



Research Article

ASSESSMENT OF THE BACTERIOLOGICAL QUALITY OF THE RAW WATER AND THE ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF BACTERIA ISOLATED IN WATER SURFACE OF A RIVER

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Abstract- Antibiotic resistance in the aquatic environment can originate from contaminants of human and animal origin such as waste, sewage, effluents, chemical and pharmaceutical products. These contaminants may select resistant organisms and/or introduce resistant bacteria directly in this environment. Thus, the aquatic environment acts as a reservoir for the spread of multidrug-resistant bacteria. The aim of this study was to evaluate the microbiological contamination and the level of antibiotic resistance in isolates from samples of raw surface water of the Meia Ponte River, in Goiás State, Brazil. It was performed the multiple-tube fermentation technique to quantify bacterial contamination of the water samples from the river, biochemical tests for the identification of bacterial isolates, and disk diffusion susceptibility test to check sensitivity to antimicrobial agents. It was found high counts of bacteria in the coliform group, mainly during rainy periods. Of the 103 isolated bacteria, the majority was capable of transmitting diseases, mostly gastroenteritis. In addition, it was observed a worrying rate of resistant bacteria present in the environment. Further researches are necessary to understand the demand of antibiotic resistance in the aquatic environment, and to verify its consequences. Some measures should be created to prevent the spread of resistant microorganisms in the environment.

Keywords- Resistance, Antibiotics, Contaminants, Aquatic environment, Bacteria

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Introduction

The microbial community present in fresh water can be impacted by anthropogenic action, resulting in changes in water quality and in functions performed by this environment [1]. In accordance with Korajkic et al. [2], the change of the microbial community occurred at an event of pollution, mainly caused by sewage, characterize as a form of water quality research.

The microbiological contamination in water of rivers can occur at pasture, runoff of rainwater, direct deposit of fecal material by animals, discharge of contaminated sewage effluents and wastewater treatment [3]. The new microorganisms introduced into the environment may be pathogenic and are capable of transmitting by direct contact such as drinking contaminated water or practicing recreational activities, or by indirect contact, for example, fishing and eating raw fruits and vegetables that are irrigated with contaminated water [4, 5]. These microorganisms cause the spread of several diseases, including gastrointestinal and breathing problems, skin and ear infections [6-8].

In addition to the great potential of transmission of waterborne illness that microorganisms can spread, the level of pollution and the use of antibiotics increased the resistance of bacteria present in the environment [6, 9]. Several researches have given attention to the spread of antibiotic-resistance gene due to the problems associated with human health, especially complications in the treatment of diseases that have been encouraging the vigilance in antibiotic administration [9]. Due to the growth of potentially pathogenic microorganisms, the increase of pollution by chemical agents causes the increase in antimicrobial resistance in bacteria that are present in the environment [10, 11].

Metals, antibiotics and chemical compounds, in general, are contaminants that may exert selective pressure on the aquatic microbiota and modify the resistance of bacteria present in water bodies, as well as promoting the transfer and dissemination of antimicrobial resistance [12, 13]. The detection of bacteria resistant to antimicrobial agents in aquatic environments can be used as a bioindicator to monitor the degree of environmental pollution [14], and then as an accessory tool for monitoring the pollution and the water quality of the Meia Ponte River, in Goiás State. The study of these bacteria promotes the knowledge of these microorganisms as potential reservoirs and disseminators of resistance to other pathogenic bacteria, as well as assess the ecological risk to the environmental health [6, 12, 15].

Therefore, the objective of this study was to evaluate the bacteriological quality of surface water of the Meia Ponte River, in Goiás State, Brazil, and to check the antimicrobial susceptibility profile of isolated bacteria.

Materials and Methods

Area of the study

The study area comprised the basin of the Meia Ponte River, in six sampling points along the course of the river, passing through some cities such as of Goiânia, Brazabrantes and Inhumas. These points were called as P01 (16°47'45.6"S; 49°09'45.5"W), P02 (16°40'27.0"S; 49°11'49.6"W), P03 (16°38'20.4"S; 49°15'51.4"W), P04 (16°34'11.4"S; 49°19'45.4"W), P05 (16°26'00.0"S; 49°23'43.9"W) and P06 (16°20'19.7"S; 49°29'43.5"W).

Sample collection and transport

The sample collections were carried out from August 2015 to September 2016, and the transport followed the Brazilian Guide for Collection and Preservation of Samples: Water, sediment, aquatic communities and liquid effluents [16]. Four samples were collected in this period (twice during the dry season and twice during the rainy season), being collected approximately 200 mL of superficial raw water at each point, and from a depth of approximately 30 cm.

Total coliforms

The multiple-tube fermentation technique was used to analyze the presence of bacterial contamination by the groups of thermotolerant coliforms, that determines and quantifies the most probable number (MPN) of bacterial groups thermotolerant coliforms. The tests were performed according to the recommendations of the APHA [18], presenting some adaptations. The water samples were homogenized, and aliquots of 1000 µL, 100 µL and 10 µL were inoculated in tubes containing 10 mL of lactose broth and inverted Durham tubes. Each aliquot was inoculated in a series of five tubes and incubated at 44°C for 48 hours. Subsequently, it was found the bacterial growth by turbidity in the culture medium and gas production in Durham tubes. In the samples that showed bacterial fermentation, aliquots of 100 µL were transferred to a tube containing brilliant green bile broth and 100 µL were transferred to EC medium broth (*Escherichia coli*), both with inverted Durham tubes. The brilliant green bile broth was incubated for 48 hours at 37 °C, and the EC medium broth was incubated for 48 hours at 44°C. The samples with fermentation in lactose broth suggest the presence of total coliforms, in brilliant green bile broth indicate the presence of total coliforms, and in EC medium broth suggest the presence of *Escherichia coli*. The results obtained were interpreted according to the data presented by APHA [17].

Bacterial isolation

The samples with fermentation in lactose broth were selected and inoculated on nutrient agar. These samples were incubated under aerobic conditions for 24 hours at 37°C. Subsequently, the plates were monitored and after the growth, the different colonies on each plate were selected and isolated. Then, the isolates were incubated under the same conditions for obtaining pure colonies by the streak plate method, being stored in freezers at -20°C in 20% glycerol.

Bacterial identification

For gender/species identification, the isolated bacteria were subjected to the characterization by morpho-colonial, morpho-tinctorial and biochemical tests, in accordance with the National Sanitary Surveillance Agency [18].

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed according to methodology for disk diffusion test by Bauer & Kirby [19]. Discs of antibiotics in agar were used with the assistance of sterile forceps. After placing the discs, the plates were inverted and incubated for 24 hours at 36 °C, latterly they were analyzed through of inhibition halos. In addition, the analysis of the result was compared by the criteria of Clinical & Laboratory Standards Institute [20], that was based on the diameter of the inhibition halos, determining if the bacterium had sensitivity, resistance or intermediate to the antibiotics tested. The antimicrobials used for gram-positive bacteria isolated were ciprofloxacin (5µg/disk), chloramphenicol (30µg/disk), erythromycin (15µg/disk), penicillin G (10 units), rifampin (5µg/disk), tetracycline (30µg/disk) and vancomycin (30µg/disk). On the other hand, the antimicrobials used for gram-negative bacteria isolated were aztreonam (30µg/disk), amikacin (30µg/disk), amoxicillin / clavulanic acid (30µg/disk), ampicillin (10µg/disk), cefepime (30µg/disk), cefoxitin (30µg/disk), ciprofloxacin (5µg/disk), cefuroxime (30µg/disk), ceftazidime (30µg/disk), meropenem (10µg/disk) and gentamicin (10µg/disk).

Statistical test

The STATISTICA software 7.0 version [21] was used to analyze the results and to determine the percentage of the identification of the isolates and the sensitivity or

resistance profile.

Results

The Brazilian legislation classifies the rivers based on water quality standards. The 357 Resolution, from March 17, 2005, of the National Environment Council (CONAMA) [22], establishes provisions for the classification of water bodies as well as environmental directives for their framework, and standards for effluent releases. In accordance with this Resolution, the waters of the Meia Ponte River are classified as class 2 [23], being designed to water supply for human consumption, after conventional treatment. For class 2, the accepted MPN is ≤ 1000 thermotolerant coliforms in 100 mL of water sample [22]. The results of this study demonstrate that all samples of raw water surface of the Meia Ponte River, collected during the rainy season, were above the standard limit by law. In relation to the dry period, only the points P01, P02 and P03 in the collection 1 and points P02 and P03 in the collection 4 were above the standard limit of Brazilian legislation [Table-1].

Table-1 Result of thermotolerant coliforms count in water samples from the Meia Ponte River - Goiás State.

Collection/Period	Sample sites	MPN/100mL	95% Confidence Limits	
			Lower	Upper
Collect 1 - dry season (August/2015).	P01	> 1600	700	-
	P02	> 1600	700	-
	P03	> 1600	700	-
	P04	< 1.8	0	10
	P05	-	-	-
	P06	< 1.8	0	10
Collect 2 - rainy period (December/2015).	P01	>1600	700	-
	P02	>1600	700	-
	P03	>1600	700	-
	P04	>1600	700	-
	P05	>1600	700	-
	P06	>1600	700	-
Collect 3 - rainy season (February/2016)	P01	>1600	700	-
	P02	>1600	700	-
	P03	>1600	700	-
	P04	>1600	700	-
	P05	>1600	700	-
	P06	>1600	700	-
Collect 4 - dry period (September 2016)	P01	17	5.9	36
	P02	>1600	400	4600
	P03	>1600	400	4600
	P04	33	10	100
	P05	79	22	220
	P06	350	100	1100

Highlighted values are above the established by 357 Resolution, from 2005, of CONAMA [23].

Analyzing six sampling points in four collections, 103 bacteria were isolated (31.06% gram-positive and 68.94% gram-negative). The [Table-2] presents the identification in percentage of these isolates. The most abundant bacterial family found was the Enterobacteriaceae.

The antibiogram verified the resistance pattern of isolates of this study. For the microorganisms gram-positive, the *Bacillus spp.* and *Planococcus spp.* were ignored, due to the CLSI [20] does not have a resistance pattern to these isolates. For the *Enterococcus spp.* (n= 21), 4.76% (n= 1) of resistance was found to vancomycin, 9.52% (n= 2) to ciprofloxacin, chloramphenicol and penicillin, 19.05% (n=4) to tetracycline, 42.86% (n=9) to erythromycin, and 57.14% (n=12) to rifampin. For intermediate resistance, 4.76% (n=1) of resistance was found to vancomycin and rifampin and 19.05% (n=4) to ciprofloxacin and erythromycin. The bacteria that had complete resistance to two or more classes of tested antimicrobial agents were considered multi-resistant bacteria. Among these bacteria, 14.29% (n=3) were resistant to two antimicrobial classes, 4.76% (n=1) were resistant to three antimicrobial classes, and 14.29% (n=3) were resistant to four antimicrobial classes.

For the microorganisms gram-negative, the *Aeromonas spp.* was ignored, because there is not any standardization in the CLSI [21] for this microorganism.

For the family *Enterobacteriaceae* and *Vibrio* spp. (n= 64), 4.69% (n= 3) of resistance was found to meropenem and gentamicin, 6.25% (n= 4) to ampicillin, 9.38% (n= 6) to ciprofloxacin, 12.50% (n= 8) to ceftazidime, 14.06% (n=9) to aztreonam and cefoxitin, 15.63% (n= 10) to cefuroxime, 20.31% (n= 13) to amikacin and amoxicillin / clavulanic acid, and 48.44% (n= 31) to cefepime. Among these, 7.81% (n=5) were resistance to two antimicrobial classes, and 1.56% (n= 1) were resistance to three antimicrobial classes.

Table-2 Identification of bacterial isolates found in samples of water from the Meia Ponte River - Goiás State.

Bacterial isolate	Quantity	Percentage
<i>Aeromonas</i> spp.	1	0.97%
<i>Citrobacter freundii</i>	1	0.97%
<i>Enterobacter aerogenes</i>	1	0.97%
<i>Morganella morganii</i>	1	0.97%
<i>Pantoea agglomerans</i>	1	0.97%
<i>Planococcus</i> spp.	1	0.97%
<i>Providencia</i> spp.	1	0.97%
<i>Pseudomonas</i> spp.	2	1.94%
<i>Vibrio</i> spp.	2	1.94%
<i>Citrobacter freundii</i>	4	3.88%
<i>Enterobacter agglomerans</i>	6	5.83%
<i>Plesiomonas</i> spp.	6	5.83%
<i>Salmonella</i> spp.	7	6.80%
<i>Enterobacter cloacae</i>	10	9.71%
<i>Enterobacter</i> spp.	12	11.65%
<i>Escherichia coli</i>	12	11.65%
<i>Bacillus</i> spp.	14	13.59%
<i>Enterococcus</i> spp.	21	20.39%
Total	103	100%

Discussion

The highlighted microbiological results in [Table-1], according to the Brazilian legislation [22], suggest that the waters of the Meia Ponte River are not recommended for the practice of recreational activities designated for primary contact, aquaculture and protection of aquatic communities. Thus, to use this river for public human consumption, it is necessary to apply the conventional or advanced treatment of water supply.

Fonseca [24] conducted a survey to establish the levels of total coliforms in the urban area of Goiânia, Goiás State. During the dry period, the results obtained in this study are coincident to the study of Fonseca [24]. However, the results are divergent during the rainy period. It could be explained for many reasons such as different types of pollutants, human activity and natural phenomena.

The coliform bacteria are commonly used to evaluate the quality of surface waters, because they include bacteria of fecal origin, being use as indicative of the water quality and risk of infectious diseases [25], such as gastrointestinal diseases, mainly [5]. The waters of the Meia Ponte River used without prior treatment for leisure activities can cause harm to people's health, when they are above the values allowed by the legislation.

The studies of Laurent & Mazumder [26] indicate that the increase in precipitation causes the elevation of concentrations of fecal coliforms in the water from rivers, that is influenced by point source pollution and problems of drainage of water to rivers caused by vegetation and artificial dams. It could explain in this study why the results of MPN indicated an increase in the concentration of total coliforms during the rainy season (points P01, P02 and P03 of the collections 2 and 3), when compared with the dry period. Nevertheless, further studies would be necessary to confirm this statement.

Abia et al. [27] suggests that there is a greater risk of infections with microorganisms associated with the water of the Apies River, in South Africa, in the rainy season when compared with the dry season. Utilizing this knowledge for this research, it is suggested that a greater risk of bacterial infections are associated with the consumption or practice of leisure activities in the waters of the Meia Ponte River, during the period of high precipitation. However, to confirm this statement, further studies are needed.

The changes in temperature and precipitation may influence the process of

dissemination of pathogens in the environment, playing an important role in the seasonality of enteric diseases [28]. Thus, the seasonal influence in this study implies a higher incidence of bacteria in the surface waters of the Meia Ponte River during the rainy season. Among all bacteria isolated, 65.17% were pathogenic and enteric species.

The resistance data demonstrated above are important because they can indicate that the environmental bacteria previously described are reservoirs of resistance to antimicrobial agents, facilitating the dissemination of resistance genes in the aquatic environment [6, 8, 12, 13, 29-31]. However, more studies are needed for this verification.

According to Wellington et al. [32], antibiotic resistance in the environment occurred by mutation or by acquisition of mobile genes that have evolved over time in bacteria. This may be an explanation of resistance found in this study, which may have been acquired by these factors. Because of it, the antimicrobial agents used in this study comprised the class of β -lactam antibiotics, aminoglycosides, quinolones, fluoroquinolones, phenicols and glycopeptides, getting resistance of all antibiotics tested by at least one of the isolates. This study found resistance and multiple resistances to antimicrobials, regardless of the period of collection.

Studies have reported the existence of bacterial resistance to antimicrobial agents in rivers, as for example, in Bangladesh [33], Pakistan [34], China [35, 36], Iran [37], Russia [38], India [39, 40], Nigeria [41], Portugal [8, 42], United States [43], Canada [44], Spain [45] and Vietnam [46]. These researches demonstrate the importance of this theme to the environmental health, revealing many risks for health and the environment.

In Brazil, there is also a concern about the risks of antibiotic resistance in the aquatic environment. A study conducted by Rocha et al. [47], in the Água Boa River, in Mato Grosso State, Brazil, detected the resistance of *Escherichia coli* to antibiotics as ampicillin and ampicillin/sulbactam, and the resistance of genus *Pseudomonas* to antibiotics as ceftazidime, cefepime, imipenem, meropenem, gentamicin and piperacillin/tazobactam. Another study conducted by Conte et al. [48], in the Iguazu River, in Curitiba City, Brazil, detected enterobacteria with resistance to β -lactamases and quinolones. In addition, Coutinho et al. [49] analyzed the bacterial diversity in aquatic environments in the Rio de Janeiro State, Brazil, highlighting the resistance to ampicillin.

The concern with the health should be constant, due to the increase and prevalence of antibiotic resistance genes in aquatic environments [50]. Berendonk et al. [51] proposes that measures for identification of antibiotic resistance and their respective genes are essential studies for the evaluation of the environment. To prevent this type of contamination, the author suggests that it may be implemented technological and policy solutions for the management. Thus, it is required greater attention and vigilance of the Meia Ponte River, due to the results of resistance obtained in this study. An integrated and multidisciplinary approach should be created to support further research connected to the problem of antibiotic resistance in the Meia Ponte River.

As well as Coutinho et al. [49], this study suggests that the conscious use of antibiotics, adequate management, reduction of environmental impacts and pollution in water environments, may be strategies to decrease the resistance in the aquatic environment, preserving the efficacy of these antimicrobial drugs. Furthermore, public policies must be implemented on the issue of antibiotic resistance in Rio Half bridge.

Conclusion

The number of total coliforms in water samples was increased in relation to the established by the legislation, demonstrating high contamination of fecal origin in analyzed samples of water from the Meia Ponte River. The bacterial diversity found in the Meia Ponte River should be considered, since the majority of the isolates can cause human diseases, requiring attention from public health. This bacterial diversity has several origins, however, in the environment where water samples were collected, there are seasonal and human influences, especially of fecal contamination which can influence the diversity of microbiological species.

In Brazil, the contamination of water bodies with antibiotic resistant bacteria is a

reality that must be monitored and studied. This study proved that, in the Meia Ponte River, there are antibiotic resistant bacteria tested. The main problem associated to this resistance in the environment, is that many bacterial strains were not cultivated yet, being responsible for the dissemination and transfer of genes conferring resistance to antimicrobial agents. This phenomenon has not been widely studied, requiring further research.

The surveys of antimicrobial resistance and their genes into rivers and water bodies in Brazil are relatively small, requiring more attention to this theme of great importance for the health of living beings. The oversight bodies should pay attention to the information presented in this work, since the large amount of chemicals and pharmaceuticals products discharged into the aquatic environment. Moreover, to complement this research, new molecular biology studies are being carried out to identify the resistance of bacterial isolates in the aquatic environment of the Meia Ponte River.

Declarations

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Competing interests

The authors declared that they have no competing interests.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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