

TITLE: EVALUATION OF *MCR* GENES AND THEIR ASSOCIATED MOBILE GENETIC ELEMENTS IN *ENTEROBACTERIACEAE* RECOVERED FROM COASTAL WATERS.

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ABSTRACT:

Polymyxins are last resource antimicrobials used for treating infections caused by multidrug resistant bacteria. However, emergence and dissemination of plasmid-mediated *mcr* genes are threatening their therapeutic efficacy. Since its first description, *mcr* variants have spread to all continents. In Brazil, studies reported *mcr*-carrying *Enterobacteriaceae* in clinical and food producing animal isolates from several States countrywide. Regarding environmental detection, *mcr*-1 has been found in bacteria isolated from beach water in São Paulo and from a mangrove in Sergipe. Such findings suggest that *mcr* might be disseminating to aquatic environments along the coast. Our aim was to detect these gene in strain isolated from Cabo Branco beach, João Pessoa, PB. Superficial water sample was collected, and different sample volumes were filtered through 0.22µm membranes that were plated onto MacConkey agar supplemented with colistin (3.5µg/ml) and the one exhibiting 20 to 50 colonies was selected for further analyses. Each colony was then subcultured and microbial identification performed by MALDI-TOF-MS. Colistin resistance screening included polymyxin NP Test, minimum inhibitory concentration and multiplex PCR for *mcr*. Plasmid incompatibility group was also assessed by PCR. Conjugation of *mcr* positive isolates was performed employing *E. coli* J53 AZI® as the recipient strain. Plasmid extraction, Southern blot and hybridization were used to confirm *mcr* genetic location within the strain and transconjugants. A total of 50 bacteria were recovered and six of them yielded positive results in polymyxin NP phenotypic. However, only *Escherichia coli* JP24 was positive for *mcr*-1. Additional analysis of JP24 revealed a colistin MIC > 128 mg/L. The *mcr*-1 gene was carried by a conjugative plasmid belonging to the IncX4 family. Antimicrobial resistance determinants found in JP24 included *bla*_{CTX-M-8}, *tet*(A), *dfrA12*, *cmIA1* and mutations in the quinolone resistance-determining region of *gyrA* (S83L and D87N) and *parC* (S80I). Our findings support that *mcr* have already reached the aquatic environment in another region of Brazil. Beaches are widely used for sports and recreation and may be an important source of contamination to the community. To acknowledge how *mcr* genes spread, not only in healthcare settings, but also in the

environment, may enable future development of measures devoted to control such dissemination.

Keywords: *Enterobacteriaceae*, Multidrug resistance, seawater, Brazilian Northeast.

Development Agency:

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