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Antibiotic Resistance Case Study: *Enterobacteriaceae* isolated from Batlama Creek in Giresun, Turkey

Tamer Akkan[*]

Department of Biology, Faculty of Arts and Sciences, Giresun University, 28200 Giresun, Turkey

A R T I C L E I N F O	A B S T R A C T
	Enterobacteriaceae were isolated from the freshwater of the Batlama Creek in Giresun, to measure their resistance levels against to antibiotics. A total of 9 antibiotics disc were applied for the resistance test. Antibiotic resistances of all isolates were at percentages for ampicillin (75%), erythromycin (64%), nalidixic acid (48%), tetracycline (39%), amikacin (34%), cefazolin and chloramphenicol (33%), cefuroxime (32%) and cefotaxime (23%), respectively. The highest resistant strain was resistant against to 9 antibiotics, while the weak resistance of 16 isolates were sensitive to all antibiotics. Moreover, Multiple antibiotic resistance index values were found to be higher than 0.2 for 77% of all isolates. High resistances of examined bacteria against to antibiotics indicated a dense and multisource pollution in the Batlama Creek. Consequently, a need for good surveillance programs to monitor antimicrobial resistance patterns in surface water bodies.
[*]Corresponding Author: E-mail: biyoloji@yahoo.com	

Introduction

Today, one of the current subjects of ecological studies is to assess the pollution of different surface water ecosystems. The accumulation of pollutants by both domestic and other sources shows increased and threatened concentrations over time without recycling feature which causing the ecological deterioration in the aquatic environment. As a result of these events, pollution is formed, which can have a negative impact on the different part of the aquatic habitats (Aydın and Sunlu, 2004; Sunlu et al., 2005; Verep et al., 2017). The negative effects of pollution on this ecosystem include algal blooms, increased of sedimentation, oxygen consumption, oxygen depletion and bacterial pollution in the surface waters. There are many studies, which show the domestic pollution in surface water environment (Verep et al., 2007; Mutlu and Uncumusaoğlu, 2016; Kurnaz et al., 2016; Mutlu et al., 2016). Moreover, bacteria are often serve as good indicators for the presence of pollutants. Also, bacteria isolated from the aquatic environment use to monitor of pollution (Lobova et al., 2008; Kalkan and Altuğ, 2015; Altuğ et al., 2016).

Recently, the aquatic environment has been contaminated by pharmaceutical substances (Brown et al., 2006). Antibiotic contamination has been identified as a concern in aquatic area, due to discharges from urban sewage and agricultural activities. Several recent studies demonstrate that uncontrolled use of antibiotics contributes to the increasing level of antibiotic resistance among the pathogenic bacteria in water ecosystems (Shah et al., 2014). Additionally, antimicrobial resistance is increasingly compromising the treatment of many infections that were until recently, controllable, and so remain the most common diseases in the world (Yang et al., 2013). Currently in different parts of the world most studies (Reinthaler et al., 2003; Ahmed et al., 2008) on antibiotic resistance in aquatic habitat have concerned bacteria of *Enterobacteriaceae,* which is thought to be associated with infectious diseases (Liu et al., 2015). A lot of microbial ecology studies in Turkey have reported that antibiotic resistance is becoming a national importance, but it has not yet been reached a sufficient level (Akkan et al., 2011; Akkan et al., 2013; Çardak et al., 2016; Altuğ et al., 2016).

The aim of the present study to examine the levels of antibiotic resistance in *Enterobacteriaceae*, isolated from freshwater, along the Batlama Creek in Giresun (Turkey), and based on the results to determine the case study which have effects on both aquatic organisms and public health.

Material and Method

Sampling

Surface freshwater samples were collected from different point in the Batlama Creek under sterile conditions (Figure 1). The water samples were collected 0-20 cm below from the surface, using 250 ml sterile bacteriological sample bottles and brought to the laboratory in an ice chest. All of these sampling were performed within 4 hours (APHA, 1992).

Bacterial Isolation and Antibiotic Resistance Test

Enterobacteriaceae from the surface water were isolated using the spread plate technique. To isolation of these bacteria were made using Mac Conkey and Eosin Methylene Blue (EMB) Agar (Merck), inoculated with appropriate dilutions of the sample homogenous, and incubated for 24–72 h at 35°C then maintained in nutrient agar (Merck). Furthermore, all isolates were screened by colonial morphology, Gram stain, Oxidation/Fermentation of glucose and motility.

Antibiotic resistance of bacterial strains was determined by the agar diffusion test using Mueller–Hinton agar (Merck) and 9 antibiotic discs representing 7 classes of antibiotics: tetracycline (TE, 30μg), erythromycin (E, 15 μg), nalidixic acid (NA, 30μg), cefazolin (CZ, 30μg), cefuroxime (CXM, 30μg), cefotaxime (CTX, 30 μg), ampicillin (AM, 10μg), amikacin (AN, 30μg) and chloramphenicol (C, 30μg). The entire surface of the Mueller–Hinton agar plate (diameter, 90 mm) (Merck) was covered with the required inoculums, and the plate was air dried for 15 min, before the discs were laid on the surface and incubation was performed for 18 h at the required temperature. The verification of the antibacterial effect as the reference strain *E. coli* K-12 was used (NCCLS, 1997).

We calculated the MAR (Multiple Antibiotic Resistance) index values for all isolates (*a/b*, where *a* represents the number of antibiotics the isolate was resistant to and *b* represents the total number of antibiotics the isolate was tested against). A MAR index value of equal or less than 0.2 was defined as those antibiotics were seldom or never used for the animal in terms of treatment, whereas the MAR index value higher than 0.2 is considered that animal have received high-risk exposure to those antibiotics (Krumperman, 1983).
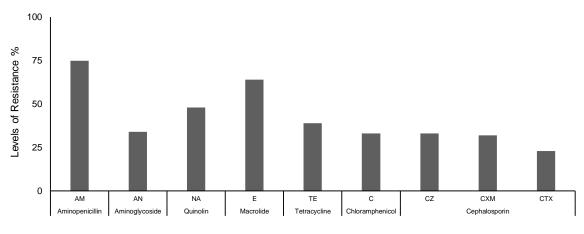
Results and Discussion

Among the surface water isolates were resistant to AM 75%, E 64%, NA 48%, TE 39%, AK 34%, CZ and C 33 %, CXM 32% and CTX 23%, respectively (Figure 2). Only two strains isolated from freshwater were resistant to 9 antibiotics, six isolated were resistant to 8 antibiotics (resistant to all antibiotics except chloramphenicol, Figure 3). Also, sixteen isolates were sensitive to all antibiotic discs. Levels of multiple antibiotic resistant isolates were examined in terms of expressions for the MAR, 77% of all isolates MAR value of 0.2. 84 % of the isolates were resistant: 7% to one antibiotic, 12% to two and three antibiotics, 16% to four, 7% to five and six antibiotics, 13% to seven, 8% to eight and 2% to nine. A high incidence of antibiotic resistance in *Enterobacteriaceae* isolates were reported by Akkan and Mutlu, (2016) from Giresun Coasts. According to this study, all isolates showed high resistance to E 82%, CZ 46.5%, CTX 50.5%, AK 41.5%, NA 34.5%, TE 30.5%, C 36.5%, CXM 35.5% and AM 15.5%, respectively. By comparison, our results indicate higher rates of NA, TE and AM. In addition, other studies from Giresun indicated that bacteria isolated from *Engraulis encrasicolus* showed high resistance to; gill: E 96.43%, CTX 92.86%, CZ 85.71%, NA and CXA 78.57%, AK 75%, AM 67.86% and TE 53.57%, intestine: E 88.89%, CZ and CTX 77.78%, AK and NA 66.67%, respectively (Sipahi et al., 2013). Another study examined that level of antibiotic resistance in *Enterobacteriaceae* isolated from seawater in Turkey respectively, kanamycin 82%, vancomycin 78% and ampicillin 60% (Çardak et al., 2016). Also, Toroglu et al., (2009) showed that antibiotic resistance levels of *Enterobacteriaceae* isolated from freshwater fish (*Achanthobrama marmid* (Heckel,1843)) catch from Sir Dam Lake, intestine: penicillin-G (63%), cefazoline (50%), cefoxitine (45%), ceftriaxone (41%) and cefotaxime (35%), gill: penicillin-G (73%), cefazoline (58%), cefoxitine (50%), ceftriaxone (38%) and cefotaxime (38%), respectively. Toroglu and Toroglu, (2009) reported that highly antibiotic resistance penicillin (100%), cefazolin (61.5%), cefoxitin (38.5%) and ceftriaxone (92.3%) in thirteen *E. coli*. The worldwide studies, like Mudryk et al., (2010) reported that levels of antibiotic resistance among the 30 isolated from the Baltic Sea, AM 12%, C 8.3%, CIP 4.2%, E 6.3% and CXM 8.3%, respectively.

The results of MAR values in this study indicate that the study area is also polluted directly or indirectly by domestic waste water. Moreover, unnatural increases in antibiotic resistant bacteria in surface water indicate that the unconscious use of different groups of antibiotics. Also, 37 isolates of the multiple antibiotic resistance indexing ranged from 0.50 to 1 and exceeded the threshold value of 0.2, suggesting the origin of the isolates to be of high antimicrobial usage. Mudryk et al., (2016) reported that the number of strain multi-resistant to several antibiotics was high in seawater, wet sand and dry sand samples containing high levels of fecal indicators. Also, a higher incidence of antibiotic resistance in fecal coliform bacteria was reported by Skorczewski et al., (2013) for two water basins.

Figure 1 The Study Area (Batlama Creek, adapted from Google Earth)

Figure 2 The percentage of antibiotic-resistant *Enterobacteriaceae*
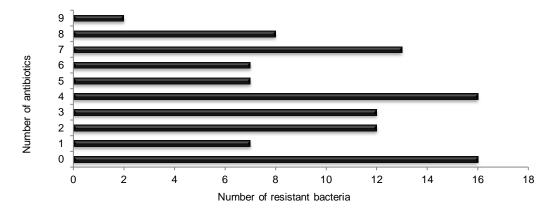

Figure 3 Results of MAR index value of multiple resistant *Enterobacteriaceae*

Conclusion

Confirmation of the presence of *Enterobacteriaceae* in the Batlama creek surface water samples in Giresun, Turkey, indicates fecal contamination and the possible presence of other enteric pathogens. Present findings showed that an increase in the incidence of antimicrobial resistance of *Enterobacteriaceae* towards conventionally used antibiotics. This is the case necessitating proper surveillance programs towards the monitoring of antimicrobial resistance determinants in all water bodies. Also, taken, the results of the our findings demonstrated that, local people encouraged to conscious consumption of antibiotics.

Acknowledgement

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