

## Intrinsic Carbapenem-Hydrolyzing Oxacillinases from Members of the Genus *Pandoraea*

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We analyzed the oxacillinases of isolates of six different species of *Pandoraea*, a genus that colonizes the respiratory tract of cystic fibrosis patients. The isolates produced carbapenem-hydrolyzing enzymes causing elevated MICs for amoxicillin, piperacillin, meropenem, and imipenem when expressed in an *Escherichia coli* host strain. Sequencing revealed nine new oxacillinases (OXA-151 to OXA-159) with a high degree of identity among isolates of the same species; however, they had much lower interspecies similarities. The intrinsic oxacillinase genes might therefore be helpful for correct identification of *Pandoraea* isolates.

xacillinases are serine  $\beta$ -lactamases of the molecular class D, which currently comprises more than 480 enzymes (ftp://ftp .ncbi.nlm.nih.gov/pathogen/betalactamases/Lahey.tab; last accessed in August 2015) showing a high degree of variability both in their amino acid sequences as well as in their activities against β-lactams. Their impact on antibiotic resistance has long been considered low; however, during the last 15 years, the prevalence of carbapenem-hydrolyzing variants has increased (1). Furthermore, the number of OXA-type enzymes, which have been found to be intrinsically produced by a broad range of bacterial species, is rising, and among them are several enzymes with carbapenem-hydrolyzing activity: e.g., OXA-51-like from Acinetobacter baumanii, OXA-228-like from Acinetobacter bereziniae, OXA-213-like from Acinetobacter calcoaceticus, OXA-214-like from Acinetobacter haemolyticus, OXA-211-like from Acinetobacter johnsonii, OXA-134 from Acinetobacter lwoffii, OXA-23-like from Acinetobacter radioresistens, OXA-54 from Shewanella oneidensis, and OXA-55 from Shewanella algae (2-6). Another intrinsic carbapenem-hydrolyzing oxacillinase is OXA-62 from *Pandoraea pnomenusa* (7).

*Pandoraea* spp. are Gram-negative, glucose-nonfermenting rods closely related to *Burkholderia* and *Ralstonia* (8). Isolates of the genus *Pandoraea* were shown to colonize the respiratory tract predominantly of cystic fibrosis patients (9–11), with the potential to cause severe deterioration of lung function or septicemia in some cases (12–16). Antimicrobial therapy of infections caused by *Pandoraea* spp. is impaired by their broad resistance to antibiotics (7, 9, 11). In this study, the oxacillinases of nine isolates of six *Pandoraea* species were analyzed.

The isolates H4-1-1, E126-13, Va8523, and HD7676 obtained from sputa of cystic fibrosis patients from Germany (7), were identified by phenotypic (API 20 NE [bioMérieux, Marcy l'Etoile, France] and additional biochemical tests) and genotypic methods (in-house PCR assay with species-specific oligonucleotides based on published 16S rRNA and *gyrB* gene sequences). *Pandoraea* isolates LMG 16407, LMG 18379, LMG 18087, LMG 18106, and LMG 18819 were obtained from Laboratorium voor Microbiologie (Universiteit Ghent, Ghent, Belgium). *Pandoraea sputorum* LMG 18100 (C4964) was provided by D. P. Speert, Vancouver, British Columbia, Canada. A previously obtained *Escherichia coli* transformant producing OXA-62 (7) was included for comparison.

MICs were determined by an agar dilution technique following CLSI guidelines (17). The  $\beta$ -lactamase inhibitors tazobactam and

BRL 42715, an inhibitor of active-site serine  $\beta$ -lactamases (18), were used at a concentration of 4  $\mu$ g/ml. Similar to the findings of several investigators (11–13, 15, 16), our *Pandoraea* isolates showed broad resistance to  $\beta$ -lactams as well as to non- $\beta$ -lactam compounds (Table 1). Tazobactam showed only a marginal inhibitory effect, while BRL 42715 had a strong inhibitory effect in combination with amoxicillin and meropenem.

Sonication of strain suspensions, isoelectric focusing, and assessment of the hydrolytic activity by a bioassay were performed as described previously (7). The *Pandoraea* isolates produced between one and three  $\beta$ -lactamases, and all isolates showed a carbapenem-hydrolyzing enzyme focusing on pIs of  $\geq$ 8.0 (Table 1).

For cloning of the  $\beta$ -lactamase genes, whole-cell DNA of the isolates was extracted using the GFX Genomic DNA purification kit (Amersham Biosciences, Freiburg, Germany), partially digested with Sau3AI, and ligated into BamHI-digested pBC-SK+ (Stratagene, La Jolla, CA). The ligation product was transformed into *E. coli* DH5 $\alpha$  by electroporation. Transformants were selected on tryptic soy agar containing ampicillin (32 µg/ml). The cloned DNA fragments were sequenced by primer walking (Eurofins MWG Operon, Ebersberg, Germany). Additionally, the *bla*<sub>OXA</sub> genes of *Pandoraea* isolates E126-13, LMG 18087, and LMG 18100 were sequenced using oligonucleotides deduced from the sequences of the cloned DNA fragments. Sequence analyses and multiple alignments were performed using Chromas Lite 2.01 (Technelysium Pty Ltd., Brisbane, Australia) and DNAMAN 4.1 (Lynnon BioSoft, Vaudreuil-Dorion, Canada).

All *E. coli* DH5 $\alpha$  transformants showed amoxicillin MICs above 256 µg/ml, which were strongly reduced by the addition of BRL 42715 (Table 2). Piperacillin MICs ranged from 32 to 256

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This article is dedicated to the memory of Adolf Bauernfeind.

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Result for:       P. pnomenusa       P. apista LMG       P. norimbergensis       P. pulmonicola       P. sputorum       Pandorac         n		
$\frac{P. pnomenusa}{P. pnomenusa} P. apista LMG P. norimbergensis P. pulmonicola P. sputorum Pandorac$		
$\mathbf{n}_{1} = \mathbf{n}_{1} $	Pandoraea	
rarameter and H4-1-1 E120-13 LMG 1006/ 1040/ LMG 183/9 LMG 10100 LMG 10019 LMG 10100 V v00323	8100 Va8523	HD7676
antibiotic" OXA-62 OXA-152 OXA-151 OXA-153 OXA-157 OXA-156 OXA-154 OXA-155 OXA-156	0XA-158	OXA-159
MIC (µg/ml)		
AMX         >512	>512	>512
AMX + BRL 32 16 8 16 8 16 16 8	8	4
PIP         >512         512         256         256         512         >512         >512         512         512         512	512	>512
PIP + TZB         >512         256         128         256         512         512         >512         128         512	512	128
CAZ         256         256         128         128         128         >256         >256         256	256	128
CAZ + BRL         256         64         64         32         128         128         >256         256         128	128	128
CTX         64         32         32         16         32         32         128         32         32	32	16
FOX         >128         >128         >128         >128         >128         >128         >128         >128         >128         >128	>128	>128
ATM >256 >256 >256 >256 >256 >256 >256 >256	>256	> 256
MEM 1024 128 64 32 128 64 128 64 64	64	64
MEM + BRL 16 4 2 2 4 16 16 4 4	4	2
IPM 64 4 1 2 2 4 2 1 2	2	4
IPM + BRL 8 0.5 0.25 0.5 0.5 2 1 0.25 2	2	0.13
GEN         >128         128         >128         >128         1	>128	>128
TOB         >128         >128         >128         64         128         >128         >128         >128         >128	>128	>128
CIP 8 16 8 64 4 16 16 8 8	8	32
SXT >256 64 32 4 16 16 32 2 16	16	32
CHL         64         32         32         32         16         64         32         32         128	128	32
TET 2 2 2 1 4 256 4 4 256	256	64
pI of β-lactamase(s) <sup>b</sup> 7.4, 8.0, >9.0 6.5, >9.0 6.7, 7.7, >9.0 7.4, 8.5 8.8 8.0, 8.4 8.4, 8.8 8.0, 8.8 7.0, 7.6, 8		→ 7.0, 7.6, 8.9
<sup>a</sup> Abbreviations: AMX, amoxicillin; BRL, BRL 42715; PIP, piperacillin; TZB, tazobactam; CAZ, ceftazidime; CTX, cefotaxime; FOX, cefoxitin; ATM, aztreonam; MEM, meropenem; IPM, imipenem; GEN, gentami	7.0, 7.6, 8.9	

tobramycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; TET, tetracycline. <sup>b</sup>The pI values of carbapenem-hydrolyzing β-lactamases identified by bioassay are indicated in boldface.

		MIC (	µg/ml) for	<sup>a</sup> :												
		<i>E. coli</i> DH5α									<i>S. enterica serovar</i> Enteritidis 104.773					
Transformant or			AMX +		PIP +		CAZ +					MEM +		IPM +		
host strain	Oxacillinase	AMX	BRL	PIP	TZB	CAZ	BRL	CTX	FOX	ATM	MEM	BRL	IMP	BRL	MEM	IMP
Transformant strain plasmids																
pT6	OXA-62	>256	0.5	64	4	0.13	0.06	0.06	2	0.03	0.06	0.03	0.25	0.25	1	1
pT89	OXA-153	>256	0.5	32	0.5	0.13	0.13	0.03	4	0.03	0.03	0.03	0.13	0.13	0.13	1
рТ90	OXA-156	>256	0.5	256	1	0.13	0.25	0.03	4	0.03	0.06	0.03	0.25	0.13	1	1
pT100	OXA-154	>256	0.5	256	4	0.06	0.06	0.03	8	0.03	0.13	0.016	0.25	0.13	1	1
pT103	OXA-157	>256	0.5	128	2	0.13	≤0.03	0.06	8	0.25	0.25	0.016	0.5	$\leq 0.06$	2	2
pT104	OXA-158	>256	0.5	256	8	0.06	0.06	0.03	4	0.03	0.13	0.03	0.5	0.13	4	2
pT106	OXA-159	>256	0.5	256	8	0.13	0.06	0.03	8	0.03	0.13	0.016	0.5	0.13	2	1
Host strains		8	0.5	0.5	0.5	0.13	0.13	0.03	4	0.03	0.016	0.016	0.13	0.13	0.016	0.13

<sup>a</sup> Abbreviations: AMX, amoxicillin; BRL, BRL 42715; PIP, piperacillin; TZB, tazobactam; CAZ, ceftazidime; CTX, cefotaxime; FOX, cefoxitin; ATM, aztreonam; MEM, meropenem; IPM, imipenem.

µg/ml and were reduced 8 to 256-fold by tazobactam. Such a strong inhibitory effect of tazobactam could not be seen for the wild-type isolates. The MICs of ceftazidime, cefotaxime, cefoxitin, and aztreonam were only marginally affected by the introduction of the recombinant plasmids into E. coli DH5α. The meropenem and imipenem MICs were elevated up to 16- or 4-fold, respectively, by the expression of the OXA enzymes. The addition of BRL 42715 reduced the MICs for meropenem and imipenem to the levels of the host strain. As the effect of the expression of  $\beta$ -lactamases on the carbapenem MICs of fully susceptible E. coli hosts is often very small, we additionally transformed the recombinant plasmids into a Salmonella enterica serovar Enteritidis host strain lacking an outer membrane protein (Table 2). This resulted in a more pronounced increase of the MICs for meropenem (256fold) and imipenem (16-fold) in comparison to those of the E. coli host.

The seven recombinant plasmids harbored inserts of 1.5 to 2.5 kb with open reading frames corresponding to class D  $\beta$ -lactamases. The G+C content of the *bla*<sub>OXA</sub> genes ranged between 60 and 65%, and that of the environmental sequences ranged between 60 and 67%, which is close to the values for *Pandoraea* spp. (61.2 to 64.3%) published by Coenye et al. (8).

The putative promoter sequences are located between 88 and 135 bp upstream of the start codon. The promoter sequences are identical for genes derived from the same species; however, they vary among the genes of different species.

The regions upstream and downstream, respectively, of the oxacillinase genes corresponded to a "hypothetical protein" and to an NAD(P)H quinone oxidoreductase gene also present in two *P. pnomenusa* genome sequences (CP006900 and CP007506). Comparable sequences upstream of  $\beta$ -lactamase genes are present in the genomes of several isolates of different species, e.g., *Ralstonia pickettii* (CP006668), *Ralstonia eutropha* (AM260480), and *Cupriavidus taiwanensis* (CU633750).

The nine *Pandoraea*-derived oxacillinase genes encoded proteins of 283 to 292 amino acids, which were found to be new oxacillinase variants, namely, OXA-151 to OXA-159. They showed the structural elements characteristic for class D  $\beta$ -lactamases (Fig. 1), as follows. At positions 70 to 73 (class D  $\beta$ -lactamasenumbering, DBL) (19) the STYK tetrad was found, which is also present in enzymes of the OXA-50 subgroup, in contrast to the STFK motif, which is most common among oxacillinases. Within the usual SXV motif (positions 118 to 120), valine was replaced by leucine, except for OXA-154 and OXA-155 from *P. sputorum*, where valine was replaced by tyrosine. The *Pandoraea* oxacillinases showed the YGN motif at positions 144 to 146 and the KTG motif at positions 216 to 218, like most of the class D $\beta$ -lactamases, in contrast to the motifs FGN and KSG, which were found in some of the carbapenem-hydrolyzing class D enzymes. The common WXXG motif at positions 232 to 235 was found as in all oxacillinases.

The amino acid sequence similarities for OXA-151 to OXA-159, including the previously described OXA-62 from *P. pnomenusa* (7) and the respective regions of the two *P. pnomenusa* genome sequences, ranged between 71.5 and 99.6%. In comparison to other oxacillinases, OXA-151 to OXA-159 showed the highest similarities (36 to 45%) to the intrinsic enzymes of *Pseudomonas aeruginosa* (OXA-50) (20) and *Shewanella algae* (OXA-55) (2), to the acquired  $\beta$ -lactamases OXA-2 and OXA-20, as well as to enzymes of several genome sequences of bacteria of different phyla: e.g., *Parvibaculum lavamentivorans* (NC009719), *Acaryochloris marina* (NC009925), *Idiomarina loihiensis* (NC006512), *Methylobacillus flagellatus* (CP000284), and *Streptosporangium roseum* (CP001814).

Accurate identification of pathogens to the species level is mandatory for epidemiological analysis and the implementation of measures to prevent spread of isolates among cystic fibrosis patients. However, members of the genus *Pandoraea* are difficult to identify by conventional biochemical methods (11, 15, 21, 22). Molecular methods based on 16S rRNA are helpful to differentiate *Pandoraea* from closely related genera like *Burkholderia* and *Ralstonia*; however, they are not able to discriminate accurately between the *Pandoraea* species (23, 24). Coenye et al. (25) found *gyrB* gene sequences helpful to differentiate among *Pandoraea* 

		10	20	30	40	50	60	70
OXA-151	MNTIISBBWBAGLWBBLVGAV	νι.ρατι.αατρααγααρυρκαα	PGRITERAL	WGKLFTAEG	VKGTTVVI.DA	BT-OTYOAY	DAARAEKR	MSPASTYKT
OXA-151	MNTIISREWRAGLWRRLVGAV	VI.PATI.AATPAAYAADVPKAA	PGRITERAL	WGKLFAAEG	VKGTIVVLDA	RT-OTYOAY	DAARAGKR	MSPASTYKI
OXA-62	MNTIISRRWRAGLWRRLVGAV	VLPATLAATPAAYAADVPKAA	LGRITERAL	WGKLFAAEG	VKGTIVVLDA	RT-OTYOAY	DAARAEKR	MSPASTYKI
OXA-153	MKKIISRWRRGVFGLRVALAVVSPM	VFAVPAHATEAAGGAAGTKAA	AVHMKERAL	WGKFFDAEG	VKGTIVVLDG	RT-OTYOAF	DTARAERR	MSPASTYKI
OXA-155	MKRILSRWRRAAVVLRLASAVVAHGLLP	SPAHALELSRASAAAAPSVAA	PVHVTERAL	WGKFFAAEN	VKGTVVVLDG	KT-QTYQAY	DSARAERR	MSPASTYKI
OXA-154	MKRILSHWCRAAVVLRLASAVVAHGLLP	SPAHALELSRASAAAAPSVAA	PVHVTERAL	WGKFFAAEN	VKGTVVVLDG	KT-QTYQAY	DSARAERR	MSPASTYKI
OXA-156	MKKTFSRWRRGALVLRILG	ALASPVVFATPGHAAEPVRPP	SVHITERAL	WGKYFADEG	VKGTVVVLDG	RT-QTYQAY	DAARAERR	LSPASTYKI
OXA-158	MKKTLSRWRRGALALRLLG	ALASPVVFAMPGHAAEPAHSS	AVRIAERAD	WGKYFADEG	VKGTVIVLDG	RT-QTYQAY	DAARAERR	MSPASTYKI
OXA-159	MKKTLSRWRRGALALRLLG	ALASPVVFAMPGHAAEPAHSS	AVRIAERAL	WGKYFADEG	VKGTVIVLDG	RT-QTYQAY	DAARAERR	MSPASTYKI
OXA-15/	MMMLSRWRRSAVVLRIAAALL	SPLAVAI PAHADAIANAANAV	APKIVERAL	WGKYFDAEG	AKGTIIVLDG	RT-GGIQAI	DSTRANQR	MSPASTIKI
OXA = 10		MKIFAAIVIIACLSSIAL	AGSITENIS	WNKEFSAEA	SOCWWINE	NK-OOCETN	NI KBYNUY	FIDASTERI
OXA-55	MNKGLHBKBLSKBLLLPMLLCLLA	OOTOAVAAEOTKVSDVCSEVT	AEGWOEVRE	WDKLFESAG	VKGSLLLWDC	KR-SLGLSN	NLSRAAEG	FIPASTEKL
OXA-51	MNIKTLLLIT	SAIFISACSPYIVTANPNHSA	SKSDEKAEK	IKNLFNEVH	TTGVLVIOOG	OT-OOSYGN	DLARASTE	YVPASTFKM
OXA-24	MKKFILPIFSI	SILVSLSACSSIKTKSEDNFH	ISSQQHEKA	IKSYFDEAQ	TQGVIIIKEG	KN-LSTYGN	ALARANKE	YVPASTFKM
OXA-23	MNKYFTCYV	VASLFLSGCTVQHNLINETPS	QIVQGHNQV	IHQYFDEKN	TSGVLVIQTE	KK-INLYGN	ALSRANTE	YVPASTFKM
OXA-134	MKIL	IFLPLLSCLGLTACSLPVSSL	PSQSISTQA	IASLFDQAÇ	SSGVLVIQRE	QQ-VQVYGN	DLNRANTE	YVPASTFKM
OXA-211	MKTLQLALIA	LITTFGSACTTIPPSVETAKN	HQQQSAQQQ	IQQAFDQLQ	TTGVIVIKDK	HG-LHSYGN	DLSRAQTP	YVPASTFKM
OXA-214	MKLSKLYTLTV	LIGFGLSGVACQHIHTPVSFN	QIENDQTKQ	DIASLFENV	TTGVLITFDG	QA-YKAYGN	DLNRAKTA	YIPASTEKI
OXA-228	MKI KTI SI VCI S	TATCACAEHAMADAKTATIDO	VNNSTTDON	INOVIENEIS TEPPENŐUČ	ADAVEVIIDG	UN-IKKACL	HI DRAKIL	VIDASTERM
OXA-50	MLSR	YSKTLAFAVVACTLAISTATA	HAFLVVRNI	I.KRVFDDAG	VSGTFVLMDI	TA-DRTYVV	DPARAARS	THPASTERT
OXA-50		MRPLLFSALLLLSGHTO	ASEWNDSOA	VDKLFGAAG	VKGTFVLYDV	OR-ORYVGH	DRERAETR	FVPASTYKV
OXA-1	МК	NTIHINFAIFLIIANIIYSSÄ	SASTDISTV	ASPLFEGTE	GCFLLYDA	STNAEIAQF	NKAKCATQ	MAPDSTFKI
				*				* ** *
	80 90 100	110 120	130	140	150	160	1	170
OXA-151	FNSLLALDSGALDNERATTPWDGKPBRT	KNWNAAMDLETAFRVSCLPCY	OVVSHKTGE	RYAOAKLNE		APD	AYWVDDSL	OTSAREOVD
OXA-152	FNSLLALDSGALDNERAIIPWDGKPRRI	KNWNAAMDLRTAFRVSCLPCY	OVVSHKIGF	OYAOAKLNE	VGYGNRTIGG	APD.	AYWVDDSL	OISAREOVD
OXA-62	FNSLLALDSGALDNERAIIPWDGKPRRI	KNWNAAMDLRTAFRVSCLPCY	QVVSHKIGF	RYAQAKLNE	VGYGNRTIGG	APD.	AYWVDDSL	QISAREQVD
OXA-153	FNSLLALESGALDNEREIIPWDGKPRRG	KYWNAAMDLRTAFRVSCLPCY	QVVSHKIAF	QFAQSKLNE	AGYGNHTIGR	AAD.	AYWVDDSL	QISAREQVD
OXA-155	FNSLLALESGALDNERETIPWDGKPRRI	KAWNAELNLRDAFRVSCYPCY	QVVSHKIPF	RAYAQAKLDA	VGYGNRTIGR	VND	TYWVDDSL	QISAREQVD
OXA-154	FNSLLALESGALDNERETIPWDGKPRRI	KAWNAELNLRDAFRVSCYPCY	QVVSHKIPF	AYAQAKLDA	VGYGNRTIGR	VND	TYWVDDSL	QISAREQVD
OXA-156	FNSLLALESGAIDNEREVIPWDGKPRSM	KAWNAALNLRDAFRVSCLPCY	QILSHKIPF	QYAQAKLNE	VGYGNRTIGH	AAD	TYWVDDSL	QISAREQVD
OXA-158	FNSLLALESGALDNEREVIPWDGKPRKV	KAWNAALDLENAF RVSCLPCI	QVVSHKIPF	QIAQAKLNE	AGIGNRIIGR	AAH.	VAIMIDDGI	OTSAREQVD
OXA-159	FNSLLALESGALDNERE I I PWDGKPRKV	KRWNAAHDERNAF RVSCLPC1	OVVSRKIAF	TYAOGKI.DA	VGYGNHTIGS	AAD	AYWVDNSL	OISAREQVD
OXA-10	PNAIIGLETGVIKNEHOVFKWDGKPRAM	KOWERDITIRGATOVSAVPVF	OOTAREVGE	VRMOKYLKK	FSYGNONISG	GTD	KFWLEGOL	RISAVNOVE
OXA-48	PNSLIALDLGVVKDEHQVFKWDGQTRDI	ATWNRDHNLITAMKYSVVPVY	QEFARQIGE	ARMSKMLHA	FDYGNEDISG	NVD	SFWLDGGI	RISATEQIS
OXA-55	PSSLIALETGAVRDETSRFSWDGKVREI	AVWNRDQSFRTAMKYSVVPVY	QQLAREIGE	KVMAAMVRQ	LEYGNQDIGG	QAD	SFWLDGQL	RITAFQQVD
OXA-51	LNALIGLEHHKATT-TEVFKWDGQKRLF	PEWEKDMTLGDAMKASAIPVY	QDLARRIGI	ELMSKEVKF	VG <mark>YGN</mark> ADIGT	QVD	NFWLVGPL	KITPQQEAQ
OXA-24	LNALIGLENHKATT-NEIFKWDGKKRTY	PMWEKDMTLGEAMALSAVPVY	QELARRTGI	ELMQKEVKF	VNFGNTNIGT	QVD	NFWLVGPL	KITPVQEVN
OXA-23	LNALIGLENQKTDI-NEIFKWKGEKRSF	TAWEKDMTLGEAMKLSAVPVY	QELARRIGI	DLMQKEVKF	IGFGNAEIGQ	QVD	NFWLVGPL	KVTPIQEVE
OXA-134	LNALIGLQHGKATT-NEIFKWDGKKRSF	TAWERDMTLGQAMQASAVPVY	QELARRIGI	LE LMQQEVQE		QVD	NEWLVGPL	AKVTPKQEVQ OTTRVOEVI
OXA-211	LNALIGIEHOKTSP-NEVEKWDGOKRAF	FSWEKDITLAFAMOASAVPV1	OALAORIGI	DIWAKEAKE	VGEGNTRIGT	OVD	NFWLVGFL NFWLTGPL	KITPIFFAO
OXA-228	LNALIGLONAKATN-TEVFHWNGEKRAF	SAWEKDMTLAEAMOASAVPVY	OELARRIGI	ELMREEVKF	VGFGNAEIGC	OVD	NFWLVGPL	KISPEOEVO
OXA-58	ANALIGLENHKATS-TEIFKWDGKPRFF	KAWDKDFTLGEAMQASTVPVY	QELARRIGE	SLMQSELQF	IGYGNMQIGT	EVD	QFWLKGPL	TITPIQEVK
OXA-60	PNSLIAFDTGAVRDDQEVLPYGGKPQPY	EQWEHDMALPEAIRLSAVPIY	QEVARRVGE	ERMQAYVDA	FDYGNRQLGS	AID	QFWLRGPL	EISAFEEAR
OXA-50	ANSLIGLSTGAVRSADEVLPYGGKPQRF	KAWEHDMSLRDAIKASNVPVY	QELARRIGI	ERMRANVSF	LGYGNAEIGÇ	VVD	NFWLVGPL	KISAMEQTR
OXA-1	ALSLMAFDAEIIDQ-KTIFKWDKTPKGM	EIWNSNHTPKTWMQFSVVWVS	QEITQKIGI	NKIKNÄTKE	FDYGNQDFSG	DKERNNGLT	EAWLESSL	KISPEEQIQ
	180 190 200	* *	* 230	240	**	260	* 270	
		210 220	230	240	250	200	270	
OXA-151	FLQRLARGTLPFSARSQD-IVRQMSIVE	ATPDYVLHGKTGWFVDKK	PDİGWWVGW	IIER-DGNIA	SVAINIDMLS	-EADAPKRA	RIVKSVLK	DLKLI
OXA-152	FVQRLARGTLPFSARSQD-IVRQMSIVE	ATPDYVLHGKTGWFVDKK	PDIGWWVGW	IER-DGNIT	SVAINIDMLS	-EADAPKRA	RIVKAVLK	DLKLI
OXA-62	FVQRLARGTLPFSARSQD-IVRQMSIVE	ATPDYVLHGKTGWFVDKK	PDIGWWVGW	ILER-DGNII	SVAINIDMLS	-EADAPKRA	RIVKAVLK	DLKLI
OXA-155	FLORIARGTLPFSARSOD-IVROISIVE	ANADYVI.HCKTCWFVEKK		ILFR-DGNII		-DADAPKRA	RIVEENI'K	NIKIT
OXA-154	FLORLARGTLPFSARSOD-IVROISIVE	ANADYVLHGKTGWFVDKK	PDIGWWVGW	LER-DGNLT	MIALNIDMNG	-DADGPKRA	RIVREVLK	NLKLI
OXA-156	FLQRLAKGTLPFSARSQD-IVRNISIVE	ANADYVLHGKTGWFTDKK	PDIGWWVGW	LER-DGNLI	MIALNIDIQS	-DADGPKRV	RIVRSVLK	DLKLI
OXA-158	FLQRLATGTLPFSARSQD-IVRNISIVE	ANVDYVLHGKTGWFTEKK	PDIGWWVGW	LER-DGNLI	MIALNIDIQT	-DADAPKRA	RIVRNVLK	DLKLI
OXA-159	FLQRLATGALPFSARSQD-IVRNISIVE	ANVDYVLHGKTGWFTEKK	PDIGWWVGW	ILER-DGNLI	MIALNIDIQI	-DADAPKRA	RIVRNVLK	DLKLI
OXA-157	FLQRLARGQLPFSARTQD-IVRQISIAE	ANMDYVLHGKTGWYVDGK	HDIGWWVGW	IIER-DGNII	TVALNMDMRS	-DADAPKRA	RIARAVLK	DLKLI
OXA-10	FLESLYLNKLSASKENQL-IVKEALVTE	AAPEYLVHSKTGFSGVGTESN	PGVAWWVGW	VEK-ETEVY	FFAFNMDIDN	-ESKLPLRK	SIPTKIME	SEGIIGG
OXA-40	FI DOT HDNKT DAREBOOD - TAKOWNT AL	ANGUITIKANIGISIKIE	PAIGWWVGW		VFAUNIDIAS	- A SOL PL PO	UINKUMIK	OFOLLP
OXA-51	FAYKLANKTLPFSPKVOD-EVOSMLETE	EKNGNKIYAKSGWGWDVD	POVGWLTGW	IVVOPOGNIU	AFSLNLEMKK	-GIPSSVRK	EITYKSI'E	OLGIL
OXA-24	FADDLAHNRLPFKLETOEVKKMLLIK	EVNGSKIYAKSGWGMGVT	PQVGWLTGW	VEQANGKKI	PFSLNLEMKE	-GMSGSIRN	EITYKSLE	NLGII
OXA-23	FVSQLAHTQLPFSEKVQA-NVKNMLLLE	ESNGYKIFGKTGWAMDIK	PQVGWLTGW	VEQPDGKIV	AFALNMEMRS	-EMPASIRN	ELLMKSLK	QLNII
OXA-134	FVSALAREQLAFDPQVQQ-QVKAMLFLQ	ERKAYRLYVKSGWGMDVE	PQVGWLTGW	VETPQAEIV	AFSLNMQMQN	-GIDPAIRL	EILQQALA	ELGLYPKAEG
OXA-211	FVEKLANTQLAFKPDVQH-TVQDMLLIE	QKPNYKLYAKSGWGMDLE	PQVGWWTGW	IVETATSEKV	YFALNMHMKT	-GISASVRE	QLVKQSLT	ALGII
OXA-214	FAYRLAKQELPFTPKTQQ-QVIDMLLVD	EIRGTKVYAKSGWGMDIT	PQVGWWTGW	IEDPNGKVI	AFSLNMEMNÇ	-PAHAAARK	EIVYQALT	QLKLL
OXA-228	FAYQLAMKQLPFDSNVQQ-QVKDMLYIE	REGUSELYAKSGWGMDVE	PQVGWYTGW	VEQPNGKVI	AFALNMNMQA	-GNDPAERK	QLTLSILD	KLGLFFYLR-
0XA-20	LATDPACOTELVERADO	OOGDAALYAKTCVATAVD	PETGWWACW	VER-ACHUV	APALNTOMOD	-GDDIALKK	DI'CKUI WD Arofnard	VPCALEAMDVD
OXA-50	FLLRLAOGELPFPAPVOS-TVRAMTILE	SGPGWELHGKTGWCFDCT	PELGWWAG	VKR-NERLY	GFALNIDMPG	GEADJGKRV	ELGKASI.K	ALGILP
OXA-1	FLRKIINHNLPVKNSAIENTIENMYLOD	LDNSTKLYGKTGAG-FTANRT	LQNGWFEGF	IISKSGHKY	VFVSALTGNI	-GSNLTSSI	KAKKNAIT	ILNTLNL
	*	* *	* *					

FIG 1 Comparison of the amino acid sequences of OXA-151 to OXA-159 with those of other carbapenem-hydrolyzing class D  $\beta$ -lactamases and those of OXA-1 and OXA-10. Identical residues are marked by asterisks, and motifs conserved among oxacillinases are shaded. The amino acid positions are numbered according to the DBL system (19).

species, while the applicability of the *recA* gene, which is also commonly used for phylogenetic analysis, seems to be limited (26). Using sequences of the NCBI database, we compared the nucleotide sequence similarities for 16S rRNA, *gyrB*, and *bla*<sub>OXA</sub> genes for isolates of different *Pandoraea* species. All three genes showed high similarities (96.1 to 100%) for isolates belonging to the same species. In contrast, interspecies similarities showed larger variations: 97.2 to 99.8% for 16S rRNA, 84.6 to 97.3% for *gyrB*, and 71.8 to 87.7% for *bla*<sub>OXA</sub>. So, the oxacillinase genes showed the broadest interspecies variability and along with their high degree of intraspecies similarities (98.5 to 99.8%), they might pose an opportunity for species identification by molecular techniques.

As the Pandoraea isolates Va8523 and HD7676 were not identifiable to the species level, a 970-bp fragment of their gyrB genes was sequenced. The sequences showed high similarity (99.8%) and formed a separate branch in a phylogenetic tree with additional 19 gyrB gene sequences from isolates of eight Pandoraea species obtained from the NCBI database (data not shown). Therefore, the isolates Va8523 and HD7676 seem to belong to the same species, which is different from the Pandoraea species described until now. The oxacillinase genes of those two isolates (OXA-158 and OXA-159) showed 99.5% nucleotide sequence identity and clearly differed from the oxacillinases of the other Pandoraea spp. (75.1 to 87.7% similarity). So, similar to the gyrB genes, the oxacillinase genes of those two isolates form a distinct branch in the oxacillinase gene homology tree (data not shown), supporting the assumption that they may belong to a separate species not yet identified.

In conclusion, our study showed the intrinsic production of carbapenem-hydrolyzing oxacillinases by *Pandoraea* isolates of various species. The oxacillinases, which contribute to the resistance to aminopenicillins and carbapenems, seem to be species specific and may therefore be helpful for the identification of members of the genus *Pandoraea* up to the species level. Our work indicates that *Pandoraea* species are contributing to the natural reservoir of carbapenem-hydrolyzing oxacillinases that may serve as progenitors of acquired  $\beta$ -lactamases, as has been the case for the OXA-23-like enzymes of *A. radioresistens* (27).

Nucleotide sequence accession numbers. The nucleotide sequences of the  $bla_{OXA}$  genes have been deposited in the GenBank database under accession no. KP771979 (OXA-151), KP771980 (OXA-152), KP771981 (OXA-153), KP771982 (OXA-154), KP771983 (OXA-155), KP771984 (OXA-156), KP771985 (OXA-157), KP771986 (OXA-158), and KP771987 (OXA-159).

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