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Letters to the Editor

Codetection of bla_{OXA-23}-Like Gene ($bla_{OXA-133}$) and bla_{OXA-58} in *Acinetobacter radioresistens*: Report from the SENTRY Antimicrobial Surveillance Program▽

We read with great interest the report from Poirel et al. (7) describing *Acinetobacter radioresistens* as a source of bla_{OXA-23}-like genes. During the 2006 SENTRY Antimicrobial Surveillance Program, the occurrence of acquired class D carbapenemases and metallo-β-lactamases in *Acinetobacter* spp. from the Asia-Pacific region was evaluated (4). In this study, one *A. radioresistens* strain (251-39C; identified by 16S rRNA gene sequencing) showing decreased susceptibility to penicillins and imipenem was observed (Manipal, India). However, since bla_{OXA-23}-like genes from all *A. radioresistens* strains described by Poirel et al. (7) were silent and chromosome borne and did not confer a resistance phenotype, we were intrigued by the elevated MICs displayed by the isolate 251-39C. Therefore, further screening for class D carbapenemase (6, 10) detected bla_{OXA-58} in addition to the intrinsic bla_{OXA-23}-like gene.

Flanking sequences of both bla_{OXA} genes were characterized by PCR using primers targeting IS*Aba1*, *-2*, or *-3* or a degenerate primer approach (5, 9). The upstream region of the bla_{OXA-23}-like gene showed the highest identity with a putative O-sialoglycoprotein endopeptidase gene detected in the *Acinetobacter baumannii* strain AYE (CU459141); no putative promoter was located in this region. Sequencing analysis of the bla_{OXA-23}-like gene revealed a new gene, named $bla_{OXA-133}$. The putative amino acid sequence displayed the most identity with OXA-102 (99.6%; one amino acid substitution difference [Leu-5 Phe]). Other close variants were OXA-103 (98.5%), OXA-23 (97.4%), OXA-73 (97.1%), OXA-27 and -49 (96.7%), and OXA-105 (96.3% [data not shown]) (7). A truncated ATPase located downstream of $bla_{OXA-133}$ was detected, consisting of the same genetic context as that reported previously (7). Sequencing confirmed the presence of bla_{OXA-58} and IS*Aba3* upstream, thus providing a promoter region.

Plasmid analysis demonstrated nine plasmid bands (ca. 54, 35, 14, 5.6, 4.0, 3.0, 2.8, 2.5, and 2.4 kb) and a bla_{OXA-58}-specific probe hybridized with the 54-, 35-, and 5.6-kb bands (5). It is worthwhile to mention that the 4.0-kb and smaller plasmid bands may represent different forms of at least two distinct plasmids. However, bla_{OXA-58} was considered to be carried by three different plasmids, since they showed greater size differences. Further experiments should be performed to determine the exact number of plasmid DNAs carried by isolate 251-39C. Curing experiments were performed to investigate whether β-lactam MICs would decrease after removing bla_{OXA-58}-carrying plasmids (3). Curing was confirmed by a negative PCR result for bla_{OXA-58}. Index and cured strains were tested for susceptibility by the broth microdilution method (2) and Etest (AB BioDisk, Solna, Sweden). The cured *A. radioresistens* isolate became susceptible and showed MICs for penicillins and carbapenems between 128- and 16-fold and 4- to 32-fold lower than the index strain, respectively (Table 1).

TABLE 1. MICs for *A. radioresistens* index and cured strains tested against several antimicrobial agents

Antimicrobial agent	MIC (μg/ml)	
	Index *A. radioresistens* 251-39C strain	Cured *A. radioresistens* strain
Amoxacillin-clavulanate	32	2
Ampicillin-sulbactam	1	1
Piperacillin-tazobactam	64	1
Ticarcillin-clavulanate	>256	4
Aztreonam	8	8
Cefoxitin	8	8
Ceftazidime	0.5	0.5
Ceftriaxone	4	2
Cefepime	0.5	0.25
Ertapenem	4	1
Imipenem	8	0.25
Meropenem	1	0.25
Ciprofloxacin	0.06	0.12
Amikacin	0.5	0.5
Tobramycin	2	2
Tetracycline	≤2	≤2
Polymyxin B	≤0.5	≤0.5
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5

In addition, we evaluated the transcriptional levels of $bla_{OXA-133}$ and bla_{OXA-58} by using quantitative real-time PCR. RNA was extracted using the RNeasy mini kit (Qiagen GmbH, Hilden, Germany). Relative quantification of target gene expression ($bla_{OXA-133}$ and bla_{OXA-58}) was performed in triplicate by normalization to an endogenous reference (16S rRNA). Quantitative real-time PCR demonstrated that $bla_{OXA-133}$ was transcribed at very low levels (mean threshold cycle = 34.74), ca. 1,500-fold lower than those for bla_{OXA-58} (mean threshold cycle = 24.00). Although the index strain harbored the chromosomal $bla_{OXA-133}$, the loss of β-lactam resistance displayed by the cured strain suggests OXA-58 as the main β-lactam resistance mechanism (8), possibly enhanced by multiple gene copies and increased production of enzyme (1). Detection of bla_{OXA-58} in the index strain, highly disseminated in *A. baumannii* (4), indicates the occurrence of DNA exchange between these two species (7). Furthermore, our findings emphasize the ability of bla_{OXA-58} mobilization.

Nucleotide sequence accession number. The nucleotide sequences of the $bla_{OXA-133}$-carrying *A. radioresistens* clinical isolate described in this paper have been submitted to the EMBL/GenBank/DNA Data Bank of Japan sequence databases and assigned the accession number EU571228.

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