Antimicrobial susceptibility profiles of multidrug-resistant aeromonads isolated from Northern Portugal freshwater ecosystem

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INTRODUCTION

Extensive use of water and anthropogenic activities contribute to water body pollution. Agricultural, urban, and animal waste, often characterized by numerous toxic and carcinogenic chemicals, pathogenic bacteria, and antibiotics, as well as antibiotic resistance genes (ARGs), loaded with microflora, can contaminate water and enter the food chain, posing a considerable danger to public health [1,2]. The inappropriate use of antibiotics, one of the causes of the high incidence of antimicrobial-resistant bacteria isolated from aquatic ecosystems, represents a risk for aquatic organisms and the welfare of humans. Infectious diseases, both human and animal, are closely related through the environment in the One World - One Medicine - One Health concept, in order to deal with the growing problem of antibiotic resistance. *Aeromonas* spp. can acquire antimicrobial resistance mechanisms, with the potential to spread via horizontal gene transfer, so they could be a good candidate as an indicator to follow antimicrobial resistance dissemination in water [3,4]. This study aimed to determine the antimicrobial resistance rates among riverine *Aeromonas* spp., taken as representative of the autochthonous microbiota, to evaluate the level of antibacterial resistance in the Tua River (Douro basin).

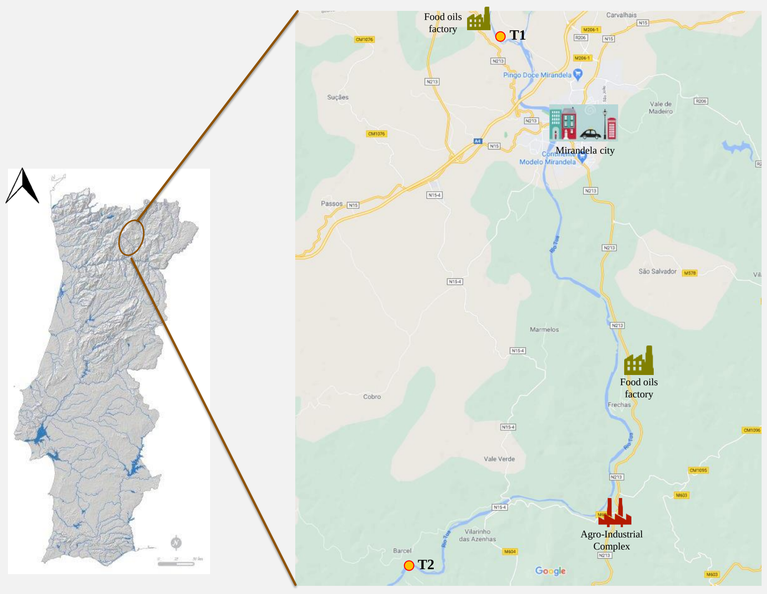

Figure 1. Map of the study area and the location of the two sampling sites (T1 and T2) in the Tua River basin. The water samples were collected in two different seasons (summer and autumn).

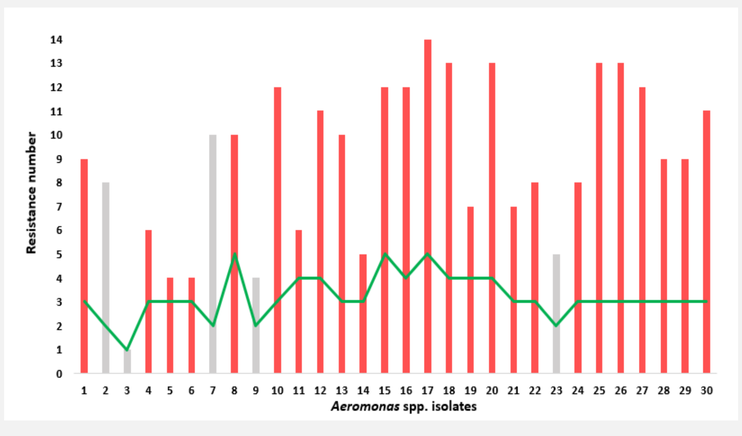

Figure 5. Multidrug-resistant (MDR) in Tua River, where 25 out of the 30 *Aeromonas* isolates (red bars) were resistant to three or more antibiotic classes. Grey bars indicate no MDR isolates (5 out of the 30). Green line represents the number of antibiotic classes of resistance.

METHODOLOGY

Two replicates of water were collected at two sites (Fig. 1) in 1 L sterile glass bottles, stored in a cold container, and transported to Laboratory. Water samples were collected from the middle sector of the river, which is most impacted area by several anthropogenic pressures. The detection and quantification of bioindicators was performed by the filter membrane method. The filter was put on solid culture media with selective and differential for growth of *Aeromonas* spp. Purified colonies were stored in aliquots of Brain Heart Infusion (BHI) medium with 17 % (v/v) glycerol at -80 °C.

All the presumptive *Aeromonas* isolates were identified by classical biochemical methods, Gram-negative staining, the presence of normally positive cytochrome oxidase, catalase reaction, and growth in nutritive broth at 0% to grow in the presence of vibri-ostatic factor O/129. Strains were maintained on Tryptone Soya Agar (TSA) (Oxoid, Thermo Scientific, UK). The prevalence and degree of antibiotic resistance was examined using motile aeromonads as a potential indicator of antimicrobial susceptibility for the aquatic environment. *Aeromonas* isolates were tested against 19 antibiotics according to the Clinical Laboratory Standards Institute (CLSI). Isolates were classified as sensitive (S) or resistant (R) based on size of the zone of bacteria growth inhibition, according to CLSI recommendations after 24 h incubation at 30 °C. Multidrug-resistant (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

RESULTS

30 *Aeromonas* isolates were collected from the sampling survey, in the summer and autumn of 2018.13 strains were isolated from the site T1 and 17 from the site T2. Antibiotic resistance rates were fosfomycin (FOS) 83.33%, nalidixic acid (NA) 60%, cefotaxime (CTX) 40%, gentamicin (CN) 26.67%, tobramycin (TOB) 26.67%, cotrimoxazole (SXT) 26.67%, chloramphenicol (C) 16.67%, and tetracycline (TE) 13.33%. Some of the nalidixic acid resistant strains were susceptible to fluoroquinolones. Resistance up to 50% to ciprofloxacin was observed and more than 70% of the *Aeromonas* isolates were found to be susceptible to chloramphenicol, trimethoprim-sulfamethoxazole and tetracycline (Fig. 2).

Antibiotic abbreviations:

AML - amoxicillin
AMC - amoxicillin/clavulanic acid
TIC - ticarcillin
TIM - ticarcillin/clavulanic acid
PRL - piperacillin
TZP - piperacillin/tazobactam
ATM - aztreonam
IPM - imipenem
KF - cephalothin
CTX - cefotaxime
NA - nalidixic acid
CIP - ciprofloxacin
CN - gentamicin
TOB - tobramycin
K - kanamycin
C - chloramphenicol
SXT- trimethoprim/sulfamethoxazole
TET - tetracycline
FOS - fosfomycin

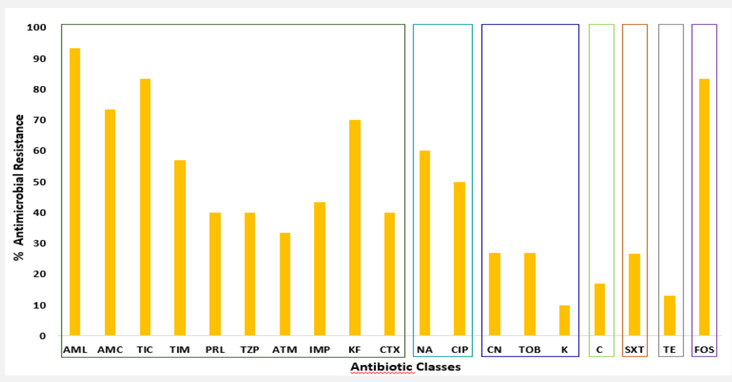

Figure 2. Susceptibility and resistance profile (%) of *Aeromonas* spp. (n=30) isolates to 19 antibiotics. Squares of different colors represents the seven families to which 19 antibiotics belong: ten β- lactams (AML, AMC, TIC, TIM, PRL, TZP, ATM, IMP, KF, CTX), two quinolones (NA, CIP), three aminoglycosides (CN, TOB, K), one phenicol (C), one sulfonamide (SXT), one tetracycline (TE) and fosfomycin (FOS).

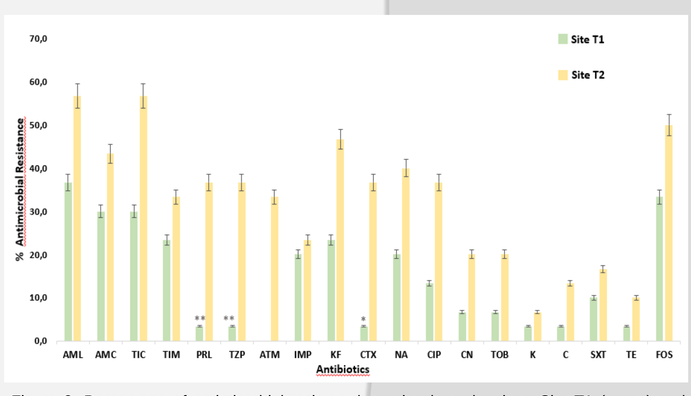

Figure 3. Percentage of antimicrobial-resistant bacteria along the river: Site T1 (green) and Site T2 (yellow). Values were expressed as mean of resistant *Aeromonas* spp. ± standard deviation, for the same antimicrobial. * and ** indicate significant differences between different locations (sites T1 and T2) within the same antimicrobial (ANOVA followed by Tukey's test at $p < 0.05$ and $p < 0.01$, respectively).

Generally, the pattern of antimicrobial-resistant *Aeromonas* spp. showed that high values were observed on site T2. Indeed, the T2 site was the one where the most resistant isolates were observed (32.46%), when compared with the T1 site (14.39%) - see Fig. 3. Comparing two sampling periods (summer vs. autumn), it was observed that the resistance to antibiotics increased in autumn, being 1.3-fold higher (Fig. 4). Multiple resistance was also observed (83.33 %) – see Fig. 5.

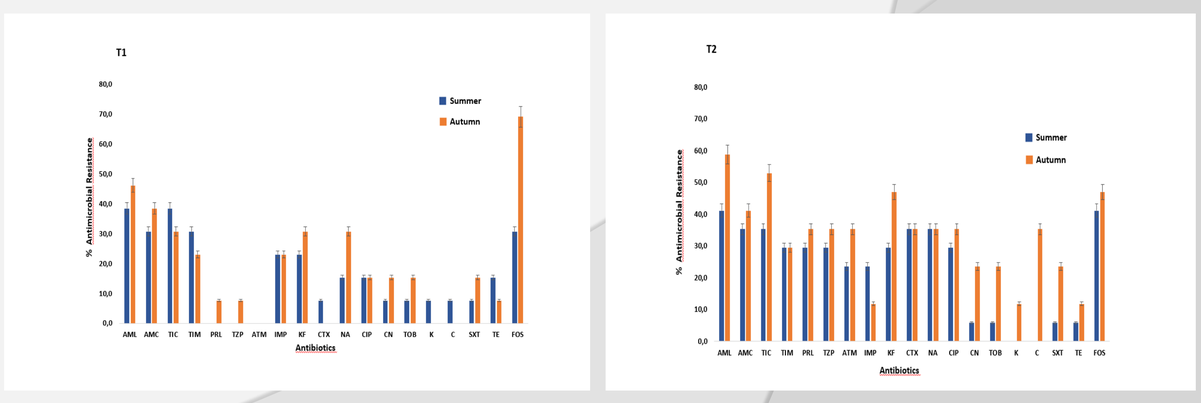

Figure 4. Percentage of antimicrobial-resistant bacteria for each sampling site (T1 *vs* T2) by seasons (summer - blue colour *vs* autumn - orange colour). Values were expressed as mean of resistant *Aeromonas* spp. ± standard deviation, for the same antimicrobial. No lettering indicates non-significant differences at a 95 % confidence level.

CONCLUSIONS

A pool of MDR aeromonads in the Tua River basin was observed. These results, together with the resistance patterns of *Aeromonas* to antibiotic tests, suggest that *Aeromonas* spp. can be an effective bioindicator organism for monitoring antimicrobial resistance in rivers. This knowledge is essential to manage and mitigate potential risks to human health emphasizing the need for predicting and preventing the spread of antibiotic-resistant pathogenic aeromonads. It is imperative a continuous monitoring surveillance in aquatic systems, considering interactions between the key elements (geographical, ecological, human activities, and the food-agricultural components) within the "*One World - One Health*" approach.

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