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Hidden dissemination of carbapenem-susceptible OXA-48-producing *Proteus mirabilis*

Rosa Pedraza[1,2], Nicolas Kieffer[3]*, Julia Guzmán-Puche[1,2,4], María José Artacho[1,2], Cristina Pitart[5,6], Marta Hernández-García[7,8], Jordi Vila[4,5,6], Rafael Cantón[4,7,8] and Luis Martinez-Martinez[1,2,4,9]

[1]*Unit of Microbiology, Reina Sofía University Hospital, Córdoba, Spain;* [2]*Maimonides Institute of Biomedical Research of Cordoba (IMIBIC), Córdoba, Spain;* [3]*Molecular Basis of Adaptation, Department of Animal Health and VISAVET, University Complutense of Madrid, Madrid, Spain;* [4]*Spanish Network for Research in Infectious Diseases (REIPI) and CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain;* [5]*Hospital Clinic, University of Barcelona, Barcelona, Spain;* [6]*Institute of Global Health of Barcelona, Barcelona, Spain;* [7]*Ramon y Cajal University Hospital, Madrid, Spain;* [8]*Ramón y Cajal Institute for Health Research, Madrid, Spain;* [9]*Department of Agricultural Chemistry, Soil Science and Microbiology, University of Córdoba, Córdoba, Spain*

*Corresponding author. E-mail: nkieffer@ucm.es

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Objectives: To detect a potential hidden dissemination of the bla_{OXA-48} gene among *Proteus mirabilis* isolates obtained from a single centre.

Methods: *P. mirabilis* from diverse clinical samples presenting an ESBL phenotype or obtained from blood cultured from 2017 to 2019 were evaluated. Bacterial identification was performed using MALDI-TOF MS. MICs were determined using International Organization for Standardization (ISO) standard microdilution and interpreted following EUCAST guidelines. WGS was performed using both short- and long-read technologies and assemblies were done using Unicycler. Resistomes were assessed using the ResFinder database. SNPs were detected using the PATRIC bioinformatics platform. Cloning experiments were performed using the pCRII-TOPO cloning kit.

Results: Thirty-one out of 108 (28.7%) isolates were positive for bla_{OXA-48} and $bla_{CTX-M-15}$. Twenty-nine out of 31 of the isolates were susceptible to temocillin, piperacillin/tazobactam, ertapenem and meropenem, whereas only 2/31 showed a resistance phenotype against these antibiotics. Both bla_{OXA-48} and $bla_{CTX-M-15}$ genes were detected within the same chromosomally integrated new transposon in all isolates. The resistant isolates displayed a single mutation located in the putative promoter upstream of bla_{OXA-48}. Cloning experiments confirmed that the mutation was responsible for the resistance phenotype.

Conclusions: The presence of a chromosomal copy of bla_{OXA-48} did not confer resistance to carbapenems, but a single mutation in the promoter could lead to an increase in resistance. This study shows a hidden circulation of OXA-48-positive, but carbapenem- and piperacillin/tazobactam-susceptible, *P. mirabilis* isolates that can become resistant to β-lactams after a single mutation.

Introduction

The rapid emergence and dissemination of antibiotic resistance represents a major concern in clinical healthcare. The circulation of carbapenemase genes among common pathogens, such as Enterobacterales, *Pseudomonas* spp. and *Acinetobacter* spp., jeopardizes the use of broad-spectrum β-lactams, such as carbapenems, and hence drastically reduces the therapeutic solutions available against common infections caused by these microorganisms. Monitoring the presence of such resistance determinants is more than ever crucial to mitigate their spread in clinical settings.[1]

Proteus mirabilis is a Gram-negative bacterium belonging to the family Morganellaceae (order Enterobacterales).[2] Recently, antimicrobial resistance has been increasingly reported for this species and resistance to β-lactams had been associated with the acquisition of extended-spectrum β-lactamases ESBLs, plasmid-mediated cephalosporinases (pAmpC) and carbapenemase genes, including bla_{KPC-2}, bla_{VIM-1}, bla_{IMP-1}, bla_{NDM-1} and, more infrequently, bla_{OXA-48}.[3–6]

The class D OXA-48 carbapenemase was first described from a *Klebsiella pneumoniae* isolate in Turkey and has now spread worldwide to other Enterobacterales, including *P. mirabilis*.[7,8] The bla_{OXA-48} gene is commonly carried on a highly conjugative

IncL plasmid (ca. 62 kb).[9] It is flanked by two IS*1999* mobile elements, forming the functional composite transposon Tn*1999*. A variant of this transposon, Tn*1999.2*, is characterized by the insertion of a copy of an IS*1R* mobile element into the IS*1999* located upstream of the bla_{OXA-48} gene and provides a hypothetical hybrid promoter.[10] Three other forms of Tn*1999* have been described previously.[11,12] Interestingly, despite the high transferability of the IncL plasmid carrying bla_{OXA-48}, only a few studies reported this gene in *P. mirabilis* isolates.[6,13]

In May 2019, we detected an OXA-48-producing *P. mirabilis* clinical isolate presenting an ESBL phenotype, but susceptible, using EUCAST breakpoints, to imipenem, ertapenem and piperacillin/tazobactam, from a blood culture sample of a patient admitted to the University Hospital Reina Sofia, in Cordoba, Spain. Given the unusual susceptibility phenotype for a carbapenemase-producing microorganism, we retrospectively analysed all *P. mirabilis* isolates from our collection since 2017 to see if they showed the same resistance profile and we used high-throughput sequencing and bioinformatics analyses to characterize and understand this phenotype.

Materials and methods

Strain collection and identification

All identified *P. mirabilis* responsible for bloodstream infection or presenting an ESBL phenotype collected in our institution from May 2017 to July 2019 were included in the study. Only one strain per patient was kept in the final collection. Isolates were identified at the species level using MALDI-TOF MS (MALDI Biotyper A System; Bruker Daltonics, Madrid, Spain).

Carbapenemase detection

Detection of carbapenemase activity was assessed using the modified carbapenem inactivation method (mCIM).[14] Detection of OXA-48-type carbapenemase production was performed using the NG-Test CARBA 5 immunochromatography assay (Biotech, Madrid, Spain).[15]

Susceptibility testing

Antimicrobial susceptibility testing was performed for all isolates using commercial microdilution panels (Sensititre™ DKMGN) (Thermo Fisher Scientific, MA, USA), including amikacin, tobramycin, gentamicin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime, ceftazidime/avibactam, ceftolozane/tazobactam, aztreonam, colistin, trimethoprim and ciprofloxacin. MICs of meropenem, imipenem, ertapenem and temocillin were determined following the International Organization for Standardization (ISO) standard for broth microdilution.[16] For each method, susceptibility results were interpreted according to the EUCAST guidelines (http://www.eucast.org).[17]

Phenotypic detection of ESBLs and AmpC-type β-lactamase production was performed using discs (Oxoid, Thermo Fisher Scientific, Madrid, Spain) of cefotaxime (30 μg), ceftazidime (30 μg) and cefepime (30 μg) alone or combined with clavulanic acid (10 μg) on both Mueller–Hinton (MH) agar and MH agar supplemented with 200 mg/L cloxacillin.

Cloning experiments

bla_{OXA-48} with the $P_{OUT-bis}$ region was amplified by PCR using the high-fidelity polymerase Phusion (Thermo Fisher, Madrid, Spain) and cloned using the pCRII-TOPO cloning kit into *Escherichia coli* TOP10, and clones were selected on LB agar plates supplemented with kanamycin (25 mg/L) and ampicillin (50 mg/L).

DNA extraction, PCR and WGS

Total DNA for Illumina sequencing and conventional PCR was extracted from colonies using the automatic MagCore® HF16 Plus system with the MagCore® Genomic DNA Bacterial Kit 502 (RBC 94 Bioscience, Taiwan). Detection of the bla_{OXA-48} gene was performed for all isolates by conventional PCR using specific primers (F, TGCGTGTATTAGCCTTATCG; R, TTTTTCCTGTTTGAGCACTTC), followed by Sanger sequencing. WGS was performed for all OXA-48-producing isolates. DNA paired-end libraries were generated using the Nextera XT DNA sample preparation kit (Illumina Inc., San Diego, CA, USA). The libraries were sequenced using the Illumina NextSeq 500 sequencer system with 2×150 bp paired-end reads (Illumina Inc.). *P. mirabilis* isolates HURS-186083 and HURS-181823 were additionally sequenced using Nanopore® long-read technology using a MinION flow cell in accordance with SQK-RBK004 sequencing procedures.

Bioinformatics analyses

Assemblies, annotations and SNP analyses were performed on the PATRIC platform (v3.6.12) (https://www.patricbrc.org/), using Spades, Prokka and the variation analysis service, respectively, using the default parameters.[18] Assembly of hybrid-sequencing outputs was performed using Unicycler (v0.4.8) via the galaxy platform.[19] Genome alignments were performed using progressiveMAUVE (v2.4.0) software.[20]

The acquired antimicrobial resistance genes were identified using ResFinder server v3.1 (https://cge.cbs.dtu.dk/services/ResFinder/) using a threshold of identity and coverage of 80 and 60%, respectively.[21] The 405 available reference genomes of *P. mirabilis* from GenBank (March 2022) were used for phylogenetic analyses. Phylogenetic analysis was performed using CSIPhylogeny v1.4.[22] The parameters used were as follows: minimum distance between SNPs at 10 bp, minimum Z-score at 1.96 and minimum depth at 10× with a relative depth at 10% per position. Phage detection was done using PHASTER online software using default parameters.[23]

Ethical approval

The Reina Sofia University Hospital Ethics Committee (code TFM-OXA48-2021) approved this study.

Accession number(s)

Sequence data have been deposited in NCBI under BioProject number PRJNA825519. Genome accession numbers of the downloaded *P. mirabilis* genomes are listed in Table S1 (available as Supplementary data at *JAC* Online).

Results

Bacterial collection and susceptibility testing

A total of 108 *P. mirabilis* isolates were recovered, of which 95/108 were responsible for infections and 13/108 were recovered from rectal swabbing. The NG-Test CARBA 5 immunochromatographic test detected the OXA-48 carbapenemase in 31/108 isolates (28.7%), which showed a positive result using the mCIM phenotypic carbapenemase detection method. PCR followed by Sanger sequencing confirmed the presence of the bla_{OXA-48} gene. Among the OXA-48-producing *P. mirabilis* isolates, 12/31 were recovered from urinary tract infections, 10/31 were recovered from exudates, 4/31 were recovered from bloodstream infections, 4/31 were recovered from rectal swabbing and a single isolate was recovered from a gluteal abscess sample. Samples were from patients admitted to emergency (n=5),

Table 1. Summary of the bacterial collection of *P. mirabilis*

				MIC (mg/L) (broth microdilution)			
Strain	Ward	Sampling site	Resistance determinants	ertapenem	meropenem	imipenem	temocillin
HURS-186083	IM	rectal swab	$bla_{CTX-M-15}$, bla_{OXA-48}	2	2	16	64
HURS-186818	outpatient	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	1	1	32	32
HURS-192886	ER	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.06	4	4
HURS-181823	IM	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	8	4
HURS-192553	outpatient	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.06	4	2
HURS-193248	ER	blood	$bla_{CTX-M-15}$, bla_{OXA-48}	0.06	0.125	2	4
HURS-191820	IM	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.06	0.25	8	8
HURS-182686	IM	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.125	0.125	2	4
HURS-170802	IM	blood	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.125	2	2
HURS-172921	ER	blood	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.125	2	2
HURS-193370	IM	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.125	0.25	4	4
HURS-175254	outpatient	exudate	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.06	4	4
HURS-193108	IM	ulcer	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	4	4
HURS-173593	IM	exudate	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.125	4	4
HURS-193099	ER	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	4	4
HURS-184881	ER	ulcer	$bla_{CTX-M-15}$, bla_{OXA-48}	0.06	0.06	8	4
HURS-173346	IM	ulcer	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.25	8	4
HURS-184042	outpatient	exudate	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	8	4
HURS-187111	IM	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.25	8	8
HURS-184299	outpatient	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	2	2
HURS-173816	outpatient	abscess	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	4	4
HURS-193353	outpatient	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.125	0.25	8	4
HURS-182260	IM	rectal swab	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.125	8	4
HURS-180335	IM	exudate	$bla_{CTX-M-15}$, bla_{OXA-48}	0.06	0.25	8	4
HURS-174247	neurology	rectal swab	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.5	8	4
HURS-187616	IM	exudate	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	8	4
HURS-193172	gastroenterology	wound	$bla_{CTX-M-15}$, bla_{OXA-48}	0.06	0.25	8	4
HURS-187387	IM	wound	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	8	8
HURS-192801	urology	blood	$bla_{CTX-M-15}$, bla_{OXA-48}	0.125	0.25	4	8
HURS-185356	outpatient	urine	$bla_{CTX-M-15}$, bla_{OXA-48}	0.03	0.25	8	4
HURS-190834	IM	rectal swab	$bla_{CTX-M-15}$, bla_{OXA-48}	0.25	0.25	8	4

ER, emergency room; IM, internal medicine.

internal medicine (n = 13), digestive (*n* = 1), urology (n = 1), neurology (*n* = 1) and nephrology (*n* = 1) wards and from outpatients from nine different healthcare facilities. The median age of the patients was 84 years.

Antimicrobial susceptibility testing results are shown in Table 1. All OXA-48-producing *P. mirabilis* isolates exhibited an ESBL phenotype, showing resistance to cefotaxime (MIC range from 4 to >8 mg/L) and amoxicillin/clavulanic acid (MIC range from 32/2 to >64/2 mg/L). The presence of an ESBL was additionally confirmed by observing an increase of more than 5 mm in the diameter of the inhibition zone of cefotaxime discs combined with clavulanic acid compared with the diameter of cefotaxime discs without an inhibitor. No AmpC activity was detected when phenotypically tested.

Notably, despite the presence of bla_{OXA-48}, 29/31 *P. mirabilis* isolates were susceptible to ertapenem, meropenem and piperacillin/tazobactam. Those isolates were even susceptible to temocillin, even though OXA-48-producers are known to be highly resistant to this antibiotic.[24] Only two isolates (HURS-186083 and HURS-186818) were resistant to piperacillin/tazobactam, temocillin and ertapenem (Table 1). Both resistant isolates were recovered from rectal swabbing and urine infection, respectively and both patients came from the same geriatric facility (Table 1).

All isolates were susceptible to ceftazidime, ceftazidime/avibactam, ceftolozane/tazobactam and meropenem, and resistant to ciprofloxacin. Almost all isolates (96.7%) were susceptible to amikacin and aztreonam and 93.5% of the isolates were susceptible to gentamicin and trimethoprim/sulfamethoxazole.

WGS and bioinformatics analyses

Hybrid assemblies were obtained from the sequencing of the temocillin-susceptible and temocillin-resistant HURS-181823 and HURS-186083 isolates, respectively. A comparison of both assembled genomes showed the two isolates showed high nucleotide identity, solely differing by 13 SNPs (Figure S1). The remaining OXA-48-producing *P. mirabilis* isolates solely sequenced

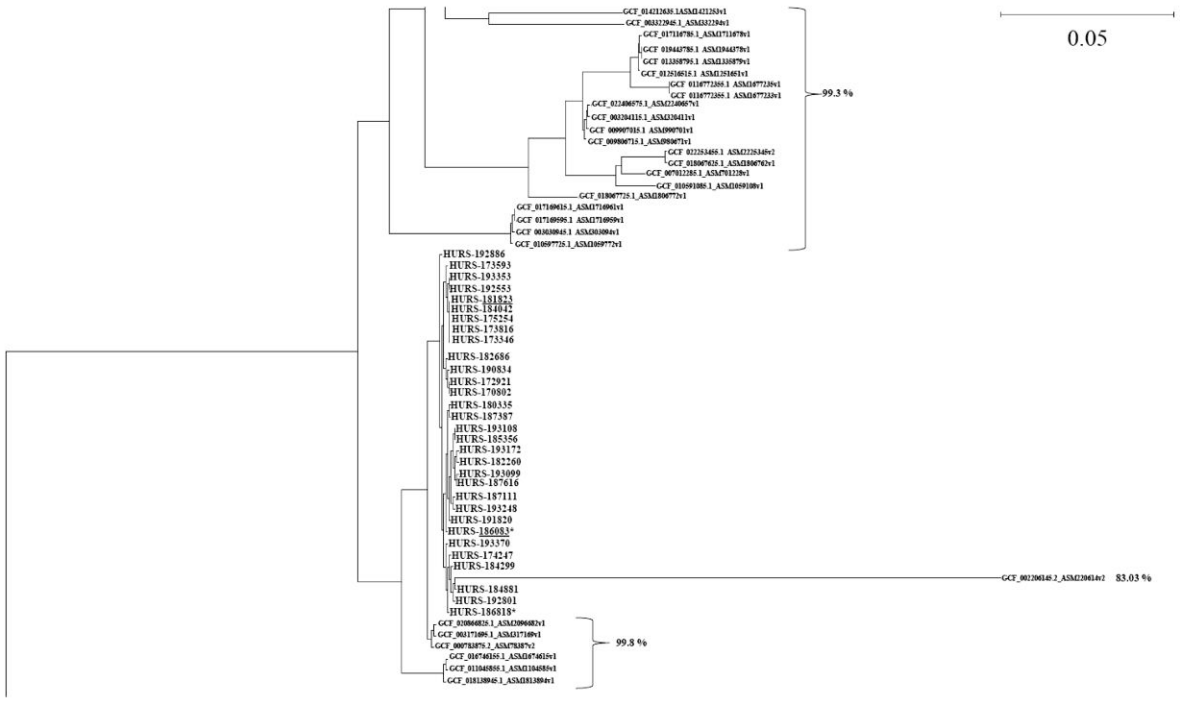

Figure 1. Phylogenetic tree; obtained using CSIPhylogeny software comparing all the genomes from this study with the 405 reference genomes of *P. mirabilis* publicly available. The tree was generated using the distance method algorithm. Branch lengths are drawn to scale and are proportional to the number of evolutionary events. The distance along the vertical axis has no significance. The figure represents an enlarged view of the complete tree for clarity reasons. The percentage indicates the gANI shared with HURS-181823 used as reference. The two isolates sequenced using both short- and long-read technologies are underlined; isolates marked by an asterisk possess the mutated version of the promoter $P_{OUT\text{-}bis}$.

by Illumina technology were compared with HURS-181823. All 29 isolates showed relatively close relatedness with HURS-181823 differing from 6 to 173 SNPs (Table S2). CSIPhylogeny results confirmed the close relatedness between the isolates of the bacterial collection, showing short evolutionary distances between them. One isolate outside of the study (accession: ASM220614v2) was homologous to the genomes from our collection but was evolutionarily more distinct in terms of whole-genome-based average nucleotide identity (gANI). That would explain why this genome was grouped in the same clade as the genomes from our collection while presenting a longer evolutionary branch in Figure 1.

Outputs from ResFinder online software showed that additionally to the $bla_{OXA\text{-}48}$ gene, all the isolates also carried the $bla_{CTX\text{-}M\text{-}15}$ gene, responsible for the observed ESBL phenotype (Table 1). Analysis of the position of $bla_{OXA\text{-}48}$ showed that the gene was chromosomally located in all isolates and 29/31 showed the same genetic context. The gene was identified in a new 9166 bp genetic structure, forming a transposon that included both $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}48}$ genes (Figure 2). The latter was embedded in a truncated version of Tn*1999.2*, whereas the $bla_{CTX\text{-}M\text{-}15}$ gene was associated with a full copy of the IS*Ecp1* mobile element in its upstream region. The presence of target site duplication (TSD) 5′-TATTT-3′ direct repeats at the extremity of the transposon indicated a mobilization event mediated by IS*Ecp1*, resulting from a one-ended transposition mechanism, as observed previously.[25] Analysis of the new transposon structure showed that IS*Ecp1* could have been involved in a previous mobilization event, resulting in the truncation of the plasmid-mediated *ltrA* gene normally present in the vicinity of Tn*1999.2* by recognizing a second inverted repeat right (IRR) called IRR^b (Figure 2a). This first transposition event may have led to the formation of an intermediate genetic structure (Figure 2b) where the orientation of IS*Ecp1* was auspicious to recognize a third putative IRR^c and performed a second one-ended transposition that led to the mobilization of the whole 9166 nt structure into the chromosome of *P. mirabilis*, precisely into the *trmB* gene at the 5′-TATTT-3′ insertion site (Figure 2c). The *trmB* gene encodes a transcriptional regulator that is present in a prophage genomic island, which was identified using PHASTER software as an intact 61.8 kb phage named ϕ_Burkho_BcepB1A_NC_005886 and identified for the first time in *Achromobacter ruhlandii*.[26] All isolates showed the same integration site for the transposon except for two isolates, HURS-173346 and HURS-184881, that differed only in their 5′ region. Indeed, even though the TSD was identified in the 3′ region, both isolates showed that their 5′ region was in the same prophage, but 9908 bp upstream, closer to the phage integrase gene.

Susceptibility testing showed that all isolates (except isolates HURS-186083 and HURS-186818) presented an unusual susceptibility profile against piperacillin/tazobactam, carbapenems and temocillin, despite the presence of the $bla_{OXA\text{-}48}$ gene. SNP analysis detected a single mutation in both strains located in the putative hybrid promoter located in the 5′ region of the IS*1R* that we named $P_{OUT\text{-}bis}$ and more precisely in the −35 box giving the following sequence: 5′-TTG **A**CA-3′ instead of 5′-TTG **G**CA-3′ in the susceptible isolates. To investigate if this mutation was

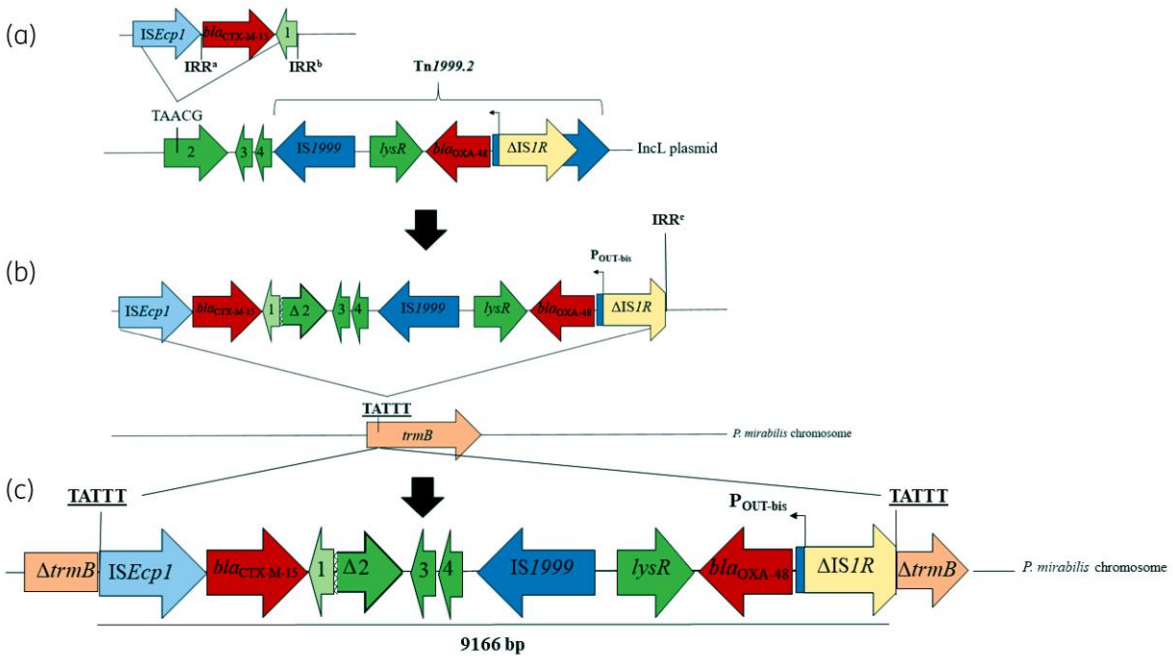

Figure 2. Schematic representation of the putative chronology of the formation of the new transposon carrying both $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}48}$. (a) Putative first mobilization of the IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$ transposon via the recognition of the putative second IRR^b into the *ltrA* gene located near the Tn*1999.2*. (b) Second IS*Ecp1*-mediated transposition by the recognition of the putative IRR^c located in the IS*1R* of Tn*1999.2* leading to the new transposon carrying both $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}48}$ in the chromosome as presented in (c). Direct repeats are indicated in bold and are underlined. 1, tryptophan synthase gene; 2, truncated *ltrA* gene; 3, *pemK*; 4, *pemI*. The 5′-TATTT-3′ sequence of the *trmB* gene is located at positions 100–104 of the gene. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 2. MICs (mg/L) determined by broth microdilution for the clinical isolates and the clones carrying the different $bla_{OXA\text{-}48}$ and 5′ regions

Antibiotic	*P. mirabilis* HURS-181823	*P. mirabilis* HURS-186083	*E. coli* TOP10 p-181823	*E. coli* TOP10 p186083	*E. coli* TOP10-pTOPO
Amoxicillin	>64	>64	1	64	1
Amoxicillin/clavulanic acid	>64	>64	2	>64	2
Cefotaxime	>64	>64	≤0.06	0.25	≤0.06
Ceftazidime	1	2	≤0.06	≤0.06	≤0.06
Cefepime	64	64	≤0.06	≤0.06	≤0.06
Cefoxitin	2	4	2	2	0.5
Piperacillin/tazobactam	0.5	>64	0.5	>64	0.5
Aztreonam	1	1	≤0.06	≤0.06	≤0.06
Temocillin	4	64	4	>128	4
Ertapenem	0.25	2	0.03	2	0.03
Imipenem	8	16	0.25	1	0.125
Meropenem	0.25	2	≤0.06	2	≤0.06

responsible for the observed resistance phenotype, two recombinant clones, p-181823 and p-186083, carrying $bla_{OXA\text{-}48}$ with its promoter region without or with the mutation were constructed, respectively. Antimicrobial susceptibility testing showed that p-186083 carrying the G→A mutation in the −35 box of $P_{OUT\text{-}bis}$ had increased MICs of β-lactams, including carbapenems, compared with p-181823 carrying the putative WT $P_{OUT\text{-}bis}$ (Table 2).

Discussion

The quick and precise identification of outbreaks in hospital facilities is a crucial point to control the dissemination of MDR bacteria. In this study, we highlighted, with both phenotypic diagnostic and bioinformatics tools, the identification of a silent outbreak of OXA-48-producing *P. mirabilis* isolates since at least 2017. Phylogenetic analyses using WGS showed that all isolates

differed from 6 to 173 SNPs. To our knowledge, no threshold has been defined for *P. mirabilis* to determine if two isolates belong to the same clone, but, according to our results, we can assess that all isolates in our study are closely related and belong to the same clonal cluster. Moreover, no other isolate from the public databases showed the same level of similarity as our collection.

All the isolates produce both OXA-48 and CTX-M-15 β-lactamases. However, only two isolates showed a typical OXA-48 resistance phenotype. This was due to the unique genetic context surrounding the resistance genes. Both $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-48}}$ genes were indeed chromosomally located in a new transposon whose mobilization probably resulted from the action of the IS*Ecp1* mobile element that mobilized $bla_{\text{CTX-M-15}}$ in the $bla_{\text{OXA-48}}$ surrounding before mobilizing the latter into the *trmB* gene of *P. mirabilis* (Figure 2). The last mobilization event resulted in the truncation of the IS*1999* copy upstream of the $bla_{\text{OXA-48}}$ gene, removing the promoter located in the latter that could explain the lack of resistance observed in most of the isolates. Poirel *et al.*[10] described a hybrid promoter created by the insertion of IS*1R* that truncates the IS*1999* element, suggesting that it would lead to a higher expression of the $bla_{\text{OXA-48}}$ gene, conferring a higher level of resistance. However, our results showed that the presence of $P_{\text{OUT-bis}}$ alone did not confer a resistance phenotype when associated with a chromosomal single copy of $bla_{\text{OXA-48}}$. We believe that the promoter created by the insertion of IS*1R* worked in synergy with the promoter located in IS*1999* and the absence of the latter led to an inefficient expression of the $bla_{\text{OXA-48}}$ gene. However, our cloning experiments showed that the single mutation in $P_{\text{OUT-bis}}$ in isolates HURS-186083 and HURS-186818 was able to restore the resistance phenotype, probably due to an increased expression level of the $bla_{\text{OXA-48}}$ gene. Altogether, we highlighted that a single copy of $bla_{\text{OXA-48}}$ without a complete copy of IS*1999* in its upstream region is not able to confer a clinical level of resistance to β-lactams, but a single mutation in $P_{\text{OUT-bis}}$ can restore the resistance phenotype.

Notably, every rapid diagnostic tool detected the production of OXA-48, including mCIM and immunochromatographic tests. This showed that, even if a β-lactamase is weakly expressed, those tools could detect it, confirming their necessity in the monitoring of the emergence of carbapenemase-producing bacteria.

The presence of the unmutated form of the transposon carrying $bla_{\text{OXA-48}}$ in the major part of the collection was intriguing. Indeed, with the use of antibiotics, we could predict that the mutated promoter would be more prevalent. However, only two isolates out of the collection recovered since 2017 showed a resistance phenotype. Our hypothesis, based on our difficulties to clone the mutated promoter in *E. coli* (data not shown), is based on its potential high fitness cost, causing a disadvantage in the competitive growth with other pathogens. More surveillance is needed in the future to monitor a possible emergence of *P. mirabilis* isolates carrying the mutated promoter.

Interestingly, we showed that the insertion of the transposon carrying $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-48}}$ was located in the *trmB* gene, itself located in a prophage genomic island. This location seemed to be a hotspot for the insertion of acquired genes, since chromosomal integration of resistance genes, including genes encoding other carbapenemases, such as $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-23}}$, has also been identified in prophage regions.[27–30] We could speculate that, since a prophage would represent a genomic platform that only possesses accessory and/or non-essential genes, it represents a potential hotspot for the mobilization of mobile elements without affecting the growth/metabolism of the bacterium.[31,32]

Finally, we hypothesized that the new transposon carrying both $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-48}}$ was mobilized via IS*Ecp1*. Additional experiments would be necessary to assess if this structure is still mobilizable, underlying the potential threat of a specific *P. mirabilis* clone as a reservoir for both ESBL and carbapenemase genes for future horizontal transfers.

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Transparency declarations

J.V. has been a consultant for MSD and Shionogi, has served as a speaker for MSD and Gilead, and has received research support from Shionogi. R.C. has served as a speaker for MSD, Pfizer and Shionogi, and has received research support from MSD, Shionogi and Venatorx. L.M.-M. has been a consultant for MSD and Shionogi, has served as a speaker for MSD, Pfizer and Shionogi, and has received research support from Janssen, MSD and Pfizer. All other authors: none to declare.

Supplementary data

Tables S1 and S2 and Figure S1 are available as Supplementary data at *JAC* Online.

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