Genetic and biochemical characterization of OXA-405, an OXA-48 type extended-spectrum β-lactamase without significant carbapenemase activity

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ABSTRACT

Epidemiology of carbapenemases worldwide is showing that OXA-48 variants are becoming the predominant carbapenemase type in *Enterobacteriaceae* in many countries. However, all OXA-48 variants do not possess significant activity towards carbapenems (*e.g.* OXA-163). Two *S. marcescens* isolates with either resistance to carbapenems or to extended-spectrum cephalosporins were successively recovered from a same patient. Genomic comparison using pulse field gel electrophoresis and automated Rep-PCR typing identified a 97.8% similarity between both isolates. Both strains were resistant to penicillins and first generation cephalosporins. The first isolate was susceptible to expanded-spectrum cephalosporins and resistant to carbapenems and had a significant carbapenemase activity (positive Carba NP test) related to expression of OXA-48. The second isolate was resistant to expanded-spectrum cephalosporins and susceptible to carbapenems and did not express a significant imipenemase activity (negative for the Carba NP test) despite possessing a *bla*$_{OXA-48}$ type gene. Sequencing identified a novel OXA-48-type β-lactamase, OXA-405, with a four amino-acids deletion as compared to OXA-48. The *bla*$_{OXA-405}$ gene was located on a ca. 46-kb plasmid identical to the prototype IncL/M *bla*$_{OXA-48}$ carrying plasmid except for a ca. 16.4-kb deletion in the *tra* operon, leading to the suppression of self-conjugation properties. Biochemical analysis showed that OXA-405 has a clavulanic acid inhibited activity towards expanded-spectrum activity without significant imipenemase activity. This is the first identification of a successive switch of catalytic activity in OXA-48-like β-lactamases suggesting their plasticity. Therefore, this report suggests that the first-line screening of carbapenemase producers in *Enterobacteriaceae* may be based on biochemical detection of carbapenemase activity in clinical settings.

INTRODUCTION

Ambler class D β-lactamase (oxacillinases) are widely disseminated among clinical relevant Gram-negatives (1). They exhibit a high degree of diversity of hydrolysis activity ranging from narrow to broad-spectrum hydrolysis activity toward β-lactams (1). Among the class D β-lactamases, several enzymes hydrolyze carbapenems. Most carbapenem-hydrolyzing class D β-lactamases (CHDLs) are from *Acinetobacter* spp. (e.g. OXA-23, OXA-40, OXA-58, OXA-143...) (2, 3), whereas OXA-48-type enzymes are identified in *Enterobacteriaceae* only (4). The OXA-48 derived CHDLs have initially been identified in Turkey (5), first in *Klebsiella pneumoniae* and then in other enterobacterial species (4). The known OXA-48 variants are currently as follows: (i) OXA-162, identified from *K. pneumoniae* isolates in Turkey (6); (ii) OXA-163 identified from *K. pneumoniae* and *E. cloacae* isolates in Argentina (7, 8); (iii) OXA-181 identified in a *K. pneumoniae* isolate from India (9); (iv) OXA-204 identified from *K. pneumoniae* isolates from patients having a link with North Africa (10); (v) OXA-232, identified in France from a *K. pneumoniae* isolate recovered from patients who had been transferred from India or Mauritius (11); (vi) OXA-244 and OXA-245 from *K. pneumoniae* isolates collected in Spain (12); (vii) OXA-247, identified in a *K. pneumoniae* isolate recovered from Argentina (13); and (viii) OXA-370 reported in a *Enterobacter hormaechei* isolate from Brazil (14). These variants differ from OXA-48 by one to five amino acid substitutions or/and by a four amino acids deletions, which result in modified β-lactam hydolysis spectrum.

Epidemiology of carbapenemases worldwide is showing that OXA-48 variants are becoming the predominant carbapenemase type in *Enterobacteriaceae* in many countries such as in North Africa, the Middle East, Turkey, France and Germany.

The aim of this study was to characterize peculiar molecular mechanisms of resistance to β-lactams made of a switch of carbapenem resistance/expanded-spectrum cephalosporins

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susceptibility profile followed by a carbapenem susceptibility/expanded-spectrum cephalosporins resistance profile among two successive *Serratia marcescens* isolates from a same patient.

MATERIAL AND METHODS

Bacterial strains.

Identification of clinical isolates were performed by using API20E system (bioMérieux, La Balme-les-Grottes, France) and confirmed by MALDI-TOF mass spectrometry (MALDI Biotyper CA system, Bruker Daltonics, Billerica, USA). *Escherichia coli* TOP10 (Invitrogen, Saint-Aubin, France) was used for cloning experiments and azide-resistant *E. coli* J53 for conjugation assays.

Susceptibility testing.

Antimicrobial susceptibilities were determined by the disc diffusion technique on Mueller-Hinton agar (BioRad, Marnes-La-Coquette, France) and interpreted according to the EUCAST breakpoints as updated 2014 (http://www.eucast.org). Minimal inhibitory concentrations (MICs) were determined using the E-test technique (bioMérieux).

Detection of carbapenemase activity.

The carbapenemase activity was searched for using two techniques: the updated Carba NP test (15), and UV-spectrophotometry (16). The updated Carba NP test that detects imipenemase activity was performed after performing culture on Trypticase soy agar medium supplemented with $ZnSO_4$ as previously described (17). The UV-spectrophotometry technique used as been detailed elsewhere (16).

PCR, cloning experiments and DNA sequencing.

Whole-cell DNAs of the two *S. marcescens* isolates and of OXA- 48 and OXA-163-producing *K. pneumoniae* isolates (8), were extracted using QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France) and were then used as a template to amplify the $bla_{\text{OXA-48-like}}$ genes. The

PCR using following primers: preOXA-48A (5’-TATATTGCATTAAGCAAGGG-3’) and preOXA-48B (5’-CACACAAATACGCGCTAACC-3’), was able to amplify *bla*$_{OXA-48}$, *bla*$_{OXA-163}$ and *bla*$_{OXA-405}$ genes. The amplicons obtained were then cloned into the pCR®-Blunt II-TOPO® (Invitrogen) downstream the pLac promoter, in the same orientation. Recombinant plasmids pTOPO-OXA were electroporated into *E. coli* TOP10 strain. Plasmid DNA extraction was performed using Qiagen Miniprep Kit (Qiagen). Both strands of the inserts of the recombinant plasmids, were sequenced using T7 promotor and M13 Reverse primers with an automated sequencer (ABI PRISM 3100; Applied Biosystems). The nucleotide sequences were analyzed using software available at the National Center of Biotechnology Information website (http: //www. ncbi.nlm.nih.gov).

Plasmid characterization and mating-out assay.

Plasmid DNA of both clinical *S. marcescens* isolates and OXA-163-producing *K. pneumoniae* 6299 were extracted using the Kieser method (18). Plasmids of ca. 154, 66, 48 and 7 kb of *Escherichia coli* NCTC 5019 were used as plasmid size markers. Plasmid DNA was analysed by agarose gel electrophoresis. Transfer of the β-lactam resistance markers was attempted by liquid mating-out assays at 37°C using *E. coli* J53 as the recipient strain and by electroporation of the plasmid DNA suspension of clinical isolates into *E. coli* TOP10. Selection of transconjugants was performed on agar -supplemented plates with ticarcillin (100 mg/L) and with azide (100 mg/L). Plasmids were typed using PCR-based replicon typing (PBRT) scheme as described previously (19), and specific primers RepA-A (5’-GACATTGAGTCAGTAGAAGG-3’) and RepA-B(5’-CGTGCAGTTCGTCTTTCGGC-3’) designed for the detection of the IncL/M OXA-48 plasmid replicase (20).

The *bla*$_{OXA-405}$ carrying plasmid was characterized by PCR mapping followed by DNA sequencing. Fourteen couples of primers were used for the mapping of the 61,881 bp IncL/M plasmid carrying *bla*$_{OXA-48}$ gene (Table 1). The *bla*$_{OXA-48}$ carrying plasmid sequence

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(GenBank accession number JN626286) was used as a positive control for PCR mapping (20).

Hydrolysis analysis.

The specific activities of the β-lactamases OXA-48, OXA-163 and OXA-405 were determined using the supernatant of a whole-cell crude extract obtained from an overnight culture of *E. coli* clones expressing those β-lactamases (pTOPO-OXA-48, pTOPO-OXA-163 and pTOPO-OXA-405 in *E. coli* TOP 10) with an UV spectrophotometer ULTROSPEC 2000 (Amersham Pharmacia Biotech), as previously described (10).

Nucleotide sequence accession number.

The nucleotide sequence of the $bla_{\text{OXA-405}}$ gene has been submitted to EMBL/GenBank nucleotide sequence database under accession number KM589641.

RESULTS

Patient features and characteristics of the *S. marcescens* clinical isolates

In January 2011, a 26 year old woman was admitted at the emergency unit of the University hospital of Besançon (East part of France) for acute pulmonary infection. After two days of hospitalization, blood cultures and a tracheal aspirate gave *S. marcescens* isolates with identical antibiotic susceptibility profile (Sm1). They were resistant to ticarcillin, ticarcillin/clavulanic acid, piperacillin/tazobactam, and temocillin (MIC > 256 mg/L), had decreased susceptibility to carbapenems (imipenem, meropenem, ertapenem, and doripenem), and remained susceptible to expanded-spectrum cephalosporins (Table 2). A positive Carba NP test indicated the expression of a carbapenemase, PCR experiments were carried out on purified DNA of Sm1 with primers specific of common carbapenemases genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and $bla_{\text{OXA-48-}}$). A $bla_{\text{OXA-48-like}}$ gene was amplified which was later identified as $bla_{\text{OXA-48}}$ according to sequencing results. The patient was successfully treated

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with cefepime and amikacin for fifteen days. Furthermore, due to the irradiation of the nasopharynx for a carcinoma at the age of 14, the patient presented important loco-regional sequellae composed of sclerosis of the thorax and cervical regions, and the persistence of a right laryngeal-cervical fistula. More than 18 months later (October 2012), another *S. marcescens* strain (Sm2) was isolated from a breast hematoma. This *S. marcescens* isolate was resistant to ticarcillin, ticarcillin/clavulanic acid, piperacillin/tazobactam, had a decreased susceptibility to ertapenem but remained susceptible to the other tested carbapenem molecules (imipenem, meropenem and doripenem). The Carba NP test did not reveal a carbapenemase activity. Unlike isolate Sm1, the isolate Sm2 was resistant to expanded-spectrum cephalosporins (cefotaxime, ceftazidime, cefepime) and aztreonam (Table 2), and recovered susceptibility to temocillin (MIC = 8 mg/L). PCR using whole-cell DNA of Sm2 as template was positive for a *bla*$_{OXA-48-like}$ gene. Sequencing results identified a novel *bla*$_{OXA-48}$ -like gene, designated as the *bla*$_{OXA-405}$ gene.

Genomic comparison using a Rep-PCR based technique (Diversilab®, bioMérieux) identified a 97.8% genomic similarity between *S. marcescens* Sm1 and Sm2 isolates (Figure 1A). Therefore, both strains were considered to be clonally related. This clonality has been confirmed by pulse field gel electrophoresis (Figure 1B).

Characterization of the β-lactamase OXA-405

This *bla*$_{OXA-405}$ gene differs from *bla*$_{OXA-48}$ gene by a 12-bp deletion leading to a four amino-acids deletion in the OXA-405 protein sequence from residues Thr213 to Glu216, as compared to the OXA-48 sequence (Figure 2). The comparison of hydrolysis spectrum of OXA-405, OXA-48 and OXA-163 was done by cloning *bla*$_{OXA-405}$, *bla*$_{OXA-48}$ and *bla*$_{OXA-163}$ genes in the pCR®-Blunt II-TOPO® (Invitrogen) and expressing into *E. coli* TOP10. OXA-405 and OXA-163 conferred a similar resistance profile made of a decreased susceptibility to expanded-spectrum cephalosporins and aztreonam as compared to that conferred by OXA-48

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(Table 2). As opposed to OXA-48, OXA-405 like OXA-163 once expressed in a reference *E. coli* strain was not associated with a decrease susceptibility to carbapenems (Table 2). Both the Carba NP test and UV spectrophotometry analysis showed that the OXA-405 and OXA-163 did not express a significant imipenemase activity (Table 3). In addition, OXA-405- and also OXA-163 producers were eight-fold more susceptible to temocillin than OXA-48-producers (Table 2).

The specific activities of OXA-405 and of OXA-163 were very similar for penicillins, broad-spectrum cephalosporins, and carbapenems. However, OXA-405 hydrolyzed less ceftazidime (8.5-fold less) than OXA-163 (Table 3). Both OXA-405 and OXA-163 have barely detectable activity against carbapenems as compared to OXA-48 (~ 25-fold less for imipenem) (Table 3). On the other hand, OXA-405 and OXA-163 hydrolyzed expanded-spectrum cephalosporins and aztreonam at much higher rates while OXA-48 did not (Table 3). This activity against expanded-spectrum cephalosporins of OXA-405 was inhibited by tazobactam addition (Table 2).

Genetic environment of the *bla*$_{\mathbf{OXA\text{-}405}}$ gene.

The *bla*$_{OXA\text{-}405}$ gene was located onto a Tn*1999* transposon as the *bla*$_{OXA\text{-}48}$ gene usually is (4, 20). Plasmid DNA of *S. marcescens* Sm1 (pOXA-48) and Sm2 (pOXA-405) were extracted and compared. A single plasmid was identified from each strain, of ca. 62-kb and ca. 46-kb for the Sm1 and Sm2, respectively. PCR-based replicon typing method revealed that these plasmids belonged to a same IncL/M incompatibility group. Whereas transformants in *E. coli* were obtained by using both plasmids, transconjugants were obtained with the pOXA-48 plasmid only. PCR mapping of plasmids pOXA-48 and pOXA-405 showed that pOXA-48 was structurally identical to the prototype IncL/M OXA-48 positive plasmid. Plasmid pOXA-405 had a similar backbone as pOXA-48 but had a 16,382 bp deletion from nucleotides 24,210 to 40,587 according to reference *bla*$_{OXA\text{-}48}$ plasmid (number JN626286, GenBank

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nucleotide database) (20). This deletion included the *ssb* gene, *mobC* and *mobA* genes, *nikB* and *nikA* genes, and a part of locus *Tra* (H, I, J, K, L and *primase* genes). This deleted DNA section was replaced by an insertion sequence IS*1R* (Figure 3B).

DISCUSSION

A novel OXA-48 type β-lactamase, OXA-405, has been identified here. OXA-405 like the other OXA-48 type β-lactamases OXA-163 and OXA-247 has a significant activity toward expanded-spectrum cephalosporins but barely none toward carbapenems. Therefore it shall be underlined that OXA-48-like β-lactamases as opposed to all known KPC, NDM, VIM or IMP β-lactamases are not all significant carbapenemases. In addition, it has been shown that OXA-48 -type producers with carbapenemase activity are mostly resistant to temocillin. Here, we confirm that this temocillin resistance trait would be a good criteria for differentiating OXA-48-type producers with and without carbapenemase activity.

Structural protein analysis of OXA-405, OXA-163 and OXA-247 showed that they possess at least a same four amino acids deletion in a specific region from Thr213 to Glu216 (8, 13). This result agrees with crystal structure analysis of OXA-48 showing that Arg 214 (which is part of a β5 strand) is critical for carbapenemase activity (21). In addition, recent studies point out the crucial of this short loop connecting β5 and β6 strands in conferring a carbapenemase activity of Ambler class D β-lactamases (22, 23).

Genetic analysis of the *S. marcescens* clinical isolates Sm1 and Sm2 producing OXA-48 and OXA-405, respectively, indicate that they are clonally related. This result suggests that the bla_{OXA405} gene may derive from a same ancestor, a bla_{OXA-48} gene. This hypothesis is reinforced by the common genetic environment of both those genes. Actually, the bla_{OXA-48} and $bla_{OXA-405}$ genes were bracketed by two copies of an identical IS element IS*1999*, forming a composite transposon Tn*1999*. This genetic environment was completely different to the

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mosaic structures made of insertion sequences and truncated mobile element that surrounds the *bla*$_{\text{OXA-163}}$ gene and its derivative *bla*$_{\text{OXA-247}}$ (Figure 3) (8, 13). In addition, the *bla*$_{\text{OXA-405}}$ gene was identified on the plasmid pOXA-405 that possessed a backbone similar to that of IncL/M *bla*$_{\text{OXA-48}}$-bearing plasmid (pOXA-48) (20), except for a deletion of ca. 16 kb replaced by an insertion sequence IS*1R*. This deletion/insertion lead to loss of conjugative genes and related self-conjugative property of pOXA-405 (20). The role of a cephalosporin-containing treatment (here cefepime) remains to be determined for selecting an OXA-48 type β-lactamase with activity against expended-spectrum cephalosporins from an OXA-48 type β-lactamase with carbapenemase activity.

As conclusion, this report underlines that OXA-48-type β-lactamases are more diverse than expected. As exemplified by OXA-405, the OXA-48-type β-lactamases are not all true carbapenemases. A same statement is valid for another group of serine β-lactamases, the GES group of enzymes for which GES-1 is an extended-spectrum β-lactamase while GES-2 is a carbapenemase (24). Therefore, the first-line screening of carbapenemase producers in *Enterobacteriaceae* may be best based on biochemical detection of carbapenemase activity in clinical settings. The molecular biology techniques, although useful, may overreport OXA-48-like producers as being all carbapenemases and, on the opposite, may fail to detect carbapenemase producers related to totally novel or slightly structurally modified carbapenemase genes.

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FIGURE LEGEND

Figure 1. A. Rep-PCR analysis by using the Diversilab technique. Dendrogram and computer-generated image of rep-PCR banding patterns of OXA-48-producing *S. marcescens* (Sm1), OXA-405-producing *S. marcescens* (Sm2) and an unrelated strain of *S. marcescens*. **B.** Pulse field gel electrophoresis of OXA-48-producing *S. marcescens* (Sm1), OXA-405-producing *S. marcescens* (Sm2) and an unrelated strain of *S. marcescens.*

Figure 2. Alignment of the amino acid sequences of OXA-48, OXA-405, OXA-163 and OXA-247. Possible conserved residues of the active site of the OXA-48 type β-lactamases are highlighted in gray.

Figure 3. A. Schematic representation of the genetic environment of the $bla_{\text{OXA-48}}$ (a), $bla_{\text{OXA-405}}$ (b), $bla_{\text{OXA-163}}$ (c) and $bla_{\text{OXA-247}}$ (d) genes. The Tn*1999* composite transposon is made of two copies of insertion sequence IS*1999* bracketing a fragment containing the $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-405}}$ genes. **B.** Major structural features of plasmid pOXA-405 from *S. marcescens* Sm2 in comparison with the prototype IncL/M $bla_{\text{OXA-48}}$ plasmid (pOXA-48) (GenBank accession number JN626286). Common structures are highlighted with a shaded grey color.

Table 1. Primers used for the mapping of the *bla*$_{OXA-48}$ type carrying plasmids

Primer name	Nucleotide sequence according to Genbank assession number JN626286			Location	Amplicon size (bp)
	Start	Stop	5'→3'		
C1F	57425	57444	ATCCGGTCCCCCTGATTATC	*IncL/M rep*	4531
C1R	55	74	GTCTGCGACTGACAGACGAT	*trbA*	
C2F	1208	1227	CGAAAGCCAAACCACATCAC	*trbA*	4469
OXA-48-3'ext	5655	5676	TATTGTCAAACAAGCCATGCTG	*bla*$_{OXA-48}$	
OXA-48-5'ext	6099	6119	ATTCCAGAGCACAACTACGCC	*bla*$_{OXA-48}$	3025
C3R	9104	9123	CCGTCGTTGTTGCTGAGAAC	*mucB*	
C4F	10248	10267	CGCAGTGGAAGGATATTCCC	*mucB*	4077
C4R	15005	15024	TTCAGGGCGCTGGATTCAAG	*orf12*	
C5F	15480	15499	GCGTGACCGCCTCAAATTCT	*orf12*	4207
C5R	19667	19686	CGAGCACTTACGGTTATCAG	*parB*	
C6F	20083	20102	CATCTGTTCCCGGATGATGA	*parB*	3892
C6R	23955	23974	TCTATGCCGCCCTGTATTCC	*orf25*	
C7F	25154	25173	CAGTGAAGGACTGAGCCACT	*orf25*	4240
C7F	29374	29393	GGCGGGTTGATTCAGTTCAG	*klcA*	
C8F	29786	29805	GATTTACCGCGCGATTGACT	*klcA*	3757
C8R	33523	33542	GACTTTTTGTCCCTTCGGCC	*mobA*	
C9F	35370	35389	GCAGGCGTATGCTCAAAACG	*mobA*	2913
C9R	38263	38282	ACGTTGGCGATCGTCAAAGG	*pri*	
C10F	41356	41375	CAGCCTCAGCATTTACAAGC	*pri*	4613
C10R	45949	45968	TCAGCAGGCTTAGCAGACAC	*traP*	
C11F	46577	46596	CAAGTAAAGGCCTTATCCGC	*traP*	4597
C11R	51154	51173	CTGACCGTTTTGCTTTTCCG	*traW*	
C12F	52321	52340	GAGTGTGAACGCGGGAGTAT	*traW*	4144
C12R	56445	56464	ATGAACTCCGGCGAAAGACC	*IncL/M rep*	

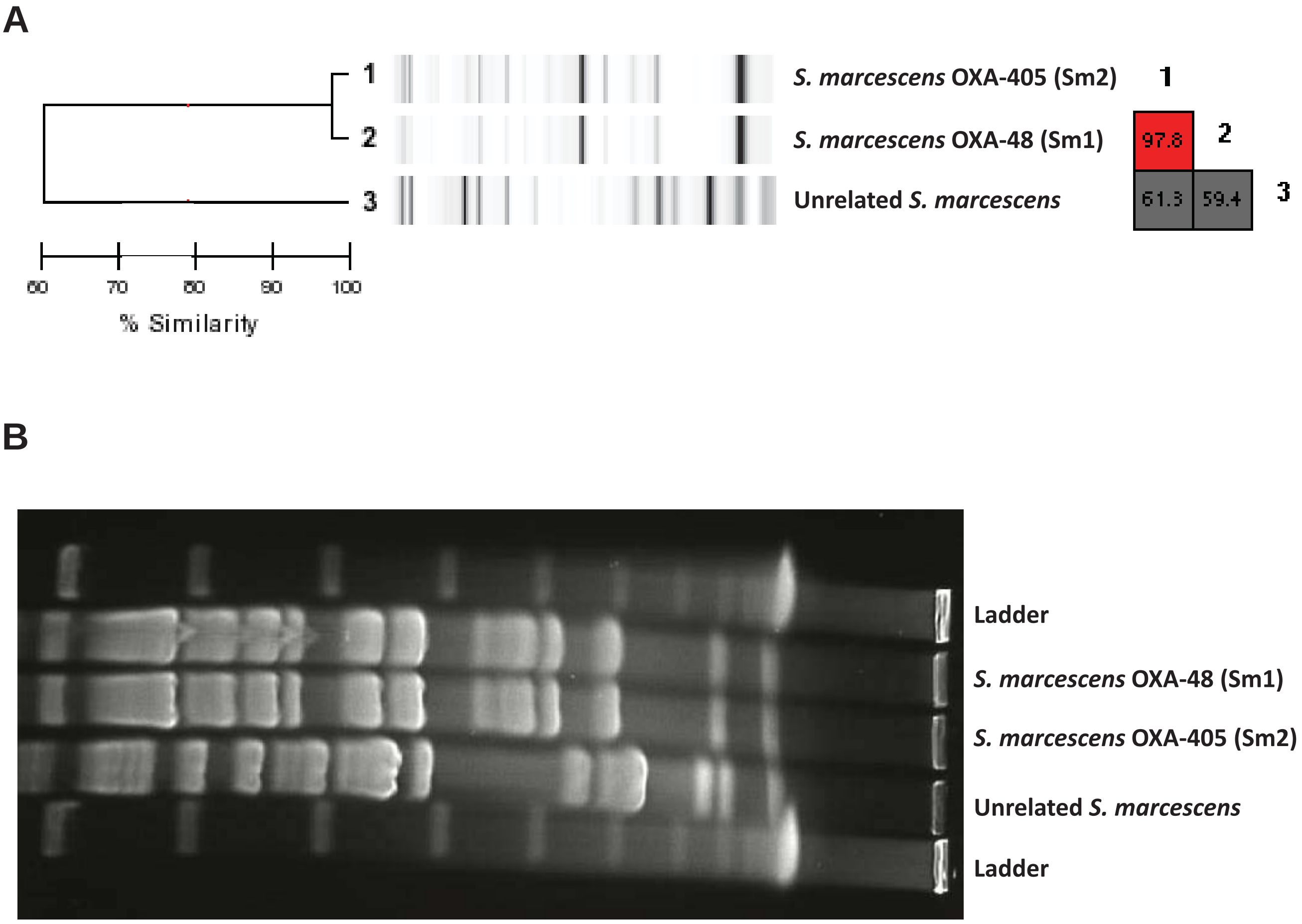
Table 2. MICs of β-lactams for *S. marcescens* OXA-48 (Sm1), *S. marcescens* OXA-405 (Sm2), *E. coli* pTOPO-OXA-48, *E. coli* pTOPO-OXA-40 *coli* pTOPO-OXA-163 and *E. coli* TOP10.

β-lactams	MIC (mg/L)					
	S. marcescens OXA-48 (Sm1)	*S. marcescens* OXA-405 (Sm2)	*E. coli* TOP10 (pTOPO-OXA-48)	*E. coli* TOP10 (pTOPO-OXA-405)	*E. coli* TOP10 (pTOPO-OXA-163)	*E. coli* TOP10
Amoxicillin	>256	>256	>256	>256	>256	2
Amoxicillin + CLA[a]	>256	>256	192	>256	96	2
Piperacillin	>256	>256	128	>256	>256	1,5
Piperacillin + TZB[b]	96	>256	12	24	32	1
Temocillin	>256	8	>256	32	32	4
Ticarcillin	>256	>256	>256	>256	>256	2
Cefalotin	>256	>256	8	32	64	2
Cefepime	0.25	3	0.032	0.5	0.5	0.023
Cefepime + TZB[b]	0.25	2	0.032	0.19	0.19	0.023
Cefotaxime	1.5	6	0.19	0.5	3	0.06
Cefotaxime + TZB[b]	1.5	4	0.19	0.19	1	0.06
Ceftazidime	0.25	4	0.25	3	16	0.12
Ceftazidime + TZB[b]	0.25	2	0.25	1	3	0.12
Imipenem	4	0.5	0.5	0.25	0.25	0.19
Meropenem	4	0.19	0.094	0.023	0.023	0.01
Ertapenem	>32	0.75	0.25	0.032	0.032	0.06
Doripenem	3	0.125	0.064	0.023	0.023	0.023
Aztreonam	0.125	4	0.064	1	2	0.047

[a]CLA, clavulanic acid at a fixed concentration of 4 mg/L; [b]TZB, tazobactam at a fixed concentration of 4 mg/L

Table 3. Specific activities of β-lactamases OXA-48, OXA-405 and OXA-163

	Specific Activity (mU/mg of protein)		
β-lactams	OXA-48	OXA-405	OXA-163
Amoxicillin	981 ± 62	485 ± 35	795 ± 81
Piperacillin	450 ± 5	436 ± 4	214 ± 2
Temocillin	11 ± 2	5 ± 0.5	5 ± 0.4
Ticarcillin	647 ± 59	63 ± 6	80 ± 7
Cefepime	5 ± 0.5	27 ± 2	30 ± 3
Cefotaxime	60 ± 6	117 ± 10	167 ± 15
Cefoxitin	2 ± 0.2	1 ± 0.1	1 ± 0.1
Ceftazidime	2 ± 0.2	9 ± 0.8	53 ± 5
Cephalotin	75 ± 8	140 ± 12	130 ± 10
Imipenem	57 ± 4	3 ± 0.2	2 ± 0.2
Meropenem	3 ± 0.1	2 ± 0.2	2 ± 0.1
Ertapenem	2 ± 0.2	1 ± 0.1	1 ± 0.1
Doripenem	2 ± 0.2	1 ± 0.1	1 ± 0.1
Aztreonam	5 ± 0.5	14 ± 1	18 ± 2


A
1
2
3
S. marcescens OXA-405 (Sm2)
S. marcescens OXA-48 (Sm1)
Unrelated S. marcescens
97.8
61.3
59.4
60
70
80
90
100
% Similarity
B
Ladder
S. marcescens OXA-48 (Sm1)
S. marcescens OXA-405 (Sm2)
Unrelated S. marcescens
Ladder

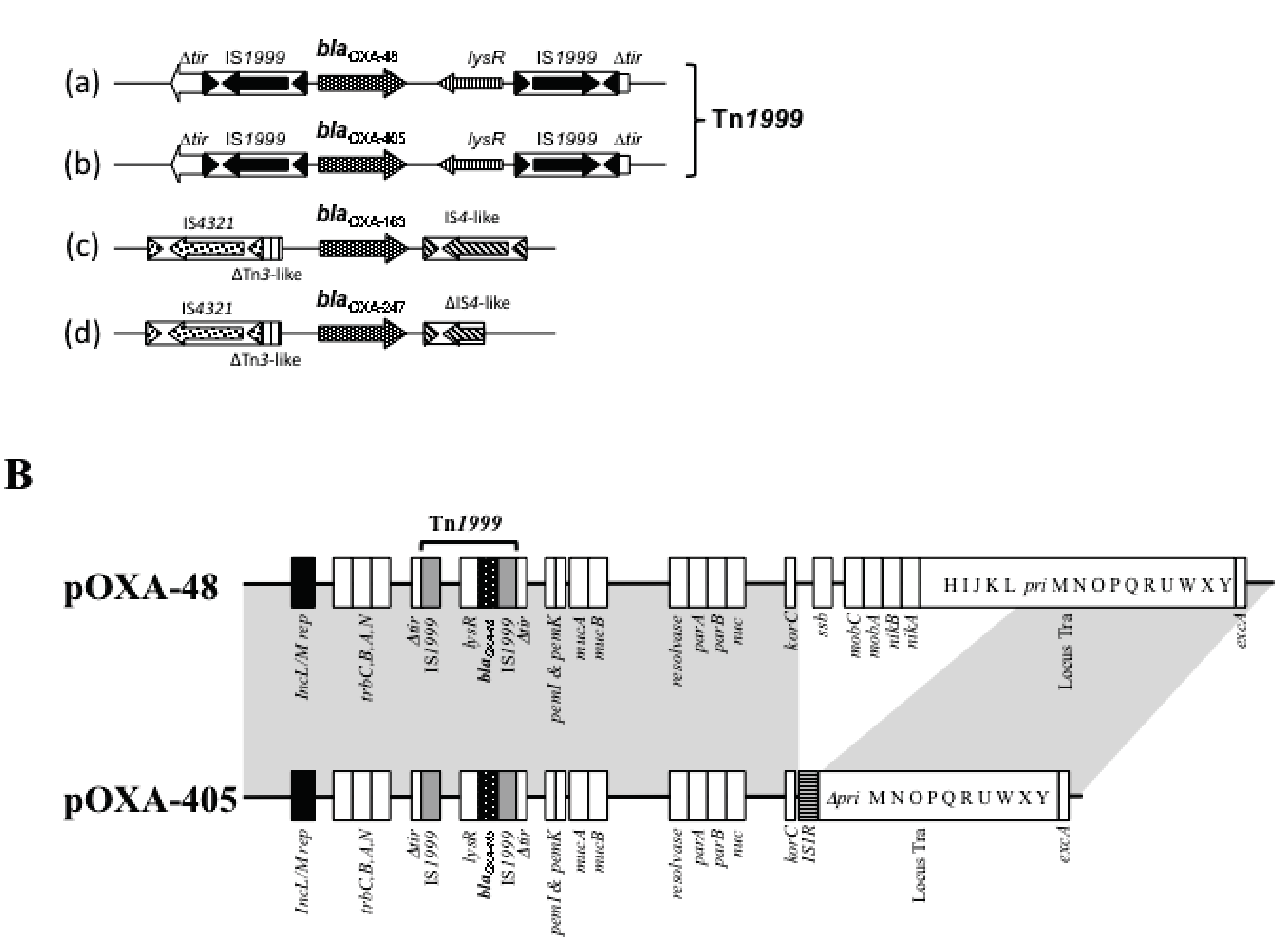
```
           1        10        20        30        40        50        60        70        80        90        100
           |        |         |         |         |         |         |         |         |         |         |
OXA-48     MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-405    MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-163    MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-247    MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR

                    110       120       130       140       150       160       170       180       190       200
                    |         |         |         |         |         |         |         |         |         |
OXA-48     DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-405    DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-163    DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-247    DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN

                    210       220       230       240       250       260
                    |         |         |         |         |         |
OXA-48     GDYIIRAKTGYSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-405    GDYIIRAKTGYS----PKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-163    GDYIIRAKTGYDT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-247    GDYIIRAKTGSNT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
```

Figure 3

A


(a)
Δtir
IS1999
bla OXA-48
lysR
IS1999
Δtir
Tn1999
(b)
bla OXA-405
(c)
IS4321
bla OXA-163
IS4-like
ΔTn3-like
(d)
IS4321
bla OXA-247
ΔIS4-like
ΔTn3-like
B
Tn1999
pOXA-48
HIJKL pri MNOPQRUWXY
IncL/M rep
trbC,B,A,N
Δtir
IS1999
lysR
bla OXA-48
IS1999
Δtir
pemI & pemK
mucA
mucB
resolvase
parA
parB
nuc
korC
ssb
mobC
mobA
nikB
nikA
Locus Tra
excA
pOXA-405
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IncL/M rep
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IS1999
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bla OXA-405
IS1999
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korC
IS1R
Locus Tra
excA