AAC Journals.ASM.org

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

Ines Schneider, Adolf Bauernfeind†

MICOER Institute, Munich, Germany

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.

Oxacillinases are serine β-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 β-lactamase inhibitors tazobactam and BRL 42715, an inhibitor of active-site serine β-lactamases (18), were used at a concentration of 4 μg/ml. Similar to the findings of several investigators (11–13, 15, 16), our *Pandoraea* isolates showed broad resistance to β-lactams as well as to non-β-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 β-lactamases, and all isolates showed a carbapenem-hydrolyzing enzyme focusing on pIs of ≥8.0 (Table 1).

For cloning of the β-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* DH5α 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_{OXA} 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* DH5α 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

Received 11 May 2015 **Returned for modification** 19 July 2015
Accepted 24 August 2015
Accepted manuscript posted online 8 September 2015
Citation Schneider I, Bauernfeind A. 2015. Intrinsic carbapenem-hydrolyzing oxacillinases from members of the genus *Pandoraea*. Antimicrob Agents Chemother 59:7136–7141. doi:10.1128/AAC.01112-15.

Address correspondence to Ines Schneider, schneider_ines@gmx.net.
† Deceased August 2014.

This article is dedicated to the memory of Adolf Bauernfeind.

TABLE 1 Antibiotic susceptibilities and pIs of β-lactamases of *Pandoraea* isolates

Parameter and antibiotic[a]	Result for:									
	P. pnomenusa			*P. apista* LMG 16407^T	*P. norimbergensis* LMG 18379^T	*P. pulmonicola* LMG 18106^T	*P. sputorum*		*Pandoraea*	
	H4-1-1 OXA-62	E126-13 OXA-152	LMG 18087^T OXA-151	OXA-153	OXA-157	OXA-156	LMG 18819^T OXA-154	LMG 18100 OXA-155	Va8523 OXA-158	HD7676 OXA-159
MIC (μg/ml)										
AMX	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
AMX + BRL	32	16	8	16	8	16	16	16	8	4
PIP	>512	512	256	256	256	512	>512	>512	512	>512
PIP + TZB	>512	256	128	256	512	512	>512	128	512	128
CAZ	256	256	256	128	128	128	>256	>256	256	128
CAZ + BRL	256	64	64	32	128	128	>256	256	128	128
CTX	64	32	32	16	32	32	128	32	32	16
FOX	>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
MEM + BRL	16	4	2	2	4	16	16	4	4	2
IPM	64	4	1	2	2	4	2	1	2	4
IPM + BRL	8	0.5	0.25	0.5	0.5	2	1	0.25	2	0.13
GEN	>128	>128	>128	128	>128	>128	>128	>128	>128	>128
TOB	>128	>128	>128	64	128	>128	>128	128	>128	>128
CIP	8	16	8	64	4	16	16	8	8	32
SXT	>256	64	32	4	16	16	32	2	16	32
CHL	64	32	32	32	16	64	32	32	128	32
TET	2	2	2	1	4	256	4	4	256	64
pI of β-lactamase(s)[b]	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, **8.9**	7.0, 7.6, **8.9**

[a] Abbreviations: AMX, amoxicillin; BRL, BRL 42715; PIP, piperacillin; TZB, tazobactam; CAZ, ceftazidime; CTX, cefotaxime; FOX, cefoxitin; ATM, aztreonam; MEM, meropenem; IPM, imipenem; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; TET, tetracycline.

[b] The pI values of carbapenem-hydrolyzing β-lactamases identified by bioassay are indicated in boldface.

TABLE 2 Antibiotic susceptibilities of transformants producing oxacillinases derived from *Pandoraea* species

Transformant or host strain	Oxacillinase	MIC (μg/ml) for[a]: *E. coli* DH5α													*S. enterica serovar* Enteritidis 104.773	
		AMX	AMX + BRL	PIP	PIP + TZB	CAZ	CAZ + BRL	CTX	FOX	ATM	MEM	MEM + BRL	IMP	IPM + 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
pT90	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	≤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

[a] 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 β-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 (256-fold) 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 β-lactamases. The G+C content of the bla_{OXA} 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 β-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 β-lactamases (Fig. 1), as follows. At positions 70 to 73 (class D β-lactamase-numbering, 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 β-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 β-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*

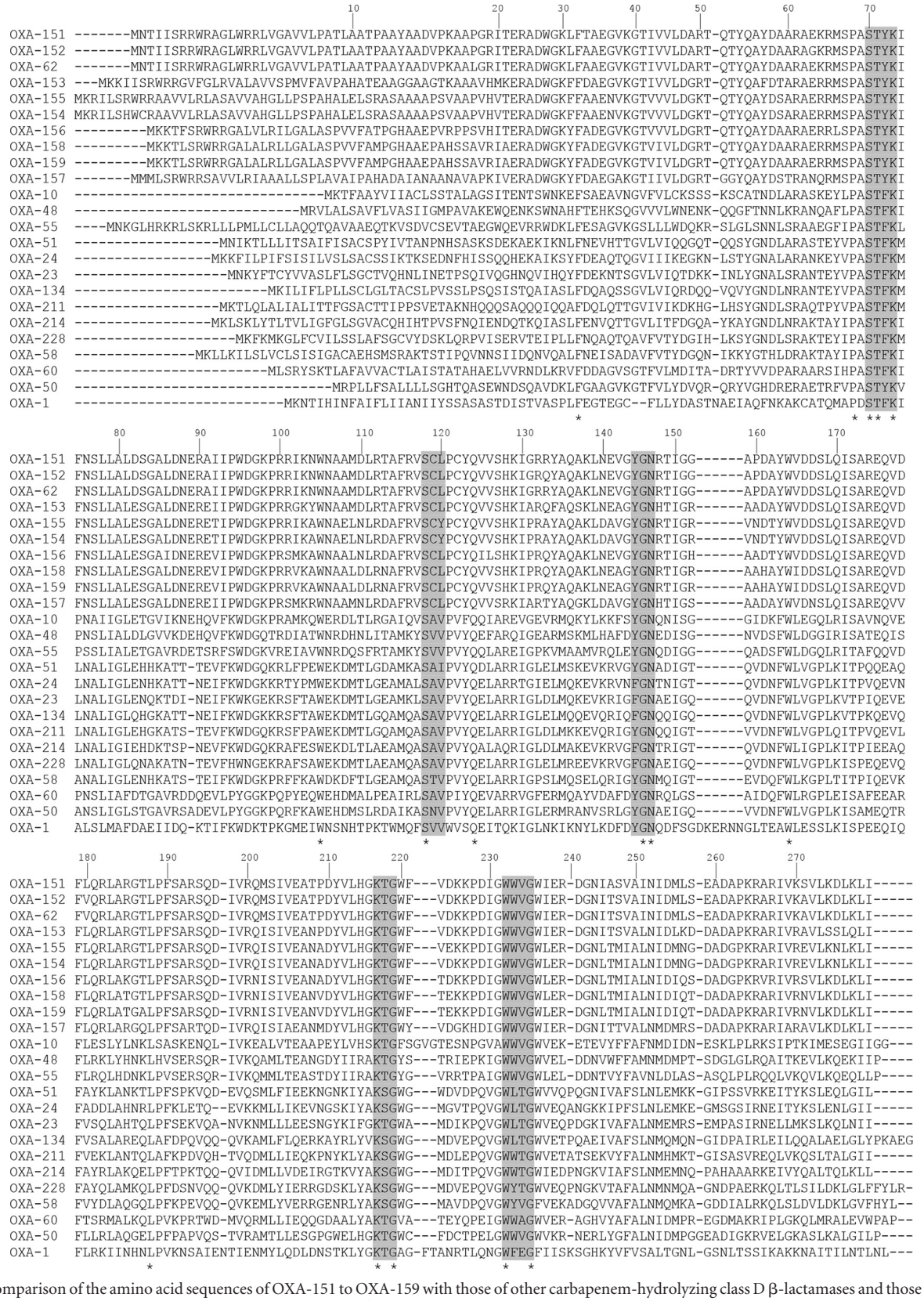

FIG 1 Comparison of the amino acid sequences of OXA-151 to OXA-159 with those of other carbapenem-hydrolyzing class D β-lactamases and those of OXA-1 and OXA-10. Identical residues are marked by asterisks, and motifs conserved among oxacillinases are shaded. The amino acid positions are numbered according 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_{OXA} 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_{OXA}. 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 β-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).

REFERENCES

1. **Patel G, Bonomo RA.** 2013. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol **4:**48. http://dx.doi.org/10.3389/fmicb.2013.00048.
2. **Héritier C, Poirel L, Nordmann P.** 2004. Genetic and biochemical characterization of a chromosome-encoded carbapenem-hydrolyzing ambler class D β-lactamase from *Shewanella algae*. Antimicrob Agents Chemother **48:**1670–1675. http://dx.doi.org/10.1128/AAC.48.5.1670-1675.2004.
3. **Poirel L, Naas T, Nordmann P.** 2010. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrob Agents Chemother **54:**24–38. http://dx.doi.org/10.1128/AAC.01512-08.
4. **Bonnin RA, Ocampo-Sosa AA, Poirel L, Guet-Revillet H, Nordmann P.** 2012. Biochemical and genetic characterization of carbapenem-hydrolyzing β-lactamase OXA-229 from *Acinetobacter bereziniae*. Antimicrob Agents Chemother **56:**3923–3927. http://dx.doi.org/10.1128/AAC.00257-12.
5. **Figueiredo S, Bonnin RA, Poirel L, Duranteau J, Nordmann P.** 2012. Identification of the naturally occurring genes encoding carbapenem-hydrolysing oxacillinases from *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, and *Acinetobacter calcoaceticus*. Clin Microbiol Infect **18:**907–913. http://dx.doi.org/10.1111/j.1469-0691.2011.03708.x.
6. **Poirel L, Héritier C, Nordmann P.** 2004. Chromosome-encoded ambler class D β-lactamase of *Shewanella oneidensis* as a progenitor of carbapenem-hydrolyzing oxacillinase. Antimicrob Agents Chemother **48:**348–351. http://dx.doi.org/10.1128/AAC.48.1.348-351.2004.
7. **Schneider I, Queenan AM, Bauernfeind A.** 2006. Novel carbapenem-hydrolyzing oxacillinase OXA-62 from *Pandoraea pnomenusa*. Antimicrob Agents Chemother **50:**1330–1335. http://dx.doi.org/10.1128/AAC.50.4.1330-1335.2006.
8. **Coenye T, Falsen E, Hoste B, Ohlén M, Goris J, Govan JR, Gillis M, Vandamme P.** 2000. Description of *Pandoraea* gen. nov. with *Pandoraea apista* sp. nov., *Pandoraea pulmonicola* sp. nov., *Pandoraea pnomenusa* sp. nov., *Pandoraea sputorum* sp. nov. and *Pandoraea norimbergensis* comb. nov. Int J Syst Evol Microbiol **50:**887–899. http://dx.doi.org/10.1099/00207713-50-2-887.
9. **Atkinson RM, LiPuma JJ, Rosenbluth DB, Dunne WM, Jr.** 2006. Chronic colonization with *Pandoraea apista* in cystic fibrosis patients determined by repetitive-element-sequence PCR. J Clin Microbiol **44:**833–836. http://dx.doi.org/10.1128/JCM.44.3.833-836.2006.
10. **Daneshvar MI, Hollis DG, Steigerwalt AG, Whitney AM, Spangler L, Douglas MP, Jordan JG, MacGregor JP, Hill BC, Tenover FC, Brenner DJ, Weyant RS.** 2001. Assignment of CDC weak oxidizer group 2 (WO-2) to the genus *Pandoraea* and characterization of three new *Pandoraea* genomospecies. J Clin Microbiol **39:**1819–1826. http://dx.doi.org/10.1128/JCM.39.5.1819-1826.2001.
11. **Fernández-Olmos A, Morosini MI, Lamas A, García-Castillo M, García-García L, Cantón R, Máiz L.** 2012. Clinical and microbiological features of a cystic fibrosis patient chronically colonized with *Pandoraea sputorum* identified by combining 16S rRNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol **50:**1096–1098. http://dx.doi.org/10.1128/JCM.05730-11.
12. **Johnson LN, Han JY, Moskowitz SM, Burns JL, Qin X, Englund JA.** 2004. *Pandoraea* bacteremia in a cystic fibrosis patient with associated systemic illness. Pediatr Infect Dis J **23:**881–882. http://dx.doi.org/10.1097/01.inf.0000136857.74561.3c.
13. **Jørgensen IM, Johansen HK, Frederiksen B, Pressler T, Hansen A, Vandamme P, Høiby N, Koch C.** 2003. Epidemic spread of *Pandoraea apista*, a new pathogen causing severe lung disease in cystic fibrosis patients. Pediatr Pulmonol **36:**439–446. http://dx.doi.org/10.1002/ppul.10383.
14. **Martínez-Lamas L, Rabade Castedo C, Martín Romero Domínguez M, Barbeito Castiñeiras G, Palacios Bartolomé A, Pérez Del Molino Bernal ML.** 2011. *Pandoraea sputorum* colonization in a patient with cystic fibrosis. Arch Bronconeumol **47:**571–574. http://dx.doi.org/10.1016/j.arbres.2011.06.015. (In Spanish.)
15. **Moore JE, Reid A, Millar BC, Jiru X, Mccaughan J, Goldsmith CE, Collins J, Murphy PG, Elborn JS.** 2002. *Pandoraea apista* isolated from a patient with cystic fibrosis: problems associated with laboratory identification. Br J Biomed Sci **59:**164–166.
16. **Stryjewski ME, LiPuma JJ, Messier RH, Jr, Reller LB, Alexander BD.** 2003. Sepsis, multiple organ failure, and death due to *Pandoraea pnomenusa* infection after lung transplantation. J Clin Microbiol **41:**2255–2257. http://dx.doi.org/10.1128/JCM.41.5.2255-2257.2003.
17. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing, 22nd informational supplement. M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.
18. **Matagne A, Ledent P, Monnaie D, Felici A, Jamin M, Raquet X, Galleni M, Klein D, François I, Frère JM.** 1995. Kinetic study of interaction between BRL 42715, beta-lactamases, and D-alanyl-D-alanine peptidases. Antimicrob Agents Chemother **39:**227–231. http://dx.doi.org/10.1128/AAC.39.1.227.
19. **Couture F, Lachapelle J, Levesque RC.** 1992. Phylogeny of LCR-1 and OXA-5 with class A and class D beta-lactamases. Mol Microbiol **6:**1693–1705. http://dx.doi.org/10.1111/j.1365-2958.1992.tb00894.x.
20. **Girlich D, Naas T, Nordmann P.** 2004. Biochemical characterization of the naturally occurring oxacillinase OXA-50 of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother **48:**2043–2048. http://dx.doi.org/10.1128/AAC.48.6.2043-2048.2004.
21. **Aravena-Román M.** 2008. Cellular fatty acid-deficient *Pandoraea* isolated from a patient with cystic fibrosis. J Med Microbiol **57:**252. http://dx.doi.org/10.1099/jmm.0.47671-0.
22. **Pimentel JD, MacLeod C.** 2008. Misidentification of *Pandoraea sputorum* isolated from sputum of a patient with cystic fibrosis and review of *Pan-*

doraea species infections in transplant patients. J Clin Microbiol **46:**3165–3168. http://dx.doi.org/10.1128/JCM.00855-08.

23. **Coenye T, Liu L, Vandamme P, LiPuma JJ.** 2001. Identification of *Pandoraea* species by 16S ribosomal DNA-based PCR assays. J Clin Microbiol **39:**4452–4455. http://dx.doi.org/10.1128/JCM.39.12.4452-4455.2001.
24. **Segonds C, Paute S, Chabanon G.** 2003. Use of amplified ribosomal DNA restriction analysis for identification of *Ralstonia* and *Pandoraea* species: interest in determination of the respiratory bacterial flora in patients with cystic fibrosis. J Clin Microbiol **41:**3415–3418. http://dx.doi.org/10.1128/JCM.41.7.3415-3418.2003.
25. **Coenye T, LiPuma JJ.** 2002. Use of the *gyrB* gene for the identification of *Pandoraea* species. FEMS Microbiol Lett **208:**15–19. http://dx.doi.org/10.1111/j.1574-6968.2002.tb11053.x.
26. **Payne GW, Vandamme P, Morgan SH, LiPuma JJ, Coenye T, Weightman AJ, Jones TH, Mahenthiralingam E.** 2005. Development of a *recA* gene-based identification approach for the entire *Burkholderia* genus. Appl Environ Microbiol **71:**3917–3927. http://dx.doi.org/10.1128/AEM.71.7.3917-3927.2005.
27. **Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P.** 2008. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. Antimicrob Agents Chemother **52:**1252–1256. http://dx.doi.org/10.1128/AAC.01304-07.