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Investigation of enterobacteria with zoonotic and multi-resistant potential in exotic parrots kept in a domestic environment¹

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ABSTRACT.- Marques A.R., Pascoal-Filho N.M., Teixeira R.S.C., Silva I.N.G., Melo L.S., Lima B.P., Campos E.M.S.Q. & Maciel W.C. 2024. **Investigation of enterobacteria with zoonotic and multi-resistant potential in exotic parrots kept in a domestic environment**. *Pesquisa Veterinária Brasileira 44:e07387, 2024*. Laboratório de Estudos Ornitológicos, Faculdade de Veterinária, Universidade Estadual do Ceará, Av. Doutor Silas Munguba 1700, Fortaleza, CE 60714-903, Brazil. E-mail: adsonribeiromarques@gmail.com

This investigation elucidated the presence of potentially zoonotic and antimicrobialresistant bacteria in domestically reared psittacines. The present study was sanctioned by the Animal Ethics Committee of the State University of Ceará (CEUA-UECE) and bears registration number 03423745/2023. A total of 111 cloacal swab samples were procured from exotic psittacines encompassing six distinct species: the Australian budgerigar (Melopsittacus undulatus), cockatiels (Nymphicus hollandicus), lovebirds (Agapornis sp.), roseringed parakeets (Psittacula krameri), red-rumped parrots (Psephotus haematonotus), and rosellas (*Platycercus eximius*). The process encompassed the isolation and characterization of enterobacteria and ascertaining their resistance profiles. Among the collected specimens. 70.2% (78/111) yielded growth indicative of one or more enterobacterial agents. The collective isolates comprised 110 strains encompassing 13 distinct bacterial species. Foremost among these was *Escherichia coli*, accounting for a significant percentage of the total isolates at 30% (33/110), followed by Pantoea agglomerans at 27.2% (30/110). The study revealed that 35.4% (39/110) of the isolates exhibited resistance to tobramycin, with tetracycline and fosfomycin showing resistance rates of 34.5% (38/110) and 30.9% (34/110), respectively. Particularly noteworthy was that *E. coli* showed a heightened propensity for tetracycline resistance at 51.5% (17/33), while resistance rates to tobramycin and gentamicin were 36.6% (12/33) and 15.1% (5/33), respectively. A noteworthy subset of the enterobacterial cohort exhibited multidrug resistance patterns (28.9%, 32/110). Collectively, these outcomes underscore not only an elevated prevalence of enterobacterial strains but also the pervasive phenomenon of antimicrobial resistance across a diverse spectrum of antimicrobial agents.

INDEX TERMS: Enteropathogens, enterobacteria, antimicrobial resistance, exotic birds.

RESUMO.- [Investigação de enterobactérias com potencial zoonótico e multirresistentes em psitacídeos exóticos mantidos em ambiente doméstico.] Este estudo investigou a presença de bactérias potencialmente zoonóticas e resistentes a antimicrobianos em psitacídeos criados em ambiente doméstico.

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Esse projeto foi aprovado pela Comissão de Ética para o Uso de Animais da Universidade Estadual do Ceará (CEUA-UECE), registrado sob o número 03423745/2023. Foram coletadas 111 amostras de suabes cloacais de psitacídeos exóticos de seis espécies, incluindo, periquitos-australianos (*Melopsittacus undulatus*), calopsitas (*Nymphicus hollandicus*), agapornis (*Agapornis* sp.), periquitos-de-colar (*Psittacula krameri*), periquitos-dorso-vermelho (*Psephotus haematonotus*) e roselas (*Platycercus eximius*). Foi realizado o isolamento e a identificação de enterobactérias e determinado o perfil de resistência. Das amostras coletadas, 70,2% (78/111) apresentaram crescimento

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para uma ou mais enterobactérias. Foram isoladas 110 cepas pertencentes a 13 espécies bacterianas. *Escherichia coli* apresentou o maior índice de isolamento, com 30% (33/110). Em segundo lugar *Pantoea agglomerans*, com um percentual de isolamento de 27,2% (30/110). Observou-se que 35,4% (39/110) das enterobactérias apresentaram resistência à tobramicina, seguidas da tetraciclina com 34,5% (38/110) e da fosfomicina com 30,9% (34/110). *E. coli* apresentou maior taxa de resistência a tetraciclina com 51,5% (17/33), seguida de 36,6% (12/33) para tobramicina e 15,1% (5/33) para gentamicina. Das enterobactérias analisadas 28,9% (32/110), apresentaram multirresistência. Os resultados indicam uma alta prevalência de enterobactérias, a resistência antimicrobiana foi constatada em diversas classes de antimicrobianos.

TERMOS DE INDEXAÇÃO: Enteropatógenos, enterobactérias, resistência antimicrobiana, aves exóticas.

INTRODUCTION

A "one health" approach recognizes the complex interplay between human, animal, and environmental ecosystems and is crucial to addressing global challenges. In this context, antimicrobial resistance (AMR) is emerging as a rising threat (Antimicrobial Resistance Collaborators 2022, WHO 2022). Over time, the dynamic relationship between humans and animals has evolved, and the growing popularity of birds as companion animals is highlighted. From this perspective, AMR proves to be a significant and unique health concern, potentially affecting both human and animal health (Freitas et al. 2018, Marques et al. 2021, Keshmiri et al. 2022).

Exotic parrots are widely adopted as pets (Damborg et al. 2016) and have been the subject of several studies that point to high rates of isolation of zoonotic bacteria and resistance to antimicrobials (Pontes et al. 2018, Legadevi et al. 2019, Calaça et al. 2020). The spread of resistant zoonotic bacterial strains due to the indiscriminate use of antimicrobials amplifies health risks, making it essential to deeply understand the dynamics to implement effective mitigation and control strategies (Bagai et al. 2024). AMR creates significant challenges in treating bacterial infections in animals and humans (Arnold et al. 2016, Furness et al. 2017, WHO 2021). This resistance contributes to increased morbidity and mortality and significantly impacts the duration and costs of treatments (Ouakrim et al. 2020). A comprehensive and cooperative approach is imperative to address this issue and preserve the effectiveness of antimicrobials in combating infections.

In this context, birds kept under human care can be carriers of several zoonotic bacteria of significant importance in public health, thus representing a risk to humans and other birds (Cupertino et al. 2020, Nupur et al. 2023). Notably, zoonotic diseases originating from pet birds tend to receive less attention than foodborne zoonoses (Damborg et al. 2016). According to Luca et al. (2018), additional investigations are necessary for a more in-depth understanding of the potential risk associated with contact with pet birds and their role as reservoirs of bacterial zoonoses. The increase in antimicrobial resistance of zoonotic bacteria has been documented in several domestic parrots. It is considered a potential source for transmitting multi-resistant zoonotic bacteria, such as those in the order Enterobacterales (Wieler et al. 2011, Marques et al. 2021). Enterobacterales are naturally found in the intestinal microbiota of animals and humans and include species such as *Escherichia coli* (Adeolu et al. 2016, Pakbin et al. 2021). However, species of this order can manifest as primary or opportunistic pathogens, mainly associated with intestinal and extraintestinal infections in humans and animals (Jenkins et al. 2017). Examples of these species include *Klebsiella oxytoca* (Singh et al. 2016), *Cronobacter sakazakii* (Song et al. 2018), *Shigella* sp. (Kotloff et al. 2018), *Hafnia* sp. (Yin et al. 2019), *Citrobacter* sp. (Liu et al. 2020), *Pantoea agglomerans* (Mirtella et al. 2021), and pathogenic strains of *E. coli* (Pakbin et al. 2021). The objective of this study was to investigate the presence of zoonotic and multi-antimicrobial resistant bacteria in exotic parrots kept in a domestic environment.

MATERIALS AND METHODS

Animal Ethics. This project was approved by the Ethics Committee for the Use of Animals of the "Universidade Estadual do Ceará" (CEUA-UECE) and registered under number 03423745/2023.

Sampling. One hundred eleven samples of cloacal swabs were collected from exotic parrots kept in a domestic environment in the city of Fortaleza/CE, and its metropolitan region. They were collected from six species, including 20 Australian parakeets (*Melopsittacus undulatus*), 20 cockatiels (*Nymphicus hollandicus*), 20 lovebirds (*Agapornis* sp.), 25 collared parakeets (*Psittacula krameri*), 20 red-backed parakeets (*Psephotus haematonotus*), and six rosellas (*Platycercus eximius*). The choice of each breed was carried out by convenience sampling (non-probability sampling), and the selection of birds, age, sex, and clinical condition was done at random.

Microbiological processing of enterobacteria. The isolation of enterobacteria was done in accordance with the methodology used by Lopes et al. (2015) with modifications as follows. The samples were collected and conditioned in Stuart Transport Medium (Laborclin®). Upon arrival at the Ornithological Studies Laboratory (LABEO), the swabs were transferred to 5mL of 1% peptone water (Kasvi[®]), which was incubated in a microbiological oven for 24h at 37°C. Subsequently, 0.5mL aliquots were transferred to Cystine Selenite (Kasvi®) and Brain Heart Infusion (Kasvi®) broths, and 0.05mL was transferred to Rappaport Vassiliadis broth (Merck®). These solutions were incubated for 24h at 37°C. After enrichment in broths, plating was performed. The samples were sown on MacConkey agar (Kasvi®), EMB Levine agar (Laborclin®), Salmonella-Shigella agar (Laborclin®), and Brilliant Green agar (Laborclin®), and then the plates were incubated for 24h at 37°C. Different colonies were collected on each plate and inoculated into tubes containing Triple Iron Sugar agar (Kasvi[®]).

To confirm the identity of the enterobacteria, a biochemical battery consisting of the following media was used: SIM Medium (Himedia[®]), lysine decarboxylase (Kasvi[®]), ornithine decarboxylase (Himedia[®]), MR-VP broth (Himedia[®]), urea (Dinâmica Formula[®]), Simmons Citrate agar (Himedia[®]), arginine decarboxylase (Êxodo Cientifica[®]), malonate broth (Himedia[®]), H₂S production, glucose fermentation (with gas production), lactose (Merck[®]), sucrose (Dinâmica[®]), mannitol (Dinâmica[®]), arabinose (Dinâmica[®]), raffinose (Dinâmica[®]), sucrose (Dinâmica[®]), adonitol (Dinâmica[®]), inositol (Sigma[®]), and sorbitol (Sigma[®]) (Winn Jr. et al. 2008). Samples suspected of *Salmonella* spp. were subjected to an agglutination test using the polyvalent "O" serum (Probac[®]) and then sent to the "Fundação Oswaldo Cruz" (Fiocruz) reference laboratory for serotyping.

Enterobacteriaceae sensitivity profile (antimicrobial susceptibility test). Bacterial resistance was studied using the Kirby-Bauer method following the standards stipulated by the Clinical and Laboratory Standards Institute (CLSI 2020). To analyze the resistance profile of the isolated strains, 12 antimicrobials from 10 pharmacological classes were used and tested: 1) folate pathway inhibitors (sulfamethoxazole with trimethoprim, 25µg); 2) fluoroquinolones (enrofloxacin, 5µg and ciprofloxacin, 5µg); 3) penicillin + beta-lactamase inhibitors (amoxicillin with clavulanate, 30µg); 4) third-generation extended spectrum cephalosporins (ceftriaxone, 30µg); 5) carbapenems (meropenem, 10µg); 6) monobactams (aztreonam, 30µg); 7) aminoglycosides (gentamicin, 30µg, and tobramycin, 10µg); 8) tetracyclines (tetracycline, 30µg); 9) phosphonic acid (fosfomycin, 200µg); and 10) amphenicols (chlorfenazole, 30µg). Multidrug resistance (RMD) was considered present when strains were resistant to at least three classes of antimicrobials (Magiorakos et al. 2012).

To this end, the samples were recovered in tubes containing 2mL brain heart infusion (BHI) broth and placed in a bacteriological oven for 24h at 37°C. Subsequently, aliquots of the broth were seeded onto MacConkey agar plates and incubated again in an oven. Two to three units of bacterial colonies present on MacConkey agar were subsequently selected and sown in 2-mL tubes of saline solution. Then, a swab unit was moistened in saline solution with a turbidity scale of 0.5 and streaked on the surface of a plate containing Mueller-Hinton agar (Kasvi[®]), on which the antimicrobial discs were arranged. The plates were incubated at 37°C for 24h and were read and interpreted according to the presence or absence of halos surrounding the drug discs. They were classified as resistant, intermediate, or sensitive.

RESULTS

Bacterial isolation

Of the 111 samples collected, 70.2% (78/111) showed growth of one or more enterobacteria. A variation in isolation rates was observed between the parrot species studied (Table 1). *Psephotus haematonotus* had the highest isolation rate, with 85% (17/20) positive samples. *Platycercus eximius, Psittacula krameri, Nymphicus hollandicus*, and *Agapornis* sp. presented isolation rates of 83.3% (5/6), 80.0% (20/25), 70.0% (14/20), and 65.0% (13/20), respectively. *Melopsittacus undulatus* had the lowest isolation rate of 45.0% (9/20).

One hundred ten strains of enterobacteria belonging to 13 different bacterial species were isolated, including Eshcerichia coli, Serratia rubidaea, Pantoea agglomerans, Serratia liquefaciens, Citrobacter freundii, Klebsiella oxytoca, Klebsiella pneumoniae, Hafnia alvei, Serratia marcescens, Cronobacter sakazakii, Shigella sp., Arizona sp., and Providencia

Table 1. Frequency of exotic psittacines raised in a domestic environment, positive for enterobacteria in cloacal swabs

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Species	Samples	Positivity		
Psephotus haematonotus	20	17 (85.0%)		
Platycercus eximius	6	5 (83.3%)		
Psittacula krameri	25	20 (80.0%)		
Nymphicus hollandicus	20	14 (70.0%)		
Agapornis sp.	20	13 (65.0%)		
Melopsittacus undulatus	20	9 (45.0%)		
TOTAL	111	78 (70.2%)		

stuartii. Among the isolated strains, *P. krameri* had the highest representation, corresponding to 29% (31/110) of the total. *P. haematonotus* represented 21.8% (24/110), *N. hollandicus* represented 19% (21/110), *Agapornis* sp. represented 14.5% (16/110), and *M. undulatus* represented 10.9% (12/110). The lowest number of isolated strains was recorded for *P. eximius* at 5.4% (6/110) (Table 2).

E. coli presented the highest isolation rate of 30% (33/110) of the samples analyzed. This was followed by *P. agglomerans* with an isolation percentage of 27.2% (30/110). *S. rubidaea* was the third most isolated at 10.9% (12/110) of the samples. *S. liquefaciens* was isolated in 10% (11/110) samples, while *H. alvei* was found in 6.3% (7/110) of samples. *K. pneumoniae* was identified in 4.5% (5/110) of the samples, followed by *C. sakazakii* in 3.6% (4/110). The bacteria *C. freundii* and *S. marcescens* were isolated in 1.8% (2/110) of the samples. *K. oxytoca, P. stuartii, Arizona* sp., and *Shigella* sp. presented an isolation percentage of 0.9% (1/110) each (Table 2).

Antimicrobial resistance

Of the 110 enterobacteria isolated, it was observed that 35.4% (39/110) were resistant to tobramycin, followed by tetracycline with 34.5% (38/110) and fosfomycin with 30.9% (34/110) resistance (Table 3). *Escherichia coli* showed the highest rate of resistance to tetracycline at 51.5% (17/33), followed by 36.6% (12/33) for tobramycin and 15.1% (5/33) for gentamicin. *P. agglomerans* showed the highest resistance rate to tetracycline and fosfomycin at 33.3% (10/30) each, followed by 30% (9/30) to tobramycin. *S. rubidaea* showed the highest resistance rate to amoxicillin with clavulanate, tetracycline, and ceftriaxone at 25% (3/12) each.

The antimicrobial meropenem had the lowest resistance rate of 0.9% (1/110), followed by ceftriaxone at 2.7% (3/110) and enrofloxacin at 6.7% (7/110). *K. oxytoca* was sensitive to all antimicrobials tested. Of the 110 strains isolated in the present study, only 24 (21.8%) did not show resistance to any of the classes of antimicrobials tested, while 32 (28.9%) showed resistance to \geq 3 classes, thus indicating multidrug resistance. One isolate stood out as it was resistant to seven classes of antimicrobials (0.9%). Among the *E. coli* isolates, six (18.1%) did not show resistance to any class, while 27 (81.6%) showed resistance to at least one class. Among the *E. coli* strains, seven (21.1%) showed multidrug resistance (Table 4).

DISCUSSION

The results of this research on bacterial growth align with previous studies that analyzed the isolation rates of enterobacteria in cloacal samples from trafficked parrots, as well as in exotic parrots kept in pet-selling establishments. These investigations showed similar results regarding the percentage of samples with growth indicative of one or more enterobacterial agents: 70.2% in this study, 71.3% according to Lopes et al. (2015), 70.0% according to Marques et al. (2021), and 98.6% according to Pascoal-Filho (2023). This demonstrates that enterobacteria in cloacal samples of parrots occur at a high rate. As the birds appear healthy, it can be assumed that these microorganisms are in balance with the intestinal microbiota without causing clinical manifestations.

The most detected microorganism in the samples of this study was *Escherichia coli*, with a prevalence of 30%. Findings

in the literature describe a wide variation in isolation rates. For example, Hidasi et al. (2013) reported 33.9%, Lopes et al. (2015) reported 46.5%, and Lima (2020) reported 73.1%. The high isolation rates are due to the commensalism of *E. coli*, which is part of the intestinal microbiota of vertebrates and one of the most well-adapted and pathologically versatile bacterial species.

The second most isolated microorganism was Pantoea agglomerans, at 27.2%. It is a ubiquitous Gram-negative bacterium that has been associated with skin and joint infections in humans (Olmos-Alpiste et al. 2022). It maintains a mutualistic relationship with plants and is not an obligatory infectious agent in humans. However, it can be a cause of opportunistic infections through mainly infection of wounds with plant material or as a hospital-acquired infection in mainly immunocompromised individuals (Dutkiewicz et al. 2016). It was the most isolated bacteria (23.5%) in samples of cloacal swabs from exotic parrots in pet stores, which were clinically healthy, according to Margues et al. (2021). There are few reports describing it as causing disease in birds. However, the isolation of this enterobacteria in exotic parrots may be related to foods of plant origin contaminated with the bacteria.

Another isolated bacterium of great relevance was *Shigella* sp., as it is a primary pathogen for humans. It was isolated from a collared parakeet (*Psittacula krameri*) specimen and is an enterobacteria frequently associated with bloody diarrheal diseases. It is also a significant cause of mortality and morbidity throughout the world (Khalil et al. 2018). The bird from which this pathogen was isolated showed no clinical manifestations. However, in vulnerable conditions that could weaken the bird's immune defenses, the presence of this pathogen could result in a potential dissemination to its flock, further increasing the risk of transmission to humans.

In this study, 78.2% of the isolated strains were resistant to at least one class of the 12 antimicrobials tested, with tobramycin, tetracycline, and fosfomycin demonstrating the highest resistance rates of 35.4%, 34.5% and 30.9%, respectively. Lopes et al. (2015) evaluated enterobacteria isolates from trafficked parrots and identified an antimicrobial resistance rate of 48.7% to tetracycline. In another study, Marques et al. (2021) analyzed parrots from pet shops in the city of Fortaleza and its metropolitan region. They found the highest rates of resistance to tetracycline (44.0%), polymyxin b (38.0%), and nalidixic acid (25.0%). In the work of Lima (2020), the highest rates of antimicrobial resistance among enterobacteria isolated from captive parrots were to sulfonamide (54.4%), followed by amoxicillin (23.4%) and tetracycline (17.7%).

In investigations related to parrots, research on the antimicrobial resistance of members of the order Enterobacterales highlights the lack of uniformity in the use of these agents, resulting in a notable variation in the substances used and in the resistance rates identified in previous studies (Lopes et al. 2015, Matias et al. 2016, Teixeira 2019, Lima 2020, Marques et al. 2021). The high rates of resistance observed in isolated bacteria indicate a need for greater caution in using these drugs. These results also highlight the relevance of the issue involving resistance to these antimicrobials, especially in the context of therapies directed at bacteria belonging to the order Enterobacterales.

An important factor in bacterial resistance to antimicrobials is the ability of bacteria to harbor resistance-related genes (Song et al. 2020, Liu et al. 2021, 2023, Wang et al. 2021). Other factors include the indiscriminate and inappropriate use of antimicrobials, lack of information among the population, the abundant use of antimicrobials in agriculture, and environmental pollution resulting from dumping drug residues in water and soil (Silva et al. 2020). It is essential that measures to combat antimicrobial resistance target the interrelationship

Isolates	Psephotus haematonotus (n=20)	Platycercus eximius (n=6)	Psittacula krameri (n=25)	Nymphicus hollandicus (n=20)	Agapornis sp. (n=20)	Melopsittacus undulatus (n=20)	Total
Escherichia coli	9	1	13	4	5	1	33 (30.0%)
Pantoea agglomerans	8	4	6	2	4	6	30 (27.2%)
Serratia rubidaea	3	-	1	5	3	-	12 (10.9%)
Serratia liquefaciens	1	-	4	3	2	1	11 (10.0%)
Hafnia alvei	1	-	-	2	1	3	7 (6.3%)
Klebsiella pneumoniae	-	1	-	3	1	-	5 (4.5%)
Cronobacter sakazakii	2	-	2	-	-	-	4 (3.6%)
Citrobacter freundii	-	-	1	1	-	-	2 (1.8%)
Serratia marcescens	-	-	1	-	-	1	2 (1.8%)
Klebsiella oxytoca	-	-	-	1	-	-	1 (0.9%)
Providencia stuartii	-	-	1	-	-	-	1 (0.9%)
Arizona sp.	-	-	1	-	-	-	1 (0.9%)
Shigella sp.	-	-	1	-	-	-	1 (0.9%)
TOTAL	24 (21.8%)	6 (5.4%)	31 (29.0%)	21 (19.0%)	16 (14.5%)	12(10.9%)	110 (100%)

Table 2. Frequency of bacteria isolated from cloacal swabs of exotic psittacines raised in a domestic environment

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	R I 12 - 36.6 - 9 - 30	I R - 1 3 - 2	<u>I</u> 2 6	R I 3 - 9
R I R	R I 12 - 36.6 - 9 - 30 -	I R - 1 3 - 2	1 2 6 2	R I 3 - 9
Escherichia - 2 4 - 3 - 1 1 5 - - 3 - 4 1 17 - coli n(%) 6 12.1 9 3 3 15.1 9 12.1 3 51.5	12 - 36.6 9 - 30	- 1 3 - 2	2 6 2	3 - 9
<i>coli</i> n(%) 6 12.1 9 3 3 15.1 9 12.1 3 51.5	36.6 9 - 30	- 2	6	9
	9 - 30	- 2	2	
Pantoea 6 - 5 - 1 1 6 4 1 2 2 - 10 -	30		2	10 1
agglomerans 20 16.6 3.3 3.3 20 13.3 3.3 6.6 6.6 33.3 n(%)		6.	6 6.6	33.3 3.3
Serratia 3 1 3 - 3 - 1 - 1 - 1 - 3 3 -	2 -	- 2	1	6 1
rubidaea 25 8.3 25 25 8.3 8.3 8.3 25 25 n(%)	16.6	16	.6 8.3	50 8.3
Serratia 6 - 1 - 2 2 1 3 3 2 -	5 -	- 1	2	5 -
liquefaciens 54.5 9 18.1 18.1 9 27.2 27.2 18.1 n(%)	45.4	9	18.1	45.4
Hafnia alvei 3 - 2 - 1 2 1 1 1 1 - 2 -	5 -		2	5 -
n(%) 42.8 28.5 14.2 28.5 14.2 14.2 14.2 14.2 28.5	71.4		28.5	5 71.4
Klebsiella 2 - - - 1 - - - pneumoniae 40 20 20 n(%)	2 - 40		1 20	2 - 40
Cronobacter - 2 2 -			-	
sakazakii 50 50 n(%)				
Citrobacter 1 - 1 - 1 1 2	1 -		1	- 1
freundii 50 50 50 50 100 n(%)	50		50	50
Serratia 2 - 1 - 1	2 -	- 1	-	1 -
marcescens 100 50 50 n(%)	100	50)	50
Klebsiella			-	
oxytoca n(%)				
Providencia 1 1 -			-	1 -
stuartii 100 100 n(%)				100
Arizona sp 1 - n(%) 100	1 - 100		-	
<i>Shigella</i> sp			-	1 - 100
TOTAL 24 3 19 - 12 2 3 2 16 - 1 8 11 3 9 1 38 - 21.9 27 172 100 18 27 10 145 00 72 10 27 14 24 24 5 24 5 24 5 10 27 10 27 10 27 10 27 10 27 10 27 10 24 5 10 24 5 10 24 5 10 24 5 10 24 5 10 24 5 10 24 5 11 3 9 1 38 - 10 24 10 24 10 24 10 24 10 24 10 24 10 24 10 24 10 24 10 24 10 24 10 24	39 -	- 7	11	35 3

Table 3. Absolute and relative (%) frequencies of antimicrobial resistance in bacteria isolated from exotic psittacines raised in a domestic environment

AMC = Amoxicillin with clavulanate, ATM = aztreonam, CRO = ceftriaxone, CLO = chloramphenicol, GEN = gentamicin, MER = meropenem, CIP = ciprofloxacin, SUT = sulfamethoxazole with trimethoprim, TET = tetracycline, TOB = tobramycin, ENO = enrofloxacin, FOS = fosfomycin, R = resistant, I = intermediate.

Table 4. Multidrug-resistant	(MDR)	Enterobacteria isolated from exotic psittacines

Tuble 1. Multiul ug resistante (MDR) Enterobacteria isolatea nom exotte psitatemes				
Number of antimicrobial classes	Strains	Escherichia coli		
0	24 (21.8%)	6 (18.1%)		
1	30 (27.2%)	12 (36.3%)		
2	24 (21.8%)	8 (24.2%)		
3	15 (13.6%)	5 (15.1%)		
4	6 (5.4%)	-		
5	6 (5.4%)	2 (6.0%)		
6	4 (3.6%)	-		
7	1 (0.9%)	-		
8	-	-		
9	-	-		
10	-	-		
TOTAL	110 (100%)	33 (100%)		

of animals, human beings, and the environment in which they live. Thus, understanding this holistic context could help formulate policies to mitigate this problem.

The antimicrobial meropenem, a member of the carbapenem class, showed the lowest resistance rate (0.9%), which deserves to be highlighted. Antimicrobials of this class encompass broadspectrum drugs, which are adopted in therapy as a response to the spread of resistance mechanisms to conventional betalactams (Brunton et al. 2006). Meropenem is restricted for use and administered parenterally in hospital settings (Mendez 2007). This restriction contributes to the low incidence of resistance as judicious prescription and diligent surveillance influence the results. Nevertheless, despite exhibiting one of the lowest rates, it is important to emphasize that its use remains limited.

The emergence of multidrug resistance in Enterobacterales makes it difficult to successfully treat possible infections caused by these pathogens. It has been well-recognized that the avian gut serves as an important reservoir for multidrugresistant bacteria (Benskin et al. 2009, Wellington et al. 2013). The multidrug resistance rate in this study was 28.9%. Lopes et al. (2015) reported a 57.8% multidrug resistance rate in samples isolated from trafficked parrots, while Lima (2020) observed nine multidrug-resistant strains out of the 117 tested strains. Hidasi et al. (2013) reported 40 samples (23.25%) with multidrug resistance conditions, which is similar to the results of the present study. The widespread overuse and abuse of antimicrobials has accelerated multidrug resistance, and AMR has become a global problem as new resistance mechanisms emerge and spread globally. As a result, the ability to treat common infectious diseases is being compromised. leading to prolonged illness and death (Lomazzi et al. 2019).

CONCLUSION

A high prevalence of enterobacteria was found in cloacal swab samples, even without evident clinical signs. Antimicrobial resistance (AMR) has been proven in several classes of antimicrobials, highlighting the need for judicious administration of these medications to exotic parrots in domestic environments. Identifying potentially pathogenic bacteria in asymptomatic birds emphasizes the importance of awareness and adoption of related hygienic practices. This study highlights the relevance of comprehensive approaches to mitigating public and animal health risks and the continued need for monitoring and research in this area.

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REFERENCES

Adeolu M., Alnajar S., Naushad S. & Gupta R.S. 2016. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. 2016. Int. J. Syst. Evol. Microbiol. 66(12):5575-5599. https://dx.doi.org/10.1099/ ijsem.0.001485> <PMid:27620848>

- Antimicrobial Resistance Collaborators 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399(10395):629-655. https://dx.doi.org/10.1016/S0140-6736(21)02724-0 <PMid:35065702>
- Arnold K.E., Williams N.J. & Bennett M. 2016. 'Disperse abroad in the land': the role of wildlife in the dissemination of antimicrobial resistance. Biol. Lett. 12(8):20160137. <https://dx.doi.org/10.1098/rsbl.2016.0137> <PMid:27531155>
- Bagai A., Kulkarni A. & Parmar M.S. 2024. Antimicrobial resistance impact on humans, p.629-642. In: Wexler P. (Ed.), Encyclopedia of Toxicology. Vol.1. Elsevier. https://dx.doi.org/10.1016/B978-0-12-824315-2.01080-0>
- Benskin C.M.H., Wilson K., Jones K. & Hartley I.R. 2009. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biol. Rev. Camb. Phil. Soc. 84(3):349-373. https://dx.doi.org/10.1111/j.1469-185X.2008.00076.x <PMid:19438430>
- Brunton L.L., Lazo J.S., Parker K.L. 2006. Goodman & Gilman: as bases farmacológicas da terapêutica. 11^ª ed. McGraw-Hill do Brasil, Rio de Janeiro. 1821p.
- Calaça K.L., Cervi R.C., Reis S.A., Nunes I.A., Jayme V.S. & Andrade M.A. 2020. Ocorrência de *Escherichia coli* em psitaciformes de cativeiro: genes de suscetibilidade e virulência a antimicrobianos. Ciênc. Anim. Bras. 21:e-60433. <https://dx.doi.org/10.1590/1809-6891v21e-60433>
- CLSI 2020. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Clinical and Laboratory Standards Institute, Wayne. 294p. (Supplement M100).
- Cupertino M.C., Resende M.B., Mayer N.A., Carvalho L.M. & Siqueira-Batista R. 2020. Emerging and re-emerging human infectious diseases: A systematic review of the role of wild animals with a focus on public health impact. Asian Pacific J. Trop. Med. 13(3):99-106. https://dx.doi.org/10.4103/1995-7645.277535
- Damborg P., Broens E.M., Chomel B.B., Guenther S., Pasmans F., Wagenaar J.A., Weese J.S., Wieler L.H., Windahl U., Vanrompay D. & Guardabassi L. 2016. Bacterial zoonoses transmitted by household pets: state-of-theart and future perspectives for targeted research and policy actions. J. Comp. Pathol. 155(1 Supl.1):S27-S40. https://dx.doi.org/10.1016/j.jcpa.2015.03.004
- Dutkiewicz J., Mackiewicz B., Lemieszek M.K., Golec M. & Milanowski J. 2016. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part III. Deleterious effects: infections of humans, animals and plants. Ann. Agric. Environ. Med. 23(2):197-205. https://dx.doi.org/10.5604/12321966.1203878 <PMid:27294620>
- Freitas A.A.R., Faria A.R., Pinto T.C.A., Merquior V.L.C., Neves D.M., Costa R.C. & Teixeira L.M. 2018. Distribution of species and antimicrobial resistance among enterococci isolated from the fecal microbiota of captive blue-fronted parrot (*Amazona aestiva*) in Rio de Janeiro, Brazil. Sci. Total Environ. 615:1428-1437. https://dx.doi.org/10.1016/j.scitotenv.2017.09.004
- Hidasi H.W., Hidasi Neto J., Moraes D.M.C., Linhares G.F.C., Jayme V.S & Andrade M.A. 2013. Enterobacterial detection and *Escherichia coli* antimicrobial resistance in parrots seized from the illegal wildlife trade. J. Zoo Wildl. Med. 44(1):1-7. https://dx.doi.org/10.1638/1042-7260-44.1.1
- Jenkins C., Rentenaar R.J., Landraud L. & Brisse S. 2017. Enterobacteriaceae. In: Ibid. (Eds), Infectious Diseases. Vol.1-2. Elsevier, Amsterdam, p.1565-1578.
- Keshmiri M.A., Nemati A., Askari Badouei M., Ashrafi Tamai I. & Zahraei Salehi T. 2022. Clonal relatedness and antimicrobial susceptibility of *Salmonella* serovars isolated from humans and domestic animals in Iran: a one health

perspective. Iran. J. Vet. Res. 23(2):104-110. https://dx.doi.org/10.22099/10/20022.40594.5881

Khalil I.A., Troeger C., Blacker B.F., Rao P.C., Brown A., Atherly D.E., Brewer T.G., Engmann C.M., Houpt E.R., Kang G., Kotloff K.L., Levine M.M., Luby S.P., MacLennan C.A., Pan W.K., Pavlinac P.B., Platts-Mills J.A., Qadri F., Riddle M.S., Ryan E.T., Shoultz D.A., Steele A.D., Walson J.L., Sanders J.W., Mokdad A.H., Murray C.J.L., Hay S.I. & Reiner Jr. R.C. 2018. Morbidity and mortality due to shigella and enterotoxigenic *Escherichia coli* diarrhoea: the Global Burden of Disease Study 1990-2016. Lancet Infect. Dis. 18(11):1229-1240. https://dx.doi.org/10.1016/S1473-3099(18)30475-4

Kotloff K.L., Riddle M.S., Platts-Mills J.A., Pavlinac P. & Zaidi A.K.M. 2018. Shigellosis. Lancet 391(10122):801-812. https://dx.doi.org/10.1016/s0140-6736(17)33296-8 PMid:29254859

- Legadevi R., Dillibabu V., Karunakaran N., Nagarajan K. & Soundararajan C. 2019. Isolation and identification of bacteria of normal flora in handfed exotic psittacine birds at Tamil Nadu, India. Ind. J. Vet. & Anim. Sci. Res. 48(2):26-34.
- Lima B.P. 2020. Isolamento, tipificação e perfil de sensibilidade de enterobactérias oriundas de psitacídeos de zoológicos e criadouros comerciais do estado do Ceará, Brasil. Dissertação de Mestrado, Universidade Estadual do Ceará, Fortaleza. 74p.
- Liu C., Feng C., Duan Y., Wang P., Peng C., Li Z., Yu L., Liu M. & Wang F. 2023. Ecological risk under the dual threat of heavy metals and antibiotic resistant *Escherichia coli* in swine-farming wastewater in Shandong Province, China. Environ. Pollut. 319:120998. <https://dx.doi.org/10.1016/j. envpol.2022.120998> <PMid:36603760>
- Liu C., Wang P., Dai Y., Liu Y., Song Y., Yu L., Feng C., Liu M., Xie Z., Shang Y., Sun S. & Wang F. 2021. Longitudinal monitoring of multidrug resistance in *Escherichia coli* on broiler chicken fattening farms in Shandong, China. Poult. Sci. 100(3):100887. https://dx.doi.org/10.1016/j.psj.2020.11.064
- Liu L., Qin L., Hao S., Lan R., Xu B., Guo Y., Jiang R., Sun H., Chen X., Lv X., Xu J. & Zhao C. 2020. Lineage, antimicrobial resistance and virulence of *Citrobacter* spp. Pathogens 9(3):195. https://dx.doi.org/10.3390/ pathogens9030195> <PMid:32155802>
- Lomazzi M., Moore M., Johnson A., Balasegaram M. & Borisch B. 2019. Antimicrobial resistance – moving forward? BMC Public Health 19:858. https://dx.doi.org/10.1186/s12889-019-7173-7

 PMid:31266477>
- Lopes E.S., Maciel W.C., Albuquerque A.H., Machado D.N., Bezerra W.G.A., Vasconcelos R.H., Lima B.P., Gonçalves G.A.M. & Teixeira R.S.C. 2015. Prevalence and antimicrobial resistance profile of enterobacteria isolated from psittaciformes of illegal wildlife trade. Acta Scient. Vet. 43:1313.
- Luca C., Niero G., Cattarossi D., Bedin M. & Piccirillo A. 2018. Pet and captive birds as potential reservoirs of zoonotic bacteria. J. Exot. Pet. Med. 27(1):17-20. https://dx.doi.org/10.1053/j.jepm.2017.10.017
- Magiorakos A.-P., Srinivasan A., Carey R.B., Carmeli Y., Falagas M.E., Giske C.G., Harbarth S., Hindler J.F., Kahlmeter G., Olsson-liljequist B., Paterson D.L., Rice L.B., Stelling J., Struelens M.J., Vatopoulos A., Weber J.T. & Monnet D.L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18(3):268-281. <https:// dx.doi.org/10.1111/j.1469-0691.2011.03570.x> <PMid:21793988>
- Marques A.R., Lima B.P., Teixeira R.S.C., Albuquerque Á.H., Lopes E.S., Maciel W.C., Beleza A.J.F. & Alencar T.R. 2021. Zoonotic bacteria research and analysis of antimicrobial resistance levels in parrot isolates from pet shops in the city of Fortaleza, Brazil. Pesq. Vet. Bras. 41:e06837. https://dx.doi.org/10.1590/1678-5150-pvb-6837>
- Matias C.A.R., Pereira I.A., Reis E.M.F., Rodrigues D.P. & Siciliano S. 2016. Frequency of zoonotic bacteria among illegally traded wild birds in Rio de Janeiro. Braz. J. Microbiol. 47(4):882-888. https://dx.doi.org/10.1016/j. bjm.2016.07.012> <PMid:27528081>

- Mendez A.S.L. 2007. Estudo de estabilidade do antibiótico meropenem. Tese de Doutorado, Universidade Federal do Rio Grande do Sul, Porto Alegre. 246p.
- Mirtella D., Fedeli P., Scendoni R., Cannovo N. & Cingolani M. 2021. Case of nosocomial outbreak of *Pantoea agglomerans* related to parenteral nutrition procedures. Healthcare 9(6):684. https://dx.doi.org/10.3390/ healthcare 9060684> <PMid:34200170>
- Nupur M.N., Afroz F., Hossain M.K., Harun-ur-Rashid S.M., Rahman M.G., Kamruzzaman M., Ferdous K.A. & Haque M.A. 2023. Prevalence of potential zoonotic bacterial pathogens isolated from household pet birds and their antimicrobial profile in northern Bangladesh. Agrobiol. Rec. 11:28-38. <https://dx.doi.org/10.47278/journal.abr/2023.005>
- Olmos-Alpiste F, Ezquerra G.M. & Pujol R.M. 2022. Wound infection by *Pantoea* agglomerans after penetrating plant injury. Ind. J. Dermatol. Venereol. Leprol. 88(5):633-635. <https://dx.doi.org/10.25259/IJDVL_1069_19> <PMid:33666040>
- Ouakrim D.A., Cassini A., Cecchini M. & Plachouras D. 2020. The health and economic burden of antimicrobial resistance, p.23-44. In: Anderson M., Cecchini M. & Mossialos E. (Eds), Challenges to Tackling Antimicrobial Resistance. Cambridge University Press, Cambridge. https://dx.doi.org/10.1017/9781108864121.003
- Pakbin B., Brück W.M. & Rossen J.W.A. 2021. Virulence factors of enteric pathogenic *Escherichia coli*: a review. Int. J. Mol. Sci. 22(18):9922. <https://dx.doi.org/10.3390/ijms22189922> <PMid:34576083>
- Pascoal-Filho N.M. 2023. Prevalência de enterobactérias e perfil de resistência a antimicrobianos em psitacídeos oriundos do tráfico no estado do Ceará, Brasil. Dissertação de Mestrado, Universidade Estadual do Ceará, Fortaleza. 66p.
- Pontes P.S., Coutinho S.D.A., Iovine R.O., Cunha M.P.V., Knöbl T. & Carvalho V.M. 2018. Survey on pathogenic *Escherichia coli* and *Salmonella* spp. in captive cockatiels (*Nymphicus hollandicus*). Braz. J. Microbiol. 49(1 Supl.1):76-82. https://dx.doi.org/10.1016/j.bjm.2018.05.003 eMid:30170962
- Silva R.A., Oliveira B.N.L., Silva L.P.A., Oliveira M.A. & Chaves G.C. 2020. Resistência a Antimicrobianos: a formulação da resposta no âmbito da saúde global. Saúde Debate 44(126):607-623. https://dx.doi.org/10.1590/0103-1104202012602>
- Singh L., Cariappa M.P. & Kaur M. 2016. *Klebsiella oxytoca*: an emerging pathogen? Med. J. Armed Forces India 72(Supl.1):S59-S61. https://dx.doi.org/10.1016/j.mjafi.2016.05.002 <a href="https://dx.doi
- Song X., Teng H., Chen L. & Kim M. 2018. Cronobacter species in powdered infant formula and their detection methods. Kor. J. Food. Sci. Anim. Resour. 38(2):376-390. https://dx.doi.org/10.5851/kosfa.2018.38.2.376
- Song Y., Yu Y., Zhang Y., Dai P., Wang C., Feng M., Liu S., Sun S., Xie Z. & Wang F. 2020. Prevalence and characteristics of multidrug-resistant mcr-1-positive *Escherichia coli* isolates from broiler chickens in Tai'an, China. Poult. Sci. 99(2):1117-1123. https://dx.doi.org/10.1016/j.psj.2019.10.044
- Teixeira R.H.F. 2019. Ocorrência de circovírus, enterobactérias e endoparasitos em psitacídeos exóticos. Tese de Doutorado, Universidade Estadual Paulista, São Paulo. 81p.
- Wang W., Yu L., Hao W., Zhang F., Jiang M., Zhao S. & Wang F. 2021. Multi-Locus sequence typing and drug resistance analysis of swine origin *Escherichia coli* in Shandong of China and its potential risk on public health. Front. Public Health 9:780700. https://dx.doi.org/10.3389/fpubh.2021.780700 PMId:34926393 https://dx.doi.org/10.3389/fpubh.2021.780700 https://dx.doi.org/10.389/fpubh.2021.780700 https://dx.doi.org/10.389/fpubh.2021.780700 https://dx.doi.38070<
- Wellington E.M.H., Boxall A.B., Cross P., Feil E.J., Gaze W.H., Hawkey P.M., Johnson-Rollings A.S., Jones D.L., Lee N.M., Otten W., Thomas C.M. & Williams A.P. 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect. Dis. 13(2):155-165. https://dx.doi.org/10.1016/S1473-3099(12)70317-1

- WHO 2021. Antimicrobial resistance. World Health Organization. Available at <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> Accessed on Jul 12, 2023.
- WHO 2022. One health. World Health Organization. Available at <https://www. who.int/health-topics/one-health#tab=tab_1> Accessed on Nov. 13, 2023.
- Wieler L.H., Ewers C., Guenther S., Walther B. & Lübke-Becker A. 2011. Methicillin-resistant staphylococci (MRS) and extended-spectrum betalactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int. J. Med. Microbiol.

301(8):635-641. <https://dx.doi.org/10.1016/j.ijmm.2011.09.009> <PMid:22000738>

- Winn Jr. W., Allen S., Janda W., Koneman E., Procop G., Schreckenberg P. & Woods G. 2008. Diagnóstico Microbiológico: texto e atlas colorido. 6ª ed. Guanabara Koogan, Rio de Janeiro. 1760p.
- Yin Z., Yuan C., Du Y., Yang P., Qian C., Wei Y., Zhang S., Huang D. & Liu B. 2019. Comparative genomic analysis of the *Hafnia* genus reveals an explicit evolutionary relationship between the species *alvei* and *paralvei* and provides insights into pathogenicity. BMC Genomics 20(1):768. https://dx.doi.org/10.1186/s12864-019-6123-1 https://dx.doi.org/10.1186/s12864-019-6123-1 https://dx.doi.org/10.1186/s12864-019-6123-1 https://dx.doi.org/10.1186/s12864-019-6123-1