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Letter to the Editor

Occurrence of the Carbapenem-Hydrolyzing β-Lactamase Gene *bla*$_{\text{OXA-48}}$ in the Environment in Morocco$^{\nabla}$

The *bla*$_{\text{OXA-48}}$ gene is plasmid borne and encodes a carbapenem-hydrolyzing class D β-lactamase that was first identified in a *Klebsiella pneumoniae* clinical isolate in Turkey in 2004 (11). The high prevalence of OXA-48 producers in Turkey is well established (3), but there are also scattered reports of OXA-48-producing members of the *Enterobacteriaceae* in several Mediterranean countries, such as Lebanon, Belgium, France, Tunisia, and Morocco, and in Senegal (2, 5–7, 9, 12). All OXA-48-producing isolates are found in hospital settings.

In order to gain insights into the occurrence of carbapenemase-producing enterobacterial isolates in the environment, four water samples were swabbed from puddles located in and around of the city of Marrakech, Morocco, and were squeezed out in 1 ml of sterile water. The clinically relevant *bla*$_{\text{OXA-48}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{NDM}}$, and *bla*$_{\text{KPC}}$ genes were searched for as described previously by PCR amplification with 2 μl of the swab suspension as the DNA template (11, 13). PCRs were positive for two samples and only for the *bla*$_{\text{OXA-48}}$ gene. Those samples were recovered from the Marrakech downtown and Plateau du Kik, which are separated by approximately 150 km. To isolate and characterize the putative *bla*$_{\text{OXA-48}}$-harboring organisms, the two swab suspensions that were positive by preliminary PCR were repeatedly spread onto Drigalski agar (Bio-Rad, Marnes-la-Coquette, France) containing 0.5 μg/ml imipenem until single colonies were obtained. Colonies that grew during overnight incubation (37°C) were screened by PCR, which gave a positive result for one type of lactose-negative colony in both samples. The corresponding strains were designated M1 and M4 and identified using the Vitek2 system (GN card) as *Serratia marcescens*. MICs were determined by Etest (AB bioMérieux, Solna, Sweden) on Mueller-Hinton agar plates at 37°C. The results of susceptibility testing were interpreted according to the updated CLSI guidelines (4). Isolates M1 and M4 were resistant to amoxicillin, amoxicillin-clavulanate combinations, imipenem, and ertapenem, had intermediate susceptibility to cefotaxime, cefepime, and meropenem, and remained susceptible to ceftazidime and aztreonam (Table 1). In addition, both isolates were resistant to fluoroquinolones, tetracycline, chloramphenicol, and sulfamethoxazole and remained susceptible to gentamicin and fosfomycin. Analysis of the plasmid content of *S. marcescens* M1 and M4 identified a single plasmid of ca. 62 kb that was successfully transferred to *Escherichia coli* TOP10 and *S. marcescens* CIP81 reference strains by mating-out assays, as described previously (3). The *E. coli* transconjugant expressing OXA-48 exhibited increased MICs of carbapenems but no additional non-β-lactam resistance, similar to other *bla*$_{\text{OXA-48}}$-positive *E. coli* transconjugants previously reported (12) (Table 1). PCR with specific primers for the replicase gene identified from plasmid pA1 in *Klebsiella pneumoniae* 11978 (3), namely, RepA (5′-GACATTGAGTCAGTAGAAGG-3′) and RepB (5′-CGTGCAGTTCGTCTTTCGGC-3′), showed that the plasmid types identified in *S. marcescens* M1 and M4 were identical. Considering the plasmid size and lack of additional resistance markers in both cases, those plasmids were very likely identical. PCR mapping performed as described previously (1) showed that the *bla*$_{\text{OXA-48}}$ gene was part of the Tn*1999* transposon, originally identified in the *bla*$_{\text{OXA-48}}$-positive *K. pneumoniae* strain 11978. Pulsed-field gel electrophoresis analysis performed as described previously (3) showed that isolates M1 and M4 were clonally related. This is very surprising considering the distance between the locations where the samples were collected and therefore suggests a wide dissemination of that carbapenemase-producing strain.

Our study constitutes the first identification of the *bla*$_{\text{OXA-48}}$ gene in *S. marcescens*, which is a waterborne rod often involved in nosocomial infections (8). Interestingly, the strains were recovered from the environment in a country where *bla*$_{\text{OXA-48}}$-positive *Enterobacter cloacae* and *K. pneumoniae* have been identified (2, 6, 12). Of note, it has been shown that the progenitor of the

TABLE 1. MICs of β-lactams for *S. marcescens* and *E. coli* strains

β-Lactam[a]	MIC (μg/ml)				
	S. marcescens M1 and M4	*E. coli* TOP10(pOXA-48)	*S. marcescens* CIP81(pOXA-48)	*E. coli* TOP10	*S. marcescens* CIP81
Amoxicillin	>256	>256	>256	4	>256
Amoxicillin + CLA	>256	>256	>256	4	>256
Ticarcillin	>256	>256	>256	4	4
Ticarcillin + CLA	>256	>256	>256	4	4
Piperacillin	64	64	64	2	2
Piperacillin + TZB	64	64	64	2	2
Cephalothin	>256	8	>256	4	>256
Ceftazidime	0.25	0.12	0.12	0.12	0.12
Cefotaxime	2	0.25	1	0.06	0.25
Aztreonam	0.25	0.06	0.06	0.06	0.06
Cefepime	4	0.25	1	0.06	0.12
Imipenem	8	0.5	1	0.06	0.25
Ertapenem	>32	0.25	0.5	0.06	0.06
Meropenem	4	0.06	0.12	0.01	0.03

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

bla$_{\text{OXA-48}}$ gene was a species of the genus *Shewanella*, an aquatic environmental species (10). In light of the recent threatening evidence of environmental isolates harboring the carbapenemase gene *bla*$_{\text{NDM-1}}$ in India (14), our study suggests that occurrence of OXA-48 producers in the environment will lead to community-acquired colonizations or even infections caused by OXA-48-producing isolates.

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