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LETTER TO THE EDITOR

Rare Detection of the *Acinetobacter* Class D Carbapenemase bla_{OXA-23} Gene in *Proteus mirabilis*

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The OXA-23, OXA24/40, and OXA-58 class D carbapenemases are typically found in *Acinetobacter* species; OXA-48-like enzymes are generally found in *Enterobacteriaceae* (1). There have been a few reports of *Acinetobacter*-type OXA genes in *Enterobacteriaceae* (2–4), but the real prevalence of such isolates may be underreported, since most surveillance efforts go into identifying the most common "big five" carbapenemases KPC, NDM, OXA-48, VIM, and IMP (5) among *Enterobacteriaceae*. Indeed, the development of fast commercial molecular diagnostic kits further feeds this bias.

Here we report the unexpected finding of a bla_{OXA-23}-positive isolate during routine surveillance. A *Proteus mirabilis* isolate was collected in September 2014 from the urine of a 74-year-old female patient with inoperable pancreatic cancer and with an unknown travel history. The patient died 20 days later; the role of the infection in her death was uncertain. The unusual phenotype of the isolate in our disk testing panel caught the eye and, combined with a borderline UV-spectrophotometric imipenem hydrolysis assay result, prompted more-detailed tests (as previously described [6], with additional MIC testing using a custom Sensititre broth microdilution panel [Thermo Fisher Scientific, Vantaa, Finland]). The isolate was susceptible to all beta-lactams, but the zone around the carbapenem disks was outside the EUCAST wild-type cutoff, although still on the susceptible side even of the EUCAST screening breakpoints (Table 1). The isolate had no extended-spectrum β-lactamase (ESBL) or transferable AmpC β-lactamases that could have caused this (PCR negative for CTX-M, TEM, SHV, CIT, DHA, MOX, FOX, ACC, and EBC), and wild-type susceptibility to third-generation cephalosporins is not typically seen in strains

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TABLE 1 Susceptibility test results for the OXA-23-positive *P. mirabilis* ESBL4969 isolate

Compound	Disk assay			MIC[a]	
	Content (μg)	Diam (mm)	Interpretation	mg/liter	Interpretation
Meropenem	10	26	S, close to EUCAST screening breakpoint[b]	2	S, outside ECOFF[c]
Ertapenem	10	25	S, close to breakpoint		
Imipenem	10	26	No MBL[d]		
Imipenem/EDTA	10/750	26	No MBL		
Cefoxitin	30	25	S		
Cefepime	30	30	S	≤1	S
Cefalexin	30	19	S		
Ceftazidime	30 (diagnostic)	30	S, no ESBL	≤1	S
Ceftazidime-clavulanic acid	30/10	30	S, no ESBL		
Cefotaxime	30 (diagnostic)	34	S, no ESBL		
Cefotaxime-clavulanic acid	30/10	35	S, no ESBL		
Temocillin	30	22	No OXA-48-like enzyme[b]		
Piperacillin-tazobactam	30/6	21	S, close to ECOFF	8	S, outside ECOFF
Ciprofloxacin				≤0.12	S
Fosfomycin				≤4	S
Gentamicin				>16	R
Amikacin				8	S
Tobramycin				16	R
Trimethoprim-sulfamethoxazole				>8	R

[a] Broth microdilution, Sensititre (ThermoFisher).
[b] EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. V 1.0, 2013. http://www.eucast.org/resistance_mechanisms/. S, sensitive.
[c] ECOFF, epidemiological cutoff, EUCAST. http://www.eucast.org/mic_distributions_and_ecoffs/.
[d] MBL, metallo-β-lactamase.

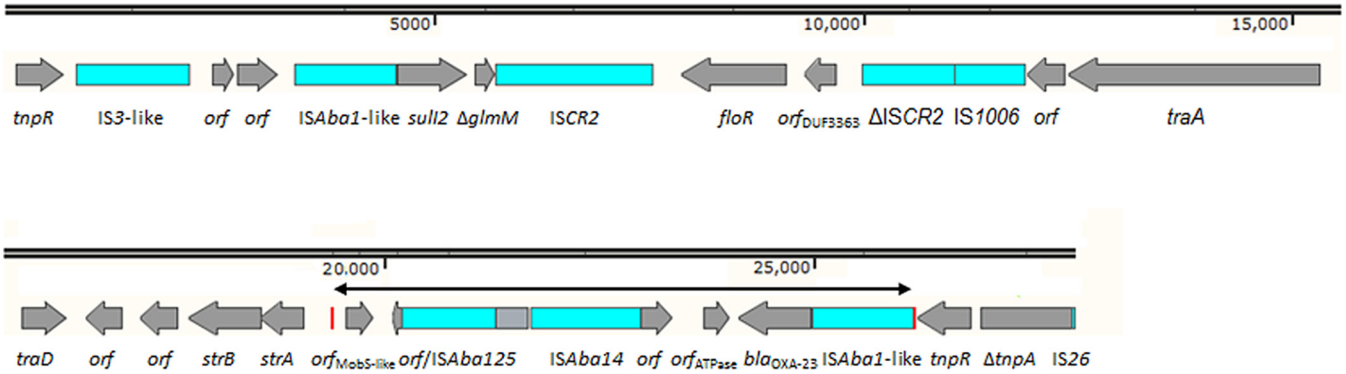

FIG 1 Genetic structure of the bla_{OXA-23} region. Genes are shown as gray-labeled arrows, with the arrowhead indicating the direction of transcription. Insertion sequence elements are shown as labeled blue boxes. The red vertical rectangles represent two 22-bp direct repeats, which might be the result of a target site duplication. GenBank accession number KU302354.

with porin mutations or upregulated efflux. A negative result from an imipenem-plus-EDTA inhibitor test (Rosco, Taastrup, Denmark) showed that no metallo-β-lactamase was involved. The isolate was PCR negative for KPC, NDM, OXA-48, VIM, IMP, and GES. PCR screening for OXA-23, OXA-24/40, OXA-51, and OXA-58 gave a positive band for OXA-23. Sanger sequencing confirmed the result. The isolate was later subjected to whole-genome sequencing, using an Illumina MiSeq sequencer (Illumina Inc.) with an Illumina Nextera XT DNA sample preparation kit and a Nextera XT index kit with 24 indices for library preparation and MiSeq reagent kit V2 (300 cycles) utilizing 150-bp paired-end sequencing. The reads were compiled using Velvet assembler version 1.1.04 (7), which is included in Ridom SeqSphere+ software (Ridom SeqSphere+ version 2.4.0; Ridom GmbH, Münster, Germany). Species identification and the presence of the OXA-23 were confirmed with the corresponding SpeciesFinder and ResFinder databases on the Center for Genomic Epidemiology (CGE) server (https://cge.cbs.dtu.dk/services/). In addition to bla_{OXA-23}, ResFinder found 9 other resistance genes [*aadA1*, *aac(3)-IIa*, *strB*, *strA*, *aph(3′)-Ic*, *floR*, *sul2*, *tet(J)*, and *dfrA1*]. The CGE Plasmid Finder found no plasmids; this result was not further confirmed.

Using primers designed in house, a number of PCR assays and Sanger sequencing were used to confirm and extend the genetic environment of bla_{OXA-23} and to fill in gaps between the involved contigs (8) (see Table S1 in the supplemental material). The bla_{OXA-23}-containing region was analyzed using Geneious 7.1 software (Biomatters Ltd., Auckland, New Zealand) and annotated with Prokka version 1.4.0 (9), which is included in Galaxy/CRS4 (Orione) (10). The OXA-23 gene was located downstream of an IS*Aba1*-like insertion sequence element, resembling the structure of transposon Tn*2008*, although not surrounded by a classic 9-bp target site duplication (Fig. 1). Instead, a flanking duplication of 22 bp (GATGAAGCGCGGA GGTGGCTCA) was detected, which could define the site of insertion in several potential backgrounds such as uncultured bacterium plasmid pHHV216, *Pseudomonas aeruginosa* plasmid pMRVIM0713, and *Bordetella bronchiseptica* plasmid R906 (GenBank accession numbers FJ012880, KP975076, and KF743818, respectively). The neighboring region also carried *sul2*, *floR*, *strB*, and *strA* and a variety of insertion elements.

There is only one previous report of OXA-23 in *P. mirabilis*, in isolates collected in the 1990s in France (2). Considering the transferable elements surrounding the gene in our isolate and that La et al. found OXA-23 in an *Escherichia coli* plasmid (3), as well as the phenotype of relative susceptibility of our isolate, OXA genes may well be spreading silently "under the radar." It is doubtful whether active screening to find these genes in relatively susceptible strains would be cost-effective, but researchers in reference laboratories should at least keep this possibility in mind.

Nucleotide sequence accession number. The sequence of the OXA-23-containing region has been deposited in GenBank with accession number KU302354.

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