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High prevalence of OXA-23 carbapenemase-producing *Proteus mirabilis* among amoxicillin-clavulanate resistant isolates in France

Amélie LOMBES^{1,2}, Rémy A. BONNIN^{2,3}, Frédéric LAURENT⁴, Hélène GUET-REVILLET⁵, Emmanuelle BILLE⁶, Vincent CATTOIR⁷, Marie-Sarah FANGOUS⁸, Cécile LE BRUN⁹, Vincent FIHMAN¹⁰, Frédéric JANVIER¹¹, Marie-Pierre Otto¹¹, Anais POTRON¹², Stéphane CORVEC¹³, Louise RUFFIER D'EPENOUX¹³, Assaf MIZRAHI^{14,15}, Laurent DORTET^{1,2,3}, on behalf of the GMC study group.

¹ CHU de Bicêtre, Laboratoire de Bactériologie-Hygiène, Assistance Publique des Hôpitaux de Paris, Le Kremlin-Bicêtre, France

² INSERM UMR 1184, Team RESIST, Faculté de Médecine, Université Paris-Saclay, Le Kremlin-Bicêtre, France

³ Centre National de Référence de la Résistance aux Antibiotiques, Le Kremlin-Bicêtre, France

⁴ Hospices Civils de Lyon, Department de Bactériologie, Institut des Agents infectieux, Lyon, France

⁵ Hôpital Purpan, Laboratoire de Bactériologie-Hygiène, Toulouse, France

⁶ CHU Necker-Enfants Malades, Laboratoire de Microbiologie, Assistance Publique des Hôpitaux de Paris, Paris, France

⁷ CHU de Rennes, Service de Bactériologie-Hygiène Hospitalière, F-35033 Rennes, France

⁸ Centre Hospitalier de Cornouaille, Laboratoire de biologie médicale, Quimper, France

⁹ CHRU de Tours, Hôpital Bretonneau, Service de Bactériologie-Virologie-Hygiène, Tours, France

¹⁰ CHU Henri Mondor, Service de Bactériologie-Virologie-Hygiène, Créteil, France

¹¹ Hôpital d'Instruction des Armées Sainte-Anne, Service de microbiologie et hygiène hospitalière, Toulon, France

¹² Centre National de Référence de la Résistance aux Antibiotiques, Laboratoire de Bactériologie, CHU de Besançon, Besançon, France

¹³ CHU de Nantes, Service de Bactériologie et des Contrôles Microbiologiques, CRCINA U1232, Nantes, France

¹⁴ Service de Microbiologie Clinique, Groupe Hospitalier Paris Saint-Joseph, Paris, France

¹⁵ Institut Micalis UMR 1319, Université Paris-Saclay, INRAe, AgroParisTech, Châtenay Malabry, France

* Corresponding author's mailing address: Service de Bactériologie-Hygiène, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France.

Fax: + 33 1 45 21 63 40.

E-mail: laurent.dortet@aphp.fr

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48 **Abstract**

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

56

57

58 *Proteus mirabilis* are gram-negative rods belonging to the *Morganellaceae* family inside the
59 Enterobacterales order. This species is widespread in the environment but is also part of the
60 gastrointestinal tract (GIT) microbiota. *P. mirabilis* clinical isolates are mainly responsible for
61 urinary tract infections (UTIs), including healthcare-associated infections (1). Intrinsically, *P.*
62 *mirabilis* is resistant to polymyxins, nitrofurantoin and tetracyclines. It does not produce any
63 β -lactamase and remains susceptible to all β -lactams except imipenem. Decreased
64 susceptibility to imipenem (but not to the other carbapenems such as meropenem and
65 ertapenem) corresponds to the expression of PBPs (penicillin binding proteins) of low affinity
66 for this molecule (2). Acquired resistance to β -lactams is mainly due to the acquisition of
67 extended-spectrum β -lactamases (ESBLs), cephalosporinases and sporadically
68 carbapenemases (3). These carbapenemases are those usually identified in Enterobacterales
69 such as: KPC (Ambler class A), metallo- β -lactamases of NDM-, VIM- or IMP-type (Ambler
70 class B), and carbapenem-hydrolyzing Ambler class D β -lactamases (CHDLs) of OXA-48
71 type. In addition, as opposed to other Enterobacterales species, the most prevalent
72 carbapenemases reported in *Acinetobacter* spp. (i.e., OXA-23, OXA-24/40 and OXA-58)
73 have also been reported in *P. mirabilis*: OXA-23 in France and Finland, OXA-24/40 in
74 Algeria and OXA-58 in Belgium and Germany (4-9). Recently, a global phylogenetic analysis
75 has demonstrated that a unique clone of *P. mirabilis* is responsible for the dissemination
76 OXA-23 or OXA-58 carbapenemases in humans and animals since 1996 (10).
77 Despite OXA-23 and OXA-58 are carbapenemases, the production of these enzymes does not
78 surprisingly lead to multidrug resistance in *P. mirabilis*. Usually, OXA-23-producing *P.*
79 *mirabilis* isolates exhibits an AST profile with only resistance to amoxicillin, ticarcillin, and
80 piperacillin with no recovery of susceptibility when combined with clavulanate or
81 tazobactam. They remain susceptible to third-generation cephalosporins and resistance to
82 carbapenems (meropenem and ertapenem) is difficult to detect due to the poor carbapenem-

83 hydrolyzing activity of OXA-23, OXA-24/-40 and OXA-58 and the common chromosomal
84 localization of the carbapenemase-encoding genes in this major clone of OXA-23/OXA-58-
85 producing *P. mirabilis* (3). Accordingly, this phenotype is very lucky to be confused with the
86 high-level production of a penicillinase or the expression of a narrow-spectrum oxacillinase
87 (e.g., OXA-1) (Figure S1). In addition, the frequency of the acquisition of carbapenemase
88 from *Acinetobacter* in *P. mirabilis* remains unknown.

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

92

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

104 All the 52 amoxicillin/clavulanate-resistant *P. mirabilis* isolates were then screened by
105 conventional PCR for the presence of *bla*_{OXA-23}, *bla*_{OXA-24/40} or *bla*_{OXA-58} genes as previously
106 described (5, 11). No strain was found to be positive for *bla*_{OXA-24/40} or *bla*_{OXA-58} gene whereas
107 26.9% (14/52) of amoxicillin/clavulanate-resistant *P. mirabilis* isolates gave a positive signal

108 for *bla*_{OXA-23}. The production of the OXA-23 carbapenemase was confirmed with the OXA-23
109 K-SeT immunochromatographic detection assay (Coris Bioconcept) performed as previously
110 described (12). These OXA-23-producing *P. mirabilis* were isolated from urines (n=7),
111 respiratory samples (n=2), abscesses and drainage (n=5). Of note, all OXA-23-producing *P.*
112 *mirabilis* isolates had an amoxicillin/clavulanate inhibition zone diameter between 7 mm and
113 11 mm (Figure 1B). It suggests that the screening cut-off for OXA-23 production in *P.*
114 *mirabilis* might be 11 mm inhibition zone diameter or less around an amoxicillin/clavulanate-
115 containing disc (20 mg amoxicillin + 10 mg clavulanate). The determination of
116 amoxicillin/clavulanate MICs by Etest (BioMérieux) confirmed that all OXA-23-producing *P.*
117 *mirabilis* isolates had MICs comprised between 12 and 24 mg/L. According to EUCAST
118 breakpoints, these OXA-23-producing *P. mirabilis* isolates are categorized as susceptible
119 (MIC \leq 32 mg/L) if they are responsible for uncomplicated UTIs. Indeed, clavulanate can
120 concentrate in urine leading to a higher breakpoint (32 mg/L) for uncomplicated UTI
121 compared to strains responsible for other infections (breakpoint at 8 mg/L). However, since
122 the OXA-23 enzyme is not inhibited by clavulanate, it might be possible that such susceptible
123 categorization leads to treatment failure. Unfortunately, we could not have access to clinical
124 data to confirm if such treatment failure occurred. Of note, among the 38
125 amoxicillin/clavulanate-resistant *P. mirabilis* isolates that were negative for *bla*_{OXA-23}, 76.3%
126 (29/38) expressed the TEM-1 penicillinase and the narrow-spectrum oxacillinase OXA-1
127 (assessed by PCR and sequencing), 13.2% (5/38) were positive only for *bla*_{OXA-1}, 7.9% (3/38)
128 were positive only for *bla*_{TEM-1} and only one isolates (2.6%) was negative for both *bla*_{TEM-1}
129 and *bla*_{OXA-1}.

130 No obvious epidemiological link could be identified between the 14 OXA-23-producing *P.*
131 *mirabilis* isolates since they were recovered in different areas. However, to assess the clonal
132 relationship between these 14 *P. mirabilis* isolates, we performed a whole genome sequencing

133 and comparison as previously described (10). The genomes of OXA-23-producing *P.*
134 *mirabilis* were submitted to GenBank (Bioproject number PRJNA780406). As previously
135 reported (10), all OXA-23-producing *P. mirabilis* isolates were part of the major lineage that
136 disseminated in France and Belgium at least since 1996 (Figure 2).

137 As conclusion, we demonstrated that nearly one quarter of the *P. mirabilis* clinical isolates
138 with an amoxicillin/clavulanate zone inhibition less than 19 mm were OXA-23-producing
139 strains. We established that an amoxicillin/clavulanate zone inhibition less than 11 mm is an
140 efficient screening cut-off to detect these OXA-23 producers. However, since currently only
141 one clone of *P. mirabilis* has been reported to vehiculate *bla*_{OXA-23}, such screening cut-off
142 might have to be adapted if the emergence of another clone is further reported. As previously
143 reported, we demonstrated that the immunochromatographic assay OXA-23 K-Set is a useful
144 tool to rapidly identify the production of OXA-23 by *P. mirabilis*. Amoxicillin/clavulanate
145 MICs of these OXA-23-producing *P. mirabilis* is comprised between 12 and 24 mg/L.
146 Accordingly, these strains might be categorized as susceptible if they were considered to be
147 responsible for uncomplicated UTIs. Since OXA-23 is not inhibited by clavulanate, clinical
148 failure might occur. However, several therapeutic options are often possible outside the β -
149 lactams family (fluoroquinolones, aminoglycosides, sulfamethoxazole-trimethoprim),
150 particularly for urinary-tract infections. In addition, OXA-23 do not hydrolyze 3rd generation
151 cephalosporins and only leads a low-level resistance that often remain undetectable for
152 carbapenems (ertapenem or meropenem) or piperacillin-tazobactam, suggesting potential use
153 of these molecules. But, complementary studies are needed to decipher this hypothesis.

154

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199

200 **LEGEND OF THE FIGURES**

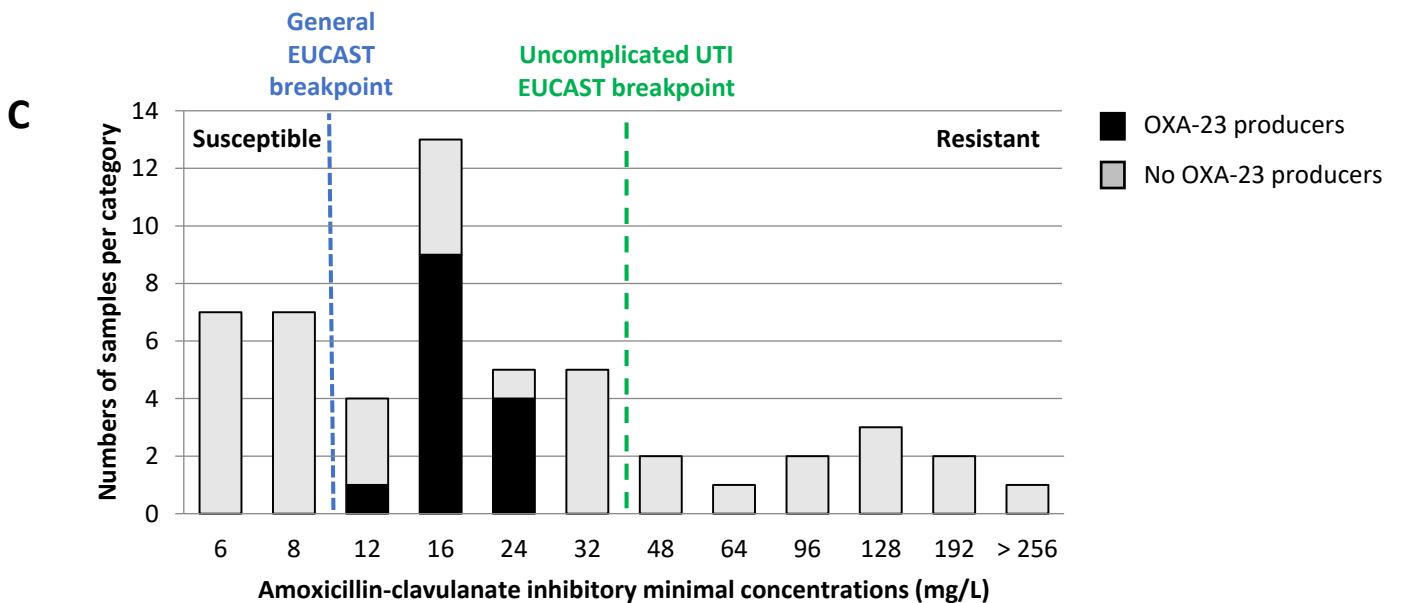
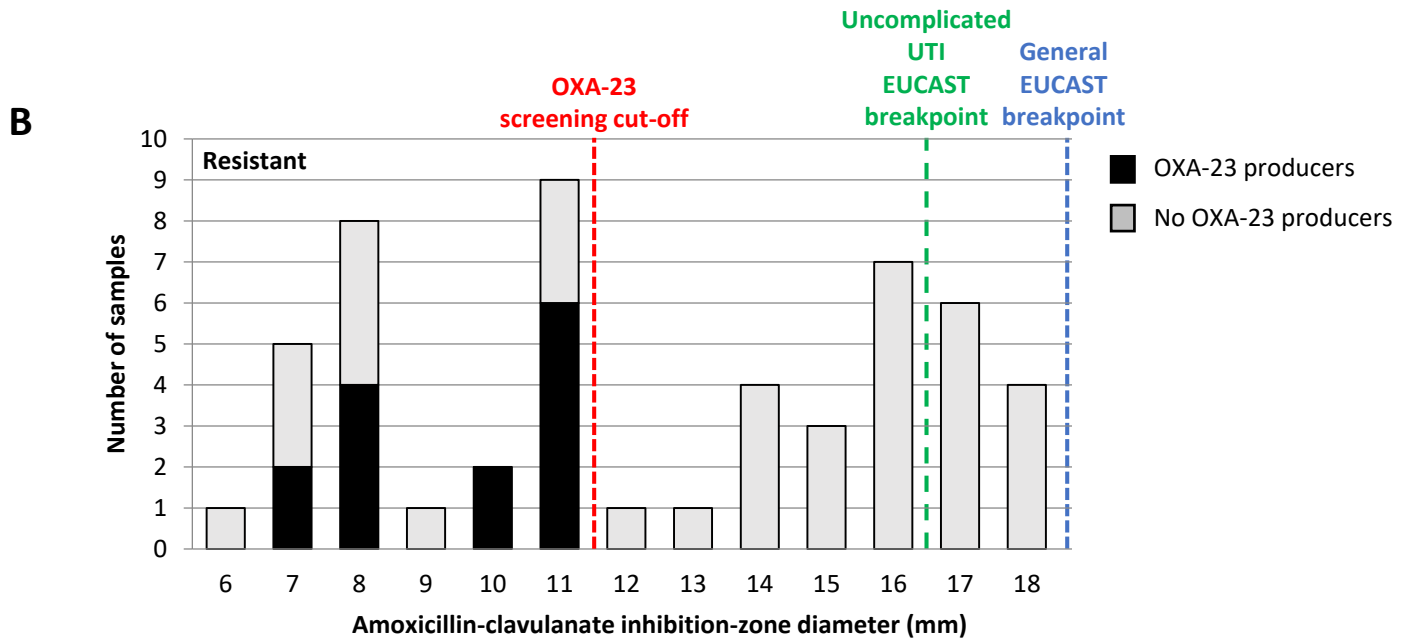
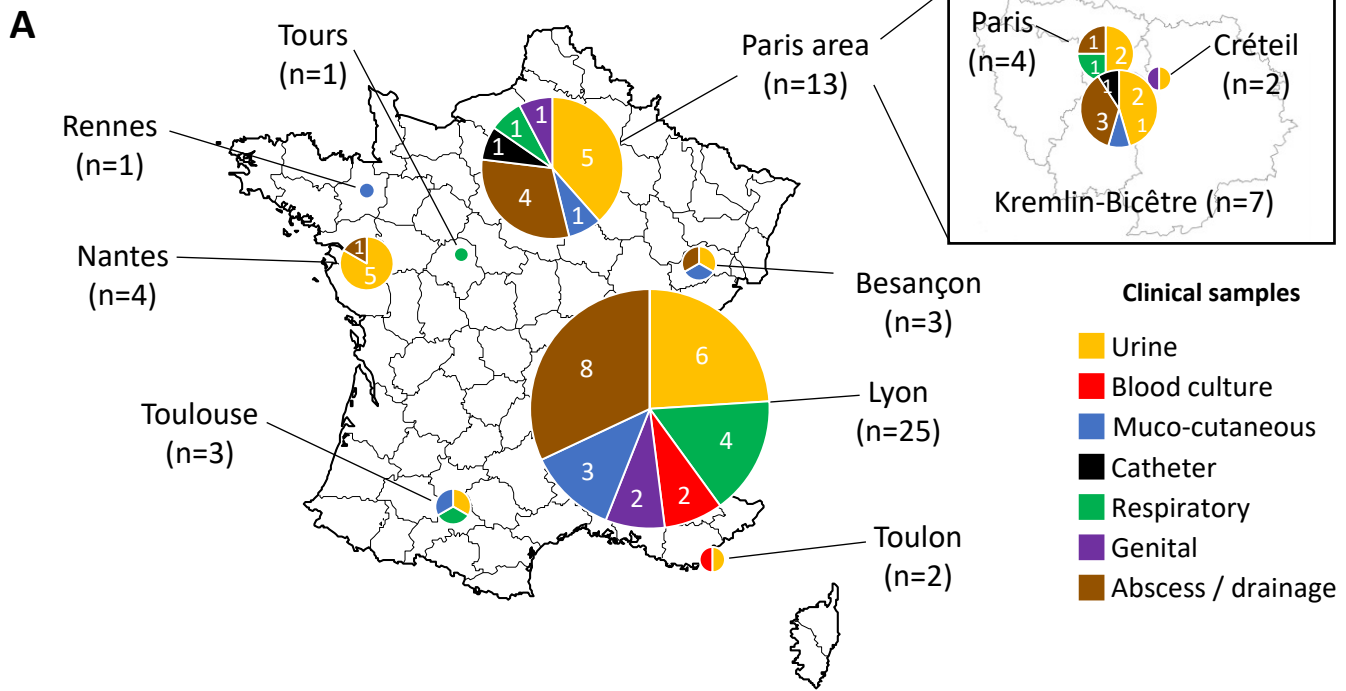
201 **Figure 1.** Characteristics of the 52 amoxicillin-clavulanate resistant *P. mirabilis* isolates **A.**
202 Geographic distribution and clinical samples. **B.** Distribution of the amoxicillin-clavulanate
203 zone inhibition diameters. **C.** Distribution of the amoxicillin-clavulanate minimal inhibition
204 concentrations.

205

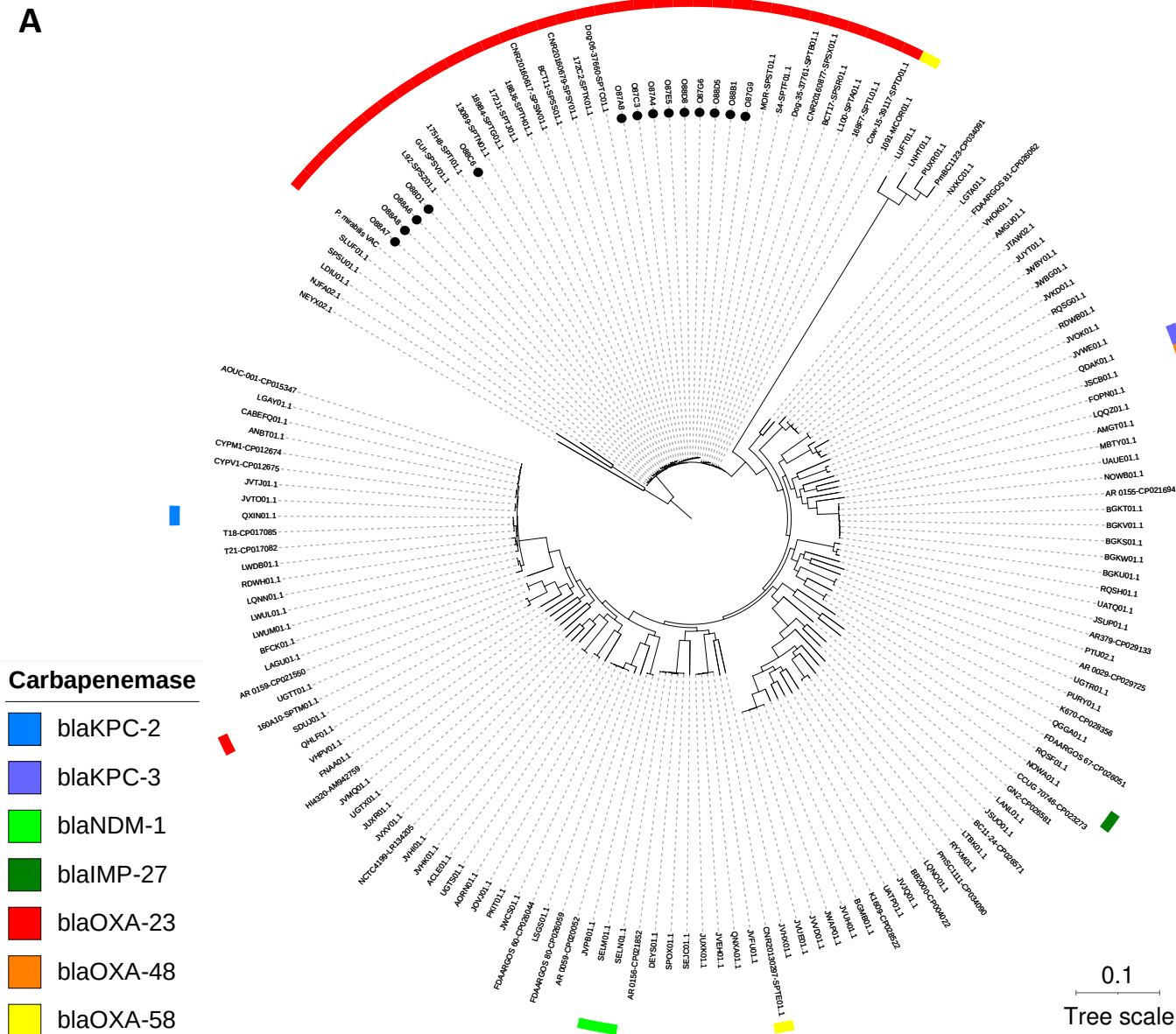
206 **Figure 2. A.** Phylogenetic relationship of the 14 OXA-23-producing *P. mirabilis* isolates with
207 the 145 reference genomes of *P. mirabilis* reported by Bonnin R.A *et al* (10). This
208 comparison was performed on 17.83% of the genome of OXA-23-producing *P. mirabilis*
209 VAC used as reference. **B.** Phylogenetic relationship of the OXA-23/OXA-58-producing *P.*
210 *mirabilis* isolates. This comparison was performed on 57,36% of the genome of OXA-23-
211 producing *P. mirabilis* VAC used as reference.

212 OXA-23-producing *P. mirabilis* from this study are marked by a black point. Scale bar on tree
213 indicates the number of single-nucleotide polymorphisms per position of common sequences.

214



A



B

