

Identification and resistance profile of gram positive bacteria from aquatic environment

Identificação e perfil de resistência de bactéria gram-positivas do ambiente aquático

Identificación y perfil de resistencia a bacterias gramopositivas en el ambiente acuático

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Abstract

The Meia Ponte River – Goiás/Brazil, is responsible for benefiting about 2 million people in Goiás State. However, the increase in pollution with the disposal of sewage, chemicals and drug remains have contributed to the increase in bacterial resistance and the exchange of resistance genes. The objective of this study was to isolate, identify and analyze the resistance profile of gram-positive bacteria present in raw water and sediment of the Meia Ponte River – Goiás. The samples were collected from four sampling points and two collections were carried out, one in the dry season and the other in the rainy season. The isolated bacteria were identified, then the antibiogram was performed. A total of 75 strains were isolated, 72.0% (54/75) of *Streptococcus* spp., 12.0% (9/75) of *Staphylococcus* spp., 9.3% (7/75) of *Bacillus* spp. and 6.7% (5/75) of *Enterococcus* spp. Furthermore, 52.0% (39/75) of the isolated strains were from raw water and 48.0% (36/75) were isolated from the sediment. Among the samples, strains of *Staphylococcus* spp. and *Bacillus* spp. showed greater resistance to antimicrobials, on the other hand, *Enterococcus* spp. showed less resistance. Some strains of *Bacillus* spp. and *Streptococcus* spp. presented multidrug resistant, *Staphylococcus* spp. showed multidrug resistant and some pan-drug resistant. In the correlation of Spearman *Staphylococcus* spp. and *Streptococcus* spp. isolated, were the ones that presented the most significant correlations ($p < 0.05$). Thus, the study shows the importance of ascertaining the resistance profile of this group of bacteria that aquatic environment.

Keywords: Correlation; Drugs; Pan-drug; Resistance genes.

Resumo

O Rio Meia Ponte - Goiás/Brasil, é responsável por beneficiar cerca de 2 milhões de pessoas no Estado de Goiás. No entanto, o aumento da poluição com o descarte de esgoto, produtos químicos e restos de drogas têm contribuído para o

aumento da resistência bacteriana e a troca de genes de resistência. O objetivo deste estudo foi isolar, identificar e analisar o perfil de resistência das bactérias gram-positivas presentes na água bruta e sedimento do Rio Meia Ponte - Goiás. As coletas foram realizadas em quatro pontos de amostragem e foram realizadas duas coletas, uma no período da seca e outra no período da chuva. As bactérias isoladas foram identificadas e, em seguida, realizado o antibiograma. Um total de 75 cepas foram isoladas, 72,0% (54/75) de *Streptococcus* spp., 12,0% (9/75) de *Staphylococcus* spp., 9,3% (7/75) de *Bacillus* spp. e 6,7% (5/75) de *Enterococcus* spp. Além disso, 52,0% (39/75) das cepas isoladas foram provenientes de água bruta e 48,0% (36/75) foram isoladas do sedimento. Dentre as amostras, cepas de *Staphylococcus* spp. e *Bacillus* spp. apresentou maior resistência aos antimicrobianos, por outro lado, *Enterococcus* spp. mostrou menos resistência. Algumas cepas de *Bacillus* spp. e *Streptococcus* spp. apresentou multirresistência, *Staphylococcus* spp. mostrou multirresistência e alguns pan-resistências. Na correlação de Spearman *Staphylococcus* spp. e *Streptococcus* spp. isolados, foram os que apresentaram as correlações mais significativas ($p < 0,05$). Dessa forma, o estudo mostra a importância de se conhecer o perfil de resistência desse grupo de bactérias nesse ambiente aquático.

Palavras-chave: Correlação; Drogas; Genes de resistência; Pan-resistências.

Resumen

El río Meia Ponte - Goiás/Brasil, es responsable de beneficiar a cerca de 2 millones de personas en el estado de Goiás. Sin embargo, el aumento de la contaminación con eliminación de aguas residuales, productos químicos y restos de medicamentos ha contribuido al aumento de resistencia bacteriana y intercambio de genes de resistencia. El objetivo de este estudio fue aislar, identificar y analizar el perfil de resistencia de bacterias grampositivas presentes en aguas crudas y sedimentos del río Meia Ponte - Goiás. Las muestras se recolectaron de cuatro puntos de muestreo y se realizaron dos recolecciones, una en época seca y otra en época de lluvias. Se identificaron las bacterias aisladas, luego se realizó el antibiograma. Se aislaron un total de 75 cepas, 72,0% (54/75) de *Streptococcus* spp., 12,0% (9/75) de *Staphylococcus* spp., 9,3% (7/75) de *Bacillus* spp. y 6,7% (5/75) de *Enterococcus* spp. Además, el 52,0% (39/75) de las cepas aisladas procedían de agua cruda y el 48,0% (36/75) se aislaron del sedimento. Entre las muestras, cepas de *Staphylococcus* spp. y *Bacillus* spp. mostró mayor resistencia a los antimicrobianos, por otro lado, *Enterococcus* spp. mostró menos resistencia. Algunas cepas de *Bacillus* spp. y *Streptococcus* spp. presentó resistentes a múltiples fármacos, *Staphylococcus* spp. se mostró multirresistente y algunos panresistentes. En la correlación de Spearman *Staphylococcus* spp. y *Streptococcus* spp. aisladas, fueron que presentaron las correlaciones más significativas ($p < 0,05$). Así, el estudio muestra la importancia de conocer el perfil de resistencia de este grupo de bacterias que el medio acuático.

Palabras clave: Correlación; Fármacos; Genes de resistencia; Panresistentes.

1. Introduction

In the mid-1940s, the fight against infections began using antibiotics and over the years this drug has been applied in agricultural, industrial and veterinary sectors. The indiscriminate use of antimicrobials contributes to the increase in resistant bacteria, including the dissemination of their resistance genes in the environment (Shao et al., 2018).

Sewage discharged into rivers is associated with an increase in resistant bacteria in the environment, due to the fact that these medications are not fully metabolized by the body, leaving traces present in both urine and feces of living beings. Other factors that also contribute to the topic addressed are the medicines applied in agriculture that end up contaminating soils and rivers, as well as industries despoiling them in the aquatic environment (Gogoi et al., 2018; Ruiz-Aguirre et al., 2017). In addition to these reported compounds, numerous others also influence the increase in environmental contamination, being called emerging contaminants (ECs). Thus, ECs have caused environmental impacts, increased bacterial resistance and generated public health problems (Gogoi et al., 2018; Mohapatra & Kirpalani, 2019).

The increase in antibiotic resistance genes (ARGs) in environmental bacteria is associated with selective pressure of antibiotics in water, together with the acquisition of resistance genes present in the environment from animals and/or humans, causing the exchange of genetic material in aquatic sites (Maruzani et al., 2020; Wang et al., 2020).

The aquatic environment is considered the largest disseminator and deposit of ARGs, this is due to pollution and the "stress" that occurs on the bacteria in the presence of antibiotics in the environment (Amarasiri et al., 2019; Dang et al., 2017). The ARGs sharing occurs through mobile genes as conjugated plasmids and transposons, integron, among others;

thus facilitating horizontal transfer and exchange of genes among bacteria (Dang et al., 2017; Sobisch et al., 2019).

The presence of resistant gram-positive bacteria in aquatic sites is of concern, as some are signs of fecal contamination, such as *Enterococcus*. Others are pathogenic to humans, such as *Staphylococcus* (Karkman et al., 2018), in which the acquisition of resistance genes makes it difficult to find effective antimicrobials against infections (Rahmani et al., 2020). Other gram-positive bacteria considered important are *Streptococcus* spp., which are responsible for being pathogenic and for developing severe infections in humans (Zhang et al., 2018), and *Bacillus* spp. that are known for spore formation, resistance to environmental changes and can cause food poisoning in the population (Ruiz-Aguirre et al., 2017).

The Meia Ponte River is one of the main water resources in the Goiás State - Brazil, being responsible for benefiting about 2 million people from Goiás and contributing to agriculture, livestock, water supply, recreation, among others. With the urbanization and with one of the tributaries running through the metropolitan region of Goiânia city, it caused an increase of pollution, interfering with its water quality (Bailão et al., 2020; Coelho et al., 2021).

Because it is an essential condition of the water supply network of Goiás and knowing that the presence of resistant bacteria that location can harm the health of the population, it becomes necessary to research and investigate the presence of these microorganisms in this aquatic environment. Thus, the aim of the study was to isolate, identify and verify the resistance profile of gram-positive bacteria taken from raw water and sediment from the aquatic environment.

2. Methodology

This study deals with an experimental research (Köche, 2016; Pereira et al., 2018) for its methodological basement.

2.1 Study area and sample collection

The collection of raw water and sediment samples were made in four sample points following the basin of the Meia Ponte River - Goiás/Brazil, at the sampling points MP01 (16°54'16.3" S, 49°07'37.8" W), MP02 (16°44'27.7" S, 49°08'35.3" W), MP03(16°39'26.0" S, 49°12'27.1" W) e MP04 (16°36'36.7" S, 49°16'58.8" W). The collection was carried out in the period of December 2018, characterized as the beginning of the rainy season and in the period of September 2019, characterized as dry period.

The collection of raw water and sediment followed the National Guide for Collection and Preservation of Samples (Brandão et al., 2011), 100 mL of raw water and 20 g of sediment from each sampling point were collected in sterile flasks, in addition to recording the water temperature with the aid of a thermometer at the time of each collection. After collection, the samples were stored and refrigerated on ice (temperature between 2 °C to 8 °C), and these samples were to be processed within a maximum of 24 hours after collection.

2.2 Bacterial count and isolation

The 20 g of sediment samples from each collection point were added in 0.5% Tween solution (200 mL of solution) and incubated in a shaker (BIOCOMPARE®) for 2 hours at 30 °C, later, 200 µL of this solution was seeded in petri dishes using the scanning technique and incubated for 48 hours at 37 °C. The raw water samples were homogenized, 200 µL of these samples were seeded in petri dishes using the scanning technique and the plates were incubated for 48 hours in a bacteriological incubator at 37 °C (APHA, 2017).

The petri dishes had specific culture media for isolation and quantification of gram-positive bacteria, using the *Azide Dextrose Broth Agar* (ADBA) and *Enterococcus Confirmatory Agar* (ECA), both from the company HIMEDIA®. The bacterial isolation and quantification process followed the protocol (APHA, 2017), being necessary to quantify the Colony Forming Units (CFU) present on the plates, select the morpho-colonial characteristics of each culture medium used, following

the respective leaflets of the HIMEDIA® to carry out the isolations of these bacteria. The preservation of pure colonies, in turn, were preserved in 2 mL cryogenic tubes, containing 20% glycerol in medium BHI (*Brain Heart Infusion*) BD® at -4°C.

2.3 Bacterial identification

The identification followed the Manual of Clinical Microbiology for the Control of Infection Related to Health Care (ANVISA, 2013). To phenotypic identification was used tests: Gram stain, catalase test, verification of spore and filament production, motility were performed, hydrogen sulfide (H₂S) production, indole production, 5% NaCl tolerance test, esculin bile test, blood agar growth and hemolysis and DNase test.

2.4 Antibiogram

The antimicrobial susceptibility test of disk diffusion method followed the National Sanitary Surveillance Agency (ANVISA, 2005), was done the suspension of the bacterial strain in 0.85% saline solution, adjusting with the turbidity on the 0.5 McFarland scale. With the aid of a swab, the bacterial suspension was evenly seeded in a Petri dish, containing Mueller-Hinton agar. Then, a set of antimicrobial discs was distributed over the agar surface. The plates were incubated in a bacteriological incubator at 30°C for 24 hours and then the inhibition halos were read.

The antibiotics used during the experiment were: Ampicillin (AMP 10 µg), Azithromycin (AZI 15 µg), Cefoxitin (CFO 30 µg), Ciprofloxacin (CIP 5 µg), Clindamycin (CLI 2 µg), Chloramphenicol (CLO 30 µg), Erythromycin (ERI 15 µg), Gentamicin (GEN 10 µg), Linezolid (LNZ 30 µg), Oxacillin (OXA 1 µg), Penicillin G (PEN 10 µg), Rifampicin (RIF 5), Sulfazotrim Sultamethoxazole/Trimethoprim (SUT 25), Tetracycline (TET 30 µg) e Vancomycin (VAN 30 µg), being both from polisensidisc 15 DME gram positive (DME®). The interpretation of the results followed the (CLSI, 2019) and (Yusuf et al., 2018). Following (Asma et al., 2019) and modifications, it was considered Multi-Drug Resistant (MDR) bacteria resistant to 3 or more classes of antibiotics tested and Pan-Drug Resistant (PDR) bacteria resistant to all tested antibiotics.

2.5 Statistic

For data tabulation and descriptive statistics, Microsoft Excel 2016 was used. To verify the association and correlation, Spearman's correlation test was used in STATISTICA software 7.0 version (StatSof©) (StatSoft I, 2012).

3. Results

The The water temperature during the collections in the dry and rainy period of all sampling points had an average of 23.9 °C, being an elevated temperature compared to the established criterion of < 20 °C (WHO, 2017).

Table 1 shows the results of counting the CFU of the culture media from the two collections.

Table 1. Bacterial quantification in culture media with samples of raw water and sediment in an aquatic environment from the Meia Ponte river, Goiás State, Brazil.

Collection and Period	Sample	Culture Mediums	Sample Points (CFU/200µL)			
			MP1	MP2	MP3	MP4
Collection 1 - rainy season	Raw water	ADBA	4	15	80	1
	Sediment	ADBA	4	126	353	61
	Raw water	ECA	1	7	29	1
	Sediment	ECA	1	51	31	16
Collection 2 - dry period	Raw water	ADBA	1	238	353	1
	Sediment	ADBA	0	5	0	0
	Raw water	ECA	0	69	16	0
	Sediment	ECA	0	2	0	4

ADBA: Azide Dextrose Broth Agar; ECA: Enterococcus Confirmatory Agar; MP1: collection point 1; MP2: collection point 2; MP3: collection point 3; MP4: collection point 4. Source: Authors.

In Table 1. it is possible to observe that at some points there was a greater amount of CFU.

With the aid of culture media, it was possible to isolate a total of 75 strains of bacteria with samples of raw water 52.0% (39/75) and sediment 48.0% (36/75), being that the ADBA medium isolated 48.9% (22/45) of the raw water and 51.1% (23/45) of the sediment, in the medium ECA isolated 56.7% (17/30) of the raw water and 43.3% (13/30) isolated of the sediment.

Comparing the identified bacteria with the total of isolates that each culture medium provided, ADBA medium isolated 82.2% (37/45) of *Streptococcus* spp., 8.8% (4/45) of *Enterococcus* spp., 6.6% (3/45) of *Staphylococcus* spp. and 2.2% (1/45) of *Bacillus* spp. The ECA medium isolated 56.7% (17/30) of *Streptococcus* spp., 20.0% (6/30) of *Staphylococcus* spp., 20.0% (6/30) of *Bacillus* spp. and 3.3% (1/30) of *Enterococcus* spp. Still following this information, the total number of isolated strains were *Streptococcus* spp. 72.0% (54/75), *Staphylococcus* spp. 12.0% (9/75), *Bacillus* spp. 9.3% (7/75) and *Enterococcus* spp. 6.7% (5/75).

The data antimicrobial susceptibility testing are shown in Table 2.

Table 2. Percentage of antimicrobial resistance for bacteria isolated from samples of raw water and sediment in an aquatic environment from the Meia Ponte river, Goiás State, Brazil.

Isolated	Antibiotics													
	AMP	AZI	CIP	CLI	CLO	ERI	GEN	LNZ	OXA	PEN	RIF	SUT	TET	VAN
<i>Streptococcus</i> spp.	NA*	48,1% (26/54)	NA*	75,9% (41/54)	9,2% (5/54)	38,9% (21/54)	NA*	7,4% (4/54)	NA*	77,7% (42/54)	NA*	NA*	29,6% (16/54)	9,2% (5/54)
<i>Enterococcus</i> spp.	0% (0/5)	NA*	20,0% (1/5)	NA*	0% (0/5)	40% (2/5)	NA*	0% (0/5)	NA*	20,0% (1/5)	NA*	NA*	60,0% (3/5)	0% (0/5)
<i>Staphylococcus</i> spp.	NA*	66,7% (6/9)	55,6% (5/9)	77,8% (7/9)	55,6% (5/9)	66,7% (6/9)	55,6% (5/9)	55,6% (5/9)	88,9% (8/9)	66,7% (6/9)	55,6% (5/9)	66,7% (6/9)	55,6% (5/9)	NA*
<i>Bacillus</i> spp.	NA*	14,3% (1/7)	14,3% (1/7)	85,7% (6/7)	14,3% (1/7)	0% (0/7)	14,3% (1/7)	14,3% (1/7)	100,0% (7/7)	100,0% (7/7)	85,7% (6/7)	100,0% (7/7)	28,6% (2/7)	NA*

AMP: ampicillin; AZI: azithromycin; CIP: ciprofloxacin; CLI: clindamycin; CLO: chloramphenicol; ERI: erythromycin; GEN: gentamicin; LNZ: linezolid; OXA: oxacillin; PEN: penicillin; RIF: rifampicin; SUT: sulfazotrim; TET: tetracycline; VAN: vancomycin. NA* (Antibiotic not analyzed). Source: Authors.

In Table 2. there is high resistance among the isolated strains, highlighting, it should be noted that the strains that presented the highest means among the percentages of resistance mentioned were *Staphylococcus* spp. 63.9% and *Bacillus* spp. 47.6%. *Streptococcus* spp. showed 36.0% and *Enterococcus* spp. had the lowest percentage among all with 17.5%.

The percentual MDR results, were from *Bacillus* spp. 100% (7/7), *Staphylococcus* spp. 77.8% (7/9), *Streptococcus* spp. 48.1% (26/54) and *Enterococcus* spp. 0% (0/5). *Staphylococcus* spp. they were the only bacteria that presented PDR, showing 55.6% (5/7).

The association/correlation among all sample data, collection, point, culture medium and antibiotic resistance/sensitivities found was verified, these are described in Table 3.

Table 3. Spearman correlation among antibiotics, medium, point, sample and collection of the Meia Ponte river, Goiás State, Brazil.

	STRAINS	AZI	CIP	CLI	CLO	ERI	GEN	OXA	LNZ	PEN	RIF	SUT	TET	VAN	Medium	Point	Sample	Collection
STRAINS	0.00																	
AZI	0.06	1.00																
CIP	0.11	0.07	1.00															
CLI	0.18	0.05	-0.13	1.00														
CLO	0.04	-0.36*	-0.04	0.00	1.00													
ERI	0.14	0.15	0.56*	-0.07	-0.16	1.00												
GEN	0.52*	0.40	0.53*	-0.41	-0.53*	0.32	1.00											
OXA	-0.03	0.29	-0.20	-0.12	0.20	0.25	-0.22	1.00										
LNZ	-0.11	0.37*	0.07	-0.23	-0.43*	0.11	0.76*	-0.20	1.00									
PEN	-0.16	-0.36*	-0.09	0.13	0.13	0.00	-0.42	0.54*	-0.24*	1.00								
RIF	0.01	-0.05	-0.52*	0.71*	0.52*	0.03	-0.58*	0.38	-0.52*	0.71*	1.00							
SUT	0.12	-0.10	-0.37	0.23	0.37	0.32	-0.42	0.54*	-0.37	0.59*	0.71	1.00						
TET	0.10	0.05	-0.09	0.37*	0.15	0.01	-0.40	0.23	-0.17	-0.10	0.59	0.42	1.00					
VAN	0.06	0.03	-0.16	0.11	-0.02	-0.10	-	-	0.57*	-0.02	-	-	0.02	1.00				
Medium	-0.03	0.20	0.28*	0.18	-0.30*	0.17	0.50*	-0.45	0.36*	-0.28*	-0.54	-0.46	0.14	0.19	1.00			
Point	0.15	0.20	0.04	-0.16	-0.08	0.08	0.53*	-0.41	0.36*	-0.22	-0.06	-0.16	0.03	0.01	0.08	1.00		
Sample	0.22	0.26*	0.19	0.17	0.05	0.23*	-0.08	-0.17	-0.02	-0.12	0.42	0.02	0.17	0.17	-0.08	0.19	1.00	
Collection	0.06	-0.06	-0.29*	0.02	-0.29*	-0.05	0.33	0.17	0.14	0.08	0.16	0.32	-0.28*	-0.15	-0.05	0.07	-0.21	1.00

AMP was removed as it was only tested for *Enterococcus* spp. is all were sensitive, with no statistical variation. *, $p < 0.05$. Source: Authors.

In Table 3. some data were extremely important for the research, for example, when comparing antibiotics and stitches, and also among samples and antibiotics.

Data only within each bacterial species were also listed. For *Bacillus* spp. only a significant correlation ($p < 0.05$) of 0.87 among point and GEN was found. For *Enterococcus* spp. a significant correlation ($p < 0.05$) was found among sample and CIP with 0.97 and among ERI and TET with 0.91. For *Streptococcus* spp. 12 significant correlations ($p < 0.05$) were obtained, with emphasis between ERI with AZI of 0.64 and VAN and LNZ of 0.57. For *Staphylococcus* spp. 34 significant correlations were obtained ($p < 0.05$) with emphasis among ERI with AZI of 0.98, AZI with CIP, CLO, GEN, LNZ, RIF and TET of -0.79.

4. Discussions

The water temperature investigation process serves as a quality parameter, and the increase in temperature is associated with pollutants and proliferation of microorganisms in the aquatic environment (WHO, 2017). In addition, the occurrence of temperature changes among the dry and rainy seasons may be associated with the flow of waste in the water brought in by the rains (Adesakin et al., 2020).

On the media chosen for isolation, it was observed (Table 1), in general, the highest bacterial count for sediment samples when compared to raw water samples. This increased amount of strains found in the sediment reports a bacterial accumulation, demonstrating that the flow of sediment into the water body can generate an increase in the amount of fecal and bacterial contamination (Pandey et al., 2018).

There was more CFU in the culture media in the rainy season when compared to the dry season, occurring due to the fact that in the rainy season the runoff brings animal feces, pollutants and soil contaminants to rivers and lakes, multiplying the amount of these microorganisms in the water (Chen et al., 2017).

The work published in 2017 (Gomes et al., 2017), also performed analyzes in the water in the same aquatic environment of the present study, collections carried out in 2015/2016 and in periods of rain and drought, found *Bacillus* spp. 13.6% (14/103) and *Enterococcus* spp. 20.4% (21/103). Comparing the previous data with the present work, it is a smaller number of the total of isolates, however, many strains of *Streptococcus* spp. were isolated and in the study in 2017 the bacterium was not found. The explanation for this distinction is because the cited authors (Gomes et al., 2017), used a no specific culture media and in this current research has chosen to use specific culture media for gram-positive bacteria.

Another study carried out in the Apies River in South Africa showed an increase in the quantification of *Enterococcus* spp. in sediment samples using specific isolation medium for this bacterium, the indication of this change being the association with the accumulation of fecal bacteria in the sediment, where this location is described as a "protection" for these microorganisms (Ekwanzala et al., 2017).

Researchers (Semedo-Lemsaddek et al., 2018), investigated the resistance of *Enterococcus* spp. in water samples from Portugal (Companhia das Lezírias) and Brazil (Rio Paranapanema) using specific culture medium to identify this bacterial genus. It was observed that *Enterococcus* spp. isolates from Brazil are more resistant when compared to isolates from Portugal, mainly to CLI, Enrofloxacin and VAN antibiotics. However, in the study carried out in the Meia Ponte River, *Enterococcus* spp. tested, showed higher resistance to ERI and TET, with different resistance results when comparing the two studies.

There was a survey conducted in Nigeria (Onuoha, 2017), finding 5.6% (1/18) of *Streptococcus* spp. bacteria isolated from this region. It was found that this isolated strain was resistant to ERI and Streptomycin, and in this study, *Streptococcus* spp. identified showed higher resistance to the antimicrobials PEN, CLI, AZI and ERI, respectively, resembling the resistance to ERI for this particular bacterium in the two studies cited.

A survey in Limpopo, South Africa using water and sediment samples, demonstrated that 37 of the resistant isolates of the *Bacillaceae* family were isolated from water samples, even so, these bacteria showed higher resistance to the

antimicrobial ceftriaxone and no resistance to OXA, PEN and SUT. Being totally divergent results of resistance profile when compared to *Bacillus* spp. described in table 2 (Jardine et al., 2019).

Another study in Dhaka, Bangladesh with mineral water samples analyzed in different climatic seasons found resistance of *Staphylococcus* spp. During the study, there is an association among the resistance to cefixime of these bacteria and the access of this microorganism with the population, warning of contamination by *Staphylococcus* spp. in water accessible to the population, consolidating the importance of inspection and research to assess these resistant microorganisms (Aditi et al., 2017).

According to the authors (Kaur et al., 2020), in hospital wastewater, the presence of strains of *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* spp. with high resistance because it is an environment with high concentrations of drugs, radioactive products, among others. The authors mention that *Staphylococcus* spp. it is the one with the greatest resistance and even multiple resistances. Comparing with the study carried out in the Meia Ponte River, the results showed that *Staphylococcus* spp. showed greater resistance among other microorganisms found at the site, so it is possible to compare the resistance of bacteria found in hospital wastewater.

In 2019, a survey of strains with MDR, collecting samples from the surfaces of a hospital in Morocco, found strains of *Staphylococcus aureus* with MDR, being a suitable place to show high resistance, however, the Meia Ponte River also presented an alarming number of MDR in *Staphylococcus* spp. being worrying results (Chaoui et al., 2019). In turn, other researchers (Palacios et al., 2017) found *Bacillus* spp. MDR in Chihuahua, Mexico, in soil samples irrigated with untreated water; the author argues that this high resistance.

Phenotypic results of MDR presented by some isolated bacteria are being related to findings of high doses of antibiotics found in sewage of a hospital in São Paulo – Brazil. This disposal found in sewers may be related to the exacerbated use of antibiotics in hospitals, because it is an environment with a patient with several and distinct infections, thus influencing the spread of bacterial resistance (Brito et al., 2020; Carvalho et al., 2021).

The strong association in the antibiotic-antibiotic correlation is characterized by being of similar antimicrobial classes (El-Din et al., 2021), being ERI and AZI, for example, belonging to the class of macrolides that have the function of preventing the protein synthesis of the bacteria (Munita & Arias, 2016). The author (Olivas, 2013), explains that *S. aureus* has resistance to the efflux pump and this can generate multiple resistances to antimicrobials, such as: TET, CIP, GEN, PEN, among others; may explain these various correlations arising from *Staphylococcus* spp. presented in the statistics.

5. Conclusion

The presence of gram-positive bacteria with resistance in the Meia Ponte River was already expected because it is a place with several different bacterial strains (animal and human), in addition to the presence of pollution and sewage disposal at the site, however, the results found for MDR and even PDR were alarming for being a water resource used by countless individuals in the state of Goiás.

Still, it is necessary to collect more data on the spot and carry out research /tests, such as genotyping to find possible resistance genes in the isolated bacteria and comparing with the phenotypic resistance found. With these data collected in this research, it can serve to alert the population that uses this water and raise awareness about possible problems with infections caused by these resistant bacteria.

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