Antimicrobial Sensitivity Profile of Bacteria Isolated in Babitonga Bay

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Abstract

Antimicrobial resistance is one of the main and growing public health concerns. However, the analysis of the expansion of this phenomenon in the environment is limited. Thus, the objective of this work was to determine the antimicrobial susceptibility profile of bacteria isolated from Baía Babitonga. Isolation and identification of microorganisms were carried out from the mangrove sediment sample, collected at two sampling points in Baía Babitonga. Microbial identification analyzes demonstrated the presence of Gram-negative microorganisms, for which the antimicrobial susceptibility profile analysis showed the presence of resistance to Aztreonam, Ciprofloxacin, Cefuroxim, Nitrofurantoin, Meropenem, Tetracyclin, Cefotaxime, and Ceftazidime. For the identified Gram-positive microorganisms, the presence of resistance to Ampicillin and Clindamycin was evidenced. The genotypic investigation showed that the resistances found were not caused by the researched genes (bla_{SHV}, bla_{CTX-M}, $bla_{OXA-48-like}$, bla_{KPC}, bla_{NDM-1}, bla_{VIM} e bla_{IMP}).

1 Introduction

The mangrove has a great diversity of plants, arthropods, molluscs, fish and birds, and constitutes a natural breeding ground for numerous species, establishing a safe place for reproduction, spawning and birth, presenting great ecological and economic importance, as it provides a means of livelihood for riverine communities (Kathiresan and Bingham 2001; Ministry of Environment 2018; Ghosh et al. 2010). The mangrove ecosystem is a transition area between the terrestrial and marine environments, where river water meets seawater, such as on the banks of bays, inlets, bars, river mouths, lagoons and coastal indentations (Ministry of the Environment, 2007).

The Babitonga Bay, located in the north of the state of Santa Catarina, is home to about 75% of the mangrove forests in the southern region of Brazil. Due to its geographic location, over the years the region has suffered from anthropogenic impacts, which result in the degradation of its ecosystems (GROSE 2013).

The presence of antimicrobial resistant bacterium, and resistance genes in the environment is of great importance in the spread of antimicrobial resistance, since the occurrence of these contaminants in water bodies can be a primary source of introduction and spread to other environmental matrices (SINGER et al. 2016).

Therefore, considering the economic and ecological importance of Babitonga Bay, this work aimed to investigate the presence of potentially pathogenic bacteria carrying antimicrobial resistance genes in sediment samples from the mangrove swamp from Babitonga Bay.

2 Materials And Methods

The mangrove studied is located in Babitonga Bay, the largest estuarine complex in the State of Santa Catarina, with an area of approximately 160 km². The collections were carried out in the city of San

Francisco of the South.

The first point of the study is called Praia do Lixo, S26º 15,701’ W048º 38,852. The second sampling point is located at Praia Avenida, S 26º 15’08.4" W 48º 38’38.3”. Both are heavily influenced by human activities, such as the construction of houses and the dumping of untreated domestic sewage.

To reduce the action of environmental variability, sampling was carried out from three sub-samples of mangrove sediment, one meter apart, obtained by a 10 cm long, 7 cm diameter sampler. These subsamples were homogenized *in situ* and stored in sterile Falcon tubes. The samples were immediately transported to the Biotechnology Laboratory of the University of the Region of Joinville (UNIVILLE).

An aliquot of 25 g from the collected samples was inoculated into 225 mL of Marine Broth and incubated at 34 °C, 150 rpm, for 72 h, to the growth of mesophilic microorganisms. After the incubation period, the samples were subcultured to selective culture media MacConkey Agar (Biobrás), Cetrimide Agar (Merck), Esculin Bile Agar (Acumedia) and Mannitol Salt Agar (Acumedia). The microorganisms that showed growth on selective media were subcultured to Nutrient Agar (Himedia),

Colonies that developed were subjected to Gram stain, with dark blue to violet stained cells considered Gram-positive and pink cells considered Gram-negative (Santos et al. 2016).

Gram-positive microorganisms were submitted for identification using the BBL Crystal Kit – BD ® – Gram-positive, and Gram-negative microorganisms were identified using the BBL Crystal Kit – BD ® – Gram-negative, as indicated by the manufacturer.

2.1 ANTIMICROBIAL SENSITIVITY PROFILE

The microorganisms isolated and identified like Gram-negative bacilli or coccobacilli were submitted to an evaluation of the antimicrobial susceptibility profile, determined according to the methodology of Bauer (1966). The isolated microorganisms were cultivated in Nutrient Agar for 24 h at 34 °C. After this period, a bacterial suspension of each microorganism was made with a turbidity equivalent to the 0.5 standard of the McFarland scale. Then, the suspension was inoculated in different directions with the help of a sterile swab on a Petri dish containing Muller-Hinton agar. After this procedure, the disks containing the antimicrobials to be tested were distributed on the plate and it was stored at 35 °C, for 24 h, for the subsequent measurement of the diameter of the inhibition zones.

For *Vibrio metschnikovii*, Muller-Hinton agar was supplemented with 3.5% NaCl.

The antimicrobials tested for each microorganism were determined according to the protocols of the “Clinical and Laboratory Standards Institute” (CLSI 2018; CLSI 2015). For all tested antimicrobials, quality control was carried out with the strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213.

For microorganisms isolated and identified like Gram-positive bacilli, the determination of the sensitivity profile to the antibiotics Penicillin, Ampicillin, Clindamycin, Erythromycin, Tetracyclin and Gentamicin was performed by the automated system Microscan Walkaway Plus (Beckman Coulter, USA), through the determination of Minimum Inhibitory Concentration (MIC) for each antibiotic.

Through this technique, the MIC for each antibiotic was obtained, which corresponded to the lowest concentration capable of promoting the inhibition of the standardized bacterial suspension. The definition of the MIC of the antibiotic Meropenem was performed using the quantitative standardized Etest® tape method (bioMérieux, Brazil) with an exponential concentration gradient. A bacterial suspension was prepared from the isolated colonies, with previous growth for 18 to 24 h. Suspension turbidity was compared to the standard 0.5 on the McFarland scale. This suspension was then seeded on a plate containing Mueller-Hinton agar culture medium (bioMérieux, Brazil) and, after five minutes, the Etest® tape was applied. Then, the plates were incubated in an inverted position in an oven at 35 ºC, for 16 to 18 h. The interpretation of the results was performed as established by CLSI, document M045 of 2015.

2.2 IDENTIFICATION OF BACTERIAL RESISTANCE CAUSING GENES

To obtain the bacterial DNA, the heat shock method was used (Vaneechoutte et al. 1995; Baratto and Megiolaro 2012), with modifications, based on solid medium (Mueller-Hinton agar). First, two to three colonies of a pure culture, collected with an inoculation loop, were resuspended in 100 µL of sterile distilled water, in a 1,5 mL microtube. The suspensions were subjected to a bath with boiling water for 5 min and then to a thermal shock in an ice bath for 5 min. The boiling and cooling procedure was repeated a total of 3 times, followed by centrifugation (Eppendorf 5415 R, Hamburg, Germany) at 8000 x g for 10 min. At the end of processing, the supernatant was collected and the precipitate discarded. The supernatant containing bacterial DNA was qualified and quantified by means of spectrophotometric analysis (readings at 260 and 280 nm) in an Epoch device (BioTek Instruments, Winooski, USA) and then stored at -20 °C until subsequent use.

To verify the viability of extracted DNAs for the purposes of subsequent genotypic analyses, the Polymerase Chain Reaction (PCR) was applied using a pair of specific primers for the 16S rRNA gene - 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GCAGAGATACCAGCAGAGATACACG-3') - which provide the amplification of a single segment of approximately 1500 bp.

The reactions were established in a final volume of 50 µL, adding approximately 50 to 500 ng of extracted DNA to the reagent mixture containing 1 U of Platinum® Taq DNA Polymerase (Invitrogen, Brazil), 200 µM of dNTPs (GE Healthcare , UK), 1X PCR Buffer (Invitrogen), 50 pmols of each primer (DNA Express, Brazil) and 1.5 mM MgCl2 (Invitrogen). Thermocycling was performed in an XP Cycler apparatus (BIOER Technology, Tokyo, Japan), with an initial denaturation step at 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min, 37 °C for 1 min, and 72°C for 2 min. A final extension was conducted at 72°C for 10 min (Hayashi et al. 2005).

2.2.1 Identification of genes encoding extended spectrum beta-lactamases (ESBL)

The choice of genes to be researched was made after knowing the resistance in phenotypic analysis.

The bla_{SHV} and $bla_{CTX\text{-}M}$ genes were investigated in all Gram-negative microorganisms using the PCR technique, in individual reactions and using the primers originated in Table 1.

All reactions were established in a final volume of 50 µL and the reagents and concentrations used in each reaction were those described to assess the viability of microbial DNA. Specific thermocyclings for target genes are described in Table 2.

2.2.2 Identification of Carbapenemase-Coding genes

The $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC}, bla_{IMP} and bla_{VIM} genes were investigated in Gram-negative microorganisms that showed resistance or intermediate resistance to carbapenems. The research was carried out using the PCR technique, in individual reactions and using the primers shown in Table 3.

The reactions for the $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC} and bla_{VIM} genes were established in a final volume of 50 µL, and the reagents and concentrations used in each reaction followed as described in item 2.2.

Thermocycling was performed with an initial denaturation step at 94 °C for 3 min. The specific thermocyclings for each investigated target gene are described in Table 4.

2.2.3 Verification of PCR products via electrophoresis

To verify the results of the PCR reactions destined to the genes of interest, submerged electrophoresis was performed in a Tris/Borate/EDTA (TBE) buffer in 1% agarose gel, containing 0.5 ug/mL of ethidium bromide.

In addition to using positive controls, described in Table 5, and blanks, the confirmation of obtaining products (amplicons) corresponding to the planned segments was performed by comparing sizes with a commercially available standard (100 bp Ladder, Fermentas, Burlington, Canada), also exposed to the same electrophoresis condition. Thus, by analyzing the electrophoretic profiles obtained, the occurrence of the investigated genetic elements was determined.

3 Results And Discussion

Replacing the samples in selective agar allowed the growth of colonies in all culture media used.

Gram-negative microorganisms were isolated: *Acinetobacter lwoffii, Vibrio metschnikovii, Shewanella putrefaciens, Pseudomonas stutzeri, Aeromonas hydrophila, Serratia* sp., *Providencia rustigianii, Enterobacter gergoviae* and *Pseudomonas* sp. Gram-positives were: *Bacillus cereus, Bacillus pumilus, Bacillus subtillis* e *Micrococcus luteus.*

3.1 DETERMINATION OF THE ANTIMICROBIAL SUSCEPTIBILITY PROFILE

Tables 6 and 7 present the results of the antimicrobial susceptibility profile found for the isolated microorganisms.

Acinetobacter lwoffii it is a Gram-negative, aerobic, non-fermenting bacillus, widely distributed in nature (González-Candelas et al. 2017). The results found for the strain of A. lwoffii isolated in this work demonstrated sensitivity to all tested antimicrobials, corroborating the results obtained by Figueiredo et al. (2010), in which all *Acinetobacter lwoffii* isolates were fully susceptible to all antibiotics tested, including penicillins, Imipenem and Meropenem. However, the authors reported that the PCR results showed that all strains had a gene similar to bla OXA-134. This gene was investigated and it was found that A. lwoffii intrinsically possesses a chromosomal gene that encodes a carbapenem hydrolysis class D β-lactamase, OXA-134. With the result of susceptibility to all antimicrobials, it is likely that the $bla_{OXA-134}$ genes were not expressed, or were expressed at a very low level, in the isolates in this study. In this way, this species becomes a reservoir for carbapenemase genes that can spread among other species of *Acinetobacter*. In contrast, Hu et al. (2011) performed the genetic sequencing of a clinical isolate of *Acinetobacter lwoffii*. This strain showed high resistance to Ampicillin, Cefazolin, Cefotaxime, Ceftazidime, Cefpiroma, Ceftriaxone, Imipenem, and Meropenem. The results of this study demonstrated the antimicrobial multiresistance of this isolate in addition to the presence of a plasmid with the NDM-1 resistance gene.

Vibrio is a genus of bacteria that occur widely in the aquatic environment, mainly in seas, estuaries, and brackish waters. Although the vast majority of species belonging to the *Vibrio* genus are considered harmless when in the marine environment, some species have been described as pathogenic for humans (Beshiru et al. 2020; Ramamurthy et al. 2014). *Vibro metschnikovi has been reported as an important cause of infections, such as postoperative infections, one of these cases being reported in the case study by Linde et al. (2004). The results of the antibiogram performed for this isolate showed resistance against the antimicrobials Cefotaxime, Ceftazidime, as well as an intermediate result for Meropenem.* The results of the present study are similar to the results of the study by Valáriková et al. (2020) in which the bacterial resistance profile of several Vibrio species, including the metschnikovi species, was evaluated. The author's results demonstrate that strains of Vibrio sp. evaluated showed broad resistance to antimicrobials, especially β-lactams. However, the study did not assess the class of 3rd generation cephalosporins, only ampicillin and penicillin, to which the isolates were resistant.

Bacteria of the genus *Shewanella* are widely distributed in the environment and can be found in soil and brackish water. *Shewanella* are gram-negative, motile, non-glucose fermenting bacilli. Among the many species of the genus *Shewanella*, only two species, *Shewanella putrefaciens* and *Shewanella algae*, are opportunistic pathogens causing skin and mucosal infections, and more rarely generalized infections such as bacteremia (Pagniez and Berche 2005). Initially when discovered, *Shewanella* was classified as *Pseudomonas putrefaciens*. However, the genotypic and phenotypic analysis of many *Shewanella* strains from very varied origins, such as fish, meat, oils and clinical samples, confirmed its great heterogeneity,

suggesting the existence of many species within this genus (Pagniez and Berche 2005). *Pseudomonas* sp. is a widely disseminated Gram-negative bacterium with the ability to survive in a wide range of environments. *Pseudomonas aeruginosa* is recognized as an opportunistic pathogen, and is the bacterium most commonly associated with nosocomial infections and ventilator-associated pneumonia. The results of the antibiogram performed for *Shewanella putrefaciens, Pseudomonas* sp. and *Pseudomonas stutzeri* demonstrate that the *Shewanella putrefaciens* strain was sensitive to all tested antimicrobials. The results obtained for *Pseudomonas* sp. show resistance of this microorganism against the antimicrobial Aztreonam, while *Pseudomonas stutzeri* showed intermediate resistance against this antimicrobial and resistance to Ciprofloxacin. The study by (Poirel et al. 2010) characterized a carbapenem-resistant strain of *Pseudomonas stutzeri* isolated from one patient. This isolate produced a metallo-β-lactamase, called DIM-1. The study concludes that the spread of the *bla*DIM-1 gene among other Gram-negative isolates, mainly among the Enterobacteriaceae, still needs to be evaluated. Several evidences indicate that environmental species such as *Pseudomonas* sp. they can act as intermediate reservoirs to capture antibiotic resistance genes from other environmental species and then exchange these genes for genes from Enterobacteriaceae.

The genus Aeromonas consists of facultative anaerobic Gram-negative bacteria that normally inhabit the aquatic environment. In Brazil, studies point to the occurrence of these pathogens isolated from aquatic ecosystems, of food origin (fish, oysters, mussels, and crabs) and skin lesions in humans (Evangelista-Barreto et al. 2006). The Aeromonas hydrophila strain showed intermediate resistance against the antimicrobials Ciprofloxacin and Meropenem and was sensitive to the other antimicrobials tested. In the study by Evangelista-Barreto et al. (2006), 48% of the studied Aeromonas isolates were resistant to at least one of the eight antibiotics tested. In a study carried out in 2010, Evangelista-Barreto et al. (2010) reported that 60% of the isolates were resistant to at least one of the antimicrobials, demonstrating that antibiotic resistance in this bacterial genus is increasing. The isolates belonging to the order Enterobacteriales were *Serratia* sp., which is mainly present in environmental habitats. Bacteria belonging to this genus are considered opportunistic nosocomial pathogens, causing many infections, including meningitis, sepsis, urinary tract infections, skin infections, bloodstream, and respiratory infections (Wu et al. 2013) in addition to being important pathogens eyepieces (Shanks et al. 2013). Providencia, which is associated with urinary tract infections, keratitis, dacryocystitis, conjunctivitis and endophthalmitis (Koreishi et al. 2006). Finally, the last isolate of that order was *Enterobacter*, which can be found in a variety of habitats, both in soil and in water. Furthermore, they are part of the normal human gut microbiota (Davin-Regli et al. 2019). In the antibiogram performed for the isolates belonging to the order Enterobacteriales, the strain of Serratia sp. showed resistance to Cefuroxime and Nitrofurantoin, in addition to intermediate resistance to the antimicrobial tetracycline. The study by Sandner-Miranda et al. (2018) evaluated the antimicrobial susceptibility profile of 32 Serratia spp. One of the results obtained in the study showed the resistance profile: 0.13% for macrolides; 0.54% for sulfonamides; 3.69% for Chloramphenicol; 3.83% for phosphonic antimicrobials; 3.96% for quinolones; 4.1% for aminocoumarin antibiotics; 7.11% associated with aminoglycoside resistance; 20.38% for β-lactams and 56.22% for polypeptides. The most pronounced resistance result in the class of polypeptide antibiotics is easily

justifiable, since this genus has intrinsic resistance to Polymyxin B. The second highest frequency corresponded to β-lactam resistance genes.

Providencia rustigianii showed resistance to Cefuroxime, Meropenem and Nitrofurantoin and also intermediate resistance to Tetracycline, Ciprofloxacino, and Chloramphenicol. According to (Santos et al., 2015) bacteria of the genus Providencia have intrinsic resistance to antimicrobials Ampicillin, Amoxicillin, Amoxicillin + Clavulanic Acid, 1st generation of Cephalosporins, Netilmycin, Tobramycin, Nitrofurantoin, Cefuroxime, Gentamicin, Polymyxin and Colistin. Virtually all *Providencia* species can produce inducible AmpC beta-lactamases, and many isolates can also produce extended-spectrum beta-lactamases (ESBLs) in nosocomial environments (Tshisevhe et al. 2017).

Enterobacter gergoviae showed resistance to Cefuroxime, Aztreonam, Tetracycline, as well as an intermediate result to Meropenem, Ciprofloxacin and Chloramphenicol. Studies have reported the production of extended-spectrum β-lactamases in bacteria of this genus (Davin-Regli et al. 2019). In the study by Freire et al. (2016), an outbreak caused by Enterobacter gergoviae was evaluated. Through DNA sequencing, this study identified $bla_{\text{IMP-1}}$ in all isolated E. gergoviae.

The genus *Bacillus* is composed of aerobic or facultative gram-positive bacteria, forming endospores. Its ability to form highly resistant endospores is key to successful colonization of a wide variety of environments (Baruzzi et al. 2011). The antibiogram performed for *Bacillus cereus, Bacillus subtillis* and *Bacillus pulmillus* showed that the isolate *Bacillus cereus* showed resistance to Ampicillin, intermediate resistance to Clindamycin and was sensitive to the other antimicrobials tested. The isolate *Bacillus pulmillus* showed resistance to Clindamycin, and was sensitive to the other antimicrobials tested. The isolate *Bacillus subtillis* showed intermediate resistance to Clindamycin, and was sensitive to the other antimicrobials tested. The study by Noor Uddin et al., (2015) evaluated the presence of antimicrobial resistance of bacteria isolated from probiotics used in shrimp cultures. This study revealed the presence of resistance among *Bacillus* spp., in particular to Ampicillin, Chloramphenicol, Clindamycin, Erythromycin and Penicillin.

Micrococcus spp. it is commonly known as normal skin microflora and its antibacterial activity has rarely been reported against different pathogens. This bacteria can be easily found in nature (Akbar et al. 2014). The antibiogram performed for *Micrococcus luteus* showed that this microorganism was sensitive to all tested antimicrobials.

3.2 IDENTIFICATION OF GENES ASSOCIATED WITH BACTERIAL RESISTANCE

Table 8 shows the possible resistance genes associated with resistant or intermediate resistant microorganisms found in this work, according to the literature.

Based on the classes of antimicrobials to which some isolated microorganisms were resistant and on the possible resistance genes associated with these microorganisms (Table 8), the genes bla_{SHV}, $bla_{\text{CTX-M}}$, $bla_{\text{NMP-1}}$, $bla_{\text{oxa-48-like}}$, bla_{kpc}, bla_{IMP} and bla_{VIM} for all Gram-negative bacilli.

The Vibrio spp. it was negative in the PCR for the 16S ribosomal rRNA gene, indicating that the extracted DNA was not viable for the continuation of the other genotypic analyses. Therefore, it was not possible to carry out the investigation of antimicrobial resistance genes.

The evaluated microorganisms did not show positivity for the investigated resistance genes; however, as shown in Fig 1, *Pseudomonas* spp. and *Enterobacter* spp. showed amplicons of unexpected size. *Pseudomonas* spp. resulted in the identification of a nonspecific size band in the search performed for the $bla_{\text{CTX-M}}$ gene, while *Enterobacter* spp. demonstrated a nonspecific band size for the bla_{SHV} gene.

The results shown in Figure 1 show that candidates Pseudomonas spp. and Enterobacter spp. did not damage the genes of interest researched. However, the presence of unspecific bands for both microorganisms is a result that deserves further investigation through genetic sequencing, in order to find out if the result presented is a variant of the researched gene, considering that the used primers performed hybridization no bacterial DNA, indicating that the nucleotide sequence is likely similar.

It was not possible to search for resistance genes in Gram-positive microorganisms due to time limitations and tolerance rates.

The study by Devarajan et al. (2017), carried out in Switzerland, Democratic Republic of Congo and India, evaluated samples of aquatic sediment from regions that receive hospital effluents and regions that receive partially treated effluents from water treatment plants, in temperate and tropical climate regions. The study looked for the presence of the bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{NDM}, $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-2}}$ genes. The bla_{SHV} gene was identified in 3, 8 and 13% and the $bla_{\text{CTX-M}}$ gene was identified in 47, 35 and 12% of the isolates from the three different regions evaluated in Switzerland, Democratic Republic of Congo and India, respectively.

In the research carried out by Griffin et al. (2019) in soil and aquatic sediment samples in the coastal region of the United States, the presence of a wide variety of antimicrobial resistance genes was found. The genes found *were*: tet_{B}, tet_{G}, tet_{L}, tet_{M}, tet_{O}, tet_{W}, amp_{C}, van_{A}, erm_{B}, mec_{A}, bla_{SHV}, bla_{PSE}, flo_{R}, aad_{A2}. All areas evaluated in the study showed the presence of at least one resistance gene.

In a study developed in China, in an estuary region, with samples collected from surface water, deep water and sediment, the presence of some antimicrobial resistance genes was evidenced, namely: tet_{B}, tet_{C}, tet_{M}, tet_{O}, tet_{W} (Chen et al. 2013). Although the genes found are from a different class than the genes researched in this study, it is worth noting that the presence of resistance genes in the environment is wide and diverse.

In Brazil, studies in this area are recent and have been growing in recent years.

The study by Conte et al. (2020) evaluated the presence of antimicrobial resistance genes in the bacterium *Aeromonas* spp., present in wastewater and hospital and domestic effluents, in the region of Curitiba, Paraná. The study found the presence of the $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{GES} genes.

A study carried out in Rio de Janeiro investigated the presence of carbapenemase genes in river and lake waters. The study evidenced the spread of different genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-370}$ and *bla*$_{GES-16}$) in two important aquatic environments (Araujo et al. 2016).

As shown, studies on the presence of antimicrobial resistance genes in the environment have shown that the dissemination of these genes is increasing and the environment has become, in fact, a large reservoir of resistance genes, which exposes human beings to settings different from hospitals.

4 Conclusion

Our study demonstrated the presence of antimicrobial resistance in bacterial strains isolated from mangrove sediment, evidenced by phenotypic analysis. However, the analysis aimed at identifying some genes associated with the resistances found was not able to determine the responsible gene.

The results found indicate that the areas studied present a microbiome with resistance to antimicrobials that deserves further studies.

The spread of antimicrobial resistance in the environment is a real public health problem that needs to be evaluated and addressed through public policies that focus especially on wastewater and effluent treatment and on the rational use and correct disposal of medicines.

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Tables

Table 1
Primers used to detect ESBL encoding genes.

Target	Sequence (5'-3')	Product (pb)	Reference
bla_{SHV}	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG	795	Weill et al. (2004)
bla_{CTX-M}	CGATGTGCAGTACCAGTAA TTAGTGACCAGAATCAGCGG	585	Batchelor et al. (2005)

Table 2
Thermocycling used to investigate the bla_{SHV} and bla_{CTX-M} genes.

Target	Desnaturation	Hybridization
	°C (time)	°C (time)
bla_{SHV}	94 (1 min)	50 (30 s)
bla_{CTX-M}	94 (30 s)	60 (30 s)

Table 3
Primers used in the detection of genes encoding carbapenemases

Target	Sequence (5' to 3')	Product (pb)	Reference
$bla_{OXA-48-like}$	TGTTTTTGGTGGCATCGAT	177	Monteiro et al. (2012)
	GTAAMRATGCTTGGTTCGC		
bla_{KPC}	TCGCTAAACTCGAACAGG	785	Monteiro et al. (2009)
	TTACTGCCCGTTGACGCCCAATCC		
bla_{NDM-1}	GGTGCATGCCCGGTGAAATC	661	Yong et al. (2009)
	ATGCTGGCCTTGGGGAACG		
bla_{VIM}	GTTTGGTCGCATATCGCAAC	382	Mendes et al. (2007)
	AATGCGCAGCACCAGGATAG		
bla_{IMP}	GGAATAGAGTGGCTTAAYTCTC	232	Poirel et al. (2011)
	GGTTTAAYAAAACAACCACC		
	ACATTATCCGCTGGAACAGG		

Table 4

Thermocycling used to investigate the $bla_{OXA-48-like}$, bla_{KPC}, bla_{NDM-1}, bla_{VIM} and bla_{IMP} genes

Target	Desnaturation	Hybridization	Extension	Cycles	Final Extension
	°C (time)	°C (time)	°C (time)	n°	°C (time)
$bla_{OXA-48-like}$	94 (1 min)	63 (40 s)	72 (30 s)	35	72 (7 min)
bla_{KPC}	94 (1 min)	60 (1 min)	72 (1 min)	40	72 (10 min)
bla_{NDM-1}	94 (1 min)	62 (1 min)	72 (1 min)	35	72 (10 min)
bla_{VIM}	94 (30 s)	68,8 (1 min)	72 (1 min)	40	72 (10 min)
bla_{IMP}	94 (30 s)	45 (1 min)	72 (1 min)	40	72 (10 min)

Table 5

Standard strains used as positive controls in genotypic tests.

Genotype	Bacterial species	Origin number	Provenance
bla_{SHV}	Klebsiella pneumoniae	CCBH6556	FIOCRUZ-RJ
bla_{CTX-M}	Klebsiella pneumoniae	CCBH6556	FIOCRUZ-RJ
$bla_{OXA-48-like}$	Klebsiella pneumoniae	12692 RM	LACEN-PR
bla_{KPC}	Klebsiella pneumoniae	CCBH6556	FIOCRUZ-RJ
bla_{NDM-1}	Acinetobacter baumannii	5379 RM	LACEN-PR
bla_{VIM}	Enterobacter cloacae	13493 RM	LACEN-PR
bla_{IMP}	Serratia marcescens	12492 RM	LACEN-PR
FIOCRUZ: Foundation Oswaldo Cruz - Strains kindly donated by Ana Paula Assef			
LACEN: Central Public Health Laboratory - Strains kindly donated by Marcelo Pillonetto			

Table 6

Isolates from mangrove sediment samples from Babitonga Bay that showed resistance (R) or intermediate resistance (I) to antibiotics according to the diameter of the inhibition zone.

Microorganism	Antimicrobial	Halo diameter according to the interpretation criteria of the method used (mm)			Diameter of halo (mm)	Type of resistance
		S	I	R		
Vibrio metschnikovi	Cefotaxim (30 µg)	≥ 26	23–25	≤ 22	20	R
	Ceftazidim (30 µg)	≥ 21	18–20	≤ 17	14	R
	Meropenem (10 µg)	≥ 23	20–22	≤ 19	20	I
Pseudomonas spp.	Aztreonam (30 µg)	≥ 22	16–21	≤ 15	13	R
Pseudomonas stutzeri	Aztreonam (30 µg)	≥ 22	16–21	≤ 15	18	I
	Ciprofloxacin (5 µg)	≥ 21	16–20	≤ 15	15	R
Aeromonas hydrophila	Meropenem (10 µg)	≥ 23	20–22	≤ 19	22	I
	Ciprofloxacin (5 µg)	≥ 21	16–20	≤ 15	18	I
Serratia spp.	Cefuroxim (30 µg)	≥ 23	15–22	≤ 14	6	R
	Nitrofurantoin (300 µg)	≥ 17	15–16	≤ 14	7	R
	Tetraciclin (30 µg)	≥ 15	12–14	≤ 11	12	I
Providencia rustigianii	Cefuroxim (30 µg)	≥ 23	15–22	≤ 14	6	R
	Meropenem (10 µg)	≥ 23	20–22	≤ 19	19	R
	Nitrofurantoín (300 µg)	≥ 17	15–16	≤ 14	6	R
	Tetraciclin (30 µg)	≥ 15	12–14	≤ 11	14	I

Microorganism	Antimicrobial	Halo diameter according to the interpretation criteria of the method used (mm)			Diameter of halo (mm)	Type of resistance
		S	I	R		
	Ciprofloxacin (5 µg)	≥ 21	16–20	≤ 15	18	I
	Cloranfenicol (30 µg)	≥ 18	13–17	≤ 12	16	I
Enterobacter gergoviae	Cefuroxim (30 µg)	≥ 23	15–22	≤ 14	6	R
	Aztreonam (30 µg)	≥ 21	18–20	≤ 17	16	R
	Tetraciclin (30 µg)	≥ 15	12–14	≤ 11	10	R
	Meropenem (10 µg)	≥ 23	20–22	≤ 19	20	I
	Ciprofloxacin (5 µg)	≥ 21	16–20	≤ 15	18	I
	Cloranfenicol (30 µg)	≥ 18	13–17	≤ 12	15	I

Table 7

Isolates from mangrove sediment samples from Babitonga Bay that showed resistance (R) or intermediate resistance (I) to antibiotics according to the Minimum Inhibitory Concentration (MIC).

Microorganism	Antimicrobial	CIM (µg/mL) according to the interpretation criteria of the method used			CIM (µg/mL) found	Type of resistance
		S	I	R		
Bacillus cereus	Ampicillin	≤ 0,25	-	≥ 0,5	> 8	R
	Clindamycin	≤ 0,5	1–2	≥ 4	2	I
Bacillus pulmillus	Clindamycin	≤ 0,5	1–2	≥ 4	> 4	R
Bacillus subtillis	Clindamycin	≤ 0,5	1–2	≥ 4	2	I

Table 8

Possible resistance genes associated with resistant or intermediate resistant microorganisms isolated from Babitonga Bay mangrove sediments, according to the literature.

Microorganism	Associated Antimicrobial Resistance Genes	Reference
Vibrio metschnikovi	bla_{AmpC}, bla_{ACT}, bla_{CMY}, bla_{FOX}, bla_{GCI}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER-1}, bla_{VEB-1}, bla_{OXA}, bla_{KPC-2}, bla_{IMI-1},bla_{SME-1}, bla_{IMP}, bla_{VIM}, bla_{CcrA}, bla_{IND-1}, bla_{L1}, bla_{CAU-1}, bla_{GOB-1}, bla_{FEZ-1}, bla_{CphA}, bla_{Sfh-1}	(Bush and Jacoby 2010)
Pseudomonas spp.	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER-1}, bla_{VEB-1}	(Bush and Jacoby 2010)
Pseudomonas stutzeri	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER-1}, $bla_{VEB-1,}$ $gyr_{A,}$ $gyr_{B,}$ $par_{C,}$ par_{E}	(Bush and Jacoby 2010) (Johnning et al. 2015)
Aeromonas hydrophila	$bla_{OXA,}$ bla_{KPC-2}, bla_{IMI-1},bla_{SME-1}, $gyr_{A,}$ $gyr_{B,}$ $par_{C,}$ par_{E}	(Bush and Jacoby 2010) (Johnning et al. 2015)
Serratia spp.	bla_{AmpC}, bla_{ACT}, bla_{CMY}, bla_{FOX}, bla_{GCI}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER-1}, bla_{VEB-1}, tet_{A}, $tet_{B,}$ nsf_{A}, nfs_{B}	(Bush and Jacoby 2010) (Vila et al. 2007) (Sandegren et al. 2008)
Providencia rustigianii	bla_{AmpC}, bla_{ACT}, bla_{CMY}, bla_{FOX}, bla_{GCI}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER-1}, bla_{VEB-1}, bla_{OXA}, bla_{KPC-2}, bla_{IMI-1},bla_{SME-1}, bla_{IMP}, bla_{VIM}, bla_{CcrA}, bla_{IND-1}, bla_{L1}, bla_{CAU-1}, bla_{GOB-1}, bla_{FEZ-1}, bla_{CphA}, $bla_{Sfh-1,}$ nsf_{A}, $nfs_{B,}$ tet_{A}, $tet_{B,}$ $par_{C,}$ $par_{E,}$ $cml_{A,}$ flo_{R}	(Bush and Jacoby 2010) (Sandegren et al. 2008) (Vila et al. 2007) (Johnning et al. 2015) (Schwarz et al. 2004)
Enterobacter gergoviae	bla_{AmpC}, bla_{ACT}, bla_{CMY}, bla_{FOX}, bla_{GCI}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER-1}, bla_{VEB-1}, bla_{OXA}, bla_{KPC-2}, bla_{IMI-1},bla_{SME-1}, bla_{IMP}, bla_{VIM}, bla_{CcrA}, bla_{IND-1}, bla_{L1}, bla_{CAU-1}, bla_{GOB-1}, bla_{FEZ-1}, bla_{CphA}, $bla_{Sfh-1,}$ tet_{A}, $tet_{B,}$ $par_{C,}$ $par_{E,}$ $cml_{A,}$ flo_{R}	(Bush and Jacoby 2010) (Vila et al. 2007) (Johnning et al. 2015) (Schwarz et al. 2004)

Microorganism	Associated Antimicrobial Resistance Genes	Reference
Bacillus cereus	bla_{TEM-1}, bla_{TEM-2}, $bla_{SHV-1,}$ erm_B	(Bush and Jacoby 2010) (Gupta et al. 2003)
Bacillus pulmillus	erm_B	(Gupta et al. 2003)
Bacillus subtillis	erm_B	(Gupta et al. 2003)

Figures

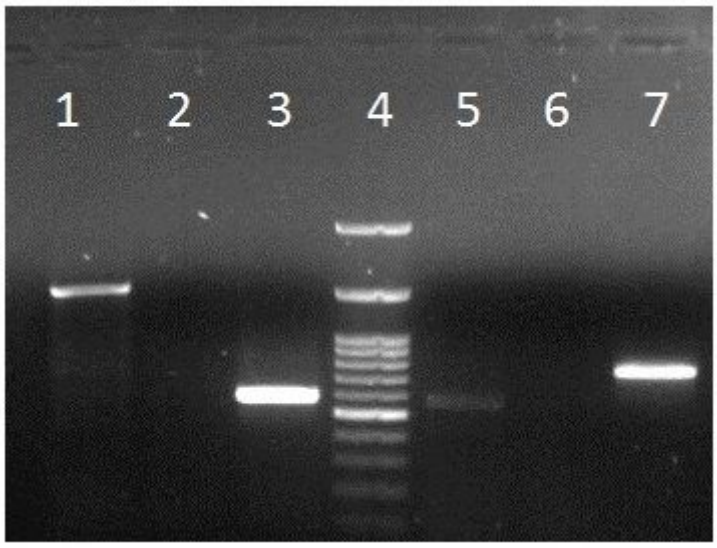

Figure 1

1: Amplicon of unexpected size generated from a strain of Pseudomonas spp. (MT CE-1-25/1); 2: Negative control; 3: Positive control (585 bp) generated from standard strain Klebsiella pneumoniae; 4: Molecular Size Marker (100 bp Ladder, Fermentas, Burlington, Canada); 5: Amplicon of unexpected size generated from a strain of Enterobacter spp. (MT BE-2-25/5); 6: Negative control; 7: Positive control (795 bp) generated from standard Klebsiella pneumoniae strain.