Isolation, identification and antimicrobial sensitivity profile of *Escherichia coli* from captive small cats and a deer at Chattogram Zoo

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Abstract

Escherichia coli (E. coli) is commonly discovered in the gastrointestinal tract of animals, that also includes wild captive felines. Many *E. coli* strains are beneficial and help maintain gut homeostasis, but pathogenic strains are threatening because they can cause pantropic infections from urinary tract infections to enteritis. This study was performed to isolate *E. coli*, identify by polymerase chain reaction (PCR) and know the antimicrobial resistance profiles of *E. coli* in captive feline population. Oral and anal samples were collected from various species, including fishing cats, leopard cat and barking deer (maya horin) from Chattogram Zoo. The identification and isolation of bacteria were confirmed through their distinctive cultural characteristics, including pink colony formation on MacConkey agar and a greenish metallic sheen on EMB agar. For molecular identification, PCR amplification of the ADK gene was performed. To identify their resistance patterns, sensitivity of isolates was performed to 10 distinct antimicrobial drugs. *E. coli* was found in 13 out of 18 samples, accounting for 72% of the total. These thirteen isolated samples showed resistance to multiple antimicrobial drugs. All isolates were resistant to ampicillin, amoxicillin + clavulanic acid, cefoxitin, nalidixic acid, and sulfamethoxazole + trimethoprim. Only two isolates were intermediately sensitive to ceftriaxone resulted with 92% resistance. The maximum sensitivity was observed in imipenem (100%), followed by gentamicin (92.31%), cefapirin (85%), and ciprofloxacin (70%). The high prevalence of multidrug-resistant *E. coli* poses a significant public health concern due to the limited treatment options available. Furthermore, the potential for cross-transmission of antimicrobial-resistant *E. coli* between humans and animals underscores the urgency for comprehensive surveillance and stringent infection control measures.

Key words: Wild captive feline, *E. coli*, bacterial culture, PCR, antimicrobial resistance profile

Introduction

Escherichia coli is the type species of *Escherichia*, a genus of gram-negative, primarily motile bacilli belonging to the tribe Escherichia and the family Enterobacteriaceae (Bettelheim et al., 1994.; Edwards et al., 1972). *E. coli* has been identified in studies on many animals using typical microbiological techniques such as growing on selective medium (e.g., MacConkey agar), biochemical testing, and molecular approaches such as 16S rRNA gene sequencing. These procedures might also be used with samples from captive felines (Said et al., 2021). The most common methods for recovering *E. coli* from feces are MacConkey or eosin methylene-blue agar, which allow enteric organisms to be differentiated based on their appearance and selectively grow members of the Enterobacteriaceae (Balows et al., 2003). Additionally, *E. coli* may cause illnesses in animals, with colibacillosis being a prominent cause of bird death and causing significant financial losses globally (Schouler et al., 2012).

Wild felines populations are quickly declining in their native environment due to a number of causes, including extensive biosphere changes, poaching, and exposure to disease agents (Thalwitzer 2010; Henschel P 2014). The International Union for Conservation of Nature and Natural Resources has designated several wild felines species as endangered due to these recent changes in biodiversity. Space, enrichment, nutrition, and care are all elements that influence the well-being of captive felines. Ethical captivity emphasizes conservation, education, and rehabilitation, whereas exploitative captivity frequently entails poor welfare standards.

The presence of *Escherichia coli*, a bacterium typically detected in the intestines of felines housed in controlled conditions such as zoos and wildlife sanctuaries. While most *E. coli* strains are innocuous, some pathogenic strains (such as Shiga toxin-producing *E. coli*) can cause severe sickness. *E. coli* infections in confined settings might occur as a result of contaminated food or water, poor hygiene, environmental stress, or interspecies transmission. *E. coli*, a common gastrointestinal bacterium is found in most mammals including dogs, cats, human but elevation level of *E. coli* in the body of human animals can cause serious and life-threating signs. *E. coli* was one of the most significant pathogens among bacteria (Achá et al., 2004).

Understanding microbial ecosystems, disease development, and zoonotic dangers has long depended on the isolation and identification of Escherichia coli from animal hosts. Cats kept in

captivity, frequently in zoological or sanctuary environments, are subjected to certain environmental and nutritional stresses that may change their microbiome.

One of the biggest global health issues for both humans and animals is the proliferation of antimicrobial-resistant (AMR) bacteria. Reducing the use of antimicrobials is a crucial strategy for stopping the development of AMR as their widespread and unjustified usage in human and veterinary care is a barrier to the fight against AMR (Hiki et al., 2015; Kurita et al., 2019).

Nowadays, antimicrobial resistance (AMR) of *E.coli* is a growing worldwide issue that has an impact on both human and animal health. Clinical therapies can be severely hampered by resistant bacterial strains that can spread resistance genes across species, especially those that are present in close animal-human interactions.

The goal of this study is:

1. To isolate and identify *E. coli* from oral and anal swabs of captive felines and deer at Chattogram Zoo by bacterial culture and PCR
2. To know the Antimicrobial resistant test (AMP) of the *E. coli* isolates

Materials and Methods

2.1. Samples

Eight wild cats (leopard cat and fishing cat) and one ill deer (a barking deer) had their anal and oral swabs taken, along with information about the animals' health, upbringing, and diet, as well as the cleanliness and upkeep of their cages, with the help of veterinarian responsible for the zoo from Chattogram Zoo.

2.2. Study area

The targeted animals were situated in Chattogram Zoo, and the experiment was performed at Poultry Research & Training Center (PRTC), CVASU. The research period lasted from September to November.

2.3. Study population

Sample collected from a wounded, juvenile, 20 kg weighted deer is fed twice a day with fruit, vegetable and concentrated types of food. Swabs taken from healthy two male (3-4.5 years old) and two female (3 years) leopard cat (weight:3-4 kg), three male (4-4.5 years old) and one female fishing cat (weight:12-14 kg) are fed once a day with raw meat. Feeding supervision is always done by zoo staff. Cage hygiene is maintained by cleaning daily with detergent and water and wastes are removed daily zoo stuff. Pest control measures are also taken occasionally. All of the captive animals' drink water which is supplied from zoos. And enrichment materials (toys, climbing structures, puzzles etc) are readily available at all times.

2.4. Sample collection

Samples were collected directly from anus and oral cavity by cotton swab and were stored into bacterial transport media – Stuart transport media. After that these samples were leveled immediately and carried to laboratory by iced container.

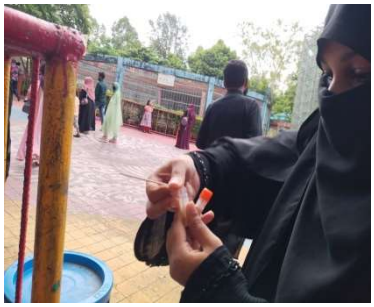

Figure 1: Sampling and storage of samples into Stuart medium at Chattogram Zoo

2.5. Bacterial culture for isolation and identification

- Agar preparation: 15.5 g MacConkey agar was mixed with 300 ml distilled water and boiled it until dissolved completely. Similarly 11.3 g EMB agar powder was mixed with 300 ml distilled water and boiled to dissolve. Then, both media was autoclaved to sterile at 121˚ C for 15 mins and cooled to about 50˚C to pour into sterile petri dish.
- *E. coli* was isolated and identified using the procedures described in Carson et al. (2001) and Quinn et al. (1999).
- According to this method, at first cotton swabs were replaced into peptone broth and incubated at 37˚C for 1 day and preserved for further use.
- Then Eosine Methylene Blue agar and MacConkey Agar media were used to isolate and identify *E. coli* in both oral and anal samples.
- Cotton swabs were first streaked over Mac Conkey Agar, then over Eosin-Methylene Blue (EMB) agar medium, and then incubated for 24 hours at 370C in order to isolate *E. coli*.

Figure:2: Measuring of agar powder

Figure:3: Streaking of bacterial colony

2.6. Molecular identification

2.6.1. DNA extraction from sample

DNA was extracted from sample by using boiling method.

- 100μl nuclear free water was taken into microcentrifuge tube.
- Then 2/3 colony of bacteria were added with nuclear free water and vortexed for 10 to 15 seconds.
- After vortex, mixture in microcentrifuge tubes were given heat shock by boiling in heat block at 99 °C temperature for 12 to 15 minutes.
- After that, cold shock was given at -20 °C for 5/6 minutes.
- The sample was centrifuged at 10,000 rpm for 5 minutes and finally preserved at -20 °C.

2.6.2. PCR analysis of ADK gene

Table 1: Primers for ADK gene amplification:

Name of primer	Primer	Sequence of primer	Length of sequence
ADK	F	ATTCTGCTTGGCGCTCCGGG	20 bp
	R	CCGTCAACTTTCGCGTATTT	20 bp

Preparation to perform PCR:

To run PCR reactions, each DNA sample was mixed with 20 µl of reagents in a PCR tube. DNA was extracted from samples by boiling method. Working primer was made from stock ADK forward and reverse primer.

Table 2: Reagents and their volume used in PCR

Components	Initial concentration	Final concentration	Total volume (20 µl)
Master mix (dNTP, $Mgcl_2$, dye, Taq polymerase)	2x	1x	10μl
Forward primer	1x	0.5x	0.5µl
Reverse primer	1x	0.5x	0.5µ
Nuclear free water			7µl
Template (extracted DNA)			2µl

2.6.3. Thermal profile for ADK gene

- Initial denaturation: 95 °C at 2 minutes
- 30 times cycles:
    - Denaturation: 95 °C at 1 minute
    - Annealing: 54 °C at 1 minute
    - Elongation: 72 °C at 2 minutes
- Final elongation: 72 °C at 5 minutes (stored at 4 °C for ∞)

Figure 4: Heat shock for DNA extraction

Figure 5: Preparation for PCR

Figure 6: Thermal cycling of DNA of *E. coli*

2.6.4. Gel electrophoresis

Tris buffer (TAE) solution and agarose were used for the electrophoresis. SYBR SAFE DNA gel strain was added to the 1.0% agarose gel to visualize the PCR result. These are fluorescent, which means they absorb invisible UV light and transfer the energy as visible orange light, and they attach to DNA forcefully by intercalating between the bases (Geurden et al., 2008).

Requirement:

- TAE buffer(1x) –Tris buffer
- Agarose powder
- SYBER SAFE DNA gel strain
- Distilled water

Procedure:

- ✓ We weighed 1.5 g of agarose into a flask and combined it with up to 100 ml of TAE buffer in a conical flask to create a 1.5% agarose gel.
- ✓ The agarose was then fully dissolved by heating in a microwave oven.
- ✓ A room temperature cooling period was subsequently given to the agarose-TAE buffer solution.
- ✓ To prepare the gel casting tray, the ends of the gel chamber were sealed with tape or the proper casting method, and the proper number of combs were added.
- ✓ The agarose-TAE buffer combination was mixed with two microliter of SYBR Green dye, shaken thoroughly, and then transferred into a gel tray.
- ✓ After that, the gel was let to cool for 15 to 30 minutes at room temperature.
- ✓ The combs were now taken out, and buffer (TAE buffer) was added to the electrophoresis chamber until the cast gel was completely submerged.
- ✓ Three microliters of the molecular weight marker (ladder) and four microliters of DNA were placed onto the gel.
- ✓ For 30 to 50 minutes, the electrophoresis was operated at 110 volts and 140 milliamperes.
- ✓ After that, the gel was placed in a UV transilluminator to capture and analyze images.

2.7. Antimicrobial sensitivity test

- ✓ Using the disc diffusion method, the isolates of the samples that tested positive for *E. coli* were put through an antibiotic sensitivity test.
- ✓ To guarantee the formation of pure colonies, the preserved organism was first cultured on blood agar and then incubated for 24 hours at 37°C as part of the cultural sensitivity (CS) test protocol.
- ✓ After that, three or four separate blood agar colonies were put into a tube with three milliliters of sterile phosphate buffer saline solution.
- ✓ A vortex machine was used to mix the inoculum. The bacterial solution was made to adhere to the McFarland standard of 0.5.
- ✓ In order to eliminate extra fluid, a pre-sterile cotton swab was inserted into the inoculum within 15 minutes of its preparation and spun against the tube's wall.
- ✓ Three times, this swab was used to streak the Mueller Hinton agar's whole dry surface while rotating the plate by around 60 degrees each time.
- ✓ Sterile discs were applied to the agar surface using sterile forceps following a 15-minute inoculation period.
- ✓ After that, the agar plates were incubated for eighteen hours at 37°C.
- ✓ A ruler was used to measure the clear area (in millimeters) surrounding each disc, including the diameter of the disk. The findings were classified as resistant, intermediate, or sensitive following the CLSI 2023 guidelines.

Figure 7: Antimicrobial sensitivity test

Table 3: List of panels of antimicrobials used and interpretation according to Clinical and Laboratory Standard Institute (CLSI) M100 document of 2023 in this study

Antibiotic Group	**Antibiotic**	**Disc Content**	**Zone diameter**		
			Sensitive(S)	**Intermediate(I)**	**Resistant(R)**
Beta-lactams					
Penicillin	Ampicillin	10 µg	≥ 17 mm	14-16 mm	≤ 13 mm
Penicillin + Beta-lactamase	Amoxicillin/Clavulanic Acid	20/10 µg	≥ 18 mm	14-17 mm	≤ 13 mm
Cephalosporins (1st gen)	Cefapirin	30 µg	≥ 18 mm	15-17 mm	≤ 14 mm
Cephalosporins (2nd gen)	Cefoxitin	30 µg	≥ 18 mm	15-17 mm	≤ 14 mm
Cephalosporins (3rd gen)	Ceftriaxone	30 µg	≥ 23 mm	20-22 mm	≤ 19 mm
Carbapenems	Imipenem	10 µg	≥ 23 mm	20-22 mm	≤ 19 mm
Fluoroquinolones	Ciprofloxacin	5 µg	≥ 21 mm	16-20 mm	≤ 15 mm
Quinolones	Nalidixic acid	30 µg	≥ 19 mm	14-18 mm	≤ 13 mm
Tetracyclines	Tetracycline	30 µg	≥ 15 mm	12-14 mm	≤ 11 mm
Aminoglycosides	Gentamicin	10 µg	≥ 15 mm	13-14 mm	≤ 12 mm
	Streptomycin	10 µg	≥ 15 mm	12-14 mm	≤ 11 mm
Folate Pathway Inhibitors	Sulfamethoxazole/ Trimethoprim	1.25/23.75 µg	≥ 16 mm	11-15 mm	≤ 10 mm

Result

3.1. Sample demographics

Nine samples total—four leopard cats, three fisher cats, and one deer—with thorough records of age, sex, and body weight are included in the study. The lone deer weighs 20 kg and is a female that is 2 years old. The leopard cats are divided into three females, ages 3, 3.5, and 3.5, weighing 3.5 kg, 4 kg, and 3.5 kg, respectively, and two males, ages 3 and 4.5, weighing 3.5 kg and 3 kg, respectively. The male fishing cats weigh 11 kg, 12 kg, and 14 kg and are between the ages of 4 and 4.5.

Table 4: Demographic data

Sample ID	Species	Age (year)	Sex	Body weight(Kg)
1	Deer	2	Female	20
2	Leopard cat	3	Male	3.5
3	Leopard cat	4.5	Male	3
4	Fishing cat	4.5	Male	14
5	Fishing cat	4	Male	12
6	Fishing cat	4	Male	11
7	Leopard cat	3	Female	3.5
8	Leopard cat	3.5	Female	4
9	Leopard cat	3.5	female	3.5

3.2. Prevalence of *E. coli*

18 samples were tested for bacterial growth using MacConkey agar, EMB agar and blood agar and Polymerase Chain Reaction (PCR). Eight samples (A1, A4, A6, O1, O2, O3, O5, O6) grew positively on MacConkey Agar, whereas ten samples (A2, A3, A5, A7, A8, A9, O4, O7, O8, O9) grew negatively. The thirteen samples (A1, A3, A4, A6, A7, A8, A9, O1, O2, O4, O5, O6, O9) showed positive growth on EMB Agar and beta hemolysis on Blood Agar (Beta hemolysis), which

indicated positive findings; the other 5 samples showed negative results. According to the PCR results, 13 samples were positive and just 5 samples (A2, A5, O3, O7, O8) were negative.

MacConkey agar: The characteristic lactose-fermenting, foul-smelling, pink colonies on eight samples (A1, A4, A6, O1, O2, O3, O5, O6) showed that *E. coli* was growing.

Figure 8: large pink colony of *E. coli* on MacConkey agar.

EMB agar: It displayed distinctive colonies with a greenish metallic shine on EMB agar plates. This characteristics was found on 13 samples(A1, A3, A4, A6, A7, A8, A9, O1, O2, O4, O5, O6, O9).

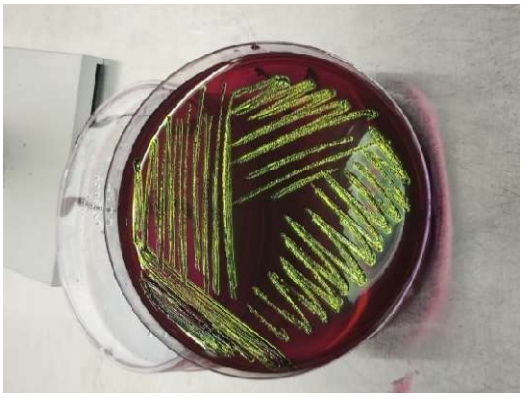

Figure 9: Greenish metallic sheen on EMB agar.

Table 5: Isolation of *E. coli* on selective agar media

Species	ID	Sample type O-oral, A-anal	Agar media		
			MacConkey Agar	EMB agar	Blood agar
Maya horin	1	O1	+	+	+
		A1	+	+	+
Leopard cat-1 (M)	2	O2	+	+	+
		A2			
L-2(M	3	O3	+		
		A3		+	+
Fishing cat-1(M	4	O4		+	+
		A4	+	+	+
F-2(M	5	O5	+	+	+
		A5			
F-3(F	6	O6	+	+	+
		A6	+	+	+
F-4(M	7	O7			
		A7		+	+
L-3(F	8	O8			
		A8		+	+
L-4(F)	9	O9		+	+
		A9		+	+

(here,+ means positive, - means negative)

3.3. Result of PCR

All bacterial samples had their entire DNA extracted for molecular identification using a PCR-based technique. Different temperature and thermal profile were followed according to protocol. Using PCR approach, ADK gene was amplified, and band was observed and bands were found at approximately 590bp . Molecular weight marker (ladder) was 100 bp. Total thirteen samples showed amplification at about 590 bp, confirming the presence of *E. coli.*

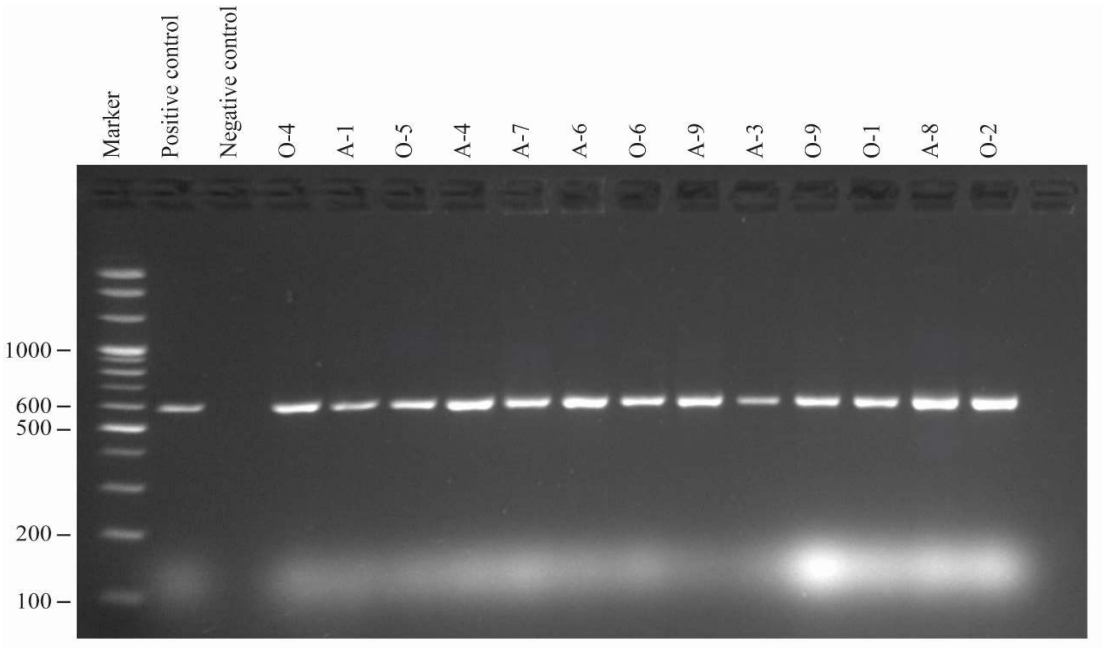

Figure 10: Electrophoretic separation of ADK gene of *E. coli* (ladder-100bp)

3.4. Antimicrobial microbial sensitivity pattern

After cultural isolation, 13 *E. coli* positive isolates were chosen for culture sensitivity (CS) testing. Antibiotic resistance in *E. coli* was detected by zone of inhibition and 14 different antimicrobial discs were employed.

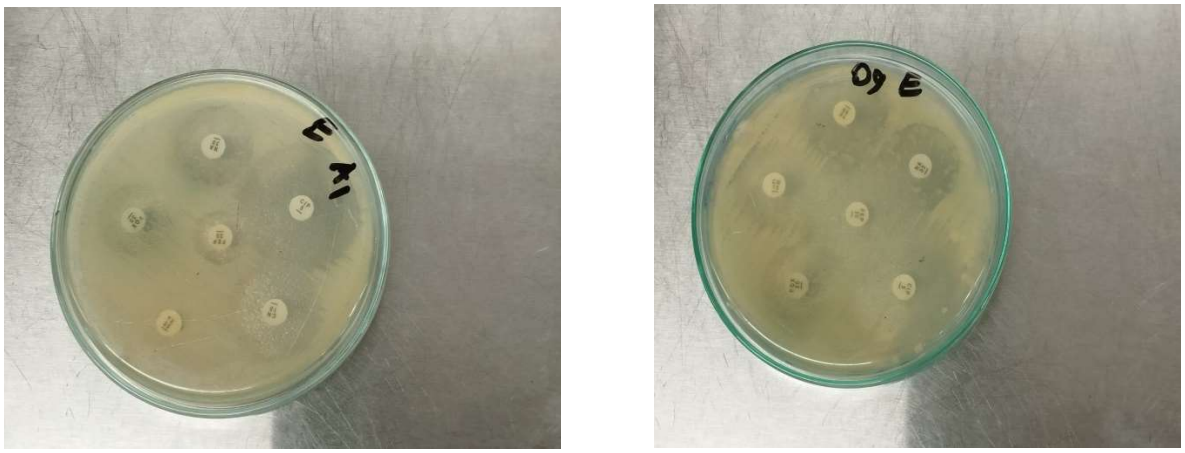

Figure 11: Zone of inhibition on Muller Hinton Agar after 18h incubation

Table 6: Antimicrobial resistance pattern of isolates

Antimicrobial	Disc code	Sample number	Resistant	Intermediate	Susceptible
Ampicillin	AMP	13	100%	-	-
Amoxicillin	AMC	13	100%	-	-
Cefapirin	FEP	13	23%	-	76%
Cefoxitin	FOX	13	100%	-	-
Ceftriaxone	CRO	13	85%	15%	-
Imipenem	IPM	13	-	-	100%
Ciprofloxacin	CIP	13	15%	15%	70%
Nalidixic acid	NAC	13	100%	-	-
Tetracycline	TE	13	54%	-	46%
Gentamicin	CN	13	8%	-	92%
Streptomycin	S	13	62%	23%	15%
Sulfamethoxazole trimethoprim	SXT	13	100%	-	-

The highest susceptibility was observed in imipenem (100%), gentamicin (92%), cefapirin (85%) and ciprofloxacin (70%); the highest resistance (100%) was observed in ampicillin, amoxicillin-clavulanic acid, cefoxitin, nalidixic acid, sulfamethoxazole-trimethoprim followed by ceftriaxone (85%), streptomycin (62%).

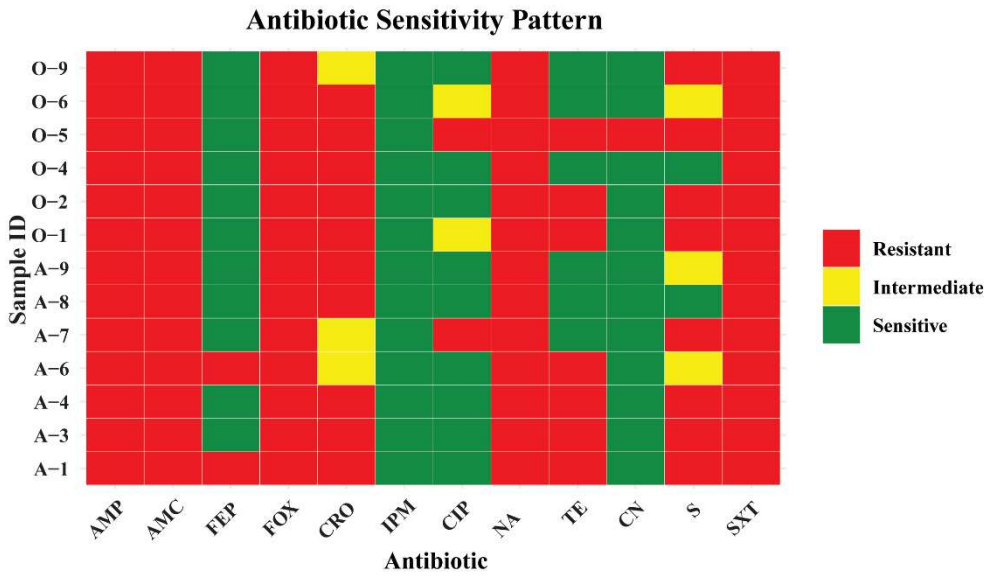

Figure 12: Antibiogram of *E. coli*

Discussion

This study focused on the isolation, identification, and antimicrobial resistance patterns of *Escherichia coli (E. coli)* isolated from wildlife samples, including various species such as Maya horin (deer), Leopard cats, and Fishing cats. The findings from our cultural, molecular, and antimicrobial susceptibility tests provide important insights into the prevalence of antibiotic resistance in wildlife, which is increasingly recognized as a significant factor influencing both public health and wildlife conservation efforts.

In total, 13 out of 18 samples were found positive for *E. coli,* confirming the presence of this bacterium across both oral (O) and anal (A) samples from the selected animals. The identification process involved the use of selective media, with *E. coli* colonies exhibiting characteristic features on MacConkey and EMB agar plates, such as lactose-fermenting, pink colonies on MacConkey agar and greenish metallic sheen on EMB agar, as shown in Figures 8 and 9. These findings are consistent with typical *E. coli* growth patterns on these selective media, which are designed to differentiate *E. coli* from other enteric bacteria based on their ability to ferment lactose and form distinctive colored colonies..

Antimicrobial resistance testing revealed concerning patterns, with 100% resistance to commonly used antibiotics such as ampicillin, amoxicillin, cefoxitin, and nalidixic acid (Table 5). This suggests that *E. coli* strains from wildlife in this study have developed resistance to several first-line antibiotics. The findings are consistent with those of other studies on *E. coli* resistance, where *E. coli* strains from wildlife, including carnivores, were found to exhibit high levels of resistance to ampicillin and other beta-lactams (Shao et al., 2015; Ranjan et al., 2017).

The observed resistance to amoxicillin-clavulanic acid, a commonly used combination therapy, may reflect the widespread use of antibiotics in veterinary and agricultural settings, leading to the selection of resistant strains. This aligns with reports that agricultural and veterinary antibiotic use contributes to the emergence of resistant bacteria in wildlife (Ghosh et al., 2018). Additionally, *E. coli* isolates from our study exhibited 85% resistance to ceftriaxone, a third-generation cephalosporin, which is a worrying indication of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strains. ESBL producers are often resistant to multiple classes of antibiotics, posing significant treatment challenges (Pitout & Laupland, 2008).

Interestingly, the study found moderate susceptibility to several other antibiotics. Imipenem and gentamicin demonstrated 100% and 92% susceptibility, respectively, while 70% of the isolates were susceptible to ciprofloxacin. This suggests that imipenem and gentamicin may still be effective treatment options for infections caused by *E. coli* in wildlife, though the emergence of resistance to other antibiotics is alarming. Ciprofloxacin, a fluoroquinolone, is often used as a second-line treatment for severe infections and its moderate resistance in 30% of isolates highlights the increasing concern regarding the spread of fluoroquinolone resistance (Andrews, 2001).

The results of this study have important implications for both public health and wildlife conservation. The presence of antibiotic-resistant *E. coli* in wildlife is a potential source of resistance transfer to humans, especially considering the zoonotic nature of *E. coli* and the close contact between humans and wildlife in certain environments (Briñas et al., 2005). For instance, wildlife may serve as a reservoir for resistant bacteria, which could be transmitted to humans through direct contact, consumption of contaminated food, or environmental exposure. The spread of antimicrobial-resistant pathogens from wildlife to humans could complicate the treatment of infections and undermine the effectiveness of current antibiotics (Marshall & Levy, 2011).

From a conservation perspective, the emergence of antibiotic resistance in wildlife may affect the health and survival of endangered species. Infected animals may suffer from chronic infections that reduce their fitness and reproductive success, potentially leading to population declines. Furthermore, the presence of resistant bacteria in wildlife habitats can contribute to the overall environmental spread of antimicrobial resistance. As such, this issue requires urgent attention from both wildlife conservationists and public health authorities to develop strategies that address the use of antibiotics in wildlife habitats and minimize the risk of resistance spread. Our findings are consistent with the results of other studies that have reported high levels of antimicrobial resistance in wildlife populations. A study by Kümmerer et al. (2000) showed that wildlife species, particularly those living in areas of high human and agricultural activity, harbor antibiotic-resistant bacteria, with *E. coli* being one of the most commonly detected pathogens. Similarly, the study by Czekalski et al. (2014) on antibiotic resistance in freshwater ecosystems found that *E. coli* from wild animals exhibited resistance to several antibiotics, with resistance levels varying depending on the location and species.

In comparison to these studies, our results show a similar pattern of high resistance to beta-lactams and other common antibiotics, but also highlight the relatively high susceptibility to imipenem and gentamicin. This variability in resistance patterns across studies may be attributed to differences in sampling methods, geographic location, and the specific species of wildlife studied. However, the overall trend of increasing antibiotic resistance in wildlife populations is consistent across studies, underscoring the need for monitoring and controlling the spread of resistant bacteria in these populations.

This study provides valuable insights into the antimicrobial resistance patterns of *E. coli* isolated from wildlife, revealing high levels of resistance to commonly used antibiotics, including ampicillin, amoxicillin, and ceftriaxone. The results underscore the importance of continued surveillance of antimicrobial resistance in wildlife and the need for strategies to mitigate the spread of resistant bacteria between wildlife, humans, and the environment. Addressing this issue is essential not only for protecting wildlife health but also for safeguarding public health against the growing threat of antimicrobial resistance.

Limitation

The study's sample size was small, and the captive feline populations studied were geographically restricted. So, the results may not accurately reflect *E. coli* prevalence or antibiotic resistance pattern in captive felines maintained in various environmental circumstances or geographies. Furthermore, the study relied on a single time-point sampling technique, which may not capture temporal variation in *E. coli* strains or the resistance profile.

Conclusion

This study highlights the presence of *Escherichia coli* in captive feline with a bacterial culture, PCR and antimicrobial resistance with 72% prevalence in the study. There is a presence of multidrug resistance of *E. coli* in these samples. The discovery of multidrug-resistant (MDR) strains highlights the possibility of these animals acting as reservoirs for resistant infections, posing a hazard to both animal and human health via zoonotic transmission. The findings highlight the necessity of establishing good antimicrobial stewardship, regular health monitoring, and better cleanliness standards in zoological settings.

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Biography

I am Sumaiya Salim Tasnim born to Mohammed Salim and Shahnaz Begum from Rangunia, Chattagram. I am presently graduating on Doctor of Veterinary Medicine at Chattagram Veterinary Animal Sciences University. I passed Secondary School Certificate examination in 2016 and Higher Secondary Certificate examination in 2018. In future I would like to work as a researcher in the field of veterinary medicine

Appendix

Questionnaire:

Title: Wild cat and primate sampling questionnaire:

Sampling date: Sample ID:

1. Animal information:
    1.1. Animal species
    1.2. Animal group:
        i. Infant
        ii. Juvenile
        iii. Adult
    1.3. Gender:
    1.4. Health condition:
    1.5. Body weight (kg):
2. Rearing and feeding practice:
    2.1. Feeding frequency:
    2.2. Types of food provided:
    2.3. Source of drinking water:
    2.4. Feeding supervision:
3. Cage hygiene and maintenance:
    3.1. Frequency of cage cleaning:
    3.2. Waste removal:
    3.3. Presence of pest control measure:
    3.4. Access to enrichment materials (e.g., toys, climbing structures):