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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Clostridioides difficile and multi-drug-resistant staphylococci in free-living rodents and marsupials in parks of Belo Horizonte, Brazil

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Abstract

The global emergence of antimicrobial resistance (AMR) has become a serious threat to human and animal health. Recent studies have shown that synanthropic animals can act as reservoirs and disseminators of pathogens and resistant bacteria. The aim of this study was to evaluate the frequency, distribution, and antimicrobial susceptibility of staphylococcal species and *Clostridioides difficile* isolated from the feces of free-living rodents and marsupials from two urban parks in Belo Horizonte, Brazil. During a 12-month period, fecal samples from 159 free-living animals, including 136 rodents and 23 marsupials, were collected from two urban parks in Belo Horizonte, Minas Gerais, Brazil. *Staphylococcus* spp. were more likely to be isolated from rodents than marsupials ($p = 0.0164$). Eight different staphylococcal species were isolated from 36 (26.5%) rodents and one marsupial (4.3%). *S. saprophyticus* (48.6%) was the most frequently isolated species, and almost a quarter of the isolates (24.3%) were resistant to at least one antimicrobial agent, four (10.8%) of which were multi-drug resistant (MDR). Two (5.4%) strains were resistant to cefoxitin and were then classified as methicillin-resistant staphylococci, and one also tested positive for the *mecA* gene. *C. difficile* was isolated from two rodents (1.5%), and one strain was toxigenic and classified as ribotype 064. One isolate was resistant to rifampicin, but both strains were susceptible to all other antimicrobials tested, including metronidazole and vancomycin. All *C. difficile* isolates and all staphylococcal strains resistant to antimicrobials were recovered from the same park. The present study suggests that free-living rodents in Belo Horizonte (Brazil) are mainly colonized by *S. saprophyticus* and may act as reservoirs of antimicrobial-resistant *Staphylococcus* spp. and *C. difficile* strains. This is the first study to evaluate the presence of staphylococci and *C. difficile* from free-living opossums and suggest a low fecal shedding of these organisms by these mammals.

Keywords Urban parks · Wild small mammals · Methicillin resistance · Antimicrobial resistance · *S. saprophyticus*

Introduction

The global emergence of antimicrobial resistance (AMR) has become a serious threat to human and animal health due to the widespread use of antimicrobials. However, there has been a significant increase in reports of AMR in bacteria isolated from environments and animals that have not been exposed to direct selective pressure from these agents [1]. Horizontal gene transfer between bacteria has been identified as an explanation for this phenomenon [2] and, in this context, staphylococci are highlighted as excellent carriers and transferors of resistance genes [3, 4] causing a wide variety of diseases in humans and animals [5–7].

Over the years, staphylococci and AMR have been extensively investigated in humans and domestic animals, but there are limited studies on wild and pest species, particularly in developing countries [8, 9]. It is known that some pest species can act as reservoirs for antimicrobial-resistant bacteria, transmitting and disseminating these microorganisms by different routes, including feces and urine [10].

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Clostridioides (prev. *Clostridium*) *difficile* is an emergent pathogen responsible for antimicrobial-associated diarrhea in humans. In the last decade, animals and the environment have been suggested as possible reservoirs of *C. difficile* strains [11]. Recent studies in Canada and European countries have shown that rats and mice are sources of *C. difficile* in both urban and farm environments [12–15]. The role of other peridomestic rodents, including *Cerradomys* and *Necromys* spp., as reservoirs of *C. difficile* strains, has not been addressed.

Given the notable lack of data on the carriage and antimicrobial resistance profile of potentially pathogenic staphylococci and *C. difficile* in wild animals living in urban areas in Brazil, this study evaluated the frequency, distribution, and antimicrobial susceptibility patterns of staphylococcal species and *C. difficile* isolated from the feces of free-living rodents and marsupials from two urban parks in Belo Horizonte, Brazil.

Material and methods

Capture sites

The capture was conducted in two urban parks in Belo Horizonte (Minas Gerais, Brazil). Park 1 was "Jacques Cousteau Municipal Park" (19°58′ S and 43°59′ W), with a total area of 335 thousand square meters. Park 2, "Mangabeiras Municipal Park" (19°56′ S and 43°54′ W), has a total area of 2.4 million square meters (Fig. 1). Previous studies on *C. difficile*, *Vaccinia virus*, and different ectoparasites and endoparasites in free-living South American coatis (*Nasua nasua*) have been performed in park 2 [16–19]. In park 1, currently only one study on the abundance and diversity of amphibians has been conducted [20].

These parks serve as recreation centers and leisure areas for the population and usually receive a large number of visitors every day throughout the year. Park 1 is located completely inside the city. The space functioned as a landfill for Belo Horizonte for 20 years (1951–1971), and then transformed into a park and horticultural garden for the production of tree and plant seedlings used for city landscaping. The site has springs and perennial watercourses that are impacted by sewage effluent from the city [21]. Park 2 is considered one of the largest urban parks in Brazil with approximately fifteen thousand visitors per month. It is located in an urban area in contact with some of the city's neighborhoods, but it is surrounded by native vegetation and other environmental preservation areas. It has water springs around and throughout, but the courses are not impacted by wastewater [22].

Animals sampled

A total of 159 free-living animals, including 136 rodents and 23 marsupials, were sampled between April 2018 and March 2019 (Table 1). For the capture, two transects were established for park 1 and three transects for park 2. Each transect had fifteen collection stations 20 m apart from each other. The stations contained a Sherman trap for capturing small rodents and marsupials, and a cage trap with suspended bait for capturing larger animals, totaling thirty traps on each transect. Each transect was surveyed once per day. The bait used was a mixture of sardines, peanuts, bananas, and corn bran. The traps were baited at the time of the survey, in the morning, between 08:00 and 09:00, on each collection day. The collections occurred for 5 consecutive days, every 2 months, totaling six campaigns in each park over a period of 1 year.

After capture, the animals were weighed. They were then anesthetized with a combination of 2% xylazine (rodents, 10 mg/kg, IP; marsupials, 5 mg/kg, IM) and ketamine hydrochloride (rodents, 100 mg/kg, IP; marsupials, 25 mg/kg, IM). After sedation, fecal samples were collected directly from the rectal ampoule of the marsupials. These animals were marked with ear tags to prevent multiple samples from the same animal, and released at the same capture site. The rodents were euthanized with an overdose of propofol (10 mg/kg) via the intracardiac route, and intestinal contents were collected from the rectum (feces) during necropsy.

The fecal samples were placed in a sterile microtube, stored in a transport box with ice packs, and transported to the Bacteriosis and Research Laboratory of the Veterinary School of the Federal University of Minas Gerais (UFMG), where they were stored at − 80 °C until laboratory processing. This study was approved by the Ethical Committee on Animal Use (CEUA) of UFMG under protocol 306/2017 and by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under protocol 12,989–2.

Staphylococci isolation and antimicrobial susceptibility

For *Staphylococcus* spp. isolation, fecal samples were suspended in 0.85% saline solution and 100 µL was streaked onto mannitol salt agar (MSA; Difco Laboratories Inc., USA) that was then incubated at 37 °C for 24 h [23]. Colonies were subcultured on brain heart infusion agar (BHI, Difco Laboratories Inc., USA) and identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Germany). The cutoff log score of 2 was used to validate

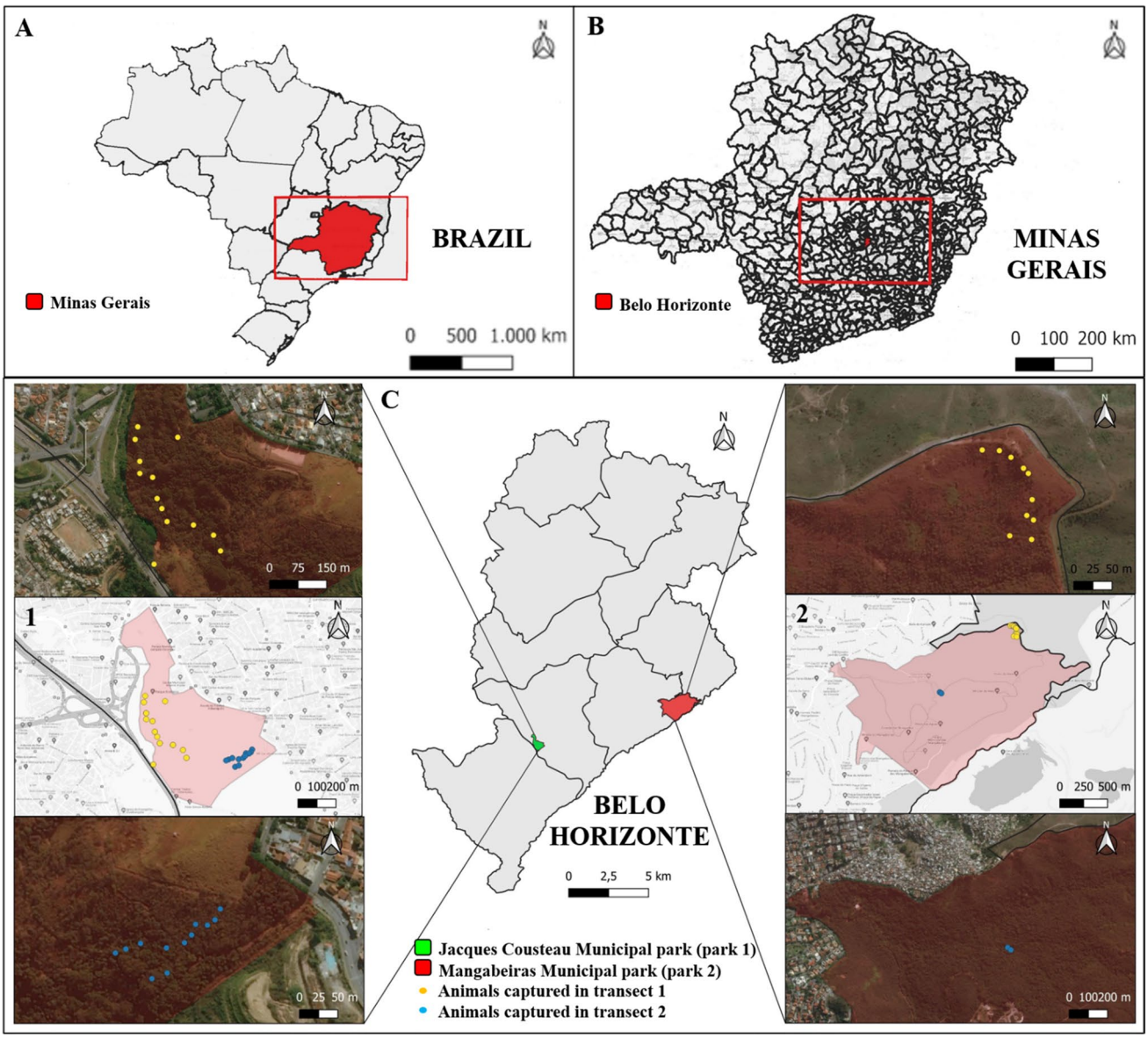

Fig. 1 Mapping of parks 1 and 2 located in the city of Belo Horizonte, Minas Gerais, Brazil. (A) Map of Brazil with the location of the Minas Gerais state. (B) Map of Minas Gerais state with the location of Belo Horizonte. (C1) Jacques Cousteau Municipal Park (park 1). (C2) Mangabeiras Municipal Park (park 2). Yellow and blue dots represent the locations where animals were captured in transects 1 and 2, respectively.
Source: http://bhmap.pbh.gov.br/v2/home.html

identification at the species level, as recommended by the manufacturer. The strains were then subjected to DNA extraction [24] and methicillin-resistant staphylococci were investigated by detection of the *mecA* gene [25]. Antimicrobial susceptibility tests were performed using disk diffusion in agar, according to the Clinical and Laboratory Standards Institute (CLSI) documents M100-S30 [26] and VET08 [27]. The following antimicrobials were tested: cefoxitin (30 µg), penicillin (10 units), tetracycline (30 µg), trimethoprim/sulfamethoxazole (25 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), and ciprofloxacin (5 µg) (DME, BRA). *Staphylococcus aureus* ATCC 25,923 was used as a control. Isolates were considered multidrug-resistant (MDR) when resistant to three or more classes of antimicrobial agents [28].

Clostridioides difficile isolation and antimicrobial susceptibility

For *C. difficile* isolation, fecal samples were incubated in 96% ethanol for 30 min (1:1) and aliquots of 10 µL were plated on

Table 1 Frequency and distribution of rodents and marsupials captured in the Jacques Cousteau Municipal Park (park 1) and Mangabeiras Municipal Park (park 2) in Belo Horizonte, Minas Gerais, Brazil

Animals	Common name (Specie)			Total	
		[2]Park 1	[3]Park 2	Sum	Group
Rodents	Genus *Cerradomys*	29	10	39	136 (85.5%)
	New world mice *(Necromys lasiurus)*	34	4	38	
	Black rat (*Rattus rattus*)	37	0	37	
	[1]Order Rodentia	18	4	22	
Marsupials	White-eared opossum (*Didelphis albiventris*)	3	9	12	23 (14.5%)
	Black-eared opossum (*Didelphis aurita*)	0	6	6	
	Gray-slender opossum (*Marmosops incanus*)	0	5	5	
Total		121 (76.1%)	38 (23.9%)	159 (100%)	159 (100%)

[1]Some rodents were not classified by genus and species and were categorized as members of the order Rodentia

[2]Park 1 (19°58′S and 43°59′W)

[3]Park 2 (19°56′S and 43°54′W)

cycloserine-cefoxitin fructose agar (CCFA) supplemented with 7% horse blood and 0.1% sodium taurocholate (Sigma, USA) [29]. After incubation in an anaerobic atmosphere at 37 °C for 96 h, *C. difficile* colonies (flat, irregular, and with ground-glass appearance) were subjected to a multiplex PCR to identify the housekeeping gene (*tpi*) and the virulence genes of toxin A (*tcdA*), toxin B (*tcdB*), and binary toxin (*cdtB*) [30]. Toxigenic *C. difficile* isolates were also subjected to PCR ribotyping, as previously described [31]. The minimal inhibitory concentrations (MIC) of metronidazole, vancomycin, clindamycin, moxifloxacin, ciprofloxacin, erythromycin, rifampicin, and tetracycline were determined using Etest strips (bioMerieux, Marcy I'Etoile, France) in *Brucella* agar (Oxoid, USA) with 5% lysed blood, supplemented with hemin (Difco Laboratories, USA) and vitamin K (Sigma-Aldrich Co., USA). The MIC values were interpreted according to the clinical breakpoints of the CLSI and EUCAST guidelines [32–34].

Statistical analysis

The association between phenotypic resistance and *Staphylococcus* species was evaluated using the chi-square or Fisher's exact tests. The chi-square test for adherence was used to evaluate the distribution of variables. All statistical analyses were performed using GraphPad Prism v.8 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $p < 0.05$.

Results

Marsupial and rodent species captured

A total of 159 free-living animals, including 136 rodents and 23 marsupials, were sampled over a 1-year period (Table 1). The number of animals sampled in park 1 (76.1%) was approximately three times higher than that in park 2 (23.9%) ($p < 0.001$). In park 1, rodents were captured more frequently (97.5%) than marsupials (2.5%), with black rats (*Rattus rattus*) the most commonly collected (31.4%) (Table 1). Among marsupials, only the white-eared opossum (*Didelphis albiventris*) was trapped. In park 2, the capture frequency was 52.6% for marsupials and 47.4% for rodents. The main representatives of marsupials and rodents in this park were white-eared opossum (*Didelphis albiventris*) (45%) and the genus *Cerradomys* (55.5%), respectively (Table 1). For marsupials (Table 1), the frequency of capture of these animals was higher (52.6%) and more species diversity was observed in park 2 than in park 1 (2.5%) ($p < 0.001$).

Staphylococcal isolation and identification

Overall, staphylococci were isolated from 36 out of 136 (26.5%) tested rodents, with one animal presenting two isolates with different *Staphylococcus* species (Table 1S). Among the 37 staphylococci isolates, 35 (94.5%) were from park 1, and only two were from park 2. There was no statistically significant difference in staphylococcal carriage by rodent species (Table 2). There was also no difference in the frequency of isolation in different seasonal periods (rain and drought) (Table 1S). Only one isolate (4.3%) was recovered from marsupials, specifically from a black-eared opossum (*Didelphis aurita*) captured in park 2. Staphylococci were more frequently isolated from rodents than from marsupials ($p = 0.0164$).

Eight different staphylococcal species were detected in rodents, with *S. saprophyticus* (48.6%) being isolated significantly more frequently than the other species ($p < 0.001$) (Table 2). *S. aureus* was isolated from three animals (8.1%). For marsupials, the only strain isolated was identified

Table 2 Frequency and distribution of staphylococcal species isolated from free-living rodents ($n=136$) from two urban parks in Belo Horizonte, Minas Gerais, Brazil

Isolate	*Cerradomys* sp. ($n=39$)	*Necromys lasiurus* ($n=38$)	*Rattus rattus* ($n=37$)	Rodentia[1] ($n=22$)	Total of isolates
S. saprophyticus	6	4	6	2	18 (48.6%)[a]
S. xylosus	0	6	0	0	6 (16.2%)[b]
S. aureus	2	0	1	0	3 (8.1%)[b]
S. sciuri	1	1	1	0	3 (8.1%)[b]
S. epidermidis	0	1	1	0	2 (5.4%)[b]
S. succinus	0	0	1	0	1 (2.7%)[b]
S. warneri	0	1	0	0	1 (2.7%)[b]
Staphylococcus sp.[2]	0	1	2	0	3 (8.1%)[b]
Total of isolates/rodent	9 (23.8%)[a]	14 (36.8%)[ab]	12 (32.4%)[ab]	2 (9.1%)[b]	37 (100%)

*Multiple comparison: different letters indicate significant differences

[1]Some rodents were not classified by genus and species and were categorized as members of the order Rodentia

[2] Only genus classification was considered for strains with scores ≤ 2 in MALDI-TOF, as recommended by the manufacturer

as *S. saprophyticus*, which was susceptible to all tested antimicrobials.

Staphylococcus spp. antimicrobial susceptibility

Nine (6.6%) rodents harbored antimicrobial-resistant staphylococci, all from park 1, and of the 37 isolates, nine (24.3%) were resistant to at least one antimicrobial agent. Four (10.8%) were classified as MDR, two (5.4%) of which were resistant to cefoxitin, and were classified as methicillin-resistant staphylococci. One of these isolates was positive for *mecA* (Table 3). Penicillin G had the highest frequency of resistance (24.3%), followed by erythromycin (8.1%), cefoxitin (5.4%), and clindamycin (5.4%). Resistance to penicillin G (cefoxitin/clindamycin: $p=0.04$; others: $p=0.002$) was significantly higher than that of the other tested antimicrobial agents, except for erythromycin ($p=0.11$). However, no significant differences were found in resistance to erythromycin and other antimicrobials. All isolates were susceptible to chloramphenicol, ciprofloxacin, gentamicin, tetracycline, and trimethoprim/sulfamethoxazole. All rodent species in the present study showed at least one antimicrobial resistant isolate, and no statistical difference was found between these species ($p=0.15$). In addition, there was no difference in the frequency of resistant isolates among transects in each park ($p=0.24$) or between the parks ($p=0.6$).

Clostridioides difficile isolation and antimicrobial susceptibility

C. difficile was isolated from two (1.7%) animals, both rodents from park 1. No association was observed between rodent species and the isolation of *C. difficile*. One strain was toxigenic (A + B + CDT-) and was classified as ribotype 064, while the other isolate was non-toxigenic (A-B-CDT-). The non-toxigenic *C. difficile* isolated in the present study was resistant to rifampicin (MIC 3.0 mg/mL). The two isolates were susceptible to all other seven antimicrobials tested.

Table 3 Characterization of staphylococcal isolates that showed antimicrobial resistance

Animal	Park	Transect	Animal	*Staphylococcus* sp.	*mecA* gene	Resistance phetotype*
JC07	1	1	Black rat (*Rattus rattus*)	*S. epidermidis*	No	PEN
JC11	1	1	Black rat (*Rattus rattus*)	*S. saprophyticus*	No	PEN
JC49	1	1	Black rat (*Rattus rattus*)	*S. aureus*	No	PEN; ERY; CLI
JC26	1	2	Genus *Cerradomys*	*S. saprophyticus*	No	PEN; ERY; CLI
JC50.2	1	2	New world mice *(Necromys lasiurus)*	*S. warneri*	No	PEN
JC54	1	2	New world mice *(Necromys lasiurus)*	*S. xylosus*	No	PEN
JC81	1	2	New world mice *(Necromys lasiurus)*	*S. epidermidis*	Yes	PEN; ERY; CEF
JC98	1	2	New world mice *(Necromys lasiurus)*	*S. sciuri*	No	PEN
JC121	1	2	Order *Rodentia*	*S. saprophyticus*	No	PEN; CEF

**PEN* penicillin, *ERY* erythromicin, *CLI* clindamycin, *CEF* cefoxitin

Discussion

Studies have suggested that wild animals living closer to humans and domestic animals may become a threat to public health because they harbor and disseminate pathogens and MDR microorganisms, including *Staphylococcus* spp. and *C. difficile* [10, 35]. However, there are few investigations on the role of rodents and marsupials in urban areas, especially in Brazil. Thus, the present study evaluated the presence of *C. difficile* and MDR staphylococci among rodents and marsupials from two urban parks in Belo Horizonte, Brazil.

Two parks were used to trap and sample rodents and marsupials. Several differences were observed in the animals sampled from each park. First, the number of animals sampled in park 1 was almost three times higher than that in park 2. In addition, differences in the species captured were also observed; black rats were the most common rodent trapped in park 1, but this animal was not captured in park 2. In contrast, the frequency and diversity of marsupials were higher in park 2 than in park 1. Ecological aspects are the main hypotheses for these differences, since park 1 is substantially smaller (335 thousand square meters versus 2.4 million square meters), is completely surrounded by urban environment (Fig. 1), has experiencing a rapidly growing of vertical urbanization, and has sewage effluent present in its waterways [21]. This environment seems more attractive to synanthropic rodents, including black rats, while the more conserved area observed in park 2 might favor the trapping of marsupials [36–38].

S. saprophyticus was the most frequently recovered species from rodents in the present study (Table 2). Staphylococcal species in rodents vary considerably between studies, with *S. xylosus*, *S. succinus*, and *S. sciuri* being the most frequently noted [39–41]. Interestingly, *S. saprophyticus* was only reported in a few animals in one study on bank voles (*Myodes glareolus*) conducted in Poland [39]. In a public health context, it is also important to remember that *S. saprophyticus* is the second highest cause of urinary tract infections in women, including in Brazil, and is typically classified as a human colonizer [42–47]. Although less frequently, *S. aureus* (8.1%) and *S. epidermidis* (5.4%) were also detected in the present study. These two species are commonly found in human microbiota and are well-known opportunistic pathogens that can cause serious infections in humans and animals [48–50].

Overall, the incidence of staphylococci in rodents in this study (27.2%) was much lower than that reported by other authors, which is generally more than 75% [39, 41]. Differences in rodent species were observed in these studies, and differences in host ecology, such as food, geographical location, and contact with different anthropogenic sources may have contributed to this large variation in carriage rate [51, 52].

Only one isolate of *S. saprophyticus* (4.3%) was recovered from marsupials, and rodents appeared to be more prone to staphylococcal colonization than marsupials ($p = 0.01$). The only study published to date that evaluated the distribution of staphylococci in marsupials, specifically in the nasal swabs of Australian wallabies (*Petrogale xanthopus*, *Petrogale lateralis*, and *Macropus eugenii*), reported a much higher isolation rate than this study, reaching 90.8%. In addition, fourteen species of staphylococci were recovered, with *S. delphini* and *S. succinus* being the predominant species. *S. saprophyticus*, also isolated in the present study, was recovered from 4.5% of the animals evaluated [53]. In that study, 70% of the wallabies lived in captivity, and the authors attributed the high frequency of isolation and diversity of staphylococcal species to environmental selection pressure and anthropogenic activity.

Staphylococci are known for their capacity to carry and disseminate antimicrobial resistance determinants, which contribute to their pathogenic potential [4, 54, 55]. In the present study, 24.3% of the rodent isolates, all from park 1, were resistant to at least one tested antimicrobial agent, including three isolates classified as MDR, one of which was also classified as methicillin-resistant *Staphylococcus*. Penicillin G and erythromycin, two drugs widely used in human and veterinary medicine, had the highest resistance rate. These results demonstrate that these rodents, although not directly exposed to antimicrobial agents, can harbor and disseminate resistant bacteria. Only animals from park 1, which is more anthropized and contains sewage effluent, showed antimicrobial resistance. It is possible that this resistance is acquired by the contact of the animals with waterways contaminated with waste from sewage effluent. Sewage effluent is known to harbor several resistant microorganisms and consequently provides a route for horizontal transfer of resistance genes, which is the main hypothesis for the higher rate of AMR in rodents in park 1 than in park 2 [52, 56–58].

The high incidence of potentially pathogenic staphylococci to humans, as well as the high rate of AMR, including methicillin-resistant and other MDR staphylococci, is of concern mainly for park 1. Methicillin-resistant staphylococci confer resistance to at least all beta-lactam antimicrobials, which excludes most of the first-choice treatment options for both animals and humans, substantially reducing therapeutic alternatives [57]. There are several reports on the colonization and infection of companion animals that transmit methicillin-resistant staphylococci and MDR [59–61], showing the relevance of studies monitoring the occurrence of these resistant bacteria in animals.

The isolation rate of *C. difficile* in the present study (1.5%) was lower than that reported previously with urban rodents,

which varied between 4.3 and 35% [13, 62, 63] and with rodents trapped in or around farms, which returned between 24 and 39.2% [63, 64]. A previous study from 2014 with South American coati (*Nasua nasua*) in park 2 also reported a low isolation rate (6.5%) of *C. difficile* [16], whereas all animals sampled in park 2 were negative for *C. difficile* in the present study. It is believed that pests reflect environmental contamination with *C. difficile* spores [65], and therefore this difference among findings is expected, suggesting that both parks have low *C. difficile* contamination.

One of the *C. difficile* strains was toxigenic and classified as ribotype 064. *C. difficile* ribotypes associated with CDI in Brazil are still largely unknown because of the lack of large-scale studies [66]. RT064 currently has not been reported in either humans or animals in Brazil, including studies specifically in Belo Horizonte, the same city where the two parks are located [66–68]. RT064 has previously been reported in animals elsewhere [69] and has also been shown to infect humans [70, 71].

Resistance to rifampicin was detected in a non-toxigenic *C. difficile* strain, but both isolates were susceptible to all other antimicrobials tested. This result contrasts with other studies with rodents, which showed high rates of MDR *C. difficile* strains isolated from rodents [65]. At the same time, the detection of non-toxigenic strains resistant to rifampicin contributes to the growing concern regarding the role of non-toxigenic strains, including isolates from rodents, in the spread of resistance patterns of *C. difficile*, which was previously only focused on toxigenic isolates [65, 72].

Conclusion

In conclusion, the present work suggests that free-living rodents in Belo Horizonte (Brazil) are commonly colonized by *S. saprophyticus* and can harbor MDR and methicillin-resistant *Staphylococcus* strains. *C. difficile* strains with antimicrobial resistance and those from a ribotype previously reported in humans were also recovered from these animals. With regard to marsupials, this is the first study to evaluate the colonization and antimicrobial resistance profile of staphylococci isolated from the feces of free-living opossums, and despite the small sample size, the results suggest low fecal elimination of staphylococci by these animals.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-021-00640-x.

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Author contribution All authors contributed to the study conception and design. Material preparation and samples collection were performed by Salene Angelini Colombo, Lara Ribeiro de Almeida, and Brendhal Almeida Silva. Laboratory analysis were performed by Jordana Almeida Santana, Salene Angelini Colombo, Brendhal Almeida Silva, Amanda Nádia Diniz, and Carlos Augusto Oliveira Junior. The first draft of the manuscript was written by Jordana Almeida Santana, Rodrigo Otávio Silveira Silva, Giliane de Souza Trindade, Adriano Pereira Paglia, and Francisco Carlos Faria Lobato. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval This study was approved by the Ethical Committee on Animal Use (CEUA) of the Federal University of Minas Gerais under protocol 306/2017 and by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under protocol 12989–2.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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