

Determination of the Presence of Antibiotic Resistance from *Vibrio* Species in Northern Italy

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Abstract : Oysters are considered filter organisms, and their raw consumption may increase health risks for consumers: it is often associated with outbreaks of gastroenteritis or enteric illnesses. Most of these foodborne diseases are caused by *Vibrio* strains, enteric pathogens also involved in the diffusion of genetic determinants of antibiotic resistance and their entrance along the food chain. The European Food Safety Authority (EFSA), during the European Union report on antimicrobial resistance in 2017, focused the attention about the role of food as a possible carrier of antibiotic-resistant bacteria or antibiotic-resistance genes that determine health risks for humans. This study wants to determine antibiotic resistance and antibiotic-resistance genes in *Vibrio* spp. isolated from *Crassostrea gigas* oysters collected in the Golfo della Spezia (Liguria, Italy). A total of 47 *Vibrio* spp. strains were isolated (ISO21872-2:2017) during the summer of 2021 from oysters of *Crassostrea gigas*. The strains were identified by MALDI-TOF (Bruker, Germany) mass spectrometry and tested for antibiotic susceptibility using a broth microdilution method (ISO20776-1:2019) using Sensititre EUVSEC plates (Thermo-Fisher Scientific) to obtain the Minimum Inhibitory Concentration (MIC). The strains were tested with PCR-based biomolecular methods, according to previous works, to define the presence of 23 resistance genes of the main classes of antibiotics used in human and veterinary medicine: tet (B), tet (C), tet (D), tet (A), tet (E), tet (G), tet (K), tet (L), tet (M), tet (O), tet (S) (tetracycline resistance); blaCTX-M, blaTEM, blaOXA, blaSHV (β -lactam resistance); mcr-1 and mcr-2 (colistin resistance); qnrA, qnrB, and qnrS (quinolone resistance); sul1, sul2 and sul3 (sulfonamide resistance). Six different species have been identified: *V. alginolyticus* 34% (n=16), *V. harveyi* 28% (n=13), *V. fortis* 15% (n=7), *V. pelagius* 8% (n=4), *V. parahaemolyticus* 11% (n=5) e *V. chagasii* 4% (n=2). The PCR assays showed the presence of the blaTEM gene on 40% of the strains (n=19). All the other genes were not detected, except for a *V. alginolyticus* positive for anrS gene. The broth microdilution method results showed a high level of resistance for ciprofloxacin (62%; n=29), ampicillin (47%; n=22), and colistin (49%; n=23). Furthermore, 32% (n=15) of strains can be considered multiresistant bacteria for the simultaneous presence of resistance for three different antibiotic classes. Susceptibility towards meropenem, azithromycin, gentamicin, ceftazidime, cefotaxime, chloramphenicol, tetracycline and sulphamethoxazole reached 100%. The *Vibrio* species identified in this study are widespread in marine environments and can cause gastrointestinal infections after the ingestion of raw fish products and bivalve molluscs. The level of resistance to antibiotics such as ampicillin, ciprofloxacin and colistin can be connected to anthropic factors (industrial, agricultural and domestic wastes) that promote the spread of resistance to these antibiotics. It can be also observed a strong correlation between phenotypic (resistant MIC) and genotypic (positive blaTEM gene) resistance for ampicillin on the same strains, probably due to the transfer of genetic material between bacterial strains. Consumption of raw bivalve molluscs can represent a risk for consumers health due to the potentially presence of foodborne pathogens, highly resistant to different antibiotics and source of transferable antibiotic-resistant genes.

Keywords : vibrio species, blaTEM genes, antimicrobial resistance, PCR

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