Microbiological analysis of Magellanic Penguins found in coastal regions of Northern Santa Catarina

Análises microbiológicas de Pinguim-de-Magalhães encontrados em regiões litorâneas do Norte catarinense

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ABSTRACT
The Magellanic penguin is the most commonly species of penguin found in temperate regions of South America. During the months of April to September, many of them are found stranded on coastal regions of Brazil, either dead or severely debilitated due to various adverse situations they face during their migratory period. Many of these debilities are due to infections caused by microorganisms present in the microbiota of these penguins, which take advantage of the immunosuppression of these birds to proliferate and generate clinical signs that can ultimately lead to their death. In the present study, several microbiological techniques were performed, including bacterial culture, antimicrobial susceptibility testing (antibiogram), fungal culture, and antifungal susceptibility testing (antifungigram) of samples that arrived from rehabilitation centers to the laboratory where the study was carried out. In the bacterial culture, the majority of the isolated bacteria belonged to the natural microbiota of the penguins, such as *Klebsiella* spp., *Pseudomonas* spp., and *Escherichia coli*. In the antibiogram, the antibiotics that showed the most resistance were cefalotin and amoxicillin, but what caught the attention in this test was the number of samples that presented multidrug resistance, being resistant to three or more classes of antimicrobials. Bacterial culture was also performed in a specific medium for the growth of Salmonella spp., but it did not yield positive results. Fungal cultures yielded growth of *Aspergillus* spp., whose antifungal susceptibility testing showed resistance to three antifungals and sensitivity to the other five antifungals tested. Further studies are necessary to understand the role of Magellanic penguins as sentinels in infections caused by environmental microorganisms. The observation of multidrug resistance to commonly used antibiotics highlights the importance of performing antimicrobial susceptibility testing for better therapeutic outcomes.

Keywords: microbial multidrug resistance, seabird, enterobactéria, *Pseudomonas* sp., *Aspergillus* sp.

RESUMO
O Pinguim-de-Magalhães é a espécie de pinguim mais encontrada em regiões de zona temperada da América do Sul. Durante os meses de abril a setembro muitos são encontrados encalhados em regiões litorâneas do Brasil, podendo estarem mortos ou bastante debilitados devido a diversas situações adversas que os mesmos sofrem durante seu período migratório. Muitas dessas debilidades são devido a infecções provocadas por microrganismos presentes na própria microbiota desses pinguins que se aproveitam da imunossupressão que essas aves se encontram para se proliferarem e gerarem sinais clínicos nesses animais que podem acabar levando-os ao óbito. No presente trabalho foi realizado diversas técnicas microbiológicas como cultura bacteriana, antibiograma, cultura fúngica e antifungigrama de amostras que chegavam de centros de reabilitações ao laboratório a qual o trabalho foi realizado. Na cultura bacteriana a grande maioria das bactérias isoladas faziam parte da própria microbiota natural dos pinguins, como a *Klebsiella* spp., *Pseudomonas* spp., e *Escherichia coli*, no antibiograma os antibióticos que mais apresentaram resistência foram a cefalotina e a amoxicilina, porém o que mais chamou a atenção nesse exame foi a quantidade de amostras que apresentaram multirresistência, sendo resistentes para três ou mais classes de antimicrobianos, foi realizada também a cultura bacteriana em meio específico para o crescimento de *Salmonella* spp., porém a mesma não obteve resultados positivos. Das culturas fúngicas realizadas obteve-se crescimento de *Aspergillus* spp., cujo antifungigrama obteve resistência para três antifúngicos e sensibilidade para os outros cinco antifúngicos testados. Estudos complementares são necessários para a compreensão do papel dos pinguins-de-Magalhães como sentinelas em infecções causadas por microrganismos ambientais. A observação de multirresistência aos antibióticos mais empregados na terapêutica ressalta a importância em se realizar antibiograma para melhores resultados terapêuticos.

Palavras-chave: multirresistência microbiana, aves marinhas, enterobactérias, *Pseudomonas* sp., *Aspergillus* sp.

1 INTRODUCTION

Penguins are considered sentinels of the oceans, as the study of these birds can provide information on how the impacts of human activities affect marine life (CAMPOS et al., 2013; DAMINELLI, 2018).

There are currently 18 species of penguins registered, with the Magellanic penguin (*Spheniscus magellanicus*) being the most commonly found in temperate regions. They are found in the Falkland Islands and in regions of Argentina and Chile that comprise the Patagonian Desert, mainly during their breeding period between September and March (VANHONI et al., 2018).

The migratory period usually begins in April and goes until mid-September. During this period, penguins leave for the ocean in search of food, traveling thousands of kilometers to the north and converging migration routes following the ocean currents to their wintering locations on the continental shelf, which are generally located in northern Argentina, Uruguay and the southernmost region of Brazil (SKEWGAR; BOERSMA; SIMEONE, 2014).

Young penguins seem to start their migratory period later than adults, usually beginning to be found stranded of Brazilian coasts, mainly in the coastal regions of the states of Rio Grande do Sul to Rio de Janeiro in mid-June, where most of the time they are dead, and when found alive, they are usually weakened due to their lack of experience in hunting for food, which causes them to get lost from the group during migration (BRANDÃO, 2013). The weakening, physical debilitation and stress generated by starvation, pollution, collision with vessels and other human actions, decrease the immunity of these young birds, leaving them predisposed to develop infections, mainly caused by microorganisms belonging to their natural microbiota or those that usually occur with asymptomatic conditions in healthy penguins (GEEVERGHESE, 2013; MINISTÉRIO DO MEIO AMBIENTE, 2010).

Although the Magellanic penguin is the most common penguin species in temperate regions, with an estimated population of 1300000 breeding pairs in 2010, the main breeding colonies in Argentina have been experiencing a population decline every year. Studies in Rio Grande do Sul have found 19500 carcasses in the coastal regions of the state in just one year, with 97% of them being young animals. These alarming data show that the population of Magellanic penguins has been drastically declining annually (VANHONI et al., 2018).

The International Union Conservation of Nature has listed the Magellanic penguin as near threatened, highlighting the need for information gathering to increase conservation and protection efforts to the species. These actions are mainly carried out by teams at marine animal rehabilitation centers located along the coastal regions from northern Brazil to Argentina. These teams rescue stranded animals from coastal areas and send them to the rehabilitation center where they are treated and may have three possible outcomes. One is that the patient does not respond to treatment and dies, the second is that the patient responds to treatment but remains unfit to release, so it is kept in captivity for environmental education and species conservation, the third, and the most desirable outcome, is that the treatment is effective for the penguin and it can be reintroduced into the ocean without further debilitation (GEEVERGHESE, 2013; VANHONI, 2018).

The aim of the study was to isolate microbiological agents from Magellanic penguin samples from rehabilitation centers in the state of Santa Catarina.

2 MATERIALS AND METHODS

During the months of May to July, the laboratory "Medivet: Centro de Diagnósticos Veterinários" located in Joinville, Santa Catarina, received nine samples with requests for bacterial culture, eight requests for *Salmonella* spp. research, and two requests for fungal culture.

The samples came from various request for exams from Magellanic penguins that were rescued on the coastal shores located between the municipalities of Itapoá and Governador Celso Ramos in the state of Santa Catarina by universities committed to the "beach monitoring project of the Santos basin (projeto de monitoramento de praias da bacia de Santos)". This project extends from the municipality of Laguna, Santa Catarina to Saquarema, Rio de Janeiro and aims to evaluate the possible impacts of oil production and flow activities on birds, turtles and marine mammals.

The examinations of rescued penguins were mainly requested by the Universidade do Vale do Itajaí (UNIVALI), which is part of section 04 of the project, corresponding to the beaches of Barra Velha, Piçarras, Penha, Navegantes, Itajaí, Balneário Camboriú, Itapema, Porto Belo, Bombinhas, Tijuca and Governador Celso Ramos, and by the Universidade da Região de Joinville (UNIVILLE), which is part of section 05 of the project, corresponding to the beaches of the municipalities of Itapoá, São Francisco do Sul, Barra do Sul and Araquari.

During the present study period, UNIVALI sent three samples requesting bacterial culture and antibiogram, corresponding to 33,33% of the total samples (3 out of 9), and five samples for *Salmonella* spp. research, corresponding to 71,43% of the total samples (5 out of 7). Regarding UNIVILLE, six samples were sent to the laboratory requesting bacterial culture and antibiogram, corresponding to 66,66% of the total samples (6 out of 9), two samples for *Salmonella* spp. research, corresponding to 28,57% of the total samples (2 out of 7), and two samples for fungal culture and antifungigram, corresponding to 100% (2 out of 2) of the total samples.

For microbiological analyses, such as bacterial and fungal culture, samples of content collected through sterile swabs were sent, mainly from the air sacs, trachea or intercostal muscles of birds placed in appropriate containers to avoid contamination that could affect the test results. Fecal samples were also sent for *Salmonella* spp. research, where samples were collected through sterile swabs of the cloacal content of the birds.

Bacterial culture was performed on Petri dishes containing Cromoclin US ® chromogenic agar from Laborclin and MacConkey agar using the streaking method with a disposable bacteriological loop. The Petri dishes were stored in a bacteriological incubator at 37ºC for 24 to 48 hours.

Samples that showed growth only on chromogenic agar were subjected to catalase, coagulase and streaking on Mannitol agar for proper differentiation of the bacterial genus. Samples that showed growth on both chromogenic agar and MacConkey agar underwent evaluation of macroscopy colony characteristics, mainly checking for lactose fermentation which gave colonies a reddish-pink color on MacConkey agar, Gram staining for microscopic analysis of the colony characteristics, oxidase test and the BacTray ® system from Laborclin.

The antibiogram was performed using the Kirby-Bauer technique on a large Petri dish containing Mueller Hinton agar, physiological solution to dilute the sample, sterile swab to spread the agent throughout the medium and antimicrobial discs which included amikacin, ciprofloxacin, gentamicin, cefalotin, penicillin, sulfazotrim, levofloxacin, amoxicillin and cephalexin. For enterobacterias isolates, a test was performed to verify the presence of extended-spectrum beta-lactamase (ESBL) enzyme by placing an amoxicillin + clavulanic acid disk in the middle of the antibiogram plate, a ceftriaxone disk to the left and a ceftazidime disk to the right, which are third-generation cephalosporins. After placing the disks, the medium was incubated in a bacteriological oven at 37ºC for 24 hours, and then the halo formed around the antibiotic disks was measured with a specific scale in millimeters. The results of the antibiogram were obtained by measuring the halos with a specific ruler with millimeters scale and classified as sensitive, intermediate or resistant, according to a specific table.

The search for *Salmonella* spp. was performed by seeding the previously enriched fecal samples using the streak plate technique on Salmonella-Shigella (SS) medium, using disposable bacteriological loops. The samples were incubated in a bacteriological oven at 37ºC for 24 to 48 hours.

Fungal cultures were performed using the laminoculture technique in the Funglab ® kit from Laborclin, which contains Sabouraud agar on one side and Micobiotic and Candida Chromogenic agar on the other. After performing the technique, the Funglab ® kit was incubated in a specific oven at 27ºC for 20 days. When filamentous fungus growth was observed on the Sabouraud agar, the samples were stained with cotton blue dye on microscope slides and viewed under an optical microscope with a 40x objective for morphological classification.

The antifungigram, like the antibiogram, was also performed using the Kirby-Bauer technique. Two small Petri dishes containing Sabouraud agar were used and four antifungal disks were placed on each of them. The antifungal agents used were amphotericin B, clotrimazole, econazole, fluconazole, itraconazole, ketoconazole, miconazole and nystatin. The plates were incubated in a specific fungus oven at 27ºC for 48 hours and the zones of inhibition were measured with a specific ruler with scales in millimeters and classified as sensitive, intermediate or resistant according to a specific table.

3 RESULTS AND DISCUSSIONS

All nine samples tested positive for bacterial culture, where a total of eleven antibiograms were performed due to two cultures that showed mixed growth. Of the eight requests for *Salmonella* spp. research, all were negative. In the fungal culture tests, only one sample showed growth, for which antifungigram was performed. The results of each analysis can be seen in the following tables:

Table 1- Results of bacterial cultures from Magellanic penguins during the period from May 16th to July 8th, 2022.

Bacterial Culture		
Animal	**Material used for analysis**	**Bacterial culture results**
1 (UNIVALI)	Tracheal content	*Pseudomonas* sp.
2 (UNIVILLE)	Intercostal muscle content	*Escherichia coli*
3 (UNIVILLE)	Tracheal content	*Klebsiella* sp.
4 (UNIVILLE)	Air sacs content	*Klebsiella* sp. e *Escherichia coli*
5 (UNIVILLE)	Mesenteric nodule content	*Proteus* sp. e *Klebsiella* sp.
6 (UNIVILLE)	Air sacs content	*Klebsiella* sp.
7 (UNIVALI)	Air sacs content	*Pseudomonas* sp.
8 (UNIVALI)	Air sacs content	*Pseudomonas* sp.
9 (UNIVILLE)	Air sacs content	*Klebsiella* sp.

Table 2 – Resistance profile of the main antibiotics tested in antibiograms performed on bacterial isolates from Magellanic penguins during the period from May 16th to July 8th, 2022.

Antibiogram				
Animal	**Bacterial Isolate**	**Amikacin**	**Ciprofloxacin**	**Gentamicin**
1 (UNIVALI)	*Pseudomonas* sp.	Sensitive	Sensitive	Sensitive
2 (UNIVILLE)	*Escherichia coli*	Sensitive	Sensitive	Sensitive
3 (UNIVILLE)	*Klebsiella* sp.	Sensitive	Resistant	Resistant
4 (UNIVILLE)	*Klebsiella* sp.	Resistant	Sensitive	Sensitive
4 (UNIVILLE)	*Escherichia coli*	Sensitive	Sensitive	Sensitive
5 (UNIVILLE)	*Proteus* sp.	Resistant	Sensitive	Sensitive
5 (UNIVILLE)	*Klebsiella* sp.	Resistant	Sensitive	Sensitive
6 (UNIVILLE)	*Klebsiella* sp.	Sensitive	Sensitive	Sensitive
7 (UNIVALI)	*Pseudomonas* sp.	Sensitive	Resistant	Sensitive
8 (UNIVALI)	*Pseudomonas* sp.	Sensitive	Sensitive	Sensitive
9 (UNIVILLE)	*Klebsiella* sp.	Sensitive	Resistant	Resistant
Animal	**Bacterial Isolate**	**Cefalotin**	**Penicillin**	**Sulfazotrim**
1 (UNIVALI)	*Pseudomonas* sp.	Resistant	Sensitive	Resistant
2 (UNIVILLE)	*Escherichia coli*	Resistant	Resistant	Resistant
3 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Resistant
4 (UNIVILLE)	*Klebsiella* sp.	Sensitive	Resistant	Sensitive
4 (UNIVILLE)	*Escherichia coli*	Resistant	Resistant	Sensitive
5 (UNIVILLE)	*Proteus* sp.	Resistant	Sensitive	Sensitive
5 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Sensitive
6 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Sensitive
7 (UNIVALI)	*Pseudomonas* sp.	Resistant	Resistant	Sensitive
8 (UNIVALI)	*Pseudomonas* sp.	Resistant	Resistant	Resistant
9 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Resistant

Table 2 – Resistance profile of the main antibiotics tested in antibiograms performed on bacterial isolates from Magellanic penguins during the period from May 16th to July 8th, 2022.

Animal	Bacterial Isolate	Levofloxacin	Amoxicillin	Cephalexin
1 (UNIVALI)	*Pseudomonas* sp.	Sensitive	Resistant	Sensitive
2 (UNIVILLE)	*Escherichia coli*	Sensitive	Resistant	Resistant
3 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Resistant
4 (UNIVILLE)	*Klebsiella* sp.	Sensitive	Sensitive	Sensitive
4 (UNIVILLE)	*Escherichia coli*	Sensitive	Resistant	Resistant
5 (UNIVILLE)	*Proteus* sp.	Resistant	Resistant	Resistant
5 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Sensitive
6 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Resistant
7 (UNIVALI)	*Pseudomonas* sp.	Sensitive	Resistant	Resistant
8 (UNIVALI)	*Pseudomonas* sp.	Sensitive	Resistant	Resistant
9 (UNIVILLE)	*Klebsiella* sp.	Resistant	Resistant	Resistant
Animal	**Bacterial Isolate**	**Extended-Spectrum Beta-Lactamase Enzymes**		
1 (UNIVALI)	*Pseudomonas* sp.	Not Performed		
2 (UNIVILLE)	*Escherichia coli*	Negative		
3 (UNIVILLE)	*Klebsiella* sp.	Not Performed		
4 (UNIVILLE)	*Klebsiella* sp.	Negative		
4 (UNIVILLE)	*Escherichia coli*	Negative		
5 (UNIVILLE)	*Proteus* sp.	Negative		
5 (UNIVILLE)	*Klebsiella* sp.	Negative		
6 (UNIVILLE)	*Klebsiella* sp.	Positive		
7 (UNIVALI)	*Pseudomonas* sp.	Not Performed		
8 (UNIVALI)	*Pseudomonas* sp.	Not Performed		
9 (UNIVILLE)	*Klebsiella* sp.	Positive		

Table 3 – Results of *Salmonella* spp. research in Magellanic penguins during the period from May 16th to July 8th, 2022.

Culture for *Salmonella* spp. research	
Animal	**Result for growth of *Salmonella* spp.**
1 (UNIVILLE)	Negative
2 (UNIVILLE)	Negative
3 (UNIVALI)	Negative
4 (UNIVALI)	Negative
5 (UNIVALI)	Negative
6 (UNIVALI)	Negative
7 (UNIVALI)	Negative

Table 4 – Results of fungal cultures from Magellanic penguins during the period from May 16th to July 8th, 2022.

Fungal Culture		
Animal	**Material used for analysis**	**Fungal culture results**
1 (UNIVILLE)	Air sacs content	*Aspergillus* sp.
2 (UNIVILLE)	Intercostal muscle content	Negative

Table 5 – Resistance profile of the antifungigram performed on a fungal isolate from Magellanic penguins between May 16th and July 8th, 2022.

Antifungigram				
Animal	**Amphotericin B**	**Clotrimazole**	**Econazole**	**Fluconazole**
	Resistant	Sensitive	Sensitive	Resistant
1 (UNIVILLE)	**Itraconazole**	**Ketoconazole**	**Miconazole**	**Nystatin**
	Sensitive	Sensitive	Sensitive	Resistant

All samples that showed bacterial growth grew on both chromogenic agar and MacConkey agar, indicating they were Gram-negative bacteria, which was confirmed by bacterioscopy. Gram-negative bacilli are the most commonly found bacteria in warm-blooded animals and can cause a range of infections from pneumonia to urinary tract infections (WOLLECK, 2022). Among the isolated Gram-negative bacteria, *Klebsiella* sp. was the most prevalent (5/9 samples), which are facultative anaerobic bacilli that reside in the gastrointestinal tract and nasopharynx of healthy animals and are considered opportunistic microorganisms. Infections occur mainly in immunosuppressed animals, such as the penguins in the present study. These bacteria are capable of forming mucoid polysaccharide capsules that aid in bacterial adhesion and provide protection against the action of bactericidal agents (FRANCISCO, 2018).

The second most prevalent bacterial agent in the bacterial cultures was *Pseudomonas* sp., growing in three out of the nine samples taken. Widmer (2016) found *Pseudomonas aeruginosa* growth in six out of nine cultures from Humboldt penguins, mainly in lesions of the oral cavity and paranasal sinuses. It is a strict aerobic Gram-negative bacillus that is oxidase positive and has polar flagella, which makes it motile. It is found in the environment, including soil and water, and also forms part of the natural microbiota of the skin and mucous membranes of most animals (WIDMER, 2016). It is considered an opportunistic pathogen and can causes local infections in a variety of birds species, mainly due to the reduction of normal microflora, immunosuppression, generalized weakness, systemic diseases or mucosal lesions. They primarily infect the oropharynx and respiratory tract, but can also cause enteritis, peritonitis, bumblefoot and sepsis.

Another enterobacteria that commonly causes weakness in penguins is *Escherichia coli*, which showed positivity in two of nine samples analyzed in this study. They are short facultative anaerobic bacilli that, although part of the enteric microbiota of most animals, including penguins, infection with it becomes of great importance due to its ability to cause sepsis and sudden death in immunosuppressed animals. This is one of the possible illness found in these penguins (OSÓRIO et al., 2017).

All bacterial agents for which the antibiogram was performed showed resistance to at least one antibiotic. The one that showed the most resistance were cefalotin and amoxicillin, both showing resistance in ten out of the eleven samples tested, followed by penicillin, which showed resistance in nine agents, and cephalexin, which showed resistance in eight agents out of the eleven tested. Cefalotin and cephalexin belong to the cephalosporin group, whose results in this present study disagree with the results presented by the study of Silva et al., (2013) that investigated the resistance profile of agents related to bumblefoot disease in Magellanic penguins, where the cephalosporin class of drugs was the one that showed the most activity against bacterial strains.

The study agrees with Silva et al., (2013) regarding the resistance of *Escherichia coli*, for which sensitivity to cephalosporin was null, similar to the findings in the present study, where both positive samples for *Escherichia coli* showed resistance to both cefalotin and cephalexin. The present study also agrees with Silva et al., (2013) regarding the penicillin class of antibiotics, which showed the highest resistance to bacterial agents at 63,3%. In the present study, the first and third antibiotics that showed the most resistance were also from the penicillin class, with the first being amoxicillin with 90,90% (10 out of the 11 samples) and the third being penicillin with 81,81% resistance (9 out of the 11 samples).

The antibiotics that showed the highest sensitivity were gentamicin, which showed resistance in only two bacterial agents out of the eleven tested, followed by amikacin and ciprofloxacin, which showed resistance in three out of the eleven bacterial agents tested for antibiotic susceptibility. Amikacin and gentamicin belong to the aminoglycoside class, while ciprofloxacin belongs to the fluoroquinolone class (WOLLECK, 2022). Wolleck (2022), who studied the genotypic and phenotypic characterization of *Escherichia coli* resistance to antimicrobials in penguins, found that aminoglycosides were the second least resistance class of antibiotics, with an average resistance of 35,76%. In the present study, both samples of *Escherichia coli* showed sensitivity to both amikacin and gentamicin, and overall, the aminoglycoside class showed the highest sensitivity, with 22,75% resistance. Regarding ciprofloxacin, Wolleck (2022) showed 78,2% resistance for *Escherichia coli* in your study, but both samples of this bacterium in the present study were sensitive to the antibiotic and ciprofloxacin was the second most sensitive antibiotic overall, demonstrating 27,27% resistance (3 out of 11 samples), tied with amikacin and behind only gentamicin, which showed 18,18% resistance (2 out of 11 samples).

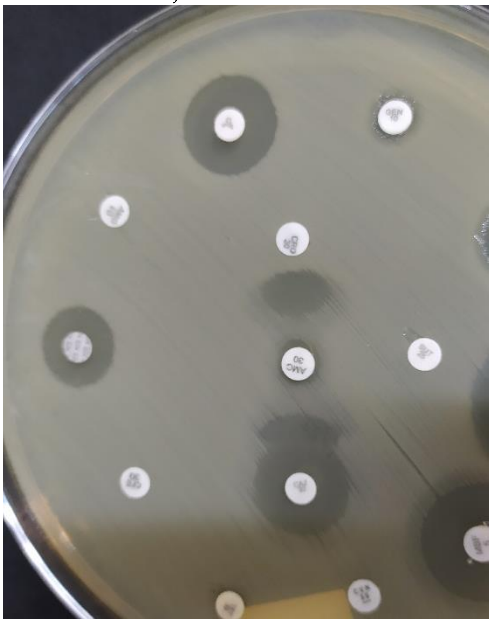
Nine out of the eleven tested bacterial agents demonstrated multidrug resistance, being resistant to three or more classes of antibiotics. Meregalli and Dewes (2019), in their study on antibiotic resistance in the microbiota of penguins, found that Magellanic penguins present more antimicrobial resistance than Antarctic penguins. Wolleck (2022), in their study on antimicrobial resistance in *Escherichia coli* isolates, found multidrug resistance in 48 out of 55 isolated samples. In the present study, one of the two *Escherichia coli* samples showed multidrug resistance, although the other also showed resistance to all tested antibiotics in the penicillin and cephalosporin classes. This multidrug resistance may be a result of human actions on the environment, such as contamination of rivers and oceans (MEREGALLI; DEWES, 2019). Anthropogenic pressure, such as coastal effluent discharges and urban surface seepage, is one of the main factors contributing to contamination of the aquatic environment, increasing the likelihood of marine life acting as vectors and reservoirs of multidrug-resistant bacteria (ELIOPULOS et al., 2022).

By using antimicrobials and developing resistance, the reservoirs of these resistance determinants remain in the host and the environment, and the movement of these genes that confer resistance occurs among the microorganisms present in the environment and the microbiota of humans and animals. Thus, it can be observed that microbial multiresistance is a complex problem involving humans, animals, and the environment (WOLLECK, 2022). Hence, the concept of One Health arises, which seeks to analyze problems from a viewpoint that prioritizes the health of humans, animals, and the environment in order to achieve the collective well-being of all individuals in the ecosystem. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) consider One Health to be an important tool for controlling microbial resistance, given that humans dump waste containing resistant bacteria into the environment, animals consume antimicrobials daily as growth promoters, and they can also act as vectors of resistant bacteria (WOLLECK, 2022).

ESBLs are a group of enzymes derived from beta-lactamases that confer resistance to bacteria against cephalosporins, penicillins, and monobactams. However, these enzymes are encoded by genes present in plasmids that can carry resistance genes to other antibiotics such as aminoglycosides, trimethoprim, sulfonamides, tetracycline, and chloramphenicol. Therefore, ESBL-producing strains are usually multidrug-resistant, but they typically remain susceptible to carbapenems, cefamycins, and the action of specific beta-lactamase inhibitors such as clavulanic acid (GIURIATTI, 2017).

ESBL production is one of the main resistance mechanisms in enterobacteria, and in the present study, two out of the seven samples tested were positive for ESBL (Figure 1). The isolates belonging to the Klebsiella genus, according to the literature, produce the greatest variety of these enzymes because they are good vectors for plasmids and allow for the evolution of genes that encode ESBL more rapidly than other enterobacteria (FRANCISCO, 2018).

Figure 1 - ESBL-positive sample showing no growth halo around the ceftriaxone disk and the appearance of phantom zones between the ceftriaxone and ceftazidime disks, as well as the amoxicillin with clavulanic acid disk.



Source: (HERRERA COSTA, 2022.)

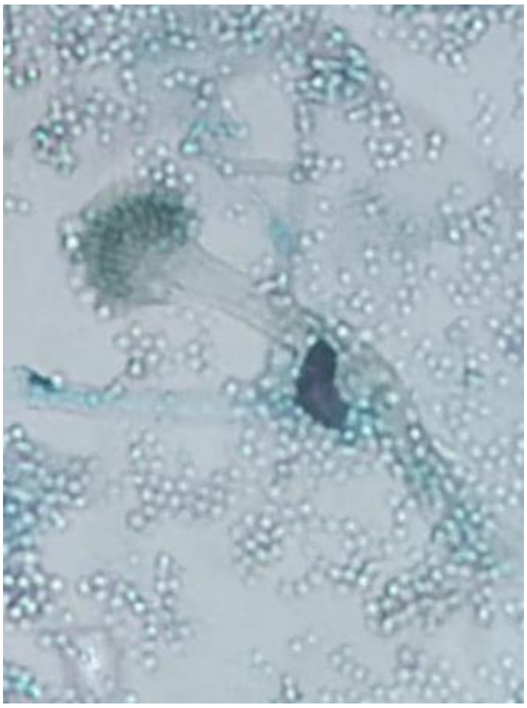
The research for *Salmonella* spp. in commercial and wild birds, such as the Magellanic penguins, is of great importance because these birds act as reservoirs, being able to eliminate the bacteria in various environments, infecting other birds and thus spreading the disease (MATOS et al., 2014). *Salmonella* spp. are known to cause severe food intoxication in various animals. In wild birds, the Salmonella genus is known to cause mortality and has a high potential for dissemination, especially in penguins, as they are free-living migratory birds that can come into contact with other animals and humans, which facilitates transmission (MATOS et al., 2014). In SS medium, *Salmonella* spp. grows with a black center due to the production of hydrogen sulfide, which can be detected due to the presence of sodium thiosulfate and ferric sulfate in the agar (ANVISA, 2004). In the present study, seven cultures were made for the research of *Salmonella* spp., but none had a positive result. Matos et al. (2014) obtained only one positive result of *Salmonella* spp. in 48 cloacal swab samples from captive Magellanic penguins. This animal may have been infected due to pollution of the seas by various types of waste and may also spread this agent to other animals, causing an imbalance in the marine ecosystem, as *Salmonella* sp. is not commonly found in this type of environment.

The samples for *Salmonella* sp. may have given negative results due to the collected material, as fecal culture does not always guarantee good sensitivity to the results. In cases of necropsies, the best samples are hepatic and biliary fragments.

Of the two fungal cultures carried out in the present study, one showed a positive result for *Aspergillus* sp. (Figure 2). Aspergillosis is an opportunistic disease, where the main species are

Aspergillus fumigatus, *Aspergillus flavus*, and *Aspergillus niger* (OLIVEIRA et al., 2013). Oliveira et al. (2013) studied the presence of fungi in 16 dead penguins in a rehabilitation center, where *Aspergillus* spp. was isolated in 11 animals, and 10 of these were classified as *Aspergillus fumigatus*. The sample in the present study was collected from the contents of the air sacs, as penguins do not have an epiglottis and diaphragm, making them easily susceptible to respiratory infections, especially immunosuppressed animals due to conditions of stress, trauma, malnutrition, vitamin A deficiency, and ingestion of oil due to contamination of the seas they face during their migratory period or rehabilitation process (OLIVEIRA et al., 2013; XAVIER et al., 2006).

Figure 2 - Presence of Aspergillus sp. in fungal culture observed under optical microscopy through a slide stained with cotton blue.



Source: (HERRERA COSTA, 2022.)

The negative result for the fungal culture lacked information about the presence of nodules in the intercostal muscles, or otherwise, why the clinician chose this location for collection. Perhaps, in isolates of collected material from other locations such as air sacs (which was the material collected in the case of the positive sample), the result could have been positive for fungal growth.

The antifungigram of the isolated sample of *Aspergillus* sp. showed resistance to amphotericin B, fluconazole, and nystatin, while demonstrating sensitivity to clotrimazole, econazole, itraconazole, ketoconazole, and miconazole, showing a higher percentage of sensitivity than nine of the eleven samples in the antibiogram.

4 CONCLUSION

The majority of bacterial agents isolated in this study are part of the natural microbiota of penguins, however, these agents can cause diseases in penguins, especially in young and immunosuppressed animals.

The observation of multidrug resistance to the most commonly used antibiotics in therapy alerts to the impact of pollution of seas and oceans on marine life and highlights the importance of performing antibiotic susceptibility testing for better therapeutic outcomes.

This study was limited by access only to laboratory analyses, with no data on the history, physical examination, and other diagnoses of the rescued animals, in addition, the short period of time in which the study was conducted limited the understanding of the role of Magellanic penguins as sentinels in infections caused by environmental microorganisms. Therefore, new studies need to be conducted encompassing the entire clinical examination of the rescued animals, to determine if these agents are indeed the causes of the infection. Additionally, longer-term studies are necessary to gather more data and obtain more precise findings for the various microbiological analyses on these animals.

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