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MICROBIOLOGY

Identification and characterization of pathogenic and multidrug-resistant bacteria in feral pigeons surrounding a veterinary hospital in Minas Gerais, Brazil

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ABSTRACT: Pigeons are known for their capacity to harbor and spread several zoonotic agents. Studies have suggested that pigeons are also relevant disseminators of multidrug-resistant strains. In this study, pigeons surrounding a veterinary hospital were sampled and tested for the presence of pathogenic *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., and *Clostridioides* (*Clostridium*) *difficile*. *E. coli* isolates from 19 (40.4%) pigeons tested positive for the *E. coli* heat-stable enterotoxin 1 (EAST1)-encoding gene. The intimin-encoding gene (*eae*) of enteropathogenic *E. coli* (EPEC) was found in one isolate (2.1%). *Salmonella* spp. were found in nine (19.1%) pigeons, all from the first capture event ($P < 000.1$). *S.* Typhimurium and *S.* Heidelberg were isolated from six and three pigeons, respectively. Enterobacterial repetitive intergenic consensus (ERIC-PCR) of the *Salmonella* spp. isolates suggested that eight of the nine strains had a high genetic similarity, supporting the hypothesis of an outbreak of salmonellosis in these pigeons. Twenty (42.5%) staphylococcal isolates were recovered from 18 (38.3%) pigeons. Eight different species were detected, with *S. xylosus* being the most frequent. Two (4.3%) *C. difficile* strains were isolated. Three isolates, one each of *S. Typhimurium*, *S. aureus*, and *C. difficile*, were classified as multidrug-resistant strains. The present research suggested that pigeons residing in urban areas can act as reservoirs and disseminators of pathogenic bacteria, including nosocomial pathogens, such as diarrheagenic *E. coli* and multidrug-resistant *Staphylococcus* spp., *C. difficile*, and *Salmonella* spp.

Key words: *Salmonella*, *Clostridioides* (*Clostridium*) *difficile*, pigeons, *Staphylococcus*, synanthropes.

Identificação de bactérias patogênicas e multirresistentes a antimicrobianos em pombos urbanos no entorno de um Hospital Veterinário em Belo Horizonte, Minas Gerais, Brasil

RESUMO: Pombos urbanos são conhecidos pela sua capacidade de carrear e disseminar diversos agentes zoonóticos. Estudos tem sugerido que pombos são também relevantes na disseminação de estirpes resistentes a múltiplas drogas. No presente estudo, pombos no ambiente de um hospital veterinário foram amostrados em três diferentes períodos e testados para a presença de *Escherichia coli* patogênica, *Salmonella* spp., *Staphylococcus* spp. e *Clostridioides* (*Clostridium*) *difficile*. Isolados de *E. coli* de 19 pombos (40.4%) foram positivos para o gene codificador da toxina EAST1. O gene codificador de intimina (*eae*) do patotipo *E. coli* enteropatogênica foi encontrada em um isolado (2.1%). *Salmonella* spp. foi encontrada em nove pombos (19.1%), sendo todos isolados do primeiro período de captura ($P < 000.1$). *S.* Typhimurium foi isolado de seis animais e *S.* Heidelberg de três. A tipagem molecular de isolados de *Salmonella* spp. por ERIC-PCR demonstrou que oito estirpes possuíam alta similaridade genética entre si, sugerindo a ocorrência de um surto de salmonelose nos animais carreadores. Vinte *Staphylococcus* (42.5%) foram isolados de 18 animais (38.3%). Oito diferentes espécies foram detectadas, sendo *S. xylosus* a mais frequente. Duas estirpes de *C. difficile* não-toxigênica (4.3%) foram isoladas. Uma estirpe de *S. Typhimurium*, uma de *S. aureus* e um isolado de *C. difficile* foram classificados como resistentes a múltiplas drogas antimicrobianas. O presente estudo sugere que pombos capturados no ambiente do hospital veterinário podem atuar como reservatórios e disseminadores de bactérias patogênicas e envolvidas em infecção hospitalar, incluindo *E. coli* diarreiogênica e *Staphylococcus* sp., *C. difficile* e *Salmonella* spp multirresistente.

Palavras-chave: *Salmonella*, *Clostridioides* (*Clostridium*) *difficile*, pestes, *Staphylococcus*, sinantrópicos.

INTRODUCTION

Urban feral pigeons (*Columba livia* f. *urbana*) are birds with a worldwide distribution commonly reported in most large cities (FERMAN et al., 2010; GARGIULO et al., 2014; SPENNEMANN & WATSON, 2017). The large population densities of urban pigeons in cities are mainly related to the large availability of food, lack or less number of predators, and the built environment resembling the original habitat of urban pigeon ancestors (SPENNEMANN & WATSON, 2017). They are known for their capacity to harbor and spread several zoonotic agents, such as *Cryptococcus neoformans* and *Salmonella*

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spp. (HAAG-WACKERNAGEL & MOCH, 2004; DUTTA et al., 2013; HAESENDONCK et al., 2016; SPENNEMANN & WATSON, 2017). Recently, studies have suggested that pigeons can also be relevant carriers of multidrug-resistant (MDR) bacteria (BORGES et al., 2017; TORRES-MEJÍA et al., 2018; CUNHA et al., 2019). Thus, close contact with pigeons and their feces is a risk for people who frequently share the same environment with these animals (HAAG-WACKERNAGEL & MOCH, 2004; TANAKA et al., 2005; GARGIULO et al., 2014). This is the scenario for employees, students, owners, and animals in the Veterinary Hospital of the Federal University of Minas Gerais (HV-UFMG), where the pigeon population has increased markedly in the last few years.

Despite the known importance of pigeons as reservoirs of several pathogens, no studies have evaluated them in a university environment, and their possible role in the epidemiology of some relevant nosocomial infections in companion animals is still unknown. In addition, the presence of *Staphylococcus* sp. and *Clostridioides* (previously *Clostridium*) *difficile* in pigeons has been reported in a few studies; however, none of the studies have been conducted in Brazil. Thus, this study investigated the occurrence and antimicrobial susceptibility of enteric bacteria isolated from pigeons captured in an urban area surrounding a veterinary hospital in Belo Horizonte, Minas Gerais, Brazil.

MATERIALS AND METHODS

Samples

This study was conducted at the Veterinary Hospital of the Federal University of Minas Gerais (HV-UFMG), which receives approximately 35,000 animals per year, including dogs, cats, horses, cattle, and wild animals. It is located inside the university campus and is surrounded by a green area (Figure 1). This study was motivated by the increase in pigeon population in the last few years in HV-UFMG, raising the need for a better understanding of the risks associated with these birds. Forty-seven

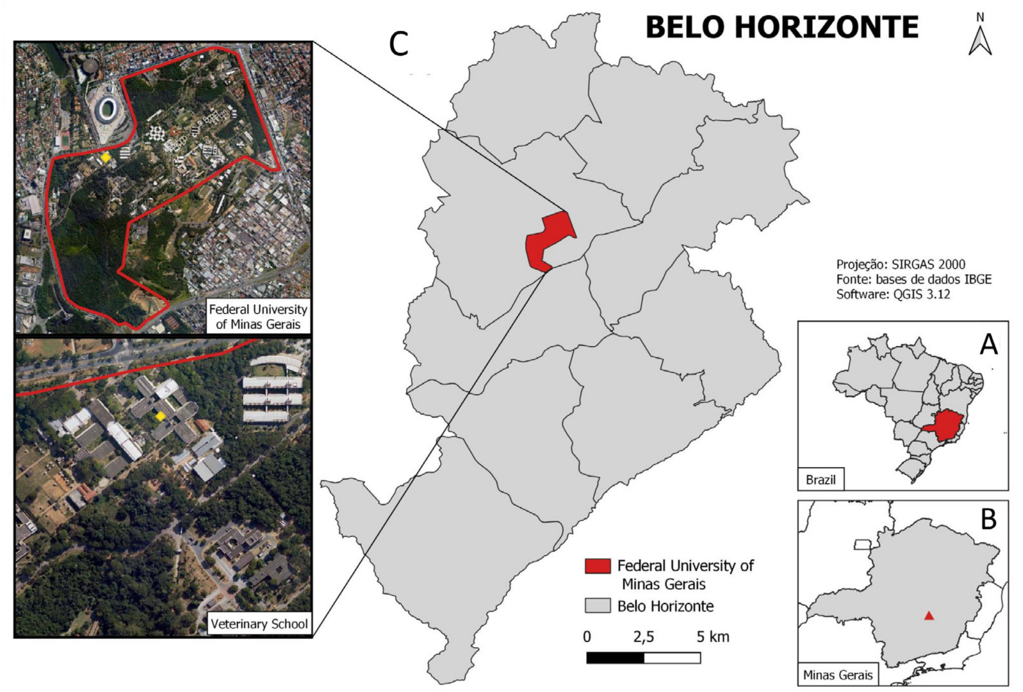

Figure 1 - Mapping showing the location of the Veterinary School of Federal University of Minas Gerais, located in the city of Belo Horizonte, Minas Gerais, Brazil. A. Map of Brazil with the location of the Minas Gerais state. B. Map of Minas Gerais state with the location of Belo Horizonte. C. Map of Belo Horizonte showing the location of the Federal University of Minas Gerais and the Veterinary School.

pigeons (*Columba livia*) were sampled after three capture campaigns (January/2019, July/2019, and January/2020), captured using mist nets (30 mm mesh size; four shelves, ap. 3 m high x 9 m length) during early mornings, in places where large animals fed. The first capture event occurred in January 2019 during the wet season, with a monthly mean precipitation of 2.3 mm and a mean temperature of 25.6 ºC (INMET, 2019), with 18 birds captured. The second capture event occurred in July of the same year during the dry season, with 0 mm of precipitation and a mean temperature of 19.2 ºC (INMET, 2019), with 13 birds captured. The third and last capture occurred in January 2020, with a monthly mean precipitation of 1.3 mm and a mean temperature of 21.5 °C (INMET, 2020), with 16 birds captured. The pigeons were physically restrained, given colored leg rings for identification and monitoring purposes, and left to rest in individual cages. Fresh feces were collected immediately after dropping and stored in microtubes using sterile spatulas (ROSARIO MEDINA et al., 2017). The samples were stored in a transport box with ice packs and sent to the Bacterial and Research Laboratory of the Veterinary School of UFMG for immediate processing. Each pigeon was returned to its environment once sample collection was completed.

Escherichia coli

For *E. coli* isolation, the samples were plated onto MacConkey agar (Difco, USA) and incubated at 37 °C for 24 h (RAMOS et al., 2019a). Three lactose-fermenting colonies were identified using polymerase chain reaction (PCR) and subjected to subsequent reactions for phylogenetic group characterization (A, B1, B2, C, D, E, and F) (MCDANIELS et al., 1996; CLERMONT et al., 2013). Virulence genes associated with pathogenic *E. coli,* such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), necrotoxigenic *E. coli* (NTEC), and enteroaggregative *E. coli* (EAEC)*,* were also identified using PCR (BLANCO et al., 1996; YAMAMOTO & NAKAZAWA, 1997; FRANCK et al., 1998). Antibiotic resistance patterns of all *E. coli* isolates that tested positive for virulence factors were evaluated using the disc diffusion method (DDM), according to the Clinical and Laboratory Standards Institute (CLSI) manual (CLSI, 2017; CLSI, 2018). The following antimicrobial agents, commonly used in human and animal clinical practice, were tested: chloramphenicol (30 µg), trimethoprim/sulfamethoxazole (25 µg), ceftriaxone (30 µg), ceftiofur (30 µg), amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), tetracycline (30 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and amikacin (30 µg) (DME, BRA).

Salmonella spp.

For *Salmonella* spp. isolation, cloacal samples were pre-enriched in Rappaport broth (Oxoid, USA) and plated onto Hektoen enteric agar (Oxoid, USA) (RAMOS et al., 2019a). Sulfite-reducing colonies were identified as *Salmonella* spp. by genus-specific PCR, according to KUANG et al. (2015). Antigenic characterization was done according to the White-Kauffmann-Le Minor Scheme (LE MINOR & POPOFF, 1987) at the Brazilian National Reference Laboratory of Enterobacteria of the Oswaldo Cruz Foundation, followed by species, subspecies, and serotype identification (GRIMONT & WEILL, 2007). *Salmonella* spp. strains were fingerprinted using enterobacterial repetitive intergenic consensus (ERIC)-PCR and analyzed using Bionumerics 7.6 software (Applied Maths NV, Belgium) to evaluate the genetic diversity between isolates from different pigeon samples (VERSALOVIC et al., 1991; RAMOS et al., 2019b). Additionally, DDM was used to evaluate the resistance patterns of *Salmonella* spp. isolates to antimicrobial agents (CLSI, 2017; CLSI, 2018) using the following drugs: chloramphenicol (30 µg), trimethoprim/sulfamethoxazole (25 µg), ceftriaxone (30 µg), ceftiofur (30 µg), amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), enrofloxacin (5 µg), and ciprofloxacin (5 µg) (DME, BRA).

Staphylococcus spp.

For *Staphylococcus* spp. isolation, fecal samples were first suspended in 0.85% saline solution. The resultant solution (100 µL) was then streaked onto mannitol salt agar (Difco Laboratories Inc., USA), which was incubated at 37 °C for 24 h. Colonies were sub-cultured on brain heart infusion agar (Difco Laboratories Inc., USA) and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Germany). A cutoff log score of 2 was used to validate identification at the species level, as recommended by the manufacturer. The strains were then subjected to DNA extraction (PITCHER et al., 1989), and methicillin-resistant staphylococci were investigated by detecting the *mecA* gene (MURAKAMI et al., 1991). In addition, isolates identified as *Staphylococcus intermedius* group (SIG) using MALDI-TOF were further confirmed by multiplex PCR of the *nuc* gene (SASAKI et al., 2010). Non-SIG isolates with MALDI-TOF score under 2.0 were subjected to sequencing of the 16S ribosomal RNA gene as described by FOX et al. (2011). Antimicrobial susceptibility tests were performed using DDM in agar, according to the Clinical and Laboratory Standards Institute (CLSI) documents M100-S30 and VET08 (CLSI 2018; CLSI, 2020). 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 25923 was used as the control strain. Isolates were considered MDR when resistant to three or more classes of antimicrobial agents (SWEENEY et al., 2018).

Clostridioides difficile

Samples were incubated in 96% ethanol for 30 min (1:1), and aliquots of 10 µL were plated on cycloserine-cefoxitin fructose agar supplemented with 7% horse blood and 0.1% sodium taurocholate (Sigma, USA) (SILVA et al., 2013). After incubation in an anaerobic atmosphere at 37 °C for 72 h, characteristic *C. difficile* isolates (flat, irregular, and with a ground-glass appearance) were subjected to multiplex PCR to identify the housekeeping gene (*tpi*) and virulence genes of toxin A (*tcdA*), toxin B (*tcdB*), and binary toxin (*cdtB*) (SILVA et al., 2011). The minimal inhibitory concentrations (MICs) of metronidazole, vancomycin, clindamycin, moxifloxacin, ciprofloxacin, erythromycin, rifampicin, and tetracycline were determined using Etest strips (bioMérieux Marcy l'Etoile, France) in Brucella agar (Oxoid, USA) with 5% lysed blood supplemented with hemin (Difco Laboratories, USA) and vitamin K (Sigma-Aldrich Co., USA). MIC values were interpreted according to the clinical breakpoints of the CLSI and European Committee on Antimicrobial Susceptibility Testing guidelines (PIRŠ et al., 2013; CLSI, 2015; EUCAST, 2019).

Statistical analysis

The association between capture events, presence of the tested pathogens, and frequency of each isolated microorganism was evaluated using chi-square and Fisher's exact tests. The chi-squared 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 statistically significant at $P < 0.05$.

RESULTS

Escherichia coli

E. coli was identified in 42 (89.3%) pigeons, with a total of 120 isolated strains (Table 1). All seven phylogenetic groups of *E. coli* were identified (Table 2) in the sampled pigeons, with B1 being the most common phylogroup (52/120 strains, 43.3%). A total of 41 strains (34%) from 19 (40.4%) pigeons tested positive for enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1) encoding gene. The intimin-encoding gene (*eae*) of EPEC was detected in one isolate (2.1%), which was also positive for EAST1. No resistance to the tested antimicrobials was observed in the 41 *E. coli* isolates that tested positive for virulence factors.

Salmonella spp.

Salmonella spp. were found in nine (19.1%) pigeons, all from the first capture event (n=18; isolation rate of 50%), indicating a strong difference between the first and the other two events ($P < 000.1$). *S.* Typhimurium and *S.* Heidelberg were isolated from six (66.7%) and three pigeons, respectively. One bird died a few days after the capture event (Figure 2; isolate "PB13"). Macroscopically, the bird had hepatomegaly, and the serosa of the small intestine was diffusely hyperemic with hemorrhagic intestinal contents. *S.* Typhimurium was isolated again from the excreta and liver of this bird. ERIC-PCR of the *Salmonella* spp. isolates suggested that eight out of the nine strains had high genetic similarity (Figure 2). These strains showed no resistance to all antimicrobials tested, while the remaining strain (*S.* Typhimurium) was resistant to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, ampicillin, tetracycline, enrofloxacin, ciprofloxacin, and nalidixic acid. Thus, it was classified as an MDR strain (MAGIORAKOS et al., 2012).

Staphylococcus spp.

A total of 20 (42.5%) staphylococcal isolates were recovered from 18 (38.3%) pigeons of the 47 captured pigeons. Eight different species were detected, with *S. xylosus* (30%) being the most common (Table 1). Overall, 7 (35%) isolates were resistant to at least one antimicrobial agent, whereas 13 (65%) were susceptible to all the tested compounds. Some isolates were resistant to tetracycline (35.5%), penicillin G (15%), erythromycin (5%), and clindamycin (5%). No significant differences were

Table 1 - Frequency of *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., and *Clostridioides difficile* isolated from free-living pigeons (n=47) in three capture events in the Veterinary Hospital of Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil).

Pathogen	Capture (%)			Total (n=47)
	First	Second	Third	
E. coli	18/18 (100)	10/13 (76.9)	14/16 (87.5)	42/47 (89.3)
EAST-1	8/18 (44.4)	6/13 (46.1)	5/16 (31.3)	19/47 (40.4)
EPEC (*eae*)	1/18 (5.5)	0/13 (0)	0/16 (0)	1/47 (2.1)
Salmonella spp.	9/18 (50)[a]	0/13 (0)[b]	0/16 (0)[b]	9/47 (12.8)
S. Typhimurium	6/18 (33.3)	0/13 (0)	0/16 (0)	6/47 (6.4)
S. Heildeberg	3/18 (18.7)	0/13 (0)	0/16 (0)	3/47 (6.4)
Staphylococcus spp.	7/18 (38.9)	6/13 (46.1)	7/16 (43.7)	20/47 (42.5)
S. xylosus	3/18 (16.7)	2/13 (15.4)	1/16 (6.3)	6/47 (12.8)
S. sciuri	2/18 (11.1)	1/13 (7.7)	2/16 (12.5)	5/47 (10.6)
S. lentus	0/18 (0)	0/13 (0)	3/16 (18.7)	3/47 (6.4)
S. haemolyticus	0/18 (0)	2/13 (15.4)	0/16 (0)	2/47 (4.2)
S. aureus	0/18 (0)	0/13 (0)	1/16 (6.3)	1/47 (2.1)
S. intermedius	1/18 (5.6)	0/13 (0)	0/16 (0)	1/47 (2.1)
S. succinus	0/18 (0)	1/13 (7.7)	0/16 (0)	1/47 (2.1)
S. schleiferi	1/18 (5.6)	0/13 (0)	0/16 (0)	1/47 (2.1)
C. difficile (Non-toxigenic)	2/18 (11.1)	0/13 (0)	0/16 (0)	2/47 (4.2)

Different lower-case letters indicate a significant difference ($P < 0.05$).

Legend: EPEC - Enteropathogenic *Escherichia coli*; EAST-1 - Enteropathogenic *Escherichia coli.*

reported in resistance to these antimicrobials. One *S. aureus* isolate showed resistance to penicillin G, erythromycin, and clindamycin; and therefore, was classified as MDR. All isolates were susceptible to cefoxitin, chloramphenicol, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole and were negative for *mecA*.

Clostridioides difficile

Two (4.3%) *C. difficile* isolates were recovered from the pigeons. Both were nontoxigenic (A-B-CDT-). One isolate was classified as MDR due to resistance to erythromycin, rifampicin, and tetracycline, while both isolates were susceptible to all other antimicrobials tested, including metronidazole and vancomycin.

DISCUSSION

Despite the known importance of pigeons as reservoirs of several pathogens, no studies have evaluated them in a university environment and their role in the epidemiology of nosocomial bacteria.

Table 2 - Phylogroups of *Escherichia coli* isolates (n=120) from pigeons (n=47) captured in the Veterinary Hospital of Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil).

E. coli	Phylogenetic groups								Total
	A	B1	B2	C	D	E	F	Clade[2]	Unassignable[1]	
Isolates (%)	11 (9.2)	52 (43.3)	12 (10) [a]	18 (15)	3 (2.5)	16 (13.3) [a]	1 (0.8)	4 (3.3)	3 (2.5)	120 (100)

[1] Identified as *E. coli* but not corresponding to any of the phylogroups according to CLERMONT et al. (2013).

[2] Clade 1, 2, 3, 4, or 5.

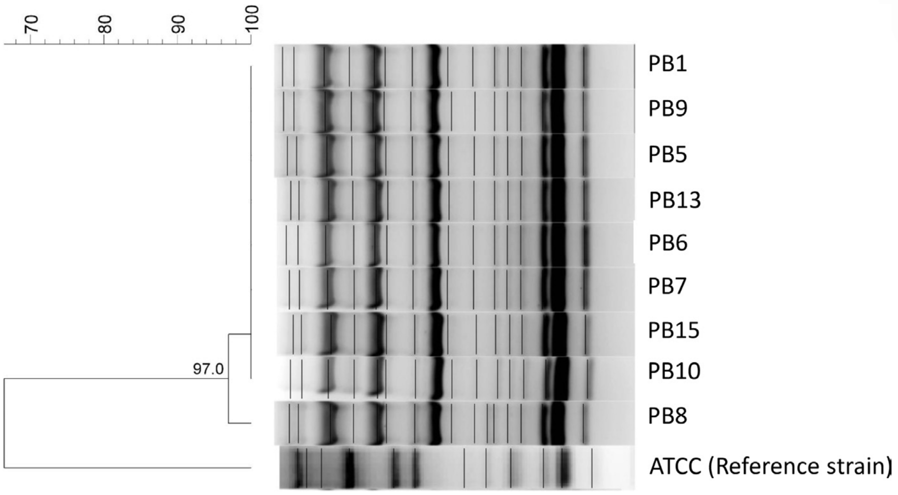

Figure 2 - Dendrogram of nine *Salmonella* spp. strains from urban pigeons and one *Salmonella* Typhimurium ATCC 14028 fingerprinted by ERIC – PCR. Clustering analysis was based on the Dice similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA) algorithms using Bionumerics 7.6 (Applied Maths, Belgium). The strains at the right side labeled as PB1, PB9, PB5, PB13, PB6, PB17, and PB10 shared 100% of genetic similarity, and 97% of similarity with PB8 strain, as demonstrated on the left of dendrogram.

This study revealed the presence of zoonotic MDR pathogens in pigeons captured in the surroundings of a veterinary hospital, suggesting that pigeons can act as reservoirs and disseminators of diarrheagenic *E. coli* and MDR *Staphylococcus* spp., *C. difficile*, and *Salmonella* spp.

The high isolation rate of *E. coli* from phylogroup B1 was similar to that reported in previous studies on urban pigeons (GORDON & COWLING, 2003; SILVA et al., 2009; GHANBARPOUR & DANESHDOOST, 2012). Interestingly, we identified two virulence factors in the *E. coli* isolates. *E. coli* isolates positive for the EAST-1 encoding gene were detected in more than 40% of the birds, whereas the *eae* encoding gene from EPEC was detected in a single isolate. The high frequency of pigeons positive for EAST-1 was surprising, since this virulence factor, previously associated with outbreaks of diarrhea in humans (ZHOU et al., 2002; SUKKUA et al., 2017), has never been reported in *E. coli* isolates from pigeons. Further, pigeons are known reservoirs of EPEC (SILVA et al., 2009; GHANBARPOUR & DANESHDOOST, 2012; SACRISTÁN et al., 2014; BORGES et al., 2017; TORRES-MEJÍA et al., 2018), a major cause of childhood diarrhea worldwide (CROXEN et al., 2013; TORRES-MEJÍA et al., 2018). Together, these results reinforce the role of pigeons as potential reservoirs of zoonotic *E. coli* pathotypes.

We tested all 42 *E. coli* isolates that were positive for virulence factors for antimicrobial resistance. Results revealed that all isolates were susceptible to all antimicrobials tested, surprisingly showing that despite living in a heavily anthropized environment, the sampled pigeons had *E. coli* isolates with no drug resistance. Our results are in contrast with previous studies that described the occurrence of MDR *E. coli* strains in pigeons in several countries (GHANBARPOUR & DANESHDOOST, 2012; BORGES et al., 2017; KARIM et al., 2020).

The fecal shedding of *Salmonella* spp. in the sampled pigeons (19.1%) was higher than that found in several previous studies, which often reported frequencies of up to 10% (DOVC et al., 2004; TANAKA et al., 2005; PEDERSEN et al., 2006; GARGIULO et al., 2014; HAESENDONCK et al., 2016; CARVALHO et al., 2020; KACZOREK-ŁUKOWSKA et al., 2020). Interestingly, the isolation of *Salmonella* spp. was significantly associated with the first capture event ($P < 000.1$) conducted during the rainy season. This result raised the hypothesis of an outbreak of salmonellosis in the pigeons during this sampling period, which was reinforced after the re-isolation of *Salmonella* from the intestinal content and liver of a pigeon that died a few days after the capture event. Furthermore, the post-mortem alterations observed in this pigeon are commonly reported in pigeons and other species with salmonellosis (SAWA & HIRAI, 1981; OLIVEIRA et al., 2019; RAMOS et al., 2021).

To better understand this possible outbreak, one *Salmonella* isolate from each animal was subjected to ERIC-PCR, a method with high discriminatory power already in use for *Salmonella* typing in outbreaks (PURIGHALLA et al., 2017; RAMOS et al., 2019b). The high similarity among the eight isolates, as suggested by ERIC-PCR, strongly suggests the occurrence of an outbreak of salmonellosis in these pigeons. Previous studies have indicated that; although, pigeons are commonly asymptomatic carriers of *Salmonella* spp., salmonellosis may occur in urban pigeons, which could significantly increase the isolation rate of this agent in the affected pigeons (SAWA & HIRAI, 1981; TIZARD, 2004; HOELZER et al., 2011; DUTTA et al., 2013; ROCHA-E-SILVA et al., 2014). Additionally, salmonellosis outbreaks in animals seem to be more common during the rainy season, which may be a risk factor for bacterial spread (PANGLOLI et al., 2008; RAMOS et al., 2019b).

Notably, we detected two well-known zoonotic *Salmonella* spp. serovars, *S.* Typhimurium *and S.* Heidelberg, in our study. Previous studies have demonstrated that *Salmonella enterica* isolates from urban pigeons commonly belong to serotype *Typhimurium* (DUTTA et al., 2013; OSMAN et al., 2013; ROCHA-E-SILVA et al., 2014; HAESENDONCK et al., 2016; TORRES-MEJÍA et al., 2018; CARVALHO et al., 2020; KACZOREK-ŁUKOWSKA et al., 2020). It is also known that *S. Typhimurium* and *S. Heidelberg* are important foodborne pathogens (CDC, 2014), which can also infect humans after direct contact with healthy or diseased animals (HALE et al., 2012; RAMOS et al., 2019b). Transmission of *Salmonella* spp. from pigeons to humans has already been reported; however, studies associating human salmonellosis with pigeon contact are scarce (HAAG-WACKERNAGEL & MOCH, 2004; SPENNEMANN & WATSON, 2017). Despite this, the common shedding of these zoonotic *Salmonella* serotypes by urban pigeons and their close contact with humans should be considered a potential risk to human health, especially for those most susceptible, such as children and immunocompromised individuals (HALE et al., 2012).

Most *Salmonella* spp. isolates showed no resistance to the antimicrobials tested. Interestingly, all isolates were genetically similar according to ERIC-PCR analysis, which also contributed to the hypothesis of an outbreak. Conversely, one *S.* Typhimurium strain was resistant to seven antimicrobials and thus, was classified as MDR (MAGIORAKOS et al., 2012). According to the World Health Organization (WHO, 2019), there are specific public health concerns related to the spread of fluoroquinolone-resistant *Salmonella* spp. In addition, β-lactams and sulfonamides are commonly used antimicrobials for the treatment of *Salmonella* spp. infections in animals and humans, and resistance to both important drugs may severely reduce the treatment options (KUANG et al., 2015).

More than one-third of the captured pigeons were positive for staphylococci and eight different species were recovered (Table 1). According to other studies, pigeons seem to harbor a high diversity of staphylococcal species, whereas other animals, such as free-living reptiles and rodents, seem to show a more homogeneous colonization pattern (SCHWARZ & WERCKENTHIN, 1994; ZIGO, 2017; SANTANA et al., 2021; SANTANA et al., 2022). It may be that the close and daily contact of the pigeons with humans and animals, combined with the hospital environment, may have influenced this large number of recovered species (SCHWARZ & WERCKENTHIN, 1994; ZIGO, 2017; KAMATHEWATTA et al., 2019).

S. xylosus, the most frequent species reported in our study, has been previously isolated from pigeons and other birds, suggesting commensalism (VELA et al., 2012; MAHMMOUD, 2013; ZIGO, 2017; MATIAS et al., 2018). This coagulase-negative *Staphylococcus* (CoNS) has also been found in different mammals and reptiles (BECKER et al., 2014; RISSI et al., 2015; MATIAS et al., 2018; SANTANA et al., 2021; SANTANA et al., 2022), and despite being mostly labeled as non-pathogenic, it has caused several opportunistic infections in animals and humans (WON et al., 2002; KOKSAL et al., 2009; AKHADDAR et al., 2010; RISSI et al., 2015).

Except for *S. succinus*, all other CoNS isolates have been reported in previous studies on pigeons (SCHWARZ & WERCKENTHIN, 1994; ZIGO, 2017). Similar to *S. xylosus*, all these species can integrate into the microbiota of skin and mucous membranes of different hosts and act as opportunistic pathogens causing distinct infections (RISSI et al., 2015). Notably, *S. haemolyticus* is highly relevant to human health and is the second most frequently isolated CoNS from nosocomial infections (SIDHU et al., 2007; CZEKAJ et al., 2015).

We also isolated *S. intermedius* and coagulase-positive *S. aureus* in this study. Both species have been frequently reported in pigeons (KIZERWETTER-ŚWIDA et al., 2015; CHROBAK-CHMIEL et al., 2021). However, *S. intermedius* is more commonly reported in wild pigeons, and its isolation from domestic pigeons is scarce (KIZERWETTER-ŚWIDA et al., 2015). In contrast, *S. aureus* is widespread among humans and different animal species, facilitating bilateral transmission between distinct hosts (WEESE & VAN DUIJKEREN, 2010). This microorganism is one of the world's most frequent causes of nosocomial infections in humans. It is also a relevant pathogen in dogs, and its treatment is often hampered by resistance to multiple antimicrobials (IPPOLITO et al., 2010; WALTHER et al., 2017; TURNER et al., 2019). Interestingly, *S. aureus* isolated in our study was an MDR strain, which reinforces the hypothesis that pigeons are potential disseminators of MDR staphylococci (KUTKOWSKA et al., 2019; CHROBAK-CHMIEL et al., 2021).

More than one-third of the staphylococci isolates showed resistance to at least one of the antimicrobials tested, mainly to tetracycline and penicillin, which are widely used in human and veterinary medicine (ARGUDÍN et al., 2017; CERBO et al., 2019). It is possible that this specific environment, where the pigeons can get directly and indirectly in contact with humans and healthy and sick animal species, may have influenced the diversity of staphylococci isolated in our study, as well as the frequency of isolates resistant to antimicrobials, as previously indicated in other studies on staphylococci in different animals and settings (FUTAGAWA-SAITO et al., 2007; HAABER et al., 2017; CERBO et al., 2019; KAMATHEWATTA et al., 2019; FROSINI et al., 2020; PALMA et al., 2020). FUTAGAWA-SAITO et al., (2007) compared the resistance profiles of staphylococci isolated from pigeons and suggested that isolates from pigeons that have direct contact with humans and other animals are more associated with resistance to several antimicrobials, which supports the idea that resistance may be acquired because of the mutual coexistence of different species.

Although, *C. difficile* is recognized as an emerging pathogen causing zoonotic diseases in humans (KNIGHT & RILEY, 2019) and has previously been reported to cause nosocomial infections in dogs (WEESE & ARMSTRONG, 2003), little is known about the role of pigeons in the epidemiology of this anaerobic microorganism. Previous studies of other avian species have suggested that *C. difficile* is either absent or present at a very low frequency (BANDELJ et al., 2011; BURT et al., 2012; BANDELJ et al., 2014). In our study, two *C. difficile* strains were isolated (4.3%), which is lower than the previously reported rate (12.5%) by ANDRÉS-LASHERAS et al. (2017) in a study on pigeons and rodents trapped within pest control programs in pig farms. Notably, the colonization of *C. difficile* in pigeons and other birds seems to be directly linked to environmental contamination (ANDRÉS-LASHERAS et al., 2017), which may explain the difference in its isolation rates. Additionally, one isolate in our study was classified as MDR due to its resistance to erythromycin, rifampicin, and tetracycline. Our results are similar to those previously reported by ANDRÉS-LASHERAS et al. (2017) and reinforce the hypothesis that pigeons may play a role in the transmission of *C. difficile*, including antimicrobial-resistant strains.

Our research suggested that pigeons captured in the surroundings of a veterinary hospital can act as reservoirs and disseminators of pathogenic and nosocomial bacteria, including diarrheagenic *E. coli* and MDR *Staphylococcus* sp., *C. difficile*, and *Salmonella* spp. Together with previous studies, our findings reinforce the importance of pigeon population control owing to their potential role in the spread of zoonotic diseases.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was approved by the Ethical Committee on Animal Use (CEUA) of the Universidade Federal de Minas Gerais (UFMG) under protocol 361/2018 and by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under protocol SISBIO 66535-2.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised and approved the final version of the manuscript.

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