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First Report of an *Enterobacter ludwigii* Isolate Coharboring NDM-1 and OXA-48 Carbapenemases

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Enterobacter spp. have emerged as pathogens responsible for hospital-acquired infections. Carbapenem resistance is increasingly being reported in this species, which is a matter of concern. *Enterobacter* spp. can produce abdominal, urinary tract, meningeal, and surgical site infections. In the present study, we report a case of *Enterobacter ludwigii* infection in a 17-year-old female who underwent surgery for an aneurysmal bone cyst in her left distal femur. She was treated with intravenous cefotaxime and amikacin postoperatively. She developed a purulent discharge from the surgical site after 72 h of surgery. Microbiological culture of the purulent material on two consecutive days grew a Gram-negative, motile bacillus in pure culture, which was identified to the species level as *Enterobacter ludwigii* by its growth on myo-inositol and 3-0-methyl-D-glucopyranose and then phenotypically confirmed by the Vitek-2 test (bioMérieux, Marcy l'Etoile, France). The antibiotic susceptibility test was performed by the disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) (1) guidelines using commercial antibiotic discs (Hi Media, Mumbai, India). MICs of antibiotics were determined by Vitek-2 per CLSI breakpoints (Table 1). MICs of meropenem and imipenem determined by the Etest were >32 μg/ml for the isolate. The production of metallo-β-lactamase (MBL) was confirmed by positive results from the double-disc synergy test, modified Hodge test, combined-disc synergy test, and MBL (IP/IPI) Etest method (bioMérieux, Marcy l'Etoile, France). PCR-based screening for beta-lactamase genes (bla_{CTXM}, bla_{SHV}, bla_{TEM}, and bla_{OXA}) and the Ambler class (B and D) carbapenemase genes bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{GIM}, bla_{SIM}, bla_{NDM-1}, bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA48} gave positive results for bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{OXA-2}, and bla_{OXA-48} (2–5), confirmed by sequencing. 16S rRNA gene sequence analysis showed a sequence identity value of 99% with GenBank accession number KC139450. PCR primers were procured from Sigma-Aldrich, India. Plasmid analysis using the Kieser technique (6) revealed that *E. ludwigii* harbored three plasmids, with sizes of 130, 50, and 35 kb. Conjugation using *E. coli* J53 (azide resistant) as the recipient strain revealed the bla_{NDM-1} gene to be present on a 130-kb plasmid. Using the PCR-based replicon typing (PBRT) (7) method, we identified an IncA/C type (amplicon size of 465 bp) that was cotransferred with two other plasmids of 35 and 50 kb. The $bla_{CTX-M-15}$ gene together with bla_{OXA-2} was identified on the 35-kb plasmid, which belonged to the IncFIB type and had an amplicon size of 702 bp. The bla_{OXA-48} gene was identified on a 50-kb plasmid that belonged to the IncFIA type and had an amplicon size of 462 bp. Although the coexistence of bla_{NDM-1} with different carbapenemase genes has been reported, we believe this to be the first report of the cooccurrence of bla_{NDM-1} and bla_{OXA-48} in a clinical isolate of *E. ludwigii* from central India. The growing emergence of carbapenem-resistant *Enterobacter* is cause for great concern, as treatment options are virtually exhausted.

TABLE 1 Antibiogram of *bla*-positive *E. ludwigii* and its transconjugant *E. coli* J53

Antibiotic[a]	MIC (μg/ml) for:	
	Enterobacter ludwigii 12	*E. coli* J53 EL 12
IPM	32	2
MEM	32	4
ETP	16	2
AMK	>64	32
GEN	>16	8
TOB	>16	4
CIP	>4	0.5
MXF	>8	0.5
TGC	<0.5	<0.5
SXT	>320	20
SAM	>128	>128
TZP	>128	>128
FEP	>64	16
AMP	>64	32
CRO	>64	16
CAZ	>64	32
CTX	>64	32
CS	<0.5	<0.5

[a] IPM, imipenem; MEM, meropenem; ETP, ertapenem; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; MXF, moxifloxacin; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; FEP, cefepime; AMP, ampicillin; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; CS, colistin.

Nucleotide sequence accession numbers. The 16S rRNA and bla_{NDM-1} nucleotide sequences from the clinical isolate of *E. ludwigii* have been assigned accession numbers KC182049 and KC182052, respectively.

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