Novel Carbapenem-Hydrolyzing Oxacillinase OXA-62 from *Pandoraea pnomenusa*

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Pandoraea spp. are gram-negative, glucose nonfermenting rods detectable in blood cultures and sputa of cystic fibrosis patients. They are resistant to various antibiotic groups, with imipenem being the only active β -lactam. We isolated an imipenem-resistant (MIC, 64 µg/ml) Pandoraea pnomenusa strain from a cystic fibrosis patient. Cloning and sequencing identified two β -lactamases of Bush group 2d, namely, the known OXA-33, located on an integron, and the novel carbapenem-hydrolyzing oxacillinase OXA-62. OXA-62 is only distantly related to other oxacillinases (OXA-50 being closest with 43% amino acid identity). It hydrolyzes penicillins, oxacillin, imipenem, and meropenem but not expanded-spectrum cephalosporins. The bla_{OXA-62} gene is chromosome located. No transposable elements were found in its genetic neighborhood. With OXA-62-specific primers, bla_{OXA-62} could be identified in all *P. pnomenusa* strains and appears to be species specific. This additional mechanism of carbapenem resistance further complicates the treatment of infections caused by *P. pnomenusa*.

The genus *Pandoraea* was established by Coenye et al. in 2000 (6) as a result of reanalyzing strains not definitely identified to the species level which were phenotypically closest to *Burkholderia cepacia*, *Ralstonia pickettii*, or *Ralstonia paucula*. The genus *Pandoraea* includes five named species (*Pandoraea apista*, *P. norimbergensis*, *P. pnomenusa*, *P. pulmonicola*, and *P. sputorum*) and four unnamed genomospecies (9). *Pandoraea* sp. strains have been isolated from patients with septicemia and respiratory tract infections (mostly cystic fibrosis), as well as from food, water, and soil (6, 9, 14, 17, 21).

Antibiotic therapy of infections caused by *Pandoraea* spp. is impaired by their resistance to multiple antibiotics, including penicillins, cephalosporins, cefoxitin, meropenem, aminoglycosides, and chloramphenicol. Their resistance to fluoroquinolones is variable. Only tetracycline, trimethoprim-sulfamethoxazole, and imipenem were found to be active against the majority of isolates (9, 14, 17, 21). We recently cultured a *P. pnomenusa* strain from a cystic fibrosis patient. For this multiresistant strain, the MIC of imipenem was unusually high. A novel carbapenemhydrolyzing oxacillinase, OXA-62, turned out to be involved in the mechanism of resistance to imipenem.

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MATERIALS AND METHODS

Strains and plasmids. The strains used in this study are characterized in Table 1. The *Pandoraea* spp. were isolated from sputa of cystic fibrosis patients between 1997 and 2004 in different centers in Germany, partly as *Burkholderia* spp. not identifiable to the species level. They were identified by phenotypic (API 20 NE [BioMérieux, Marcy l'Etoile, France] and additional biochemical tests) and genotypic methods (PCR with species-specific oligonucleotides) with *Pandoraea* sp. type strains obtained from the Laboratorium voor Microbiologie (Universiteit

Ghent, Ghent, Belgium) bacterial collection as references. *Escherichia coli* C600 R⁻ was the recipient strain in conjugation experiments. *E. coli* DH5 α and the plasmid pBC (containing a chloramphenicol resistance gene; Stratagene) were used for cloning experiments.

Susceptibility testing. The following antibiotics were obtained from the respective manufacturers: amoxicillin, Gruenenthal GmbH, Stolberg, Germany; ceftazidime, cefuroxime, clavulanate, and BRL 42715, GlaxoSmithKline GmbH & Co. KG, Munich, Germany; cefotaxime, Hoechst AG, Frankfurt/Main, Germany; cefepime and aztreonam, Bristol-Myers Squibb, Munich, Germany; cefoxitin and imipenem, Merck Sharp & Dohme, Munich, Germany; faropenem, Bayer AG, Wuppertal, Germany; meropenem, AstraZeneca GmbH, Plankstadt, Germany; nalidixic acid, reserpine, rifampin, and sodium azide, Sigma-Aldrich Chemie GmbH, Steinheim, Germany; piperacillin and tazobactam, Wyeth Pharma GmbH, Münster, Germany; chloramphenicol, Boehringer, Mannheim, Germany. The B-lactamase inhibitors clavulanate, tazobactam, and BRL 42715 (7, 16) were used at a fixed concentration of 4 µg/ml, and the efflux pump inhibitor reserpine was used at a fixed concentration of 20 µg/ml. MICs were determined by an agar dilution technique on Mueller-Hinton agar inoculated with 10⁴ CFU/spot with a multipoint inoculator following Clinical and Laboratory Standards Institute (formerly National Institute for Clinical and Laboratory Standards) guidelines (18). E. coli ATCC 25922 was used as the MIC reference strain.

Conjugation. Conjugation experiments with *P. pnomenusa* H4-1-1 as the donor strain and *E. coli* C600 R⁻ as the recipient strain were performed as described previously (2). Selecting agents were amoxicillin (32 μ g/ml), ceftazidime (2 μ g/ml), imipenem (2 μ g/ml), and meropenem (0.5 μ g/ml). MacConkey agar was used to counterselect as *P. pnomenusa* H4-1-1 was resistant to rifampin, nalidixic acid, and sodium azide.

IEF and induction studies. Sonication of strain suspensions and isoelectric focusing (IEF) were performed as previously described (3), except that suspensions of Pandoraea spp. were exposed to lysozyme (5 mg/ml; SERVA Electrophoresis GmbH, Heidelberg, Germany) for 1 h at 37°C prior to sonication. After focusing, the alkaline rim of the gel was neutralized by replacing the NaOH electrode strip with a strip soaked with 1 N HCl prior to visualization of the β-lactamase bands with nitrocefin. The hydrolytic activities of individual β -lactamase bands were assessed by a bioassay with two consecutive overlays. The first one, with 0.75% tryptic soy agar (TSA) containing the respective β -lactam, was followed, after 2 h of incubation at 35°C, by a second TSA overlay containing a susceptible indicator strain (1.2×10^7) CFU/ml). After overnight incubation at 35°C, growth of the indicator strain on the gel localized the β -lactamase band by which the β -lactam had been inactivated. For induction, an over night culture of P. pnomenusa H4-1-1 was diluted 1:20 and grown for 3 h on a shaker at 37°C. After addition of the potential inducer cefoxitin (64 µg/ml), clavulanate (64 µg/ml), or imipenem (16 µg/ml), the cultures were incubated for an additional 3 h.

Cloning and sequencing. Cloning was performed as described previously (3). Whole-cell DNA of *P. pnomenusa* H4-1-1 was extracted with the GFX genomic

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TABLE 1. Bacterial strains used in this study

Organism	Designation	Source or description
P. pnomenusa	H4-1-1	CF ^{<i>a</i>} sputum (Hannover, Germany)
P. pnomenusa	B-31	CF sputum (Berlin, Germany)
P. pnomenusa	E126-13	CF sputum (Essen, Germany)
P. pnomenusa	MUN 66-59	CF sputum (Munich, Germany)
P. pnomenusa	PA 2228	CF sputum (Passau, Germany)
P. pnomenusa	BUC	CF sputum (Magdeburg, Germany)
P. pnomenusa	GRO	CF sputum (Magdeburg, Germany)
P. pnomenusa	KRU	CF sputum (Magdeburg, Germany)
Pandoraea sp.	Va8523	CF sputum (Frankfurt, Germany)
Pandoraea sp.	HD 7676	CF sputum (Heidelberg, Germany)
P. apista	LMG 16407 ^T	CF sputum (Denmark)
P. norimbergensis	LMG 18379 ^T	Oxic water layer above a sulfide-
		containing sediment (Nürnberg,
		Germany)
P. pnomenusa	LMG 18087 ^T	CF sputum (United Kingdom)
P. pulmonicola	LMG 18106 ^T	CF sputum (Canada)
P. sputorum	LMG 18819 ^T	CF sputum (United States)
<i>E. coli</i> C600 R ⁻		Recipient strain for conjugation experiments
E. coli DH5α		Host strain for cloning experiments
E. coli DH5α	$T1^+$	Transformant harboring plasmid
		pT1 (vector pBC plus 5-kb insert)
E. coli DH5α	T6 ⁺	Transformant harboring plasmid pT6 (vector pBC plus 2.6-kb insert)

^a CF, cystic fibrosis patient.

DNA purification kit (Amersham Pharmacia Biotech), partially digested with Sau3AI or PstI, and ligated into BamHI- or PstI-digested pBC. The ligation product was transformed into *E. coli* DH5 α made competent by the calcium chloride method. Transformants were selected on TSA containing amoxicillin (32 µg/ml) and chloramphenicol (16 µg/ml). Because of its large insert (>7kb), one recombinant plasmid was subjected to subcloning by partial Sau3AI digestion of the insert and ligation into pBC.

Sequencing was performed with consecutive primers by the dideoxy chain termination procedure with an automatic sequencer (ABI 3700). The nucleotide and deduced amino acid sequences were analyzed, and multiple alignments were performed with the DNAMAN 4.1 Software (Lynnon BioSoft).

Kinetic analysis. Three liters of TSB containing 100-µg/ml amoxicillin was inoculated with E. coli transformant strain T6+ carrying the recombinant plasmid pT6 and shaken overnight at 37°C and 200 rpm. Cells were harvested by centrifugation, washed with 50 mM phosphate buffer (pH 7.0), and resuspended in 5 ml of the same buffer. Five freeze-thaw cycles on dry ice-ethanol were followed by centrifugation at 27,000 \times g for 30 min. Several purification schemes were attempted, but OXA-62 did not behave as expected on either gel filtration or ion-exchange chromatography, and a pure preparation was not obtained. For partial purification of the OXA-62 β-lactamase, the supernatant was filtered, loaded onto a Sephacryl S-100 gel filtration column, and eluted with 50 mM phosphate buffer. All nitrocefin-hydrolyzing activity came out in the void volume. The two fractions with the highest nitrocefin-hydrolyzing activity were pooled, precipitated with 90% ammonium sulfate, and then resuspended and dialyzed in 25 mM Tris (pH 8.15)-200 mM K₂SO₄. Gel filtration on S-100 was repeated. Coomassie-stained polyacrylamide gel electrophoresis gel showed a purification of about 50% at this point. Initial hydrolysis rates were measured spectrophotometrically in 50 mM phosphate buffer containing 10 mM sodium bicarbonate at 25°C (22). K_m and V_{max} values were obtained with a Hanes plot.

PCR amplification of the oxacillinase gene. With whole-cell DNA as the template and primers 5'-ACGCACGCAAACCTATCA-3' and 5'-ATGTTGAT CGCGACGCTG-3' (based on the sequence of the cloned gene), PCR was carried out under the following conditions: 95°C for 1 min; 35 cycles of 95°C for 45 s, 57°C for 45 s, and 72°C for 45 s; and a final elongation step of 72°C for 10 min.

Nucleotide sequence accession number. The nucleotide sequence of bla_{OXA-62} has been submitted to the GenBank database and has been assigned accession no. AY423074.

TABLE 2. Antibiotic susceptibilities of *P. pnomenusa* H4-1-1 and *E. coli* DH5 α host and recombinant plasmid-harboring strains

	MIC (µg/ml) for:							
$Compound(s)^a$	D	E.						
1 · · · (1)	P. pnomenusa H4-1-1	pT1OXA-33	pT6OXA-62	Host strain				
Amoxicillin	>512	128	256	4				
Plus CLA	>512	16	64	4				
Plus TZB	512	32	64	4				
Plus BRL	32	2	1	2				
Piperacillin	>512	16	16	1				
Plus TZB	>512	4	16	1				
Cefuroxime	256	4	4	4				
Ceftazidime	256	0.06	0.13	0.13				
Plus CLA	256	0.06	0.13	0.13				
Plus BRL	256	0.06	0.06	0.13				
Plus Res	256	0.06	0.13	0.13				
Cefotaxime	64	0.06	0.06	0.13				
Cefepime	256	0.06	0.06	0.06				
Cefoxitin	>128	4	4	8				
Aztreonam	>256	≤0.06	≤0.06	≤0.06				
Faropenem	256	0.5	2	0.5				
Imipenem	64	0.25	0.25	0.25				
Plus BRL	8	0.25	0.25	0.25				
Plus Res	64	0.5	0.5	0.5				
Plus BRL and Res	8	ND	0.5	0.5				
Meropenem	1,024	0.03	0.13	0.03				
Plus BRL	16	0.06	0.06	0.06				
Plus Res	128	0.03	0.06	0.06				
Plus BRL and Res	8	ND	0.03	0.03				

^{*a*} Abbreviations: CLA, clavulanate; TZB, tazobactam; BRL, BRL 42715 (inhibits all active-site serine β-lactamases); Res, reserpine.

RESULTS AND DISCUSSION

Antibiotic resistance of P. pnomenusa H4-1-1. P. pnomenusa H4-1-1 was resistant to all of the β -lactams tested (Table 2). Clavulanate and tazobactam reduced the MICs of the penicillins and ceftazidime only weakly. BRL 42715, an inhibitor of activesite serine β-lactamases, lowered the MIC of amoxicillin more than 16 times, that of imipenem 8 times, and that of meropenem 64 times but had no effect in combination with ceftazidime. Reserpine, an efflux pump inhibitor, did not change the MICs of ceftazidime and imipenem but reduced the MIC of meropenem 8 times. The combination of BRL 42715 and reserpine with imipenem or meropenem showed no superior activity compared to the combination with BRL 42715 alone. So the resistance of P. pnomenusa H4-1-1 to meropenem appeared to be the result of two mechanisms, namely, hydrolysis and extrusion by an efflux pump. Their proportion can be roughly estimated from inhibition of the enzymatic component by BRL 42715, which reduces the meropenem MIC from 1,024 to 16 µg/ml (a factor of 64), while inhibition of the efflux by reserpine reduces the MIC to 128 µg/ml (a factor of 8). The MIC of imipenem was reduced by BRL 42715

Organism		MIC (µg/ml)			Presence of OXA-62-specific	
	Strain designation	IMI^b	MER ^c	pl(s) of β-lactamase"	PCR product (570 bp)	
P. pnomenusa	H4-1-1	64	1,024	7.4, 8.0, >9.0	+	
P. pnomenusa	B-31	16	1,024	8.0, >9.0	+	
P. pnomenusa	E126-13	4	128	6.5, >9.0	+	
P. pnomenusa	MUN 66-59	1	64	7.7, 8.0, >9.0	+	
P. pnomenusa	PA 2228	1	64	7.7, >9.0	+	
P. pnomenusa	BUC	16	256	8.0, >9.0	+	
P. pnomenusa	GRO	16	128	8.0, >9.0	+	
P. pnomenusa	KRU	2	64	8.0, >9.0	+	
P. pnomenusa	LMG 18087 ^T	1	64	6.7, 7.7, >9.0	+	
Pandoraea sp.	Va8523	2	64	7.7, 8.9	_	
Pandoraea sp.	HD 7676	4	64	7.0, 7.5, 8.9	_	
P. apista	LMG 16407 ^T	2	32	7.4, 8.5	_	
P. norimbergensis	LMG 18379 ^T	2	128	8.8	_	
P. pulmonicola	LMG 18106 ^T	4	64	8.0 , 8.4	_	
P. sputorum	LMG 18819 ^T	2	128	8.4, 8.8	-	

TABLE 3. Characteristics of *Pandoraea* sp. β-lactamases

^{*a*} The values for carbapenem-hydrolyzing β -lactamases identified by bioassay are in bold.

^b IMI, imipenem.

^c MER, meropenem.

from 64 to 8 μ g/ml (a factor of 8) but remained unchanged in combination with reserpine. The resistance to imipenem appeared to be the result of hydrolysis and possibly impaired expression of outer membrane proteins, while efflux seems not to be involved. No transconjugants could be produced in conjugation experiments.

Isoelectric points. In the crude homogenate of *P. pnomenusa* H4-1-1, three β -lactamases with pIs of 7.4, 8.0, and >9.0 were detected (Table 3). Only the β -lactamase with a pI of >9.0 hydrolyzed imipenem and meropenem in the bioassay.

Analysis of cloned genes and characterization of β -lactamases. After ligation of partially digested genomic DNA of *P. pnomenusa* H4-1-1 into pBC and transformation into *E. coli* DH5 α , we selected the amoxicillin-resistant transformants T1⁺ and T6⁺ harboring recombinant plasmids pT1 and pT6 with inserts of about 5.2 and 2.6 kb, respectively.

E. coli DH5 α T1⁺ produced a single β-lactamase with a pI of 7.4, and for this strain, the MICs of amoxicillin and piperacillin, but not those of the carbapenems, were elevated (Table 2). MICs of the penicillins were reduced 4 to 8 times by clavulanate or tazobactam and 64 times by BRL 42715. Sequencing of the 5.2-kb insert of pT1 identified a class 1 integron similar to In40 and In111 (20, 23) containing four gene cassettes in its variable region: *aac*(6')-*Ib*, *aac*(3)-*Ia*, *qacF*, and *bla*_{OXA-33} coding for the class D β-lactamase OXA-33.

E. coli DH5 α T6⁺ produced a β-lactamase with a pI of >9.0, and for this strain, in comparison with those for the T⁻ strain, the MICs of amoxicillin (64 times) and piperacillin (16 times) were elevated. Clavulanate and tazobactam reduced the MIC of amoxicillin from 256 to 64 µg/ml, and BRL 42715 reduced it from 256 to 1 µg/ml. No (imipenem) or only a slight increase (meropenem, faropenem) in the MICs of carbapenems were observed. However, in a bioassay following IEF, homogenates of this strain showed high hydrolytic activity for imipenem and meropenem comparable to that of the wild-type strain. Similar observations have been described for several oxacillinases (5, 12, 13).

Sequencing of the insert of pT6 revealed an open reading frame (ORF) of 858 bp encoding a protein of 285 amino acids (Fig. 1). The protein showed, at positions 70 to 73 according to the class D β -lactamase (DBL) numbering system (8), an STYK tetrad representing the active site of serine β -lactamases. Among class D β-lactamases, the STYK motif has so far been described only for enzymes of the OXA-50 class D carbapenemase subgroup, while the STFK motif is the most common. Five structural elements characteristic of class D β-lactamases were identified (Fig. 1). Within the SXV motif (DBL positions 118 to 120), valine (V) was replaced by leucine (L), which was otherwise found only in β -lactamase mll0916 from Mesorhizobium loti (15). At positions 144 to 146, we found the YGN motif common to all oxacillinases except enzymes of the OXA-23 and OXA-24 carbapenemase subgroups, where it is replaced by FGN. This motif had recently been associated with the resistance to NaCl inhibition (12). At positions 164 to 172, the motif WXXXXLXIS was observed, as in most of the oxacillinases. The KTG motif (positions 216 to 218), necessary for function, was found as in the OXA-23, OXA-50, and OXA-60 subgroups, whereas it was replaced by KSG in the other two class D carbapenemase subgroups of OXA-24 and OXA-51. The motif WXXG at positions 232 to 235 was found as in all other oxacillinases.

Multiple alignment of protein sequences revealed no close relationship to any of the oxacillinases already known. The highest sequence similarities were found with enzymes of the OXA-50 subgroup (43.1 to 43.6%), class D carbapenemases produced by *Pseudomonas aeruginosa* (10), and with OXA-36 (41.4%), an oxacillinase closely related to OXA-2 found in *P. aeruginosa* (4).

We propose the designation OXA-62 for the novel oxacillinase. The full-length protein has a predicted molecular mass of 31.7 kDa. The G+C content of the ORF is 65.3%, which is close to the G+C content of *Pandoraea* spp. of 61.2 to 64.3% (6). In the genetic neighborhood of *bla*_{OXA-62}, no transposable elements could be found. Sequencing of the regions up- and downstream

				10		20	30	4	0 5	50
OXA-62	MNTII	SRRWRAGLWE	RRLVGAVVLP	ATLAATPAA	AYAADVPKAA	LGRIT	ERADWGK	LFAAEGVK	GTIVVLDA	ARTQTYQA
OXA-50				-MRPLLFSA	ALLLLSGHTQ	ASEWN	DSQAVDK	LFGAAGVK	GTFVLYD	VQRQRYVG
OXA-60			MLSRYSK	TLAFAVVA	CTLAISTATA	HAELV	VRNDLKR	RVF'DDAGVS	GTFVLMDI	LTADRTYV
OXA-48			MRV	LALSAVFL	/ASIIGMPAV	AKEWQ	ENKSWNA	HFTEHKSQ	GVVVLWNE	ENKQQGFT
OXA-55	MNKGLHRK	RLSKRLLLPN	MLLCLLAQQT	QAVAAEQTH	KVSDVCSEVI	AEGWQ	EVRRWDK	LFESAGVK	GSLLLWDQ	QKRSLGLS
OXA-24		MKKF]	ILPIFSISIL	VSLSACSSI	IKTKSEDNFF	IISSQQ	HEKAIKS	YFDEAQTQ	GVIIIKEC	GKNLSTYG
OXA-51		MNIE	KTLLLITSAI	FISACSPYI	IVTANPNHSA	SKSDE	KAEKIKN	ILFNEVHTT	GVLVIQQO	GQTQQSYG
OXA-23		- MNI	KYFTCYVVAS	LFLSGCTV	QHNLINETPS	SQIVQG	HNQVIHÇ	YFDEKNTS	GVLVIQTI	DKKINLYG
OXA-58		MKLLKII	LSLVCLSISI	GACAEHSMS	SRAKTSTIPQ	VNNSI	IDQNVQA	LFNEISAD	AVFVTYDO	GQNIKKYG
OXA-61				Mł	KKITLFLLFI	NLVFG	QDKILNN	WFKEYNTS	GTFVFYDO	GKTWAS
OXA-1			MKNTI	HINFAIFL	LIANIIYSSA	SASTD	ISTVASP	PLFEGTEGC	FLLYDAS1	ſNAEIAQF
							110	10	0	1 2 0
	60	70	80	90	10	0	110	12	0	130
									navoi na	
OXA-62	YDAARAEK	RMSPASTYK.	IFNSLLALDS	GALDNERA	LIPWD-GKPF	RIKNW	NAAMDLE	TAFRV5CL	PCIQVVSP	HKIGRRIAQ
OXA-50	HDRERAET	REVPASTYK	VANSLIGLSI	GAVRSADE	/LPYG-GKPC	2RF KAW	EHDMSLR	CDAIKASNV	PVIQELA	RIGLERMR
OXA-60	VDPARAAR	SIHPASTEK.	IPNSLIAFDI	GAVRDDQE	/LPYG-GKPQ	PYEQW	EHDMALP	EAIRLSAV	PIYQEVAR	RVGFERMQ
OXA-48	NNLKRANQ	AFLPA STFK I	IPNSLIALDI	GVVKDEHQV	/FKWD-GQTF	RDIATW	NRDHNLI	TAMKYSVV	PVYQEFAI	RQIGEARMS
OXA-55	NNLSRAAE	GFIPA STFK I	LPSSLIALET	GAVRDETSE	RFSWD-GKVF	REIAVW	NRDQSFF	TAMKYSVV	PVYQQLAH	REIGPKVMA
OXA-24	NALARANK	EYVPASTEKN	MLNALIGLEN	I-HKATTNEI	IFKWD-GKKF	TYPMW	EKDMTLG	EAMALSAV	PVYQELAH	RTGIELMQ
OXA-51	NDLARAST	EYVPASTEKI	MLNALIGLEH	-HKATTTE\	/FKWD-GQKF	RLFPEW	EKDMTLG	DAMKASAL	PVYQDLAF	RIGLELMS
OXA-23	NALSRANT	EYVPASTEKI	MLNALIGLEN	-QKTDINE:	IFKWK-GEKF	RSFTAW	EKDMTLC	EAMKLSAV	PVYQELAH	RRIGLDLMQ
OXA-58	THLDRAKT	AYIPA STFK	IANALIGLEN	I-HKATSTE	IFKWD-GKPF	RFFKAW	DKDFTLG	GEAMQASIV	PVYQELAF	RRIGPSLMQ
OXA-61	NDFSRAME	TFSPA STFK I	IFNALIALDS	GVIKTKKE.	IFYHYRGEKV	/FLSSW	AQDMNLS	SAIKYSNV	LAFKEVAI	RRIGIKTMQ
OXA-1	NKAKCA-T	OMAPDSTEK.	TALSLMAFDA	-ETTDOKT	IFKWD-KTPM	GMETW	NSNHTPR	TWMOF'SVV	WVSOEFIC)KIGLNKIK
		5		L DIIPQUI.			NOMILLI			
	140	150	160	170	180		190	200	21	10
	140	150	160	170	180		190	200	21	10
084-62	140		160			RLARGT	190		21 SIVEATPI	10 DYVLHG KTG
OXA-62	140 AKLNEVGY ANVSBLGY	150 GNRTIGG	160 APDAYM VVDNFM	170 VDDSLOIS/	180 AREQVDFVQF	LARGT	190 LPFSARS	200 200 SQD-IVROM ZOS-TVRAM	21 SIVEATPI TLLESGPO	10 DYVLHGKTG SWELHGKTG
OXA-62 OXA-50 OXA-60	140 AKLNEVGY ANVSRLGY AYVDAFDY	150 GNRTIGG GNAEIGQ SNBOLGS	160 APDAYN VVDNFN AIDOFN	170 IVDDSLQISA LVGPLKISA	180 AREQVDFVQF AMEQTRFLLF	LARGT LAQGE	190 LPFSARS LPFPAPV LPVKPRI	200 J SQD-IVRQM ZQS-TVRAM WD-MVORM	21 SIVEATPI TLLESGPO	10 DYVLHGKTG GWELHGKTG DAALYAKTG
OXA-62 OXA-50 OXA-60 OXA-48	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY	150 GNRTIGG GNAEIGQ GNRQLGS GNEDISG	160 apdaym VVDNFW aidQFM	170 VDDSLQIS/ LVGPLKIS/ LRGPLEIS/ LRGPLEIS/	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEOISFLBF	LARGT LAQGE MALKQ	190 LPFSARS LPFPAPV LPVKPRI LHVSERS	200 SQD-IVRQM QS-TVRAM WD-MVQRM SOR-IVKOA	21 SIVEATPI TLLESGPO LLIEQQGI MLTEANGI	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVROLEY	150 GNRTIGG GNAEIGQ GNRQLGS GNEDISG GNEDISG	160 apdaym vvdnfm aidQfm nvdsfm oadsfm	170 VVDDSLQIS/ ILVGPLKIS/ ILRGPLEIS/ ILDGGIRIS/ ILDGOLBIT/	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFOOVDFLR(RLARGT RLAQGE RMALKQ KLYHNK	190 LPFSARS LPFPAPV LPVKPRI LHVSERS LPVSERS	200 J GQD-IVRQM VQS-TVRAM WD-MVQRM GQR-IVKQA GOR-IVKQM	21 SIVEATPI TLLESGP(LLIEQQGI MLTEANGI MLTEASTI	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVNE	150 GNRTIGG GNREIGQ GNRQLGS GNEDISG GNQDIGG GNQDIGG	160 apdaym vvdnfw aidQfw vvdsfw QadSfm QadSfm	170 IVDDSLQIS/ ILVGPLKIS/ ILDGGIRIS/ ILDGGLRIT/ ILDGQLRIT/ ILVGPLKIT	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ 2VOEVNFADI	RLARGT RLAQGE RMALKQ KLYHNK QLHDNK DLAHNR	190 LPFSARS LPFPAPV LPVKPRI LHVSERS LPVSERS LPFKLEI	200 J QQD-IVRQM QQS-TVRAM WD-MVQRM GQR-IVKQA GQR-IVKQM COEVKKM	21 SIVEATPI TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNG	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVNE KEVKRVGY	150 GNRTIGG GNAEIGQ GNRQLGS GNQDIGG GNQDIGG GNTNIGT GNADIGT	160 APDAYW VVDNFW AIDQFW QADSFW QVDNFW QVDNFW	170 IVDDSLQIS/ ILVGPLKIS/ ILGGPLEIS/ ILDGQLRIT/ ILVGPLKITI	180 AREQVDFVQF AMEQTRFLLF AFEQISFLRF AFQQVDFLRQ 200EA0FAYF	RLARGT RLAQGE RMALKQ KLYHNK QLHDNK DLAHNR KLANKT	190 LPFSARS LPFPAPV LPVKPRI LHVSERS LPFKLEI LPFKLEI LPFSPKV	200 SQD-IVRQM VQS-TVRAM WD-MVQRM SQR-IVKQM SQR-IVKQM CQEVKKM VOD-EVOSM	21 SIVEATPI TLLESGP(LLIEQQGI MLTEANGI MLTEASTI LLIKEVN(LFIEEKN(10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GNKIYAKSG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVDY KEVKRVGY KEVKRIGF	150 GNRTIGG GNRQLGS GNQDIGG GNQDIGG GNADIGT GNADIGT GNADIGT GNADIGT GNADIGT GNAEIGO	160 VVDNFW APDAYW 	170 UVGDLSLQISJ LUGGLKISJ LLGGLRISJ LLGGLRITJ LVGPLKITI LVGPLKVT	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ PVQEVNFADI 20QEEAQFAYF 210EVEFVSC	ALARGT ALAQGE MALKQ ALYHNK ALYHNK ALYHNK ALANKT ALANKT ALANTO	190 LPFSARS LPFPAPV LPVKPRI LPVSERS LPFKLEI LPFSPKV LPFSEKV	200 SQD-IVRQM VQS-IVRAM WD-MVQRM SQR-IVKQA SQR-IVKQA SQR-IVKQM VQD-EVQSM VQA-NVKNM	21 SIVEATPI TLLESGPC LLIEQQGI MLTEASTI LLIKEVNC LLIKEVNC LLIEEKNC LLLEESNC	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG GSKIYAKSG GYKIYAKSG GYKIFGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVGF KEVKRVGF SELORIGY	150 GNRTIGG GNAEIGQ GNEDISG GNQDIGG GNUIGG GNADIGT GNAEIGQ GNAEIGQ GNMOIGT	160 APDAYM APDAYM AIDQFW 	170 IVDDSLQIS/ ILVGPLKIS/ ILGGLRIS/ ILDGGLRIT/ ILVGPLKITI ILVGPLKITI ILVGPLKITI	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ PVQEVNFADI PQQEAQFAYF PIQEVEFVSQ PIQEVEFVSQ	ALARGT ALAQGE MALKQ ALYHNK ALYHNK ALYHNK ALANKT ALANKT ALANTQ ALATQ ALAGGO	190 LPFSARS LPFPAPV LPVKPRI LHVSERS LPFKLEI LPFSPKV LPFSEKV LPFKPEV	200 200- 2	21 SIVEATPI TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNC LFIEEKNC LLLEESNC LVVERRGI	10 DYVLHGKTG DWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GNKIYAKSG GYKIFGKTG ENRLYAKSG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVNF KEVKRVGY KEVKRIGF SELQRIGY EYLNKLHY	150 GNRTIGG GNRQLGS GNPQLGS GNQDIGG GNDIGT GNADIGT GNADIGT GNMQIGT GNAKIS	160 APDAYM AIDQFM AIDQFM QADSFM QVDNFM QVDNFM QVDNFM QVDNFM QVDNFM 	170 VDDSLQIS/ ULVGPLKIS/ ULDGGLRIS/ ULDGQLRIT/ ULVGPLKITI ULVGPLKITI ULVGPLKITI ULKGPLTITI ULKGPLTITI	180 AREQVDFVQF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ PVQEVNFADI PQEAQFAYF PIQEVEFVSY AKEOAILLFF	LARGT LAQGE MALKQ LYHNK LHDNK LAHNKT LAHKT DLAHTQ LSONS	190 LPFSARS LPVKPRI LHVSERS LPVKERS LPFKLEI LPFSPKV LPFSPEKV LPFSPEKPEV LPFSOEA	200 J QQ-IVRQM WD-MVQRM QR-IVKQA QR-IVKQA QQ-EVKKM YQA-NVKNM YQA-NVKNM MM-SVKEM	21 SIVEATPH TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNC LFIEEKNC LLLEESNC LYVERRGH IYLKNMEN	10 DYVLHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GNKIYAKSG GYKIFGKTG ENRLYAKSG NLELFGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVNF KEVKRVGY KEVKRIGF SELQRIGY EYLNKLHY NYLKDFDY	150 GNRTIGG GNRQLGS GNRQLGS GNQDIGG GNDIGT GNADIGT GNADIGT GNACIGQ GNAVIS GNAKIS GNQDFSGDKI	160 APDAYK AIDQFK QADSFK QVDNFK QVDNFK QVDNFK QVDNFK QVDNFK QVDNFK QVDNFK 	170 IVDDSLQISJ ILVGPLKISJ ILRGPLEISJ ILDGQLRITJ ILVGPLKITI ILVGPLKITI ILVGPLKITI ILKGPLTITI ILCNSLKISJ ILESSLKISJ	180 AREQVDFVQF AFEEARFILF AFEEARFISF ATEQISFLRF AFQQVDFLRQ PVQEVNFADI PQEAQFAYF PIQEVEFVSI AKEQAILLFF PEEQIQFLRF	RLARGT RLAQGE MALKQ LYHNK 2LHDNK DLAHNR (LANKT 2LAHTQ 2LAHTQ 2LAHTQ XLSQNS (IINHN	190 LPFSARS LPFSARS LPVKPRI LHVSERS LPVSERS LPFKLEI LPFSPKV LPFSPEV LPFSQEA LPVKNSA	200 J QQ-IVRQM WD-MVQRM QR-IVKQA QQ-IVKQM QQ-EVQSM VQQ-QVKEM VQQ-QVKEM LIENTIENM	21 SIVEATPI TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNC LFIEEKNC LLIEESNC LYLERGH IYLKNMEN YLQDLDNS	10 DYVLHGKTG GWELHGKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GNKIYAKSG GYKIFGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1	140 AKLNEVGY ANVSRLGY AYVDAFDM KMLHAFDY KEVKRVNF KEVKRVGY KEVKRVGY SELQRIGY EYLNKLHY NYLKDFDY	150 GNRTIGG GNAEIGQ GNEQLGS GNQDIGG GNADIGT GNADIGT GNAEIGQ GNAKIS GNAKIS GNQDFSGDKH	160 APDAYM VUDNFM QADSFM QVDNFM QVDNFM QVDNFM QVDNFM QVDNFM 	170 IVDDSLQISJ ILVGPLKISJ ILRGPLEISJ ILDGQLRITJ ILVGPLKITI ILVGPLKITI ILVGPLKITI ILKGPLTITI ILGNSLKISJ	180 AREQVDFVQF AFEEARFTSF AFQUVDFLRQ PVQEVNFADI PQQEAQFAYH PIQEVEFVSQ PIQEVKFVYI AKEQAILLFF PEEQIQFLRH	RLARGT RLAQGE KLAUGE KLYHNK RLHDNK RLANKT RLANKT RLANKT RLAUGQ RLSQNS KLSQNS	190 LPFSARS LPFPAPV LPVKPRI LHVSERS LPFKLEI LPFSPKV LPFSEKV LPFSQEA LPVKNSA	200 J QD-IVRQM WD-MVQRM QR-IVKQA QR-IVKQM QD-EVQSM QD-EVQSM QD-EVQSM QD-EVQSM MN-SVKEM MN-SVKEM LIENTIENM	21 SIVEATPI TLLESGPG MLTEANGI MLTEASTI LLIKEVNG LFIEEKNG LLLEESNG LYVERRGH IYLKNMEN YLQDLDNS	10 DYVLHGKTG GWELHGKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GSKIYAKSG GYKIYGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY KEVKRVGY KEVKRVGY KEVKRVGY KEVKRIGF SELQRIGY EYLNKLHY NYLKDFDY 220	150 GNRTIGG	160 APDAYW AIDQFW QADSFW QVDNFW QVDNFW QVDNFW QVDNFW QVDNFW ERNNGLTEAW 240	170 VVDDSLQIS/ LVGPLKIS/ LDGGIRIS/ LUGGLRIT LVGPLKIT LVGPLKIT LVGPLKVT	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ PVQEVNFAD PVQEVACAQFAYF PIQEVEFVSQ PIQEVEFVSQ PIQEVKFVYI AKEQAILLFF PEEQIQFLRF 260	RLARGT RLAQGE RMALKQ (LYHNK 2LHDNK 2LHDNK 2LAHTQ 2LAHTQ 2LAHTQ 2LAHTQ 2LAHTQ 2LAHTQ 2LAHTQ 27	190 LPFSARS LPFPAPV LPVKPRI LPVSERS LPFKLEI LPFSPKV LPFSEKV LPFSQEA LPVKNSA 0	200 J QD-IVRQM VQS-TVRAM VQD-MVQRM QR-IVKQA QQ-EVKKM VQD-EVQSM VQA-NVKNM VQQ-QVKEM MN-SVKEM AIENTIENM	21 SIVEATPI TLLESGP(LLIEQQGI MLTEANGI MLTEASTI LLIKEVN(LLIKEVN(LLLEESN(LYVERRGI IYLKNME) YLQDLDN;	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG GYKIRAKTG GSKIYAKSG GYKIFGKTG GYKIFGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVGY KEVKRVGY KEVKRVGY KEVKRIGF SELQRIGY EYLNKLHY NYLKDFDY 220	150 GNRTIGG	160 	170 VDDSLQISJ LKGPLKISJ LDGGIRISJ LDGQLRITJ LVGPLKITI LVGPLKITI LVGPLKITI LVGPLKITI LVGPLKITI LSSLKISJ LESSLKISJ 	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRC PVQEVNFADF PIQEVEFVSC PIQEVEFVSC PIQEVKFVYI AKEQAILLFF PEEQIQFLRF 260	RLARGT RLAQGE MALKQ (LYHNK DLAHNR (LANKT DLAHNZ DLAQGQ RLSQNS (IINHN 27	190 LPFPAPV LPFVKPRI LHVSERS LPFKLEI LPFSPKV LPFSEKV LPFSQEA LPFSQEA LPFVKNSA 0	200 J QD-IVRQM WD-MVQRM QR-IVKQA QR-IVKQA QD-EVKKM VQD-EVKSM VQA-NVKNM VQQ-QVKEM MN-SVKEM	21 SIVEATPH TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNG LLIEESNC LLLEESNC LLVERRGH IYLKNMEN YLQDLDNS	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG GYKIYAKSG GYKIYAKSG GYKIFGKTG ENRLYAKSG NIELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVNF KEVKRVNF KEVKRIGF SELQRIGY EYLNKLHY NYLKDFDY 220	150 GNRTIGG GNAEIGQ GNEDISG GNEDISG GNADIGT GNADIGT GNAEIGQ GNAKIS GNAKIS GNAKIS GNAKIS GNAKIS GNAKIS GNAKIS GNAKIS GNAKIS	160 	170 IVODSLQISA ILVGPLKISA ILGGIRISA ILDGQLRITA ILUGPLKITA ILVGPLKITA ILVGPLKITA ILVGPLKITA ILVSLKISA ILSSLKISA 250 ILSSLNIDA	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ PVQEVNFADI PQQEAQFAYF PIQEVEFVSC PIQEVEFVSC PIQEVKFVYI AKEQAILLFF PEEQIQFLRF 260 MLSEADAPKF	RLARGT RLAQGE MALKQ (LYHNK 2LHDNK 2LHDNK 2LAHNZ 2LAHTQ DLAQGQ RLSQNS (IINHN 27 RARIVK	190 LPFSARS LPFVAPV LPVKPRI LHVSERS LPFSEKV LPFSEKV LPFSQEA LPFKPEV LPFSQEA LPFVKNSA 0 AVLKDLK	200 J QD-IVRQM VQS-TVRAM WD-MVQRM SQR-IVKQA SQR-IVKQA QD-EVKSM VQD-EVKSM VQD-VKEM MAN-SVKEM LII	21 SIVEATPH TLLESGP(LLIEQGGI MLTEANGI MLTEASTI LLIKEVN(LFIEEKN(LLLEESN(LLLEESN(LYVERRGH IYLKNMEN YLQDLDN(10 SWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG SKIYAKSG GYKIFGKTG GYKIFGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY AMVRQLEY KEVKRVGY KEVKRIGF SELQRIGY EYLNKLHY NYLKDFDY 220 WFVDKKP- WCFDCTP-	150 GNRTIGG GNAEIGQ GNRQLGS GNQDIGG GNADIGT GNADIGT GNAUGT GNAVIGT GNAKIS GNQDFSGDKH 230 	160 	170 IVDDSLQIS/ ILVGPLKIS/ ILRGPLEIS/ ILDGQLRIT/ ILUGPLKITI ILVGPLKITI ILVGPLKITI ILVGPLKITI ILSSLKIS/ ILSSLK/	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRC PVQEAQFAYF PIQEVEFVSC PIQEVEFVSC PIQEVKFVYI AKEQAILLFF PEEQIQFLRF 260 MLSEADAPKF MPGGEADIGF	ALARGT RLAQGE MALKQ (LYHNK DLAHNR (LANKT DLAHQ DLAHQ DLAHQ DLAQGQ RLSQNS (IINHN 27 ARIVK (RVELG	190 LPFSARS LPFVAPV LPVKPRI LHVSERS LPVSERS LPFSEKV LPFSEKV LPFSQEA LPFKPEV LPFSQEA LPVKNSA 0 AVLKDLK KASLKAL	200 J QD-IVRQM VQS-TVRAM WD-MVQRM SQR-IVKQA SQR-IVKQA SQR-IVKQM QD-EVQSM QQ-QVKEM MN-SVKEM MN-SVKEM MN-SVKEM MIENTIENM (LI GILP	21 SIVEATPH TLLESGPC LLIEQGGI MLTEANGI MLTEASTI LLIEESNC LFIEEKNC LLLEESNC LYVERRGH IYLKNMEN YLQDLDNS	10 DYVLHGKTG DWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GNKIYAKSG GYKIFGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-61 OXA-61 OXA-62 OXA-50 OXA-60	140 AKLNEVGY ANVSRLGY AVVDAFDY KMLHAFDY AMVRQLEY KEVKRVNF KEVKRVGF SELQRIGY EYLNKLHY NYLKDFDY 220 WFVDKKP- WCFDCTP- VATEYOP-	150 GNRTIGG GNAEIGQ GNPQLGS GNPQLGG GNQDIGG GNAIGT GNALGQ GNALGQ GNALGQ GNAKIS GNQDFSGDKH 230 	160 	170 IVDDSLQIS/ ILVGPLKIS/ ILGGLRIS/ ILGGLRIT/ ILVGPLKITI ILVGPLKITI ILVGPLKITI ILVGPLKITI ILSGPLTITI ILSSLKIS/	180 AREQVDFVQF AFEEARFTSF AFEQISFLRF AFQUVDFLRQ PVQEVNFADI PQQEAQFAYF PIQEVEFVSQ PIQEVKFVYI AKEQAILLFF PEEQIQFLRF 260 MLSEADAPKF MPGGEADIGF MPGGEADIGF	RLARGT RLAQGE MALKQ (LYHNK DLAHNR DLAHNR QLAHUQ RLSQNS (IINHN 27 RARIVK RVELG RIPLGK	190 LPFSARS LPFPAPV LPVSERS LPVSERS LPFKLEI LPFSPEV LPFSPEV LPFSQEA LPFVKNSA 0 AVLKDLK KASLKAI QLMRALE	200 J QD-IVRQM VQS-TVRAM WD-MVQRM SQR-IVKQA QQ-EVKEM VQD-EVQSM VQD-VKEM VQD-VKEM MN-SVKEM LIENTIENM KLI .GILP .WPAP	21 SIVEATPH TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNC LFIEEKNC LLLEESNC LLLEESNC LLLEESNC LYVERRGI IYLKNMEN YLQDLDNS	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG GXKIYAKSG GNKIYAKSG GYKIFGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-55 OXA-25 OXA-24 OXA-51 OXA-23 OXA-58 OXA-61 OXA-1 OXA-1 OXA-62 OXA-60 OXA-60 OXA-60	140 AKLNEVGY ANVSRLGY AYVDAFDM KMLHAFDY KEVKRVNF KEVKRVGY KEVKRUGY SELQRIGY EYLNKLHY NYLKDFDY 220 WFVDKKP- WCFDCTP- VATEYQP- YSTRIEP-	150 GNRTIGG GNAEIGQ GNRQLGS GNPQLGG GNUGG GNALIGT GNALIGT GNAKIS GNQLGT GNQLGT GNQLGT GNQLGWVGW 230 	160 	170 IVDDSLQIS/ ILVGPLKIS/ ILDGGLRIS/ ILDGGLRIS/ ILUGPLKITI ILVGPLKITI ILVGPLKITI ILVGPLKITI ILVGPLKITI ILSGPLTITI ILGNSLKIS/ ILSSLKIS/ ILSSLKIS/ ISVAINIDI YGFALNIDI WFFAMMMDI	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRQ PVQEVNFADI PQQEAQFAYF PIQEVEFVSQ PIQEVKFVYI AKEQAILLFF PEEQIQFLRF 260 MISEADAPKF MPGGEADIGF MPREGEMAKF	RLARGT RLAQGE RMALKQ LLAURK DLHDNK DLAHNR CLANKT DLAGQ RLSQNS CIINHN 27 RARIVK RVELG RVELG RQAITK	190 LPFSARS LPFSARS LPVKPRI LHVSERS LPVKRES LPFKLEI LPFSPEKV LPFSQEA LPFSQEA LPFVKNSA 0 AVLKDLK KASLKAI QLMRALE EVLKQEF	200 200 20D-IVRQM 20S-TVRAM 20D-MVQRM 20D-VQRM 20D-EVQSM 20	21 SIVEATPH TLLESGPO LLIEQQGI MLTEANGI MLTEASTI LLIKEVNO LFIEEKNO LLIEESNO LLLEESNO IYLKNMEN YLQDLDNS	10 DYVLHGKTG DWELHGKTG DAALYAKTG DYIIRAKTG DYIIRAKTG GSKIYAKSG GYKIFGKTG SYKIFGKTG STKLYGKTG
OXA-62 OXA-50 OXA-60 OXA-48 OXA-55 OXA-24 OXA-51 OXA-23 OXA-61 OXA-61 OXA-1 OXA-61 OXA-60 OXA-60 OXA-60 OXA-60	140 AKLNEVGY ANVSRLGY AYVDAFDY KMLHAFDY KEVKRVGY KEVKRVGY KEVKRVGY KEVKRUGF SELQRIGY EYLNKLHY NYLKDFDY 220 WFVDKKP- WCFDCTP- VATEYQP- YSTRIEP- YGVRRTP-	150 GNRTIGG	160 APDAYM AIDQFM QVDNFM QVDNFM QVDNFM 	170 VDDSLQISJ LVGPLKISJ LDGGIRISJ LDGGLRITJ LVGPLKITJ LVGPLKITJ LVGPLKITJ LVGPLKTI LVGPLKTI LVGPLKTI LVGPLKTI VGFALNIDN VGFALNIDN VVFFANNDN VVFFAVNLDN	180 AREQVDFVQF AMEQTRFLLF AFEEARFTSF ATEQISFLRF AFQQVDFLRC PVQEVNFAU PUQEVAQFAYF PIQEVEFVSC PIQEVEFVSC PIQEVEFVSC PIQEVEFVSC PIQEVEFVSC PIQEVAFVYI AKEQAILLFF PEEQIQFLRF 260 MLSEADAPKF MPGGEADIGF MPGGEADIGF MPTSDGLGLF LASASQLPLF	ALARGT ALAQGE MALKQ (LYHNK DLAHNR (LANKT DLAHNR (LANKT DLAQGQ LAGQ LAGQQ LAGQQ LAGQQ LAGQQ LAGQQ ALSQNS (INHN 27 ARIVK (RVELG (RVELG AQAITK QQAITK	190 LPFPAPV LPFVKPRI LHVSERS LPFKLEI LPFSPKV LPFSEKV LPFSQEA LPFKPEV LPFSQEA 0 AVLKDLK KASLKAI QLMRALE EVLKQEF	200 J QD-IVRQM VQS-TVRAM VQD-VVQRM QQ-IVKQA VQD-EVKKM VQD-EVQSM VQD-VKEM MIN-SVKEM AIENTIENM SULI	21 SIVEATPH TLLESGPC LLIEQQGI MLTEANGI MLTEASTI LLIKEVNG LLIEESNG LLLEESNG LYVERRGH IYLKNMEN YLQDLDNS	10 DYVLHGKTG GWELHGKTG DAALYAKTG DYIIRAKTG GYKIYAKSG GYKIYAKSG GYKIFGKTG ENRLYAKSG NLELFGKTG STKLYGKTG
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FIG. 1. Comparison of the amino acid sequence of OXA-62 with those of other carbapenem-hydrolyzing oxacillinases and that of OXA-1. GenBank accession numbers were obtained from www.lahey.org/studies/webt.htm. Conserved residues of oxacillinases are shaded. Numbering of β -lactamase amino acid positions is according to the DBL system (8).

of the OXA-62 gene revealed four further ORFs; BLAST searches with the ORFs alone (nucleotide and amino acid sequences) or the whole up- and downstream regions gave only scores of low homology for short sequences. The G+C content of the environment of bla_{OXA-62} is similar to that of the OXA-62 gene. These findings suggest a chromosomal location of the OXA-62 gene.

Biochemical properties of OXA-62. After purification from *E. coli* DH5 α harboring recombinant plasmid pT6, the purity of the β -lactamase OXA-62 was estimated as ~50% by NuPAGE gels. There were at least eight other bands visible, and for this reason, we did not calculate k_{cat} values. The kinetic parameters of OXA-62 obtained with the partially purified

extract are shown in Table 4. The hydrolysis profile of OXA-62 includes penicillins and, to a lesser extent, carbapenems, cephaloridine, and cefoxitin, but not ticarcillin, ceftazidime, cefotaxime, and aztreonam. The rate of hydrolysis of oxacillin was higher than that of benzylpenicillin, which is characteristic for oxacillinases. However, most of the other carbapenem-hydrolyzing oxacillinases have weak or no oxacillin-hydrolyzing activity, except OXA-25, -26, and -55 (1, 13). OXA-62 showed $V_{\rm max}$ values six times higher for ampicillin than for benzylpenicillin. However, because of its low affinity for ampicillin (K_m , 160 μ M), its hydrolytic efficiency is about the same as that for benzylpenicillin. The weak hydrolytic efficiency of OXA-62 for cephaloridine resulted from its very low affinity (K_m , 3200 μ M)

Substrate	$K_m \; (\mu \mathrm{M})$	V _{max} (nmol/min/mg)	Relative V_{\max}^{a} (%)	$V_{\rm max}/K_m$ ratio	Relative V_{max}/K_m^a (%)
Benzylpenicillin	28 ± 2.3	31 ± 0.8	100	1.1	100
Cephaloridine	$3,200 \pm 500$	14 ± 2.0	44	0.004	0.36
Ampicillin	160 ± 2.1	190 ± 8.0	600	1.2	109
Piperacillin	13 ± 5.7	2.1 ± 0.14	6.8	0.16	14.5
Oxacillin	35 ± 5.5	67 ± 2.5	220	1.9	172
Ticarcillin ^b	ND^{c}	≤0.31	≤1.0	ND	ND
Imipenem	13 ± 1.4	0.044 ± 0.0	0.14	0.003	0.27
Meropenem	8.0 ± 2.8	0.058 ± 0.0	0.18	0.007	0.64
Cefoxitin	68 ± 1.4	0.23 ± 0.016	0.74	0.003	0.27
Ceftazidime ^b	ND	≤0.01	≤0.026	ND	ND
Cefotaxime ^b	ND	≤0.01	≤0.036	ND	ND
Aztreonam ^b	ND	≤0.50	≤1.6	ND	ND

TABLE 4. Kinetic parameters of partially purified β-lactamase OXA-62

^a The benzylpenicillin value was taken as 100%.

 $^{b}V_{\text{max}}$ was estimated due to slow hydrolysis.

^c ND, not determined. Hydrolysis was too slow to determine the K_m .

for this substrate. OXA-62 showed weak hydrolysis of imipenem, as seen for most of the class D carbapenemases. In contrast to other carbapenem-hydrolyzing oxacillinases, for which hydrolysis of meropenem was less efficient than that of imipenem or even undetectable (like that for OXA-40 and OXA-60 [11, 12]), OXA-62 showed a hydrolytic efficiency two times higher for meropenem than for imipenem. Hydrolysis of the expanded-spectrum cephalosporins ceftazidime and cefotaxime, as well as aztreonam, was very weak, as seen for most of the OXA carbapenemases (1, 5, 10, 11, 12, 13, 19). OXA-62 hydrolyzed the cephamycin cefoxitin at low but measurable rates.

Hydrolysis of nitrocefin, indicated by a color change from yellow to red, by crude extracts of *E*. *coli* DH5 α harboring the bla_{OXA-62} gene was significantly delayed when the β -lactamase-containing extract was incubated with 0.1 M NaCl (37°C, 10 min) prior to the addition to nitrocefin. This indicates that OXA-62 is inhibited by NaCl, as expected from the presence of the YGN motif at DBL positions 144 to 146. In contrast, pretreatment of the homogenates of E. coli DH5 α T6⁺ with meropenem or imipenem (64 µg/ml) only slightly delayed the reaction while no effect was detectable in the presence of cefoxitin (64 μ g/ml). These results indicate that OXA-62 is inhibited poorly by carbapenems and not at all by cephamycins. Crude homogenates of P. pnomenusa H4-1-1 obtained from cultures induced with cefoxitin, imipenem, or clavulanate showed slightly greater amounts of β -lactamase activity for OXA-62 in bioassays than the preparation from an uninduced culture.

Distribution of OXA-62 among *Pandoraea* **spp.** All *Pandoraea* sp. strains were resistant to piperacillin, piperacillin-tazobactam, cefuroxime, ceftazidime, cefoxitin, aztreonam, and meropenem and intermediately resistant or resistant to cefotaxime (except one strain) and cefepime (data not shown). Four strains were resistant to imipenem (MIC, $\geq 16 \ \mu g/ml$); all others were susceptible. Like *P. pnomenusa* H4-1-1, all *Pandoraea* sp. isolates were 8 to 64 times more resistant to meropenem than to imipenem, and 11 of the 15 strains were classified as imipenem sensitive and meropenem resistant (Table 3). This has been described by several authors (9, 14, 17, 21). Reduction of the MICs of imipenem (2 to 32 times) and meropenem (4 to 256 times) was achieved in combination with BRL

42715. All *Pandoraea* spp. harbored meropenem-hydrolyzing β -lactamases. However, only the β -lactamases of the *P. pnomenusa* isolates focused at a pI of >9.0, comigrating with OXA-62. The meropenem-hydrolyzing β -lactamases of the non-*P. pnomenusa* strains showed variable pIs between 7.5 and 8.9 (Table 3). With oligonucleotides derived from the sequence of the *OXA-62* gene, a 570-bp fragment could be amplified from the whole-cell DNAs of all of the *P. pnomenusa* wild-type strains but not from those of the other *Pandoraea* spp. (Table 3). All *Pandoraea* spp. seemed to produce a meropenem-hydrolyzing oxacillinase, while OXA-62 appeared to be specific for the species *P. pnomenusa*.

The first carbapenem-hydrolyzing oxacillinase was described in 1993 in an *A. baumannii* strain isolated in 1985 (19). Since then, more than 25 class D carbapenemases have been found. They form at least six different homology groups with only low identity. Most of their genes were identified on the chromosome of *A. baumannii* isolates. Their origin is still unknown. Recently, gram-negative environmental species like *R. pickettii*, *P. aeruginosa*, *Shewanella algae*, and *Shewanella oneidensis* were found to be a natural reservoir of carbapenem-hydrolyzing oxacillinases (10, 11, 13).

OXA-62 of *P. pnomenusa* is a further carbapenem-hydrolyzing class D β -lactamase produced by a glucose-nonfermenting environmental species. It appears to be species specific and therefore helpful for identification of *P. pnomenusa*. With benzylpenicillin, amoxicillin, and oxacillin as preferred substrates, slow hydrolysis of carbapenems, and no activity against ceftazidime, cefotaxime, and aztreonam, the biochemical profile of OXA-62 resembles that of other class D carbapenemases (1, 5, 10, 11, 12, 13, 19). The β -lactamase OXA-62 contributes to the resistance of *P. pnomenusa* H4-1-1 to amoxicillin, imipenem, and meropenem. In addition, the presence of an efflux mechanism for meropenem could be shown. The presence of multiple mechanisms of carbapenem resistance further complicates the treatment of infections caused by *P. pnomenusa*.

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