

Master Thesis: Characterization of antibiotic resistance in strains isolated from different environmental reservoirs

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Abstract

Antibiotic resistance is an emerging Public Health problem that causes high mortality due to the emergence of multidrug-resistant clones for which there is no effective treatment. Despite this problem being mostly associated with healthcare facilities, other antibiotic resistance reservoirs have gained some relevance such as animals, water, and soils, in a One Health perspective. The aim of this study was to characterize the antibiotic resistance in strains isolated from different aquatic environmental reservoirs. In total, 143 strains belonging to 45 different species were isolated. Characterization of antibiotic resistance was performed in *Enterobacter cloacae* complex strains due to their importance in Public Health and for being one of the most represented species in the analyzed reservoirs (n=29, in five reservoirs: river water, and affluent, surface water, sewage sludge and effluent of a wastewater treatment plant). Phenotypic characterization (by disk diffusion and minimum inhibitory concentration) was done for seven antibiotic classes, allowing the oriented search of antibiotic resistance genes by PCR (Polymerase Chain Reaction). The genetic diversity was studied using MLST (Multilocus Sequence Typing). Whole Genome Sequencing was performed for a subset of 12 strains presenting the most relevant phenotypes. Freeware web-based tools were used to identify antibiotic resistance genes, virulence factors, plasmids and respective pMLST, and other mobile genetic elements (MGE), and determine the pathogenicity for humans. Phylogenetic analysis was performed by using CSI Phylogeny v1.4 with default options. Most of the strains were susceptible to all antibiotics tested except the natural resistances. However, 86% were resistant to at least one carbapenem and 93% were resistant to colistin. Globally we identified genes related with a decreased susceptibility to β -lactams (*blaFRI-8*, *blaIMI-6*, and *blaACT*-type), quinolones (*oqxAB*-type), fosfomicin (*fosA2*-type), macrolides (*mdfA*-type) and several efflux pumps. We also detected virulence factor (*iroN*), plasmids [*IncFII(Yp)*, *Col(MG828)*, *Col(pHAD28)*], and different MGE. The study of the genetic diversity allowed identifying five different STs: ST1688 was the most represented, being identified in 83% of strains, in four of the five studied environmental reservoirs. The phylogenetic analysis identified three main clades, differing between 6 and 49517 SNPs, with closely related strains from different reservoirs and seasons. In conclusion, this study demonstrated the diversity of antibiotic resistance genes (ARGs) and MGE within *E. cloacae* complex strains isolated from different aquatic environments, confirming that they can act as a reservoir of antibiotic resistant bacteria and ARGs that can be pathogenic to humans. Thus, it is necessary to implement a monitoring and surveillance network not only in water, but also in other environmental reservoirs to avoid their dissemination.