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ASSESSMENT OF MULTIDRUG RESISTANCE IN FALCON BACTERIAL ISOLATES: IMPLICATIONS FOR THERAPEUTIC APPROACHES

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ABSTRACT
The emergence of bacterial resistance to antimicrobials employed in veterinary medicine poses a significant threat to the efficacy of human antibiotic therapy. This study primarily investigates the efficacy of antibiotics against bacterial strains isolated from Falcons. A retrospective analysis was conducted using samples collected from various swab sites of falcons admitted to Souq Waqif Falcon hospital during the falconry season. Bacterial strains were identified using biochemical assays and the Vitek-2 system, with susceptibility to a range of antibiotics assessed. Data analysis followed the guidelines outlined by the Clinical and Laboratory Standards Institute. A total of 564 bacterial strains were isolated from falcons, with Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli being the most prevalent. Resistance to at least one antibiotic was observed in 93.6% of the isolates, with 55.4% exhibiting multidrug resistance (MDR). Notably, oxacillin, erythromycin, and amoxicillin showed the highest rates of resistance, while clindamycin, ampicillin, and amikacin demonstrated the most favorable efficacy. These findings suggest that clindamycin, ampicillin, and amikacin could be viable treatment options for bacterial infections in falcons. Nonetheless, further research is warranted to validate these results and establish comprehensive treatment protocols for bacterial infections in falcons.

Keywords: resistance, infections, multidrug, antimicrobials

INTRODUCTION
Wild birds, due to their widespread distribution and close interaction with humans, have the potential to act as reservoirs for bacteria carrying antibiotic resistance genes. Contamination from various sources, including chemical agents, antibiotics, sulfonamides, and other pollutants, coupled with their proximity to human settlements, can significantly impact the microbial composition. Research by Cao et al. (2020) and Sun et al. (2022) indicates that the bacterial microbiota of wild migratory birds in temperate regions exhibits the highest diversity among avian species. Commonly isolated bacteria from these birds include strains of Salmonella, Campylobacter, Pasteurella multocida, Borrelia

burgdorferi, and Escherichia coli. There is speculation that wild birds may harbor and disseminate antibiotic-resistant genes. Numerous studies have demonstrated that free-living birds contribute to the dissemination of multidrug-resistant bacteria, suggesting their potential role in transmitting these pathogens to other animals and humans (Ramey and Ahlstrom, 2020; Marcelino et al., 2019). Tracking the temporal and geographical distribution of disease-causing bacteria in wild birds is essential in combating the global challenge of antibiotic resistance, providing valuable diagnostic insights for managing vectors of drug resistance transmission.

Among the most ubiquitous raptors globally, the falcon inhabits every continent, even Antarctica. Currently, falcons are predominantly found in southwestern and western Europe, encompassing southern France, Spain, Great Britain, as well as northern Scandinavia and Russia (Gu et al., 2021; Wilcox et al., 2019). Studies by Smith et al. (2020), De Luca et al. (2018), and Lawhon et al. (2023) highlight that falcons can encounter human pathogenic microorganisms such as Campylobacter spp., Salmonella, E. coli, and Mycoplasma through various means including contaminated food sources, close proximity to livestock or companion animals, and indirect contact with humans.

Numerous investigations have been carried out in Saudi Arabia and Qatar to explore the prevalence of bacterial and fungal infections in falcons, which pose a significant threat to avian health and foster the emergence of drug-resistant bacterial strains (De Oliveira et al., 2020). Research conducted in Saudi Arabia has primarily concentrated on the correlation between parasitic infestations and Aspergillosis in avian species, notably falcons (Arné et al., 2021). Aspergillosis and Candidiasis are recognized for inducing immunosuppression, thereby predisposing birds to recurrent bacterial infections. Furthermore, oral infections resulting from the ingestion of sharp bones serve as potential entry points for bacterial pathogens (Mahadevia & Brandwein-Gensler, 2019).

Concurrently, investigations carried out in Qatar between 2011 and 2013 have emphasized the isolation and antimicrobial susceptibility patterns of various bacterial and fungal strains in falcons. The results from these studies revealed Pseudomonas aeruginosa as the predominant bacterial isolate, alongside Aspergillus spp. and diverse yeast species. These co-infections were identified across different falcon species, underscoring the necessity for comprehensive monitoring of multiple infections (Saleh, 2021; Nourani et al., 2022).

The present study endeavors to compile data concerning bacterial strains sampled from different anatomical sites and to analyze clinically significant findings reported during the falconry season. Additionally, meticulous observation of wound and post-operative care protocols at the hospital was conducted to identify bacterial infections while excluding contaminants and commensal flora. Furthermore, comprehensive data on antibiotic susceptibility profiles was documented for bacterial characterization, with drug sensitivity assessments documented for hospital records.

METHODOLOGY

The present study was conducted at the Souq Waqif Falcon Hospital in Qatar. A total of 564 avian samples were collected over the period spanning 2014 to 2017.

Sample collection

Birds exhibiting signs of respiratory distress, such as dyspnea and sneezing, along with low flying performance, were admitted to the hospital. Upon endoscopic examination, pharyngeal granulomas were observed, alongside indications of air sacculitis or white lesions in the trachea. Swab samples were obtained from various sites including the right and left air sacs, trachea, choana slit, and nose. Histories suggestive of Trichomoniasis or Serrato-speculum infections were documented in certain cases. Additionally, crop and cloacae swabs were taken in response to reports of mucus presence, blood in stool, enteritis, delayed digestion, food impaction, and parasite infestation. Notably, eye and ear swabs were obtained from birds exhibiting symptoms such as eye swelling, conjunctivitis, and ear discharge, with positive identification of Candida in some cases (Hofacre et al., 2013). A pie chart illustrating the distribution of sample collection from different anatomical regions of falcons revealed that the highest number of samples were collected from the air sacs (33%) (Figure 1).

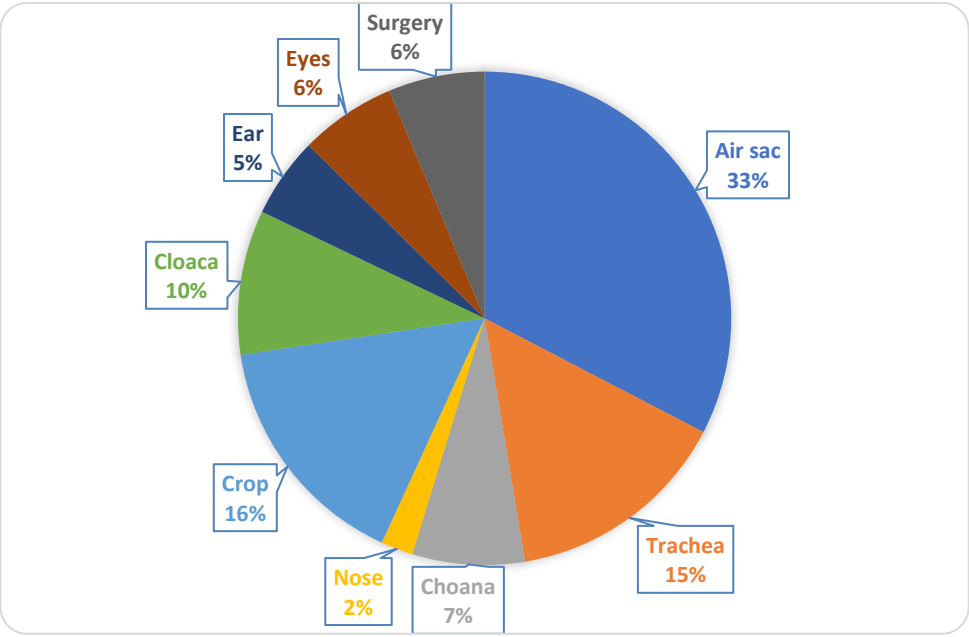

Fig 1. The pie chart depicts the distribution of samples received in the Microbiology laboratory for culture and sensitivity procedures, presented as percentages. It is pertinent to emphasize that the data depicted in the pie chart reflects an average, indicating potential variability in the number of samples collected from each location over the years. Furthermore, it is imperative to recognize that the pie chart offers a relative portrayal of sample distribution and does not include precise numerical values for the quantity of samples obtained from individual locations.

Isolation and characterization of microorganisms

Microbiological streaking was performed under aseptic conditions within a Class II Biosafety Cabinet (Labconco, USA). Citoswabs were employed for sample collection, and subsequent streaking was conducted on commercially available agar plates, including Blood agar, MacConkey agar, and Nutrient Agar media (Berhe et al., 2020; et al., 2020). The inoculated plates were then placed in an incubator set to 37°C under aerobic conditions for 24 hours, after which growth was assessed. Cultures displaying growth were subjected to Gram staining for initial identification. Further characterization was achieved using the Vitek-2 Compact System (Konicek et al., 2016).

- **Gram staining**

A Gram staining procedure was conducted following standard protocols. A smear was prepared from the bacterial sample and allowed to air dry. Subsequently, the smear was immersed in crystal violet solution for one minute. Excess crystal violet was removed by rinsing the slide with distilled water. Gram's iodine solution was then applied to the smear, followed by another round of rinsing with water. Next, the slide was treated with 95% ethyl alcohol to decolorize the stain. Safranin was utilized as a counterstain, with the slide immersed in the solution for 45 seconds and then rinsed. Finally, the stained smear was examined under a microscope to determine the Gram reaction of the bacteria (Tjoa et al., 2013).

- **VITEK 2 automated biochemical identification**

Following the protocol described by Al-Enawey et al. (2020), the Vitek® 2 system was directly inoculated. A 4 ml aliquot obtained from the positive blood culture bottle was aseptically transferred to a serum separator tube. Centrifugation was conducted at 1,525 g for 10 minutes, after which the supernatant was carefully aspirated. Subsequently, a cotton swab was utilized to remove the bacterial film from the gel layer. The bacteria were then resuspended in 3 milliliters of 0.45% saline solution until reaching a density corresponding to 0.6–0.8 McFarland standard. This particular density was chosen following pilot investigations and consultations with the manufacturer. The suspension was

processed using ID-GNB and AST-N064 cards, following the standard inoculation method recommended for the Vitek® 2 system.

Antibiotic sensitivity

The antibiotic susceptibility of the isolated bacteria was assessed using the Kirby-Bauer disk diffusion method as described by Venkadesan and Sumathi (2015). Briefly, sterile disks impregnated with specific antibiotics including Ampicillin/sulbactam, Erythromycin, Marbofloxacin, Clindamycin, Oxacillin, Amoxicillin/clavulanic acid, Amikacin, Piperacillin, Trimethoprim, and Cefpodoxime were placed onto Mueller-Hinton agar plates (Hardy Diagnostics) previously inoculated with the test microorganism. Following incubation, the diffusion of antibiotics from the disks into the surrounding agar resulted in the formation of zones of inhibition. The diameter of these zones was measured and interpreted to determine the sensitivity profile of the bacteria to each antibiotic.

Statistical analysis

The obtained data were subjected to statistical analysis using the ANOVA along with post hoc test using graph pad prism 7.

RESULTS

Biochemical characterization of bacterial isolates

The collected samples underwent Gram staining analysis. Microscopic inspection identified the presence of both gram-positive (Staphylococcus) and gram-negative (Escherichia coli and Pseudomonas aeruginosa) colonies, as illustrated in Figure 2a-c, respectively. Salmonella spp colonies were discerned through antigen testing, with positive outcomes depicted in Figure 2d.

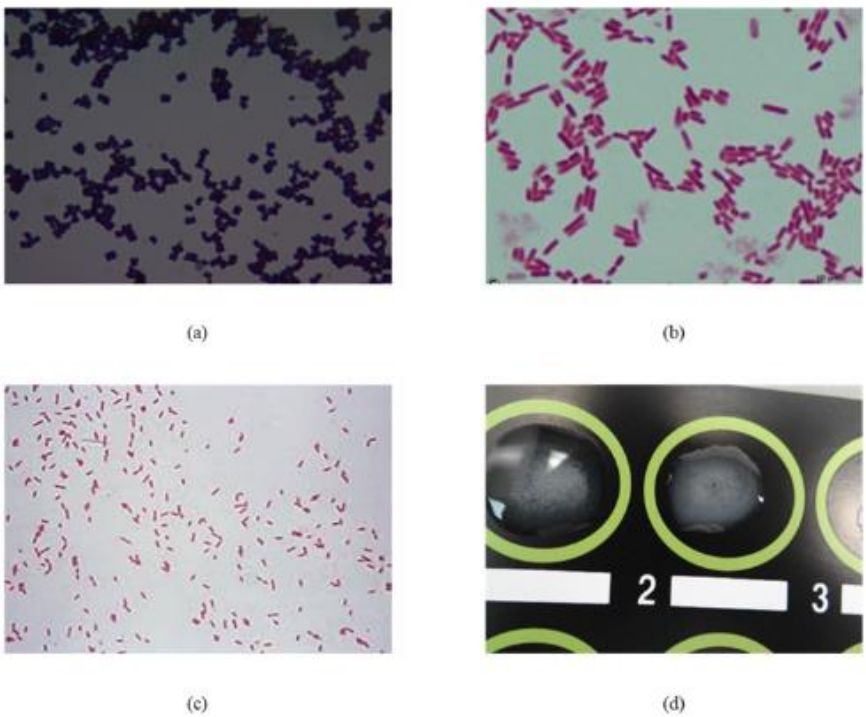

Figure 2: Biochemical characterization of bacterial colonies. (a) Gram positive Staphylococcus colony. (b) Gram negative Escherichia coli colony (c) Gram negative Pseudomonas aeruginosa colony (d) Antigen positive test for Salmonella spp colony

Characterization through VITEK-2 system

The bacterial isolates obtained from collected samples underwent further characterization using the VITEK-2 system. Table 1 presents the distribution of bacterial isolates across samples collected from different body parts. Among the 140 samples analyzed, Pseudomonas was the most frequently

isolated genus, followed by Staphylococcus with 16 different species identified, totaling 136 cases. Additionally, Enterococcus (n=44) and Kocuria (n=32) were observed. The distribution of the Enterobacteriaceae group varied significantly. Escherichia coli (n=99) was the predominant species identified. Sphingomonas was detected specifically in surgical wounds (n=20). Various species of Bacilli were isolated, primarily from air sac swabs (n=10). During characterization three distinct types of Aspergillus were also isolated, namely Niger, Brazellians, and fumigatus (n=20), along with Candida albicans (n=20).

Table 1. Microbial Isolates Collected at Souq Waqif Falcon Hospital with respect to different sample types

Bacteria	Air sac	Trachea	Choana	Crop	Cloaca	Ear	Eye	Nose	Surgery	Total
Staph. aureus	12	1		3	2	1			5	**24**
Staph. warneri	8			1	1					**10**
Staph. capitus	5								1	**6**
Staph. Chromogenesis	1	1	1			1			1	**5**
Staph. schleifer	3	1	1	1			1	1	1	**9**
Staph. hominis	4									**4**
Staph. lentus	7	1					1			**9**
Staph. aglactiae								2		**2**
Staph. lugdinensis	3									**3**
Staph. epidermis	24	1					2		2	**29**
Staph. pseudointermedius		2	1				2	1		**6**
Staph hemolyticus	8		1	1			1		1	**12**
Staph xylosus	4							1		**5**
Staph sciuri	1	2	1	1			1			**6**
Staph gallinarum	1			1		1	1			**4**
Staph simulans				1	1					**2**
Pseudomonas aeruginosa	25	37	17	16	9	13	12	2	5	**136**
Pseudomonas lstutzeri	2									**2**
P. putida		1				1				**2**
E. coli	7	15	8	33	21	7	3		5	**99**
Kocuria kristinae	7	7	3			3	10	2		**32**
Enterococcus faecalis	19	4	1	4	4	1	2	2	4	**41**
Enterococcus gallinarum	1	1							1	**3**
Aspergillus Niger	1									**1**
Aspergillus fumigatus	6	2		7					1	**16**
Aspergillus Brazellians	3									**3**
Candida albicans	2	1		4	12		1			**20**
Sphingomonas paucimobilis	4	4	1	2		3		1	5	**20**
Proteus mirabilis			2	1	3	1	2	1		**10**
Klebsiella pneumonia spp(oxytoca)	3		2	6		1		1	1	**14**
Salmonella spp	1			6						**7**
Serratia fonticola				2						**2**
Pantoea	4				1				1	**6**
Bacillus species	9		1							**10**
Yersinia spp kristenseii	2	1							1	**4**
Total	**177**	**82**	**40**	**90**	**54**	**33**	**39**	**14**	**35**	**564**

Antibiotic profiling of bacterial isolates

Figure 3 (a-c) presents the antibiotic susceptibility profiles of various strains of Staphylococcus, Escherichia coli, and Pseudomonas, respectively. As illustrated in Figure 3a, Staphylococcus species displayed the greatest resistance to oxacillin (56%), while exhibiting the highest sensitivity to clindamycin (65%) and ampicillin (61%). In contrast, Figure 3b reveals that E. coli strains showed the highest resistance to trimethoprim (21%) but were most susceptible to amikacin (33%). Similarly, Figure 3c indicates that Pseudomonas species exhibited the highest resistance to amoxicillin and ampicillin (46%), yet demonstrated the highest sensitivity to amikacin (46%).

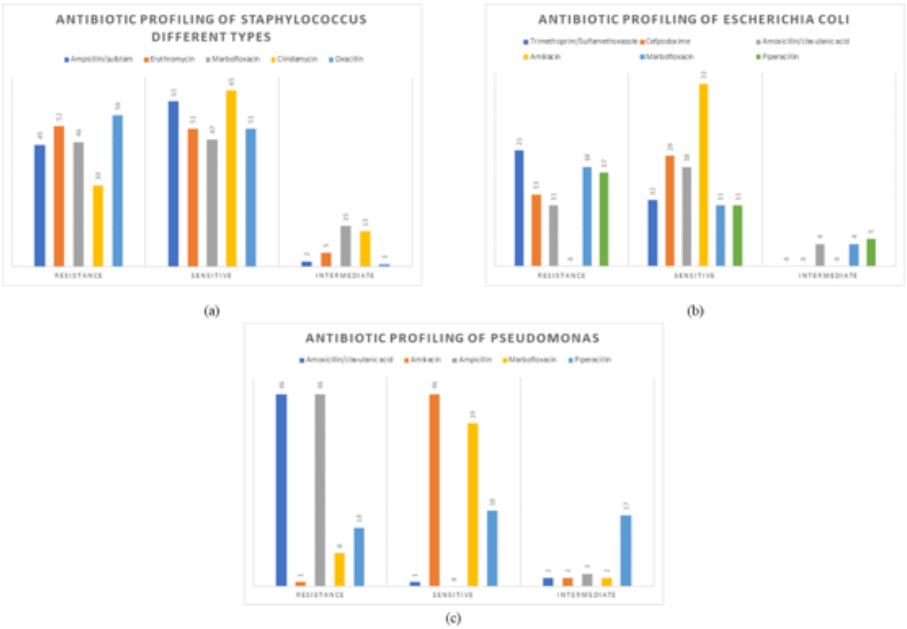

Figure 3: Antibiotic profile of different species of (a) Staphylococcus (b) Escherichia coli (c) Pseudomonas

DISCUSSION

Free-living avian populations inhabiting urban environments are at an increased risk of exposure to various zoonotic pathogens, posing significant threats to both animal and human health (Nowaczek et al., 2021). Recent research has underscored the importance of understanding the presence of bacterial agents in free-living birds, as they may serve as potential reservoirs of infectious agents, thereby amplifying the risk to domestic animals and humans.

This study aimed to investigate bacterial isolates obtained from different anatomical sites of falcons, particularly focusing on individuals presenting clinical symptoms such as vomiting, food impaction, and respiratory distress. Biochemical analysis of the samples revealed the presence of both gram-positive and gram-negative bacterial species. These findings corroborate earlier research by Pyzik et al. (2021), which similarly identified diverse bacterial species in falcons.

Among the isolated bacteria, Staphylococcus species were predominant, with 16 different species of Staphylococcus identified, primarily in tracheal and air sac samples, comprising 24% of the total isolates. This aligns with the observations of Nemeth et al. (2024), who highlighted the prevalence of staphylococcal infections in falcons. Staphylococcus aureus, found in air sac samples, may pose a risk due to its ability to bind to host receptor cells. While Staphylococcus epidermidis is not typically pathogenic in falcons, nosocomial strains could still pose health risks.

Escherichia coli, the second most prevalent isolate, was predominantly found in crop samples (33 cases) and cloacal swabs (21 cases). Its pathogenicity in birds, characterized by shedding of microorganisms and toxins leading to septicemia, renal involvement, and neurological symptoms, has been well-documented (Christensen et al., 2021). Notably, the isolation of Escherichia coli from tracheal samples was associated with the clinical manifestation of food impaction in the crop, highlighting the potential severity of its impact on falcon health. The isolation of Enterobacteriaceae from the respiratory tract, though uncommon, suggests the possibility of systemic bacterial infections, underscoring the invasive nature of primary bacterial pathogens (Nowaczek et al., 2021). Understanding the prevalence and distribution of these bacterial species in falcons is crucial for devising effective management and intervention strategies to mitigate the risks associated with zoonotic pathogen transmission.

In this investigation, Pseudomonas species constituted the third most prevalent isolates, with a predominant presence of Pseudomonas aeruginosa obtained primarily from respiratory tract samples. Notably, Pseudomonas aeruginosa is a common constituent of the normal intestinal flora in both avian and mammalian species (Grond et al., 2018). Surveillance data from the CDC revealed P. aeruginosa

as the fifth most frequently encountered nosocomial pathogen, contributing to 9% of all hospital-acquired infections in the United States (Yetkin et al., 2018).

Salmonella species comprised the fourth most prevalent isolates, with evidence suggesting potential sexual transmission during the breeding season via the cloacae passage in falcons, a phenomenon also observed in amphibians (Mohan et al., 2023). Research by Ugur Parin in 2017 highlighted the association between Salmonella infections in falcons and the consumption of infected prey (Ahmad et al., 2020). Additionally, significant pathogens such as Clostridium haemolyticum and Clostridium burnati were isolated, indicating their potential role in enteritis development in falcons. Various bacterial species, including Bacillus spp., Proteus, Serratia, and Klebsiella pneumonia spp., were identified from the crop and nose, with some strains exhibiting Extended Spectrum Beta-Lactamase (ESBL) positivity, underscoring their virulence potential.

Antibiotic susceptibility profiling revealed Clindamycin, Ampicillin, and Amikacin as effective treatments for respiratory infections, with Amikacin demonstrating efficacy against conjunctivitis and otitis as well. Pseudomonas aeruginosa, the predominant isolate, exhibited high susceptibility to Amikacin and marbofloxacin, while resistance and intermediate responses were observed against piperacillin, contrary to previous recommendations. For Staphylococcus infections, Clindamycin and ampicillin/sulbactam exhibited good sensitivity, while oxacillin showed moderate efficacy. Escherichia coli infections were susceptible to Amikacin and displayed mild sensitivity to cefpodoxime, while resistance was noted against Trimethoprim/sulfamethoxazole, with intermediate responses to piperacillin. Consistent findings were reported by Pimenov et al. (2020).

Further investigations are warranted to enhance the understanding of bacteriological profiles in captive birds and to identify more efficacious antimicrobial agents. The selection of appropriate antibiotics for falcon infections presents a challenge due to variations in infection sites impacting drug penetration and efficacy. Veterinarians must carefully consider factors such as bacteriostatic and bactericidal properties, site-specific homeostasis, cell wall permeability, as well as variations in oxygen, pH, and tension levels.

CONCLUSION

In conclusion, the findings of these studies emphasize the significance of monitoring bacterial infections in falcons, especially those with zoonotic potential. To mitigate the risk of bacterial transmission and uphold the well-being of these majestic birds, it is imperative to enforce effective disease prevention measures, such as prudent prey management and meticulous hygiene practices. This investigation furnishes crucial insights into the effectiveness of diverse antimicrobial agents in addressing the spectrum of bacterial and fungal infections frequently observed in captive avian species, including falcons.

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