

BACTERIOLOGICAL ISOLATION AND THEIR ANTIBIOGRAM AND MYCOLOGICAL ANALYSIS OF PIGEON DROPPINGS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: Pigeon feces are a major source of infectious pathogens spreading to the surrounding environment in yards and live-bird markets. The aim of this study is to isolate, identify, and characterize the bacterial and fungal species present in pigeon droppings, and to evaluate the antibiotic susceptibility patterns (antibiogram) of the bacterial isolates.

Methods: The present study is a cross-sectional, descriptive, laboratory-based study conducted for a period of eight months from Jan 2025 to Aug 2025 in a Tertiary care teaching hospital, Chevella. 110 pigeon dropping samples were taken using sterile cotton swabs from various locations, including temples, historic places, and public parks. Pigeon droppings were cultured on Nutrient, MacConkey and Blood agar and incubated at 37 °C for 24–48 h to isolate bacteria. Identification was based on colony morphology, Gram staining, and standard biochemical tests. Antimicrobial susceptibility was determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar following CLSI guidelines. Fungal isolates were obtained on Sabouraud Dextrose Agar at 25–28 °C and identified by their macroscopic and microscopic features.

Results: In the present study, the maximum bacterial count was identified in 10⁻⁴ dilution which ranged from 41 x 10⁵ cfu/ml. The major isolates obtained were CONS 38.45%, *Pseudomonas aeruginosa* 27.6%, *E. coli* 16.9%. Among the fungal isolates, *Candida* species 33.3% followed by *Aspergillus niger* 29.16% from pigeon droppings. Most of the CONS were resistant to penicillin antibiotic 80% and *Pseudomonas* isolates were resistant to CXM and COT 77.8% followed by 72.3% to AMC and third-generation cephalosporins. Most of the bacterial isolates obtained in the study were multidrug-resistant isolates.

Conclusion: Overall, pigeon droppings represent a potential public health hazard, contributing to environmental contamination, source of MDR isolates and possible zoonotic transmission and hence preventive measures should be followed to prevent environmental contamination and Zoonotic transmission.

Keywords: Pigeon droppings, Bacteria, Fungi, MDR, Antibiotics

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INTRODUCTION

Humans who have frequent contact with pigeons at home, live bird markets, and on farms are at serious risk of contracting diseases, including *Salmonella* spp., *Campylobacter*, *Escherichia coli*, and *Chlamydia*, while they are around them. Healthy pigeons are a significant human salmonellosis source [1]. Pigeon feces are a major source of infectious pathogens spreading to the surrounding environment in yards and live-bird markets. Pigeons are a source of *E. coli* and are very good at spreading the bacteria to animals, birds, and people. It has been proposed that *E. coli* poses a potential zoonotic risk to humans, causing urinary tract infections [2]. In domestic animal species, and poultry specifically, antimicrobial resistance (AMR) restricts the therapeutic options for treating bacterial illnesses [3]. AMR disease strains may be hosted and spread by birds, endangering human health [4]. With respect to the main bacterial infections, the prevalence of AMR has been increasing [5]. A growing problem worldwide is the presence of multidrug-resistant (MDR) *Salmonella* spp. in several animal species that are bred for food. Through the food chain, antibiotic-resistant zoonotic agents from an animal host may infect humans [6].

Pigeons are found all throughout the world, in both rural and urban areas, and they have intimate interactions with both people and other animals. Because pigeon feces may carry a variety of microorganisms, they may aid in the spread of infectious diseases in the environment. Strong human pathogens, such strains of diarrheagenic *Escherichia coli* may be among them, increasing the risk of exposure and infection in humans [7, 8]. Clinically and economically, the development of antibiotic resistance in Gram-negative bacteria poses a serious threat to the treatment of humans and animals. *Pseudomonas*, *Stenotrophomonas*, *Aeromonas*, and

Enterobacteriaceae are examples of environmental microbes that are multi-resistant opportunistic pathogens that have been connected to severe illnesses in both humans and animals [8].

Pigeons can act as potential carriers of antimicrobial resistance (AMR) due to their frequent exposure to environments contaminated with antibiotic residues and resistant bacteria, such as urban areas, hospitals, farms, and landfills. These birds may harbor resistant bacteria like *Escherichia coli* or *Salmonella* in their digestive systems without showing any symptoms, making them silent reservoirs. Studies have identified resistance genes such as *bla*_{CTX-M} and *mcr-1* in bacteria isolated from pigeons, indicating their role in the environmental spread of AMR. Although pigeons are not a primary source of human infection, close or indirect contact with them, particularly through their droppings, can contribute to the transmission of resistant bacteria. This highlights the importance of including urban wildlife in AMR surveillance programs, as part of the One Health approach that connects human, animal, and environmental health. Pigeon droppings can cause several human infections. Histoplasmosis and *Cryptococcus*'s are fungal infections caused by inhaling spores from dried droppings, primarily affecting the lungs and posing a risk to immunocompromised individuals. Psittacosis, a bacterial infection caused by *Chlamydia psittaci*, can lead to flu-like symptoms and pneumonia after inhaling dust from droppings. Salmonellosis and *E. coli* infections may occur through contact with contaminated surfaces or ingestion, leading to gastrointestinal illness. Less commonly, *Campylobacteriosis* can also result from exposure to contaminated areas. Fungi commonly isolated from pigeon droppings include *Cryptococcus neoformans*, *Aspergillus* species, *Candida* species, *Cladosporium*, and *Penicillium*. These fungi thrive in the nutrient-rich droppings and can pose health risks, especially

Cryptococcus neoformans, which is a known pathogen causing cryptococcosis in immunocompromised individuals. The pigeon population in Hyderabad is high due to its warm climate, abundant food sources from human leftovers and temple offerings, plenty of nesting spots in old buildings, and general human tolerance and feeding habits. The aim of this study is to isolate, identify, and characterize the bacterial and fungal species present in pigeon droppings, and to evaluate the antibiotic susceptibility patterns (antibiogram) of the bacterial isolates. This investigation seeks to assess the public health risks associated with microbial contaminants found in pigeon excreta and to provide scientific data for developing effective control and preventive measures against possible zoonotic infections and antimicrobial resistance dissemination.

MATERIALS AND METHODS

Study design and study period

The present study is a cross-sectional, descriptive, laboratory-based study conducted for a period of eight months from Jan 2025 to Aug 2025 in a tertiary care teaching hospital.

Sampling

In Chevella, Ranga Reddy District, Telangana, 110 pigeon feces samples were taken using sterile cotton swabs from various locations, including temples, historic places, and public parks. After being properly sealed and labeled with the date and location of collection, pigeon droppings were gathered in sterile, clean plastic bags (plastic packets with disposable hand gloves). Nearly 100 g of sample was taken from each site. After being finely powdered by a grinding machine, the pigeon droppings were gathered in sterile, airtight containers and sent straight to the microbiology lab for examination.

Preparation of pigeon dropping suspension

In 9 ml of saline, 1g of the dry dropping powder was completely vortexed. After being moved into the next screw-capped tubes, the aliquot (1.0 ml) was serially diluted in tenth to tenth-of-a-dilution increments. A sterile spreader was used to spread a 0.1 ml aliquot from each dilution onto newly made nutritional agar plates after it had been transferred aseptically. For twenty-four hours, the inoculation plates were kept in a bacteriological incubator at 37 °C. The number of colonies that formed on the plate was measured and reported as cfu/ml. The streak plate method was used to create pure cultures of bacteria. Based on the macro-morphological colony characteristics, discrete bacteria colonies that developed were subcultured on nutrient agar and incubated at 37 °C for 24 h.

Isolation and identification of microorganisms

The bacterial isolates were identified using morphological characterization, Gram staining, cultural and biochemical characteristics by using standard microbiological procedures. MacConkey agar, Nutrient agar and Blood agar plates were used for the bacteria isolation from pigeon droppings and Sabouraud Dextrose Agar media plates for fungal isolation.

The identification of fungi was based on macro and microscopic characteristics. The surface and the reverse of the colonies were observed, as well as diameter, colony morphology, texture and pigmentation were identified. For isolation and identification, of the bacterial and fungal culture on the surface of the agar plate are

incubated and inoculated in petri plates and kept at 37 °C for bacteria and 28 °C for fungi. Bacterial morphology characteristics, such as colony diameter, growth, colour, form, were observed on culture medium. Morphological features such as shape and arrangement were observed after Gram's staining according to standard protocol. The fungal morphology was studied macroscopically by observing the colony features (colour, shape, size and hyphae) and microscopically by a compound microscope using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium. For the identification of bacterial isolates routine standard biochemical tests such as Indole test, Triple sugar test, Citrate utilization test, Urease test, Nitrite reduction test, Mannitol motility, carbohydrate fermentation, catalase test, coagulase test etc. were performed. Isolates were inoculated in these medium and incubated at 37 °C for 24 h.

Antimicrobial susceptibility test

All bacteria isolates were tested for antimicrobial susceptibility against routinely used antibiotics, meropenem (10 µg), ceftazidime (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), amoxicillin+clavulanic acid (25/10 µg), ciprofloxacin (5 µg), Amikacin (10 µg), gentamicin (10 µg) etc., using disk diffusion technique developed by Bauer *et al.* To determine whether the isolates were resistant or sensitive to the tested antimicrobial agents, the zone of growth inhibition was measured and compared to standards given by the Clinical and Laboratory Standards Institute. Resistance to three or more antimicrobial groups is considered multidrug resistance or MDR.

RESULTS

Table 1 showed the bacterial count for serial dilutions 10⁻¹ to 10⁻⁹ of pigeon droppings. The highest bacterial count was reported from 10⁻⁴ to 10⁻⁷ serial dilutions. Out of 110 pigeon samples, 65 samples showed bacterial growth and 24 samples showed fungal growth. Among the 65 samples showed bacterial growth, the highest number of samples 25 (38.4%) showed Coagulase-negative Staphylococci followed by 18 samples showed Staphylococci 27.6% (table 2). Out 24 fungal isolates screened from the pigeon dropping samples, the major isolates were *Aspergillus niger* in 07 samples 29.16% followed by *Aspergillus fumigatus* 20.83% (table 3). Table 4 depicted the antimicrobial susceptibility findings of bacterial isolates. Most of the *E. coli* isolates were highly susceptible to meropenem 72.7% and showed resistance to most of the tested antibiotics like AMC, CXM, CIP81.1% followed by resistance to all third-generation cephalosporins 72.7%.50% of the *Klebsiella* species were sensitive to MRP, AK, GEN and COT. All the 4 strains were resistant to AMC100%, and 75% strains were resistant to second and third-generation cephalosporins and ciprofloxacin. Out of 18 isolates of *Pseudomonas aeruginosa* 72.2% strains were highly susceptible to meropenem followed by 55.5% to Amikacin. Most of the *Pseudomonas* isolates were resistant to CXM and COT 77.8% followed by 72.3% to AMC and third-generation cephalosporins. Among the 7 isolates of *Acinetobacter* species 71.4% isolates showed maximum susceptibility to meropenem and 57.2% isolates to gentamycin and amikacin and most of the isolates were resistant to CIP 85.8% and COT 71.4%. All the 25 CONS isolated were sensitive to linezolid and teicoplanin 100% and 88% strains were sensitive to clindamycin and erythromycin, 72% to tetracycline. Most of the strains were resistant to penicillin antibiotic 80% (table 5).

Table 1: Bacterial count by serial dilution technique

Serial dilution	Bacterial count CFU/ml
10 ⁻¹	19 cfu/ml
10 ⁻²	23 cfu/ml
10 ⁻³	35 cfu/ml
10 ⁻⁴	41 x 10 ⁵ cfu/ml
10 ⁻⁵	20 x 10 ⁶ cfu/ml
10 ⁻⁶	8 x 10 ⁷ cfu/ml
10 ⁻⁷	3 x 10 ⁸ cfu/ml
10 ⁻⁸	5 cfu/ml
10 ⁻⁹	3 cfu/ml

Table 2: Bacterial isolates from pigeon samples

Bacteria	Number of isolates from pigeon samples	Percentage %
<i>E. coli</i>	11	16.9
Klebsiella sps.	04	6.15
Pseudomonas sps.	18	27.6
CONS	25	38.4
Acinetobacter sps.	07	10.76
Total	65	

Table 3: Fungal isolates from pigeon samples

Fungi	Number of isolates from pigeon samples	Percentages
<i>Aspergillus niger</i>	07	29.16%
<i>Aspergillus fumigatus</i>	05	20.83%
<i>Mucor</i>	04	16.6%
<i>Candida</i>	08	33.3%
Total	24	

Table 4: Antimicrobial susceptibility of bacterial isolates

Bacterial Isolate	Number of Isolates	Antimicrobial susceptibility	AMC	CXM	CTR	CTX	GEN	AK	MRP	COT	CIP	CAZ	
<i>E. coli</i>	11	Number of sensitive isolates	02 (18.9%)	02 (18.9%)	03 (27.3%)	03 (27.3%)	05 (45.5%)	05 (45.5%)	08 (72.7%)	03 (27.3%)	02 (18.9%)	03 (27.3%)	
		Number of resistance isolates	09 (81.1%)	09 (81.1%)	08 (72.7%)	08 (72.7%)	06 (54.5%)	06 (54.5%)	03 (27.3%)	08 (72.7%)	09 (81.1%)	08 (72.7%)	
		Number of sensitive isolates	00 (0.00%)	01 (25%)	01 (25%)	01 (25%)	02 (50%)	02 (50%)	02 (50%)	02 (50%)	02 (50%)	01 (25%)	01 (25%)
Klebsiella sps.	04	Number of sensitive isolates	00 (0.00%)	03 (75%)	03 (75%)	03 (75%)	02 (50%)	02 (50%)	02 (50%)	02 (50%)	03 (75%)	03 (75%)	
		Number of resistance isolates	04 (100%)	01 (25%)	01 (25%)	01 (25%)	02 (50%)	02 (50%)	02 (50%)	02 (50%)	02 (50%)	01 (25%)	01 (25%)
		Number of sensitive isolates	04 (100%)	05 (72.7%)	04 (57.1%)	05 (72.7%)	09 (55.5%)	10 (72.2%)	13 (50%)	04 (22.2%)	05 (27.7%)	05 (27.7%)	05 (27.7%)
Pseudomonas sps.	18	Number of sensitive isolates	05 (27.7%)	04 (22.2%)	05 (27.7%)	05 (27.7%)	09 (50%)	10 (55.5%)	13 (72.2%)	04 (22.2%)	05 (27.7%)	05 (27.7%)	
		Number of resistance isolates	13 (72.3%)	14 (77.8%)	13 (72.3%)	13 (72.3%)	09 (44.5%)	08 (27.8%)	05 (17.8%)	14 (77.8%)	13 (72.3%)	13 (72.3%)	
		Number of sensitive isolates	03 (42.8%)	03 (42.8%)	03 (42.8%)	03 (42.8%)	04 (57.2%)	04 (57.2%)	05 (71.4%)	02 (28.6%)	01 (14.2%)	03 (42.8%)	
Acinetobacter sps.	07	Number of sensitive isolates	04 (57.2%)	04 (57.2%)	04 (57.2%)	04 (57.2%)	03 (42.8%)	03 (42.8%)	02 (28.6%)	05 (71.4%)	06 (85.8%)	04 (57.2%)	
		Number of resistance isolates	03 (42.8%)	03 (42.8%)	03 (42.8%)	03 (42.8%)	04 (57.2%)	04 (57.2%)	05 (71.4%)	02 (28.6%)	01 (14.2%)	03 (42.8%)	
		Number of sensitive isolates	04 (57.2%)	04 (57.2%)	04 (57.2%)	04 (57.2%)	03 (42.8%)	03 (42.8%)	02 (28.6%)	05 (71.4%)	06 (85.8%)	04 (57.2%)	

Table 5: Coagulase-negative staphylococci antibiotic susceptibility pattern

Antibiotics	CONS (n=25)	
	Number and percentage of sensitive strains	Number and percentage of resistance strains
Gentamycin	15 (60%)	10 (40%)
Tetracycline	18 (72%)	07 (28%)
Clindamycin	22 (88%)	03 (12%)
Linezolid	25 (100%)	00 (0.00%)
Cotrimoxazole	13 (52%)	12 (48%)
Erythromycin	22 (88%)	03 (12%)
Cefoxitin	10 (40%)	15 (60%)
Penicillin	05 (20%)	20 (80%)
Ciprofloxacin	10 (40%)	15 (60%)
Teicoplanin	25 (100%)	00 (0.00%)



Fig. 1a: Growth of aspergillus species



Fig. 1b: Growth of candida on subculture from plate count method

Fig. 1c: Growth of *E. coli* on subculture from plate count methodFig. 1d: Growth of *Klebsiella* spp. on subculture from plate count method

Fig. 1 a-d: Microbial isolates from pigeon droppings

DISCUSSION

Esakkiammal and Sheeba Rajakumari in 2024 [9] performed similar study and found that, maximum number of bacterial population was exhibited in dilution 10^{-4} which ranged from 76×10^5 cfu/m. In the present study, the maximum bacterial count was identified in 10^{-4} dilution which ranged from 41×10^5 cfu/ml. Esakkiammal and Sheeba Rajakumari (2024) [9] isolated bacteria isolate of *Campylobacter* sp., *Salmonella* sp., and *Micrococcus* species. However, in the present study, most of the isolates were CONS 38.45%, *Pseudomonas aeruginosa* 27.6%, *E. coli* 16.9%, *Acinetobacter* species 10.76% and *Klebsiella* species. They also isolated different fungal isolates *Fusarium* sp., *Aspergillus* sp., and *Candida* sp. In the present study, we got major fungal isolates *Candida* species 33.3%, followed by *Aspergillus niger* 29.16% from pigeon droppings. Dutta *et al.* (2013) studied on similar work and revealed that *E. coli* 60.7% was the major isolate; however, in the present study we isolated 16.9% *E. coli* isolates [10]. The use of antibiotics is crucial for lowering mortality and illness rates associated with communicable diseases in both humans and animals, according to Tadesse *et al.* and Hur *et al.* [11-12] Intestinal disease is one of the most prevalent infectious diseases in animals that are treated with antibiotics. However, widespread use of antibiotics in both humans and animals has led to the emergence of antibiotic-resistant bacteria [11-12]. The emergence of resistant and multidrug-resistant diseases among animal isolates is a medical concern, even though few antibiotics used in animal husbandry treat human infections and many animal pathogens are zoonotic [13]. As a result, we looked at the sensitivity profile of *K. pneumoniae*, *E. coli*, and *E. cloacae* that were found in pigeon excrement in the present investigation. Elsheikh *et al.* [8] in 2024 studied about the presence of enteric bacteria from pigeon feces in Jeddah and their antimicrobial susceptibility and they found that, 50 antibiotic-resistant isolates, 28% (14/50) were *Klebsiella pneumoniae*, 24% (12/50) were *Enterobacter cloacae*, and 48% (24/50) were *Escherichia coli*. Ninety percent (90%) of the isolates showed resistance to cefuroxime, 56% to gentamicin, 52% to amoxicillin/clavulanic acid, and 100% to meropenem. The results were quite differed from the present study. In the present study, we obtained highest number of CONS 38.45% and *Pseudomonas aeruginosa* 27.6% in the pigeon droppings. All the 25 CONS isolated were sensitive to linezolid and teicoplanin 100% and 88% strains were sensitive to clindamycin and erythromycin, 72% to tetracycline. Most of the strains were resistant to penicillin antibiotic 80%. Out of 18 isolates of *Pseudomonas aeruginosa* 72.2% strains were highly susceptible to meropenem, followed by 55.5% to Amikacin. Most of the *Pseudomonas* isolates were resistant to CXM and COT 77.8% followed by 72.3% to AMC and third-generation cephalosporins. It was concluded from the study that, pigeon droppings pose a significant human health risk and act as a potential source for MDR isolates. The accumulation of pigeon droppings constitutes a public health danger and causes diseases.

CONCLUSION

The bacteriological and mycological examination of pigeon droppings revealed that these excreta serve as potential reservoirs of various pathogenic microorganisms, including both bacteria and fungi of public health significance. Common bacterial isolates may include *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* etc. while fungal isolates often include *Aspergillus* spp., *Candida* spp., *Mucor*. The antibiogram typically indicates that many of these bacterial isolates exhibit resistance to multiple antibiotics, underscoring the risk of environmental dissemination of antimicrobial-resistant strains. Similarly, the presence of opportunistic and pathogenic fungi highlights a risk of respiratory or systemic infections, especially in immunocompromised individuals. Overall, pigeon droppings represent a potential public health hazard, contributing to environmental contamination and possible zoonotic transmission and hence preventive measures should be followed to prevent environmental contamination and Zoonotic transmission like regular cleaning and disinfection at areas contaminated with pigeon droppings. Bird deterrents like nets and spikes can be installed to reduce pigeon roosting near human dwellings. Collected droppings should be properly disposed of to prevent environmental contamination. Public education on the health risks associated with pigeon droppings and routine monitoring for pathogenic microbes and antimicrobial resistance are also essential preventive measures.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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