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Prevalence of pathogenic *Enterobacteriaceae* in the excreta of free living wild birds in Agroecosystems

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Abstract

The occurrence and population density of Enterobacteriaceae in the excreta of six bird species: blue rock pigeon (*Columba livia*), rose-ringed parakeet (*Psittacula krameri*), common myna (*Acridotheres tristis*), house crow (*Corvus splendens*), cattle egret (*Bubulcus ibis*), and red-wattled lapwing (*Vanellus indicus*) was studied. A total of 12 excreta samples were analyzed from approximately 60 pellets. Bacterial isolates were identified through biochemical characterization using a standard biochemical test kit. The isolates belonged to *Escherichia coli*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Klebsiella pneumoniae*, all of which are members of the Enterobacteriaceae family and are known to be pathogenic to humans. Among pigeon samples, *E. coli* showed the highest prevalence (75%), while *C. freundii*, *E. cloacae*, and *K. pneumoniae* were detected in 25% of samples. House crow excreta showed 100% prevalence of *K. pneumoniae*. The bacterial counts(cfu/g) varied among bird species. Overall, 73% of excreta samples were positive for *E. coli*, while 64% showed the presence of *C. freundii*, *E. cloacae*, and *K. pneumoniae*. These findings indicate that bird excreta may serve as a significant source of zoonotic bacterial pathogens, posing a potential health risk to themselves and human beings.

Keywords: Free living birds, excreta, Enterobacteriaceae, bacteria, pathogens

Introduction

The excreta of free-living birds harbour a variety of pathogens such as viruses, bacteria, and fungi and these pathogens can be transferred during the movements of these and result in intra- and interspecies transmission (Gogu-Bogdan *et al.*, 2014) [1]. Although many bacteria are a part of the normal intestinal flora of birds, bird species associating with potentially contaminated environments, such as refuse dumps, sewage treatment facilities and agricultural sites are more likely to harbor pathogenic bacteria in their intestinal tracts. Most of the birds infected with zoonotic bacteria do not display clinical symptoms and are asymptomatic carriers, however, they pose a significant risk to human health (Kobuszewska, and Wysok, 2024) [2]. Of particular concern in the farm environment are the Gram-negative bacteria within the family Enterobacteriaceae that includes *Salmonella* species, *Escherichia coli*, *Enterobacter aerogenes* and *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Providencia alcalifaciens* which are pathogenic to humans (Vilcins *et al.*, 2002) [3]. Additionally, antibiotic-resistant strains of these bacteria have been detected in wild bird excreta, raising concerns about the role of wildlife in the dissemination of resistance genes (Mansoor *et al.*, 2024) [4]. The high prevalence of Enterobacteriaceae members in the digestive tract and fecal material of birds is driven by a combination of unique avian physiology, environmental exposure, and dietary factors that favor facultative anaerobes as the birds have a shorter digestive tract, faster transit times, and a distinct anatomy where the digestive and urogenital tracts meet at the cloaca, creating a unique, oxygen-accessible environment suitable for these bacteria (Pan and Xu, 2014) [5]. The wild birds harbor antibiotic-resistant strains of *Campylobacter jejuni* and *Escherichia coli* which can be transmitted to humans through direct contact or environmental contamination and posing significant health risks. Besides this, *Salmonella enteric*, a multidrug-resistant, Gram-negative, facultatively anaerobic or aerobic bacteria, often found to be present in the digestive tract of birds and humans can spread through faecal material of birds and lead to contamination of water and food sources (Ebani *et al.*, 2021) [6]. The literature reported presence of bacteria in fecal samples of birds of agroecosystems such as pigeons, house crow, myna, parakeet etc.

The excreta of free-living wild birds may act as reservoirs of pathogenic bacteria and pose a risk to public health and other wildlife. The present study was thus planned to isolate and identify the pathogenic bacteria (family *Enterobacteriaceae*) from the excreta of free living wild birds commonly found in agroecosystems of Ludhiana so that appropriate management practices could be followed to minimize exposure to these pathogenic bacteria.

Materials and Methods

Collection of excreta of birds

Fresh excreta of different bird species (blue rock pigeon, *Columba livia*; rose ringed parakeet, *Psittacula krameri*; common myna, *Acridotheres tristis*; house crow, *Corvus splendens*; cattle egret, *Bubulcus ibis* and red wattled lapwing, *Vanellus indicus*) was collected from their roosting, foraging and nesting sites in Punjab Agricultural University (PAU), dairy farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) and residences of Ludhiana city. After collecting the excreta samples in sterile plastic bags with the help of spatula these were labeled appropriately with the source, time and date of collection and then stored at 4 °C till further processing.

Isolation and characterization of bacteria

One gram of fresh excreta sample of each species was thoroughly mixed in 9 ml of MacConkey broth and kept at 37°C for 24 hours for pre-enrichment of sample. Aliquots (0.1 ml) from serial logarithmic dilutions of each suspension were pipetted onto the surface of duplicate Petriplates containing MacConkey agar. The inoculum was spread evenly with a sterile T-shaped glass applicator. The Petriplates were incubated at 37 °C for 24 - 48 hours. The mean colony count was determined and represented as colony forming units (cfu/g) of each sample.

Identification criteria of bacteria

The bacteria isolated were identified using Himedia *Enterobacteriaceae* identification kit which was provided with two sterile white opaque strips with 12 wells each for various biochemical and carbohydrate utilization tests. The tests were based on the principle of pH change and substrate utilization. On incubation the bacteria undergo metabolic changes which were indicated by a colour change in the media that was either visible spontaneously or after addition of a reagent (Table 1, Table 2).

Results and Discussion

The excreta of six bird species was tested for the presence of bacteria (*Enterobacteriaceae* family). Few genus of bacteria were isolated from 12 excreta samples collected from about 60 pellets of six different species i.e. blue rock pigeon (n=4), rose ringed parakeet (n=1), common myna (n=1), house crow (n=3), cattle egret (n=1) and red wattled lapwing (n=1). This may be due to the reason bacteria need a temperature of 37 °C for their growth, but in the present studies the collection was done in fields after the elimination from the bird body where the temperature is quite different from the growth temperature of bacteria. The amount of bacteria decreased with age of feces. Birds are widely recognized as important reservoirs and disseminators of microorganisms, including several bacterial pathogens of

public health concern. Due to their mobility, feeding habits, and frequent proximity to human settlements, birds can play a significant role in the environmental spread of enteric bacteria (Ebani *et al.*, 2021) ^[6].

The bacterial colonies were identified by biochemical characterization. These isolates belonged to *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae* strains based on the results of biochemical tests in the kit (Fig. 1). The members of the family *Enterobacteriaceae* including *Salmonella* species, *Escherichia coli*, *Enterobacter aerogenes* and *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Providencia alcalifaciens* are pathogenic to humans (Janda and Abbott, 2021) ^[7]. Thus excreta of all bird species studied in present work indicated a source of infection to humans.

Out of the 4 samples of pigeon excreta samples tested for *Enterobacteriaceae* family, 3 were positive for *E. coli* indicating its prevalence in 75% of the samples and *C. freundii*, *E. cloacae* and *K. pneumoniae* was detected in only one sample revealing 25% of the samples showing positive isolation (Table 2). The occurrence of *E. cloacae* and *E. coli* was highest (2×10^9 cfu/ g) followed by *K. pneumoniae* (1.2×10^9 cfu/ g) and *C. freundii* (8.2×10^8 cfu/ g) (Table 3). Earlier, 2.5% of 400 fresh fecal samples of rock pigeons found to be positive for shiga-toxin producing *E. coli* 0157 (Abulreesh, 2011) ^[8]. Shiga -toxin producing *E. coli* were found in fecal droppings and/or cloacal swabs of pigeons that live in urban and rural areas around the world (Kobayashi *et al* 2009, Kowalczyk and Wójcik-Fatla, 2025) ^[9,10]. The pigeons act as a possible reservoir of Shiga toxin 2f-producing *E. coli* associated with human disease (Wani *et al.*, 2004, Sonntag *et al.*, 2005) ^[11, 12].

The excreta of rose ringed parakeet carried highest bacterial count of *E. coli* (7×10^9 cfu/ g) followed by *C. freundii* (6.5×10^9 cfu/ g), *K. pneumoniae* (2.7×10^9 cfu/ g) and *E. cloacae* (1.4×10^9 cfu/ g). *E. coli* (64%) was also isolated from caged parrots of dhaka zoo of Bangladesh (Akhter *et al.*, 2010) ^[13].

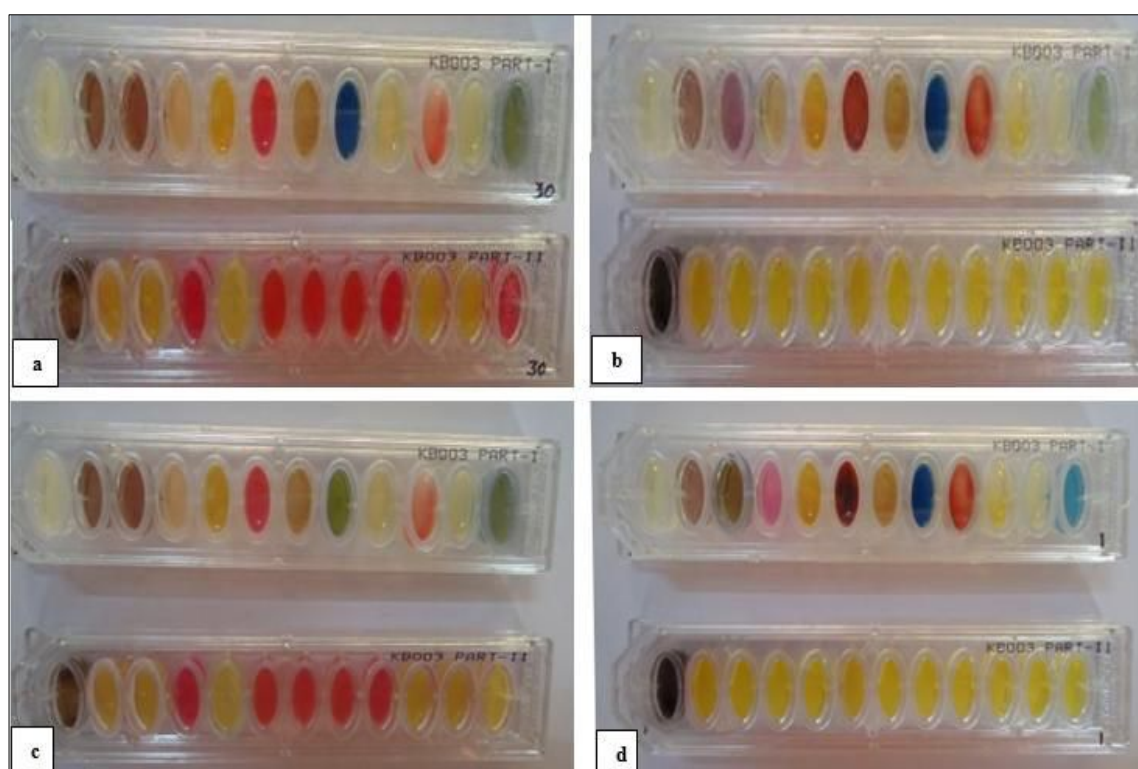
Out of the 3 samples of house crow excreta tested for *Enterobacteriaceae* family, 3 were positive for *K. pneumoniae* indicating its prevalence in 100% of the samples *E. cloacae* was isolated from 2 samples only showing its prevalence in 66% of samples and *C. freundii* and *E. coli* was detected in only one sample revealing 33% of the samples showing positive isolation. The excreta of house crow carries highest bacterial count of *E. cloacae* (1.7×10^9 cfu/ g) followed by *C. freundii* (3.4×10^8 cfu/ g), *E. coli* (3×10^8 cfu/ g) and *K. pneumoniae* (2.3×10^8 cfu/ g). Earlier, 9 different species of bacteria belonging to the family *Enterobacteriaceae* (*Citrobacter freundii*, *Enterobacter cloacae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, *Salmonella arizonae*, *Salmonella typhi*, *Shigella flexneri* and *Shigella sonnei*) in fresh fecal samples of the large-billed crow (*Corvus* sp.) collected from the study site at Bangsar, an urban setting in Kuala Lumpur, Malaysia (Yong *et al.*, 2010) ^[14]. Crows being scavengers with very versatile feeding habits that will take food from the ground or might have fed on items which have been contaminated by infectious droppings from another infected and become infected or be carriers of these pathogens.

The excreta of common myna carries highest bacterial count of *K. pneumoniae* (3.5×10^9 cfu/ g) followed by *E.coli*. (2.4×10^9 cfu/ g), *C. freundii* (1.4×10^9 cfu/ g) and *E. cloacae* (8×10^8 cfu/ g). The excreta of cattle egret showed highest count of *E. cloacae* (1.6×10^9 cfu/ g) followed by *E. coli* (2×10^8 cfu/ g) and *C. freundii* (1.7×10^7 cfu/ g). The excreta of red wattled lapwing carried highest population density of *E. cloacae* (4×10^9 cfu/ g) followed by *E. coli* (2.4×10^9 cfu/ g), *K. pneumoniae* (1×10^9 cfu/ g) and *C. freundii* (1.2×10^7 cfu/ g). These findings suggested that excreta of common myna, cattle egret and red-wattled lapwing act as significant carriers of pathogenic enteric bacteria and may contribute to environmental contamination and zoonotic transmission (Cocoş *et al.*, 2025)^[15].

In the present studies the population density of *C. freundii* was highest in rose ringed parakeet, *E. cloacae* in red wattled lapwing, *E.coli* in rose ringed parakeet and *K. pneumoniae* in common myna and 73% of excreta samples showed presence of *E.coli* and 64% were positive for *C. freundii*, *E. cloacae* and *K. pneumoniae* (Fig. 2). *Escherichia coli*, is a common inhabitant of the intestinal tract of numerous vertebrate species. In the past *E. coli* also has been reported from bird species. While most strains of *E. coli* are considered commensal, some strains are responsible for a variety of illnesses, including urinary tract infections (UTIs), gastroenteritis, and bacteremia but more specifically, the Shiga toxin-producing *E. coli* (STEC)

serotype 0157:H7 has been associated with human outbreaks of hemorrhagic colitis and hemolytic uremic syndrome after the consumption of contaminated food, especially beef (Adams *et al.*, 2016, Nemati *et al.*, 2024)^[16, 17]. *Enterobacter* species, particularly *E. cloacae* and *E. aerogenes*, are important nosocomial pathogens responsible for various infections, including bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, CNS, and ophthalmic infections. *Enterobacter* species can also cause various community-acquired infections, including UTIs, skin and soft-tissue infections, and wound infections, among others. The bacteria, *Klebsiella pneumoniae* is a major cause of pneumonia (Jezzini *et al.*, 2024)^[18], urinary tract infections (Filev *et al.*, 2025)^[19], bloodstream infections and sepsis associated with high morbidity and mortality, particularly in cases involving multidrug-resistant strains (Shi *et al.*, 2025)^[20].

Thus, excreta of different bird species in agroecosystems harbor a wide range of pathogenic members of the Enterobacteriaceae family. As these birds are free living, so spreads of infectious agents may occur through excreta of these birds which serve as a significant source of environmental contamination and pose potential zoonotic risks to humans and emphasizing the need for proper hygiene and following of appropriate monitoring measures.



- Citrobacter freundii*
- Enterobacter cloacae*
- Escherichia coli*
- Klebsiella pneumoniae*

Fig 1: Biochemical characterization of Bacteria isolated from excreta of different bird species

Table 1: Reactions shown by different isolated bacteria on identification kit

Strip No.	Well No.	Name of Biochemical test	Positive reaction	Negative reaction	<i>Citrobacter freundii</i>	<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>	<i>Kleibsellia pneumoniae</i>
1	1	ONPG Test	Yellow colour	No colour change	+	+	V	+
	2	Lysine utilization	Purple / Dark Purple colour	No colour change or yellow colour	-	-	V	+
	3	Ornithine utilization	Purple / Dark Purple colour	No colour change or yellow colour	V	+	V	-
	4	Urease Test	Pink colour	No colour change	V	V	-	+
	5	Phenylalanine Deamination Test	Dark green colour	No colour change	-	-	-	-
	6	Nitrate Reduction Test	Pinkish red colour	No colour change	+	+	+	+
	7	H ₂ S production	Orange yellow colour to black	No color change or slight yellowish brown	V	-	-	-
	8	Citrate utilization	Blue colour	Green or no colour change	+	+	-	+
	9	Voges-Proskauer's Test	Pinkish red colour	No colour change or a copper colour	-	+	-	+
	10	Methyl red Test	Red colour	Yellowish orange colour	+	-	+	V
	11	Indole Test	Appearance of pinkish red ring	No red ring	-	-	V	-
	12	Malonate utilization	Blue colour	Light green or no colour change	V	V	-	+
2	1	Esculin hydrolysis	Cream colour changes to black colour	No colour change or brownish yellow colour	-	V	-	+
	2	Arabinose	Colour change to yellow colour	Red/Pink or no colour change	+	+	V	+
	3	Xylose			+	+	V	+
	4	Adonitol			-	V	-	+
	5	Rhamnose			+	+	V	+
	6	Cellobiose			V	+	-	+
	7	Melibiose			V	+	V	+
	8	Saccharose			V	+	V	+
	9	Raffinose			V	+	V	+
	10	Trehalose			+	+	+	+
	11	Glucose			+	+	+	+
	12	Lactose			V	+	V	+

+ indicates positive reaction; - indicates a negative reaction and V indicates a variable reaction

Table 2: Distribution of bacteria in excreta of different bird species

S. No.	Bird species	Total no. of samples examined	Number of samples yielded			
			<i>Citrobacter freundii</i>	<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>	<i>Kleibsellia pneumoniae</i>
1.	Blue rock mpigeon	4	1	1	3	1
2.	Rose ringed parakeet	1	*	*	*	*
3.	Common myna	1	*	*	*	*
4.	House crow	3	1	2	1	3
5.	Cattle egret	1	*	*	*	*
6.	Red wattled lapwing	1	*	*	*	*

ND refers to not detected,

*Only one sample tested, thus no intraspecies comparison made

Table 3: Population density (mean CFU/ g of excreta) of pathogenic bacteria in different bird species

S. No.	Bird species	CFU/ g			
		<i>Citrobacter freundii</i>	<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>	<i>Kleibsellia pneumoniae</i>
1.	Blue rock pigeon	8.2 x 10 ⁸	2 x 10 ⁹	2 x 10 ⁹	1.2 x 10 ⁹
2.	Rose ringed parakeet	6.5 x 10 ⁹	1.4 x 10 ⁹	7 x 10 ⁹	2.7 x 10 ⁹
3.	Common myna	1.4 x 10 ⁹	8 x 10 ⁸	2.4 x 10 ⁹	3.5 x 10 ⁹
4.	House crow	3.4 x 10 ⁸	1.7 x 10 ⁹	3 x 10 ⁸	2.3 x 10 ⁸
5.	Cattle egret	1.7 x 10 ⁷	1.6 x 10 ⁹	2 x 10 ⁸	ND
6.	Red wattled lapwing	1.2 x 10 ⁷	4 x 10 ⁹	2.4 x 10 ⁹	1 x 10 ⁹

ND refers to not detected

cfu= colony forming units

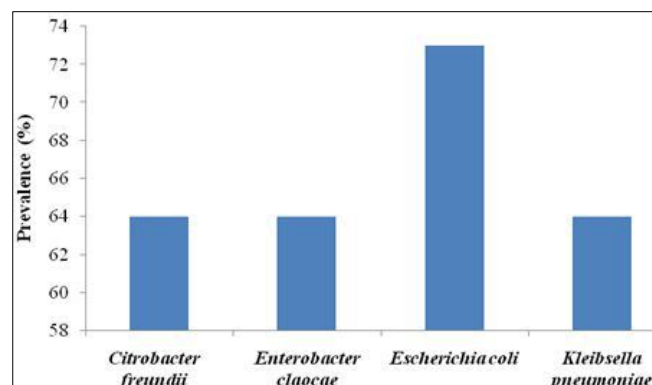


Fig 2: Prevalence of bacteria in excreta of different bird species

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