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Prevalence and Molecular Characterization of Extended-Spectrum-β Lactamase and Carbapenemase-Producing Enterobacterales from Tunisian Seafood

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Abstract : Multi-resistance to antibiotics in gram-negative bacilli and particularly in enterobacteriaceae, has become frequent in hospitals in Tunisia. However, data on antibiotic resistant bacteria in aquatic products are scarce. The aims of this study are to estimate the proportion of ESBL- and carbapenemase-producing Enterobacterales in seafood (clams and fish) in Tunisia and to molecularly characterize the collected isolates. Two types of seafood were sampled in unrelated markets in four different regions in Tunisia (641 pieces of farmed fish and 1075 mediterranean clams divided into 215 pools, and each pool contained 5 pieces). Once purchased, all samples were incubated in tubes containing peptone salt broth for 24 to 48h at 37°C. After incubation, overnight cultures were isolated on selective MacConkey agar plates supplemented with either imipenem or cefotaxime, identified using API20E test strips (bioMérieux, Marcy-l'Étoile, France) and confirmed by Maldi-TOF MS. Antimicrobial susceptibility was determined by the disk diffusion method on Mueller-Hinton agar plates and results were interpreted according to CA-SFM 2021. ESBL-producing Enterobacterales were detected using the Double Disc Synergy Test (DDST). Carbapenem-resistance was detected using an ertapenem disk and was respectively confirmed using the ROSCO KPC/MBL and OXA-48 Confirm Kit (ROSCO Diagnostica, Taastrup, Denmark). DNA was extracted using a NucleoSpin Microbial DNA extraction kit (Macherey-Nagel, Hoerdt, France), according to the manufacturer's instructions. Resistance genes were determined using the CGE online tools. The replicon content and plasmid formula were identified from the WGS data using PlasmidFinder 2.0.1 and pMLST 2.0. From farmed fishes, nine ESBL-producing strains (9/641, 1.4%) were isolated, which were identified as E. coli (n=6) and K. pneumoniae (n=3). Among the 215 pools of 5 clams analyzed, 18 ESBL-producing isolates were identified, including 14 E. coli and 4 K. pneumoniae. A low isolation rate of ESBL-producing Enterobacterales was detected 1.6% (18/1075) in clam pools. In fish, the ESBL phenotype was due to the presence of the blaCTX-M-15 gene in all nine isolates, but no carbapenemase gene was identified. In clams, the predominant ESBL phenotype was blaCTX-M-1 (n=6/18). blaCPE (NDM1, OXA48) was detected only in 3 isolates 'K. pneumoniae isolates'. Replicon typing on the strains carring the ESBL and carbapenemase gene revelead that the major type plasmid carried ESBL were IncF (42.3%) [n=11/26]. In all, our results suggest that seafood can be a reservoir of multi-drug resistant bacteria, most probably of human origin but also by the selection pressure of antibiotic. Our findings raise concerns that seafood bought for consumption may serve as potential reservoirs of AMR genes and pose serious threat to public health.

Keywords : BLSE, carbapenemase, enterobacterales, tunisian seafood
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