LMB-1, a novel subclass B3 metallo-β-lactamase from a carbapenem-resistant *Enterobacter cloacae* isolate

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Nosocomial infections pose a growing challenge to general healthcare as antimicrobial resistance spreads rapidly among bacteria. β-lactam antibiotics are of essential importance for the treatment of infections with gram negative bacilli. Resistance against β-lactams in gram negative species is commonly conferred by expression of β-lactamases. Metallo-β-lactamases are carbapenemases conferring resistance to almost all β-lactam antibiotics. As no efficient inhibitors of metallo-β-lactamase activity are available for clinical use, expression of metallo-β-lactamase strongly diminishes treatment options for bacterial infections.

Here we report a clinical isolate of *Enterobacter cloacae* of sequence type 443 yielding an entirely novel metallo-β-lactamase. Resistance against penicillins, cephalosporins and carbapenems was detected. Carbapenemase detection was performed by the modified Hodge test and a bioassay based on cell-free extracts. Inhibition of carbapenemase activity could be achieved by EDTA but not by clavulanic acid or boronic acid. No metallo-β-lactamase gene could be detected by PCR. Whole genome sequencing of the isolate revealed a sequence with low homology to the subclass B3 metallo-β-lactamase L1 of *Stenotrophomonas maltophilia.* The novel gene was heterologously expressed *in Escherichia coli* Top 10 and were tested for beta-lactam resistance by disc diffusion and Etest. As the isolate was found in Linz, Austria, the novel metallo-β-lactamase was named Linz metallo-β-lactamase (LMB-1).

Table 1: Inhibition zone diameters (mm) and MICs (mg/l) of the strain carrying pBK-CMV-*bla*$_{LMB-1}$ compared to of the strain carrying pBK-CMV.

	E. coli TOP10			
	Disc diffusion		**Etest**	
	pBK-CMV	pBK-CMV-LMB-1	pBK-CMV	pBK-CMV-LMB-1
ampicillin	19	6	2	>256
ampicillin/sulbactam	16	6	2	>256
amoxicillin/clavulanic acid	20	6	3	>256
piperacillin	27	6	3	192
cefuroxime	22	6	4	>256
cefoxitine	25	6	64	>256
cefotaxime	35	6	0,094	16
ceftazidime	30	6	0,38	32
cefepime	40	29	0,032	0,5
imipenem	35	18	0,25	2
meropenem	40	24	0,023	0,5
ertapenem	40	21	0,004	1,5
aztreonam	39	39	0,094	0,125

LMB-1 confers resistance against penicillins, cephalosporins and carbapenems but not aztreonam. LMB-1 is insensitive against β-lactamase inhibitors.

LMB-1 showed highest identity (55%) to EFM-1, a metallo-β-lactamase found in *Erythrobacter flavus*, a marine bacterium.

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EFM-1    1 ----msarc---lpaltlplalaacappiqtpdplppreivtpqqwaqqc
LMB-1    1 mtlaksfrcfclvttlssmamlagcggtapvtvlspp-----padwvnsc

EFM-1   44 tdwdewdkpappyrihgstyyvgtcgiaailvagdgghilidsgteagad
LMB-1   46 kdwddwdkagppyriygnsyyvgtcgisailitgdnghilidgateagak

EFM-1   94 vvldnvgklgfrpneiasllhshehfdhtgghalvrresgahvvaspqaa
LMB-1   96 viaanidrlgfslrdvklllqshehfdhvaglaqlqqqsgakllaspaaa

EFM-1  144 pvlrtgeddpadpqhglhdamepvpvdvivrdgetvrsesatltaiatpg
LMB-1  146 pvltsgvvaaadpqagmhepfpavrvdglvtagqvvtlgklsllpvatpg

EFM-1  194 htpgalswawqscdeagdclsivyadslspisrkgyrfgdhpeylegfra
LMB-1  196 htpgalswqwssc-eagqcqvlvyadslspvssdsyrfsehltylnayra

EFM-1  244 gleklravdcdilltphpsasdmvtra-aagsmvggmscvdyadavearl
LMB-1  245 slhklaaldcqilltphpsasnmrtrlqssagltdtqgcvvyadaitqrl

EFM-1  293 darlveeagd
LMB-1  295 eqrlikettq
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Figure 1: Amino acid sequence alignment between LMB-1 and EFM-1

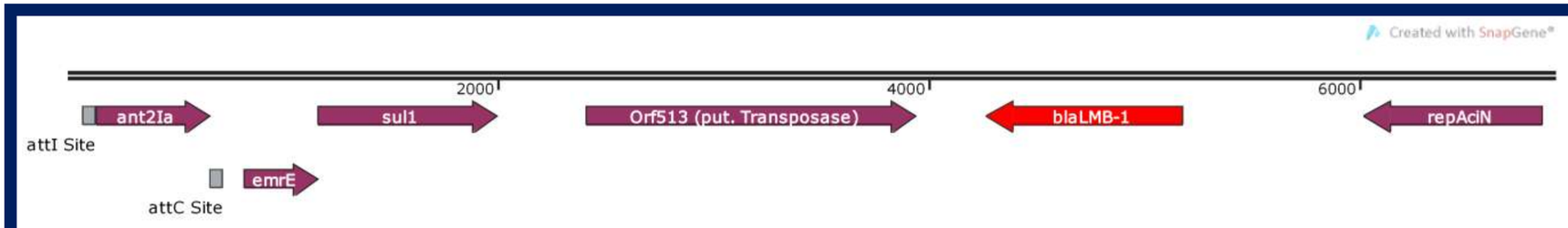

Figure 2: The proximate genetic environment of *bla*$_{LMB-1}$ comprised the 3‘ end of an integron and *Orf513*, a putative transposase often associated with Integrons. It is possible that *bla*$_{LMB-1}$ can be mobilised by *Orf513*. Upstream of *bla*$_{LMB-1}$, a replicase gene was located.

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