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Avaliação do perfil de resistência aos antimicrobianos em cepas de *Escherichia coli* e *Staphylococcus* spp. no canal do Marambaia em Balneário Camboriú, Santa Catarina

Evaluation of the antimicrobial resistance profile of isolated strains of *Escherichia coli* and *Staphylococcus spp.* in the Marambaia river in Balneário Camboriú, Santa Catarina

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ABSTRACT

Introduction: During the last few decades, various organic micropollutants, including antibiotics, have been released into the environment because of anthropogenic activities. These drugs are poorly biodegradable and very persistent, such that when they accumulate in aquatic systems, they provide a favorable environment for the selection and proliferation of resistant microorganisms. **Objective:** evaluate the antimicrobial resistance profile of isolated strains of *Escherichia coli* and *Staphylococcus* spp taken from the Marambaia river and its adjacent beach, in Balneário Camboriú, in the Brazilian state of Santa Catarina. **Method:** The sampling was divided into six collections, with a total of thirty-six samples, across all seasons of the year. Subsequently, microbiological analyses and antibiotic sensitivity tests were performed. Data analysis was performed using the software programs Excel and Past, version 4.03, through exploratory and descriptive analyses. **Results:** The results showed the presence of total and thermotolerant coliforms at all sampling points, indicating the influence of the river outflow on the beach. Moreover, the concentrations of *E. coli* found in the sampling points are not compliant with Conama Resolution 357/2005, except for the points Marambaia 1 and Praia 3. The MAR index for *Staphylococcus* showed high risks for multidrug resistance, with no differences for the time and place of collection. Ninety-six strains were resistant to at least one antibiotic. The isolated strains showed greater resistance to antibiotics erythromycin and clindamycin. **Conclusions:** The presence of *E. coli* is worrisome because it is a thermotolerant coliform, indicative of recent fecal contamination. The presence of resistance mechanisms in these bacteria can modify the local microbiota, putting the health of the population at risk. As for *Staphylococcus* spp., through the evaluation of the MAR index, high risks of multidrug resistance were found for most of the sampled locations and at any time of collection. In addition, few strains were sensitive to all antibiotics tested. The resistance mechanisms that occur in Gram-positives, despite differing from those that occur in Gram-negatives, are equally worrisome, whether from a microbiological point of view or in terms of public health.

KEYWORDS: Bacterial Resistance; Antibiotics; Wastewater Treatment

RESUMO

Introdução: Durante as últimas décadas, diversos micropoluentes orgânicos foram liberados no meio ambiente como resultado de atividades antropogênicas. Entre eles, se destacam os antibióticos, substâncias pouco biodegradáveis e muito persistentes que, ao se acumularem nos sistemas aquáticos, proporcionam um ambiente favorável à seleção e proliferação de microrganismos resistentes a antimicrobianos. **Objetivo:** Avaliar o perfil de resistência aos antimicrobianos em cepas de *Escherichia coli* e *Staphylococcus* spp., isoladas no rio Marambaia e na praia adjacente, em Balneário Camboriú, Santa Catarina. **Método:** A amostragem foi dividida em seis coletas, totalizando 36 amostras e abrangendo todas as estações do ano. Foram realizadas análises microbiológicas fenotípicas, incluindo morfologia e testes de susceptibilidade aos antimicrobianos. A análise de dados foi realizada com o auxílio dos *softwares* Excel e Past, versão 4.03, através de análise explorativa e descritiva. **Resultados:** Foi verificada a presença de coliformes totais e termotolerantes, em todos os pontos amostrais, indicando a influência da desembocadura do rio na praia, além disso, a concentração de *E. coli* encontrada nos pontos amostrais está em desacordo com a Resolução Conama n° 357/2005, exceto para os pontos Marambaia 1 e Praia 3. De forma geral, cinco cepas apresentaram resistência a pelo menos três antibióticos, sendo a mais expressiva a ampicilina, com 14 cepas resistentes. O índice MAR para *Staphylococcus* spp. mostrou altos riscos para multirresistência, não apresentando diferenças para os fatores momento e local de coleta, sendo que 96 cepas apresentaram resistência para pelo menos um antibiótico. Foi encontrado um número maior de cepas resistentes aos antimicrobianos eritromicina e clindamicina. **Conclusões:** A presença de *E. coli* é preocupante por se tratar de um coliforme termotolerante, indicativo de contaminação fecal recente. A existência de mecanismos de resistência nessas bactérias pode modificar a microbiota local, colocando em risco a saúde da população. Quanto aos *Staphylococcus* spp., através da avaliação dos dados do índice MAR, verificou-se riscos altos de multirresistência para a maioria dos locais amostrados e em qualquer momento das coletas. In addition, few strains were sensitive to all antibiotics tested. A análise do antibiograma revelou que 96 cepas apresentaram resistência para pelo menos uma classe de antimicrobiano. Os mecanismos de resistência que ocorrem em Gram-positivos, apesar de divergirem daqueles ocorridos em Gram-negativos, são igualmente preocupantes, seja do ponto de vista microbiológico ou em questões de saúde pública.

PALAVRAS-CHAVE: Resistência Bacteriana; Antibióticos; Tratamento de Efluentes

INTRODUCTION

Urban sprawl often occurs without proper planning, so services such as sewage collection and treatment end up receiving limited service.

According to the National System of Sanitation Information (SNIS)[1], in Brazil, around 98.2% of the urban population is served by the water supply network and only 61.9% is served by sewage collection. Only 49.1% of the sewage generated undergoes some type of treatment, demonstrating that more than 50.0% of all domestic sewage in Brazil is released *in natura* into the environment.

Many domestic septic tanks, due to negligence or lack of financial resources of the population, overflow and invade the rainwater collection systems, which are not treated and end up flowing directly into water bodies. On the other hand, it is known that a good portion of the population not assisted by sewage treatment performs clandestine connections in storm sewers or discharges sewage *in natura* directly into streams in cities[2].

Among the drugs found as environmental pollutants, antibiotics stand out, capable of providing a favorable environment for the proliferation of bacterial resistance. Antibiotics are natural, synthetic or semi-synthetic compounds, classified as bactericidal, when they cause the death of bacteria, or bacteriostatic, when they promote the inhibition of microbial growth[3].

Antibiotics contribute to increasing the life expectancy of human populations[4]. However, this can be compromised by bacterial resistance to antibiotics, by reducing the therapeutic options of drugs effective against bacterial pathogens[5].

However, the use of antibiotics is not restricted to treatments aimed at human or veterinary health, they are also used in agriculture, livestock, and fish farming for prophylactic and treatment purposes. Misuse represents a risk to public health, as it causes the release of these substances into the environment through domestic sewage, and hospital and industrial effluents, in addition to the disposal of expired medicines and domestic leftovers of those not used, selecting resistant strains[5,6].

The increase in antimicrobial-resistant bacteria can harm humans, in addition to the toxic effect on aquatic organisms[7,8].

Resistant organisms have been observed in diverse aquatic environments including rivers and coastal areas, surface waters and sediments, lakes, oceans, drinking water, and domestic and hospital sewage[9].

Resistance can be considered an ecological phenomenon, the microorganism's response to the widespread use of antibiotics and their presence in the environment[3].

Bacteria have complex resistance mechanisms. These mechanisms can coexist in the same bacterial strain, making it resistant to different classes of antimicrobials and generating a profile of multiresistance[11].

A bacterium is considered multidrug resistant if it is resistant to three or more classes of antibiotics[12]. The increase in antibiotic resistance compromises the treatment of several infections that, until recently, were controllable[12]. Consequently, the choice of many drugs important in the treatment of bacterial infections has become limited and can lead to unpredictable results[13].

Bacteria may have intrinsic or acquired resistance to antibiotics. Intrinsic resistance is characterized by an innate response in which all individuals of the same genus or species show resistance to a particular antimicrobial agent, due to structural or functional particularities. Acquired resistance occurs when a bacterium previously sensitive to a particular antimicrobial develops resistance, which may be a consequence of mutations in chromosomal genes or the acquisition of mobile genetic elements, such as plasmids and transposons, by horizontal gene transfer[11].

Among the most clinically important bacteria is *Escherichia coli*, a Gram-negative bacillus of the Enterobacteriaceae family. It is the only species of the thermotolerant coliform group whose exclusive *habitat* is the gastrointestinal tract of humans and homeothermic animals and is present in large quantities in sewage, effluents, natural waters, and soils that have recently received fecal contamination, being used as a bioindicator of environmental quality[14].

Another prominent genus is *Staphylococcus* spp., which makes up the microbiota of humans and animals, inhabiting the skin and mucous membranes. They are opportunistic pathogens, associated with mild infections, such as skin infections and food poisoning, but which can also cause serious, potentially fatal infections, such as pneumonia, bacteremia, and infections related to invasive medical devices. Among the species, *Staphylococcus aureus* is the most pathogenic because it has a diversity of virulence factors[11].

Antibiotic-resistant bacteria have been found in diverse aquatic environments, including treated and untreated effluents, rivers, and the ocean[12]. However, the direct and continuous supply of these compounds in natural waters provides an environment that can favor the selection of resistance genes in pathogenic microorganisms, making them resistant[15,16].

Since these bodies of water can be used by the population, the assessment of the effects of the presence of antibiotics in water sources and the possible development of resistance in bacteria is essential to support public policies to protect human life and the environment. In this context, the present study aimed to evaluate the profile of bacterial resistance to antibiotics, in the water of the Marambaia river and on the adjacent beach, in Balneário Camboriú, Santa Catarina (SC). The focus of the study were bacteria of the species *Escherichia coli* and bacteria of the genus *Staphylococcus*.

METHOD

Sampling

Sampling was divided into six collections, in a total of six samples per collection, in the Marambaia river, in Balneário Camboriú. The collections were carried out on 10/28/2020, 11/25/2020, 01/28/2021, 04/14/2021, 07/26/2021, and 10/29/2021. In each collection, three samples came from points along the river, and three from points along the beach, following the deck. The total course was about 1 km. Altogether, six collections were carried out, totaling 36 samples.

Enumeration and isolation of *Escherichia coli*

For the isolation of *E. coli*, the analysis of coliforms was used by means of the method of multiple tubes, being established, at the end of the analysis, the most probable number (MPN) of microorganisms per 100 mL of sample. The culture medium used in this step was Chromogenic agar.

The next step, for isolating *E. consisted of sowing by depletion of positive aliquots for thermotolerant coliforms, in solid MacConkey culture medium, aiming at the isolation of strains of E. coli* through the morphology of the colonies, which present a reddish pink color, characteristic of lactose-fermenting bacteria, and may present the formation of a veil or swarming, due to the presence of motility through flagella[17].

The identification of colonies and confirmation of the result elapsed from specific biochemical tests, such as SIM semi-solid medium and solid medium in a Simmons Citrate inclined tube. In SIM medium, the species *E. coli* does not produce sulfide, is mobile and produces indole. In Simmons Citrate medium, *E. coli* does not use citrate as the only carbon source[18].

Enumeration and isolation of Staphylococcus spp.

For the isolation of *Staphylococcus* spp., aliquots from the Marambaia river were inoculated into two solid culture media: Mannitol Salts agar and Baird-Parker agar. For the samples from the points defined for the beach, aliquots were inoculated in both media, through the filtering membrane method, aiming at a better recovery of the microorganisms, since the samples showed greater natural dilution at these points.

On Mannitol Salts medium, colonies suggestive of *Staphylococcus aureus*, mannitol fermenters, are larger and surrounded by a yellow zone. Colonies suggestive of coagulase-negative *Staphylococci*, in turn, are smaller and surrounded by a red or pink zone. Some species, such as *Staphylococcus epidermidis*, may have white colonies[19].

On Baird Parker medium, colonies suggestive of *S. aureus* show shiny black colonies surrounded by a lecithinase halo (opaque zone surrounded by a light halo), whereas colonies suggestive of coagulase-negative *Staphylococci* show dark gray to black coloration and do not develop a halo around the colonies[20].

Antibiogram

The Antibiotic Susceptibility Testing (AST) or antibiogram, consists of the *in vitro* determination of bacterial sensitivity through the disc diffusion methodology, defined in 1966 by Kirby and Bauer. The measurements obtained were compared to the reference value established by the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST)[21], being able to establish sensitive, intermediate, and resistant results[21].

Antibiotics were chosen according to their importance in the treatment of human and animal infections caused by

Gram-negative bacilli of the Enterobacteriacea family and bacteria of the *Staphylococcus* genus.

For *E. coli strains, antibiotics ampicillin* (10 µg), *aztreonam* (30 µg), *cefoxitin* (30 µg), *ciprofloxacin* (5 µg), *gentamicin* (10 µg), *imipenem* (10 µg), *piperacycline + tazobactam* (110 µg), and *sulfamethoxazole + trimethoprim* (25 µg) were tested.

For staphylococcal strains, cefoxitin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), and gentamicin (10 µg) were tested.

For the staphylococcal strains, the vancomycin disk (30 µg) was also used, however the reference value used to read the inhibition halos was established by the Clinical & Laboratory Standards Institute (CLSI)[22], as a form of screening, since resistant results need confirmation through the methodology of minimum inhibitory concentration (MIC)[21,22].

Data analysis

To conduct the statistical analyses, data normality was initially tested using the Shapiro-Wilk test. In this test, the data are considered normal when the values obtained for p exceed 0.05.

To compare *Staphylococcus* spp. counts from different culture media, the nonparametric Wilcoxon test for paired samples was used. Counts were compared for each site separately, taking samples from different collection dates as replicates. For the *S. aureus* counts, only samples M1, M2, and M3 were tested, since it was not possible to distinguish the *S. aureus* colonies in the Baird-Parker medium from the remaining samples in the other samples.

To compare different sites over time with respect to antimicrobial resistance of *Staphylococcus* spp. *strains*, a two-factor PERMANOVA was performed. The variables evaluated were the diameters of the inhibition halos for each of the tested strains.

For the same comparisons, but based on *E. coli* strains, the two factors were tested independently. This was necessary because, due to the smaller number of *E. coli* strains isolated and tested, it was not possible to obtain replicate measures for each combination of the different levels of location and time factors. Thus, in the first one-factor PERMANOVA, the strains were grouped by the local factor; in the second, by the time factor.

The multiple antibiotic resistance (MAR) indices for the analyzed samples were calculated by the formula MAR = a/(b*c), with a being the combined number of resistances for all isolates and antibiotics in the sample, b the number of antibiotics tested, and c the number of isolates analyzed using the Krumperman method[24]. The indices were calculated only for *Staphylococcus* spp. isolates, since for *E. coli*, the number of isolates obtained was insufficient to obtain indices for all samples.

RESULTS

Enumeration of total and thermotolerant coliforms

Total coliforms were detected at all sampled points (Table 1). Despite the natural dilution that occurred at the beach points, they presented similar results to the channel. The point with the lowest concentration of total coliforms was Beach 3.

The highest concentrations observed for all points occurred in the period from January to February 2021. In the collection for the period of July 2021, the Marambaia 2 point showed lower concentrations of total coliforms in relation to the other points. There was greater variability over time for points Marambaia 1 and Beach 2.

Table 1. Total and thermotolerant coliform concentrations (MPN/100 mL) for each sampling point in each collection performed.

Microorganism	Date	Collection points					
		M1	M2	M3	B1	B2	B3
Total coliforms	10/28/20	4,600	11,000	4,600	2,400	11,000	230
	11/25/20	> 11,000	>11,000	4,600	4,600	2,400	930
	01/28/21	> 11,000	>11,000	> 11,000	> 11,000	> 11,000	> 11,000
	04/14/21	750	>11,000	> 11,000	> 11,000	5,300	> 11,000
	07/26/21	> 11,000	4,600	> 11,000	> 11,000	11,000	4,600
	10/29/21	4,600	11,000	> 11,000	> 11,000	4,600	4,600
Thermotolerant coliforms	10/28/20	1,500	2,400	930	430	1,200	92
	11/25/20	4,600	4,600	930	2,400	930	930
	01/28/21	2,100	11,000	930	> 11,000	4,600	2,400
	04/14/21	230	1,500	> 11,000	4,600	4,400	1,500
	07/26/21	4,600	2,400	> 11,000	> 11,000	2,400	2,400
	10/29/21	930	4,600	11,000	> 11,000	2,400	930

Source: Elaborated by the authors, 2021.
M: Marambaia; B: Beach.

The highest concentrations of thermotolerant coliforms were observed at points Marambaia 2, Marambaia 3, and Beach 1. Considering the Marambaia River as a class 2 brackish water body, the Resolution of the National Environment Council (Conama) No. 357, of March 17, 2005, establishes that the limits for thermotolerant coliforms should not exceed 2,500 per 100 mL in 80% or more, from at least six samples collected during a period of one year, with bimonthly frequency. It was found that the sampling points Marambaia 2 and Marambaia 3 did not reach the established standards[25].

For the beach points, the standards established for class 2 saline water bodies were considered, whose limits must not exceed 2,500/100 mL. Thus, sampling points 1 and 2 do not meet the established standards.

Temporarily, there was great variation between the points of Marambaia. The same occurs for Beach 1, when compared to points 2 and 3 of the beach, in which a more similar pattern can be observed over time. In general, point 3 of the beach presented the lowest variability, as well as the lowest concentrations of thermotolerant coliforms.

Enumeration of Staphylococcus

The highest values of *Staphylococcus* spp. occurred in the Marambaia points in January and July 2021, in both culture media (Table 2) However, through statistical analysis it was not possible to verify the difference between the culture media evaluated, with the exception of the Marambaia 2 point (p = 0.03125). At the beach points, higher counts of *Staphyloccus* spp. were observed in January 2021, which may also consider slightly increased values in the period of April 2021. By means of statistical analysis, no difference was observed between the media.

Enumeration of Staphylococcus aureus

The analysis of colony morphology allowed the identification of microorganisms suggestive of *S. aureus* to Marambaia points on Mannitol Salts and Baird Parker culture media. As for the beach aliquots, the morphological differentiation was only possible for the solid medium Mannitol Salts, since the filtering membrane made it impossible to visualize the possible lecithinase halos, characteristic of *S. aureus*. Therefore, the use of Baird Parker solid medium presented some limitation regarding the isolation and differentiation of microorganisms suggestive of *S. aureus*, and the counting of this microorganism for this culture medium was only possible for the Marambaia points (Table 3).

At the beach points, a greater number of microorganisms isolated from January to April 2021 was observed, with emphasis on the Beach 3 point. In the Marambaia canal, the highest values were found at points 1 and 3 in January 2021. However, there were no differences between the counts obtained from the two culture media.

Escherichia coli antibiograms

Of the 34 evaluated strains of *E. coli*, 19 showed sensitivity to all antibiotics tested. The isolated strains showed higher rates of resistance to the antibiotics ampicillin (41.2%) and sulfamethoxazole + trimethoprim (26.5%). Intermediate results could be observed in certain strains for the antibiotics imipenem (11.8%),

Table 2. *Staphylococcus* spp. concentrations (UFC/mL) for each sampling point and collection performed, for both culture media.

Medium	Date	Collection points					
		M1	M2	M3	B1	B2	B3
Mannitol Salts Agar	10/28/20	0.00	30.00	50.00	5.50	6.00	7.50
	11/25/20	6.67	10.00	36.67	3.05	3.65	1.75
	01/28/21	1,180.00	173.33	1,206.67	50.00	60.00	> 100.00
	04/14/21	0.00	0.00	135.00	15.00	11.00	6.75
	07/26/21	233.33	460.00	375.00	4.50	14.00	8.50
	10/29/21	16.67	66.67	186.67	12.40	4.25	2.30
Baird-Parker Agar	10/28/20	600.00	275.00	40.00	4.50	4.65	3.00
	11/25/20	626.67	483.33	525.00	6.00	1.50	0.30
	01/28/21	1.175,00	1,511.67	770.00	55.00	65.00	> 100.00
	04/14/21	105.00	600.00	1,155.00	32.00	> 100.00	5.00
	07/26/21	1,431.67	1,135.00	680.00	20.00	0.93	3.00
	10/29/21	465.83	693.33	595.00	42.50	3.20	2.50

Source: Elaborated by the authors, 2021.
M: Marambaia; B: Beach.

Table 3. *S. aureus* concentrations (UFC/mL) at each sampling point and collection performed, for both culture media.

Culture medium	Date	Collection points					
		M1	M2	M3	B1	B2	B3
Mannitol Salts Agar	10/28/20	0.00	30.00	50.00	3.35	3.25	5.75
	11/25/20	6.67	10.00	16.67	3.00	3.50	1.75
	01/28/21	408.33	88.33	550.00	30.00	30.00	> 100.00
	04/14/21	0.00	0.00	130.00	12.00	8.00	6.50
	07/26/21	75.00	70.00	33.33	2.00	1.50	1.00
	10/29/21	6.67	50.00	126.67	11.20	4.00	2.00
Baird-Parker Agar	10/28/20	30.00	30.00	0.00	ND	ND	ND
	11/25/20	3.33	6.67	20.00	ND	ND	ND
	01/28/21	283.33	136.67	55.00	ND	ND	ND
	04/14/21	5.00	75.00	30.00	ND	ND	ND
	07/26/21	33.33	36.67	26.67	ND	ND	ND
	10/29/21	20.00	3.33	13.33	ND	ND	ND

Source: Elaborated by the authors, 2021.
ND: not determined.
M: Marambaia; B: Beach.

ciprofloxacin (8.8%), and piperacycline + tazobactam (2.9%). The strains isolated at points 1 of Marambaia and 2 of the beach did not present intermediate results against any antibiotic tested.

Using the Shapiro-Wilk test, it was found that the data do not follow a normal distribution ($p < 0.05$). In the PERMANOVA analysis, it was found that antimicrobial resistance did not differ in relation to the place of collection ($F = 1.38$; $p = 0.1759$) nor in relation to the time of collection ($F = 1.308$; $p = 0.2197$).

Fourteen strains showed resistance to at least one antibiotic tested. Two strains were resistant to five antibiotics, one strain to four antibiotics, and two strains to three antibiotics. Most of these strains, with the exception of one, originated from samples from the Marambaia canal.

Staphylococcus spp. antibiograms

In total, 117 *Staphylococcus* spp. strains were evaluated. The strains showed more resistance to antibiotics of the macrolide and lincosamide classes. Seventy-five strains were resistant to erythromycin (64.1%) and 73 strains were resistant to clindamycin (62.4%), with three strains showing induced resistance, in which resistance to erythromycin and false sensitivity to clindamycin are found, resulting in a flattening of the zone of inhibition, adjacent to the erythromycin disk, in the shape of the letter D (D-test). On the other hand, the highest sensitivity was for gentamicin, at all evaluated points. Intermediate results appeared more frequently when strains were tested against ciprofloxacin.

With the aid of the Shapiro-Wilk test, it was verified that the data do not follow a normal distribution ($p < 0.05$). By PERMANOVA, it was concluded that antibiotic resistance does not differ in the factors time ($F = 1.0542$, $p = 0.0997$) or place of collection ($F = 0.6331$, $p = 0.4981$). It was also found that there is no interaction between these factors ($F = -0.50935$, $p = 0.0541$).

Ninety-six *Staphylococcus* spp. strains were resistant to at least one antibiotic. One strain showed resistance to all antibiotics tested. Six strains were resistant to four antibiotics and 29 strains to three antibiotics. These strains originated from both the canal and the beach points.

Using the results of *Staphylococcus* spp. strains, MAR indices were calculated for all analyzed samples (Table 4). According to Krumperman[24], who considers those with values greater than 0.20 as the greatest risk of multidrug resistance. It was found that most of the samples analyzed present a high risk of multidrug resistance, considering the results of the antibiograms of this bacterial genus.

The samples were compared in relation to the MAR indexes calculated from the antibiograms of *Staphylococcus* spp. strains. After verifying that the data were normally distributed ($p > 0.05$), it was observed that there were no differences considering the factors moment ($F = 0.6397$, $p = 0.6721$) and place of collection ($F = 1.182$, $p = 0.3486$).

DISCUSSION

The sampling point with the highest average concentration of total coliforms was Marambaia 2, a place that passes through the most urbanized area of the river, receiving greater influences from civil and commercial construction. On the other hand, the sampling point with the lowest average concentration was Beach 3, indicating the highest natural dilution, since it is more distant, receiving less influences from both the

Table 4. Multiple antibiotic resistance (MAR) indices in *Staphylococcus* spp. for collection sites. The highlighted values indicate the samples that showed a low risk of multidrug resistance, according to the criterion established by Krumperman[24].

Collection points	Season of the year/year of collection				
	Spring 2020	Summer 2021	Autumn 2021	Winter 2021	Spring 2021
M 1	0.38	0.57	**0.17**	0.25	0.25
M 2	0.60	0.33	0.33	0.33	0.42
M 3	0.52	0.67	0.39	0.46	**0.20**
B 1	0.42	0.42	0.38	0.22	**0.17**
B 2	0.43	0.33	**0.00**	0.42	0.39
B 3	0.27	**0.11**	0.33	0.50	0.50

Source: Elaborated by the authors, 2021.
M: Marambaia; B: Beach.

river and the Barra Norte breakwater. It is noteworthy that the points Marambaia 3 and Beach 1 showed similar patterns when observed over time, indicating the influence of the river mouth on the microbiological quality of the beach.

The highest concentrations observed for all points occurred in January to February 2021, which may be associated with the leaching of microorganisms from the soil to the water. In July 2021, the Marambaia 2 point showed lower concentrations of total coliforms compared to the others, which may have been caused by the strong anthropogenic pressure of the contribution of civil construction waste observed at the time of collection. Alluding to the results of point 2 of the beach, the variability of the data over time can be explained by the greater presence of recreational use of the site.

According to Conama Resolution No. 357/2005, the parameters must meet the conditions and quality standards according to the use of the water body. The framework of water bodies, in turn, should not be based on their current state, but on the quality levels they should reach to meet the needs of the community[25]. Considering the Marambaia river as a class 2 brackish water body, the necessary conditions for quality assurance were not met, given that the average count of thermotolerant coliforms exceeded the limit of 2,500 MPN/100 mL in most points, with the exception of Marambaia 1 and Beach 3. This fact may be related to the Marambaia River at point 1 being closer to the underground portions, receiving smaller amounts of effluents and the beach at point 3 having higher rates of natural dilution. The data obtained for the points Marambaia 3 and Praia 1 indicate the influence of the river contamination until the mouth at Pontal Norte.

Also according to Conama Resolution No. 357/2005, effluents from any polluting source can only be released, directly or indirectly, into bodies of water after proper treatment[25]. Hospital effluents contain a multitude of drugs and metabolites, including antibiotics. Grenni et al.[26] emphasized that the highest concentrations of antibiotics generally occur in areas with strong anthropogenic pressure, such as hospital effluents, sewage, and treated domestic effluents, in addition to soils managed with compost or used for livestock.

It should be noted that the municipality of Balneário Camboriú is served by four hospitals. If the treatment of hospital effluents is ineffective, it can cause several damages to human health and the environment, considering that the continuous supply of these substances with antimicrobial properties can favor the selection of resistant microorganisms. This prevalence can change the local microbiota, in addition to jeopardizing the fine line between infections and effective treatment, aggravating the so-called “superbug crisis”, putting an end to the era of antibiotics.

Multiresistant bacteria have already been observed outside the hospital environment, such as in seawater and treated sewage in Antarctica[27], in rivers in Nigeria[12], at different points in the Godavari River, in India[28], and in Brazil, in water and sediments[29] and hospital effluents[10,30].

E. coli strains do not show intrinsic resistance to any antibiotic, however, cases of acquired resistance have been reported both in hospitalized patients and disseminated in the general community[31,32]. The main mechanisms of resistance in enterobacteria are described in strains producing extended-spectrum β-lactamase (ESBL), β-lactamase AmpC, and producing carbapenemases.

ESBL enzymes are produced in many Gram-negative bacilli and confer resistance to penicillins, cephalosporins, and aztreonam. They are frequently detected in *E. coli* and *Klebsiella* spp. strains[31,32].

Carbapenemases were initially found in *Klebsiella pneumoniae*, receiving the classification of KPC (*Klebsiella pneumoniae* carbapenemase), however, this resistance mechanism quickly spread to other Enterobacteriaceae[11]. According to de Paula et al.[33], carbapenem antibiotics are used in the treatment of serious infections caused by multidrug-resistant microorganisms, including ESBL enzyme producers.

The present study evaluated 34 strains of *E. coli*. The results obtained showed higher resistance rates for ampicillin and sulfamethoxazole + trimethoprim, highlighting the need for studies focused on the resistance mechanism and possible genes involved. For the tested carbapenem, imipenem, the results found demonstrate strains in general sensitive, with some intermediate results and a resistant strain.

The MAR index was not evaluated in *E. coli*, due to the low *n* sample. However, in general, bacteria that show resistance to three or more classes of antibiotics are considered multidrug-resistant. Through the results obtained, an alert is raised regarding the selection of resistant microorganisms, considering that five strains showed resistance to at least three antibiotics.

The genus *Staphylococcus* spp. it is not used as a quality parameter in effluents and water. However, it represents an emerging risk to human health, as it is an opportunistic pathogen, usually associated with infections acquired in the community and in the hospital environment.

Initially, two culture media were evaluated, seeking to highlight the one that would best allow the isolation and recovery of *S. aureus* strains and coagulase-negative *Staphylococci*. Mannitol Salts medium is widely used in clinical practice, aiming at the isolation of *Staphylococcus* spp. in biological samples, while Baird Parker medium is normally used in the analysis of foods. For this study, the statistical analysis showed no difference for the media, with the exception of point Marambaia 2, in which the Baird Parker medium showed better recovery of the isolated microorganisms. Both Baird Parker and Mannitol Salts showed satisfactory results for samples sown by scattering, that is, those from the Marambaia river. For the beach samples, the methodology chosen, by filtering membrane, indicated a certain limitation for the Baird Parker medium, considering the isolation of *S. since the membrane made it impossible to visualize the lecithinase halo. For the isolation of Staphylococcus* spp., without distinction of species, both culture media were satisfactory.

For the assessment of antibiotic sensitivity according to the BrCAST clinical cut-off table, sensitivity to cephalosporins and carbapenems in *Staphylococcus* is inferred by sensitivity to cefoxitin. The disk diffusion test is reliable for predicting methicillin and oxacillin resistance. In the present study, cefoxitin was tested in 117 *Staphylococcus* spp. strains, showing resistant results in 37 strains (31.6%), characterizing them as MRSA.

Discovered in 1960, methicillin was the first semi-synthetic penicillin to be in clinical use[34,35]. However, in the early 1970s, methicillin-resistant strains of *S. aureus* sprung up, identified by the acronym MRSA (*Staphylococcus aureus* resistant to methicillin), also showing resistance to the other *β*-lactams[36].

MRSA strains have already been described on beaches in Florida, being associated with skin infections, in Hawaii and in effluents before treatment[37,38,39].

The main mechanism of resistance to oxacillin/methicillin is related to the alteration of the site of β-lactams action. The Brazilian National Health Surveillance Agency (Anvisa) determines that resistance to oxacillin is often accompanied by resistance to other classes of antimicrobials, such as clindamycin, erythromycin, and tetracycline, reducing the therapeutic options[11].

Erythromycin and clindamycin are widely used to fight infections with Gram-positive bacteria. However, resistance mechanisms have already been described. According to Anvisa, resistance to macrolides, lincosamines, and streptogramin B (MLS B) can be constitutive or inducible, depending on the exposure. In constitutive expression, resistance to erythromycin and clindamycin is detected on the antibiogram. The inducible form may show resistance to erythromycin and false sensitivity to clindamycin. The detection of inducible resistance can be done by the D-test or disk-approximation, in which the erythromycin disk is placed next to the clindamycin disk on the antibiogram plate, according to BrCAST, the distance needs to be 12-20 mm edge-to-edge. As erythromycin diffuses through the agar, resistance to clindamycin is induced, resulting in a flattening of the zone of inhibition, adjacent to the erythromycin disk, in the shape of the letter D[11].

In this study, three strains were found with inducible resistance, verified through the positive D-test. Overall, 73 strains were resistant to clindamycin and 75 strains were resistant to erythromycin. By the disk diffusion method, erythromycin can be used to determine the sensitivity to azithromycin and clarithromycin, since they belong to the same class of drugs (macrolides). It is worth noting that the collections were carried out between October 2020 and October 2021, a period in which the SARS-COV-2 pandemic led a large part of the population to self-medication, including the misuse of azithromycin, often indicated in kits without medical prescription and without correct guidance for use. The spread of antimicrobial resistance is intrinsically linked to the increase in the consumption of antibiotics, their irrational use, and subsequent disposal in the environment.

Due to the spread of MRSA/ORSA and MLS B strains, vancomycin, known since 1956, started to be used as an alternative treatment[34]. Vancomycin is a glycopeptide whose spectrum of action encompasses Gram-positive cocci and bacilli; its use is restricted to the hospital environment, with the parenteral route as the main route of administration[11].

However, in 1997, the first vancomycin-resistant *S. aureus* strain was described in Japan[34]. According to Anvisa, resistance to glycopeptides can be expressed by two different phenotypes: Vancomycin-intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA)[11]. In general, this event is rare, but measures to detect this resistance must be implemented by microbiology laboratories.

For this study, the 117 *Staphylococcus* spp. strains were tested against vancomycin in a preliminary way, considering that the disc diffusion method is not the most indicated, because according to BrCAST, it does not distinguish wild-type isolates from those with resistance not mediated by the *vanA* gene. It is necessary to implement the MIC methodology to obtain more reliable clinical results[21].

Therefore, the screening of vancomycin-resistant strains in this study used the reference value for the disc diffusion method established by the CLSI for the year 2009, finding 54 vancomycin-resistant strains. The result found is not consistent with that established by BrCAST/EUCAST, CLSI, and Anvisa, which determine that non-susceptible isolates are rare[11,21,22,23].

The evaluation of the MAR index in *Staphylococcus* spp. allowed the verification of the risk of multidrug resistance for the isolated strains. There were no differences considering the time and place of collection factors. In all sampling points, it was possible to detect indices greater than 0.20 with few moments presenting a low risk of multidrug resistance. It is necessary to evaluate the mechanisms of resistance in order to understand the multiresistance present in strains in the environment, since the MRSA and MLS B strains have already been described in treated and untreated effluents, whether domestic or hospital, in addition to various aquatic environments, in which the alert for recreational use or supply for human and animal consumption is raised, as they may be associated with various infections. Also noteworthy are the VISA or VRSA strains, because, despite the resistance described in the literature being uncommon for vancomycin, the selection of resistant strains, even if few so far, is extremely worrying, as it is one of the last therapeutic options available for multidrug-resistant strains.

CONCLUSIONS

The so-called superbacteria, microorganisms that present multi-resistance to antibiotics, were described, at first, strictly in hospital environments. However, they soon reached the community, being widely found in the environment. Infections by multidrug-resistant bacteria compromise pharmacological treatment, as they reduce therapeutic options and increase hospital stay, which ensures that these strains circulate longer in healthcare environments, harming immunocompromised patients and increasing nosocomial infections.

This study analyzed *E. coli* and *Staphylococcus* spp. strains regarding their presence in the water of the Marambaia river and on the adjacent beach; in addition to evaluating antimicrobial resistance for the isolated strains. The presence of *E. coli* in these environments is already worrying in itself, as it is a thermotolerant coliform, indicative of recent fecal contamination. Although the results found indicate few multidrug-resistant strains, the presence of just one resistance mechanism already serves to raise awareness, as the associated genes may be present in water samples or in effluents released by the community, and may be transmitted to other microorganisms, modifying the local microbiota and putting the health of the population at risk.

The evaluation of MAR index data for *Staphylococcus* spp. is alarming. There were high risks of multidrug resistance for most of the sampled sites and at any time during the collections. In addition, few strains were sensitive to all antibiotics tested. The resistance mechanisms that occur in Gram-positives, despite differing from those that occur in Gram-negatives, are equally worrisome, whether from a microbiological point of view or in terms of public health.

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Author's Contributions

Lima ACD, Resgalla Junior C, da Silva MAC - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. All authors approved the final version of the work.

Conflict of Interests

The authors inform that there is no potential conflict of interest with peers and institutions, politicians, or financial in this study.