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Original Study

Antimicrobial Sensitivity Profile in Psittacine Birds at an Avian Teaching Hospital: A Retrospective Study, 2015-2022

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Abstract: Veterinary hospitals house patient populations with diverse infectious statuses, microbiota, and histories of prior antibiotic therapy. Choanal swabs are commonly used for assessing the upper respiratory tract of birds for bacterial disease, with the samples submitted for cytologic testing and/or culture and antimicrobial sensitivity testing. The aim of this retrospective study was to identify and quantify bacteria isolated from choanal swabs collected from psittacine patients at a veterinary teaching hospital in Mexico City, Mexico. Data regarding bacterial isolates from choanal swabs were obtained from the medical records of companion psittacines suspected of upper respiratory bacterial disease that presented between November 2015 and December 2022. A total of 47.8% (175 of 366) of the bacterial isolates were from specimens obtained from red-lored Amazons (*Amazona autumnalis*). Gram-negative bacteria predominated, with 27 different genera identified. *Klebsiella*, *Staphylococcus,* and *Escherichia* were the most frequently isolated genera. A total of 90.4% (331 of 366) of the isolates were resistant to at least 1 antibiotic tested in the sensitivity panel, and a single *Klebsiella* isolate was resistant to 13 different antibiotics. Gentamicin had a high percentage of efficacy (79.5%; 182 of 229) against the bacterial isolates, whereas isolates tested against sulfonamide-trimethoprim (46.7%, 98 of 210), streptomycin (43.8%; 88 of 201), and clindamycin (12.9%; 15 of 116) had susceptibilities $<50\%$. This is the first study to report common bacterial isolates and their antimicrobial susceptibility patterns from choanal swab samples collected from companion psittacines suspected of upper respiratory disease in Mexico. Clinicians can use the information presented in this study as a guide for therapeutic decision-making when managing upper respiratory bacterial infections in companion psittacine patients.

Key words: antimicrobial resistance, bacterial culture, choanal swab, avian, *Amazona autumnalis*, red-lored Amazon parrot

INTRODUCTION

Antimicrobial resistance is the ability of bacteria, parasites, viruses, and fungi to grow and spread in the presence of anti-infective drugs that are normally active against them.[1] Antimicrobial resistance is a natural process that has been observed since the first antibiotics were developed, and the genes that confer drug resistance in some strains of bacteria date back millions of years before the use of antibiotics.[2] Antimicrobial resistance has become an increasingly serious problem in recent years due to the excessive use of antimicrobials, which has increased the rate at which resistance develops and spreads.[1,2]

Large veterinary teaching hospitals house a transitory population of animals with diverse resident microbiota, infectious disease statuses, and previous histories of exposure to antibiotics. The most recent sustainable practices against antimicrobial resistance in veterinary medicine encourage prescribing antibiotics only when necessary and avoiding prophylactic use. Furthermore, a sample for culture should be collected before starting antibacterial therapy whenever possible.[3]

Choanal swabs are commonly used for assessing the upper respiratory tract of birds for bacterial disease, and

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as a helpful tool for screening healthy birds.[4] Samples obtained from the choana can be submitted for cytology, culture, and antimicrobial sensitivity testing. Gram-positive cocci and rods are common findings in healthy birds, and Gram-negative bacteria are scarce when this technique is performed in birds without signs of disease.[4] Therefore, an increased presence of Gram-negative bacteria may indicate a primary bacterial disease of the upper respiratory tract, or an overgrowth of these organisms secondary to other pathologic processes.[5] Because antibiotics are frequently used for the treatment of bacterial infections in psittacine birds, antimicrobial sensitivity testing should be pursued for any concerning isolates. If this is not possible, or drug selection must occur before the test results are known, then historical susceptibility data can be used to guide empirical drug selection.[6]

The objectives of this study were to 1) identify and quantify the bacteria isolated from choanal swabs collected from companion psittacines presented to a veterinary teaching hospital in Mexico and 2) characterize the antimicrobial susceptibility patterns of these bacterial isolates. To the authors' knowledge, there have been no studies pursuing this type of research in Mexico. Our hypotheses for this study were that 1) the choanal swabs would reveal predominantly Gram-negative bacterial isolates from the psittacine birds suspected of respiratory disease of the upper respiratory tract, and that these bacteria would presumably be involved in the etiology of the disease; and 2) due to their excellent spectrum against Gram-negative bacteria, we expected that the aminoglycosides would stand out with a greater efficacy against the isolated bacteria.

MATERIALS AND METHODS

The bacterial data used for this study were obtained from the medical records (SmartZooft, Squenda, Mexico City, Mexico) of companion psittacines presented to the Hospital de Aves de Ornato, Compañía y Silvestres (Facultad de Medicina Veterinaria y Zootecnia of Universidad Nacional Autónoma de México; HAOCS-UNAM). The medical record search included cases from November 6, 2015, to December 7, 2022. Aerobic bacterial culture and antimicrobial sensitivity testing were performed by the microbiology laboratory service at the university. Aerobic bacterial culture was performed with blood agar (MCD LAB, Tlalnepantla, Mexico) and McConkey agar (Becton Dickinson and Company, Madrid, Spain). This laboratory used a Kirby-Bauer disc-diffusion method (Becton Dickinson and Company), but the number and type of antibiotics evaluated in each panel were not under the control of the authors. Only avian patients that had a positive bacterial culture that was successfully characterized to genus were included in the analysis.

The following data were obtained from the laboratory report in the medical record: bird species, genus of bacteria, and antimicrobial sensitivity test results. For data processing, bacterial isolates with intermediate responses to antibiotics were considered resistant. A database (Microsoft Excel 15.31, Microsoft Corporation, Redmond, WA, USA) was constructed to house the collected information. Each bacterial isolate was considered a unit of analysis because there were patients with more than 1 genus of bacteria isolated from the sample.

RESULTS

A total of 366 different bacterial isolates were obtained from 172 choanal swabs collected from companion psittacines suspected of having upper respiratory disease. The total number of avian patients that were presented to the hospital during the study period were distributed in different taxonomical orders as follows: Psittaciformes (n = 172), Galliformes (n = 17), Passeriformes (n = 3), Anseriformes (n = 2), Strigiformes (n = 1), and Columbiformes (n = 1).

A total of 47.8% (175 of 366) of the bacterial isolates were obtained from red-lored Amazon parrots (*Amazona autumnalis*; n = 77 patients), which, according to the HAOCS-UNAM medical records, is the most frequently treated species at the clinic. The second most frequently treated species was the white-fronted Amazon parrot (*Amazona albifrons*; n = 17; n = 38 bacterial isolates). Overall, 21 different psittacine species were included in this study, including other Amazon parrots (*Amazona* spp.), cockatiels (*Nymphicus hollandicus*), budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis* spp.), and Pionus parrots (*Pionus* spp.).

Twenty-seven different genera of bacteria were isolated from the birds, and *Klebsiella* spp. (n = 69; 18.9%; 95% CI, 14.9–22.9%), *Staphylococcus* spp. (n = 51; 13.9%; 95% CI, 10.3–17.4%), and *Escherichia* spp. (n = 49; 13.4%; 95% CI, 9.9–16.9%) were the most frequently isolated genera (Table 1). Based on the 95% confidence intervals, *Klebsiella* was more frequently isolated than all genera except for *Staphylococcus* and *Escherichia*. These latter 2 genera were also more common than all others except *Pseudomonas*. Regarding their staining affinity, Gram-negative bacteria (258 of 366; 70.5%; 95% CI, 65.8–75.2%) were more commonly isolated than Gram-positive bacteria (108 of 366; 29.5%; 95% CI, 24.8–34.2%). As mentioned previously, more than 1 genus of bacteria was isolated from some patient swabs; 81 samples (47.1%) had 2 different genera

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Table 1. Genera of bacteria (n = 366) isolated from choanal swabs in companion psittacines presented to the Universidad Nacional Autónoma de México, Mexico City, Mexico. Based on the 95% CIs, *Klebsiella* was more frequently isolated than all species except for *Staphylococcus* and *Escherichia*. These latter 2 bacteria were also more common than all species except *Pseudomonas*.

Genus	Number of isolates	%	95% CI
Klebsiella	69	18.9	14.9–22.9
Staphylococcus	51	13.9	10.3–17.4
Escherichia	49	13.4	9.9–16.9
Pseudomonas	31	8.5	5.6–11.3
Proteus	25	6.8	4.2–8.6
Enterobacter	23	6.3	3.8–8.8
Streptococcus	20	5.5	3.2–7.8
Trueperella	17	4.6	2.4–6.7
Aeromonas	15	4.1	2.1–6.4
Pasteurella	15	4.1	2.1–6.1
Enterococcus	10	2.7	1–4.4
Citrobacter	7	1.9	0.5–3.3
Corynebacterium	6	1.6	0.6–2.6
Acinetobacter	5	1.4	0.2–2.6
Moraxella	4	1.1	0.03–2.2
Other	19	5.2	2.9–7.5

isolated. In 41 samples, only one genus of bacteria could be isolated (23.8%).

Combining all the sensitivity panels, 2590 antibiotic sensitivity tests were recorded. In 54.3% (n = 1406) of the tests, the bacteria were susceptible to the antibiotic. The antibiotic sensitivity disc evaluated most frequently was gentamicin (8.8%; n = 229), followed by sulfonamide-trimethoprim (8.1%; n = 210) and streptomycin (7.8%; n = 201) (Table 2).

A total of 90.4% (331 of 366) of the bacterial isolates showed resistance to at least 1 antibiotic in the sensitivity panel, and 9.6% (35 of 366) of the isolates were susceptible to all the antibiotics in the panel. A *Klebsiella* isolate from an orange-fronted parakeet (*Eupsittula canicularis*) was resistant to 13 different antibiotics. Other isolates were resistant to up to 11 different antibiotics. Gentamicin had a high percentage of efficacy (79.5%; 182 of 229) against the bacterial isolates, whereas isolates tested against sulfonamide-trimethoprim (46.7%; 98 of 210), streptomycin (43.8%; 88 of 201), and clindamycin (12.9%; 15 of 116) had efficacies $<50\%$ (Table 2).

DISCUSSION

The present study identified bacteria isolated from choanal swabs and their antibiotic sensitivities from a representative population of companion psittacines in Mexico. Other aerobic culture and antimicrobial sensitivity studies reported from other avian hospitals showed a predominance of birds belonging to other taxonomic orders and were likely associated with the purpose of these birds in the study populations. For example, the pet and semipoultry bird populations studied by Ola-Fadunsin et al[7] showed a greater representation of Galliformes.[7] A retrospective study by a veterinary diagnostic service that evaluated aerobic cultures from their facility reported that Psittaciformes represented 37% of the samples,[8] whereas in another study 64.5% of the patients were Psittaciformes.[9] According to Boseret et al,[10] both Passeriformes and Psittaciformes are frequently identified as avian companion pets. Because of differences in diet, housing, and general exposure to the environment among studies, comparisons of results across studies should consider the influence of these potential risk factors and be used as a guide for interpreting results within our own facility.

Table 2. Susceptibility and resistance patterns for the 10 most frequently tested antibiotics against bacterial isolates from choanal swabs from companion psittacines at the Hospital de Aves de Ornato, Compañía y Silvestres, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico (n = 229).

Antibiotic	No. of susceptible isolates (%)	No. of resistant isolates (%)
Gentamicin	182 (79.5)	47 (20.5)
Sulfonamide-trimethoprim	98 (46.7)	112 (53.3)
Streptomycin	88 (43.8)	113 (56.2)
Kanamycin	118 (61.1)	75 (38.9)
Carbenicillin	103 (53.9)	88 (46.1)
Amoxicillin-clavulanate	126 (67)	62 (33)
Neomycin	93 (62)	57 (38)
Norfloxacin	91 (63.6)	52 (36.4)
Cefotaxime	98 (69.5)	43 (30.5)
Clindamycin	15 (12.9)	101 (87.1)

By analyzing choanal swabs, it was expected that the genera of bacteria commonly reported in the avian upper respiratory tract would be the most frequently identified in cultures, in addition to the presence of bacteria from the gastrointestinal tract due to the communication between the nasal cavity and the oropharynx.[11–13] Regardless, previous studies have shown that Gram-positive rods and cocci are the most common bacteria identified in both the upper respiratory[4] and the gastrointestinal tracts of healthy parrots. Considering the limitations for sample collection by swabbing, and given the size and behavior of the patients in some cases, it is difficult to determine if the isolated bacteria represented the etiological agent of the disease that was suspected in the bird, or if the isolation was the result of contamination of the sample by commensal bacteria or bacteria present in the environment.[4]

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The genus of bacteria most frequently isolated in this population was *Klebsiella*. This finding is consistent with studies in which fecal samples and respiratory tract swabs were collected from various species of parrots and passerines.[14] *Klebsiella* belongs to the Enterobacteriaceae family and is a Gram-negative, facultatively anaerobic bacterium.[15] *Klebsiella pneumoniae* and *Klebsiella oxytoca* are frequently isolated from birds and have been found to serve as primary pathogens or as opportunistic agents in immunosuppressed or stressed individuals.[16] However, *Klebsiella* has also been isolated from healthy birds.[17] Enterobacteriaceae are ubiquitous and are considered part of the indigenous gut microbiota of many species of birds, especially raptors. Enterobacteriaceae isolates from the respiratory or reproductive tract should be considered abnormal, especially when clinical disease is present, and are also considered important avian intestinal pathogens.[16]

In 2016, Nemeth et al[9] found that 17.6% of the bacteria isolated from avian patients with clinical disease at a diagnostic pathology service were Gram negative. Within that population was a broad set of cohorts, including companion birds and birds from zoological, educational, and backyard collections. In our study, 5 of the 6 most frequent genera of bacteria isolated were also Gram negative; the exception was *Staphylococcus*. The authors suggest that the difference in the prevalence of Gram-negative bacteria between the studies is most likely associated with the types of birds sampled, and possibly changes caused by sample handling and postmortem bacterial proliferation. *Staphylococcus*, a Gram-positive bacteria, has been associated with infections that can induce sporadic or enzootic diseases in many species of birds. Moreover, *Staphylococcus aureus* has been found to participate as a primary agent or complicate other infections as a secondary pathogen.[16]

The finding that the birds in this study showed clinical signs associated with the upper respiratory system only partially explains the change in the distribution between Gram-negative and Gram-positive bacteria. In a recent publication by Rueanghiran et al,[18] *Streptococcus* species, *Staphylococcus* species, *Escherichia coli*, *Pseudomonas* spp., *K pneumoniae*, *Pasteurella* spp., and *Enterobacter* spp. were all isolated from choanal swabs of parrots with respiratory disease. There is the possibility that bacterial contamination occurred and the primary etiologic agent in our patients was not identified. This still does not rule out that the isolated bacteria participated in antimicrobial resistance in their interaction with the bird's microbiota, so their finding remains relevant to describe the profile of the bacterial population present in the birds in this study. It is important to remark that after becoming established, many secondary invaders can maintain a disease process independent of other infectious agents or predisposing conditions. Also, laboratory tests rarely help to differentiate primary from secondary invaders.[16] In 23.8% of the samples, the presence of a single bacterium in an almost pure culture indicated that the isolated agent was a component in the disease process.[16] Clinical interpretation and therapeutic decision-making based on a choanal culture must consider the findings in cytology, Gram's stain, and physical examination of the bird.[4]

Escherichia was the third most frequently isolated genus of bacteria in our study. This is in contrast with the results reported in a study by Sigirci et al[19] in which *E coli* was isolated from 37.7% of cloacal swabs from clinically healthy parrots. *Escherichia coli* belongs to the Enterobacteriaceae family and is considered the most common commensal inhabitant of the gastrointestinal tract of humans and animals. *Escherichia coli* typically lives in a mutually beneficial association with its host and rarely causes illness in mammals; however, it is also one of the most common human and animal pathogens and is responsible for a wide spectrum of diseases.[20] Recent reports suggest that infections by *E coli*, as well as *Klebsiella* spp. and *Pseudomonas aeruginosa*, are especially common in birds; these are often identified as etiologic agents responsible for respiratory, alimentary tract, and multiorgan infections.[6]

Multidrug-resistant organisms (MDROs) are defined as microorganisms, predominantly bacteria, that are resistant to 1 or more classes of antimicrobial agents. Although the names of certain MDROs describe resistance to a specific agent (eg, methicillin-resistant *S aureus* and vancomycin-resistant enterococci), these pathogens are usually resistant to most of the available antimicrobial agents.[21] In our study, we found bacteria resistant to up to 13 different antibiotics. In general, MDROs of high importance in human medicine are proportionally distributed between Gram-positive and Gram-negative bacteria.[22] However, the ability to develop resistance to antimicrobials has significantly accelerated in Gram-negative microorganisms in recent years.[23] One of the explanations for this phenomenon is the high capacity for genetic exchange of these bacteria, not only with other Gram-negative bacteria but also through the transfer of resistance genes from Gram-positive to Gram-negative bacteria.[24]

Clindamycin is often used for the treatment of anaerobic infections in birds.[6] In this study, the drug showed a low efficacy, with only 12.9% of the isolates being susceptible; however, it is important to point out that only aerobic bacteria were evaluated in this study.

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Clindamycin has been associated with rapid rates of antimicrobial resistance. The most widely distributed mechanism of resistance to clindamycin among Gram-negative bacteria involves the active expulsion of the antibiotic from the periplasmic space.[25]

Gentamicin had the highest percentage of efficacy, and Gram-negative isolates were susceptible to this aminoglycoside in 57.2% of the sensitivity tests. For years, the use of gentamicin has been reserved to treat severe infections caused by Gram-negative organisms in humans and small animal medicine.[26,27] It has rapid bactericidal activity with low levels of resistance in most Gram-negative pathogens.[26] Gentamicin toxicity remains a major clinical problem and thus gentamicin is not considered by many clinicians as a first-line antibiotic, even for short-term therapy.[26] However, gentamicin remains a secondary option over other antibiotics with fewer adverse effects on the body, such as amikacin.[6] Gentamicin is only US Food and Drug Administration–approved for use in turkeys and chickens, and despite its appearance in some formularies, its use in psittacine birds is off-label. Thus, clinicians treating birds with this antibiotic should consider both its adverse effects and therapy stewardship.[27]

Oral treatment with sulfonamide-trimethoprim for companion birds with suspected bacterial infection is often considered a reasonable treatment choice.[6] Our study showed a concerning low percentage of sensitivity of the isolates to this antibiotic (46.7%), questioning its empirical use in avian patients. Resistance to sulfonamide-trimethoprim has been previously reported as a rapid and irreversible process.[28]

Fluoroquinolones are a group of antibiotics that are commonly used to treat debilitated birds with suspected systemic Gram-negative bacterial infections.[6] Norfloxacin was the fluoroquinolone with the greatest representation in the sensitivity tests and showed a 62% efficacy against the isolates. Norfloxacin has a wide spectrum against Gram-negative and some Gram-positive bacteria as well as activity against *Chlamydia* and *Mycoplasma*.[29] However, its use in avian medicine is not widespread in such a way that it can stand out as a priority therapeutic option compared with other available fluoroquinolones such as enrofloxacin.[6] In the US, it is illegal to use any fluoroquinolone in food or food-producing animals, such as chickens, turkeys, and waterfowl.

At this point, the selection criteria for sensitivity evaluations established by the laboratory are noteworthy. The laboratory used sets of susceptibility panels made up of antibiotic combinations that changed randomly over time. During the period of our study, the laboratory tested up to 36 different antibiotics in multiple combinations, sometimes testing antibiotics not available in the hospital or not commonly used in avian medicine. Clinicians should be able to request sensitivity evaluations that correspond to the needs of their patient and the availability of drugs in their clinic. The standardized use of predetermined mammalian sensitivity disc packs (laboratory protocol because of high mammalian caseload) may prove inadequate to provide valuable assessments in avian medicine for therapeutic decision-making.

One of the limitations of our study was the lack of a clinical history regarding the previous use of antibiotics; most patients presented to HAOCS-UNAM come for a second-opinion consultation and the clinicians often obtain imprecise information from the guardians of the birds regarding previous treatments. In the study by Williams and Newman,[30] a statistically significant association between antibiotic resistance and prior treatment was reported, where resistant organisms were 2 to 36 times more likely to come from a bird that had received prior antibiotic therapy. A future prospective study with a large sample size would be valuable for increasing our understanding of the impact that previous drug administration has on bacterial sensitivity to antimicrobials.

The present study is the first description of common bacterial isolates and their antimicrobial sensitivity profiles from the choana of companion psittacine birds (suspected of having upper respiratory disease) in Mexico. This information can be used to assist clinicians in their decision-making on treatment for these types of infections. Although this work is meant as a guide, the authors want to emphasize the importance of performing culture and antimicrobial sensitivity tests on each case and prior to starting any antimicrobial therapy to practice good antimicrobial stewardship.

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