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Antimicrobial Resistance and Plasmid Characterization of *Escherichia coli* Isolated in *In natura* Water

¹A. Carnot, ¹J.S. Guerra, ¹T.S. Souza and ²L.C. Carneiro

¹State University of Goiás UEG, Unu Morrinhos, Brazil

²Molecular Biology Institute of Paraná, IBMP, State University of Goiás UEG, Unu Morrinhos, 129 Avenida das Indústrias Avenue, Block 2, Cidade Industrial Neighborhood, Curitiba, People's Republic, Brazil

Corresponding Author: L.C. Carneiro, State University of Goiás UEG, Unu Morrinhos, 129 Avenida das Indústrias Avenue, Block 2, Cidade Industrial Neighborhood, Curitiba, People's Republic, Brazil Zip Code: 81310-060, Tel: +55-41-98622906 Fax: +55 62 32292266

ABSTRACT

The constant use of antibiotics in human and animal medicines is causing a rise in *Escherichia coli* resistance to these medicines, interfering with the effective treatment of infections caused by this agent. For this study, we isolated and identified *E. coli* from in nature water samples and verified the presence of plasmids that caused bacteria resistance. We collated 24 water samples from two different stations (a water treatment station and a water captation station). From the contaminated samples, we evaluated antibiotic resistance to amoxicillin (5 µg) cephalixin (5 µg), azitromicin (5 µg), ampicilin (5 µg), tetracycline (5 µg) and ciprofloxacin (10 µg). We also evaluated whether the resistance was plasmidial or chromosomal. The results showed a frequent occurrence of multiple resistance to the main antimicrobials utilized, including cephalixin (67.44%), amoxicilin (62.79%), ampicilin (58.13%), tetracycline (37.20%), azithromycin (32.55%), ciproflaxacin (18.60%). We observed the presence of different plasmidial profiles, including occurrences of amoxicillin (30%), ampicillin (30%), tetracycline (30%) and ciprofloxacin (10%). The study showed that the samples presented plasmids with genes resistant to important antibiotics used for public health.

Key words: Microbiology, coliforms, antibiotics, bacterial resistance

INTRODUCTION

Fecal coliform bacteria may occur in water contaminated with sewage or animal waste (Tundisi, 2003). *Escherichia coli* is a fecal coliform (Silva *et al.*, 2009). Its 20 existing species are present in the gastrointestinal tracts of humans and warm-blooded animals. It belongs to a group of emerging pathogens that are associated with a broad spectrum of human diseases (Russo and Johnson, 2003).

Around 95% of coliforms in faeces are *E. coli* and this bacterium is considered to be the best indicator of fecal contamination (Silva *et al.*, 2009). This microorganism has been used in quality monitoring for drinking water and water used in the bathroom (Lebaron *et al.*, 2005).

E. coli is associated with virulence factors (Johnson, 1991). The plasmid virulence characteristics can be transferred by Brito *et al.* (2004). The capacity of *E. coli* to harbor and transmit resistance genes is of huge ecological importance in the dispersal of these genes (Saenz *et al.*, 2001). The genes that encode antimicrobial resistance may be located on chromosomes and moving parts such as plasmids (Ishii *et al.*, 2007).

Bacterial resistance has been increasing, presenting resistance to multiple drugs (Pidcock, 2006). The two main factors involved in the development of this resistance are selective pressure and the presence of resistant genes (Witte, 2000). Bacterial resistance mechanisms always reflect genetic changes that can be classified as their own species or result of mutations or some form of gene transfer (Silva *et al.*, 2009).

In general, plasmids contain one or two genes that confer a selective advantage to the bacteria, such as the ability to build resistance to antibiotics (Dulebohn *et al.*, 2013). It is assumed that various strains of antibiotic-resistant *E. coli* have been isolated from drinking water and may constitute a direct threat to humans, eventually replacing normal intestinal microbiota. Infections caused by resistant microorganisms hinder therapies and restrict the use of antibiotics. Thus, there is an interest in antimicrobial susceptibility testing for bacterial strains such as *E. coli* that survive in different environmental conditions (Melo, 2006).

This study aimed to evaluate fecal contamination levels using *E. coli* as an indicator for fresh water collected in a water captation station and a water treatment station. We evaluated the bacteria's plasmid DNA profiles and the antibacterial resistance conferred by these plasmids.

MATERIALS AND METHODS

The samples were collected in two instances. Twelve samples were collected at the water captation station and 12 at the water treatment station, for a total of 24 samples. For water analysis, we used the techniques described by APHA, AWWA, WEF (2013).

The samples were inoculated according to the multiple tube procedure (Silva *et al.*, 2010), inoculating three sets of triplicate (0.1, 1.0 and 10.0 mL). We used a lactose broth medium with bromocresol purple (BCP-CL) (Amin, 1997) and incubated it at 37°C for 48 h. Positive tubes were transferred in quintuplicate to an EC broth medium with inverted Durham tubes and incubated in a water bath at 45°C for 24 h. The data were recorded using the Hokins Table (Speck, 1992).

Following antibiotics were tested: Amoxicillin (5 µg), cephalexin (5 µg), azithromycin (5 µg), ampicillin (5 µg), tetracycline (5 µg) and ciprofloxacin (10 mg). The susceptibility profile of the *E. coli* isolates was determined by the antimicrobial resistance technique in a liquid medium (Atlas, 2004).

For plasmid extraction, we used the alkaline lysis method. The plasmid DNA samples were analyzed in 1% agarose gel. The plasmid DNA bands were viewed under ultraviolet irradiation (Sambrook and Russell, 2001). Subsequently, the *Eco* R1 digestion system was prepared according to the manufacturer's instructions (Biosystems ®).

RESULTS

The bacteriological parameters observed showed the occurrence of 12 fecal coliforms at the water captation station and 12 at the water treatment station, as shown in Table 1. The presence of *E. coli* indicates unsatisfactory hygienic conditions.

All isolates showed resistance to at least one of the six antimicrobials or to more than one antimicrobial while showing a high resistance index (Table 2).

Samples contaminated with *E. coli* showed low levels of ciprofloxacin resistance (18.60%) and azithromycin resistance (32.55%). These findings may be related to the low intake of these drugs. The highest levels of antimicrobial resistance were found in samples from the water treatment station, using betalactamics, cephalexin, amoxicillin and ampicillin (Fig. 1).

Table 1: Occurrence of fecal coliforms

Station (UFC mL ⁻¹)	Most probably No. (MPN 100 mL ⁻¹)											
	1	2	3	4	5	6	7	8	9	10	11	12
Station1*	6	4	6	6	<2	2	<2	4	4	6	2	4
Station2*	4	6	4	4	4	2	2	2	4	6	4	2

Station1*: Water captation station; Station2*: Water treatment station

Table 2: *E. coli* resistance isolates to antibiotics in both stations

Antibiotic	Resistance cultures			
	No. of samples	No. of isolates cultures	No. of resistance	Percentual
AMX*	72	43	27	62.79
AMP*	72	43	25	58.13
CEF*	72	43	29	67.44
AZ*	72	43	14	32.55
TE*	72	43	16	37.20
CP*	72	43	08	18.60

*AMX: Amoxicillin, AMP: Ampicillin, CEF: Cephalexin, AZ: Azithromycin, TE: Tetracyclin, CP: Ciprofloxacin

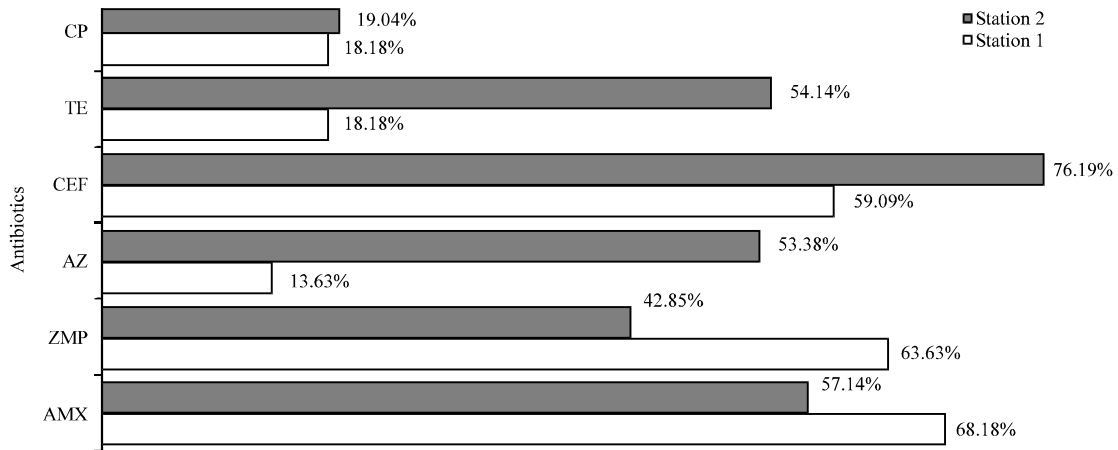


Fig. 1: Comparison of the antibacterial resistance of samples, *AMX: Amoxicillin, AMP: Ampicillin, CEF: Cephalexin, AZ: Azithromycin, TE: Tetracyclin, CP: Ciprofloxacin, Station1*: Water captation station, Station2*: Water treatment station

We studied the antibiotic resistance of profile plasmids from bacteria tested and it is clear that there was no azithromycin or cephalexin resistance (data not shown). Bacterial resistance to ampicillin and amoxicillin showed mostly low molecular weight. Samples from *E. coli* strains were digested with the *Eco* R1 enzyme and we observed the presence of plasmid profiles above 2.5 kb (data not shown).

DISCUSSION

The coliform results obtained from the water captation station and water treatment station are within the normal range according to the 357 CONAMA Resolution (Brasil, 2005). *E. coli* is associated with virulence factors (Johnson, 1991). Plasmid virulence characteristics can be

transferred by plasmids from pathogenic samples to non-pathogenic samples. Similar results were obtained in this study within the parameters set by the resolution.

Antimicrobial resistance studies were developed in isolates from sewage in coastal regions of Espírito Santo state, where the highest rates of ampicillin resistance was observed. Similar data were found in this study. The high rates of ampicillin resistance may be related to the earlier emergence of this drug, which has been used by the population for a longer period of time and has greater bacterial resistance (Ribeiro *et al.*, 2000).

Bechtluft (1999) described an association between increased bacterial resistance and the number of plasmids in a gradient of organic pollution at a water treatment station. The susceptibility results confirm that some of these multidrug-resistant bacteria are plasmid-mediated. The plasmids may have the same profile and are not identical. Therefore, they are differentiated by enzyme digestion restriction and molecular characterization (Lazaro, 2004).

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