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Evaluating Antibiotic Resistance in the Bacterial Pathogens Isolated from Avian Species

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ABSTRACT

The avian population plays a vital role as both a reservoir and sentinel for numerous pathogens. Antimicrobials are widely used for the treatment and control of infections in avian species. However, inappropriate selection, irrational use of these antibiotics may contribute to the development of drug resistance in population. This research evaluates the Antibiogram of *Chlamydia psittaci*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus epidermidis* and *Pasteurella multocida* isolated from caged and free-living birds. Antimicrobial susceptibility was tested using the disk diffusion method on Mueller Hinton Agar, according to instructions by the Clinical and Laboratory Standards Institute. *Chlamydia psittaci* exhibited sensitivity to sulfamethoxazole (22mm), azithromycin (21mm), doxycycline (20mm) and gentamicin (15mm). In contrast, penicillin G, oxacillin, amoxicillin and chloramphenicol were ineffective. *Escherichia coli* shown azithromycin (20mm), doxycycline (16mm) and gentamicin (15mm) were most effective. *Klebsiella pneumoniae* demonstrated high sensitivity to sulfamethoxazole (20mm) and doxycycline (18mm), and showing complete resistance to oxacillin and rifampin. *Pasteurella multocida* exhibited the highest susceptibility to doxycycline (21mm), sulfamethoxazole (20mm), amoxicillin (20mm), chloramphenicol (19mm), norfloxacin (18mm) and gentamicin (16mm). *Staphylococcus epidermidis* demonstrated high sensitivity to norfloxacin (23mm), doxycycline (23mm), oxacillin (22mm), chloramphenicol (22mm), sulfamethoxazole (17mm) and gentamicin (15mm). The current study reveals sulfamethoxazole, doxycycline, and azithromycin were highly effective antibiotics against the bacterial pathogens infecting avian species. Penicillin G, amoxicillin, oxacillin, and ampicillin were resistant against the identified bacterial species.

Keywords: Antimicrobials, Susceptibility, Bacteria, Resistance, Birds

INTRODUCTION

Antimicrobial resistance poses a severe threat to both human and animal health. Wild animals may develop into bio indicators or reservoirs of resistant bacteria if they come into possession of resistant bacteria (Bhattarai *et al.*, 2024). Additionally, they have the ability to reintroduce resistant bacteria to domestic animals and people through contaminated shared water and soil sources (Carroll *et al.*, 2015). Contact with an environment that has been impacted by humans, however, continues to be a significant determinant in the development of resistant bacteria. Because of their mobility, wild birds can come into touch with humans and other animals rather frequently, which make them perfect for studying the transfer of germs at the human-wildlife interface (Radhouani *et al.*, 2014).

Antimicrobial resistance (AMR) presents a major obstacle for the fields of veterinary and human medicines and is a global health issue (Mohamed, 2019). For species of birds, this problem is especially serious as wild and domestic birds intimate contact between them as well people and birds contact can lead to pathogen resistance spread (Liao *et al.*, 2020). Historically a broad range of antimicrobials has primarily been used to treat a variety of bacterial infections (Christensen *et al.*, 2021). In poultry, antimicrobial resistance poses a major health issue because pathogen posses' resistance against routinely used antibiotics and poultry is a major source of zoonosis (Munangandu *et al.*, 2012). Antimicrobial resistance bacteria horizontal transmission in farms and flocks of chicken as well as zoonotic transfer through food chain has drawn a lot of attention in the modern era (Christensen *et al.*, 2021). Therefore, keeping an eye on the AMR profiles of pathogenic bacteria is essential for maximizing the effectiveness of antimicrobial therapies in poultry and tracking the advancement of bacterial drug resistance. Antibiotic-resistant bacteria have been the subject of much research due to their potential health risks as well as their potential economic effects demonstrated that bacteria with antibiotic-resistance mechanisms are increasingly using free-living birds as hosts (Carroll *et al.,* 2015).

Wild birds are known to have enteric bacteria such as Salmonella sp., *E. coli*, and Enterococcus spp. as well as zoonotic enteric diseases that may infect people (Smith *et al.,* 2020). Being one of the most frequently identified Gram-negative nosocomial pathogens in health settings worldwide, Enterococcus spp. have garnered a lot of interest because of the impact species like *E. faecalis* and *E. faecium* had in clinical settings (Gao *et al.,* 2018). Because of their extremely mobile behavior, migratory shorebirds have the potential to be a significant vector of emerging illnesses and AMR. Every year, a large number of Australian shorebird species migrate between the northern and southern hemispheres, making stops along the way on land masses that are home to around one-third of the world's human population (Yong *et al.*, 2018). About millions of migratory birds regularly travel between the Arctic and Australia, where they often interact with people (Old land *et al.* (2009). The amount of interaction that these migratory birds have with people is increased by encroaching human development at important stopping places like the Yellow Sea and hunting shorebirds for food (Piersma *et al.*, 2017).

The possibility of zoonotic disease transmission and the spread of AMR bacteria between birds, people, and other wild animals is therefore increased (Altizer *et al.,* 2011). There is little information available about the occurrence of clinically significant bacterial species and related AMR in both caged and free-living birds. Few studies have been done on shorebirds and terns worldwide and those that have been done have mostly looked on the possibility of pathogenic Enterobacteriaceae (Keeler & Huffman., 2009). Therefore, it's critical to establish baseline both AMR measurements for both residents and migrant species in these communities (Weller & Lee., 2017). The purpose of this work is to examine the antimicrobial susceptibility profiles of bacterial species that have been isolated from both free-living and confined birds. Slow the emergence of antimicrobial resistance (AMR) in avian populations require an understanding of the resistance patterns shown by these diseases.

MATERIAL AND METHODS

Sample collection: The samples were obtained from free-living birds including, sparrow (*Passer domesticus*), duck (*Bucephala albeola*), dove (*Columbidae*), peacock (*Pavo cristatus*), backyard chicken (Golden Missri, Desi and Aseel,). Bulbul (*Pycnonotidae*), quail (*Synoicus ypsilophorus*), geese (*Anser anser domesticus*), myna (*Acridotheres tristi*) and crow (Corvus). The sampling from caged birds including pahari parakeet (*Psittacula eupatria*), crimson rosella (*Platycercus elegans*), cockatiels (*Nymphicus hollandicus*), canaries (*Serinus canaria domestica*), pigeons (*Columba livia domestica*), lutino (*Melopsittacus undulates*) and fisher (*Agapornis fischeri*). The random sampling was carried out for the screening of microbial infections in birds living in free and caged environment. A total of 600, fecal (n=190), cloacal (n=150), nasal (120), conjunctival (n=90), oropharyngeal/tracheal swabs (n=150), and blood (n=95) samples (each sample along with other samples such as fecal, cloacal, nasal, conjunctival, and tracheal swabs collected from a bird counted as one sample) were obtained randomly from caged (n=400) and free-living birds (n=200) of regions; Hyderabad (n=75), Thatta (n=75), Badin (n=75), Dadu (n=75), and Karachi (n=75), Sindh Province, and Mirpur ((n=75), Bhimber (n=75) and Kotli (n=75), Azad Jammu and Kashmir (n=75).

At the time of collection, physical condition, signs, and symptoms included; fever (n=21), diarrhea (n=14), nasal and ocular discharges (n=11), respiratory distress (n=13), feather loss/ruffled (n=07), reduced feed and water intake (n=17), oral lesions (n=08), dryness of mouth (n=2), lameness (n=2) and weight loss (n=08), and death (n=12) of the sampled birds were recorded. Faecal samples were collected aseptically from the cloacal region using cloacal swabs/sterile tubes, while for nasal, tracheal, and conjunctival samples, sterile cotton wool swabs containing phosphate buffer saline (PBS) were used to maintain the pH. Carefully, with the consent of owners, samples were taken from commercially sold apparently healthy and sick pet avian species for screening of bacterial pathogens. During the collection of samples from pet birds, they were handled carefully to avoid causing harm or stress to the birds.

Laboratory work: The collected samples were brought in cold chain container to Department of Veterinary Microbiology, Sindh Agriculture University, Tandojam and subsequently transferred to Veterinary Research Institute Peshawar, Khyber Pakhtunkhwa, for further processing and analyses.

Culture and Sub-culture: For surveillance of bacterial infections, the samples were inoculated on different bacteriological laboratory medium and incubated overnight at 37ºC. The streak plate method, swabbing method, and spread plate method were used for cultivation of bacterial pathogens. The collected samples were cultured and sub-cultured on nutrient agar, blood agar, and MacConkey agar, Eosin, methylene blue agar, Brain heart infusion agar, Brilliant green agar, Mannitol salt agar, Lowenstein Jensen agar (L&J medium), and Simmon's citrate agar, (Oxoid, UK), for isolation and identification of bacterial organisms. The cultural and colonies characteristics, staining features, and biochemical tests for identification of bacterial species of isolated organisms were performed using the instructions described by (Khalil & Gabbar, 1992).

Antimicrobial susceptibility: The different commercially available antibiotics were used for the antibiotic efficacy against isolated organisms. Antimicrobial susceptibility was determined by disk diffusion method on Mueller Hinton Agar according to the Clinical and Laboratory Standard Institute (CLSI, 2018) guideline using different antimicrobial discs belonging to different groups. Twelve antibiotics of different classes were tested i.e. (norfloxacin, ciprofloxacin, ceftriaxone, penicillin G, oxacillin, azithromycin, doxycycline, sulfamethoxazole, amoxicillin, ampicillin, linezolid, gentamicin and chloramphenicol). Further, the organisms were cultured on Mueller Hinton Agar, antibiotic discs were placed on the inoculated plates at an appropriate distance from each other and zone of sensitivity against each antibiotic were recorded. The clear zone of inhibition appeared around the antibiotic discs after 24hrs of incubation at 37ºC. The diameter of inhibitory zones surrounding the antimicrobial disk were measured in millimetres (mm) and then interpreted according to the Clinical and Laboratory Standard Institute guideline (CLSI, 2018).

Table No 1. Cut off values for interpretation of results on the basis of zone diameter

Antibiotic disc used	Resistant	Intermediate	Susceptible
Amoxicillin	≤13	14-16	≥17
Ampicillin	≤28	-	≥29
Azithromycin	≤13	14-17	≥18
Ceftriaxone	≤24	25-26	≥27
Ciprofloxacin	≤19	20-25	≥26
Doxycycline	≤10	11-13	≥14
Gentamicin	≤12	13-14	≥15
Penicillin G	≤26	27-46	≥47
Rifampin	≤16	17-19	≥20
Sulfamethoxazole	≤10	11-15	≥16
Norfloxacin	≤12	13-16	≥17
Chloramphenicol	≤13	14-17	≥18
Oxacillin	≤9	-	≥10

Source: CLSI, (2018)

RESULTS

Out of six hundreds (600) bird samples screened; 37 samples were detected positive for 12 bacterial pathogens. However, this research was carried out to investigate the antibiotic resistance of the bacterial pathogens including *Chlamydia psittaci, Klebsiella pneumonia, Escherichia coli, Pasteurella multocida*, and *Staphylococcus epidermidis* isolated from caged and free-living birds.

Antibiogram pattern against *Chlamydia psittaci*: Antibiotic response to *Chlamydia psittaci* isolated from both caged and free-living birds was determined. Sulfamethoxazole (22mm), Azithromycin (21mm), Doxycycline (20mm) and Gentamicin (15mm) demonstrated susceptible against *Chlamydia psittaci* (Table 2). In contrast, Ciprofloxacin (14mm), Ceftriaxone (12mm), Rifampin (12mm), Ampicillin (06mm), Norfloxacin (02mm) were found resistant against *Chlamydia psittaci*. Penicillin G, Oxacillin, Amoxicillin and Chloramphenicol showed complete resistant against the organism. Overall, Sulfamethoxazole, Azithromycin, Doxycycline and Gentamicin potentially were detected highly effective antibiotics against the isolated *Chlamydia psittaci* from the avian species living free and cage conditions.

Antibiogram pattern against *Escherichia coli*: Antimicrobial profile of *Escherichia coli* isolated from both caged and free-living birds were observed (Table 3). Azithromycin (20mm), Doxycycline (16mm) and Gentamicin (15mm) were found the susceptible drugs against the isolated *Escherichia coli.* Whereas, Norfloxacin (14mm), Sulfamethoxazole (12mm) and Oxacillin (09mm) were shown intermediate resistance response to the microbe. While, Ceftriaxone (19mm), Ciprofloxacin (13mm), Chloramphenicol (11mm), Amoxicillin (04mm), Ampicillin (04mm) shown resistant against the isolated species. Penicillin G and Rifampin showed complete resistant against the pathogen. In general, Azithromycin, Doxycycline and Gentamicin were detected the highly efficient antibiotics against the organism isolated from the birds living in different environments.

Antibiogram pattern against *Klebsiella pneumonia*: *Klebsiella pneumoniae* isolated from caged and free-living birds was analyzed for antibiotic resistance profile for the various antibiotics. The organism was observed susceptible to Sulfamethoxazole (20mm) and Doxycycline (18mm). The Norfloxacin (16mm) and Azithromycin (15mm) shown intermediate resistant against the organism, Ceftriaxone (21mm), Ciprofloxacin (18mm), Penicillin G (07mm), Ampicillin (06mm), Chloramphenicol (06mm), Amoxicillin (04 mm) and Gentamicin (03mm) exhibited resistant to the isolated species. The pathogen showed complete resistance to Oxacillin and Rifampin. Overall, Sulfamethoxazole and Doxycycline were found highly efficient against the isolated *Klebsiella pneumonia* from bird species.

Table.2. Antibiogram pattern against *Chlamydia psittaci* detected from caged and free-living birds

S. No.	Antibiotic used	Symbol & disc potency (µg)	Inhibited zone diameter (mm)	Level of susceptibility
1	Norfloxacin	NOR-10	02	Resistant
2	Ciprofloxacin	CIP-5	14	Resistant
3	Ceftriaxone	CRO-30	12	Resistant
4	Penicillin G	P-5	0	Resistant
5	Oxacillin	OX-1	0	Resistant
6	Azithromycin	AZM-15	21	Susceptible
7	Doxycycline	DO-30	20	Susceptible
8	Sulfamethoxazole	SXT-25	22	Susceptible
9	Amoxicillin	ANC-30	0	Resistant
10	Ampicillin	AMP-10	06	Resistant
11	Rifampin	RD-5	12	Resistant
12	Gentamicin	CN-10	15	Susceptible
13	Chloramphenicol	C-30	0	Resistant

Table. 3. Antibiogram pattern against *Escherichia coli* detected from caged and free-living birds

S.No.	Antibiotic used	Symbol & disc potency (µg)	Inhibited zone diameter (mm)	Level of susceptibility
1	Norfloxacin	NOR-10	14	Intermediate
2	Ciprofloxacin	CIP-5	13	Resistant
3	Ceftriaxone	CRO-30	19	Resistant
4	Penicillin G	P-5	0	Resistant
5	Oxacillin	OX-1	9	Intermediate
6	Azithromycin	AZM-15	20	Susceptible
7	Doxycycline	DO-30	16	Susceptible
8	Sulfamethoxazole	SXT-25	12	Intermediate
9	Amoxicillin	ANC-30	04	Resistant
10	Ampicillin	AMP-10	04	Resistant
11	Rifampin	RD-5	0	Resistant
12	Gentamicin	CN-10	15	Susceptible
13	Chloramphenicol	C-30	11	Resistant

Table. 4. Antibiogram pattern against *Klebsiella pneumoniae* detected from caged and free-living birds

S.No.	Antibiotic used	Symbol & disc potency (µg)	Inhibited zone diameter (mm)	Level of susceptibility
1	Norfloxacin	NOR-10	16	Intermediate
2	Ciprofloxacin	CIP-5	18	Resistant
3	Ceftriaxone	CRO-30	21	Resistant
4	Penicillin G	P-5	07	Resistant
5	Oxacillin	OX-1	0	Resistant
6	Azithromycin	AZM-15	15	Intermediate
7	Doxycycline	DO-30	18	Susceptible
8	Sulfamethoxazole	SXT-25	20	Susceptible
9	Amoxicillin	ANC-30	04	Resistant
10	Ampicillin	AMP-10	06	Resistant
11	Rifampin	RD-5	0	Resistant
12	Gentamicin	CN-10	03	Resistant
13	Chloramphenicol	C-30	06	Resistant

Table No 5. Antibiogram pattern against *Pasteurella multocida* detected from caged and free-living birds

S.No.	Antibiotic used	Symbol & disc potency (µg)	Inhibited zone diameter (mm)	Level of susceptibility
1	Norfloxacin	NOR-10	18	Susceptible
2	Ciprofloxacin	CIP-5	23	Intermediate
3	Ceftriaxone	CRO-30	22	Resistant
4	Penicillin G	P-5	10	Resistant
5	Oxacillin	OX-1	08	Resistant
6	Azithromycin	AZM-15	17	Intermediate
7	Doxycycline	DO-30	21	Susceptible
8	Sulfamethoxazole	SXT-25	20	Susceptible
9	Amoxicillin	ANC-30	20	Susceptible
10	Ampicillin	AMP-10	14	Resistant
11	Rifampin	RD-5	0	Resistant
12	Gentamicin	CN-10	16	Susceptible
13	Chloramphenicol	C-30	19	Susceptible

Antibiogram pattern against *Pasteurella multocida*: Antibiotic response of *Pasteurella multocida* isolated from both caged and free-living birds was determined. The Doxycycline (21mm), Sulfamethoxazole (20mm), Amoxicillin (20mm), Chloramphenicol (19mm), Norfloxacin (18mm) and Gentamicin (16mm) were shown susceptible against the isolated species (Table 5). Ciprofloxacin (23mm) and Azithromycin (17mm) exhibited intermediate resistant against the isolated *Pasteurella multocida* from avian species living in the different environmental conditions. Ceftriaxone (22mm), Ampicillin (14mm), Penicillin G (10mm) and Oxacillin (08mm) were detected resistant to isolated *Pasteurella multocida*. Rifampin shows complete resistant against the pathogen. In general, Doxycycline, Sulfamethoxazole, Amoxicillin were observed as the most effective antibiotics against the pathogen.

Antibiogram pattern against *Staphylococcus epidermidis*: *Staphylococcus epidermidis* isolated from caged and free-living birds was analyzed for antibiotic resistance profile for the various antibiotics. Norfloxacin (23mm), Doxycycline (23mm), Oxacillin (22mm), Chloramphenicol (22mm), Sulfamethoxazole (17mm) and Gentamicin (15mm) were susceptible against the isolated pathogen (Table 6). Ciprofloxacin (24mm) were recorded intermediate resistant against the isolated species. Penicillin G (13mm), Ampicillin (12mm), Rifampin (11mm), Amoxicillin (10mm) and Azithromycin (05mm) were observed resistant against the isolated *Staphylococcus epidermidis*. Ceftriaxone shows complete resistant against the pathogen. Overall, Doxycycline, Norfloxacin, Oxacillin, Chloramphenicol were determined highly efficient antibiotics against the isolated *Staphylococcus epidermidis* from the avian species.

Table. 6. Antibiogram pattern against *Staphylococcus epidermidis* detected from caged and free-living birds

S.No.	Antibiotic used	Symbol & disc potency (µg)	Inhibited zone diameter (mm)	Level of susceptibility
1	Norfloxacin	NOR-10	23	Susceptible
2	Ciprofloxacin	CIP-5	24	Intermediate
3	Ceftriaxone	CRO-30	0	Resistant
4	Penicillin G	P-5	13	Resistant
5	Oxacillin	OX-1	22	Susceptible
6	Azithromycin	AZM-15	05	Resistant
7	Doxycycline	DO-30	23	Susceptible
8	Sulfamethoxazole	SXT-25	17	Susceptible
9	Amoxicillin	ANC-30	10	Resistant
10	Ampicillin	AMP-10	12	Resistant
11	Rifampin	RD-5	11	Resistant
12	Gentamicin	CN-10	15	Susceptible
13	Chloramphenicol	C-30	22	Susceptible

Discussions

Findings of this study contribute to our knowledge of prospective treatment options and resistance patterns by shedding light on the antimicrobial susceptibility profiles of different bacterial species isolated from both caged and free-living birds. The antibiotics sulfamethoxazole, azithromycin and doxycycline were indicating high efficiency against the isolated *Chlamydia psittaci*. It is generally, believed to have effective against the Chlamydia species because its capability of doxycycline to target and penetrate within the cell (Kong et al., 2015). In concurrent to our findings, azithromycin have been proved successful in control of *Chlamydia psittaci* infections in cockatiels (Guzman et al., 2010). Findings of current study indicates that doxycycline, azithromycin, gentamicin, and sulfamethoxazole were susceptible against *Chlamydia psittaci*. Results of the present study are close to reported by Hidasi *et al.,* (2013); Davies *et al.,* (2016), they stated that these antibiotics showed efficacy against chlamydial infections in avian species. Our study indicated that norfloxacin, ceftriaxone and ciprofloxacin showed resistance against *Chlamydia psittaci*. Results are in accordance with reports of previous studies, Hu *et al.,* (2013); Lopes *et al.,* (2015), they demonstrated that these antibiotics are not effective against *C. psittaci* *Chlamydia psittaci*, the bacterium that is responsible for psittacosis in birds, is resistant to various antibiotics and physical agents. The members of chlamydia genus contains cysteine-rich outer membrane proteins that are involved in the mechanism for synthesis of peptidoglycan. The cysteine-rich outer membrane proteins may interact and associated in replacement of peptidoglycan to produce an osmotically stable and rigid extracellular spore that is resistant to several physical and environmental agents (Hatch, 1996). Azithromycin and doxycycline were detected the highly efficient antibiotics against the *E. coli* isolated from the birds living in different environments. In contrast to results of current, the previous study detected developing resistance of Azithromycin in isolated *E. coli* (Ahsan et al., 2017). This finding is according to research Szmolka & Nagy., (2013); Smith *et al.,* (2014), their studies support the antimicrobial resistance growing trend among *E. coli* strains in both veterinary. The observed sensitivity to gentamicin and norfloxacin against *E. coli* strains are in accordance with earlier studies of Ievy *et al.,* (2020); Bhattarai *et al.,* (2024), they stated that the use of norfloxacin and gentamicin are still effective despite the fact that resistance is growing against a large number of *E. coli* strains. The current research results indicated that sulfamethoxazole and doxycycline were indication of high efficiency against the isolated *Klebsiella pneumonia* from bird species. Previously the similar effective antibiotic response of doxycycline, cotrimoxazole, and ciprofloxacin were measured using Time-curve analysis (Setiwan et al., 2022). The resistance to oxacillin, and penicillin G were observed against *Klebsiella pneumoniae*, indicating multi-drug resistance in animals (Awad *et al.,* 2016). Ciprofloxacin, ceftriaxone, and gentamicin shown resistance to *K. pneumoniae* in this investigation is consistent with findings of Johar *et al.,* (2021); Ghorbani *et al.,* (2022), they stated that these drugs ongoing albeit declining, effectiveness against a few strains of bacteria which causes infections in avian species.

In this study, doxycycline, sulfamethoxazole, amoxicillin, chloramphenicol, norfloxacin and gentamicin were observed the most effective antibiotics against isolated *Pasteurella multocida*. Previous research studies of Clemente *et al.,* (2015) and Diren Sigirci *et al.,* (2019), stated that these antibiotics work against respiratory infections caused by *P. multocida*. In current finding, rifampin, penicillin G, ceftriaxone were detected resistant to isolated *Pasteurella multocida*. The research explore ciprofloxacin, and azithromycin exhibited intermediate susceptibility against the isolated organism. However, Giacopello *et al.,* (2015), they indicating that these antibiotics may not be option best for treating *P. multocida* infections in birds. In this study, norfloxacin, doxycycline, oxacillin, chloramphenicol, sulfamethoxazole and gentamicin were shown enhanced activity against the isolated *Staphylococcus epidermidis* from the avian species. The findings are in line with Hidasi *et al.,* (2013); Yilmaz & Dolar, (2017), they showing that these antibiotics were effective treatment for staphylococcal infections. Amoxicillin, ampicillin, ceftriaxone, and penicillin G were resistance to these bacterial species and this study observations are in accordance with findings of Pomba *et al.,* (2017); Machado *et al.,* (2018), they reported that most of the staphylococcal infections were found resistance against methicillin-resistant staphylococcus strains (MRSA) around the world.

CONCLUSIONS

It is concluded from present study that antibiotic resistance profile indicated high efficiency of sulfamethoxazole, doxycycline and azithromycin against *Chlamydia psittaci Klebsiella pneumonia, Escherichia coli, Staphylococcus epidermidis* and *Pasteurella multocida*. Penicillin G, amoxicillin, oxacillin, and ampicillin were resistant against the identified pathogens. It is inferred that the current findings will be valuable in identifying and effectively managing bacterial pathogens in both confined and free-living birds, providing valuable insights for improving treatment strategies and enhancing bird's health outcomes.

ETHICAL STATEMENT

The samples were collected from both caged and free-living birds following ethical guidelines to ensure minimal distress and harm. The collection process was conducted with respect for animal welfare and in compliance with relevant regulations.

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AUTHORS' CONTRIBUTION

Concept: SHA, RA, AMUD. Plan: AMUD, SHA, MS. Data Analysis: AMUD, SHA, DHK, & RA. Writing, Review and Editing: AMUD, SHA, DHK, MS, RA and All authors have reviewed and consented to the final version of the manuscript for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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