

# OXA-134, a Naturally Occurring Carbapenem-Hydrolyzing Class D $\beta$ -Lactamase from *Acinetobacter lwoffii*<sup>∇</sup>

Samy Figueiredo,<sup>1,2</sup> Laurent Poirel,<sup>1\*</sup> Harald Seifert,<sup>3</sup> Pauline Mugnier,<sup>1</sup>  
 Dan Benhamou,<sup>2</sup> and Patrice Nordmann<sup>1</sup>

Service de Bactériologie-Virologie, INSERM U914, Emerging Resistance to Antibiotics,<sup>1</sup> and Département Anesthésie-Réanimation,<sup>2</sup> Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, 94275 Le Kremlin-Bicêtre, France, and Institute for Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany<sup>3</sup>

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*Acinetobacter lwoffii*, a species whose natural habitat is the human skin, intrinsically possesses a chromosomal gene encoding a carbapenem-hydrolyzing class D  $\beta$ -lactamase, OXA-134. This species may therefore constitute a reservoir for carbapenemase genes that may spread among other *Acinetobacter* species.

*Acinetobacter baumannii*, the most common *Acinetobacter* species isolated from humans, is an opportunistic pathogen for which resistance to carbapenems is increasing worldwide (13–15). Carbapenem resistance in *A. baumannii* is associated mostly with acquired carbapenem-hydrolyzing class D  $\beta$ -lactamases (CHDLs) (19). Four groups of acquired CHDLs in *A. baumannii*, OXA-23, OXA-40, OXA-58, and OXA-143, have been identified (9, 18). In addition, *A. baumannii* possesses a

naturally occurring *bla*<sub>OXA-51</sub> or *bla*<sub>OXA-69</sub> CHDL-encoding gene that reduces the efficacy of carbapenems when it is over-expressed (4, 5, 7, 22). Identification of the sources of acquired and clinically relevant CHDLs is important to better understand the way and the reason why these resistance determinants are spreading. *Acinetobacter radioresistens* has recently been identified as the natural carrier of *bla*<sub>OXA-23</sub>, a gene encoding one of the most commonly acquired CHDLs in *A.*

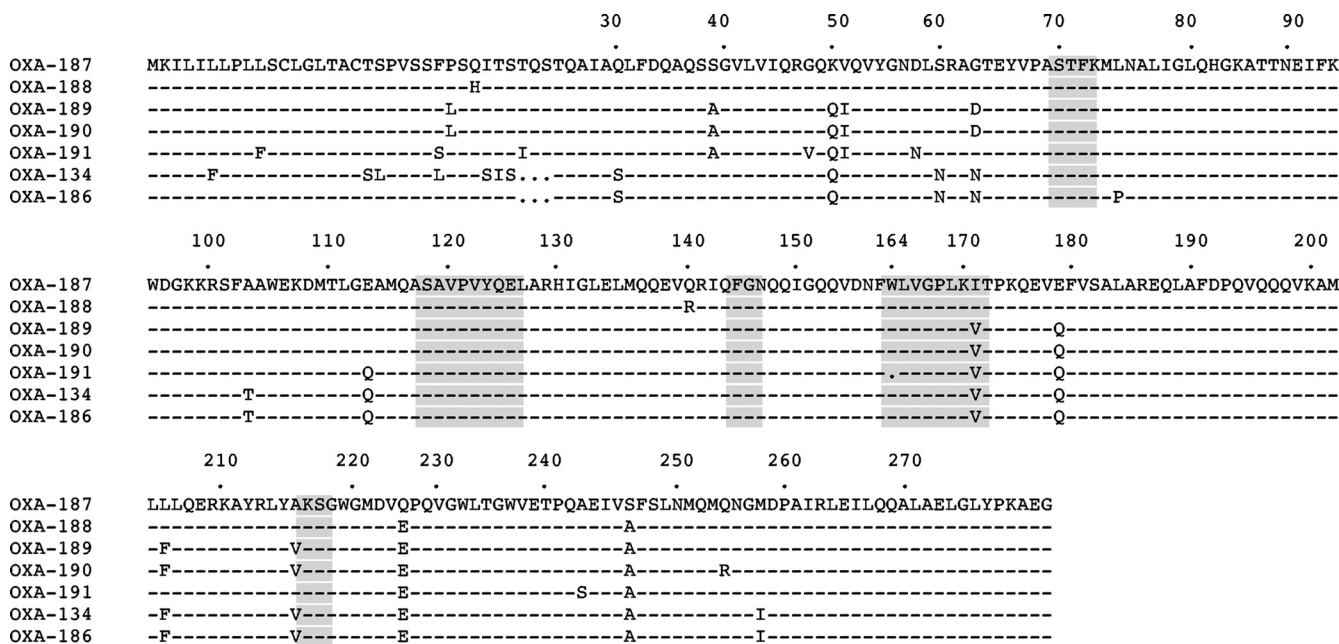


FIG. 1. Amino acid alignment of the seven OXA-134-like class D  $\beta$ -lactamases identified in this study. Dashes indicate amino acids identical to those in the OXA-187 sequence. Amino acid motifs which are well conserved (even if possibly variable) among class D  $\beta$ -lactamases are shaded in gray. Numbering is according to DBL numbering (2).

\* Corresponding author. Mailing address: Service de Bactériologie-Virologie, INSERM U914, Emerging Resistance to Antibiotics, Hôpital de Bicêtre, 78 rue du General Leclerc, 94275 Le Kremlin-Bicêtre, France. Phone: 33 1 45 21 36 24. Fax: 33 1 45 21 63 40. E-mail: laurent.poirel@bct.aphp.fr.

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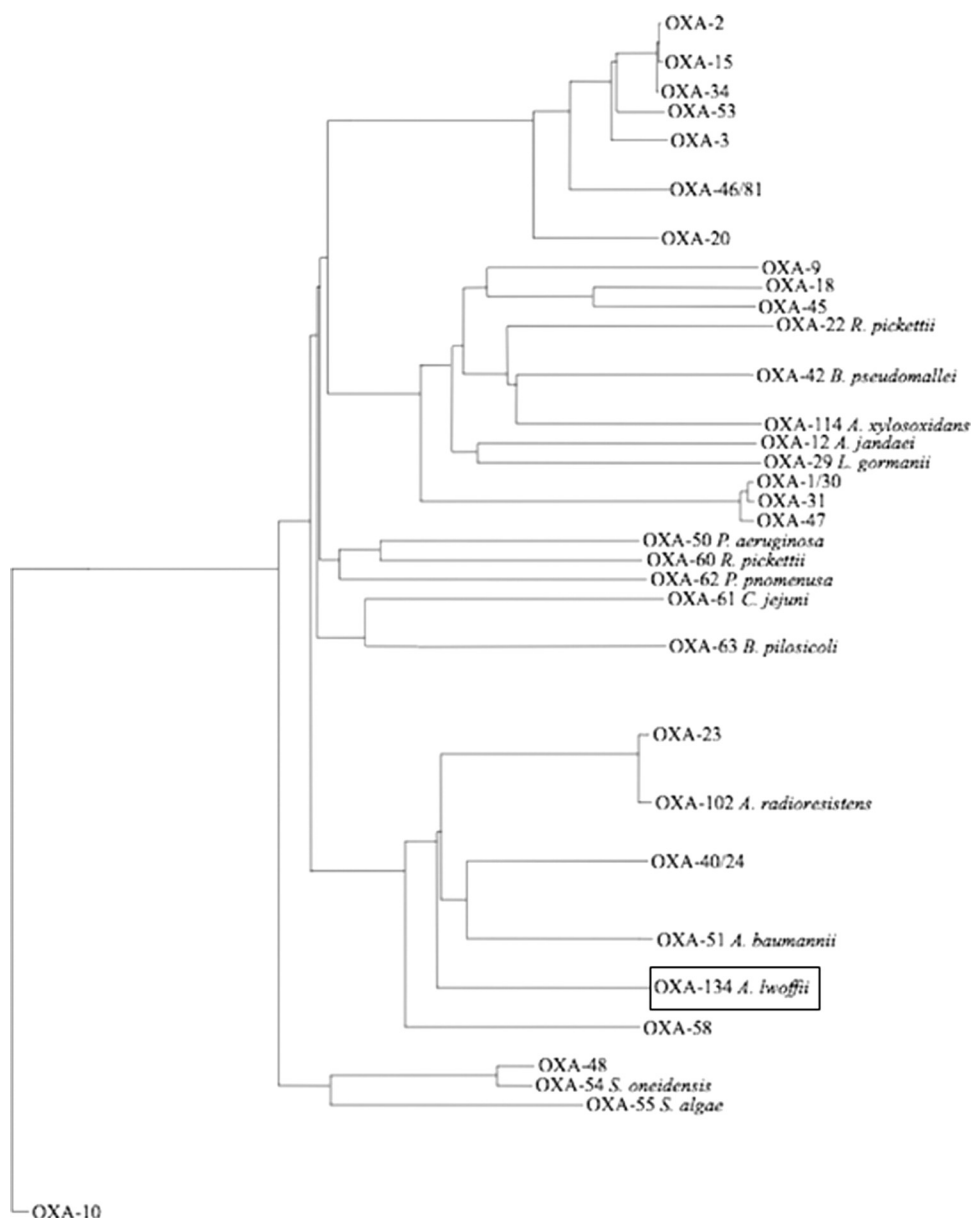


FIG. 2. Dendrogram obtained for 32 class D  $\beta$ -lactamases by neighbor-joining analysis. The alignment used for tree calculation was performed with ClustalX. Branch lengths are drawn to scale and are proportional to the number of amino acid changes. The distance along the vertical axis has no significance. The different clusters identified allowed the identification of nine main groups, considering that proteins from the same group have more than 80% amino acid identity. The class D  $\beta$ -lactamases which are considered to be naturally occurring are indicated together with the names of the corresponding species. *R. pickettii*, *Ralstonia pickettii*; *B. pseudomallei*, *Burkholderia pseudomallei*; *A. xylosoxidans*, *Alcaligenes xylosoxidans*; *A. jandaei*, *Aeromonas jandaei*; *L. gormanii*, *Legionella gormanii*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. pnomenususa*, *Pandoraea pnomenususa*; *C. jejuni*, *Campylobacter jejuni*; *B. pilosicoli*, *Brachyspira pilosicoli*; *S. oneidensis*, *Shewanella oneidensis*; *S. algae*, *Shewanella algae*.

*baumannii* (16). However, the progenitors of the other acquired CHDLs identified in *Acinetobacter* species remain unknown. Our study aimed to evaluate whether other *Acinetobacter* species may represent additional reservoirs of CHDL-encoding genes.

The screening panel included strains belonging to 23 *Acinetobacter* species, including *A. junii*, *A. johnsonii*, *A. haemolyticus*, *A. baylyi*, *A. lwoffii*, *A. radioresistens*, *A. schindleri*, *A. ursingii*, *A. calcoaceticus*, *A. gernerii*, *A. tjernbergiae*, *A. bouvetii*, *A. tandoii*, *A. grimontii*, *A. townneri*, *A. parvus*, and *Acinetobacter*

genomospecies 3, 6, 9, 10, 13, 15, 16, and 17. *Acinetobacter* genomospecies 9 is now classified as *A. lwoffii* (15). Strains were identified at the species level by using 16S rRNA sequencing (3). Susceptibility testing was analyzed by the disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (1), and MICs were determined by using Etest strips (AB bioMérieux, Solna, Sweden).

Screening for the known CHDL-encoding *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-40</sub>, *bla*<sub>OXA-58</sub>, and *bla*<sub>OXA-143</sub> genes was performed by

TABLE 1. MICs of  $\beta$ -lactams for the different *A. lwoffii* isolates, *E. coli* TOP10 harboring recombinant plasmid pOXA-134, and the *E. coli* TOP10 reference strain

$\beta$ -Lactam(s) <sup>a</sup>	MIC ( $\mu$ g/ml) for:		
	<i>A. lwoffii</i> isolates	<i>E. coli</i> TOP10(pOXA-134)	<i>E. coli</i> TOP10
Amoxicillin	0.5–1	>512	4
Amoxicillin + CLA	0.5–1	128	4
Ticarcillin	0.5–1	>512	4
Ticarcillin + CLA	0.5–1	256	4
Piperacillin	1.5–4	8	1
Piperacillin + TZB	1.5–4	8	1
Cephalothin	4–8	8	2
Cefuroxime	4–8	4	2
Ceftazidime	1–4	0.12	0.06
Cefotaxime	0.75–3	0.12	0.12
Cefepime	0.25–1	0.12	0.06
Moxalactam	1–4	0.12	0.06
Aztreonam	4–8	0.12	0.12
Imipenem	0.12–0.5	0.5	0.06
Meropenem	0.12–0.5	0.5	0.06

<sup>a</sup> CLA, clavulanic acid at a fixed concentration of 4  $\mu$ g/ml; TZB, tazobactam at a fixed concentration of 4  $\mu$ g/ml.

PCR using internal primers (8, 9). This screening was positive only for the *bla*<sub>OXA-23</sub> gene and only for *A. radioresistens* strain 3 and *A. lwoffii* strain 1. After sequencing, *A. radioresistens* strain 3 was found to possess the *bla*<sub>OXA-23</sub> gene, in accordance with previous results (16). Sequencing of the amplicon obtained from *A. lwoffii* strain 1 identified a gene encoding a novel OXA-type  $\beta$ -lactamase. Thermal asymmetric interlaced (TAIL) PCR experiments were performed in order to obtain the entire sequence of this gene (11, 12). It encoded a 273-amino-acid protein named OXA-134 that shared 63, 58, 57, and 53% amino acid identity with OXA-23, OXA-40, OXA-51, and OXA-58, respectively. OXA-134 possessed the typical features of a class D  $\beta$ -lactamase, including the STFK tetrad at positions 70 to 73 according to class D  $\beta$ -lactamase (DBL) numbering (Fig. 1) (2). Also, as observed for other CHDLs (except for OXA-58), an FGN motif at DBL positions 144 to 146 replaced the usual YGN motif of classical class D  $\beta$ -lactamases (18). Finally, a KSG element was identified at DBL positions 216 to 218, as observed in the CHDLs OXA-40 and OXA-51, whereas a KTG motif is present in most class D  $\beta$ -lactamases, including the CHDLs OXA-23 and OXA-58 (18). A phylogenetic analysis showed that OXA-134-like  $\beta$ -lactamases were constituting a separate subgroup of CHDLs but that this subgroup was more closely related to the identified class D  $\beta$ -lactamases from *Acinetobacter* spp. than to other known CHDLs (Fig. 2).

*A. lwoffii* is a commensal organism found on human skin, the perineum, and the oropharynx. It has been associated with catheter-related bloodstream infections in immunocompromised patients and with bacteremia associated with community-acquired gastroenteritis and gastritis (20, 21). All the *Acinetobacter* genomospecies 9/*A. lwoffii* isolates we included in our study were fully susceptible to all antibiotics tested, including penicillins, imipenem, and meropenem. It is therefore likely that the *bla*<sub>OXA-134</sub>-like genes were not expressed (or were expressed at an insignificant level) in these hosts.

In order to study the biochemical properties of OXA-134,

TABLE 2. Kinetic parameters for purified  $\beta$ -lactamase OXA-134<sup>a</sup>

Substrate	$k_{\text{cat}}$ (s <sup>-1</sup> )	$K_m$ ( $\mu$ M)	$k_{\text{cat}}/K_m$ ratio (s <sup>-1</sup> · mM <sup>-1</sup> )
Benzylpenicillin	70	50	1,400
Ampicillin	150	250	600
Ticarcillin	0.2	200	1
Piperacillin	30	200	150
Ceftazidime	<0.01	ND <sup>b</sup>	
Cefotaxime	<0.01	ND	
Cefepime	<0.01	ND	
Cefoxitin	<0.01	ND	
Aztreonam	<0.01	ND	
Imipenem	0.1	10	10
Meropenem	0.05	250	0.2

<sup>a</sup> Data are means of results from three independent experiments. Standard deviations were within 10% of the means.

<sup>b</sup> ND, no detectable hydrolysis (<0.01 s<sup>-1</sup>).

cloning of the *bla*<sub>OXA-134</sub> gene into the kanamycin-resistant plasmid pCR-BluntII-TOPO (Invitrogen, Life Technologies, Cergy-Pontoise, France) was performed using PCR products generated with primers PreOXA-134A (5'-GAAAAATGACC AAAATTTGATCG-3') and PreOXA-134B (5'-TATTTGCA TCATCCTTCAGC-3') as described previously (16). *Escherichia coli* TOP10(pOXA-134) showed reduced susceptibility to imipenem and meropenem and resistance to most penicillins that was not inhibited by  $\beta$ -lactamase inhibitors (Table 1).

To further characterize the activity of OXA-134, the enzyme was purified from *E. coli* cultures containing recombinant plasmid pOXA-134 as described previously (17). After DNase treatment and ultracentrifugation at 40,000  $\times$  g for 1 h, the extract was loaded successively onto two Q-Sepharose columns with 20 mM diethanolamine (pH 8.5) and 20 mM diethanolamine (pH 9.5) buffers. The specific activity of the purified  $\beta$ -lactamase OXA-134, measured with 100  $\mu$ M imipenem as the substrate, was 116 U · mg of protein<sup>-1</sup>, with a 20-fold purification factor. The kinetic measurements of the purified enzymes were carried out at 25°C in 50 mM sodium phosphate (pH 7.0), and  $K_m$  and  $k_{\text{cat}}$  values were determined as described previously (6).  $\beta$ -Lactamase OXA-134 showed a narrow-spectrum hydrolysis profile, including mostly penicillins (Table 2). The rates of imipenem and meropenem hydrolysis were low, whereas the MICs of both carbapenems for *E. coli* TOP10 expressing OXA-134 were increased by 3-fold (Table 1). Overall, the catalytic activities obtained for OXA-134 were similar to those for OXA-58 and OXA-40, taken as references for CHDL activity (17).

In order to assess whether the *bla*<sub>OXA-134</sub>-like gene was naturally present in *A. lwoffii*, a *bla*<sub>OXA-134</sub>-specific PCR was performed using whole-cell DNA samples from a collection of 10 *A. lwoffii* isolates recovered from clinical specimens, including blood cultures, urine samples, cerebrospinal fluids, and central venous catheter tips, from Bicêtre and Cologne hospitals. PCR results showed that all strains possessed a *bla*<sub>OXA-134</sub>-like gene. Sequencing of the amplicons allowed the identification of six additional OXA-134 derivatives (named OXA-186 to OXA-191) (see www.lahey.org/Studies) differing by 3 to 18 amino acid substitutions (Fig. 1). Noteworthy, OXA-134 and OXA-186 each possessed 273 amino acids whereas the five other variants each possessed an additional

3-amino-acid stretch (Fig. 1). In three isolates, the *bla*<sub>OXA-134</sub>-like gene was disrupted by nucleotide substitutions located in the center of the gene and likely leading to interruption of the open reading frame (data not shown). In order to evaluate whether an OXA-134-like variant possessing additional amino acids may confer a different  $\beta$ -lactam resistance pattern, the *bla*<sub>OXA-187</sub> gene chosen as a representative was cloned and expressed in the same manner as the *bla*<sub>OXA-134</sub> gene. MICs of  $\beta$ -lactams for *E. coli*(pOXA-187) were similar to those for *E. coli*(pOXA-134) (data not shown), showing that those additional amino acids did not play any significant role in hydrolysis. The chromosomal locations of the *bla*<sub>OXA-134</sub>-like genes in these *A. lwoffii* isolates were confirmed by using the endonuclease I-CeuI technique, as described previously (10).

In order to evaluate whether *bla*<sub>OXA-134</sub>-like genes might have disseminated among *A. baumannii* strains, a collection of 100 *A. baumannii* isolates (with variable susceptibilities to imipenem, including 50 carbapenem-resistant isolates) were screened by PCR. None of the screened *A. baumannii* isolates harbored a *bla*<sub>OXA-134</sub>-like gene.

*A. lwoffii* was found to be a reservoir of a novel type of CHDL-encoding gene. Detection of that  $\beta$ -lactamase gene might be used as a tool for rapid and accurate identification of the *A. lwoffii* species.

**Nucleotide sequence accession number.** The nucleotide sequence of the *bla*<sub>OXA-134</sub> gene described in this work is available in the GenBank nucleotide database under accession number HQ122933.

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