

**TITLE:** *Citrobacter freundii* CO-PRODUCING KPC-2 AND OXA-370 IN RECREATIONAL COASTAL WATERS

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**ABSTRACT:** KPC and OXA-48-like  $\beta$ -lactamases are important determinants of carbapenem resistance among *Enterobacteriaceae*. The spread of carbapenemase-producing isolates on hospital settings is considered a major health concern. Nonetheless, these microorganisms have also been reported in polluted aquatic matrices. Here we characterized *Citrobacter freundii* co-producing KPC-2 and OXA-370, isolate LB\_887. It was recovered on June 2014, from Leblon's coastal water, Rio de Janeiro, Brazil. Counts of fecal indicator bacteria classified water sample as suitable for primary contact. *bla*<sub>KPC-2</sub> and *bla*<sub>OXA-370</sub> were identified with PCR and amplicon sequencing. LB\_887 had a MDR phenotype, only susceptible to tigecycline, fosfomicin and colistin. Its whole genome sequence was determined using Illumina MiSeq platform. De novo assembly was conducted using SPAdes and annotation was accomplished using RAST server and Geneious software v.R9. MLST analysis revealed *C. freundii* LB\_887 belonged to a new ST. ResFinder identified further acquired resistance determinants to  $\beta$ -lactams (*bla*<sub>OXA-1</sub>, *bla*<sub>OXA-2</sub>), aminoglycosides (*aph3'-Ia*, *aadA1*, *aadB*, *aacA4*, *aac(6')Ib-cr*), sulfonamides/trimethoprim (*sul1*, *dfrA19*), tetracyclines [*tet(C)*], chloramphenicol (*catA1*, *catB3*), macrolides [*mph(A)*], rifampicin (*arr-3*) and fluoroquinolones (*aac(6')Ib-cr*, *qnrB-34*, *qnrB-2*). *In vitro* and *in silico* experiments suggested that *bla*<sub>KPC-2</sub> was not inserted on a Tn4401 structure, whereas *bla*<sub>OXA-370</sub> was flanked by IS5075-like and IS4-like insertion sequences. Electrophoresis of S1-treated total DNA revealed seven plasmids (ranging from about 5 to 170 kb), and hybridization experiment showed *bla*<sub>KPC-2</sub> harbored on a small plasmid of about 9 kb. Moreover, the incompatibility groups IncA/C<sub>2</sub>, IncR, Col-family and IncQ-family were identified by PlasmidFinder. Repetitive mating-out assays failed to provide transconjugants carrying either *bla*<sub>KPC-2</sub> or *bla*<sub>OXA-370</sub>. Carbapenemase-producing *Enterobacteriaceae* in coastal waters reinforces that aquatic environments are reservoirs and possible vectors of resistance genes. Recreational activities, common at this beach, may facilitate the spread of these antimicrobial resistance threats throughout the community. Although these microorganisms frequently colonize and/or cause serious infections in immunocompromised patients, the real impact of this occurrence to health of beach frequenters remains undetermined.

**Key-words:** Enterobacteriaceae, Carbapenemase, Environmental dissemination, Antimicrobial resistance genes

**Financial support:** CNPq, CAPES, FAPERJ