ANTIMICROBIAL SUSCEPTIBILITY OF *Eschericia coli* ISOLATED FROM ARIEŞ RIVER (ROMANIA)

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Abstract. We studied the prevalence of antimicrobial resistance (AR) and multiple antimicrobial resistances (MAR) among the faecal bacteria found in the Arieş river (Romania) affected by strong anthropogenic pressures. Isolation and identification of *E. coli* were done by using enrichment media, selective media, and biochemical tests. Antimicrobial susceptibility testing by the disk diffusion method was conducted for 12 antimicrobial agents: ciprofloxacin, gentamicin, streptomycin, ceftazidin, ofloxacin, sulfamethoxazole, ticarcycline, ampicillin, nalidixic acid, nitrofurantoin, erythromycin, and norfloxacin. The data of the antimicrobial susceptibility reviled that all the studied *E. coli* strains were resistant to most of the tested antibiotics. The analysis of antibiotic resistance frequencies has showed an incidence of 46.66% strains resistant to more than 4 different antibiotics. Moreover, a high incidence of multiple antibiotic resistances was detected in each of the studied samples.

Keywords: *Escherichia coli*, antibiotic resistance, multiple antibiotic resistances, Arieş River, water.

INTRODUCTION

The aim of the bacteriological tests it is not only to discover the pathogenic germs, but in the same time to establish if this microorganisms could be destroyed by some chemicals or antibiotics. The broadly uses of antibiotics constitute a high risk of bacterial development. Antibiotics are substances produced by living organisms, which are able to kill or inhibit the growth of microorganisms. Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine.

The increase in the number of resistant and multiresistant strains of bacteria is a major concern of health officials worldwide, particularly with the decline in the number of new antibiotics available for treatment. While much effort has been directed toward management and monitoring of antibiotic use and the prevalence of bacterial within communities, bacterial resistance to antibiotics in the aquatic environment has received comparatively little attention. Bacterial contamination of surface waters, particularly contamination with fecally derived bacteria, has long been a water quality issue due to the potential for disease transmission. Because of this and the potential for antibiotic resistance, there is a new level of risk associated with these bacteria. Recent studies have also identified antibiotics themselves in surface waters [4, 9, 11, 19], and the role of these antibiotics in the development, transfer, and maintenance of resistance is largely unknown.

The number of antimicrobial-resistant (AMR) bacteria in the environment increases exponentially with the use of antimicrobials, as a result of increasing selective pressure on bacterial populations [25, 30, 36]. Furthermore, AMR is increasing, and its spread between different bacterial strains in different habitats has been demonstrated [31, 35, 37].

In a limited number of studies workers have identified antibiotic-resistant bacteria in the aquatic environment. In a study of 16 United States Rivers, antibiotic-resistant bacteria were found to be widespread, and the resistance included to chemically modified and synthesized antibiotics [2].

E. coli has been the foremost indicator of faecal contamination in water quality monitoring for many decades. *E. coli* has also been shown to be a significant reservoir of genes coding for antimicrobial drug resistance and therefore is a useful indicator for resistance in bacterial communities [8]. Although there are several studies assessing AMR in *Escherichia coli* populations of animal origin, not much work has been done on the ecology of AMR [7, 39, 41]. The spread of AMR into environments where antibiotics are not used is a possibility that has not yet been well researched, although it has been postulated that water could disseminate AMR [40].

The potential exchange of AMR elements during waste treatment has been recognized [12, 14, 22].

In fact, some researchers [28] observed that AMR was more common in effluents from sewage treatment plants than in those before treatment, indicating that there is a possibility of the exchange of resistance genes in sewage treatment plants.

All *E. coli* strains isolated from river and polluted waters show a high incidence of multiple antibiotic resistance (MAR) phenotype. Many investigators have recognized that wastewater treatment plants are the principal recipients of enteric bacteria with multiple antibiotic resistance [17, 32] and an important site for horizontal gene transfer, by containing nutrients and high concentrations of microorganisms [3, 43]. Biological treatment processes at sewage treatment plants could produce selective elimination, and / or changes in the proportions of phenotypes within effluent bacterial populations [23, 33]. Furthermore the disposal of treated sewage into rivers, lakes, or elsewhere may or may not influence environmental bacterial populations [42]. Some studies have found that wastewater treatment can raise or lower the proportions of antibiotic resistant bacteria which carry antibiotic resistance plasmids [26, 33].

The aim of our study was to realize a better understanding of mechanisms by which the pollution of the Arieş River affects the microbial populations and the antibiotic resistance profiles of *E. coli* isolates to complete the previously realized studies on this aquatic ecosystem [5, 6].

MATERIALS AND METHODS

The survey was performed on the water of the river Arieş. Water samples were collected in summer of 2009 from five sampling sites along the river course (taking into consideration the main town that the river passing through - Abrud, Baia de Arieş, Sălciua, Turda and Luncani) in sterile 250 ml polypropylene bottles, according to STAS 3001-91 [34]. Samples were taken at 4 ºC until their arrival to laboratory.

This study was undertaken to determine the incidence and antibiotic resistant patterns of *E. coli* strains isolated from water samples through the membrane filtration method according to ISO 9308-1:2004 [16. 37 isolates of *E. coli* were collected and tested against 12 commonly used antimicrobial agents.

Isolation, identification and confirmation of E. coli isolates: Standard methods were used for the enrichment, isolation, identification and biochemical confirmation (indole, methyl-red, Voges-Proskauer and Simmons citrate tests) of *E. coli* isolates [13]. Only the bacterial isolates that were confirmed to be *E. coli* based on the results of the biochemical tests were selected for antimicrobial agent sensitivity testing.

Antibiotic susceptibility testing: The antibiotic resistance was determined by a standard disc diffusion technique using Mueller-Hinton agar (Difco) according with the recommendations of National Commitee for Clinical Laboratory Standards (NCCLS 2008) [24] including *Escherichia coli* ATCC 25922 as a control strain.

The antimicrobial drugs tested and their sensidisk concentrations were: ampicillin (AM), 10 µg; ophloxacin (Ofx) 5 µg; nalidixic acid (NA), 30 µg; sulphametoxazol-trimethoprim (SXT), 25 µg; and ticarcycline (TIC) 75 µg; gentamicin (CN) 120 µg; erythromycin (E) 5 µg; nitrofurantoin (F) 300 µg; norfloxacin (NOR) 10 µg; streptomicin (STR) 10 µg; ciprofloxacin (CIP) 5 µg and ceftazidin (CF) 30 µg.

Within 15 min of the application of the discs, the plates were inverted and incubated at 37 °C. After 24 h of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into sensitive, intermediate and resistant categories.

These antimicrobial agents were chosen based on their importance in treating human or animal *E. coli* infections and their use as feed additives to promote growth in animals in agriculture, zootechny and aquaculture [1, 18, 21].

Multiple Antibiotic Resistance (MAR) indexing: The MAR index of profile was performed to evaluate the heath risk of the environments. Multiple antibiotic resistance index (MAR) (number of antibiotics to which test isolate displayed resistance divided by total number of antibiotic to which the test organism has been evaluated for sensitivity) for each test isolate was calculated as recommended by Krumperman (1983) [20].

RESULTS

The resulted obtained in this study for each tested antimicrobial agent, have been reported to NCCLS/CLSI standards (2008). According to this, the strains were classified as sensitive, intermediate or resistant based on the inhibition zone diameter. Patterns of antibiotic resistance were determined, reviewing the order of importance (as %) of each type of resistance for each sample evaluated. Results obtained in the case of the 12 tested antibiotics are represented in table 1.

Tabel 1. Antibiotic resistance of *E. coli* isolates profiles recovered from Arieş river water samples based on the diameter of inhibition reported to the NCCLS/CLSI standards (2008).

Antimicrobial agents	Disk contents (µg)	Antimicrobial sensitivity				
		1	2	3	4	5
Ticarcillin	75	S	S	I	R	R
Ceftazidin	30	R	R	S	R	R
Gentamicin	10	S	S	S	S	S
Streptomicin	10	S	S	S	S	S
Ophloxacin	5	R	R	S	R	R
Nitrofurantoin	300	S	R	R	R	S
Sulfamethoxazol	25	S	S	S	S	R
Ampicillin	10	R	S	R	R	R
Norfloxacin	10	S	S	S	S	S
Nalidixic acid	30	S	R	S	S	R
Ciprofloxacin	5	R	R	S	R	S
Erythromycin	15	R	R	R	R	R

S = sensitive; I = intermediate; R = resistant; 1 – Abrud; 2 – Baia de Arieş; 3 – Sălciua; 4 – Turda; 5 – Luncani

Data obatined in this work suggest that 48.33% from the *E. coli* isolates recovered from Arieş river water samples were resistant to the 12 tested antibiotics, 50% of them were sensitive and 1.66% have shown medium sensitivity to the tested antibiotics.

At a more accurately analysis, according with the class of antimicrobial agents can be observed that 60%

from the tested strains were resistant to the antibiotics from the Penicillin group (ticarcylin and ampicillin), 30% were sensitive, and 10% of them were medium sensitive to the used antibiotics.

In what view the ceftazidin, majority of the tested strains have shown resistance to this antibiotic. In the same time, gentamycin, streptomycin and norfloxacin proved to be much more effective because they inhibit the microbial development of the isolated strains at the level of the each sampling point. Analyzing the effect of ophloxacin on the isolated strains it can be observed a decreased susceptibility of these strains, *E. coli* showing high resistance (80%) against this antibiotic. The bacterial sensitivity for this antibiotic has been observed in the case of the strains isolated from Sălciua sampling point, respectively a medium sensitivity in the case of the isolates that came from Turda sampling point. In what view the nitrafurantoin the isolated *E. coli* strains showed a high sensitivity in the case of the samples collected from at the level of the two tails of the river (Abrud and Luncani), meanwhile, in the case of the isolates collected from the river course have been registered a resistance to this antibiotic.

High sensitivity was detected in the case of sulphametoxazol; a bacterial resistance was observed only in the case of the isolates collected from Luncani sampling point. Regarding the resistance of the isolates to the ciprofloxacin and nalidixic acid it was observed a relatively high resistance without accentuated differences between the sensitive isolates (47.67%) and the resistant ones (52.33%). The less inhibitory effect was registered in the case of the erythromycin where each isolates showed resistance.

Following the microbial sensitivity of the isolates recovered from the downstream of the river Arieş was observed a high incidence of resistance of the studied *Escherichia coli* strains regarding the used antimicrobial agents, which can be explained as a result of urbanization. The increasing resistance and multiple resistances of the microbial strains in this area may be due to the inefficiency of the water treatment plant in this area, faecal residues and discharge resulted by the house-holding activities and hospital wastes, which are overflowed in the Arieş river through the effluents Racilor and Racoşa. More than 46.66% from the studied isolates highlighted resistance to more than 4 antibiotics.

DISCUSSIONS

Our results have been compared with those from literature [10, 27, 29, 38] who report a high resistance of the studied *E. coli* strains to ampicillin and gentamicin (76%), and an 85% of to ceftazidin. The same authors report the resistance of the studied strains in the 99% to the tetracicline.

Also, this study wants to highlight the applicability of the multiple antibiotic resistance (MAR) with the aim to identify the origin of the faecal pollution, offering information about the source of water pollution, a very useful tool for water management.

An isolate with a value of MAR > 0.2 it is considered as being part from an area with a high risk of contamination (e.g. animal farms, increased human population) where the antibiotics are frequently used.

A high incidence of *E. coli* strains with MAR, was observed in each of the studied samples. In Sălciua sampling point only 25% from the isolates present multiresistance to the tested antibiotics, the rest of the strains were resistant. In the case of the sampling points situated on the downstream of the river, 58.33% from the studied isolates shown multiresistance, meanwhile, the rest of 41.66% being sensible at least 4 antibiotics.

The obtained results in the case of the MAR are represented in table 2.

Based on the calculation of the MAR index it can be recorded in each sampling point the presence of a source of faecal pollution with a high risk of contamination where *E. coli* strains shows a high incidence of resistance and also, a multiple antibiotics resistance that highlighting the existence along the river course of some area where the antibiotics are largely used in the treatment of humans and domestic animals illnesses.

Tabel 2. The establishment of the sourse of the Arieş river water faecal pollution bazed on the MAR index calculation.

MAR value	Sampling points				
	1	2	3	4	5
	0.41	0.50	0.25	0.58	0.59

1 – Abrud; 2 – Baia de Arieş; 3 – Salciua; 4 – Turda; 5 – Luncani

Water pollution with such microbial strains with high incidence of antibiotic resistance as a result of overflowed waste waters, waters resulted from the treatment plants that function inadequately in this area, the practice of intensive grazing on the river bed and also, as a results of an abatoir function upstream of the Turda sampling point. The obtained results has been compared with those from the literature [15], and it can be observed a similarity with these, where the authors show a high incidence of multiple resistance of the *E. coli* isolates recovered from Bhavari river, where the MAR indice shows that 95% of isolates was by human or bovine origin.

The data of the antimicrobial susceptibility reviled that all the studied *E. coli* strains were resistant to most of the tested antibiotics. Moreover, a high incidence of multiple antibiotic resistances was detected in each of the studied samples. The analysis of antibiotic resistance frequencies has showed an incidence of 46.66% strains resistant to more than 4 different antibiotics, demonstrating the existence of some area on the course of the river where antibiotics are broadly used in animals and humans for control of bacterial

infections and incorporated into commercial livestock for growth promotion.

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