

MCR-1-, KPC-2-, and CTX-M-producing Enterobacteriaceae in Marine Ecosystem: an Infectious Threat Emerging in Brazilian Coastal Waters

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Background: The presence of multidrug-resistant (MDR) Enterobacteriaceae in aquatic environments has generated an important public health problem, creating an urgent need to strengthen surveillance. Urbanized and densely populated coast lines have produced a considerable amount of untreated sewage and organic matter containing antimicrobial drug residues as well as MDR bacteria, which are frequently dumped into watercourses that flow down to the sea. In this study, we have monitored the dissemination of MCR-1, KPC-2 and CTX-M-producing Enterobacteriaceae in the marine ecosystem of the southeast coast of Brazil. **Methods:** We screened 170 samples including, bivalves ($n= 30$), osseous fishes ($n= 30$), marine rays ($n= 40$), infected ($n= 13$) and healthy ($n = 20$) penguins, green turtles ($n= 25$), and seawater ($n= 12$), collected from different coastal points of Santos and São Vicente Cities, São Paulo state. The samples were processed according to standardized methods and then streaked onto MacConkey agar plates supplemented with colistin (2 μ g/ml), meropenem (2 μ g/ml), or ceftriaxone (2 μ g/ml). Screening for *mcr-1*, *bla*_{KPC}, and *bla*_{ESBL} genes was performed by PCR. Strains positive for such genes were submitted

to whole genome sequencing predicted by MLSTFinder, ResFinder, and PlasmidFinder, respectively. **Results:** Twenty-one (12.4%) Enterobacteriaceae isolates exhibited a MDR profile, which were obtained from seawater (8/12, 66.6%), bivalves (8/30, 26.6%), penguins' footpad infection (3/13; 23%), and osseous fishes (2/30; 6.66%). *E. coli* strains carrying *bla*_{CTX-M} genes were found in seawater samples, bivalves, infected penguins, and fishes. These strains belonged to ST10, ST38, ST46, ST131, ST155, ST302, ST457, ST466, ST1284, ST1638, and ST4012. Interestingly, *E. coli* carrying IncX4 plasmid-mediated *mcr-1* was found in two seawater samples and one infected penguin. The strain isolated from infected penguin displayed 100% of identity with an *E. coli* ST10 strain isolated from seawater, confirming an epidemiological link. Two *bla*_{CTX-M-15}-producing *Klebsiella pneumoniae* belonging to ST307 and ST2646 were recovered from bivalves, whereas an *Enterobacter cloacae* ST520 co-producing *bla*_{KPC-2} and *bla*_{CTX-M-15} was recovered from a seawater sample. **Conclusion:** Polluted marine environments can act as potential reservoirs for MDR bacteria carrying clinically significant resistance genes, exposing coastal inhabitants, tourists, and wildlife animals to an eminent risk.