) using the environmental strains and transconjugants. Further analysis showed that the $bla_{\text{VIM-1}}$ and $bla_{\text{IMP-8}}$ genes were carried on a ca. 150-kb plasmid. Molecular typing of plasmids was performed using the PCR-based replicon typing (Diatheva, Fano, Italy) (7), revealing that all plasmids bearing the carbapenemase genes belonged to the IncFIB group.

The failure to obtain a transconjugant or transformant from strain E202 suggests a possible chromosomal location of the $bla_{\text{VIM-34}}$ gene. It is noteworthy that the $bla_{\text{VIM-34}}$ gene was previously identified from *Klebsiella pneumoniae* in Portugal in 2013 and was found to be located on the chromosome (8). PCR mapping followed by sequencing revealed that the $bla_{\text{VIM-34}}$ gene was embedded in class 1 integron In817 in strain E202, the exact same structure as identified in the *K. pneumoniae* isolate from Portugal (8).

The occurrence of carbapenemase-producing *E. coli* strains in the aquatic environment remains worrying. Interestingly, we first identified a KPC-producing *E. coli* in Portugal from the same

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Address correspondence to Laurent Poirel, laurent.poirel@unifr.ch.



**TABLE 1** MICs of carbapenems for the environmental isolates and *E. coli* J53 harboring the carbapenemase genes

| | MIC (μg/ml)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Environmental isolates | | | | *E. coli* J53 transconjugants | | | |
| Carbapenem or parameter | E61 | E201 | E202 | E203 | E61TC | E201TC | E203TC | *E. coli* J53 |
| Imipenem | ≥32 | 16 | 6 | 32 | 1 | 1.5 | 6 | 0.125 |
| Meropenem | ≥32 | 8 | 4 | 16 | 0.38 | 1 | 2 | 0.016 |
| Ertapenem | ≥32 | 16 | 4 | 32 | 0.38 | 0.5 | 3 | 0.012 |
| Sequence type | 167 | 2612 | 354 | 2612 | | | | |

[a] MICs of carbapenems are shown unless specified otherwise.

river, as early as 2010 (9). This river may be a source of carbapenemase-producing *E. coli* or, conversely, humans might be the source of this environmental contamination. Note that IMP-8 producers have been reported in Portugal (10) in *K. pneumoniae* and *Pseudomonas aeruginosa* clinical isolates (11), suggesting a wide spread of this carbapenemase, which is not commonly identified in other European countries.

Finally, monitoring the occurrence of carbapenemase-producing members of the family *Enterobacteriaceae* in the environment can be of interest, since it may contribute to better control of the occurrence and spread of these bacteria. It is therefore mandatory to screen for carbapenemase producers not only among the hospitalized populations but also among healthy people and potential environmental cradles in order to identify possible hidden reservoirs.

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