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EPIDEMIOLOGY AND SURVEILLANCE

Carbapenem-Susceptible OXA-23-Producing *Proteus mirabilis* in the French Community

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ABSTRACT Nineteen *Proteus mirabilis* isolates producing the carbapenemase OXA-23 were recovered over a 2-year period in 19 French hospitalized patients, of whom 12 had community onset infections. The isolates exhibited a slightly reduced susceptibility to carbapenems. Whole-genome analysis revealed that all 19 isolates formed a cluster compared to 149 other *P. mirabilis* isolates. Because of its susceptibility to carbapenems, this clone may be misidentified as a penicillinase producer while it constitutes a reservoir of the OXA-23-encoding gene in the community.

KEYWORDS carbapenemase, OXA-23, *Proteus mirabilis*, spread, clonality

The emergence of carbapenem resistance in the *Enterobacteriaceae* is mainly linked to horizontal diffusion of carbapenemases belonging to Ambler classes A, B, and D (1). While the OXA-48-like enzymes are the most prevalent carbapenemases in several European countries, including France, carbapenem-hydrolyzing enzymes of OXA-23 and OXA-58 types are generally confined to *Acinetobacter* species (2). The bla_{OXA-23} and bla_{OXA-58} genes have occasionally been reported in *Enterobacteriaceae* species, especially in *Proteus mirabilis*. The spread of an OXA-23-producing clone of *P. mirabilis* was first revealed during a survey conducted between 1996 and 1999 in France (3). Later, the bla_{OXA-23} gene was detected sporadically in *Escherichia coli* in Singapore and India and, more recently, in *P. mirabilis* from Finland (4–6). Besides, *P. mirabilis* strains with a chromosomally integrated or plasmid-borne bla_{OXA-58} gene were characterized in Belgium and in Germany, respectively (7, 8).

Here, we report on the regional spread of a bla_{OXA-23}-positive *P. mirabilis* clone. Between November 2016 and May 2018, 19 isolates of *P. mirabilis* were found to exhibit an unusual penicillinase-like resistance phenotype (see below). The isolates were recovered from 19 patients hospitalized in ten different wards at the University Hospital of Besançon, France (Table 1). None of these patients had travelled abroad, and three had received amoxicillin or the amoxicillin-clavulanate combination within the 2 months before the isolation of *P. mirabilis* strain. Twelve patients were detected positive within the first 2 days following their admission, among whom seven had no history of hospitalization within the preceding 6 months (Table 1). An epidemiologic link between the cases could not be found. Together, these elements strongly suggested an acquisition of the *P. mirabilis* isolates within the community. Their antimicrobial susceptibility was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France) and interpreted according to current EUCAST guidelines (http://www.eucast.org). All of the isolates were resistant to amoxicillin and ticarcillin, with no recovery of their susceptibility with the addition of clavulanate. They also displayed decreased susceptibility to piperacillin-tazobactam but remained susceptible to

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TABLE 1 Clinical and microbiological features associated with bla_{OXA-23}-positive *P. mirabilis* isolates

	Isolate data[a]							Presence of antibiotic resistance gene														
Isolate	Hospitalization ward	Patient gender	Age range (yrs)	Clinical sample	Date of isolation	Strain isolated within 48 hours of admission	History of hospitalization within the preceding 6 mo	*aac*(3)-*IIa*	*aac*(3)-*IVa*	*aadA1*	*aph*(3″)-*Ib*	*aph*(3′)-*Ia*	*aph*(4)-*Ia*	*aph*(6)-*Id*	bla_{OXA-23}	*cat*	*dfrA1*	*floR*	*lnu*(G)	*sul2*	*tet*(J)
PmOXA23-1	Geriatric medicine	F	90–99	Urine	November 2016	No	No			+	+			+	+	+	+	+		+	+
PmOXA23-17	Surgery ward	F	60–69	Urine	February 2017	No	No	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-2	Hematology	M	50–59	Urine	February 2017	No	Yes	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-19	Surgery ward	M	70–79	Bone	January 2017	No	Yes			+	+	+		+	+	+	+	+		+	+
PmOXA23-3	Medical ICU	F	60–69	Wound	March 2017	Yes	Yes	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PmOXA23-18	Surgery ward	F	60–69	Abscess	March 2017	Yes	Yes	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-4	Geriatric medicine	F	90–99	Bone	March 2017	No	Yes			+	+	+		+	+	+	+	+		+	+
PmOXA23-5	Surgery ward	F	90–99	Urine	April 2017	Yes	No	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PmOXA23-6	Geriatric medicine	M	80–89	Urine	July 2017	Yes	Yes			+	+			+	+	+	+	+		+	+
PmOXA23-7	Pediatrics	F	0–9	Urine	August 2017	Yes	Yes	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-8	Medical ICU	F	50–59	Urine	October 2017	Yes	No			+	+	+		+	+	+	+	+		+	+
PmOXA23-9	Surgery ward	F	90–99	Joint fluid	November 2017	Yes	No	+		+	+	+		+	+	+	+				+
PmOXA23-10	Cardiology	M	80–89	Urine	November 2017	Yes	No	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-11	Gynecology department	F	40–49	Vagina	January 2018	Yes	No	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-12	Urology	M	70–79	Urine	February 2018	Yes	Yes	+		+	+	+		+	+	+	+			+	+
PmOXA23-13	Reproductive Biology	M	30–39	Sperm	February 2018	Yes	No	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-14	Surgery ward	M	70–79	Bone	March 2018	No	No			+	+	+		+	+	+	+	+		+	+
PmOXA23-15	Cardiology	F	80–89	Urine	May 2018	No	Yes	+		+	+	+		+	+	+	+	+		+	+
PmOXA23-16	Emergency room	F	80–89	Urine	May 2018	Yes	No			+	+			+	+	+	+	+		+	+

[a]ICU, intensive care unit; F, female; M, male.

expanded-spectrum cephalosporins (cefotaxime, ceftazidime, and cefepime). All isolates appeared to be susceptible to ertapenem but with inhibition zone diameters around the disk close to the breakpoint (i.e., 25 to 26 mm). This unusual antibiotic resistance profile evoked production of a class D β-lactamase expressing a poor carbapenem-hydrolyzing activity. All of the isolates were thus screened by PCR for the Ambler class D carbapenemase-encoding genes $bla_{\text{OXA-48-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$ (9, 10), and finally turned out to harbor $bla_{\text{OXA-23}}$. We tested the ability of ChromID Carba Smart medium (bioMérieux, La Balme-les-Grottes, France) to grow the *P. mirabilis* isolates carrying $bla_{\text{OXA-23}}$ by plating ~10^7 CFU of five representative isolates (PmOXA23-1 to PmOXA23-5). However, none of them could develop on that selective chromogenic medium. Of note, enterobacteria that produce other class D carbapenemases, such as OXA-244, are also not detected by this method (11). The transferability of $bla_{\text{OXA-23}}$, using *E. coli* and *Acinetobacter baumannii* as recipient strains, was tested as described previously (12). No transconjugants were obtained despite several attempts under different conditions (not shown), suggesting a chromosomal location for the resistance determinant. In order to identify other antibiotic resistance genes, the total DNA of each *P. mirabilis* strain was fully sequenced on an Illumina NextSeq platform. The DNA libraries for whole-genome sequencing were prepared using the Nextera XT kit with a 2 × 150-bp paired-end approach (BioProject accession number PRJNA490489). *De novo* assembly of the contigs was performed with SPAdes v3.11 (13), while the resistance genes were identified by using BLAT software with the ResFinder database (http://cge.cbs.dtu.dk/services/ResFinder, accessed 22 November 2018) (14). The mean size of the *P. mirabilis* genomes was 3.99 Mb, with a G+C content of 38.8%. We found that, in addition to $bla_{\text{OXA-23}}$, all isolates harbored genes conferring resistance to aminoglycosides [*aadA1*, *aph*(3″)-*Ib*, and *aph*(6)-*Id*], sulfamides (*sul2*, except in isolate PmOXA23-9), trimethoprim (*drfA*), chloramphenicol (*cat*), and tetracyclines [*tet*(J)] (Table 1). Most isolates also possessed the phenicol resistance gene *floR* and the aminoglycoside resistance genes *aac*(3)-*IIa* and *aph*(3′)-*Ia*. Finally, isolates PmOXA23-3 and PmOXA23-5 contained the genes *aac*(3)-*IVa* and *aph*(4)-*Ia* and the lincosamide nucleotidyltransferase-encoding gene *lnu*(G) (Table 1).

To assess the clonal relationship between the 19 *P. mirabilis* isolates, we compared their genomes with those of 149 *P. mirabilis* strains available in the NCBI database (Table S1) by whole-genome multilocus sequence typing (wgMLST; https://github.com/bvalot/pyMLST). Multilocus sequence typing (MLST) alleles were assigned with respect to 3,686 genes present in the core genome of the reference strain *P. mirabilis* HI4320 (15). From the 2,660 genes identified in ≥95% of the genomes, we built a distance matrix that showed the relative genomic divergence between the isolates. This revealed that our 19 isolates formed a cluster (rectangle in Fig. 1). This cluster also included the $bla_{\text{OXA-23}}$-positive isolate ESBL4969, identified in Finland in 2014, and the $bla_{\text{OXA-58}}$-positive isolate 1091, collected in 2015 in Belgium (6, 7). Overall, the wgMLST-based phylogeny revealed a notable diversity within the 149 *P. mirabilis* isolates. Another cluster of 19 *P. mirabilis* isolates was evidenced (triangle in Fig. 1), corresponding to CMY-2-positive isolates responsible for community-acquired infections in Ireland (16). The phylogenetic network analysis also highlighted two other genomic branches evolving from a common ancestor, which included 6 and 19 strains, respectively (represented as circles in Fig. 1).

Examination of the $bla_{\text{OXA-23}}$ genomic environment showed that the gene was embedded in a transposon-like structure that was itself inserted in a truncated Tn*5393* transposon (Fig. 2). The structure was identical in the 19 isolates and exhibited 100% sequence identity with that of $bla_{\text{OXA-23}}$-positive *P. mirabilis* ESBL4969 (GenBank accession number KU302354). Interestingly, only one IS*Aba1*-like copy bounded by canonical 9-bp direct repeats (DRs) (CGCTTCATC) is inserted in Tn*5393* in the clonally related OXA-58-positive strain *P. mirabilis* 1091 (Fig. 2). In the PmOXA-23 isolates, a 6,766-bp genetic element mapped at the same position and was bracketed by additional 13-bp DRs (TGAGCCACCTCCG), which together with the aforementioned 9-bp DRs formed a

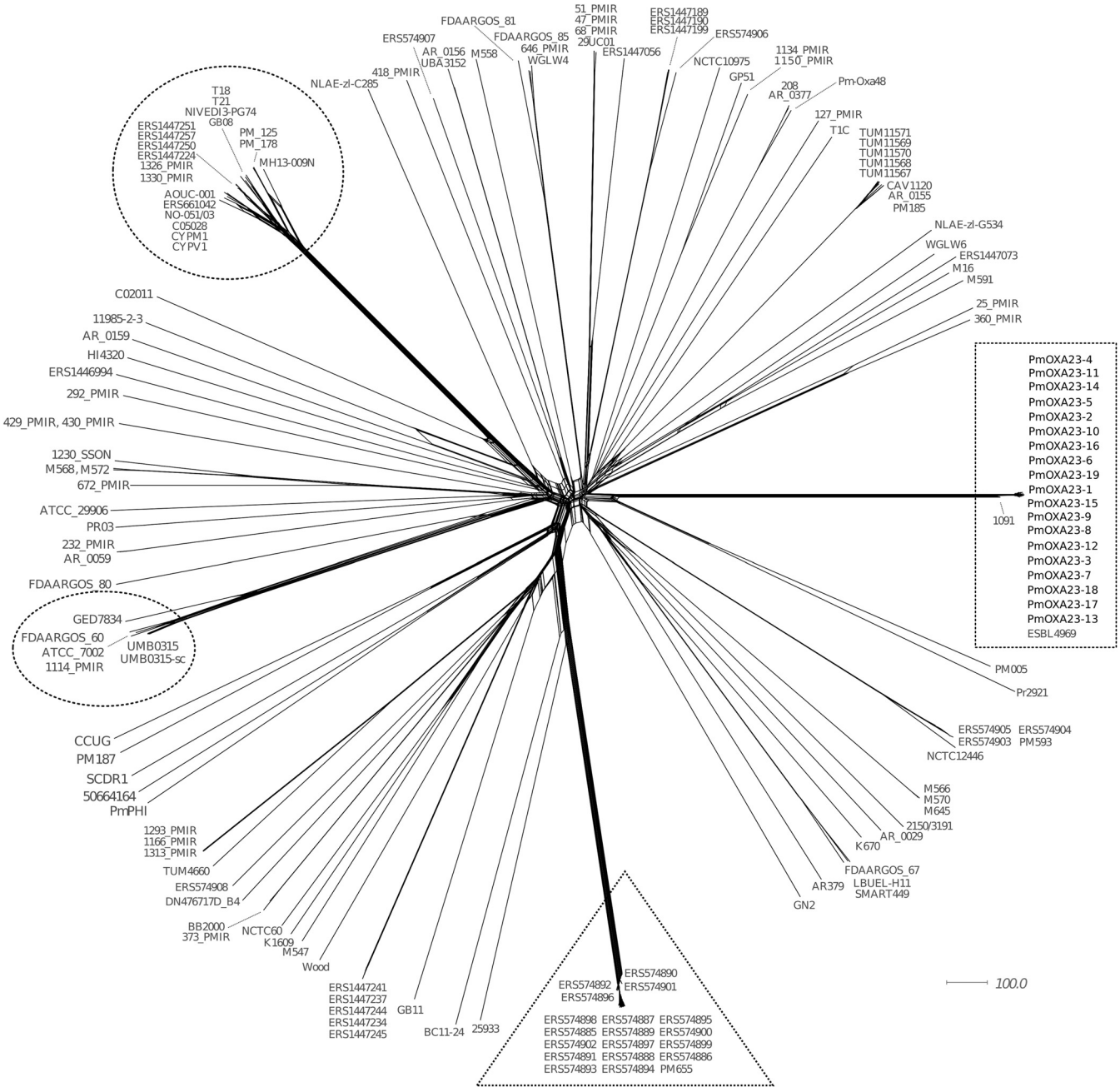

FIG 1 Phylogenetic network of the genomes of 168 *P. mirabilis* isolates. The genomic comparison included the 19 bla_{OXA-23} isolates collected at the University Hospital of Besançon (France) and 149 *P. mirabilis* isolates available in the NCBI database (Table S1). The core genome was defined as genes shared by ≥95% of the selected genomes (≥160/168 genomes). It was composed of 2,660 genes of the 3,686 genes annotated in reference strain *P. mirabilis* HI4320. The network was built using core genome multilocus sequence typing (cgMLST) distances with the neighborNet method in SplitTree4 (17). OXA-23- and CMY-2-positive *P. mirabilis* clusters are surrounded by a rectangle and a triangle, respectively. Circles represent two additional genomic branches evolving from a common ancestor.

22-bp duplication signature identical to that of isolate ESBL4969 (6). This genetic element was composed of transposon Tn*2008*, two other insertion sequences (IS*Aba14* and an IS*Aba125*-like sequence), and a gene encoding a truncated peptide related to the RepB family of plasmid replication initiators. Despite the presence of the 22-bp target site duplication suggesting a classical insertion of a Tn*2008*-containing transposon-like structure, the hypothesis of a genetic recombination cannot be ruled out. The identification of the same insertion site in the *P. mirabilis* 1091 genome and the fact that the sequencing depth of the bla_{OXA-23}-carrying contig was similar to that of

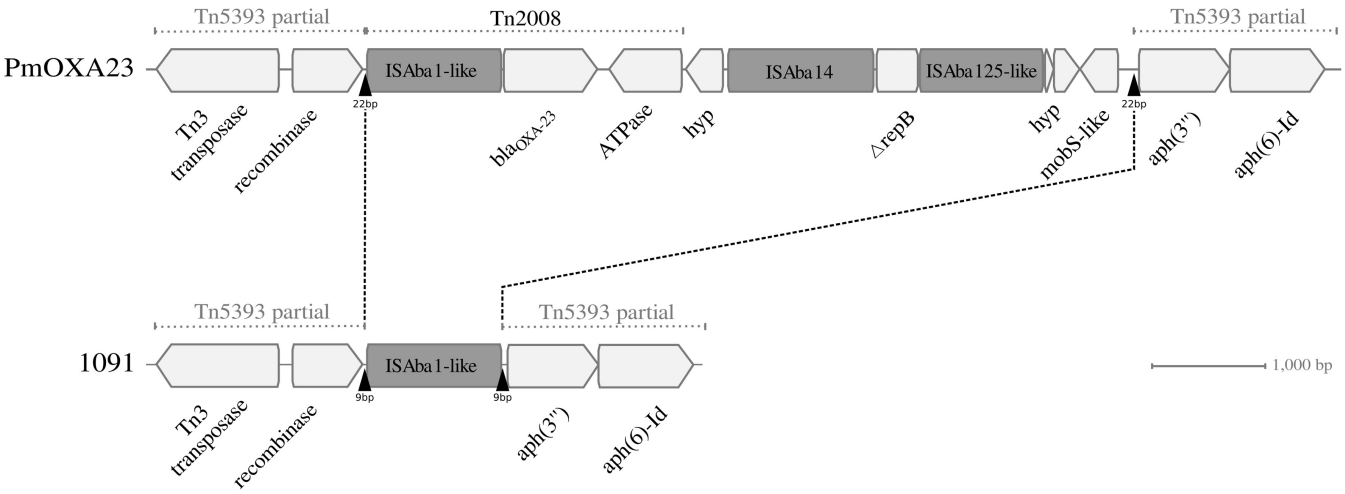

FIG 2 Schematic representation of bla_{OXA-23} insertion in the PmOXA23 clone. Genes are represented by light gray arrows indicating the direction of transcription. The predicted functions of genes are shown under the arrows. Gray boxes and black triangles represent insertion sequences and direct repeats, respectively. The OXA-23-carrying transposon-like structure of strain PmOXA23-13 (GenBank accession number SLUF00000000) was compared to that of an OXA-58-positive *P. mirabilis* 1091 isolate (accession number MCOR00000000).

the whole genome strongly support the notion that the element is integrated in the chromosome and not in a plasmid. Furthermore, as noted for isolate ESBL4969, the vicinity of this element is a hot spot for integration of various insertion sequences (IS*Aba1*-like, IS*Aba14*, IS*Aba125*-like, IS*Vsa3*, and IS*3*-like) and is subject to significant rearrangements in antibiotic resistance genes. Hence, the gene *sul2* associated or not with the gene *floR* was absent in isolates PmOXA23-12 and PmOXA23-9, while *aph*(4), *aac*(3)-*IVa*, and *lnu*(G) were present in PmOXA23-3 and PmOXA23-5. This high degree of genetic polymorphism suggests a propensity of this region to collect genes by horizontal transfer.

In summary, the present study highlights the diffusion of an OXA-23-positive *P. mirabilis* clone among epidemiologically unrelated patients. Because of its very low level of resistance to carbapenems, this clone is likely to be underrecognized by medical laboratories analyzing samples from outpatients, especially if antibiotic susceptibility tests are performed with automated systems based on the broth microdilution method, which uses breakpoint concentrations of drugs only. Prevalence of the clone in the French community remains unknown and warrants more extensive investigations. The attention of microbiologists should be drawn by ertapenem-susceptible strains of *P. mirabilis* having inhibition zones around the ertapenem disk close to the EUCAST susceptibility breakpoint diameter (25 mm).

Accession number(s). The DNA libraries for whole-genome sequencing in this study have been deposited in GenBank under the BioProject accession number PRJNA490489. The whole-genome shotgun project for PmOXA23-13 has been deposited in GenBank under the accession number SLUF00000000.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00191-19.

SUPPLEMENTAL FILE 1, XLSX file, 0.01 MB.

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We have no conflict of interest to declare.

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