

Companion psittacine birds as reservoir of gentamicin and vancomycin-resistant *Enterococcus* spp.¹

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ABSTRACT.- Cabral B.G., Davies Y.M., Menão M.C., Saidenberg A.B.S., Gomes V.T.M., Moreno L.Z., Sato M.I.Z., Moreno A.M. & Knöbl T. 2020. **Companion psittacine birds as reservoir of gentamicin and vancomycin-resistant *Enterococcus* spp.** *Pesquisa Veterinária Brasileira* 40(2):129-133. Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenida Prof. Dr. Orlando Marques de Paiva 87, Vila Universitária, São Paulo, SP 05508-270, Brazil. E-mail: tknobl@usp.br

Enterococcus are recognized worldwide as significant nosocomial agents that have been continuously evolving to adapt to different niches and acquire resistance to several antibiotic classes. Vancomycin and gentamicin-resistant strains of *E. faecalis* and *E. faecium* have been associated with nosocomial human infections. Some epidemiological studies suggest the participation of pets as reservoirs of vancomycin and gentamicin-resistant *Enterococcus* strains. However, the role of companion birds as reservoirs of these strains has been poorly studied. In this study, 126 psittacine birds were evaluated and 26.9% carried *Enterococcus* spp., including the species *E. faecalis*, *E. faecium*, *E. hirae*, *E. phoeniculicola*, *E. gallinarum* and *E. casseliflavus*. The antibiotic resistance profile showed four high-level gentamicin-resistance (HLGR) strains. In addition, two strains presented intermediate levels of vancomycin resistance. Resistant strains were isolated from fecal and oropharynx samples of sick and clinically healthy birds, suggesting that psittacine birds may act as reservoirs of HLGR *Enterococcus* spp. However, sick birds appear to be more implicated in the enterococci transmission than healthy birds.

INDEX TERMS: Psittacine birds, gentamicin, vancomycin, resistance, *Enterococcus* spp., avian diseases, antibiotic resistance, parrots, birds, wildlife animals, zoonosis.

RESUMO.- [Psitacídeos de companhia como reservatórios de *Enterococcus* spp. gentamicina e vancomicina-resistentes.]

Enterococcus são reconhecidos mundialmente como significantes agentes nosocomiais, que têm continuamente se adaptado a diferentes nichos e adquirido resistência a várias classes de antibióticos. Cepas de *E. faecalis* e *E. faecium* vancomicina e gentamicina-resistentes têm sido associadas a infecções nosocomiais em humanos. Alguns estudos epidemiológicos sugerem a participação de aves como reservatórios de cepas de *Enterococcus* vancomicina e gentamicina-resistentes. Entretanto, a relação das aves de companhia como reservatórios destas cepas

tem sido pouco estudada. Neste estudo, 126 psitacídeos foram avaliados, e 26,9% destes eram portadores de *Enterococcus* spp., incluindo as espécies *E. faecalis*, *E. faecium*, *E. hirae*, *E. phoeniculicola*, *E. gallinarum* e *E. casseliflavus*. O perfil de resistência antibiótica mostrou quatro cepas com alto nível de resistência a gentamicina (ANRG). Além de duas cepas com nível intermediário de resistência a vancomicina. As cepas resistentes foram isoladas de amostras fecais e de orofaringe de aves doentes e clinicamente saudáveis, sugerindo que psitacídeos podem estar atuando como reservatórios para *Enterococcus* spp. com ANRG. Contudo, Aves doentes parecem estar mais relacionadas à transmissão de enterococcus, do que aves saudáveis.

TERMOS DE INDEXAÇÃO: Psitacídeos, *Enterococcus* spp., gentamicina, vancomicina, doenças aviárias, resistência antimicrobiana, psitacídeos, pássaros, animais selvagens, zoonoses.

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INTRODUCTION

Enterococcus infections associated with healthcare settings emerged in the 1970s and 1980s as a leading cause of antibiotic-resistant infections such as bacteraemia and urinary tract infection (UTIs) (Gilmore et al. 2013). These bacteria generally present low virulence potential as evidenced by their presence in enteric microbiota of healthy humans and animals (Arias & Murray et al. 2012, Hammerum 2012, Gilmore et al. 2013). Despite this, the importance of *Enterococcus* infections is highlighted by the intrinsic resistance to first-line antimicrobial agents (e.g. low-level resistance to β -lactams and aminoglycosides) and the capacity to acquire resistance to several other antimicrobial agents, including last resort antibiotics (glycopeptide) (van den Bogaard & Stobberingh, 2000, Hammerum et al. 2010).

Starting in 1979, the frequent finding of high-level gentamicin resistance (HLGR) in isolates from clinical conditions led to further challenges for the treatment of life-threatening enterococcal infections. The only new effective antibiotic available at that time was developed in the 1990s in the form of vancomycin. Nevertheless, throughout the last decades there has been a rising prevalence of vancomycin-resistant *Enterococcus* (VRE) (Woodford et al. 1998). VRE were reported as the second most common cause of human nosocomial infections in the USA, being responsible for up to approximately 25,000 deaths each year (McKinnell et al. 2012). However, the prevalence of VRE in South America is still relatively low, although the clonality of the strains follows the pattern described in other parts of the world with trends to become more disseminated (Panesso et al. 2010).

Several studies have been conducted concerning the epidemiology of antimicrobial resistance between production animals and humans, and the role of growth promoters in livestock resulting in drug-resistant strains which might have zoonotic potential (Hammerum 2012). In the domestic environment, pets such as dogs and cats are reported as potential reservoirs for resistant microorganisms to humans (Guardabassi et al. 2004, Gülhan et al. 2006, Iseppi et al. 2015). It has been shown that resistant bacteria that colonize the intestinal tract of healthy pets could represent a risk to humans as well as transmitting their resistance genes located on mobile genetic elements to the human intestinal microbiota. Similarly, companion pets can get colonized by resistant bacteria after human contact (Simjee et al. 2002, De Leener et al. 2005).

Free-ranging wild birds have also been identified as possible reservoirs for vancomycin and gentamicin antimicrobial-resistant enterococci isolates (Oravcova et al. 2013, Roberts et al. 2016). Recently, Freitas et al. (2018) reported the identification of captive blue-fronted-parrot (*Amazona aestiva*) as potential zoonotic sources of multidrug-resistant *Enterococcus* spp. in two wild animal screening centers. Parrots are popular pets throughout the world. Their close contact with humans in captivity may lead to the transmission of several microorganisms with zoonotic potential (Boseret et al. 2013). We aimed to address this question by identifying enterococci species isolated from psittacine birds maintained as companion pets, both from clinical cases and asymptomatic individuals, establishing the phenotypic antibiotic resistance profile to gentamicin and vancomycin.

MATERIALS AND METHODS

Ethics statement. This study was approved by Ethics Committee of “Universidade de São Paulo” and authorized for scientific purposes (5174111215).

Samples. A total of 126 captive psittacine pet birds belonging to 13 species from the state of São Paulo (Brazil) were sampled in this study (Table 1). The sampled birds included native and exotic adult psittacine kept as companion pets. The age and gender were undetermined, as well the history of antimicrobial therapeutic use and the contact with other species. The collection of birds were grouped into apparently healthy animals (n=109) and sick birds (with respiratory disease, n=17). Cloacal and oropharynx swabs were collected from asymptomatic individuals and sick birds.

Isolation and identification of *Enterococcus*. The samples were collected with sterile swabs and inoculated in BHI broth (Brain heart-infusion) and incubated at 37°C for 18 hours. Standard culture procedures were performed using Columbia agar with 5% sheep blood and Chromagar VRE culture media (OXOID™, Basingstoke, UK). Isolated colonies were screened according to Gram stain morphology and identified through MALDI-TOF MS technique (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry); the acquired spectra were loaded in MALDI BioTyper™ 3.0 software (Bruker Daltonik) and compared to the manufacturer database accepting scores ≥ 2.0 for species identification. Also, the software Graph Pad was employed to compare the health and sick groups by Fisher’s Exact Test 84 (with 95% confidence interval and $p < 0.05$).

Phenotypic resistance profile. The phenotypic resistance profile was determined using the disc diffusion technique for high-concentration gentamicin (120 μ g) and vancomycin (30 μ g), following the recommendations of the Clinical Laboratory Standards Institute (CLSI 2019).

Table 1. Species of *Enterococcus* spp. isolated from psittacine birds kept as pet in São Paulo, Brazil, 2018

Psittacine birds	<i>Amazona Aestiva</i> n=80	<i>Amazona amazonica</i> n=7	<i>Anodorhynchus hyacinthinus</i> n=3	<i>Amazona xanthops</i> n=1	<i>Nymphicus hollandicus</i> n=25	<i>Pionus maximiliani</i> n=1	Total of <i>Enterococcus</i> spp. strains
<i>E. faecalis</i>	9	1	2	1	2		15
<i>E. hirae</i>	10	2					12
<i>E. faecium</i>	4					1	5
<i>E. phoeniculicola</i>		2					2
<i>E. galinarum</i>	1						1
<i>E. casseliflavus</i>	1						1
TOTAL	25	5	2	1	2	1	36

Enterococcus spp. were not isolated in the following species of birds: *Amazona rhodocorytha* (n=1), *Psittacus erithacus* (n=1), *Alisterus scapularis* (n=1), *Psittacula krameria* (n=1), *Ectetus roratus* (n=1), *Cacatuidae* (n=3) and *Loriinae* (n=1).

RESULTS

Enterococci isolates

Enterococci isolates (36 in total) were obtained from 34 birds (26.9%). Isolates were obtained statistically more frequently from the 17 birds presenting respiratory disease (70.58%) than from the 109 healthy birds (22.01%) (p values 0.0001).

Enterococci species

Enterococcus faecalis was the most frequent specie identified (15/36), followed by *E. hirae* (12/36), *E. faecalis* (5/36), *E. phoeniculicola*, (2/36), *E. gallinarum* (1/36), and *E. casseliflavus* (1/36), (Table 1). In clinically healthy birds *E. hirae* was the most prevalent species (11/24), followed by *E. faecalis* (9/24), *E. faecium* (3/24) and *E. phoeniculicola* (1/24). However, among birds with respiratory disease the species *E. faecalis* and *E. faecium* were more prevalent, with 6/12 and 2/12 strains, respectively. The species *E. gallinarum* (1/12), *E. casseliflavus* (1/12) *E. hirae* (1/12) and *E. phoeniculicola* (1/12) were also detected in sick birds.

Antibiogram results

The antibiogram results are presented in Table 2. The analysis of the 36 *Enterococcus* spp. isolates revealed that 4 strains presented high-level gentamicin resistance (HLGR). Two HLGR strains were identified as *E. faecalis* and the other two as *E. faecium*. In addition, resistant strains were identified in both healthy and sick birds.

A total of two isolates of *E. faecalis* and *E. hirae* showed intermediate levels of resistance to vancomycin (Table 2). The intrinsic resistance of *E. gallinarum* was disregarded. No concomitant resistance among gentamicin high level and vancomycin was found among these isolates.

DISCUSSION

The results of our study showed that 26.9% of 126 sampled birds presented *Enterococcus* spp. However, while the healthy birds presented a frequency of 22.01% of *Enterococcus* spp., the sick birds presented 70.58%. The species identified were *E. faecalis*, *E. hirae*, *E. faecium*, *E. phoeniculicola*, *E. gallinarum* and *E. casseliflavus* (Table 1). In healthy birds, the most frequently isolated species in our study was *E. hirae* (10.09%). *E. hirae* has been described as microbiota of asymptomatic parrots as well as associated with disease in parrots; however, it seems to be rarely isolated from humans (Devriese et al. 1995, Freitas et al. 2018). The most prevalent species among symptomatic individuals in our study were *E. faecalis* and *E. faecium* (Table 1). In avian species, it has been shown that *E. faecium* can be a cause of septicemia and that *Enterococcus faecalis* can also be associated with amyloid arthropathy (Shivaprasad 2014). Here, we report a frequency of 35.2% for

E. faecalis and 11.7% for *E. faecium* in psittacine birds with respiratory disease, and 8.25% for *E. faecalis* and 2.75% for *E. faecium* from asymptomatic birds.

It is now suggested that *E. faecium* from animal origin are less likely to be transferred to humans, but they may still represent a risk due to the transfer of resistance genes to virulent enterococci while *E. faecalis* is a more capable zoonotic agent with the same clones being recovered from human and animal sources (Hammerum et al. 2010). *E. faecalis* has also been strongly suggested as a zoonotic agent that can cause urinary-tract infections in humans either by having close contact working with pig, poultry or by ingestion of pork or poultry meat (Larsen et al. 2010, Poulsen et al. 2012, Abat et al. 2016).

De Leener et al. (2005) studied enterococci isolated from dogs and cats and determined that HLGR was present in rectal swab samples besides resistance to several other antibiotics (De Leener et al. 2005). The authors concluded that the enterococci present in the intestinal microbiota of pets might act as a reservoir of resistance genes for humans and animals alike (De Leener et al. 2005). In dogs, Simjee et al. (2002) identified a high prevalence of HLGR *E. faecium* (54%) and a sole vancomycin-resistant *E. faecium* strain (Simjee et al. 2002).

Data on the occurrence and epidemiology of HLGR in captive psittacine pet birds are very scarce. Freitas et al. (2018) reported a survey with 88 captive blue-fronted parrots (*Amazona aestiva*) and highlight the high proportion (48%) of multidrug-resistant enterococci, but only one strain of *E. faecalis* were classified as HLGR (Freitas et al. 2018). Our results showed that four strains (*E. faecalis*/*E. faecium*) were classified as HLGR (Table 2). HLGR is frequently found in association with the bifunctional aminoglycoside modifying enzyme AAC6'-APH2" (Hammerum et al. 2010, Arias & Murray 2012). The combined use of gentamicin with β -lactam antibiotics or glycopeptides is of high importance for treating severe enterococci infections in humans. Still, this synergistic effect is lost if high-level aminoglycoside resistance is present. In the present study, we report 11.1% of the *Enterococcus* isolates presented resistance to high level gentamicin (120 μ g), which is of concern regarding the zoonotic potential of these isolates. Aminoglycosides are often used in specific conditions of pet birds, in particularly infections of the respiratory tract that are difficult to eradicate. Therefore, it is imperative to carefully consider this type of resistance when deciding to prescribe aminoglycosides treatments in companion birds (van den Bogaard & Stobberingh 2000, Arias & Murray 2012).

The hypothesis that animals could also be sources of zoonotic VRE has been explored in several studies (Woodford et al. 1998, Stobberingh et al. 1999, van den Bogaard & Stobberingh, 2000, Gülhan et al. 2006, Arias & Murray 2012, Simjee et al. 2002). Concerns for its use as a feed additive in livestock

Table 2. Antimicrobial resistance of *Enterococcus* spp. isolated from pet parrots in São Paulo, Brazil, 2018

Birds	<i>Enterococcus</i> species	High level gentamicin	Vancomycin
<i>Amazona amazonica</i>	<i>E. hirae</i>		Intermediary
<i>Anodorhynchus hyacinthinus</i>	<i>E. faecalis</i>	Resistant	
<i>A. hyacinthinus</i>	<i>E. faecalis</i>		Intermediary
<i>Nymphicus hollandicus</i>	<i>E. faecalis</i>	Resistant	
<i>N. hollandicus</i>	<i>E. faecium</i>	Resistant	
<i>Pionus maximiliani</i>	<i>E. faecium</i>	Resistant	

leading to cross-resistance between avoparcin and an analogue vancomycin resulted in the ban of its use in agriculture. These measures were organized by the European Union and followed by several countries around the world, including Brazil, after the first report of VRE in 1998 (Hammerum et al. 2010). Unlike several countries around the world, the USA has never allowed the use of avoparcin in livestock and the observed resistance to vancomycin in that country was in the past related almost exclusively to hospital settings (Guardabassi et al. 2004). However, a shift has been noticed and several studies in the USA have also shown the presence of VRE in migrating and synanthropic birds as well as in pet dogs and cats. These reports suggest that the acquisition of resistant enterococci in non-livestock sources might be linked to human sources either as environmental pollution or due to direct close contact (Guardabassi et al. 2004, Gülhan et al. 2006, Roberts et al. 2016). Another possible cause for the presence of pets carrying vancomycin resistance is the co-selection of VRE due to the use of other antimicrobials such as tetracyclines, macrolides and aminoglycosides. Indeed, VRE isolates in pets are also frequently found showing resistance to these antibiotics classes (Guardabassi et al. 2004). In the current study, we have not identified VRE, but one strain of *E. faecalis* and one of *E. hirae* presented intermediate resistance to this antibiotic (Table 2).

There are no other studies at present showing the contribution of pet birds as reservoirs for resistant *Enterococcus* spp. and this work shows the possible role that these animals may have as important, yet underrated, infection sources in the domestic environment.

CONCLUSIONS

We describe in this study that 26% of companion birds were colonized by *Enterococcus* spp., with *E. faecalis* and *E. faecium* being most frequently detected in sick birds.

High levels of gentamicin resistance isolates (4/36) were detected in sick and apparently healthy birds. Intermediary resistance to vancomycin was found among two isolates (2/36).

The data here presented show the potential that pet birds have as possible reservoirs for resistant gentamicin *Enterococcus* spp., which is of grave concern regarding the maintenance of these birds as companion animals, although the risks regarding the capacity to transmit these isolates and/or of their mobile genetic material remains to be further evaluated.

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Conflict of interest statement.- The authors have no competing interests.

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