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Occurrence of esbl-producing *Escherichia coli* and Methicillin-resistant *Staphylococcus sciuri* in the faecal droppings of free-ranging Indian vultures (*Gyps indicus*)

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Abstract

Aim: Vultures are considered as sentinel species at the human-wildlife interface. As it is important to monitor such species for the presence of antimicrobial resistance (AMR), the current study focused on the characterisation of two such bacteria, which were identified from the faecal droppings of the Indian vulture (*Gyps indicus*).

Methodology: Conventional culture methods were used to isolate *Escherichia coli* and *Staphylococcus sciuri,* followed by confirmation using BD Phoenix M50 bacterial identification system and 16S rRNA gene amplification. Antibiotic susceptibility was performed using Kirby-Bauer disc diffusion method and BD phoenix M50 bacterial antibiotic susceptibility system.

Results: Extended spectrum beta-lactamase producing *Escherichia coli* and methicillin-resistant and mupirocin high level resistant *Staphylococcus sciuri* was identified in the study. *Staphylococcus sciuri* was also resistant to linezolid.

Interpretation: The findings of the study show that vulture and other scavenger species do carry multi-drug resistant organisms and may contribute to environmental dissemination. The study highlights the urgent need to reduce the misuse of antibiotics at the clinical setting, thereby implementing strict rules and regulations for the use of antibiotics. Therefore, following "One health approach" is required to tackle such problem.

Key words: ESBL, Indian vulture (*Gyps indicus*), Multidrug-resistant



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Introduction

Indian vulture (*Gyps indicus*) is a critically endangered species of vulture is known for their socio-cultural and religious significance in Indian subcontinent (Jha *et al.,* 2020; Birdlife International, 2021). Their scavenging nature helps reduce the risk of disease spread like brucellosis, tuberculosis and anthrax by disposing off the animal carcassesquickly before making the carcass available to the opportunistic scavengers such as feral dogs (Kanaujia and Kushwaha, 2013; Plaza *et al.,* 2020). The high acidic pH of the vulture's stomach, immune system adaptions and stable protective intestinal microbiome may contribute to the coping mechanism of vultures against harmful pathogens (Plaza *et al.,* 2020). Although performing essential ecosystem services, the vultures may act as carriers for certain pathogens. They have been reported to carry *Bacillus anthracis* spores within the gastro-intestinal tract and externally on the beak, talons, feathers and other body surfaces. However, the vegetative form of the bacterium is rapidly inactivated during passage through the digestive tract (Bellan *et al*., 2013).

The role of wild animals in carriage and spreading of antimicrobial resistance is poorly understood (Espunyes *et al.,* 2022). Vultures are sentinel species connecting the domestic and sylvatic cycle. They may carry antimicrobial resistant organisms across both cycles. Their behaviour of feeding ondisposal areas and also on carcasses of livestock, which may be treated with antibiotics, contribute to the emergence of antimicrobial resistant organisms (Vela *et al.,* 2014; Blanco *et al.,* 2018; Carvalho *et al.,* 2020). Antimicrobial resistance (AMR) is not a local phenomenon, but it is a global, ecological and genetic problem. Bacterial resistance genes can move and multiply across humans, animals and the environment they may either be inherent to a bacterial lineage through vertical inheritance or from unrelated microbes through horizontal gene transfer. Even bacteria that are not normally pathogenic can pose a public health concern if they carry AMR genes, since these genetic elements can be passed on to disease-causing bacteria through transmission route. (McDanel, 2017; Partridge *et al.,* 2018).

Antimicrobial resistance (AMR) has been recognized as one of the ten most urgent global health threats by WHO (Walsh *et al.,* 2023). The extensive use of antibiotics leads to the dissemination of resistant bacteria and resistance genes in animals and humans (Tang *et al.,* 2023). Although several studies have highlighted the dual role of vultures in limiting disease transmission through efficient carcass disposal, while also acting as potential reservoirs or disseminators of antimicrobial-resistant organisms, significant research gaps remain regarding antimicrobial resistance in vultures, particularly in relation to its molecular epidemiology and genetic mechanisms underlying resistance. At the 78th World Health Assembly (May 2025), the countries have agreed to update the Global Action Plan on AMR and discuss a revised version at the 79th World Health Assembly in 2026. One of the key goals of this initiative is to strengthen systems for monitoring and surveillance (WHO, 2026). The current study characterises the multidrug resistant *Escherichia coli* and *Staphylococcus sciuri* isolated from wild rescued Indian vulture brought to the School of Wildlife Forensic and Health, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh. The study emphasizes that the vulture species in the wild are carriers of AMR bacteria and can potentially spread such bacteria to humans increasingthe likelihood of zoonotic transmission, particularly where vultures congregate near human settlements, landfills or livestock areas, thereby bridging the gap between wildlife and the human food chain.

Materials and Methods

The present study was conducted at the School of Wildlife Forensic and Health (SWFH), Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. The investigation was designed to assess the occurrence and characteristics of microorganisms and antimicrobial resistance determinants present in the faecal droppings of vultures maintained under different environmental conditions. Vultures (n=20), rescued from wild (n=3/20) and captive vultures (n=17/20) from Gandhi Zoological Park, Gwalior, Madhya Pradesh (N=8/17) and Kamla Nehru Prani Sangrahalaya, Indore, Madhya Pradesh (N=9/17) were included in this study.Indian vulture – 1 (IV -1) and Indian vulture – 2 (IV -2) were brought by Madhya Pradesh Forest Department from Tikariya, Katni, and Chargawan, Jabalpur, Madhya Pradesh, respectively. Fresh faecal droppings of vultures were collected using sterile bacteriological swabs (HiMedia Laboratories Pvt. Ltd.). Swab samples were transported to the laboratory by maintaining a strict cold chain (4°C). Bacterial isolates were obtained using conventional culture methods described as per Markey *et al.* (2013).

The swabs were pre-cultured in Brain-heartinfusion (BHI) broth (HiMedia Laboratories Pvt. Ltd.) for 18 hrs at 37°C. For isolation of *E. coli*, a loopful of BHI culture was inoculated on MacConkey lactose agar (HiMedia Laboratories Pvt. Ltd.). Pink colonies observed on MacConkey lactose a garwere further sub-cultured on Eosin Methylene Blue agar (EMB) agar (HiMedia Laboratories Pvt. Ltd.) to observe green metallic sheen. Colonies with green metallic sheen were inoculated on EMB agar for 18 hrs at 37°C to isolate pure culture of *E. coli.* For isolation of *Staphylococcus sciuri*, a loopful of BHI culture was inoculated on Mannitol Salt agar (MSA) (HiMedia Laboratories Pvt. Ltd.) and the pure colonies isolated were again inoculated on blood agar (HiMedia Laboratories Pvt. Ltd.) to check for the haemolysis activity of bacteria. Gram's staining was carried out following the procedure of Tille (2022). Identification of bacterial species was done on BD Phoenix M50 bacterial identification system (Becton, Dickinson and Company) using commercially available kitsmanufacturer's instructions. The results were further confirmed by performing PCR (Proflex PCR system, Thermo Fisher Scientific Inc.) for amplification of 16S rRNA gene by using published universal primers (Williamson *et al.,* 2021). The primers (Barcode Biosciences Pvt. Ltd.) used in the study were forward primer (27F) - AGAGTTTATCCTGGCTCAG and reverse primer

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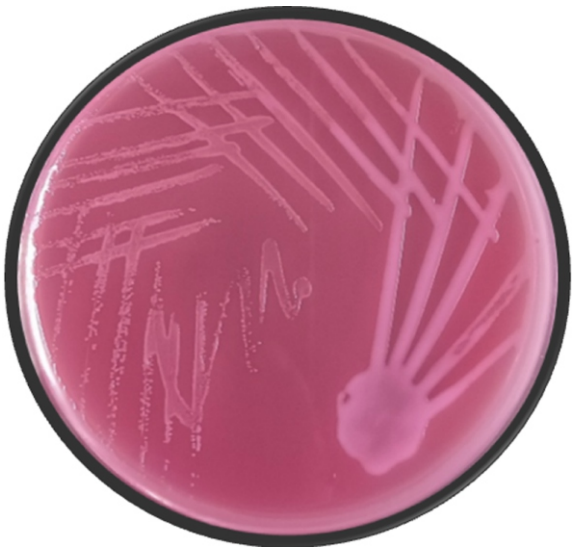


Fig. 1: Pink lactose fermenting colonies suspected for *Escherichia coli* on MacConkey lactose agar.

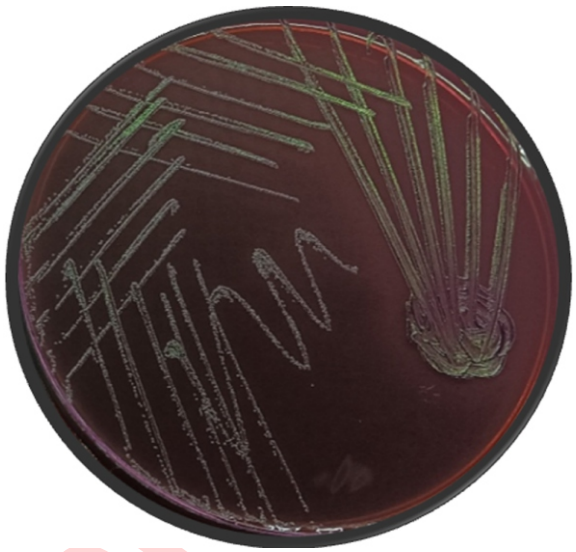


Fig. 2: Dark colonies showing 'green metallic sheen' on Eosin Methylene Blue (EMB) agar suspected for *Escherichia coli.*

(338R) - TGCTGCCTCCCGTAGGAGT. The results were visualized on gel documentation unit (SYNGENE InGenius3) after gel electrophoresis (Hoefer Inc.) Antibiotic susceptibility profile of both bacterias were performed by BD Phoenix M50 bacterial antimicrobial susceptibility system using commercially available kits and using Kirby-Bauer disc diffusion method as per the standard methods described by CLSI (2024a) against 17 antimicrobials. Zone of inhibition diameter interpretation was done using the breakpoints mentioned in CLSI (2024b). No descriptive statistical analysis was carried out in this study. Calculation of multiple antibiotic resistance (MAR) index was done using the formula given by Krumperman (1983).

Results and Discussion

Escherichia coli was isolated from Indian vulture (IV -1) brought to SWFH from Tikariya, Katni, Madhya Pradesh. Pink lactose fermenting colonies were observed on MacConkey lactose agar (Fig. 1). Medium sized colonies producing 'green metallic sheen' were observed on EMB agar (Fig. 2). InGram's staining, the culture revealed short to medium sized straight Gram-negative rods under 1000X magnification (Fig. 3). *Staphylococcus sciuri* was isolated from Indian vulture (IV -2) which was brought to SWFH from Chargawan, Jabalpur. Yellow coloured colonies indicating mannitol fermentation and growth in high salinity of the medium indicated possibility of *Staphylococcus* spp. (Fig. 4). Golden coloured colonies were observed on Blood agarwith no haemolysis (Fig. 5). Gram's staining of the visibly pure colonies revealed Gram-positive cocci under 1000X magnification (Fig. 6). Both the isolates were further confirmed to species level on BD Phoenix M50 bacterial identification system using commercially available kits. The amplification of 16S rRNA gene using conventional PCR following published primers (27F and 338R) was positive for band production at approximately 330bp. Antibiotic susceptibility profile of each isolate using Kirby-Bauer disc diffusion method (CLSI, 2024a) was performed against 17 antimicrobials discs selected. For *E. coli*, the antibiotics selected were meropenem (MRP), nalidixic acid (NA), amoxycillin and clavulanic acid (AMC), Levofloxacin (LE), Ciprofloxacin (CIP), Tetracycline (TE), Ceftazidime (CAZ), Imipenem (IPM), Ceftizoxime (CZX), Cefoxitin (CX), Cefpodoxime (CPD), Ampicillin (AMP), Amikacin (AK), Ceftriaxone (CTR), Aztreonam (AT), Cefepime (CPM) and Piperacillin and tazobactam (PIT). The isolate showed resistance to nalidixic acid, ceftazidime, ceftizoxime, cefoxitin, cefpodoxime, ceftriaxone, cefepime, aztreonam and ampicillin using Kirby-Bauer disc diffusion method, while resistance to ceftazidime, ceftizoxime, cefoxitin, ceftriaxone, cefepime, cefazolin, cefuroxime and ampicillin was seen in broth microdilution method done by BD Phoenix M50 bacterial antimicrobial susceptibility system. Although discrepancies were found for sensitivity against aztreonam in both the mentioned methods.

Antibiotics selected for *S. sciuri* using Kirby-Bauer disc diffusion method were Erythromycin (E), Linezolid (LZ), Gentamicin (GEN), Rifampicin (RIF), Chloramphenicol (C), Penicillin-G (P), Clindamycin (CD), Ceftriaxone (CTR), Cefotaxime (CTX), Co-Trimoxazole (COT), Oxacillin (OX), Ciprofloxacin (CIP), Ampicillin (AMP), Ceftazidime (CAZ), Levofloxacin (LE), Tetracycline (TE) and Meropenem (MRP). The isolate showed resistance to linezolid, penicillin-G, oxacillin, ampicillin and tetracycline, while resistance to oxacillin, penicillin-G, ampicillin, cefoxitin, cefazolin, mupirocin and mupirocin high level was seen in broth microdilution method done by BD Phoenix M50 bacterial antimicrobial susceptibility system.

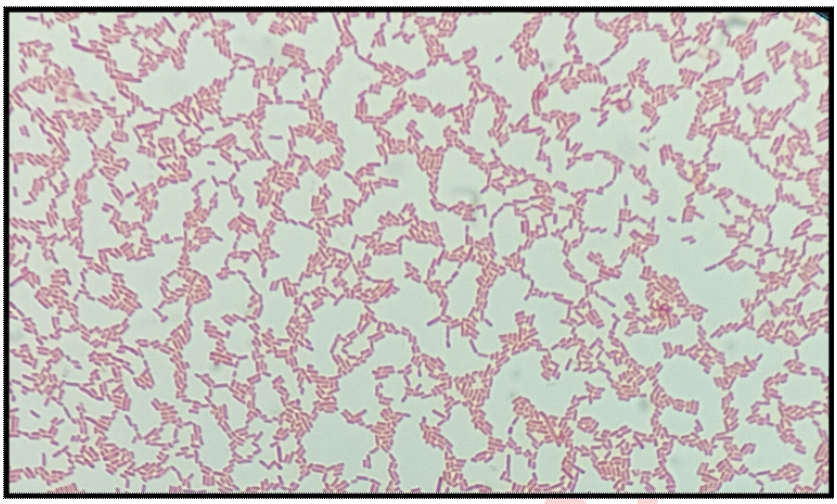


Fig. 3: Gram negative straight rods of *Escherichia coli* observed under 1000x magnification.

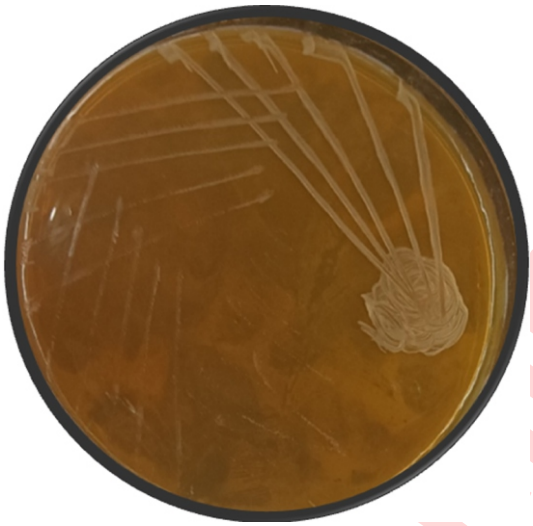


Fig. 4: Yellow coloured colonies showing mannitol fermentation on Mannitol Salt agar (MSA) suspected for *Staphylococcus* spp.



Fig. 5: Golden coloured colonies suspected for *Staphylococcus aureus* on blood agar.

The calculated MAR index value for the isolate was 0.29, which indicates origin of pathogen from region of high antibiotic exposure environment (Krumperman, 1983). The resistance profile of *E. coli* (Table 1) showed resistance to second, third and fourth generation cephalosporins along with resistance to monobactam and penicillin, which is highly suggestive of extended spectrum beta-lactamase (ESBL) producing characteristics in the isolate. The calculated multiple antibiotic resistance (MAR) index was 0.53, which indicates a high-risk pathogen (Krumperman, 1983). The ESBL producing characteristic of the isolate has been associated with blaCTX-M-15 gene in previous studies (Haenni *et al.,* 2023).

Also, the occurrence of ESBL-producing *E. coli* was found to be 22.7% (n=5/22), of which all isolates carried blaCTX-M-15gene (Carvalho *et al.,* 2020). There is a strong correlation between ESBL-producing and AmpC-producing characteristics of *E. coli* isolates (11.8%) identified in Eurasian griffon vultures (*Gyps fulvus*) in studies done by Haenni *et al.* (2023). Also, the occurrence of ESBL-producing *E. coli* in this study strongly correlates with prevalence of more than 50% of ESBL-producing E.coli in Gambian Hooded vultures (*Necrosyrtes monachus*) of Gambia (Woksepp *et al.,* 2023). Not only vultures, but other wild birds also carry ESBL producing *E. coli,* which was 16% of the tested birds (n=100) in Northern Spain (Alcalá *et al.,* 2016).

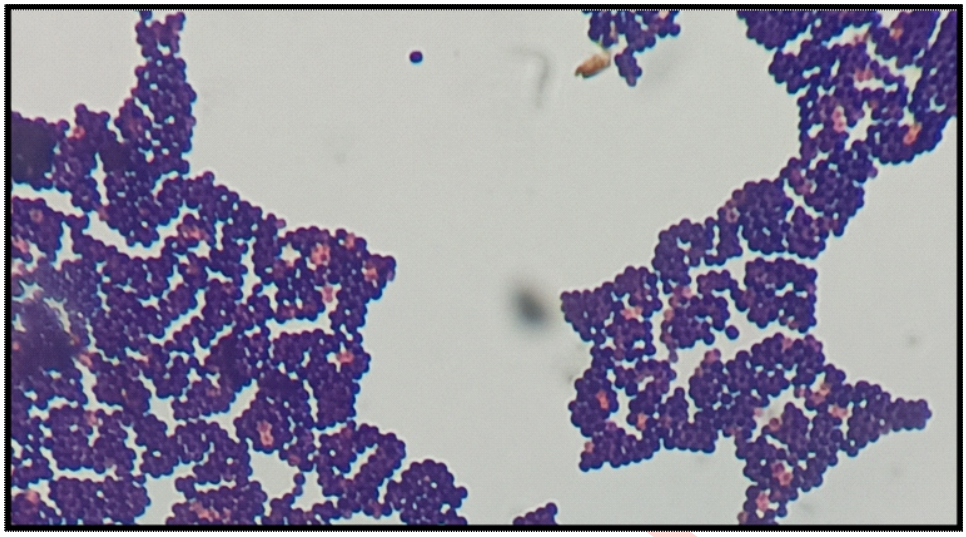


Fig. 6: Gram-positive cocci of *Staphylococcus sciuri* observed under 1000x magnification.

Table 1: Antibiotic susceptibility results using Kirby-Bauer disc diffusion method and BD phoenix M50 bacterial antimicrobial susceptibility system for *Escherichia coli*

Antibiotics	Symbol	IV-1	IV -1[a]
Meropenem	MRP	S	S
Nalidixic acid	NA	R	-
Levofloxacin	LE	S	S
Amoxycillin and clavulanic acid	AMC	S	-
Ciprofloxacin	CIP	S	S
Tetracycline	TE	S	S
Ceftazidime	CAZ	R	R
Imipenem	IPM	S	S
Ceftizoxime	CZX	R	R
Cefoxitin	CX	R	R
Cefpodoxime	CPD	R	-
Ampicillin	AMP	R	R
Amikacin	AK	I	S
Ceftriaxone	CTR	R	R
Aztreonam	AT	R	R
Cefepime	CPM	R	R
Piperacillin and tazobactam	PIT	S	S
Cefazolin	CZ	-	R
Cefuroxime	CXM	-	R
Nitrofurantoin	FM	-	S

S: Sensitive, I: Intermediate, R: Resistant, IV-1: Kirby-Bauer disc diffusion method and IV-1[a]: BD phoenix M50 bacterial antimicrobial susceptibility system

Raghav *et al.* (2021) reported occurrence of multidrug resistant E.coli in the faecal samples of wild birds of Madhya Pradesh, which strengthens the findings of this study. Recent study by Garcia *et al.* (2025) shows occurrence of high-risk clones of ESBL-producing *E. coli* strains, from a rusty-barred owl and a black vulture, undergoing rehabilitation at a zoological park in South-east Brazil. Genes encoding for ESBL production were related to blaCTX−M−15 gene. Study done by Cerezo-Caro *et al.* (2025) reported occurrence of multiclass resistant *E. coli* (236/282), which showed resistance to atleast three classes of antimicrobials. Approximately, 65.4% of the ESBL-producing *E. coli* isolates recovered from the captive wild birds of seven major zoological gardens of Nigeriacarried blaCTX-M gene, while blaTEM and blaSHV gene were detected in 15.4% and 34.6% of the isolates, respectively (Adesola *et al.,* 2025).

Discrepancies between the two methods is seen for some antibiotics which can be attributed to various reasons including poor diffusion rates of some antibiotics or diffuse zone of inhibition leading to errors in measurement (Sader *et al.,* 2020; Anita *et al.,* 2023). The multidrug-resistant *E. coli* isolate recovered from an Indian vulture exhibited a resistance pattern consistent with extended-spectrum β-lactamase (ESBL)-producing strains, indicating the presence of clinically significant antimicrobial resistance traits in free-ranging scavenging birds. The high MAR index further suggests exposure to environments subjected to substantial antimicrobial selection pressure. Similar findings reported from vultures and other wild birds across different geographical regions support the role of scavenging species as potential reservoirs and disseminators of antimicrobial resistance determinants. The resistance profile for *S. sciuri* (Table 2) showed resistance to oxacillin and cefoxitin, which is an indicator for phenotypically methicillin-resistant *Staphylococcus* (MRS). It also showed resistance to cephalosporins and mupirocin high level. Mupirocin is a topical antibiotic used for treating bacterial skin infections (Das *et al.,* 2025). The resistance of the isolate can be sourced from presence of mecA gene (Ruiz-Ripa *et al.,* 2019). The resistance of the isolate to linezolid is to be noted here, although further confirmation with molecular techniques for

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Table 2: Antibiotic susceptibility results using Kirby-Bauer disc diffusion method and BD phoenix M50 bacterial antimicrobial susceptibility system for *Staphylococcus sciuri*

Antibiotics	Symbol	IV-2	IV-2[a]
Erythromycin	E	S	S
Linezolid	LZ	R	-
Gentamicin	GEN	I	S
Rifampicin	RIF	S	S
Chloramphenicol	C	I	S
Penicillin-G	P	R	R
Clindamycin	CD	S	S
Ceftriaxone	CTR	S	S
Cefotaxime	CTX	S	-
Co-Trimoxazole	COT	S	-
Oxacillin	OX	R	R
Ciprofloxacin	CIP	S	S
Ampicillin	AMP	R	R
Ceftazidime	CAZ	S	-
Levofloxacin	LE	S	-
Tetracycline	TE	R	-
Meropenem	MRP	S	-
Cefoxitin	CX	-	R
Cefazolin	CZ	-	R
Mupirocin	MUP	-	R
Mupirocin high level	MUH	-	R
Trimethoprim	TMP	-	R

S: Sensitive, I: Intermediate, R: Resistant IV-2: Kirby-Bauer disc diffusion method and IV-2[a]: BD phoenix M50 bacterial antimicrobial susceptibility system

presence of resistance genes needs to be undertaken, as the antimicrobial agent is seen as "last resort" antibiotic for treatment of MRSA organisms (Stefani *et al.,* 2010). The resistance can be attributed to the cfr gene present in the organism which can be transferred to other bacteria horizontally (Stefani *et al.,* 2010).

These findings the reports of Raghav *et al.* (2021) who reported 11.4% resistance against oxacillin for *Staphylococcus* spp. isolated from wild birds of Madhya Pradesh. The presence of methicillin-resistant Staphylococcus aureus (MRSA) in wild birds of Poland was found to be 22% (Kutkowska *et al.,* 2019) and 5% int the Eurasian Griffon vulture of Spain (Porrero *et al.,* 2013), which further supports the results of this study and suggests that wild birds especially scavenger birds can potentially carry harmful, organisms, indicating possible bidirectional transmission. Isolation of methicillin-resistant and high-level mupirocin- resistant *Staphylococcus sciuri* from an Indian vulture highlights the emergence of clinically important antimicrobial resistance mechanisms in wildlife species. Resistance to oxacillin, cefoxitin and linezolid raises concerns regarding the potential presence and dissemination of transferable resistance genes, including mecA and cfr. Similar observations in wild birds suggest that scavenging species may contribute to the environmental circulation of multidrug-resistant bacteria and their associated resistance determinants.

The findings of this study suggest that vultures may serve as an indicator species for the transfer of multidrug-resistant bacteria between humans and wildlife. The resistance profiles of both isolates are particularly concerning. The *E. coli* isolate demonstrated extensive resistance to beta-lactams, including third and fourth-generation cephalosporins (Ceftriaxone, Ceftazidime, Cefepime) and the monobactam (Aztreonam). In the case of *S. sciuri,* the observed resistance to Linezolid was highly significant. Linezolid is considered a "last-resort" antibiotic for treating serious Gram-positive infections in humans. Thus, the vultures (especially free-ranging) become a source of contamination and agents for spreading multi-drug resistant bacteria. These multidrug-resistant bacteria are often result of exploited use of antibiotics in clinical settings and disposing of such organic waste and carcasses in the areas where vultures breed and feed in large numbers. Such multi-drug resistant bacteria can potentially spread these genes to further generation vertically or to other bacteria horizontally which further intensifies already serious concern. Not only animal carcasses but human hospital waste is also responsible for such multi-drug resistant bacteria. So, a comprehensive 'One-health approach encompassing human, animal and environmental health is the need of the hour for tackling such issues.

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