**ABSTRACT:** Antibiotic-resistant bacteria pose a significant global health threat due to their widespread use not only in human medical settings but also in animal clinics and animal production, where they are employed for growth promotion and prophylaxis. The aquatic environment plays a crucial role in disseminating these resistant bacteria, with marine species serving as reservoirs. Seabirds, such as the brown booby, a coastal species, are effective bioindicators of environmental quality. This study aims to evaluate the resistance profiles of different antimicrobial agents in bacterial strains collected from brown booby samples treated at the stabilization unit in Rio de Janeiro.
Janeiro, Brazil, using the SIMBA platform. Samples from both live and deceased animals were collected for antibiogram testing. The most commonly identified species were *Escherichia coli* and *Pseudomonas aeruginosa*, primarily from the respiratory tract. The highest number of resistant isolates was observed in the β-lactams and fluoroquinolones classes. Medical significance was noted in the identified species and resistance genes, highlighting the impact of pollution on the environment and confirming the utility of brown boobies as bioindicators.

**KEYWORDS:** public health, bioindicator species, resistant bacteria, marine animals.

**RESUMO:** As bactérias resistentes aos antibióticos representam uma ameaça significativa para a saúde mundial devido à sua utilização generalizada, não só em ambientes médicos humanos, mas também em clínicas e na produção animal, onde são empregues para a promoção do crescimento e a profilaxia. O ambiente aquático desempenha um papel crucial na disseminação destas bactérias resistentes, com as espécies marinhas a servirem de reservatórios. As aves marinhas, como o atobá castanho, uma espécie costeira, são bioindicadores eficazes da qualidade ambiental. Este estudo tem como objetivo avaliar os perfis de resistência de diferentes agentes antimicrobianos em cepas bacterianas coletadas de amostras de atobá-marrom tratadas na unidade de estabilização no Rio de Janeiro, Brasil, utilizando a plataforma SIMBA. Amostras de animais vivos e mortos foram coletadas para testes de antibiograma. As espécies mais comumente identificadas foram *Escherichia coli* e *Pseudomonas aeruginosa*, principalmente do trato respiratório. O maior número de isolados resistentes foi observado nas classes dos β-lactâmicos e das fluoroquinolonas. A importância médica das espécies e dos genes de resistência identificados foi notada, salientando o impacto da poluição no ambiente e confirmando a utilidade dos mochos castanhos como bioindicadores.

**PALAVRAS-CHAVE:** saúde pública, espécies bioindicadoras, bactérias resistentes, animais marinhes.

**RESUMEN:** Las bacterias resistentes a los antibióticos suponen una importante amenaza para la salud mundial debido a su uso generalizado, no sólo en entornos médicos humanos, sino también en clínicas y en la producción animal, donde se utilizan para la promoción del crecimiento y la profilaxis. El medio acuático desempeña un papel crucial en la propagación de estas bacterias resistentes, y las especies marinas sirven de reservorio. Las aves marinas, como el piquero pardo, una especie costera, son eficaces bioindicadores de la calidad del medio ambiente. Este estudio pretende evaluar los perfiles de resistencia a diferentes agentes antimicrobianos en
cepas bacterianas recogidas de muestras de piqueros pardos tratados en la planta de estabilización de Río de Janeiro (Brasil), utilizando la plataforma SIMBA. Se recogieron muestras de animales vivos y muertos para realizar pruebas de antibiograma. Las especies más comúnmente identificadas fueron *Escherichia coli* y *Pseudomonas aeruginosa*, principalmente del tracto respiratorio. El mayor número de aislados resistentes se observó en las clases de β-lactámicos y fluoroquinolonas. Se señaló la importancia médica de las especies y los genes de resistencia identificados, lo que pone de relieve el impacto de la contaminación en el medio ambiente y confirma la utilidad de los búhos pardos como bioindicadores.

**PALABRAS CLAVE:** salud pública, especies bioindicadoras, bacterias resistentes, animales marinos.

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### 1. Introduction

Antimicrobial resistance (AMR) is a public health problem and has been designated as a focal point for global public health efforts in the year 2020 (WHO, 2021). AMR can cause deaths, increase healthcare costs and complicate the control of infectious diseases. Furthermore, it also harms the country’s economic activity and stability, mainly due to the substantial costs associated with treating these resilient infections (Dadgostar, 2019).

Antibiotic resistance is a natural process that enables bacterial survival in the environment and is not solely an indicator of human impact. However, the excessive and inappropriate use of antimicrobial agents in human and veterinary medicine, livestock farming, and industrial settings, along with their subsequent release into wastewater treatment plants (WWTP), has significantly contributed to the emergence and spread of resistant microorganisms in the environment. These resistant microorganisms can cause harm to humans and infections in animals (D’Costa *et al*., 2011; Bhullar *et al*., 2012; Marcelino *et al*., 2019), directly impacting the One
Health interface (Aarestrup, 2005; Queenan et al., 2016; Ewbank et al., 2022). The aquatic environment is a major conduit for the propagation of antimicrobial resistance genes (ARGs) due to effluent discharge, aquaculture, and agricultural runoff. Additionally, aquatic sediments play a crucial role in the dissemination of resistant genes through genetic transfer and recombination (Cabello, 2006; Marti et al., 2014).

The marine environment, characterized by its rich biodiversity, serves as a crucial source of oxygen, water, and biomass (Moura et al., 2012). However, it faces significant threats from anthropogenic activities, with tons of waste being dumped into the oceans daily (Barnes et al., 2009; Roman et al., 2020), along with extensive commercial activities such as fishing (Aguirre-Guzmán et al., 2012). Human impact through habitat fragmentation and pollution from sources like landfills, wastewater treatment plants, and sewers intensifies interactions among bacterial communities from wildlife, domestic animals, and humans, thereby increasing the spread of antimicrobial resistance genes (Ahlstrom et al., 2018; Marcelino et al., 2019; Sacristán et al., 2020). These activities degrade ecosystem quality, often with devastating consequences. Seabirds, which rely on the oceans for survival (Schreiber & Burger, 2001), are particularly vulnerable. Their contact with wastewater treatment effluents can lead to increased contamination with resistant bacteria, making them valuable bioindicators of antibiotic-resistant bacteria and overall environmental health (Parsons et al., 2008; Hasan et al., 2012; Atterby et al., 2016; Marcelino et al., 2019).

The brown booby (Sula leucogaster) is a species that relies on the sea for feeding. These birds exhibit coastal habits, residing on islands and coastal rocks. Studies have shown that this species interacts with debris, such as the presence of plastic in their nests (Lavers, 2013; Verlis, 2014). These wastes can harbor resistant microorganisms, making it crucial to identify bacterial species and assess their susceptibility profiles. This analysis can be considered as an indicator of both animal and environmental health. The
The present study aimed to evaluate the resistance profiles of various antimicrobial agents in bacterial strains collected from brown booby samples in a Rio de Janeiro basin (Brazil) using the SIMBA platform.

2. Methodology

All data was analyzed based on the Aquatic Biota Monitoring Information System platform – SIMBA – Petrobras. These data were collected by the Beach Monitoring Program (Programa de Monitoramento de Praias da Bacia de Santos – PMP-BS) along the Santos Basin, Rio de Janeiro, Brazil, and stored in SIMBA platform for public domain and access. The results are based on information collected from SIMBA following these steps: 1) Occurrence of brown booby in Estabilization Unity – Rio de Janeiro; 2) Laboratory exams – culture and antibiogram. Data referring to samples collected from live and dead animals were considered in this study, 38 animals were analyzed from november 2019 to april 2023. Of the 38 animals, 65 samples were collected for culture and antibiograma for analysis of resistance, the resistance profile were based on Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) and Clinical and Laboratory Standards Institute (CLSI).

3. Results and Discussions

3.1 Identification of Bacteria Isolates from Different Samples

Samples were collected from various sources, including both live and deceased animals. In total, 12 species were identified, with Escherichia coli being the only species found in all types of samples. E. coli emerged as the predominant bacteria, closely followed by Pseudomonas aeruginosa. The respiratory and digestive tracts exhibited the highest diversity of species.
Specifically, the respiratory tract featured six isolates each of *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa*. The digestive tract also showed a notable presence of *E. coli*, with five isolates (Figure 1).

Figure 1: Species of bacteria identified from several types of samples collected from brown boobies.

3.2 Antimicrobial Resistance Profile

Among the 65 samples collected, only one did not present cell growth. Of the remaining 64 samples, 63 demonstrated resistance to at least one class of antimicrobial agents. The analysis revealed bacterial isolates resistant to seven different classes of antimicrobials, with a majority showing resistance to β-lactams (61%). Notably, every species had at least one isolate resistant to β-lactams. The other two classes with the most prevalent resistance were aminoglycosides (15.2%) and fluoroquinolones (11.1%). Multidrug-resistant species were observed in 38 out of 65 samples (58.5%).
(Supplementary Table 1). The species with the highest number of resistant isolates were *E. coli*, followed by *Acinetobacter baumannii* and *P. aeruginosa* (Figure 2).

Figure 2: Antimicrobial resistance profile of bacteria found in samples collected from brown boobies.

![Antimicrobial resistance profile](chart)

Source: The Authors.

3.3 Resistance to Cephalosporin

Cephalosporin, a member of the β-lactam class, exhibits the highest resistance prevalence among antimicrobials. Resistance was identified across all four generations of cephalosporin, with the third generation displaying the highest number of resistant isolates, totaling 93 bacteria. Among all species exhibiting resistance, each demonstrated resistance to at least one generation of cephalosporin (Table 1).
Table 1: Number of resistant bacterial isolates to different generations of cephalosporin.

<table>
<thead>
<tr>
<th>Species</th>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
<th>Fourth generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>1</td>
<td>19</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>4</td>
<td>1</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>2</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>16</td>
<td>7</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5</td>
<td>21</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Source: The Authors.

Our findings show a diversity of bacterial species from different sites collected from the brown booby. We observed that the class of antimicrobial that was found to have the highest resistance profile was β-lactams. Our results revealed bacteria resistant to four different generations of cephalosporins, which could prove worrying in the future.

The most prevalent bacteria identified in the present study was *E. coli*, which were mostly found from two different tracts: respiratory and digestive. Furthermore, this species presented a greater number of resistance genes for different classes of antimicrobials. *E. coli*, classified under Enterobacteriales, is a part of the normal intestinal microbiota in animals and humans (Tenaillon *et al.*, 2010; Cardoso *et al.*, 2023). However, this species can be pathogenic, causing diarrhea and other intestinal disorders. Additionally, its abundant presence in the environment is a bioindicator of contamination and anthropogenic activity (Pesapane *et al.*, 2013; Tenaillon *et al.*, 2010).
Other studies have reported a high prevalence of *E. coli* in seabird samples from Brazil (Saviolli *et al*., 2016; Cardoso *et al*., 2023), the USA (Steele *et al*., 2005; Atterby *et al*., 2016), and Morocco (Barguigua *et al*., 2019). Gordon and Cowling (2003) demonstrated that the prevalence of *E. coli* in various vertebrates in Australia is due to the proximity of human-inhabited areas, which increases seabirds' vulnerability to *E. coli* infection. Typically, *E. coli* is less common in birds than in other animals. While these studies report a prevalence of 70% or more, our findings showed that only 26% of the samples contained *E. coli* strains. This relatively low number is likely due to the study's inclusion of samples from both living and deceased animals, in contrast to previous studies that exclusively examined samples from live animals.

In the present study, *K. pneumoniae* and *P. aeruginosa* were also identified in samples from the respiratory tract. Both species are part of the ESKAPE group, which comprises six opportunistic pathogens: *Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp. Clinically, these bacteria are considered a global threat due to their resistance to a wide range of drugs, including multidrug resistance (MDR), extensive drug resistance (XDR), and even pan-drug resistance (PDR) (Panda *et al*., 2022). Consequently, they are listed as high priority for research and development of new treatments by the World Health Organization (WHO) (Denissen *et al*., 2022).

In this analysis, the seabirds sampled in Brazil can be considered opportunistic hosts of human pathogens, capable of carrying resistance genes. Among the 12 different species found, 11 exhibited resistance to at least one class of antimicrobials, spanning a total of seven classes. The classes with the highest number of resistant bacteria were β-lactams, aminoglycosides, and fluoroquinolones. β-lactams are one of the most commonly used antibiotic classes in both human and veterinary medicine and are often employed as one of the last resort treatment against multidrug-
resistant infections in humans (Guenther et al., 2011; Sharland et al., 2018; Ewbank et al., 2022).

Although the use of tetracyclines, β-lactams, sulfonamides and quinolones as food additives for farm animals is prohibited in Brazil (Brazil, 2009) regrettably, this practice continues (Regitano & Leal, 2010). Furthermore, these medications are still used in veterinary therapy and remain prevalent in human medicine (Dougnac et al., 2015; ANVISA, 2021; Cardoso et al., 2023). Because of this, finding resistance genes to these classes in nature may not be unlikely. Cardoso et al. (2023) demonstrated the prevalence of resistance to tetracycline (22%), fluoroquinolones (15%) and β-lactamics (12%) in samples of E. coli collected from seabirds in Rio de Janeiro state and Rio Grande do Sul state, Brazil. On the other hand, another study in Rio Grande do Norte state in Brazil, demonstrated a significantly lower prevalence of genes encoding resistance to β-lactams and quinolones in brown boobies, but despite finding them in smaller numbers, the incidence of resistance genes to β-lactams was higher in migratory birds (Ewbank et al., 2021). The predominance of resistance in this study corroborates previous findings, most prevalent being resistance to β-lactam (61%), followed by aminoglycoside (15%) and fluoroquinolone (11%).

Cephalosporins are a type of semi-synthetic β-lactam antibiotic widely used in both human and veterinary medicine, and they are considered a highly important class within the β-lactam group (Ribeiro et al., 2018; Das et al., 2019). In human medicine, cephalosporins are primarily used as second- or third-line therapy. They are crucial for treating specific infections of the human reproductive system, such as gonorrhea, as well as serious infections like meningitis (Dancer, 2001; BPAC, 2011). Conversely, in veterinary medicine, cephalosporins are commonly used to treat respiratory infections and intramammary diseases in animals (EMA, 2016; WHO, 2017; Ribeiro et al., 2018).
The extensive use of cephalosporins, along with other antibiotics, may contribute to the increased prevalence and dissemination of antibiotic-resistant bacteria. The presence of cephalosporins and resistance genes in the environment has been previously documented (Ribeiro et al., 2018; Das et al., 2019).

In this study, bacterial strains resistant to four generations of cephalosporins were identified, encompassing three species (C. freundii, E. coli, and K. pneumoniae) displaying resistance to at least one antibiotic from each generation. These species, of significant medical relevance, are members of the ESKAPE group. The high prevalence of antimicrobial resistance can be influenced by an insufficient treatment of effluents in Brazil, most of the residues are thrown into the sea without a proper treatment (Dantas et al., 2021). Organic matter, human waste, hospital wastes are released in marine environments considered hotspots for the spread of antimicrobial resistance, promoting the selection of resistant strains and the spread of resistance at the bacterial level to colonize wildlife.

Our results indicate that brown boobies may harbor bacterial strains containing antimicrobial resistance genes, likely due to contamination from anthropogenic activities. These bacteria could potentially have zoonotic implications.

4. Conclusion

Brown boobies, seabirds inhabiting rocky shores, face constant exposure to anthropogenic influences. Due to anthropization and the indiscriminate use of antibiotics, these animals in close proximity to human populations may become contaminated with antibiotic-resistant bacteria. Notably, in booby samples collected at the stabilization center, approximately 97% exhibited resistance, of which 69% demonstrated multi-resistance. These findings underscore the environmental impact of pollution,
emphasizing brown boobies as bioindicator species. Monitoring the animals throughout their rehabilitation, including sample collection upon entry and exit from the center, is recommended. Further studies are necessary to assess the presence of resistance genes in the environment.

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References


