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Environmental KPC-Producing *Escherichia coli* Isolates in Portugal

Carbapenemase-producing isolates of the *Enterobacteriaceae* are reported increasingly worldwide (9). Besides the emergence of OXA-48 and NDM-1 producers in specific geographical areas, KPC-producing isolates are endemic in many places (8). Those KPCs hydrolyze all β-lactams, including carbapenems at a significant level, with the exception of cephamycins. The bla_{KPC}-like genes have been reported most often for *Klebsiella pneumoniae*, but they have been additionally reported repeatedly for other enterobacterial species. Moreover, some KPC-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates have been reported (8, 9). Besides the United States, KPC-producing enterobacterial isolates are at least endemic in Columbia, Greece, and Italy (9).

Our study was conducted in order to evaluate whether the aquatic environment in Portugal could be a reservoir of carbapenem-resistant enterobacterial isolates. Five water samples were collected in December 2010 at different locations from one river crossing the city of Santo Tirso, north Portugal. Samples of 100 ml (each) were filtered on a 0.45-μm sterile filter, and the corresponding filter was placed on an imipenem (1 μg/ml)-containing Drigalski plate. Only one type of colony grew, corresponding to *Escherichia coli*. MICs of *E. coli* strain MAS were determined by the Etest method (AB bioMérieux, Solna, Sweden) and interpreted according to updated CLSI breakpoints (2). It was resistant to all β-lactams, including to all carbapenems (Table 1). That strain remained susceptible only to tetracycline, fosfomycin, and colistin, being resistant to all fluoroquinolones and aminoglycosides (Table 1).

TABLE 1 MICs of β-lactams for *E. coli* MAS clinical isolate, *E. coli* TOJ53 strain harboring the natural bla_{KPC-2}-positive plasmid pMAS from *E. coli* MAS, and *E. coli* J53 recipient strain

β-Lactam[a]	MIC for strain		
	E. coli MAS (KPC-2)	*E. coli* J53 (pMAS)	*E. coli* J53
Amoxicillin	>256	>256	4
Amoxicillin + CLA	128	32	4
Ticarcillin	>256	>256	2
Ticarcillin + CLA	256	>256	2
Piperacillin	>256	128	1
Piperacillin + TZB	>256	128	1
Cefuroxime	>256	256	2
Ceftazidime	256	32	0.06
Cefotaxime	128	4	0.12
Cefepime	128	2	0.06
Cefoxitin	256	2	2
Aztreonam	>256	8	0.06
Imipenem	8	2	0.06
Meropenem	16	1	0.03
Ertapenem	256	0.5	0.03
Ciprofloxacin	>256	0.06	0.06
Gentamicin	64	4	0.12
Amikacin	64	4	0.12
Fosfomycin	0.25	0.06	0.06
Tetracycline	0.5	0.03	0.03
Tigecycline	0.25	0.03	0.03
Colistin	0.25	0.12	0.12

[a] CLA, clavulanic acid; TZB, tazobactam (both at a concentration of 4 μg/ml).

Molecular investigations were then performed using PCR in order to search for carbapenemase genes, followed by sequencing (6). This allowed the identification of the bla_{KPC-2} β-lactamase gene. Analysis of the plasmid content of *E. coli* isolate MAS identified a single plasmid of ca. 150 kb that was successfully transferred to *E. coli* J53 by conjugation, with selection performed on amoxicillin (100 μg/ml) and azide (100 μg/ml)-containing agar plates (6). The bla_{KPC-2}-positive plasmid was identified as an IncF plasmid by using PCR-based replicon typing (1). It cotransferred reduced susceptibility to gentamicin and amikacin. PCR mapping performed as described previously (6) showed that the bla_{KPC-2} gene was part of transposon Tn*4401a*.

Multilocus sequence typing (MLST) performed according to the protocol described on the *E. coli* MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html) showed that *E. coli* MAS belonged to the ST410 type.

Further samplings were obtained in June 2011 at the same place, and selection was performed under the same conditions, but no carbapenem-nonsusceptible *E. coli* grew.

This is the first identification of a KPC-producing *E. coli* in Portugal. It is noteworthy that the bla_{KPC-2} gene was identified in *E. coli*, in which it has been rarely found, with only a few reports from the United States, Israel, Brazil, and France (3–5, 7). Surprisingly, it has been recovered from an environmental sample, whereas no human case has been reported so far, corresponding therefore to the very first identification of KPC in this environment.

To explain those findings, we might speculate that some people living in the neighboring area could spread these KPC producers in the environment. Another possibility is that the aquatic environment could actually contain KPC producers, suggesting that it might represent the source of a future human colonization. Of particular interest is the identification of the strain as belonging to the ST410 type, considering that extended-spectrum β-lactamase (ESBL)-producing ST410 *E. coli* was recently identified in Brazil, where KPC enzymes are widespread (10). Based on the close relationship between Portugal and Brazil in terms of population exchange, it could therefore be speculated that a link might exist.

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