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EPIDEMIOLOGY AND SURVEILLANCE

High Prevalence of OXA-23 Carbapenemase-Producing *Proteus mirabilis* among Amoxicillin-Clavulanate-Resistant Isolates in France

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ABSTRACT In this multicentric study performed in 12 French hospitals, we reported that 26.9% (14/52) of the amoxicillin-clavulanate-resistant *Proteus mirabilis* isolates produced the OXA-23 carbapenemase. We found that an inhibition zone diameter of <11 mm around the amoxicillin-clavulanate disc was an accurate screening cutoff to detect these OXA-23 producers. We confirmed by whole-genome sequencing that these OXA-23-producers all belonged to the same lineage that has been demonstrated to disseminate OXA-23 or OXA-58 in *P. mirabilis*.

KEYWORDS OXA-23, epidemiology, *Proteae*, *Acinetobacter*, carbapenemase, infectious clones

Proteus mirabilis are Gram-negative rods belonging to the *Morganellaceae* family inside the *Enterobacterales* order. This species is widespread in the environment but is also part of the gastrointestinal tract (GIT) microbiota. *P. mirabilis* clinical isolates are mainly responsible for urinary tract infections (UTIs), including health care-associated infections (1). Intrinsically, *P. mirabilis* is resistant to polymyxins, nitrofurantoin and tetracyclines. It does not produce any β-lactamase and remains susceptible to all β-lactams except imipenem. Decreased susceptibility to imipenem (but not to the other carbapenems such as meropenem and ertapenem) corresponds to the expression of PBPs (penicillin-binding proteins) of low affinity for this molecule (2). Acquired resistance to β-lactams is mainly due to the acquisition of extended-spectrum β-lactamases (ESBLs), cephalosporinases and sporadically carbapenemases (3). These carbapenemases are those usually identified in *Enterobacterales* such as KPC (Ambler class A), metallo-β-lactamases of NDM-, VIM-, or IMP-type (Ambler class B) and carbapenem-hydrolyzing Ambler class D β-lactamases (CHDLs) of OXA-48 type.

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In addition, as opposed to other *Enterobacterales* species, the most prevalent carbapenemases reported in *Acinetobacter* spp. (i.e., OXA-23, OXA-24/40, and OXA-58) have also been reported in *P. mirabilis*: OXA-23 in France and Finland, OXA-24/40 in Algeria, and OXA-58 in Belgium and Germany (4–9). Recently, a global phylogenetic analysis demonstrated that a unique clone of *P. mirabilis* is responsible for the dissemination OXA-23 or OXA-58 carbapenemases in humans and animals since 1996 (10).

Despite OXA-23 and OXA-58 are carbapenemases, the production of these enzymes surprisingly does not lead to multidrug resistance in *P. mirabilis*. Usually, OXA-23-producing *P. mirabilis* isolates exhibits an AST profile with only resistance to amoxicillin, ticarcillin, and piperacillin with no recovery of susceptibility when combined with clavulanate or tazobactam. They remain susceptible to third-generation cephalosporins, and resistance to carbapenems (meropenem and ertapenem) is difficult to detect due to the poor carbapenem-hydrolyzing activity of OXA-23, OXA-24/-40, and OXA-58 and the common chromosomal localization of the carbapenemase-encoding genes in this major clone of OXA-23/OXA-58-producing *P. mirabilis* (3). Accordingly, this phenotype is very lucky to be confused with the high-level production of a penicillinase or the expression of a narrow-spectrum oxacillinase (e.g., OXA-1) (see Fig. S1 in the supplemental material). In addition, the frequency of the acquisition of carbapenemase from *Acinetobacter* in *P. mirabilis* remains unknown.

Here, we aimed to determine the prevalence of *Acinetobacter* main carbapenemases (i.e., OXA-23, OXA-58, and OXA-24/40) in *P. mirabilis* clinical isolates resistant to amoxicillin-clavulanate collected in a French multicentric cohort.

From 1 January to 31 December 2019, 139 *P. mirabilis* isolates recovered from human clinical samples (no screening sample) collected in 12 French hospitals were analyzed. Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton (MH) agar (Bio-Rad, Marnes-La-Coquette, France) and interpreted according to EUCAST guidelines. Among these *P. mirabilis* isolates, 52 strains with an inhibition diameter zone below 16 mm for urinary samples or below 19 mm for all other clinical samples were included as amoxicillin-clavulanate-resistant isolates, according to EUCAST breakpoints. These strains were isolated from urine samples ($n = 19$), blood cultures ($n = 3$), mucocutaneous samples ($n = 7$), catheters ($n = 1$), respiratory samples ($n = 7$), genital samples ($n = 3$), and abscesses and drainage ($n = 14$) (Fig. 1A). One additional ESBL-producing isolate was excluded from the study.

All 52 amoxicillin-clavulanate-resistant *P. mirabilis* isolates were then screened by conventional PCR for the presence of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, or $bla_{\text{OXA-58}}$ genes, as previously described (5, 11). No strain was found to be positive for $bla_{\text{OXA-24/40}}$ or $bla_{\text{OXA-58}}$ gene, whereas 26.9% (14/52) of amoxicillin-clavulanate-resistant *P. mirabilis* isolates gave a positive signal for $bla_{\text{OXA-23}}$. The production of the OXA-23 carbapenemase was confirmed with an OXA-23 K-SeT immunochromatographic detection assay (Coris Bioconcept) performed as previously described (12). These OXA-23-producing *P. mirabilis* were isolated from urine samples ($n = 7$), respiratory samples ($n = 2$), and abscesses and drainage ($n = 5$). Of note, all OXA-23-producing *P. mirabilis* isolates had an amoxicillin-clavulanate inhibition zone diameter between 7 and 11 mm (Fig. 1B). This suggests that the screening cutoff for OXA-23 production in *P. mirabilis* might be a $\leq$11-mm inhibition zone diameter around an amoxicillin-clavulanate-containing disc (20 mg amoxicillin plus 10 mg clavulanate). The determination of amoxicillin-clavulanate MICs by Etest (bioMérieux) confirmed that all OXA-23-producing *P. mirabilis* isolates had MICs comprised between 12 and 24 mg/L. According to EUCAST breakpoints, these OXA-23-producing *P. mirabilis* isolates are categorized as susceptible (MIC $\leq$32 mg/L) if they are responsible for uncomplicated UTIs. Indeed, clavulanate can concentrate in urine, leading to a higher breakpoint (32 mg/L) for uncomplicated UTI compared to strains responsible for other infections (breakpoint at 8 mg/L). However, since the OXA-23 enzyme is not inhibited by clavulanate, it might be possible that such susceptible categorization leads to treatment failure. Unfortunately, we could not have access to clinical data to confirm if such treatment failure occurred. Of note, among the 38 amoxicillin-clavulanate-resistant *P. mirabilis* isolates that were negative for $bla_{\text{OXA-23}}$, 76.3% (29/38) expressed

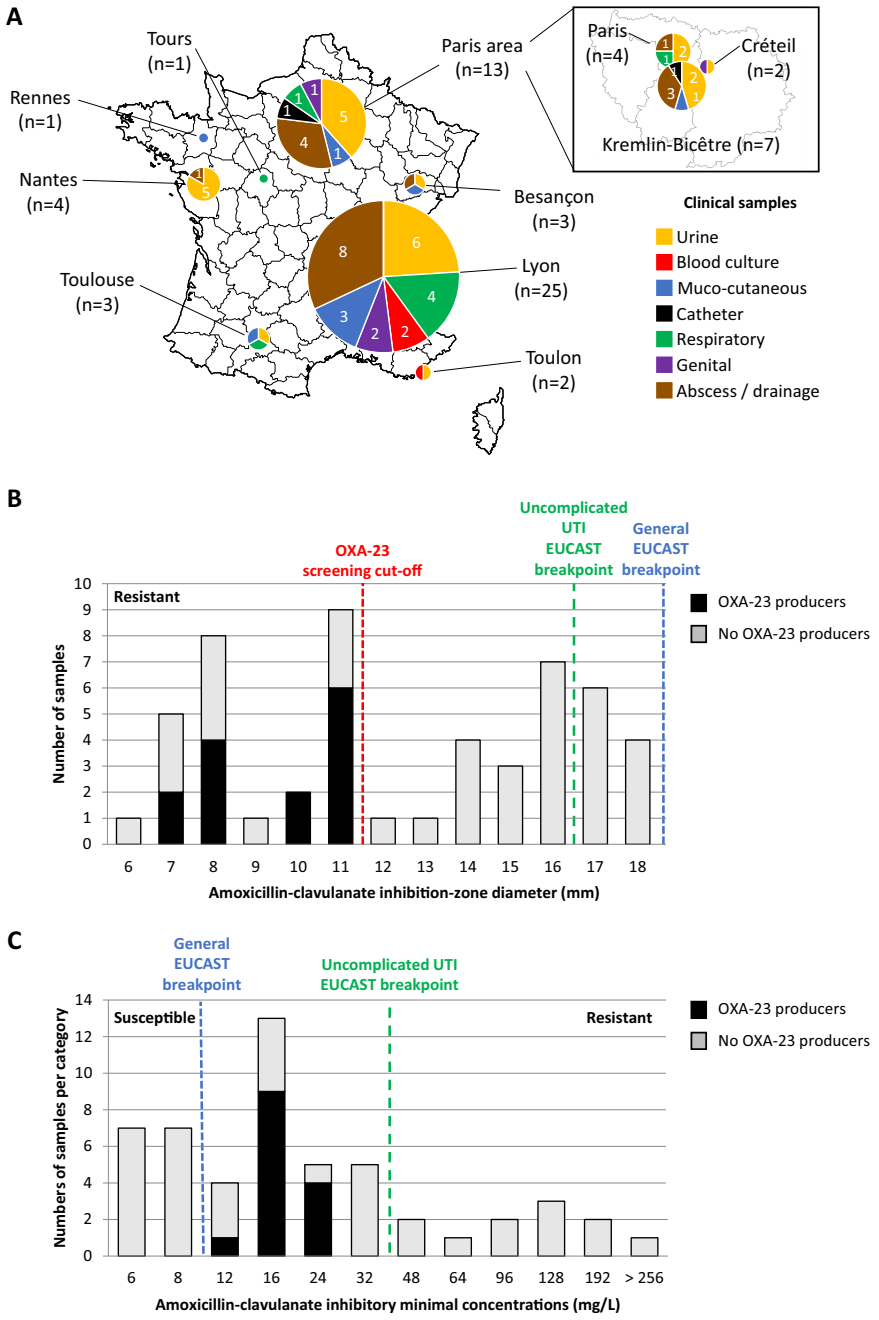

FIG 1 Characteristics of the 52 amoxicillin-clavulanate-resistant *P. mirabilis* isolates. (A) Geographic distribution and clinical samples. (B) Distribution of the amoxicillin-clavulanate zone inhibition diameters. (C) Distribution of the amoxicillin-clavulanate minimal inhibition concentrations.

the TEM-1 penicillinase and the narrow-spectrum oxacillinase OXA-1 (assessed by PCR and sequencing), 13.2% (5/38) were positive only for bla_{OXA-1}, 7.9% (3/38) were positive only for bla_{TEM-1}, and only one isolate (2.6%) was negative for both bla_{TEM-1} and bla_{OXA-1}.

No obvious epidemiological link could be identified between the 14 OXA-23-producing *P. mirabilis* isolates since they were recovered in different areas. However, to assess the clonal relationship between these 14 *P. mirabilis* isolates, we performed a whole-genome sequencing and comparison as previously described (10). The genomes of OXA-23-producing *P. mirabilis* were submitted to GenBank (BioProject PRJNA780406). As previously reported (10), all OXA-23-producing *P. mirabilis* isolates were part of the major lineage that disseminated in France and Belgium at least since 1996 (Fig. 2).

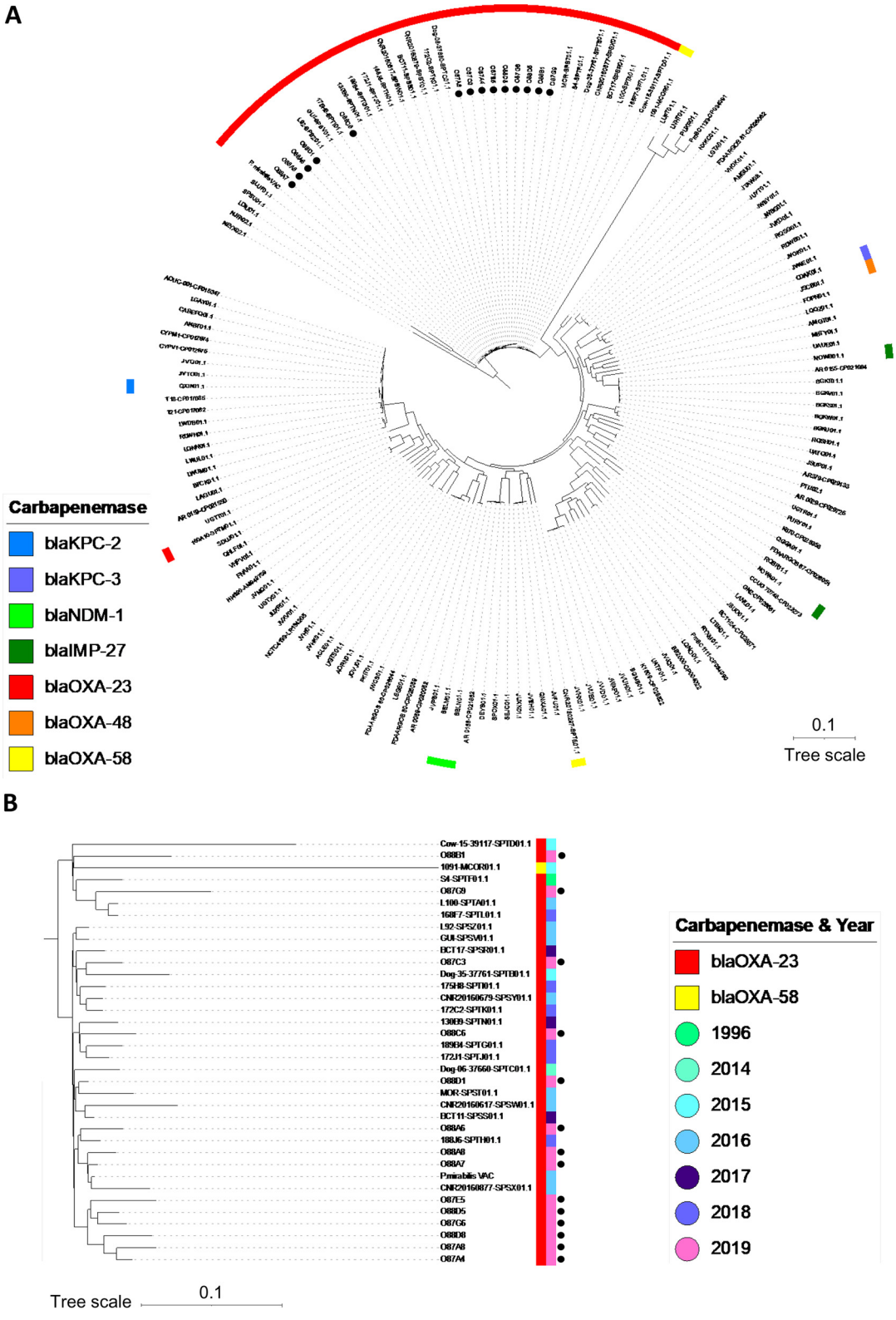

FIG 2 (A) Phylogenetic relationship of the 14 OXA-23-producing *P. mirabilis* isolates with the 145 reference genomes of *P. mirabilis* reported by Bonnin et al. (10). This comparison was performed on 17.83% of the genome of OXA-23-producing *P. mirabilis* VAC used as a reference. (B) Phylogenetic relationship of the OXA-23/OXA-58-producing *P. mirabilis* isolates. This comparison was performed on 57.36% of the genome of OXA-23-producing *P. mirabilis* VAC used as a reference. OXA-23-producing *P. mirabilis* from this study are marked by a black point. Scale bar on tree indicates the number of single-nucleotide polymorphisms per position of common sequences.

In conclusion, we demonstrated that nearly one-quarter of the *P. mirabilis* clinical isolates with an amoxicillin-clavulanate zone inhibition of <19 mm were OXA-23-producing strains. We established that an amoxicillin-clavulanate zone inhibition of <11 mm is an efficient screening cutoff to detect these OXA-23 producers. However, since currently only one clone of *P. mirabilis* has been reported to vehiculate bla_{OXA-23}, such a screening cutoff might have to be adapted if the emergence of another clone is further reported. As previously reported, we demonstrated that the immunochromatographic assay OXA-23 K-Set is a useful tool to rapidly identify the production of OXA-23 by *P. mirabilis*. The amoxicillin-clavulanate MICs for these OXA-23-producing *P. mirabilis* strains are between 12 and 24 mg/L. Accordingly, these strains might be categorized as susceptible if they were considered to be responsible for uncomplicated UTIs. Since OXA-23 is not inhibited by clavulanate, clinical failure might occur. However, several therapeutic options are often possible outside the β-lactams family (fluoroquinolones, aminoglycosides, and sulfamethoxazole-trimethoprim), particularly for UTIs. In addition, OXA-23 do not hydrolyze third-generation cephalosporins and only leads to a low-level resistance that is often remain undetectable for carbapenems (ertapenem or meropenem) or piperacillin-tazobactam, suggesting a potential use of these molecules. However, complementary studies are needed to decipher this hypothesis.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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