

TITLE: CO-OCCURRENCE OF bla_{KPC-2} AND *fosA* GENES IN *Enterobacter cloacae* ISOLATED FROM AN URBAN RIVER IN PARAÍBA, BRAZIL.

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ABSTRACT:

The increased prevalence of extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* in the environment has been attributed to anthropogenic activities related to the overuse of antimicrobial agents. Environmental reservoirs of ESBL-producing *Enterobacteriaceae* is a major issue in public health since they could be transmitted to humans and other animals. Currently, the bla_{KPC-2} gene is no longer limited to *K. pneumoniae* species but has been identified in a wide range of Gram-negative bacteria, and in regard to CTX-M β -lactamases, isolates carrying CTX-M-8 and other genes have been found worldwide. This study reports the occurrence of an ESBL-producing *Enterobacter cloacae* harboring bla_{KPC-2} gene isolated from an urban river (Jagaribe River) crossing the metropolitan area of João Pessoa city, northeastern Brazil. The isolated was recovered in a study to monitor the occurrence of multidrug-resistant (MDR) Gram-negative bacteria from urban river at water samples were collected from different locations along the Jaguaribe River and filtered using a sterile filter membrane (0.45 μ m) pore size. The membranes were placed in 20 ml of BHI broth (Falcon tubes) and vortexed for 10 sec. Aliquots (100 μ l) from each samples were streaked onto McConkey agar (McA) in paralel with McA supplemented with ceftriaxone (16 μ g/ml); and also McA supplemented with meropenem (1 μ g/ml) plus 70 μ g/ml ZnSO₄ in order to detect carbapenemase producing samples (KPC, Metalo) and incubated at 37^oC/18-24h. The samples were also inoculated in Chomagar ESBL and Chomagar KPC (Probac). Isolates grown on the selective plates containing ceftriaxone were screened for ESBL by the standardized disk approximation test. Isolates grown on selective plates supplemented with meropenem were screened for carbapenemase using the Carba NP test (Biomerieux). The isolates were identified by routine biochemical test and confirmed using MALDI-TOF (Bruker). Analysis and the antimicrobial resistance patterns were determined by disc diffusion method using antimicrobials belonging to four different classes: beta-lactams (carbapenems and cephalosporins); quinolones; aminoglycosides; fosfomicyn and sulfamethoxazole-trimethoprim, according EUCAST. The carbapenemase-encoding genes were screened by PCR and selected strains were analysed by Whole Genome Sequence (WGS) using a MiSeq platform (Illumina Inc., San Diego, CA). An *Enterobacter cloacae* strain ST 1 was shown to harbor both bla_{KPC-2} and *fosA* genes, as well as several other resistance genes, such as aminoglycoside-modifying enzymes [*aadA1*, *aac(6')-Ib*, *aph(3')-V*], β -lactamase [*bla*_{CTX-M-8}, *bla*_{CMH-3}, *bla*_{TEM-1A}, *bla*_{OXA-9}] and quinolones [*qnrE*, *qnrB19*, *aac(6')Ib-cr*] encoding genes. The detection of bla_{KPC-2} and *fosA* genes in a single isolate suggest that some commensal Gram negative strains found in the environmental might can be highly resistant to antimicrobials and pose a risk to public health. In addition, our findings underscore the distribution of resistant bacteria and highlight a new possible reservoir of bla_{KPC-2} harboring *Enterobacteriaceae* strains, as well as, several other resistance genes from environmental source. In summary, this is the first report on the occurrence of bla_{KPC-2} and *fosA* in enterobacteria cultured from an urban river in northeastern Brazil. Surveillance of antimicrobial resistance in microbes from the environmental in urban regions needs to be established as a priority in order to, establish strategies to control high-risk multiresistant bacteria into the environment.

Key words: Enviromental microbiology, Urban river, resistance genes