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European Journal of Experimental Biology, 2011, 1 (4):158-163



### Occurrence and Antibiotic Susceptibility of Some Bacteria in Saricay Stream (Canakkale, Turkey)

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#### ABSTRACT

*In this study microbial quality and prevalence of antimicrobial resistance among the enteric bacteria found in Saricay stream. For microbial quality total and faecal coliform and faecal enterococci parameters were determined. From isolated enteric bacteria Escherichia coli was the prevalent bacteria (63.33%) followed by Citrobacter freundii (10%), Enterobacter spp. (10%), Proteus vulgaris (8.33%), Klebsiella pneumoniae (5%) and Salmonella typhimurium (3.33%). Antimicrobial activity test was performed for 14 antibiotics. The highest rates of resistance were showed against erythromycin (91.66%), and Multiple Antibiotic Resistance Index of five stations vary between 0.21 – 0.33 values.*

**Key words:** Antibiotic resistance, enteric bacteria, MAR Index.

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#### INTRODUCTION

Antimicrobial agents are the most important in the treatment of bacterial infections and thus the worldwide increase in antibiotic-resistant bacteria is of major concern and antibiotic use is suggested to be a major risk factor for the development antibiotic resistant [1]. Antibiotic resistance has been detected in various aquatic environments including river, sewage, ocean water and drinking water [2, 3].

Most of the work about surveillance on antimicrobial resistance have been carried out in bacteria isolated from clinical samples: however, studies should also be expanded to those bacteria recovered from environmental samples in order to evaluate their role as possible reservoir of resistance genes and their capacity to transfer them to human pathogenic organisms [4].

River water is the main receptacle reservoir of antibiotics and antibiotic resistant bacteria in the environment. They are directly introduced into surface water through fisheries, animal farms and

agricultural practices. A large volume of sewage and effluent containing antibiotic resistant bacteria is discharged into rivers, streams, lakes and sea water. The antibiotic resistance bacteria in drinking water are a prime concern to public health [5]. The development of resistance is that bacteria in the guts of humans and animals are subjected to different types, concentrations and frequencies of antimicrobial agents. Enterobacteriaceae family (*Enterobacter* spp., *Escherichia coli*, *Citrobacter* spp., etc) is included that the main commensally bacterial species in the gut flora, unlike other microorganisms, enteric bacteria able to acquire resistance easily and are commonly found in many different animal species, therefore they are a good bioindicator model for surveillance studies antimicrobial resistance [1]. The aims of this investigation were to determine the water microbial quality of Sarıca stream (total, faecal coliform and faecal enterococci counts) and antimicrobial resistant profiles of isolated enteric bacteria from Sarıca stream.

## MATERIALS AND METHODS

### Study area

The Sarıca stream is located in the southwest region of Marmara, latitude 39° 40' – 40° 45' N and longitude 25° 37' – 27° 45' E, in Canakkale, Turkey (Fig 1). This water resource is used for agriculture as on irrigation water and drinking water for animals [6]. Atıkhisar dam which was building on Sarıca stream, was used as drinking water. Sampling sites are five sites and showed in Figure 1.

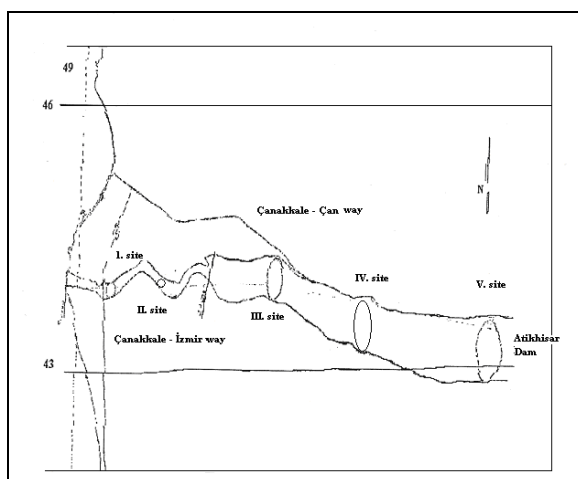


Fig 1 Map of Sarıca Stream indicating the sampling site [6]

### Water quality analysis

Sampling for water quality parameters were carried out in the five study sites at monthly intervals between January – December 2010, covering dry and rainy seasons (Fig 1). Total (TC), faecal coliforms (FC) and faecal enterococci (FE) were counted according to the most probable number techniques [7]. Complete identification of enteric bacteria were achieved by use of the tests in Bergey's Manual of Determinative Bacteriology [8].

### Antibiotic susceptibility

The antibiotic susceptibility test was performed by standard disc diffusion method [9]. The following antibiotics were used: Trimethoprim (TR10 µg/mL), Tobramycin (TB10 µg/mL), Kanamycin (K30 µg/mL), Amoxicillin (AM10 µg/mL), Oxytetracycline (O30 µg/mL), Cephalothin (CH30 µg/mL), Cefmetazole (CMZ30 µg/mL), Gentamicin (G10 µg/mL), Furazolidone (FR50 µg/mL), Erythromycin (E15 µg/mL), Cefoxitin (CN30 µg/mL), Ampicillin

(A10 µg/mL), Cefotaxime (CE30 µg/mL) and Chloramphenicol (C30 µg/mL). Organisms were reported as either resistant, intermediate or sensitive to each antimicrobial tested.

## RESULTS AND DISCUSSION

Table 1 is a summary of arithmetic means of microbiological parameters (TC, FC, FE) and percentage frequency of antibiotic resistants of Enterobacteriaceae strains are showed in Table 2. Multiple antibiotic resistance among coliforms (% resistance) and MAR Index of stations are showed in Table 3.

### Microbiological quality parameters:

The TC, FC and FE bacteria tests are a primary indicator of potability that is suitability for consumption. These tests measure the concentration of total, faecal coliform and faecal enterococci associated with the possible presence of disease causing organisms [10]. In the present study the means of TC, FC and FE are  $30252 \pm 19916$  MPN/100 mL,  $20355 \pm 31112$  MPN/100 mL,  $17855 \pm 20248$  MPN/100 mL, respectively. According to Turkish legislation [Water Pollution Control Regulation Official Gazette (WPCR)] [11], water quality of inland waters is classified into four groups as: high quality waters (Class 1), moderated quality waters (Class 2), polluted waters (Class 3), and highly polluted waters (Class 4). Based on results of comparison of data with WPCR, it seen that waters of Saricay stream for TC at all sites belonged to class 3, except site 5 (class 2); for FC, at all sites belonged to class 4. Results which were found in the present investigation had shown similarity previous studies [6, 12 - 14]. Also, there is no data about FE in WPCR. But, it appears from our results that the impact of all these factors means together contribute to the hierarchy of abundance  $TC > FC > FE$ .

FC/FE ratio is widely used to determine the origin of contamination. For human faecal contamination,  $FC/FE > 4$ , whereas with animal faecal contamination the  $FC/FE < 0.7$  [15]. In this study, FC/FE showed human faecal contamination at sites 1,2 and 5; faecal contamination of animal origin at sites 3 - 4. These findings proved that the role of human in contributing significantly to faecal contamination of Saricay stream. The bacteriological quality of the Saricay stream posed an increased risk of infectious disease transmission to the communities that were dependent on the stream.

**Table 1 Values of microbiological parameters (Mean  $\pm$  SD) Saricay Stream**

| Parameter          | Site              |                   |                   |                   |                   | Average           |
|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                    | I                 | II                | III               | IV                | V                 |                   |
| TC<br>(MPN/100 mL) | 20983 $\pm$ 31073 | 20692 $\pm$ 29395 | 26600 $\pm$ 40830 | 65400 $\pm$ 48724 | 17583 $\pm$ 19149 | 30252 $\pm$ 19916 |
| FC<br>(MPN /100mL) | 14150 $\pm$ 31436 | 17492 $\pm$ 30149 | 18850 $\pm$ 30140 | 22633 $\pm$ 41284 | 28650 $\pm$ 49060 | 20355 $\pm$ 31112 |
| FE<br>(MPN/100 mL) | 1967 $\pm$ 2433   | 2125 $\pm$ 124    | 33633 $\pm$ 46405 | 45342 $\pm$ 50456 | 6208 $\pm$ 12585  | 17855 $\pm$ 20248 |

### Isolation and identification of enteric bacteria:

Total sixty enteric bacteria isolates were identified from five sites of Saricay stream. Among enteric bacteria 38 strains of *E.coli* (63.33%) were the most frequent isolates. These were followed by 6 strains of *C. freundii* (10%) and *Enterobacter* spp. (10%), 5 strains of *Proteus vulgaris* (8.33%), 3 strains of *Klebsiella pneumoniae* (5%) and 2 strains of *Salmonella typhimurium* (3.33%) (Table 2). All these organisms are potential pathogens causing a variety of diseases. *E.coli*, *Proteus* spp., *Enterobacter* spp. and *K. pneumoniae* causes diarrhea, urinary tract, bacteremia, wound infection, pneumoniae, nosocomial infections and kidney infections etc. [16 - 19].

**Antimicrobial resistant of enteric bacteria:**

The highest rates of resistance were against erythromycin was the most commonly reported antimicrobial agent (91.66%), followed by ampicillin (73.33%), cefoxitin (46.66), cephalothin (38.33), amoxicillin (25.00%), oxytetracyclin (23.33%). Also resistant to other antibiotics observed in varying proportions. But all isolates sensitivity only one antibiotic – gentamicin – except one *E.coli* isolate (Table 2). Akinbowale *et al.* [20], reported an incidence for ampicillin and amoxicillin resistances 54.8%, cephalothin resistance 23.1%, erythromycin resistance 47.1%, oxytetracyclin resistance 19.2% in Enterobacteriaceae family, while in another study, the highest number of *E.coli* isolates were observed for tetracyclin 81.4%, kanamycin 81.4%, chloramphenicol 75.7%, gentamicin 74.3% and ampicillin 72.9% [1].

**Table 2 Percentage frequency of antibiotic resistant Enterobacteriaceae strains**

| Antibiotic class | Antibiotic      | Antibiotic resistant no. (%) |                  |                    |                         |                  |                     | Total (n=60) |
|------------------|-----------------|------------------------------|------------------|--------------------|-------------------------|------------------|---------------------|--------------|
|                  |                 | E.coli (n=38)                | C.freundii (n=6) | K.pneumoniae (n=3) | Enterobacter spp. (n=6) | P.vulgaris (n=5) | S.typhimurium (n=2) |              |
| Macrolide        | Erythromycin    | 36(94.73)                    | 5(83.33)         | 3(100)             | 6(100)                  | 5(100)           | 0(0)                | 55(91.66)    |
| Nitrofurantoin   | Furazolidone    | 2(5.26)                      | 0(0)             | 0(0)               | 0(0)                    | 1(20)            | 0(0)                | 3(5.00)      |
| Phenolics        | Chloramphenicol | 4(10.52)                     | 2(33.33)         | 2(66.66)           | 0(0)                    | 0(0)             | 0(0)                | 8(13.33)     |
| Sulphonamide     | Trimethoprim    | 4(10.52)                     | 1(16.66)         | 0(0)               | 1(16.66)                | 1(20)            | 0(0)                | 7(11.66)     |
| B- lactam        | Amoxicillin     | 7(18.42)                     | 1(16.66)         | 1(33.33)           | 3(50)                   | 3(60)            | 0(0)                | 15(25.00)    |
|                  | Ampicillin      | 27(71.05)                    | 5(83.33)         | 3(100)             | 6(100)                  | 3(60)            | 0(0)                | 44(73.33)    |
| Tetracycline     | Oxytetracycline | 8(21.05)                     | 2(33.33)         | 1(33.33)           | 1(16.66)                | 2(40)            | 0(0)                | 14(23.33)    |
| Cephalosporins   | Cephalothin     | 18(47.36)                    | 1(16.66)         | 0(0)               | 2(33.33)                | 2(40)            | 0(0)                | 23(38.33)    |
|                  | Cefmetazole     | 1(2.63)                      | 0(0)             | 0(0)               | 1(16.66)                | 1(20)            | 0(0)                | 3(5.00)      |
|                  | Cefoxitin       | 16(42.10)                    | 4(66.66)         | 1(33.33)           | 5(83.33)                | 2(40)            | 0(0)                | 28(46.66)    |
|                  | Cefotaxime      | 3(7.89)                      | 1(16.66)         | 1(33.33)           | 2(33.33)                | 0(0)             | 0(0)                | 7(11.66)     |
| Aminoglycosides  | Tobramycin      | 3(7.89)                      | 0(0)             | 0(0)               | 0(0)                    | 1(20)            | 0(0)                | 4(6.66)      |
|                  | Kanamycin       | 3(7.89)                      | 0(0)             | 0(0)               | 0(0)                    | 1(20)            | 0(0)                | 4(6.66)      |
|                  | Gentamicin      | 1(2.63)                      | 0(0)             | 0(0)               | 0(0)                    | 0(0)             | 0(0)                | 1(1.66)      |

**Table 3. Multiple antibiotic resistance among coliforms (% resistance) and MAR Index of stations**

| Bacteria                     | No Antibiotic | One Antibiotic | Two Antibiotic | Three Antibiotic | >Three Antibiotic |
|------------------------------|---------------|----------------|----------------|------------------|-------------------|
| <i>E.coli</i>                | 0(0)          | 4(10.52)       | 7(18.42)       | 10(26.31)        | 17(44.73)         |
| <i>C.freundii</i>            | 0(0)          | 0(0)           | 2(33.33)       | 2(33.33)         | 2(33.33)          |
| <i>K.pneumoniae</i>          | 0(0)          | 0(0)           | 1(33.33)       | 0(0)             | 2(66.66)          |
| <i>Enterobacter spp.</i>     | 0(0)          | 0(0)           | 0(0)           | 2(33.33)         | 4(66.66)          |
| <i>P.vulgaris</i>            | 0(0)          | 0(0)           | 0(0)           | 0(0)             | 5(100)            |
| <i>S.typhimurium</i>         | 0(0)          | 0(0)           | 0(0)           | 0(0)             | 0(0)              |
| <b>MAR Index of stations</b> |               |                |                |                  |                   |
| <b>Sampling sites</b>        |               |                |                |                  |                   |
|                              | <b>I</b>      | <b>II</b>      | <b>III</b>     | <b>IV</b>        | <b>V</b>          |
| MAR value                    | 0.33          | 0.26           | 0.27           | 0.26             | 0.21              |

Changes in antibiotic resistance among coliform species revealed that *E.coli* isolates showed greater resistance towards all the tested antibiotics than other coliforms (Table 2). But resistances to more than three antibiotics was observed in *P. vulgaris* (100%) than *K. pneumoniae* (66.66%), *Enterobacter spp.* (66.66%), *E.coli* (44.73%), *C. freundii* (33.33%). There is no resistant against any antibiotic in all *S. typhimurium* isolates. The obtained results has been compared with those from literature [5, 16, 21 – 23], and it can be observed a similarity with these, where the authors show high incidence of antibiotic resistance of enteric bacteria isolated different water resources.

**Determination of MAR Index of enteric bacteria:**

The significant increases of Multiple Antibiotic Resistant (MAR) bacteria observed in various aquatic systems may be of significant importance of health since human infections caused by such organisms could be difficult to treat with drugs [24].

MAR Index of the samples was calculated by the formula

$$\text{MAR Index} = y/nx$$

Where, y = Total number of resistance scored;

n = number of isolates;

x = Total number of antibiotics tested [5].

MAR indexing was below arbitrary value of risk contamination of 0.200 indicating low risk contamination sites. Indices of between 0.200 and 0.250 are in a range require careful scrutiny [25]. In our study seen that MAR Index of five stations changing between 0.21 – 0.33 values (Table 3). The data strongly suggest that, within the geographic limits of this study, faecal contamination from these high risk sources can be distinguished. The ability to make this distinction has obvious usefulness to the food industry, federal and state regulatory authorities, and public health agencies [5, 23].

## CONCLUSION

The result obtained from this study indicate that understanding the aquatic bacterial ecology and estimation of bacterial antibiotic resistance profiles because of can be an important tool for those who are responsible for public health and environmental protection and are charged with reducing pollution, protecting public health, and improving water quality. So, in our study, Enterobacteriaceae family was chosen routinely as representative of the faecal contamination indicator. In the next studies, are required to investigate the extent of antibiotic use in Canakkale aquaculture farms and environments and to determine molecular basis of antimicrobial resistants and the potential for transfer of resistance genes from aquatic isolates to human pathogens, some assessment of the risk of transfer of resistant organisms to human via the food chains.

## Acknowledgment

The authors thank to Research Fund of Canakkale Onsekiz Mart University, Canakkale, Turkey (Project number: 2010-36) for the financial support.

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