# 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 LAURENT[4], Hélène GUET-REVILLET[5], Emmanuelle BILLE[6], Vincent CATTOIR[7], Marie-Sarah FANGOUS[8], Cécile LE BRUN[9], Vincent FIHMAN[10], Frédéric JANVIER[11], Marie-Pierre Otto[11], Anais POTRON[12], Stéphane CORVEC[13], Louise RUFFIER D'EPENOUX[13], 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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**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 inhibition zone diameter less than 11 mm around amoxicillin/clavulanate disc was an accurate screening cut-off 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*.

*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 healthcare-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. 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 has 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 does not surprisingly 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) (Figure S1). 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 January 1$^{st}$ to December 31$^{th}$ 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 urines (n=19), blood cultures (n=3), muco-cutaneous samples (n=7), catheter (n=1), respiratory samples (n=7), genital samples (n=3), abscesses and drainage (n=14) (Figure 1A). One additional ESBL-producing isolate was excluded from the study.

All the 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*$_{OXA-23}$. The production of the OXA-23 carbapenemase was confirmed with the OXA-23 K-SeT immunochromatographic detection assay (Coris Bioconcept) performed as previously described (12). These OXA-23-producing *P. mirabilis* were isolated from urines (n=7), respiratory samples (n=2), abscesses and drainage (n=5). Of note, all OXA-23-producing *P. mirabilis* isolates had an amoxicillin/clavulanate inhibition zone diameter between 7 mm and 11 mm (Figure 1B). It suggests that the screening cut-off for OXA-23 production in *P. mirabilis* might be 11 mm inhibition zone diameter or less around an amoxicillin/clavulanate-containing disc (20 mg amoxicillin + 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 ≤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*$_{OXA-23}$, 76.3% (29/38) expressed 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 isolates (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 number 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 (Figure 2).

As conclusion, we demonstrated that nearly one quarter of the *P. mirabilis* clinical isolates with an amoxicillin/clavulanate zone inhibition less than 19 mm were OXA-23-producing strains. We established that an amoxicillin/clavulanate zone inhibition less than 11 mm is an efficient screening cut-off to detect these OXA-23 producers. However, since currently only one clone of *P. mirabilis* has been reported to vehiculate $bla_{\text{OXA-23}}$, such screening cut-off 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*. Amoxicillin/clavulanate MICs of these OXA-23-producing *P. mirabilis* is comprised 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, sulfamethoxazole-trimethoprim), particularly for urinary-tract infections. In addition, OXA-23 do not hydrolyze 3$^{rd}$ generation cephalosporins and only leads a low-level resistance that often remain undetectable for carbapenems (ertapenem or meropenem) or piperacillin-tazobactam, suggesting potential use of these molecules. But, complementary studies are needed to decipher this hypothesis.

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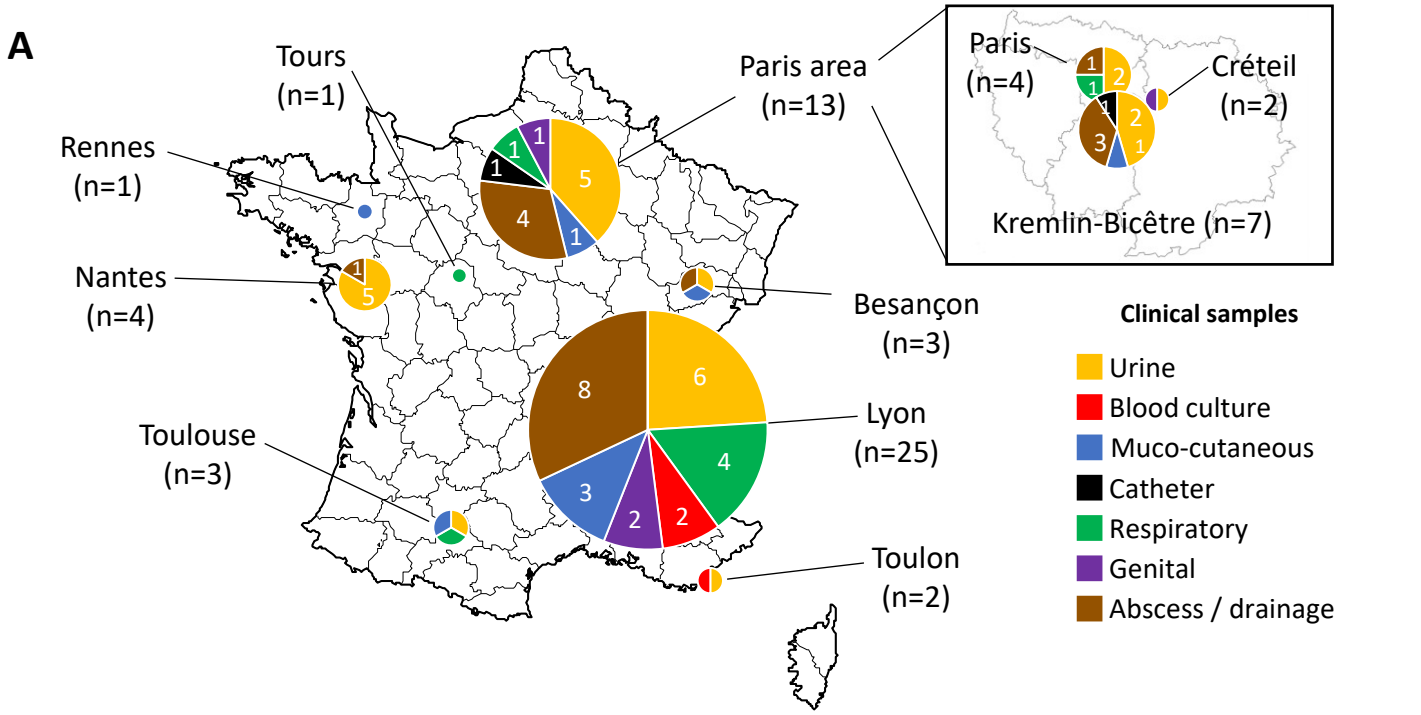
## LEGEND OF THE FIGURES

**Figure 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.

**Figure 2. A.** Phylogenetic relationship of the 14 OXA-23-producing *P. mirabilis* isolates with the 145 reference genomes of *P. mirabilis* reported by Bonnin R.A *et al* (10). This comparison was performed on 17.83% of the genome of OXA-23-producing *P. mirabilis* VAC used as 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 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.


A
Tours
(n=1)
Rennes
(n=1)
Nantes
(n=4)
Toulouse
(n=3)
Paris area
(n=13)
Besançon
(n=3)
Lyon
(n=25)
Toulon
(n=2)
Paris
(n=4)
Créteil
(n=2)
Kremlin-Bicêtre (n=7)
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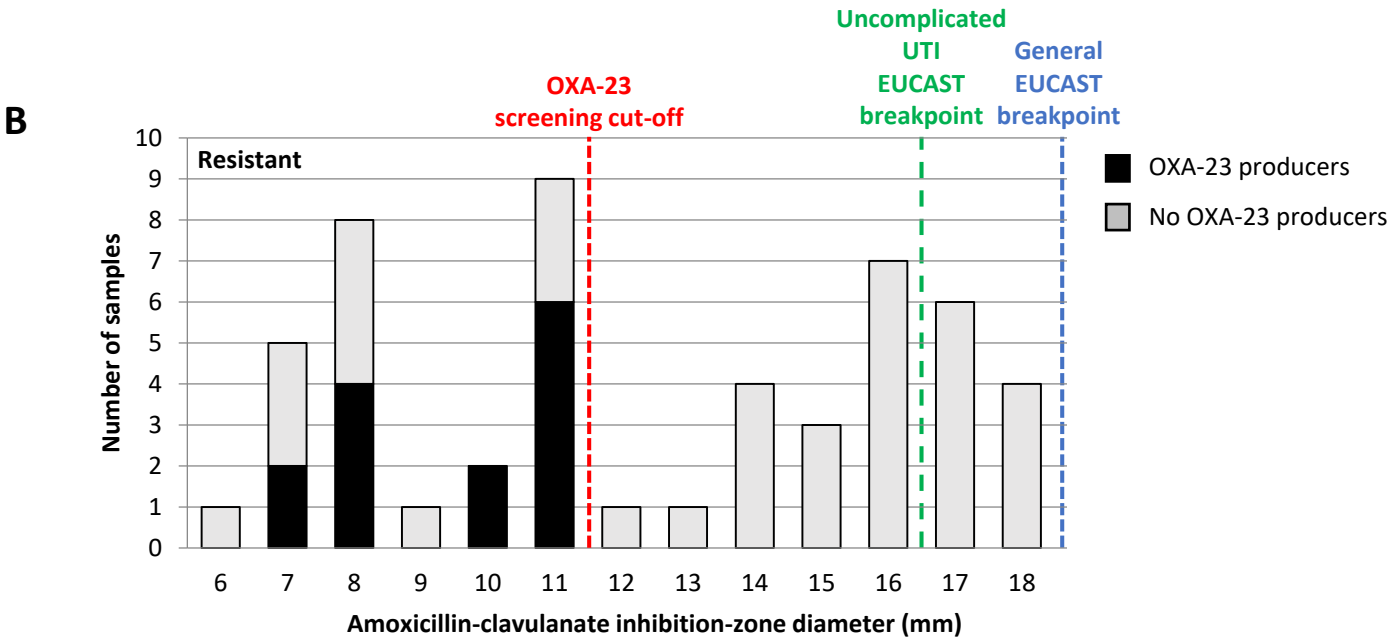
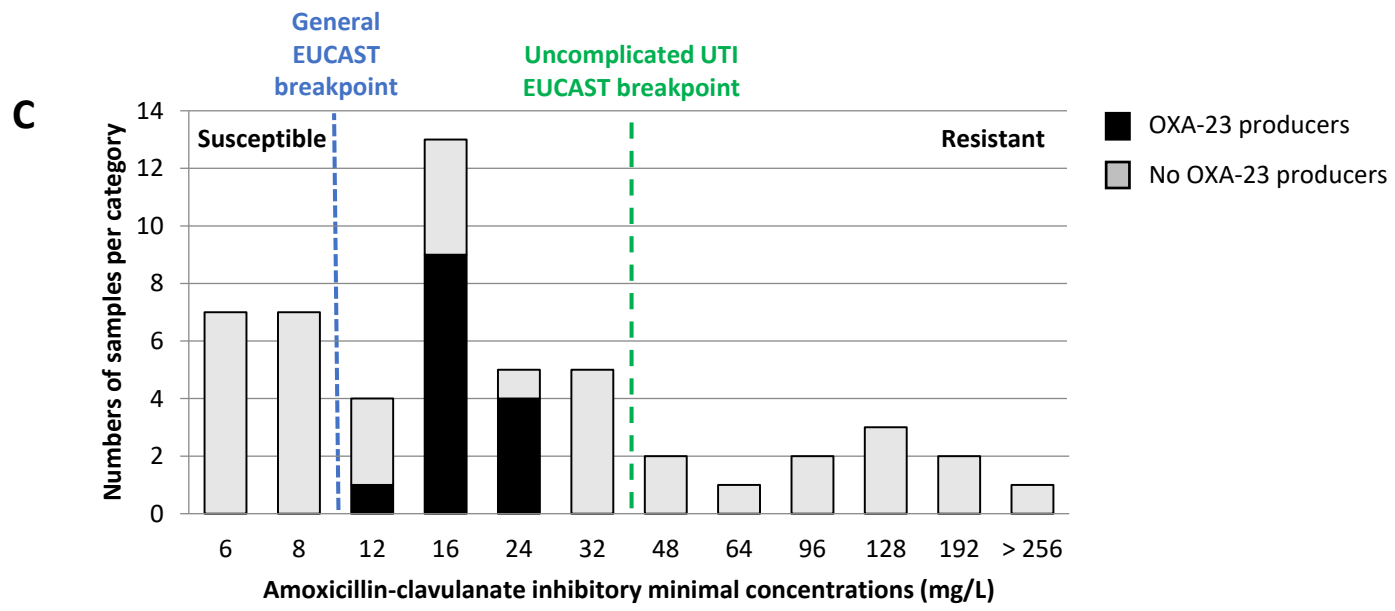
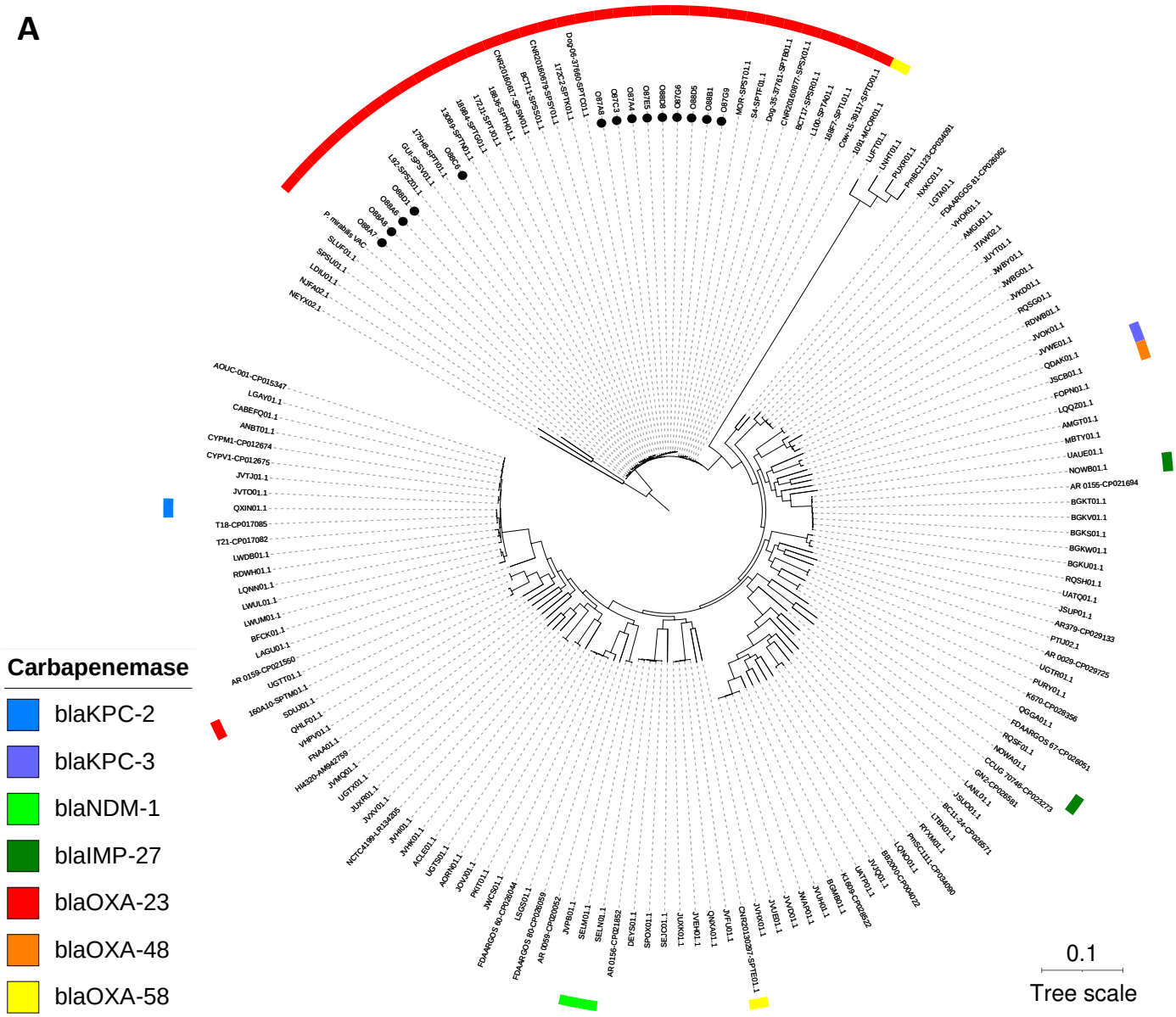
Blood culture
Muco-cutaneous
Catheter
Respiratory
Genital
Abscess / drainage


B
OXA-23
screening cut-off
Uncomplicated
UTI
EUCAST
breakpoint
General
EUCAST
breakpoint
Resistant
Number of samples
Amoxicillin-clavulanate inhibition-zone diameter (mm)
OXA-23 producers
No OXA-23 producers


C
General
EUCAST
breakpoint
Uncomplicated UTI
EUCAST breakpoint
Susceptible
Resistant
Numbers of samples per category
Amoxicillin-clavulanate inhibitory minimal concentrations (mg/L)
OXA-23 producers
No OXA-23 producers


A
Carbapenemase
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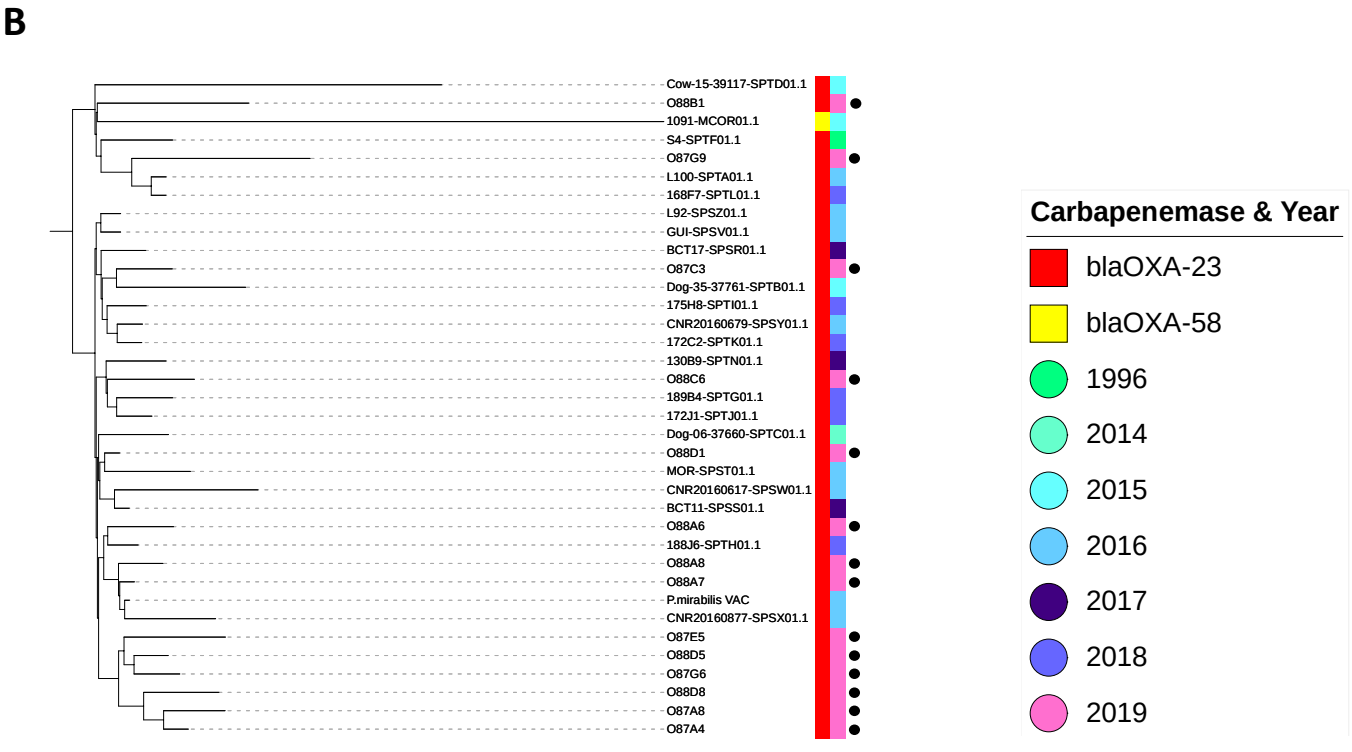
blaKPC-3
blaNDM-1
blaIMP-27
blaOXA-23
blaOXA-48
blaOXA-58
0.1
Tree scale


B
Cow-15-39117-SPTD01.1
O88B1
1091-MCOR01.1
S4-SPTF01.1
O87G9
L100-SPTA01.1
168F7-SPTL01.1
L92-SPSZ01.1
GUI-SPSV01.1
BCT17-SPSR01.1
O87C3
Dog-35-37761-SPTB01.1
175H8-SPTI01.1
CNR20160679-SPSY01.1
172C2-SPTK01.1
130B9-SPTN01.1
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189B4-SPTG01.1
172J1-SPTJ01.1
Dog-06-37660-SPTC01.1
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CNR20160617-SPSW01.1
BCT11-SPSS01.1
O88A6
188J6-SPTH01.1
O88A8
O88A7
P.mirabilis VAC
CNR20160877-SPSX01.1
O87E5
O88D5
O87G6
O88D8
O87A8
O87A4
Carbapenemase & Year
blaOXA-23
blaOXA-58
1996
2014
2015
2016
2017
2018
2019
0.1
Tree scale