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Salmonella spp. profiles isolated from seabird samples from the Brazilian coast

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ABSTRACT

In view of growing concerns, in a One Health context, regarding the transport and dissemination of pathogenic microorganisms among seabirds and other vertebrate animals, including humans, the aim of this study was to identify *Salmonella* spp. in stranded and non-stranded resident and migratory wild seabirds from the Brazilian coast. Antimicrobial susceptibility and molecular profiles, quinolone resistance genes and antigenic characterization of the isolates were also carried out. Fresh faeces and cloacal swabs were obtained totaling 122 seabirds sampled throughout different Brazilian coast regions. At the laboratory, sample culturing, *Salmonella* spp. isolation and biochemical identification were performed, followed by antigenic profile identification by serum agglutination, susceptibility profile characterization by the agar disc diffusion technique, detection of quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*) using the multiplex polymerase chain reaction technique (multiplex PCR) and, finally, isolates profiles identification by pulsed field gel electrophoresis (PFGE). *Salmonella enterica* subsp. *enterica* was identified in 7% of the studied birds, comprising three different serovars: Panama (63 %), Typhimurium (25 %) and Newport (13 %). The most important findings reported herein are the first description of *Salmonella panama* in seabirds and the totality of isolates being resistant (or intermediate) to at least one tested antimicrobial, with emphasis on quinolone resistance. The molecular results suggest that the observed resistance cannot be explained by the presence of plasmid-mediated quinolone resistance genes. The PFGE suggests that the Panama and Newport profiles detected herein are not yet widespread in Brazil, unlike Typhimurium, which is already well distributed throughout the country. Considering this finding, we suggest that seabirds are an important link in the epidemiological chain of this serovar. The monitoring of these bacteria in seabirds, as well

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as of their susceptibility profiles to antimicrobials, must be continuous, strengthening the role of these animals as environmental health indicators and sentinels.

1. Introduction

Currently, it is impossible to dissociate the interconnections between humans, pets, farm animals, wild animals and the ecological and social environment in which they are inserted (Zinsstag et al., 2011). This close integration strengthens the demand for studies from a One Health perspective, aimed at elucidating the epidemiological chain of zoonoses and preventing the spread of pathogens between animals and humans.

In an environmental health context, human health is constantly threatened by pathogenic microorganisms present in the marine environment, presenting greater risks when ecosystem imbalances occur (Moura et al., 2008).

Salmonella spp. is a non-native marine microorganism that can reach these marine ecosystems through effluent discharges, since it survives in fecal material and water for long periods of time (Ministério da Saúde, 2011; Jajere, 2019). There are three categories of *Salmonella*, where some subspecies are highly adapted to humans, others are highly adapted to animals, and a third group (made up of most serovars) affects both humans and animals, and is, thus, termed zoonotic salmonella (Jajere, 2019). In addition to its importance in effluents, this bacterium can remain viable in bird faeces for months (Ministério da Saúde, 2011). This microorganism is the etiological agent of salmonellosis, which is one of the most widespread zoonosis worldwide, especially in low- and middle-income countries, and responsible – among others – for gastroenteritis in humans (Jajere, 2019; Antilles et al., 2021).

Specific concerns regarding the transport and spread of pathogenic microorganisms by seabirds to other vertebrate animals, including humans, have been noted (Hubálek, 2004; Halpern et al., 2008). In this context, the aim of the present study was to assess the occurrence and identify *Salmonella* spp. in stranded and non-stranded, resident and migratory, wild seabirds from the Brazilian coast. Susceptibility profiles to antimicrobials, antigenic characterization, molecular profile identification and the presence of quinolone resistance genes in the isolates were also performed.

2. Material and methods

The samples were obtained in 2015 and 2016 from the following Brazilian coastal areas: Niterói - RJ; Rio de Janeiro - RJ; Açu, São João da Barra - RJ; Paraná; Rio Grande - RS; and Marajó Island - PA (Fig. 1).

All sampling protocols were approved by the Oswaldo Cruz Institute Ethics Committee on the Use of Animals (CEUA/IOC: L-005/2016). Stranded birds comprised those found on the beaches sands weakened, dying or unable to fly or swim, while non-stranded birds consisted of apparently healthy birds, without compromised flight or swimming abilities.

2.1. Biological samples

2.1.1. Cloacal swab from stranded, resident and migratory seabirds

These samplings took place through collaborations with the Ecology and Conservation Laboratory of the Center for Marine Studies, belonging to the Federal University of Paraná (UFPR), the Center for the Recovery of Marine Animals, belonging to the Rio Grande Univeristy Foundation (CRAM/FURG), and the Wild Fauna Recovery Center, belonging to the Estácio de Sá University (CRAS/UNESA).

Cloacal swabs, were collected from living stranded migratory and resident seabirds from the beaches located within the study area (opportunistic sampling). These animals are constantly found lying and dying on the beaches by bathers, workers and research groups, rescued

Fig. 1. Sample collection points for migratory and resident seabirds, where: A = Açu, São João da Barra - RJ (non-stranded birds); B = Niterói - RJ (non-stranded birds); C = Rio de Janeiro - RJ (stranded birds); D = Paraná (stranded birds); E = Rio Grande - RS (stranded birds); F = Soure, Marajó Island - PA (stranded birds).

and sent for veterinary treatment. Swabs were obtained while still on the beach during monitoring activities by the research institutions or as soon as the birds arrived at the rehabilitation centers, before any antibiotic therapy was administered. In all cases, the swabs were kept at room temperature in Cary-Blair transport medium until dispatched to the analytical laboratory. All samples were collected in duplicate to guarantee analytical replicates (duplicate results were grouped by each animal).

2.1.2. Cloacal swab collected from non-stranded resident seabirds

Cloacal swabs were collected in duplicate following the same methodology indicated in (2.1.1), but directed at *Sterna hirundinacea* and *Thalasseus acuflavidus* nestlings from a nesting site located in Açu, São João da Barra. This collection took place in partnership with the Animal Health Laboratory team, belonging to the Center for Agricultural Sciences and Technologies at the Darcy Ribeiro North Fluminense State University (UENF) (SISBIO License - ICMBio nº 3222−5-5).

2.1.3. Samplings from fresh faeces from non-stranded resident seabirds

Fresh faeces of non-stranded and apparently healthy resident species from around Guanabara Bay, located in the municipality of Niterói, were obtained in duplicate as soon as the birds defecated. The samples were then identified according to species and stored at room temperature in a Cary-Blair transport medium until being dispatched to the analytical laboratory.

2.2. Laboratory analyses

The collected samples (swabs and faeces) were sent at room temperature Cary-Blair transport medium to the Bacterial Enteroinfections National Reference Laboratory, belonging to the Oswaldo Cruz Institute, Oswaldo Cruz Foundation (LRNEB/IOC/FIOCRUZ), where *Salmonella* culturing, isolation, biochemical identification, antimicrobial susceptibility testing, antigenic characterization and molecular identification were carried out.

The samples were inoculated in an enrichment media composed of Nutrient Broth (DIFCO) and Muller-Kauffmann Tetrathionate (OXOID), both incubated at 37 °C for 18–24 hours, and Rappaport-Vassiliadis (OXOID), incubated at 42 °C overnight. Subsequently, isolation was carried out in Hektoen Enteric Agar (OXOID) and in Methylene Blue Eosin (OXOID) at 37 °C for 18–24 hours. Suspected colonies were then transferred to the Costa & Vernin (OXOID) sorting medium, followed by biochemical tests to isolate and identify *Salmonella* spp., according to the current Brazilian Ministry of Health methodology (2011).

The antigenic profiles of all isolates were identified through the rapid slide agglutination technique using somatic (O) and flagellar (H) poly and monovalent antisera, both prepared at the LRNEB/IOC/FIOCRUZ. Specific serovar identifications were carried out based on the Kauffmann-White and Le Minor serological scheme and represented according to Grimont and Weill (2007) and Guibourdenche et al. (2010) criteria.

All identified isolates were subjected to antimicrobial susceptibility profile characterization, according to the Clinical and Laboratory Standards Institute (CLSI) agar disc diffusion methodology (CLSI, 2017), using standard breakpoints pointed out in CLSI guidelines, directed to the following antibiotics: Ampicillin (AMP), Cefoxitin (FOX), Ceftazidime (CAZ), Imipenem (IMP), Ciprofloxacin (CIP), Nalidixic Acid (NAL), Streptomycin (STR), Gentamicin (GEN), Nitrofurantoin (NIT), Chloramphenicol (CHL), Sulfamethoxazole-Trimethoprim (SXT) and Tetracycline (TCY).

After observing a high isolate resistance frequency to quinolones, noteworthy due to the fact that these are the drugs of choice in the treatment of salmonellosis in humans and animals (Dalhoff, 2012), all isolates displaying resistance to ciprofloxacin and/or nalidixic acid, even if only intermediate, were subjected to multiplex polymerase chain reaction (multiplex PCR), in order to diagnose quinolone resistance genes. *Qnr* genes are plasmid-born resistances genes, related to decreased susceptibility to quinolones in bacteria, comprising three families, *qnrA*, *qnrB*, and *qnrS* (Jacoby et al., 2008).

For molecular identification, DNA was extracted from each isolate by boiling and a multiplex PCR was performed for the diagnosis of quinolone resistance genes in according to Jacoby et al. (2008). The *rss* gene was used as the reaction control. The assessed genes, the applied primers and the product sizes are presented in Table 1.

Finally, the pulsed field gel electrophoresis (PFGE) technique was applied to track circulating clones among the detected *Salmonella* serovars, considering isolates origin. The PulseNet protocol (Centers for Disease Control and Prevention (CDC), 2017) was adopted for DNA preparation and digestion with restriction enzymes, using *Xba*I as the first enzyme, followed by *Bln*I and *Spe*I in isolates presenting dubious results, outlining the profile according to each serovar, which was then used for sample assessments. The CHEF DR III system was used for restriction fragment separation, implementing specific temperature, cycle, voltage and time conditions.

Regarding gel visualization and molecular profile analyses, gels containing the S.Braenderup H9812 molecular weight marker were stained using ethidium bromide and then washed and visualized under UV light, followed by digitalization. The obtained information was inserted in the "Bionumerics IV" database (Applied Maths), based on scripts developed by the US Centers for Disease Control (CDC). After generating a matrix using the Dice coefficient, the patterns were gathered in groups by the UPGMA method, resulting in a dendrogram, whose clone definition was based on guidelines recommended by Tenover et al. (1997); Barrett et al. (2006) and the laboratory's participation in the PULSENET/CDC/WHO program. Results were compared with the LRNEB database profiles, in order to identify previous identical profiles in human, animal and environmental sources in Brazil.

3. Results and discussion

In accordance with the three collection methodologies, samples were obtained from 122 birds, namely 51 non-stranded and resident animals and 71 stranded seabirds. Concerning the stranded animals, 50 were residents and 21 were visiting migratory species, with only one visitor from the northern hemisphere and the rest comprising of visitors from the southern hemisphere. All investigated species are listed in Table 2, alongside their migratory status, conservation status according to the International Union for the Conservation of Nature (IUCN) and their stranded status. Although the focus of this study was on seabird species, *Ardea alba*, *A. cocoi* and *Nannopterum brasilianus* were also investigated, as they frequent and feed in marine environments (Votier and Sherley, 2017). As their samples were collected in a coastal zone, the samples were grouped with the seabird samples.

The prevalence of *Salmonella* spp. was 7%. It was only identified in stranded and resident birds: *Croicocephalus maculipennis* (1), *Nannopterum brasilianus* (5) and *Sula leucogaster* (2) sampled in Rio Grande (*C. maculipennis*), Rio de Janeiro (*N. brasilianus* and *S. leucogaster*) and Niterói (*N. brasilianus*) (see Table 3 for detailed information). No positive samples were detected in the other three study regions (São João da Barra, Paraná and Marajó Island). The highest frequency was noted in Rio de Janeiro (75 %), followed by Rio Grande (13 %) and Niterói (12

Table 1
Primer sequence and product sizes for *Salmonella* spp. quinolone resistance genes.

Gene	Sequence	Amplicon	Reference
qnrA	F 5′-ATTTCTCACGCCAGGATTTG-3′ R 5′-GATCGGCAAAGGTTAGGTCA-3′	516bp	Jacoby et al. (2008)
qnrB	F 5′-GATCGTGAAAGCCAGAAAGG-3′ R 5′-ACGATGCCTGGTAGTTGTCC-3′	469bp	Jacoby et al. (2008)
qnrS	F 5′-ACGACATTCGTCAACTGCAA-3′ R 5′-TAAATTGGCACCCTGTAGGC-3′	417bp	Jacoby et al. (2008)

Table 2
General description and sample number of seabirds assessed concerning the presence of *Salmonella* spp. in different Brazilian coast regions.

Species		Migratory status	Conservation status	Stranded		Total
Scientific name	**Common name**			**Yes**	**No**	
Ardea alba	Great egret	R	LC	0	8	8
Ardea cocoi	Cocoi heron	R	LC	1	0	1
Croicocephalus maculipennis	Brown-hooded gull	R	NE	9	0	9
Fregata magnificens	Magnificent frigatebird	R	LC	1	0	1
Larus dominicanus	Kelp gull	R	LC	3	0	3
Leucophaeus atricilla	Laughing gull	VN	LC	1	0	1
Macronectes giganteus	Southern giant petrel	VS	LC	1	0	1
Nannopterum brasilianus	Neotropic cormorant	R	LC	10	18	28
Procellaria aequinoctialis	White-chinned petrel	VS	VU	3	0	3
Pterodroma incerta	Atlantic petrel	VS	EN	1	0	1
Spheniscus magellanicus	Magellanic penguin	VS	NT	14	0	14
Sterna hirundinacea	South American tern	R	LC	0	12	12
Sula leucogaster	Brown booby	R	LC	26	0	26
Thalassarche melanophris	Black-browed albatross	VS	LC	1	0	1
Thalasseus acuflavidus	Cabot's tern	R	NE	0	13	13

R = Resident; VS = Seasonal Visitor from the Southern Hemisphere; VN = Seasonal Visitor from the Northern Hemisphere (Piacentini et al., 2015). EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = least Concern; NE = Not Evaluated (International Union for Conservation of Nature and Natural Resources (IUCN), 2017).

%). *Salmonella* is commonly associated with anthropogenic contamination of the environment, according to Dimitrov et al. (2009) and corroborated by Ebert et al. (2016). The municipality of Rio de Janeiro is the 26th most populous municipality in the world (Demographia, 2017), which may explain the higher *Salmonella* spp. frequency in this location. Furthermore, the aformentioned birds are an important epidemiological link in the spread of this bacterium among humans, other wild animals and farm animals, due to the anthropogenic contamination of both coastal and marine ecosystems (Silva et al., 2010; Antilles et al., 2021).

Salmonella spp. is widespread among birds and has even been detected in seabirds from Antarctica (Dimitrov et al., 2009; Vigo et al., 2011; Cerdà-Cuéllar et al., 2019). However, as reported herein, a generally low frequency is noted (Duartea et al., 2002; Steele et al., 2005; Palmgren et al., 2006; Cížek et al., 2007; Kinzelman et al., 2008; Dolejská et al., 2009; Ramos et al., 2010; Vigo et al., 2011; Amin et al., 2013; Fresno et al., 2013; Dougnac et al., 2015; Dolejska et al., 2016; Ebert et al., 2016; Masarikova et al., 2016; Tardone et al., 2020; Russo et al., 2021), except for the study of Antilles et al. (2021), which described a prevalence of 20 % in European gulls.

All isolates identified in the present study comprised *Salmonella enterica*, which is normally transmitted to humans through contaminated food items (Hendriksen et al., 2011), in addition to person-to-person transmission and close contact with animals (Newell et al., 2010). Over 1500 *Salmonella enterica* subsp. *enterica* serovars have been identified to date (Issenhuth-Jeanjean et al., 2014), and three, namely Panama (63 %), Typhimurium (25 %), and Newport (13 %), were identified herein. These serovars belong to the zoonotic salmonella category, which affect both humans and animals, causing gastroenteritis (Jajere, 2019).

Table 3
Description of each *Salmonella* isolate, considering the serovars, species of origin, sample site and resistance profile.

Serovar isolate	Seabird species	Sample site	Resistance profile
S. typhimurium	*N. brasilianus*	Niterói	STR
S. typhimurium	*C. maculipennis*	Rio Grande	CIP; STR (INT*)
S. panama	*S. leucogaster*	Rio de Janeiro	NAL
S. panama	*S. leucogaster*	Rio de Janeiro	CIP; NAL
S. panama	*N. brasilianus*	Rio de Janeiro	NAL
S. panama	*N. brasilianus*	Rio de Janeiro	NAL [a]
S. panama	*N. brasilianus*	Rio de Janeiro	NAL; IMP [a]
S. panama	*N. brasilianus*	Rio de Janeiro	NAL [b]
S. panama	*N. brasilianus*	Rio de Janeiro	CIP; NAL [b]
S. newport	*N. brasilianus*	Rio de Janeiro	CIP (INT*)

* Intermediate resistance.
[a] Duplicated sample from the same animal.
[b] Duplicated sample from the same animal.

Despite being one of the most frequent serovars, and the ninth most prevalent in Latin America (Hendriksen et al., 2011), this is the first report to describe *Salmonella panama* in seabirds, specifically in brown boobies and cormorants. The other two serovars are more commonly reported in seabirds (Duartea et al., 2002; Steele et al., 2005; Palmgren et al., 2006; Cížek et al., 2007; Dolejská et al., 2009; Ramos et al., 2010; Amin et al., 2013; Dolejska et al., 2016; Masarikova et al., 2016; Migura-Garcia et al., 2017; Uhart et al., 2020; Antilles et al., 2021), even from Antarctica (Dimitrov et al., 2009; Vigo et al., 2011).

Some serovars are ubiquitous and found in high amounts in host species (Palmgren et al., 2006). *Salmonella typhimurium*, the second most frequent detected herein in cormorants, and gulls, is the most common serovar detected in birds (Palmgren et al., 2006). Typhimurium is also one of the most common serovars associated with human salmonellosis (Masarikova et al., 2016; Antilles et al., 2021). Hendriksen et al. (2011) describe this serovar as the second most prevalent worldwide in human, animal, environmental and food samples, and indicate an increase in the occurrence of this serovar in Latin America.

Newport is described, together with the previously discussed Typhimurium, as one of the most important non-typhoidal *Salmonella* serovars worldwide, in terms of human salmonellosis burden (Jajere, 2019). Latin America and North America display the highest prevalence of *Salmonella newport*, which is the sixth most predominant serovar in these regions (Hendriksen et al., 2011). In the present study, however, it was identified only in one cormorant.

Hendriksen et al. (2011) draw attention to the importance of determining serovars in non-human sources in order to identify possible reservoirs, with the aim of developing prevention and control measures. In this context, *Salmonella* spp. was identified herein only in resident birds, with a prevalence of 11 % among *Croicocephalus maculipennis* specimens, 18 % in *Nannopterum brasilianus*, and 8% in *Sula leucogaster*. This may be due to the fact that the infection process is short in birds (Palmgren et al., 2006). Therefore, it is possible that the animals could have eliminated the bacteria prior to arriving at the sample collection site.

Seabirds are generally asymptomatic *Salmonella* spp. carriers (Palmgren et al., 2006). The bacterium was only identified in stranded birds, as reported by Tardone et al. (2020), but this alone does not allow for the conclusion that salmonellosis was the stranding cause. However, this is an important indicator for the rehabilitation centers that receive these animals to be better prepared for prophylactic measures and for the protection of their employees and other captive animals, since there

is a significant possibility that these birds excrete pathogenic bacteria (Steele et al., 2005). Enteropathogens are considered the most important microorganisms in rehabilitation centers from a zoonotic point of view, as they are resistant when in the environment and transmitted via the fecal-oral route. It is, therefore, extremely important that the technical teams linked to these locations be made aware of hygiene, ventilation and personal protective equipment measures (Siembieda et al., 2011).

3.1. Antimicrobial susceptibility profile characterization

The resistance profile of each isolate from the present study is presented in Table 3. None of the detected isolates was multidrug-resistant and no resistance to AMP, FOX, CAZ, GEN, NIT, CHL, SXT and TCY was observed. However, all isolates detected herein presented some type of resistance to antimicrobials (even if intermediate), mainly to nalidixic acid and ciprofloxacin.

All *S. typhimurium* were resistant to STR, while all *S. panama* were resistant to quinolones, especially NAL. Isolates from Rio de Janeiro presented a high quinolone resistance frequency, mainly to NAL. *N. brasilianus* presented the widest distribution of serovars. These animals were sampled in Rio de Janeiro and Niterói, thus, they inhabit the Guanabara Bay. This environment receives effluents from 16 municipalities in the state of Rio de Janeiro, with an estimated discharge of $18m^3$ per second of untreated sewage (Fistarol et al., 2015). The three seabird species were infected with isolates presenting quinolone resistance.

The frequency of antimicrobial resistance (AMR) in isolates from seabirds around the world is usually low (Duartea et al., 2002; Palmgren et al., 2006; Cížek et al., 2007; Dolejská et al., 2009; Vigo et al., 2011; Dolejska et al., 2016; Masarikova et al., 2016; Tardone et al., 2020; Russo et al., 2021) or not detected (Cerdà-Cuéllar et al., 2019), except for the study of Antilles et al. (2021), which described 50 % of AMR in the isolates from gulls.

Different *Salmonella* spp. resistance patterns in other countries throughout South America, Australia and Europe have been reported, with low resistance frequencies to quinolones (Duartea et al., 2002; Palmgren et al., 2006; Fresno et al., 2013; Dougnac et al., 2015; Retamal et al., 2015; Dolejska et al., 2016; Masarikova et al., 2016; Migura-Garcia et al., 2017; Tardone et al., 2020; Russo et al., 2021), with the exception of Dolejská et al. (2009) and Antilles et al. (2021), who reported high resistance frequency concerning fluoroquinolones. In this regard, Cížek et al. (2007) had already noted an increased resistance to nalidixic acid in *Salmonella* isolates isolated from seagulls in the Czech Republic, while increased resistance to fluoroquinolones has been reported since the 1980s (Ministério da Saúde, 2011).

Most antibiotics that the detected isolates were resistant to in the present study are classified as critically important antimicrobials for human medicine by WHO (World Health Organitzation) (2019), in a document recommending the prudent use of these substances in both veterinary and human medicine. Antibiotic resistance in *Salmonella* spp. is generally related to the irresponsible and imprudent use of these substances in animal production (Crump et al., 2011; Jajere, 2019). In Brazil, fluoroquinolones are intensively and preventatively used in farm animals (Regitano and Leal, 2010), even though their use as an additive (growth promotor) in animal feed is legally prohibited (Brasil, 2009). Fluoroquinolones are also one of the therapeutic groups most applied in farm animals (Regitano and Leal, 2010). Quinolones (especially fluoroquinolones) are the most applied drugs for specific salmonellosis treatment, both in veterinary and human medicine (Dalhoff, 2012). This may explain the important resistance to this class of antibiotics described herein.

Free-living wild animals are rarely treated with antibiotics (Hasan et al., 2014). Thus, a high frequency of antimicrobial resistance in strains isolated from seabirds suggests an anthropogenic source of contamination, due to the high use of antibiotics in humans and farm animals, which are discharged alongside effluents into the environment (Duartea et al., 2002; Tardone et al., 2020; Antilles et al., 2021). In this context, seabirds have been pointed out as adequate environmental antibiotic resistance indicators (Russo et al., 2021).

The high frequencies of antimicrobial resistance detected herein may be related to inefficient effluent treatment in Brazil, especially in Rio de Janeiro. This type of waste is, sometimes, discharged directly into oceanic environments (IBGE (Instituto Brasileiro de Geografia e Estatística), 2010), which are considered antimicrobial resistance transmission hotspots, facilitating resistant strain selection and horizontal resistance transmission among bacteria that, in turn, colonize wild animals (Piotrowska and Popowska, 2015; Masarikova et al., 2016).

Imipenem, which belongs to the carbapenem class, is considered a state-of-the-art drug to treat infections caused by multi-resistant gram-negative bacteria (Dolejska et al., 2016). In the present study, one investigated seabird, a *N. brasilianus* collected from the populous city of Rio de Janeiro, as discussed previously, was shown to carry an imipenem-resistant isolate (Panama, which has never been isolated from seabirds before). So far, resistance to imipenem in strains isolated from the sea and water birds has only been documented in two studies, one indicating intermediate resistance (Dolejska et al., 2016; Giacopello et al., 2016). This finding signals the importance of monitoring imipenem resistance in Brazil and reinforces the relevance of monitoring drug use in farm animals and in human health, while also indicating the need for improved effluent treatment systems.

3.2. Molecular characterization

No *qnr* genes were identified in the isolates, which means that quinolone resistance detected in the present study cannot be explained by the presence of plasmid-mediated quinolone resistance genes. Therefore, future studies are required to identify possible mutations in regions that determine resistance to quinolones in specific genes (*gyrA, gyrB, parC* and *parE*).

A similar result was reported for the Czech Republic, where *qnr* genes were not identified in *Salmonella* spp. isolated from seagulls (Masarikova et al., 2016).

The LRNEB is a reference laboratory for bacterial enteroinfections in Brazil. Thus, clinical, animal and environmental samples from the entire country are received and processed in our facilities. We maintain a database containing all the profiles detected from theses samples by PFGE, so we could compare them with the isolate profiles detected herein.

The molecular profiles of Panama and Typhimurium isolates analyzed by PFGE are presented in Fig. 2. The *S. panama* isolate profiles are 100 % similar to each other, as well as *S. typhimurium*. *S. newport* is not presented in Fig. 2 because only a single isolate was detected herein, impairing comparisons.

The *S. panama* isolates were detected in 2016 in stranded cormorants (*Nannopterum brasilianus*) and brown boobies (*Sula leucogaster*) from the municipality of Rio de Janeiro, exhibiting 100 % similarity, *i.e.,* with the same clonal origin. According to the LRNEB database, this profile had not yet been identified in animal, environmental, food or human samples. Therefore, it is possible that this is a profile that is not yet widely disseminated in Brazil and it is also possible that these animals came into contact with the same source of contamination. The single Newport isolate detected herein is not similar to any isolate available in the LRNEB database, which may also indicate that this profile is not yet widespread throughout Brazil. Therefore, we suggest that these Panama and Newport profiles have recently been introduced in the region and, have not, therefore, yet been detected in other animal, human or environmental sources, as reported by Vigo et al. (2011) for strains isolated from Antartica seabirds.

The *S. typhimurium* isolates detected in a stranded cormorant (*Nannopterum brasilianus*) from Rio de Janeiro and in a stranded gull (*Croicocephalus maculipennis*) from Rio Grande, in the state of Rio Grande do Sul also displayed 100 % similarity to each other. These samples were

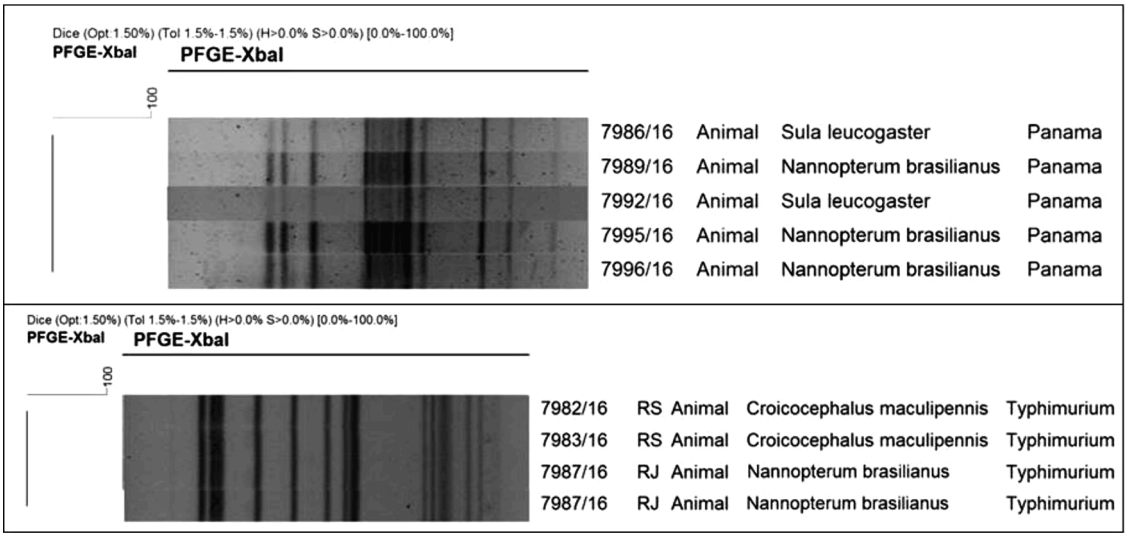

Fig. 2. Panama and Typhimurium profile dendrograms identified in seabird samples in the present study.

collected in different and distant states but exhibit the same clonal origin. According to the LRNEB database, this profile is already well disseminated in Brazil's South and Southeast regions, in animal, human and environmental origin samples, unlike the other two aforementioned serovars.

The fact that the identified Typhimurium profile is widespread suggests possible anthropogenic contamination and interspecific transmission, which was also discussed by Retamal et al. (2015) and Cerdà-Cuéllar (2019), with regard to another serovar (Enteritidis). This highlights the close relationship between human, animal and environmental health in a One Health context (Zinsstag et al., 2011; Tardone et al., 2020; Antilles et al., 2021), and also underlines the potential of using seabirds as environmental health sentinels or indicators.

4. Conclusions

Salmonella spp. was only detected in resident seabirds. Environments persistently contaminated by bird droppings can transform animals that frequent these places into asymptomatic salmonellosis carriers. Therefore, these birds may become a source of constant *Salmonella* spp. contamination to humans.

This study also reports the first detection of *S. panama* in seabirds, alongside high antimicrobial-resistance frequencies, with 100 % of resistant or intermediary resistant isolates, including to quinolones, and imipenem resistance.

Future studies should search for mutations in regions that determine quinolone resistance in specific genes (*gyrA, gyrB, parC* and *parE*), since the quinolone resistance genes investigated herein were not identified. We also suggest continuous seabird bacteria and antimicrobial susceptibility profile monitoring, to further establish these animals as environmental health indicators.

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Declaration of Competing Interest

No conflict of interest declared.

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