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ASSESSMENT OF THE RESISTANCE PROFILE OF *Escherichia coli* TO ANTIBIOTICS FROM AGRICULTURAL MICROBASIN LOCATED IN THE NORTHWEST OF THE STATE OF RIO GRANDE DO SUL, BRAZIL

AVALIAÇÃO DO PERFIL DE RESISTÊNCIA DE *Escherichia coli* A ANTIBIÓTICOS ORIUNDAS DA MICROBACIA AGRÍCOLA LOCALIZADA NO NOROESTE DO ESTADO DO RIO GRANDE DO SUL, BRASIL

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ABSTRACT
Antimicrobial resistance acquired by bacteria is a central topic in discussions about the environment and emerging waterborne diseases. Therefore, the objective of this study was to determine the in vitro bacterial sensitivity, specifically of *Escherichia coli* (*E. coli*) strains previously isolated from Lajeado Pardo, located in the municipality of Frederico Westphalen, in the northwest region of Rio Grande do Sul state, Brazil. Five antimicrobials were tested using the Antimicrobial Sensitivity Test (AST) on isolated bacteria samples and evaluated according to their resistance (R) and sensitivity (S) profiles: ampicillin (AMP), amoxicillin-clavulanate (AMC), gentamicin (GEN), chloramphenicol (CHLO), and ciprofloxacin (CIP). The analysis of the selective isolation media (LSB, Colilert, and EC) showed statistically significant differences among them ($p<0.05$). Therefore, it can be inferred that the selective medium for bacterial isolation influenced the AST results. The AST revealed that out of the 12 tested bacterial strains, none showed the same behavior towards the antibiotics. AMP had the highest resistance rate (100.0%), followed by AMC (67.0%), CIP (50.0%), CHLO (50.0%), and GEN (42.0%). GEN proved to be the efficient drug for treatment and can be initially classified as satisfactory monotherapy. Out of the total n=12 samples from the four sectors, 83.33% (n=10) showed resistance to multiple drugs. The high rate of multidrug-resistant *E. coli* suggests a potential risk to public health when using untreated raw water.

Keywords: Hydro basin. Antibiotics. *E. coli*. Water resources. Public health.

RESUMO
A resistência antimicrobiana adquirida por bactérias é um tema central nas discussões sobre meio ambiente e doenças emergentes transmitidas pela água. Portanto, o objetivo deste estudo foi determinar a sensibilidade bacteriana *in vitro*, especificamente de cepas de *Escherichia coli* (*E. coli*) previamente isoladas de Lajeado Pardo, região noroeste do estado do Rio Grande do Sul, Brasil. Cinco antimicrobianos foram testados pelo Teste de Sensibilidade Antimicrobiana (TSA) em amostras isoladas de bactérias e avaliados de acordo com seus perfis de resistência (R) e sensibilidade (S): ampicilina (AMP), amoxicilina-clavulanato (AMC), gentamicina (GEN), cloranfenicol (CLO) e ciprofloxacina (CIP). A análise dos meios de isolamento seletivo (LSB, Colilert e EC) mostrou diferenças estatisticamente significativas entre eles ($p<0,05$). Portanto, pode-se inferir que o meio seletivo para isolamento bacteriano influenciou os resultados do TSA. O TSA revelou que das 12 cepas bacterianas testadas, nenhuma apresentou o mesmo comportamento em relação aos antibióticos. AMP apresentou a maior taxa de resistência (100,0%), seguido por AMC (67,0%), CIP (50,0%), CLO (50,0%) e GEN (42,0%). A GEN mostrou-se um medicamento eficiente para o tratamento, podendo ser inicialmente classificado como monoterapia satisfatória. Do total das amostras (n=12) dos quatro setores, 83,33% (n=10) apresentaram resistência a múltiplos

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medicamentos. A elevada taxa de *E. coli* multirresistente sugere um risco potencial para a saúde pública quando se utiliza água bruta não tratada.

Palavras-chave: Bacia hidrelétrica. Antibióticos. *E. coli*. Recursos hídricos. Saúde pública.

INTRODUCTION

Infectious diseases still remain one of the leading causes of death worldwide since the discovery of antibiotics by Alexander Fleming in 1928, which increased exponentially over the past 15 years (WHO, 2017). Bacteria have an expressive role in the spread of nosocomial and community diseases, being linked to the high rate of hospitalizations of the population, especially in underdeveloped countries (Fard e Aali, 2019).

Antimicrobial resistance acquired by bacteria is a central topic in the debate about the environment and emerging water-related diseases. The increased use of antibiotics leads to the emergence and spread of antimicrobial resistance (AMR), which is a major challenge for global health (Lucien et al., 2021), making the effective treatment of a variety of infectious diseases very challenging (Jojura et al., 2020).

Microorganisms are subjected to *Darwinian* selection over time to develop some strict mechanisms for escaping the lethal effects of antimicrobial substances (Aslam et al., 2018). However, resistance has been linked mainly to the effects of human relations with the environment and its consequent imbalance (LI et al., 2023).

Overuse and the fact that antibiotics cannot be completely metabolized results in unchanged excrement that will end up in the waste systems (Watkinson et al., 2007), sewage treatments, or, at worst, in water springs. The situation becomes even more worrying as traditional wastewater treatment processes can only remove 20% of pharmaceutical products and their metabolites (kovalova et al., 2012), causing antibiotics to be directly or indirectly emitted into the environment (Chen et al., 2021), including by wastewater.

The contamination of surface water, especially with bacteria derived from feces, has long raised a concern about water quality due to the potential for disease transmission (Titilawo et al., 2015), phenomenon commonly observed in microbasins, which are areas susceptible to human activities Wuijts et al. (2017)

The development and spread of antibiotic resistance is progressively limiting treatment and prophylaxis options for most bacterial pathogens, threatening essential components of modern medicine (Hutinel et al., 2019). The global burden of AMR has no signs of decrease, instead it increases the pressure on human and veterinary medicine. Similar to global warming, AMR is an ecological calamity of undefined magnitude and has no apparent solution (Aslam et al., 2018). According to Miranda et al. (2020), AMR is already known worldwide, linked to growing political concerns, with serious social, economic, human, and animal public health repercussions.

In recent years, incidence of critical infections in which the etiological agent are gram-negative bacteria resistant to multiple drugs has increased, especially Enterobacteriaceae resistant to cephalosporins and carbapenem (Johura et al., 2020). In fact, *Escherichia coli (E. coli)* is considered one of the most dangerous pathogens. Some of its strains can cause serious diseases, including severe diarrhea, urinary tract infections, inflammations, and peritonitis (Cimafonte et al., 2020). The up-to-date surveillance of antibiotic resistance rates (Hutinel et al., 2019), mainly in environmental samples, is a key piece in guiding first-line treatment due to the increase in problems with antibiotic resistance.

Therefore, the present study aimed to investigate the in vitro bacterial sensitivity, specifically of *E. coli* strains previously isolated from Lajeado Pardo, located in the municipality of Frederico Westphalen, in the northwest region of Rio Grande do Sul state, Brazil.

MATERIAL AND METHODS

Bacterial isolation of water samples

Initially, water was sampled from the Lajeado Pardo located in the municipality of Frederico Westphalen, in the northwest region of the State of Rio Grande do Sul, Brazil, which has the contribution of five agricultural microbasins. The microbasin studied is characterized as agricultural (EMBRAPA, 2011), which flows into the main river of the Uruguay Basin, called the Uruguay River, which forms the border between two states of Brazil (Santa Catarina and Rio Grande do Sul), as well as being cross-border with the countries of Argentina and Uruguay. Focusing on the hydrographic region is breeding of chickens, pigs, cattle, irrigation, among others. It has an extension of 5,700 m, with a spring located in the coordinates 27^0 25' 43''S, 53^0 43' 25'' W, with an average altitude of 488 m. Four sectors were determined from the spring to the dam for water collection that supplies the municipalities of Frederico Westphalen and Caiçara.

It defined three strategic points of water sampling in each sector, considering the division of the points by the extension of the basin. Thus, sector 1 was located in the water dam, used for water capture and subsequent treatment. Sectors 2 and 3 are intermediate sectors of the route, and sector 4 is located at the spring of the basin.

A single sampling campaign was carried out, composing three samples collected in each of the four sectors sampled, totaling 12 points along the route. Sampling was performed in January 2021 and took place from downstream to upstream of the course of the Basin (against the flow).

Samples were filtered and planted in EC medium. Subsequently a bacterial colony characteristic of *E. coli* was picked in subcultured in three different selective media (EC, LSB and Colilert). The inoculations were prepared in Mueller Hinton (MH) broth and subsequently replicated in the respective selective media.

The subcultured in three different selective offering important insights into the isolation of the target species (*E. coli*) since each selective medium presents a different formulation, which favors the specific growth of microorganisms, we offer a wider range of selective specificity, maximizing the chance that the target strain isolated is *E. coli*. Subsequently, biochemical catalase tests and Gram staining were carried out to confirm whether the species of the isolated strains had the characteristic of *E. coli*.

The pricks were performed until obtaining the isolation of well-defined colonies in each sample compared to the standard of *E. coli* ATCC® 25922™. Some biochemical tests (catalase and Gram staining) for the putative identification of colonies from raw water were conducted. The putative *E. coli* colonies were submitted to the AST test when there was confirmation in all the above-mentioned tests.

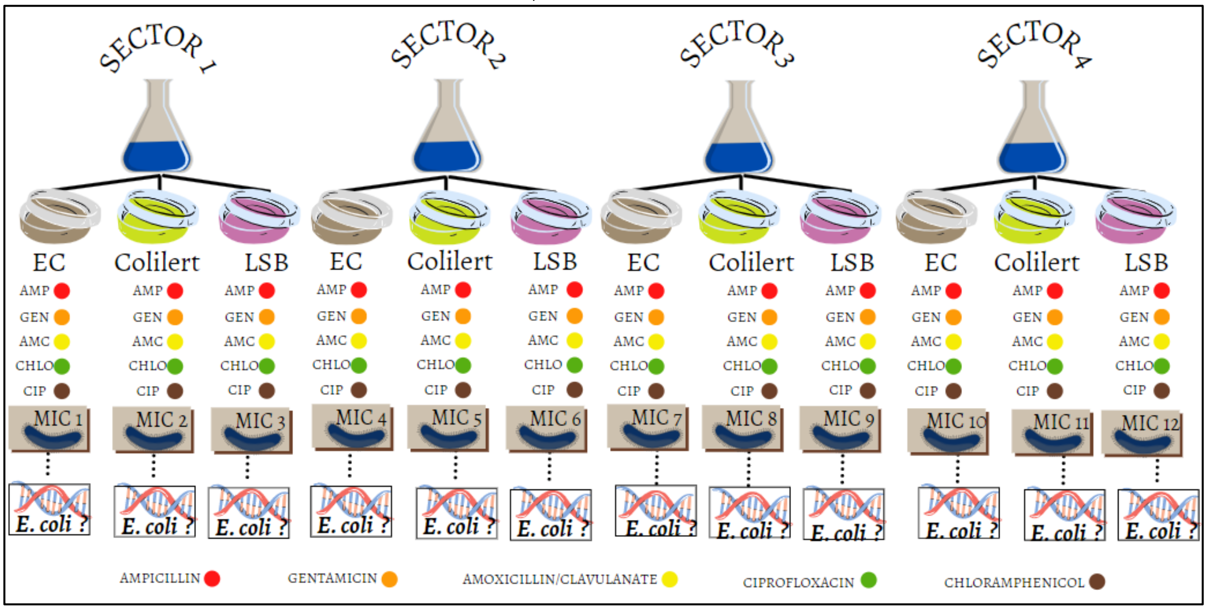
Antibiotic Sensitivity Test (AST)

This technique is aimed at determining bacterial sensitivity in vitro, indicating antimicrobial agents. The antibiogram was performed once in possession of the selection and isolation of the bacterial strains of the positive samples from the microbasin, through three distinct selective means (Bauer et al., 1966). There were three strains from each study sector, each isolated from a distinct selective medium (LSB, Colilert, and EC). Based on this assumption and considering the four sampled sectors, there were 12 presumptive strains of *E. coli* that proceeded to the AST. Subsequently, triplicate tests were performed for each of the 12 strains. The *E. coli* strain ATCC 25922 was used as a quality control strain.

The pattern of sensitivity to antibiotics was determined against five antibiotics used for the group of *Enterobacteriaceae* employing the disk diffusion method proposed by Kirby-Bauer (BAUER et al., 1966). The choice of antibiotics obeyed the suggestions of the Federal Drug Administration (FDA) (CLSI, 2019) standards adopted in the United States and also indicated by the Agência Nacional de Vigilância Sanitária (ANVISA, National Health Surveillance Agency) in Brazil. The antibiotics chosen and their respective concentrations were: ampicillin (AMP) – 10 mcg, gentamicin (GEN) – 10 mcg, ciprofloxacin (CIP) – 5 mcg, amoxicillin/clavulanate (AMC) – 20/10 mcg, and chloramphenicol (CHLO) – 30 mcg.

After the incubation period, the inhibition halos formed around the disc of each antibiotic were measured in millimeters (mm) using a digital caliper. Subsequently, the measured diameters were compared with the reference diameters for the sensitivy (S), resistant (R), susceptible, increased exposure (I) or area of technical uncertainty (ATU) classification, provided by the antibiotic disc manufacturer (Brazilian Committee on Antimicrobial Susceptibility Testing, 2023). Briefly, *E. coli* isolations from water samples were carried out for submission to the AST (Figure 1).

Figure 1 – Methodological summary of *E. coli* isolates from water samples from Lajeado Pardo-RS, Brazil, submitted to AST



Source: Prepared by the authors.

Escherichia coli resistant to multiple drugs

Resistance to multiple drugs can be defined as resistance to at least two antibiotics (Odonkor; Addo, 2018) to which a strain is subjected. Therefore, we prepared table presenting the number of strains with multiple resistance in relation to the total number (n=12) of isolated strains.

Stock Culture

The stock cultures of the 12 isolated strains were stored in brain and heart infusion broth (BHI) with glycerol at 20% at -80 °C for future use (Kouadio-Ngbesso et al., 2019).

Statistical analysis

The data were compiled through two statistical analyses, both employing the analysis of variance ANOVA and the means compared through the Scott-Knott test ($p<0.05$), using the SISVAR computer program.

The first statistical analysis correlated the three selective means of *E. coli* isolation in water samples (LSB, Colilert, and EC) in relation to the five antibiotics used in the AST, namely AMP, AMC, CIP, CHLO, and GEN. The ramifications were performed when there was significant interaction.

The second statistical analysis correlated the four sectors sampled in relation to the three selective means of *E. coli* isolation in water samples (LSB, Colilert, and EC) based on the diameters of the halo derived from the AST. The ramifications were performed when there was significant interaction.

RESULTS AND DISCUSSION

Prevalence of E. coli resistant to antibiotic and antibiotic resistance profiles in RAW WATER

Selective media are carefully formulated to preferentially promote the growth of specific bacterial strains, aiming for efficient detection and isolation of target organisms. In this context, the environment exerts a significant influence on bacterial physiology, modulating it through variables such as temperature, salinity, pH and the presence of organic compounds (Nguyen et al., 2021). However, the complexity and variability of uncontrolled environments present substantial challenges to the empirical validation of this influence. Therefore, investigating the impact of different formulations of selective media on target strains under controlled environmental conditions facilitates the elucidation of possible behavioral variations of strains in response to these media (Bonnet et al., 2020).

Charts 1 presents a summary of the response of the strains from the four sampled sectors, inoculated in each of the three selective media for the isolation of *E. coli*, against AST. From the analysis it is inferred that the response to selective media within each sector differed in all 12 cases, with no culture media showing similar behavior. Furthermore, the difference in behavior of the strains in the four sectors can be observed when comparing the bacterial cultivation media for enterobacteria. Therefore, it is possible to suggest, in the first analysis, that these are different subgroups of *E. coli*, requiring, however, confirmation of the species through molecular analysis.

Charts 1 – Summary of antimicrobial resistance the behavior of the 12 *E. coli* strains from the 4 sectors sampled and isolated in the 3 selective media. Lajeado Pardo, Frederico Westphalen-RS, Brazil

Meio	Sector 1					Sector 2				
	AMP	GEN	AMC	CHLO	CIP	AMP	GEN	AMC	CHLO	CIP
EC	R	S	S	R	S	R	S	S	S	S
LSB	R	R	R	S	R	R	S	R	R	R
Colil	R	R	R	R	R	R	S	R	S	R
Meio	**Sector 3**					**Sector 4**				
	AMP	GEN	AMC	CHLO	CIP	AMP	GEN	AMC	CHLO	CIP
EC	R	S	S	S	S	R	R	S	S	S
LSB	R	S	R	S	S	R	R	R	R	R
Colil	R	R	R	R	R	R	S	R	R	S

*sensitivy (S), resistant (R).
Source: Prepared by the authors.

Table 1 shows the results of the AST, where the least effective antibiotic was AMP, with all (100.0%) bacterial samples submitted to AST presenting resistance, followed by AMC (67.0%), CIP (50.0%), CHLO (50.0%), and GEN (42.0%). No point analyzed presented values in the ATU.

Table 1 – Antimicrobial susceptibility test with *E. coli* isolates from raw water samples of this study

Antibiotic/concentration	Nº of susceptible isolates (%)	Nº of resistant isolates (%)
AMP (10µg)	0 (0.0)	12 (100.0)
AMC (10µg)	4 (33.0)	8 (67.0)
GEN (10µg)	7 (58.0)	5 (42.0)
CIP (05µg)	6 (50.0)	6 (50.0)
CHLO (30µg)	6 (50.0)	6 (50.0)

Source: Prepared by the authors.

This data corroborates the findings of Jorgensen et al. (2017), who analyzed the resistance action of antibiotics against *E. coli* strains isolated from recreational waters in Norway and determined that the bacteria presented resistance to AMP, CIP, and GEN of 100%, 35%, and 20%, respectively. Swedan and Alrub (2019), who tested *E. coli* strains from drinking water sources in Jordan, found that the bacteria presented resistance to AMP, AMC, and CIP of 93.6%, 6.4%, and 16.5%, respectively.

Knowing that many of the drugs tested in the present study are also commonly used in human medicine, such as CHLO and CIP that are used against gastroenteritis (Gebremedhin et al., 2021), the considerable antimicrobial resistance observed in Table 1, both for CIP and CHLO, is highly significant, suggesting that conventional therapies may not be effective in cases where bacteria have acquired resistance profiles and infect humans, leading to diseases.

This further enhances the importance of global surveillance and monitoring, since the prevalence of resistant strains is increasing. For this reason, it is important to draw attention to the AMP antibiotic that has proved highly resistant in this study and other studies previously conducted (Chen et al., 2017; Gebremedhin et al., 2021; Jorgensen et al., 2017; Maal-bared et al., 2013; Swedan e Alrub, 2019). According to Ribeiro (2019), AMP has as its mechanism of action the inhibition of cell wall synthesis. Gram-negative bacteria have a more complex wall compared to Gram-positive bacteria since they have a limited permeability of their external membranes, composed of lipopolysaccharides that confer the intrinsic resistance of Gram-negative bacilli to penicillins, as is the case of AMP.

In addition to intrinsic resistance to an antibiotic, strains may have a multi-resistance pattern, which makes bacterial surveillance even more important. Of the total samples (n=12) in the four sectors, 83.33 % (n=10) presented resistance to multiple drugs, i.e., resistance to at least two antibiotics (Odonkor e Addo, 2018).

Of the total multi-resistant strains (Table 2), 30 % (n=3) were resistant to the five antibiotics tested, demonstrating high rate of acquired resistance, two of which were isolated in Colilert and one in LSB. The analysis of these data is pertinent since the strains studied are in a spring mainly used for public supply and recreation, and may infect people by the fecal-oral route through the consumption of contaminated water and primary contact in recreational water. These contacts subject people to developing diseases, which may compromise the usual treatments of choice (antibiotics).

Table 2 – Multiple antimicrobial resistance patterns in environmental *E. coli* isolated from water samples

Nº	Antimicrobial multi-resistance pattern	Selective Media	*E. coli* isolates (*n* = 10) number of resistant isolates (%)
Two	AMP, AMC	LSB	1 (10%)
	AMP, CHLO	EC	1 (10%)
	AMP, GEN	EC	1 (10%)
Three	AMP, AMC, CIP	COLILERT	1 (10%)
	AMP, AMC, CHLO	COLILERT	1 (10%)
Four	AMP, AMC, CIP, GEN	LSB	1 (10%)
	AMP, AMC, CIP, CHLO	LSB	1 (10%)
Five	AMP, GEN, AMC, CHLO, CIP	LSB (1) COLILERT (2)	3 (30%)
Total			**10 (100%)**

Source: Prepared by the authors.

Other studies have also investigated resistance to multiple drugs by *E. coli* strains from raw water samples. The study conducted by Odonkor e Addo (2018) analyzed the resistance of *E. coli* isolates from raw water sources to multiple drugs and found that 48 *E. coli* isolates (49.48% of the total isolates) exhibited resistance against two or more antibiotics, therefore classified as multi-resistant.

Additionally, Jorgensen et al., (2017) found multiple antibiotic resistance in 52% of recreational water samples (n = 82). The study highlights that, even though it was conducted in Norway, where the consumption of antibiotics in humans and animals is highly restricted, multi-resistance by *E. coli* is easily detected in aquatic environments.

The high rates of antimicrobial resistance observed in the present study for some drugs may have occurred due to natural processes, i.e., genetically decided with chromosomal mutations (Gebremedhin et al., 2021), or due to the facilitated and abusive access of antimicrobial agents for human and animal treatment, leading to a pressure of selection of resistant strains. At this point, it is important to mention that the five antibiotics used in the present study for the AST (GEN, AMP, AMC, CIP, CHLO) are part of the list from The Brazilian Government (Normative Instruction nº 83 of 2021), which defines the substances classified as antimicrobials for use under medical prescription (Brasil, 2021), and should therefore only be used under the prescription of a health professional in Brazil. However, it is known that there are many cases of misuse and without medical guidance, which can lead to the incorrect use and disposal of these antibiotics.

ResultS of statistical analyses against the Antimicrobial Sensitivity Test (AST)

Statistical analysis correlated the five antibiotics used for the AST regarding strains of bacteria isolated from raw water samples for each of the three selective isolation media used. As there was a significant interaction ($p<0.05$) between the analyzed variables. We proceeded with the ramifications of the treatment when f = 0.00.

The first ramification is presented in Table 3, which correlates the three selective media in relation to antibiotics for the isolation of bacteria in raw water. Given the data, it is possible to infer that the three selective media differed statistically from each other, with the strains isolated in EC medium showing the highest inhibition halo values, characterizing these strains with greater sensitivity to the antibiotics tested. On the other hand, the strains isolated in Colilert chromogenic medium showed the lowest inhibition halo values, characterizing greater resistance to the antibiotics tested. In view of the above, it is concluded that the strains isolated in different selective media present different values for the average diameters of the antibiotic halos.

Table 3 – Correlation of three selective media in relation to antibiotics for the isolation of bacteria in raw water

Treatments	Means (mm)	Test Result*
COLILERT	8.59	a
LSB	11.76	b
EC	18.24	c

*Means followed by the same lowercase letter for mean inhibition halos presented by the three selective isolation media tested do not differ statistically by the Scott-Knott test at p<0.05.
Source: Prepared by the authors.

The following ramification correlating the behavior of antibiotics is presented in Table 4. All antibiotics used in the AST also showed significant differences in the formation of inhibition halos compared with each other, with the exception of CHLO and GEN, which presented no significant difference between them.

Table 4 - Correlation of antibiotics used for the AST regarding the three selective bacterial isolation media in raw water

Treatments	Means (mm)	Test Result*
AMP	0.98	a
AMC	9.92	b
CHLO	14.07	c
GEN	17.04	c
CIP	22.32	d

*Means followed by the same lowercase letter for mean inhibition halos presented by the five antibiotics tested do not differ statistically by the Scott-Knott test at p<0.05.
Source: Prepared by the authors.

However, it is important to highlight the lowest mean halo value of AMP and AMC of 0.98 mm and 9.92 mm, respectively (Table 4). Both AMP and AMC differed statistically (p<0.05) compared with other antibiotics, also differed among themselves. In this regard, the values considerably below the average for sensitivity draw attention, and it can be inferred that the drug AMP does not present satisfactory results in the first analysis for the treatment of the strains tested.
The mean halo diameter value of CHLO (14.07 mm) was also below the mean halo inhibition for sensitivity, indicating resistance of the tested strains to this antibiotic. On the other hand, the CIP exhibited a mean halo diameter value that falls within the area of technical uncertainty (ATU), suggesting the need for increased exposure (I). This I classification is characterized by a reduction in the effectiveness of the tested antimicrobial at that specific concentration against the target microbiota, which may require higher concentrations of these drugs in the treatment of infections.
Additionally, the CIP differed statistically from all the antibiotics analyzed, including the CHLO. On the other hand, CHLO differed statistically from all antibiotics except GEN. It is possible to infer that the mean halo of GEN inhibition (17.04 mm) was above the mean halo predicted by the standard for sensitivity. Therefore, in a first analysis, GEN would be an efficient drug for treating *E. coli* strains from raw water samples.
This differentiation between the behaviors of antibiotics is predicted because the diameters of halos of standard inhibition that each antibiotic presents in the face of *E. coli* also differ, as shown in Table 5.

Table 5 indicates that the strains were normally sensitive to GEN. On the other hand, there was intrinsic resistance for the other antibiotics, except CIP which was classified as sensitive, increasing exhibition. Although the GEN and CHLO presented no statistical difference, they presented varied behavior concerning sensitivity since, as already mentioned, the GEN was sensitive and the CHLO resistant.

Table 5 - Expected inhibitory halo values and observed values for five antibiotics tested and demonstration of sensitivity and resistance to the AST test

Antibiotic	Sensitivity values for *Enterobacteriacea**	Halos Observed**	Sensitivity (S) or resistance (R)
AMP	≥14	0.98a	R
AMC	≥19	9.92b	R
CHLO	≥17	14.07c	R
GEN	≥17	17.04c	S
CIP	≥25	22.32d	S***

* Adapted from Brazilian Committee on Antimicrobial Susceptibility Testing (2023).
**Means followed by the same lowercase letter for mean inhibition halos presented by the five antibiotics tested do not differ statistically by the *Scott-Knott* test at $p<0.05$.
*** Sensitive, increasing exhibition.
Source: Prepared by the authors.

It was possible to analyze the behavior of the selective isolation media for each antibiotic used for analyzing the sensitivity of antibiotics in relation to *E. coli* by continuing with the ramification analysis (Figure 1). Figure 2A shows that the three selective media differ statistically ($p<0.05$) from each other for the AMC antibiotic. The strains isolated in LSB medium presented resistance responses to the antibiotic in question, as did the strains isolated in Colilert medium, which presented slightly higher halo values but were configured entirely resistant to the antibiotic. On the other hand, strains isolated in the EC medium presented halo values that characterized sensitivity.

Thus, it is important to highlight that when antibiotics have a significant difference in halo diameter means, it does not mean that both are sensitive or resistant. Thus, the statistical correlation does not explain the AST. Although AST has its cutoff standards established in regulations, the focus of this research was to assess the disparities in the inhibition zone of the strains concerning the medium in which they were cultivated. In light of this, a statistical analysis was conducted to determine whether this behavior changed according to the growth medium.

Figure 2B shows that all selective media differed statistically from each other. The mean halo diameter formed for the strains isolated in Colilert and LSB medium showed a resistance response to the CIP antibiotic. Additionally, it is possible to observe that the standard deviation value observed for the strains isolated in Colilert indicates that no data exceeded the minimum halo line for sensitivity, thus characterizing the resistance.

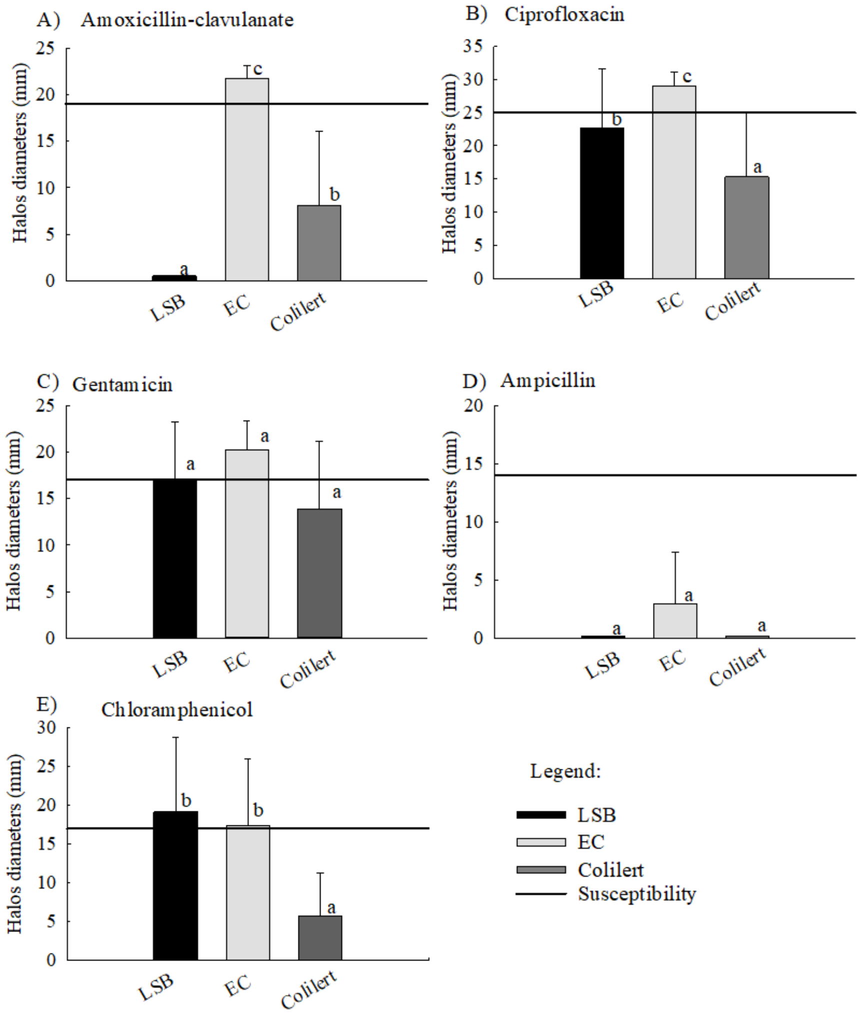
On the other hand, the strains isolated in EC medium presented sensitivity when halo means were analyzed, and it can be inferred that CIP is a satisfactory antibiotic for treating *E. coli* strains isolated in the study Micro Basin. The standard deviations considered high, presented in Figure 2, are due to the fact that strains were sampled from the four study sectors, justifying the variability of the data and standard deviation values. Also, because the n=12 was low.

Furthermore, when analyzing the resistance profile of *E. coli* strains nosocomial, to make a parallel to the environmental analyzes presented here, it was found between 8% and 65% of *E. coli* associated with urinary tract infections, showed resistance to CIP, an antibiotic commonly used to treat this condition (WHO, 2018). , and the present study found 58.2% resistance to CIP in samples collected in the waters of Lajeado Pardo. However, it is important to highlight that the spectrum of antibiotic resistance varies widely from region to region (Lucien et al., 2021). The resistance to CIP for urinary tract infections ranges from 22.5% to 58.6% for *E. coli* when considering the global picture and using data from the Global Surveillance System of Antimicrobial Use and Resistance (WHO, 2021).

Figure 2C shows a difference in sensitivity and resistance for the GEN antibiotic, although no statistical difference was observed between the selective means. In other words, the bacteria isolated in the Colilert and LSB medium proved to be resistant to the antibiotic. On the other hand, bacteria inoculated in EC were normally sensitive to GEN. There was no abrupt difference between the means of the halo of the three selective media. However, the media influenced the halo. It is also important to note that it is possible to perceive resistant and sensitive strains when analyzing the standard deviation within the same selective medium and that this fluctuation is normal, given that four distinct sectors were sampled. The strains isolated in EC medium were the only ones that obtained all points (samples) when considering the standard deviation, classified as sensitive to the GEN antibiotic.

Figure 2D shows the means and standard deviations of the isolated strains in the three selective media with respect to the AMP antibiotic.There was no statistical difference between the strains isolated in the three selective isolation media, and the strains in LSB and Colilert did not present a halo for the AMP antibiotic. Also, the strains isolated in EC presented a mean halo of 2.95 mm. However, this value is below the minimum threshold for sensitivity and characterized as resistant. In general, it was found that the AMP presented the lowest mean inhibition halo, demonstrating the highest levels of resistance when compared to other antibiotics. For this reason, we classified it as the least efficient antibiotic for treating *E. coli* strains from raw water samples.

Figure 2 – Correlation of means and standard deviations of the three selective isolation media for the five antibiotics tested



*Means followed by the same lowercase letter for mean inhibition halos presented for each of the three selective culture media tested do not differ by the Scott-Knott test at p<0.05.
Source: Prepared by the authors.

Many studies on water quality have also found a high resistance index of bacteria of the *E. coli* species bound to antibiotic AMP (Jorgensen et al., 2017; Swedan; Alrub, 2019), as infered in the present work. In this sense, the study conducted by Chen et al., (2017) in the city of Hangzhou, China, investigated the distribution of *E. coli* resistance to AMP against 18 antimicrobial agents tested, concluding that most isolates were resistant to AMP. The investigation conducted by Maal-Bared et al., (2013) in British Columbia, Canada aimed to examine the distribution of *E. coli* resistant to antibiotics isolated from water in a watershed of intensive agriculture. The study concluded there was a high frequency of resistance to AMP among the antibiotics tested. According to the authors, the highest frequency of resistance was observed in strains isolated from the sites most impacted by agriculture, while the lowest frequency of resistance was found in the headwaters.

Figure 2E shows the LSB and EC media did not differ statistically from each other, but differed from Colilert. In addition, microorganisms generally showed sensitivity to CHLO antibiotic in LSB and EC medium. On the other hand, resistance was observed in the Colilert medium.

The strains isolated in LSB and EC mediums presented sensitivity and resistance points, the LSB standard deviation being the largest of all, showing that there were different responses to the CHLO. This difference is justified because we tested strains from the four sampled sectors, which consequently presented different values when analyzed isolated by sector, thus highlighting that the strains present are exposed to different environmental conditions.

The mean halos formed in the Colilert medium showed a resistance pattern to CHLO, not even the observed standard deviation values exceeded the resistance limit. Thus, it can be inferred that the microorganisms inoculated in the Colilert medium showed resistance to the CHLO antibiotic.

Table 6, shows the analysis of halos means of the average antibiotic halos in the three selective isolation media. On average, all strains isolated in Colilert medium were resistant to all antibiotics, when compared to standard sensitivity halos. Additionally, the antibiotic AMP presented the lowest values of mean halos diameter, indicating resistance of the strains analyzed in the four sampled sectors.

Table 6 – Halo diameter means of antibiotics within selective *E. coli* isolation medium and sensitivity pattern

ANTIBIOTIC	HALO DIAMETER (mm)			
	Colilert	EC	LSB	Standard susceptibility
AMP	0,20	2,94	0,20	14,00
AMO	8,05	21,69	0,20	19,00
CIP	15,30	28,99	22,67*	25,00
CHLO	5,67	17,35	19,18	17,00
GEN	13,92	20,23	16,97	17,00

*Susceptibility, increasing exhibition.
Source: Prepared by the authors.

Within the EC selective isolation medium, the only antibiotic that provided mean inhibition halo values below the minimum sensitivity value was AMP. Thus, AMP is generally not effective for treating the strains studied. On the other hand, the other four antibiotics had inhibition halos superior to the minimum required for sensitivity when analyzed by their means, therefore, it is inferred that they were efficient for treating the strains studied.

The AMP and AMC antibiotics showed no inhibition halo diameter for the strains isolated in LSB medium, thus configuring total resistance to both antibiotics, as observed in Table 6. Resistance response to the antibiotic was also obtained when considering the mean of GEN. On the other hand, the antibiotic CHLO exhibited, on average, sensitivity values in relation to the strains inoculated in LSB. In the case of CIP, a mean value within the sensitivity range was observed with increased dosage. This indicates that the tested bacterial strain initially showed resistance to the antibiotic at standard concentrations but may become sensitive when exposed to higher doses of the medication. This finding suggests that sensitivity can be achieved with increased concentration. This information is relevant for adjusting the dosage of the antibiotic in the treatment of infections caused by this specific bacterial strain.

The second statistical analysis employed in the present study correlated the strains of bacteria isolated from the water samples of each of the four sampled sectors, in each of the three selective media used for isolation. From the analysis of variance, we can observe the significant interaction ($p<0.05$) between the analyzed variables, with $f = 0.0004$. Given this, there was a ramification of the treatments.

The significant difference between treatment 1 (sector 1) compared to the other treatments (sector 2, 3, and 4) with bacteria inoculated in three different selective media by the *Scott-Knott* test at 95% confidence, shows the sectors 2, 3, and 4 showed no significant differences. Sector 2 presented the highest mean halo diameter among the sectors, that is, the antibiotics used resulted in a greater effect against the *E. coli* strains.

The lowest mean of halos was observed in sector 1. The strains present in this sector showed greater resistance to the antibiotics tested. This can be explained because sector 1 is downstream of the other sectors of Lajeado Pardo, where the water collection for further treatment by the Companhia Riograndense de Saneamento (CORSAN, Sanitation Company of Rio Grande do Sul) occurs, using this spring for public supply of the municipalities of Frederico Westphalen and Caiçara – RS. In this sector, the environment is considered Lentic because of the Corsan dam for water capture, so this environment has still water and no currents.

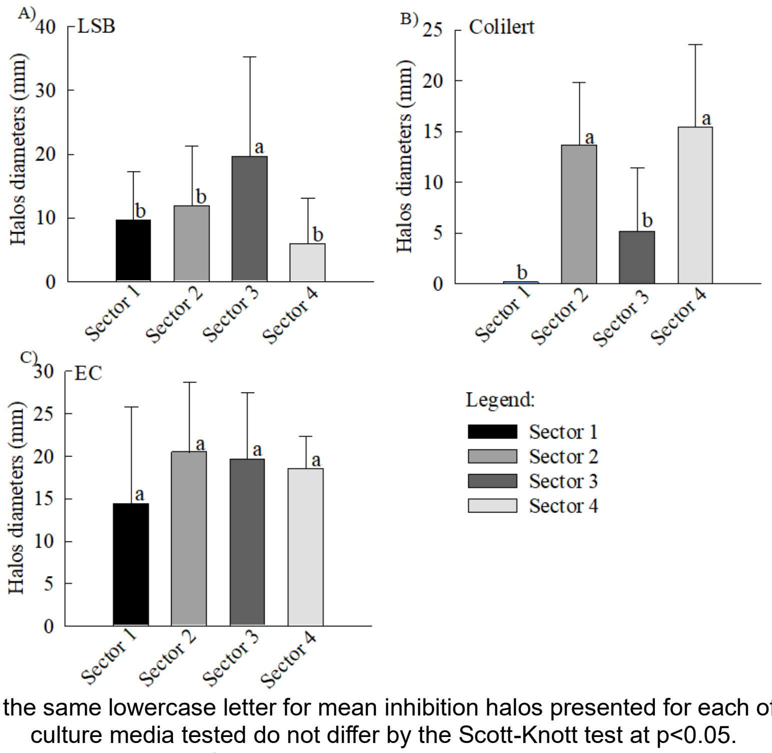
The results found by the present study regarding the microbiological conditions in sector 1, awakened a specific look at the worrying indices of the presence of antimicrobial-resistant bacteria at the point of water capture for further treatment by CORSAN. It is important to mention that bacteria resistant to antibiotics can have a pathogenic profile and trigger diseases in the population that uses water without proper treatment. In addition, many studies point to the presence of resistant bacteria in tap water after water treatment (Becerra-Castro et al., 2015; He et al., 2016; Su et al., 2018), since conventional systems do not always guarantee total removal of these pathogens.

Some subgroups of *E. coli* (enterotoxigenic; enteroinvasive, and enterohemorrhagic) (Ministério da Saúde, 2021) are etiological agents of acute diarrheal diseases (ADD) of clinical manifestation. According to the Ministério da Saúde (MS, Ministry of Health), one can cite the ingestion of water without adequate treatment and poor sanitation and hygiene conditions among the risk factors for ADDs that can put people at risk and facilitate contamination. The State of RS and Brazil presented between 2007 and 2019, 137,539 and 4,369,609 cases reported, respectively. In this sense, more caution should be given to monitoring pathogenic strains found in the environment due to the resistance results found by the isolated bacteria in the present study, as well as the cases of ADD that may have *E. coli* as an etiological agent.

Subsequently, statistical ramification was performed, where the correlations between the selective media for *E. coli* isolation and the four collection sectors in relation to the halos diameters observed in the AST were analyzed (Figure 3). It should be noted that the high standard deviations presented by most sectors when analyzed in each selective isolation medium are justifiable by the analysis of the inhibition halo diameters means for AST. The standard deviation will be high compared to the average since each antibiotic responds with different halos. However, these values far from the mean do not characterize a problem for the study.

Sector 3 statistically differed from the other sectors for the LSB medium and presented the highest inhibition halo diameter mean (Figure 3A). On the other hand, sectors 1, 2, and 4 did not differ statistically from each other, but differed from sector 3, as shown in Figure below.

Figure 3 – Correlation of means and standard deviations of halo diameter (mm) of sectors for water collection in each selective insulation medium



*Means followed by the same lowercase letter for mean inhibition halos presented for each of the three selective culture media tested do not differ by the Scott-Knott test at p<0.05.
Source: Prepared by the authors.

Sectors 1 and 3 did not differ statistically from each other for the Colilert medium, but differed from the others, as shown in Figure 3B. On the other hand, sectors 2 and 4 did not differ from each other but from the others. The highest halo means were found in sector 4, characterized by the spring.

On the other hand, it is observed that sector 1 (water capture point by CORSAN) presented mean halos of practically zero diameter. Based on this, it can be inferred that the strains isolated in this sector through Colilert may exhibit significant levels of resistance, as the antibiotics were unable to form inhibition zones of sufficient size to be classified as sensitive.

Concerning the EC selective isolation medium, it is possible to infer that the four sectors did not differ statistically from each other, and sector 1 presented the lowest inhibition halo means, as shown in Figure 3C. This finding was the same as in the Colilet selective medium. Thus, it is important to consider that sector 1 is the most downstream point of the route sampled in the present study.

It is possible to infer that there was a divergent resistance behavior/pattern of E. coli strains, depending on the culture medium in which they were isolated. The lowest halo diameter values for the Colilert and EC media occurred in Sector 1. On the other hand, the lowest means occurred in Sector 4 for the LSB selective medium. Additionally, sector 4 presented the largest halo diameters for the Colilert medium. However, the largest halo diameter values for the EC medium occurred in sector 2.

In view of these observations, some notes on selective isolation media are pertinent. It is possible to infer that *E. coli* showed variable behavior, depending on the environment in which it was isolated. Therefore, further investigation is necessary to effectively conclude which is the selective medium for isolating *E. coli* that has the greatest similarity with the natural environment, so that there are no biased sensitivity test results. This condition is very important to standardize isolation tests and subsequent AST. Combining genetic tests with putative isolates would bring greater certainty for the identification of environmental *E. coli.*

However, it is also important to mention that variations in results found from different selection methods may occur due to their composition. This corroborates with the notes of Marquezi et al. (2010), who explain that the bacteria do not normally multiply in water, only survive and are disseminated through it heterogeneously dispersed.

The performance of the analytical method depends on the yield of the target bacteria (Marquezi et al., 2010). However, if the species composition of the target group varies in the different methods, the interpretation of water quality may be affected by the analysis method (Niemi et al., 2001). As observed in the present study, a distinct response to the AST was obtained depending on the culture method used to isolate the bacteria in the study sectors.

Given the data obtained in the present research, it is inferred that as selective media are composed of different formulations with the intention of promoting the growth of specific bacterial groups, the isolation of a bacterial species, or even sub-group, depends on its viability when removed from the environment (Bartelme et al., 2020).

Substrates can play a role in favoring the manifestation of a dormant gene, since they can be at concentrations many times higher than those available in the environment (Chebotar et al., 2021). Thus, a factor that may be influencing the heterogeneous behavior of a strain when isolated in different media against AST is the composition of the medium.

It is also important to highlight the protocol of enrichment and identification of *E. coli* strains developed specifically for this project, choosing the LSB, EC, and Colilert primary enrichment media. As reported by (Cooley et al., 2014) in their study on the prevalence of *E. coli* in public places in an agricultural region of California, this is the first survey for the isolation and identification of *E. coli* in an agricultural Micro Basin in the northwest region of the State of Rio Grande do Sul, Brazil. It is not yet known how much the wildlife, soil, and vegetation contribute to the potential persistence of these microorganisms.

The lack of standardization in the selective means for isolating *E. coli* is a factor that allows biased results when carrying out the antibiogram, since there are commercially different selective means used in the step that precedes the AST (Cooley e tal., 2014; Chen et al., 2017; Jørgensen *et al*., 2017) Here we raise a question about the difficulty of comparing data between studies due to the lack of standardization, since our data show that there may be different behavior when considering the medium. Thus, it is unclear whether the predominance of *E. coli* isolates reflects on the bias of the isolation methods used or the ecology of the study region. This raises a very important issue: that future research will evaluate the influence of isolation methods of environmental microorganisms to ensure a reliable comparison with other studies using other specific media.

This research, as well as other studies, allows public policy makers to take the necessary actions to improve the safety of raw water since it is still widely used. Therefore, monitoring water and the occurrence of serious and drug-resistant pathogens is indispensable (Swedan e Alrub, 2019) since monitoring preventively guarantees prospects for improving the management of water resources, so important for the maintenance of life on Earth.

CONCLUSIONS

This study summarizes the findings of resistance to some strains of bacteria isolated in a Micro Basin located in the northwest of the State of RS, Brazil. Greater resistance was observed for the antibiotic AMP (100%), followed by AMC (67.00%), CIP (50.00%), CHLO (50.00%), and GEN (42.00%) in this study. GEN proved to be the most efficient drug for treating most strains, and the study contributes to a global analysis of susceptibility patterns in *E. coli* bacteria. Further studies are needed to confirm contingency interventions in confirmed clinical cases. Antimicrobial resistance is an indication of pathogenic bacteria and a warning sign for potential water-related diseases, posing a serious health risk to communities dependent on this water for various uses. Therefore, policies for monitoring raw water sources and reducing the indiscriminate use of antimicrobials are relevant to mitigate the risks associated with water-related diseases.

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