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Prevalence and molecular characterization of multi-drug and extreme drug resistant *Escherichia coli* in companion animals in Bangladesh

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The study aimed to investigate multi-drug resistant (MDR) *Escherichia coli (E. coli)* in companion animals in Bangladesh, with a focus on the resistance profiles of isolates from non-food-producing animals. In 2023, the studied samples were from cats, dogs, and environmental sources linked with companion animal hospitals in Dhaka city, Bangladesh. *E. coli* was isolated using standard techniques and its antimicrobial resistance (AMR) was assessed against 23 antibiotics following the CLSI protocols. Metallo-beta-lactamase genes (bla_{NDM-1} and bla_{NDM-5}) and mobile genetic elements (class 1 integron) were detected by multiplex PCR. The overall prevalence of *E. coli* was 70%, 76% in cats, 65.71% in dogs, and 65.71% in the environmental samples. Cefuroxime exhibited the highest resistance at 25%, while imipenem and nitrofurantoin showed the highest sensitivity at 100%, followed by ceftazidime at 95%. MDR strains made up 38.10%, while 11.90% were extremely drug-resistant (XDR). Additionally, 29% of *E. coli* were extended-spectrum beta-lactamase (ESBL) producers. The prevalence and association among class 1 integron and the resistant genes including bla_{NDM-1} and bla_{NDM-5} were also notable. This highlights the complex AMR challenges in these settings, including the presence of class 1 integron—a key element involved in capturing and transferring antimicrobial resistance genes.

Keywords *E. coli*, Antimicrobial resistance, MDR, XDR, ESBL, Companion animals, Antimicrobial resistance genes, Veterinary medicine, Bangladesh

Abbreviations

MDR	Multi-drug resistant
XDR	Extreme drug resistant
PDR	Pan drug resistant
E. coli	*Escherichia coli*
AMR	Antimicrobial resistance
CLSI	Clinical and Laboratory Standards Institute
bla_{NDM-1}	New Delhi metallo-beta-lactamase
UTIs	Urinary tract infections
EHEC	Enterohemorrhagic *E. coli*
HUS	hemolytic uremic syndrome
bla_{NDM-5}	New Delhi metallo-beta-lactamase-5
MGEs	Mobile genetic elements
ESBL	Extended spectrum beta-lactamase
ARGs	Antimicrobial resistance genes
AST	Antimicrobial susceptibility testing

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BPW Buffered peptone water

AMR is a pressing global health concern, posing significant challenges to both human and animal health[1]. In recent years, the emergence and spread of MDR bacteria have intensified this threat, leading to increased morbidity, mortality, and healthcare costs worldwide. Among the various pathogens contributing to AMR, *E. coli* stands out as a significant concern due to its widespread distribution and versatile pathogenic potential[2,3].

E. coli causes a range of intestinal and extra-intestinal infections in humans and animals, including diarrhea, urinary tract infections (UTIs), meningitis, and sepsis. Some strains, like enterohemorrhagic *E. coli* (EHEC), can lead to severe complications such as hemolytic uremic syndrome (HUS)[4,5]. Due to its widespread presence, frequent exposure to antibiotics, and ability to acquire resistance genes, *E. coli* is a key indicator for AMR surveillance[6]. It can develop resistance to multiple antibiotic classes through mechanisms like extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, carbapenemases, and plasmid-mediated genes[4,5,7]. Therefore, studying *E. coli* in the context of AMR provides critical insights into resistance evolution, transmission pathways, and the effectiveness of current treatment strategies.

The environment plays a crucial role in the spread of AMR in *E. coli* among companion animals. Companion animals, such as dogs and cats, are often exposed to a variety of environmental sources, including soil, water, and human contact, which may harbor resistant strains of *E. coli*[8]. The improper use or overuse of antibiotics in veterinary medicine can further contribute to the development of AMR in these animals. Resistant *E. coli* strains in companion animals pose a significant risk, not only to animal health but also to human health, as resistant bacteria can be transferred between pets and humans through direct contact or shared environments[9,10]. This highlights the need for a One Health approach, integrating environmental, animal, and human health strategies to combat AMR in companion animals and limit its spread in broader ecosystems.

Studying *E. coli* in companion animals holds significant importance due to several key factors[11]. Companion animals, such as dogs and cats, often share close living quarters and frequent interactions with humans, creating opportunities for the exchange of bacterial pathogens, including *E. coli*[12,13]. Additionally, companion animals may serve as reservoirs for AMR strains of *E. coli*, which can pose a direct risk to human health through zoonotic transmission or indirectly through environmental contamination. Furthermore, the study of *E. coli* in companion animals provides insights into the broader ecology of AMR, shedding light on the impact of factors such as antimicrobial usage practices, veterinary care, and environmental influences on the emergence and dissemination of resistant strains[3,14]. By investigating *E. coli* in companion animals, researchers can contribute to the development of evidence-based strategies for mitigating the spread of AMR and safeguarding both companion animal and human health in shared environments[15]. In regions like Bangladesh, where companion animal ownership is rapidly growing and antimicrobial usage is largely unregulated, the investigation of MDR *E. coli* in this population becomes imperative for understanding the dynamics of AMR transmission and devising effective control strategies[16,17].

This study aimed to investigate the prevalence, antimicrobial susceptibility test (AST) patterns, and molecular characteristics of MDR and XDR pathogenic *E. coli* isolates in companion animals in Bangladesh[18,19]. This study hypothesizes that MDR and XDR *E. coli* isolates are prevalent in companion animals in Bangladesh, with specific molecular resistance profiles associated with mobile genetic elements such as class 1 integrons and resistant genes ($bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$). The risk of MDR *E. coli* transmission from animals to humans is notably high in Bangladesh[20,21]. Understanding the prevalence, characteristics, and transmission dynamics of MDR *E. coli* in companion animals is essential for curbing AMR and protecting public health. This study uncovered the complex scenario of factors driving AMR in companion animals and its potential implications for human health[2]. The research fills a crucial knowledge gap regarding AMR in companion animals in Bangladesh, offering insights to inform evidence-based strategies for antimicrobial stewardship and infection control. Ultimately, this work contributes to safeguarding both animal and human health by preserving effective antimicrobial therapy and preventing the widespread dissemination of resistance.

Materials and methods

Study design and study area

The study area for this research comprises commonly visited veterinary hospitals in Dhaka city of Bangladesh. For this cross-sectional study, samples were collected from asymptomatic dogs (*Canis lupus familiaris*), cats (*Felis catus*), and the surrounding environments of the selected veterinary hospitals in the month of August to December, 2023.

Sample collection

A total of 120 samples were collected aseptically using sterile cotton swabs. This included 100 swab samples (nasal, oral, and rectal) from cats ($n = 50$) and dogs ($n = 50$), as well as 20 environmental swab samples from the surrounding areas of the selected hospitals during the study period. The samples were placed in 15 ml sterile screw-capped Falcon tubes (Corning, USA) containing 5 ml of buffered peptone water (BPW, Oxoid, UK). Immediately after collection, the samples were placed in a cool box (4 °C) and transported to the AMR Reference Laboratory at Bangladesh Livestock Research Institute, Savar, Dhaka for further processing within 12 h[22].

Bacteriological analyses

Overnight pre-enrichment of the samples in BPW was conducted at 37 °C before inoculation onto agar media. Each sample was then inoculated onto MacConkey agar (Oxoid, UK) and incubated at 37 °C for 24 h. Large pink colonies from MacConkey agar were selected and further streaked onto Eosin Methylene Blue (EMB) agar (Oxoid, UK), followed by incubation at 37 °C for 24 h. Suspected *E. coli* colonies were identified by their characteristic black centered green metallic sheen[23]. VITEK®2 (Biomerieux, USA) was used to confirm

the presence of E. coli using VITEK®2 GN cards. Further molecular confirmation was carried out using PCR targeting the E. coli-specific gene uspA. Each positive sample was considered as one isolate. Finally, the positive isolates were preserved in 15% glycerol at − 80 °C for future use.

Antimicrobial susceptibility testing (AST)

The susceptibility of the isolated pathogen to 23 clinically relevant antibiotics (Oxoid, UK) from various classes. See details in supplementary section (Table S1). was determined using the disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines[22,24,25]. The breakpoints were measured using an automatic zone reader (Interscience, USA). *E. coli* ATCC 25,922 and deionized Milli-Q water were used as positive and negative controls, respectively, for antimicrobial susceptibility testing (AST) and PCR analyses.

Grouping of the MDR, XDR, PDR (pan-drug resistant) isolates

Isolates resistant to three or more antibiotic classes were classified as MDR. XDR bacteria were sensitive to drugs from only one or two antibiotic classes, while PDR bacteria were resistant to all antibiotic classes[26,27].

Detection of extended-spectrum beta-lactamase (ESBL) producing *E. coli*

Detection of ESBL producing *E. Coli* was done following the previously published protocol[28]. The isolates displayed resistant to ESBL groups in the AST test that were considered for ESBL confirmatory tests.

Double disc synergy test (DDST) and Combined disc test were conducted for ESBL confirmation. The DDST used in this study was modified from Jarlier's double-disc synergy (DDS) method[25,28]. Discs of cefotaxime, cefepime, ceftazidime, and aztreonam were placed around an amoxicillin/clavulanic acid disc at a distance of 20 mm[28]. Positive ESBL production was marked by the observation of a keyhole.

The combined test, recommended by CLSI[29], requires the use of ceftazidime and cefotaxime, with and without clavulanic acid. Discs used were ceftazidime (30 µg), ceftazidime-clavulanic acid (30/10 µg), cefotaxime (30 µg), and cefotaxime-clavulanic acid (30/10 µg). If the zone diameter increased by ≥ 5 mm after either antibiotic was combined with clavulanic acid, this was taken as evidence for the presence of an ESBL production[30,31].

Molecular identification of ESBL genotype using PCR

PCR was used to confirm the *E. coli* and to detect the presence of antibiotic resistance genes and class 1 integron in *E. coli* showing phenotypic resistance to metallo-beta-lactamase. Bacterial colonies were boiled in TE buffer for 5 min, flash centrifuged, and then the supernatant of boiled colonies was used as a PCR template[32]. PCR reactions were conducted using a thermocycler (2720 Thermal Cycler, Applied Biosystems, USA) with a total volume of 25 µL. The reaction mixture comprised 12.5 µL of Dream Taq PCR Master Mix (Thermo Scientific, USA), 1 µL of each primer, 2 µL of template DNA, and 8.5 µL of deionized water. The PCR conditions for the specific genes included an initial denaturation step at 94 °C for 30 s, followed by 34 cycles consisting of denaturation at 94 °C for 30 s, annealing at the specific temperature for the primers for a designated time, and elongation at 72 °C for 30 s. A final extension was performed at 72 °C for 7 min. Each of the gene specific PCR was performed at optimum conditions following pre-described methods with defined annealing temperature of each primer (Table S2). Finally, the amplified products were visualized after running at 100 V with 500 mA for 30 min in 1.5% agarose gel containing ethidium bromide (0.5 µg/mL).

Statistical analysis

Microsoft Excel (MS-2021) was used to compile all the information, and R programming Language (Rstudio, version 2024.12.1 + 563) was used to analyze the results. The heatmap was generated from AST diameters using the R package 'pheatmap' to visualize correlations among antibiotics and samples. A bivariate (Chi-Square) test was conducted to assess the association between the presence of resistance genes and class 1 integron.

Ethics statement

This research was conducted in strict accordance with ARRIVE guidelines to ensure the integrity and welfare of all involved. The study protocols were reviewed and approved by the Bangladesh Livestock Research Center's Ethical Review Committee, ensuring compliance with national and international standards for the ethical treatment of biological samples. All procedures, including sample collection, handling, and laboratory analyses, were carried out with the utmost care to avoid harm and ensure the confidentiality of the data. The research team was committed to maintaining the highest ethical standards throughout the study, ensuring that the research was conducted responsibly and transparently.

Results

Prevalence of *E. coli* isolates in companion animals

A total of 120 samples were examined for the presence of *E. coli*. Out of 120 samples, 84 (38 from cats, 23 from dogs and 23 from environment linked to hospital) were confirmed to be *E. coli* by standard laboratory methods. The overall prevalence of *E. coli* was 70%. 76% in cats, 65.71% in dogs, and 65.71% in the environmental samples (Fig. 1).

Resistance pattern of *E. coli* isolated from companion animals

The results of antimicrobial susceptibility testing for different antibiotics are presented in Fig. 2. This figure shows the sensitivity, intermediate, and resistance percentages of 23 antibiotics against the tested isolates. Imipenem and nitrofurantoin demonstrated the highest sensitivity at 100%, followed by ceftazidime with 95%, making them the most effective antibiotics. In contrast, aztreonam had the lowest sensitivity at 10%, followed by cefepime at 60%. Regarding resistance, cefuroxime showed the highest resistance at 25%, while several antibiotics,

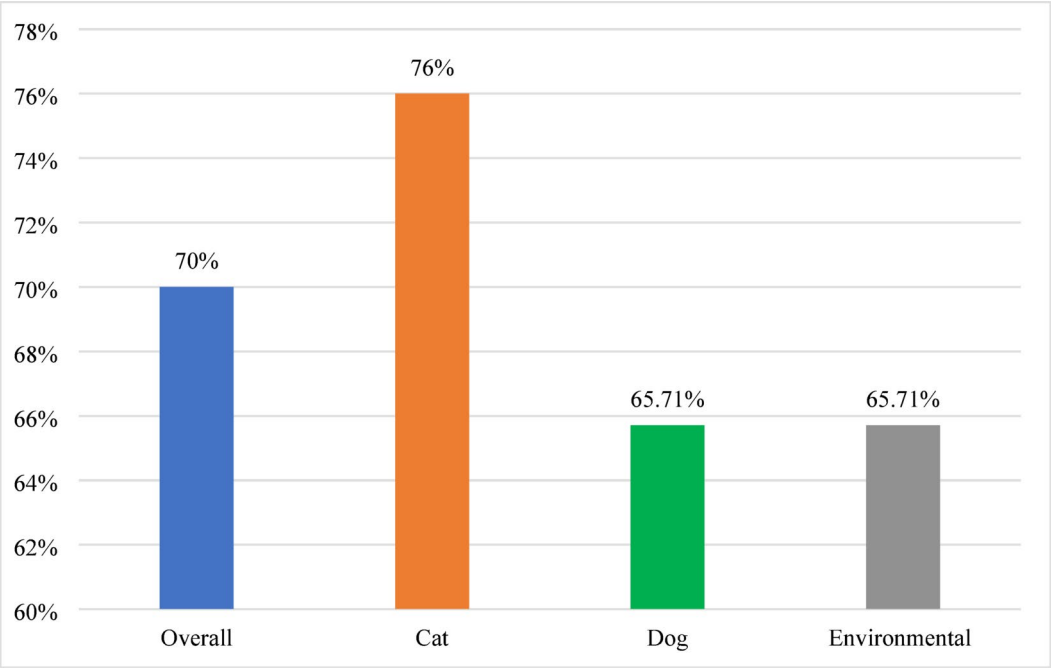

Fig. 1. Prevalence of *E. coli* among all samples.

including piperacillin, ampicillin, amoxicillin-clavulanate, ciprofloxacin, chloramphenicol, trimethoprim, STX, and aztreonam, had a resistance rate of 20%. The lowest resistance was observed with ceftazidime, imipenem, and nitrofurantoin, all of which showed 0% resistance. For intermediate, aztreonam had the highest at 70%, followed by cefotaxime at 20%, while most other antibiotics exhibited negligible or no intermediate. Overall, imipenem, nitrofurantoin, and ceftazidime were the most effective antibiotics, while aztreonam and cefuroxime performed poorly in this study.

Resistance patterns and their correlations are shown in the heatmap (Fig. 3). A strong correlation in resistance patterns was observed between ceftazidime and ceftriaxone, aztreonam and nitrofurantoin, levofloxacin and ciprofloxacin, tobramycin and gentamicin, etc. among others. The number of isolates classified as resistant, sensitive, or intermediate for each antibiotic is provided in Table 1.

Isolates from cats showed the highest sensitivity to tobramycin and nitrofurantoin (90%), followed by amikacin and amoxycillin-clavulanate (75%). Moderate sensitivity was observed with gentamycin, piperacillin, ampicillin, and levofloxacin (70%), while ciprofloxacin had the lowest sensitivity (65%) with 7% intermediate. The highest resistance was seen with gentamycin, piperacillin, and ampicillin (30%), while tobramycin, nitrofurantoin, and ciprofloxacin showed the lowest resistance (10%).

E. coli isolates from dogs showed high sensitivity to nitrofurantoin (95%), followed by ampicillin (90%) and cefepim and SXT (80%). Moderate sensitivity was observed with amoxycillin-clavulanate and cephalexin (75%) and cefoxitin (70%), while ciprofloxacin and ceftriaxone showed lower sensitivity (65%). The lowest sensitivity was seen with aztreonam (10%), which also exhibited the highest intermediate (60%). Resistance was highest in ciprofloxacin and aztreonam (30%), followed by amoxycillin-clavulanate, cephalexin, cefoxitin, and ceftriaxone (25%).

Multi-drug resistance (MDR) and extreme drug resistance (XDR) *E. coli* isolated from companion animals

Following antimicrobial susceptibility test, screening of drug resistance category (MDR, XDR and PDR) were performed according to the previously described method[26]. Overall, MDR is observed in 38.1% of isolates, with cats showing the highest proportion at 46.34%. Dogs exhibit 35.13% MDR and 10.81% XDR, while environmental samples show 80% XDR but no MDR. Notably, no PDR is detected across any source (Fig. 4).

ESBL production of *E. coli*

ESBL testing revealed that 35% and 29% of the total isolates were identified as ESBL producers using the DDST and the combined disk test, respectively. Specifically, in cat samples, 28% and 19% of *E. coli* were identified as ESBL producers using the DDST and the combined disk test, respectively. In dog samples, 35% and 22% of *E. coli* were ESBL producers in the DDST and the combined disk test, respectively.

Genotypic resistant patterns

Overall, 47.61% of isolates harbored class 1 integron, 25% carried the $bla_{\text{NDM-1}}$ gene, and 37% carried the $bla_{\text{NDM-5}}$ gene. In cat isolates, class 1 integron were present in 43.90% of isolates, with $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$ detected in 24% and 37%, respectively. Dog isolates showed the highest prevalence of class 1 integron (51.35%), with

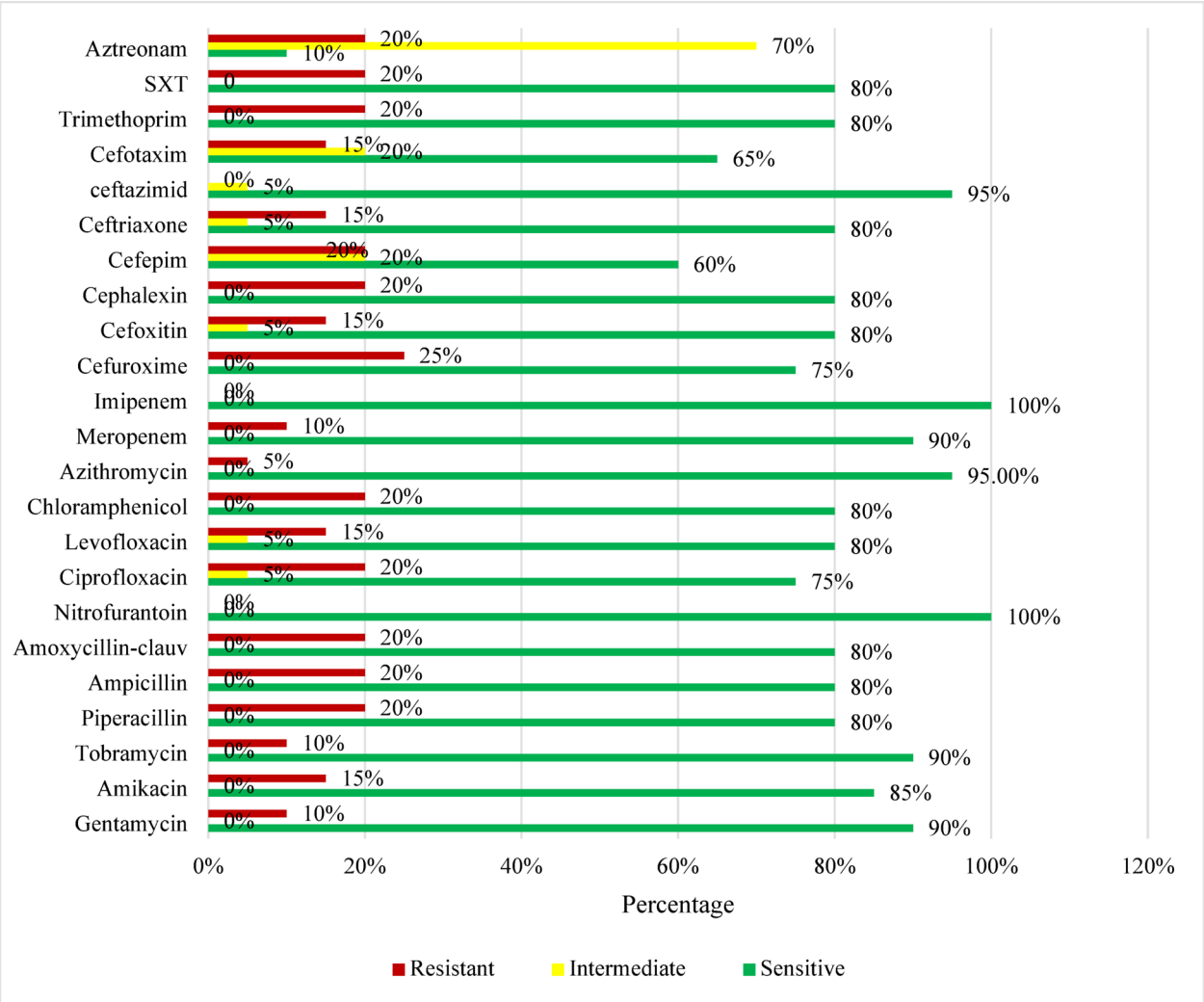

Fig. 2. AST result of all isolates.

bla_{NDM-1} at 27.02% and bla_{NDM-5} at 38%. Environmental samples exhibited 20% class 1 integron, no bla_{NDM-1}, and the highest prevalence of bla_{NDM-5} (40%) (Fig. 5).

Association among class 1 integron and resistant genes

A bivariate analysis (Chi-Square) test was performed to determine the association between the presence of the resistant genes and class 1 integron. The results revealed statistically significant associations between class 1 integron positivity and the presence of the resistance genes bla_{NDM-1} and bla_{NDM-5}. For bla_{NDM-1}, 75.0% of the isolates positive for the gene were also positive for class 1 integron ($\chi^2 = 11.41$, $p = 0.001$). Similarly, a highly significant association was observed with bla_{NDM-5}, where 80.8% of bla_{NDM-5} positive isolates were class 1 integron positive ($\chi^2 = 29.07$, $p < 0.001$). These findings highlight the strong linkage between class 1 integron and the dissemination of these resistance genes (Table 2).

Discussion

The results of the AST conducted in this study revealed varying degrees of resistance among the tested bacterial isolates from companion animals in Dhaka city of Bangladesh[27]. Notably, amikacin and tobramycin exhibited a resistance rate around 15%, while cefuroxime, piperacillin, and ampicillin showed a higher resistance rate of around 20%. Conversely, nitrofurantoin demonstrated complete sensitivity, with no observed resistance[33]. The fluoroquinolones ciprofloxacin and levofloxacin displayed resistance rates of 20% and 15% respectively[33]. Similarly, chloramphenicol exhibited a resistance rate of 20%, while azithromycin showed a lower resistance rate of 5%. Encouragingly, the carbapenems like imipenem exhibited very no resistance, indicating full sensitivity[34]. However, a notable proportion of isolates showed resistance to cephalosporins, with cefuroxime, cephalexin, cefepime, and ceftriaxone each displaying around 20% resistance. Additionally, ceftazidime showed no resistance, while cefoxitin exhibited a resistance rate of 15%[35]. Trimethoprim had a resistance rate of 20%, whereas sulfamethoxazole-trimethoprim (SXT) exhibited a slightly higher resistance rate of 20%. Aztreonam displayed 20% resistance, with a significant proportion of isolates showing intermediate (70%)[36].

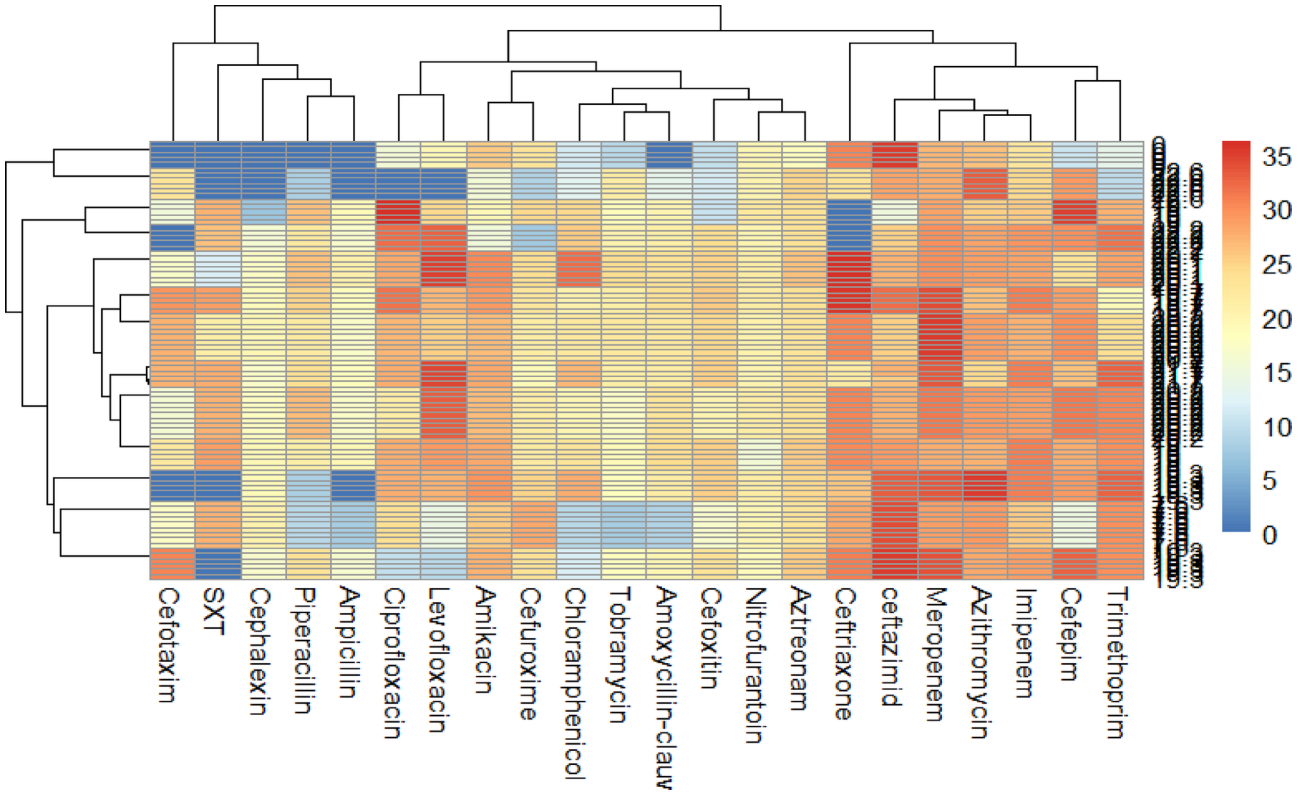

Fig. 3. Heatmap showing correlations of AMR patterns for *E. coli*.

When comparing with studies from other regions, important differences in antimicrobial resistance trends emerge. For example, a studies conducted in India reported significantly higher resistance rates to fluoroquinolones and third-generation cephalosporins among companion animal isolates, often exceeding 40%, suggesting a higher level of selective pressure, potentially due to more frequent or inappropriate use of these antibiotics in veterinary settings[37,38]. In contrast, studies from parts of Europe, such as Germany and the Netherlands, have shown notably lower resistance levels to aminoglycosides, fluoroquinolones, and β-lactams, likely due to stricter antibiotic stewardship policies and regulated veterinary antibiotic use[39,40]. In Southeast Asia, particularly in Vietnam and Thailand, resistance trends appear more aligned with the findings from Bangladesh, indicating shared challenges in antibiotic regulation, access to veterinary services, and over-the-counter availability of antimicrobials[41,42]. These regional discrepancies underscore the influence of local antimicrobial usage practices, regulatory environments, and infection control measures in shaping resistance patterns. The possible mechanisms driving AMR in Bangladesh include overuse and misuse of antibiotics in both human and veterinary medicine, inadequate infection control measures, poor sanitation, and environmental contamination, as well as the rapid spread of resistant genes through mobile genetic elements[43].

The detection of MDR in a substantial portion of isolates, defined by resistance to three or more classes of antibiotics, underscores the critical need for prudent antibiotic use and the implementation of robust antimicrobial stewardship programs in veterinary practice[11,44]. Similarly, a Chinese study found high prevalence of MDR in isolated from dogs[45]. These findings underscore the importance of continued surveillance and research to better understand and address antibiotic resistance in companion animals, ultimately safeguarding both animal and human health.

The findings revealed the prevalence of MDR and XDR among cats, dogs, and environmental samples. Cats showed the highest prevalence of MDR at 46.34%, which is consistent with studies linking frequent antibiotic use in companion animals to high MDR rates. A study conducted in Saudi Arabia found high prevalence of MDR and resistant genes in cat samples[46]. The high prevalence of MDR in cats may be attributed to the frequent and often unregulated use of antibiotics in veterinary practices to treat common infections in companion animals[47]. Dogs exhibited 35.13% MDR and 10.81% XDR isolates, reflecting similar concerns about antimicrobial overuse in veterinary care[48]. In contrast, environmental samples showed an alarming 80% XDR prevalence but no MDR, likely due to selective pressure from environmental contamination with antibiotics and resistant bacteria[49]. These variations highlight the role of improper antibiotic use and environmental pollution in driving resistance, emphasizing the need for targeted interventions to address these sources[50]. Further studies are needed to clarify these findings across a wider range of sample sources, including a broader geographical area.

The study found a high prevalence of ESBL-producing *E. coli* in companion animals in Bangladesh, posing a public health concern. Among cat samples, 28% and 19% of *E. coli* were ESBL producers in the DDST and

	Gentamycin	Amikacin	Tobramycin	Piperacillin	Ampicillin	Amoxycillin	Nitrofurantoin	Ciprofloxacin	Levofloxacin	Chloramphenicol	Azithromycin	Meropenem
Resistant	8	13	8	17	17	17	0	17	13	17	4	8
Intermediate	0	0	0	0	0	0	0	4	4	0	0	0
Sensitive	76	71	76	67	67	67	84	63	67	67	80	76

	Imipenem	Cefuroxime	Cefoxitin	Cephalexin	Cefepime	Ceftriaxone	Ceftazimid	Cefotaxime	Trimethoprim	SXT	Aztreonam
Resistant	0	21	13	17	17	13	0	13	17	17	16
Intermediate	0	0	4	0	17	4	4	17	0	0	59
Sensitive	84	63	67	67	50	67	80	54	67	67	9

Table 1. Frequency of resistant, intermediate, and sensitive isolates.

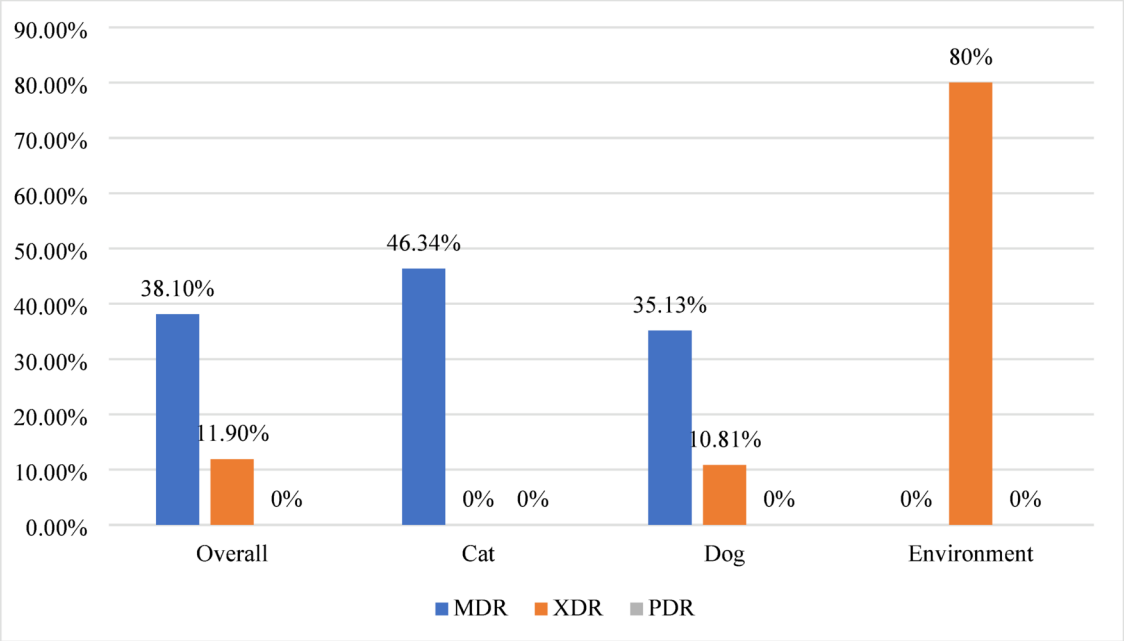

Fig. 4. Prevalence of MDR, XDR, and PDR among isolates.

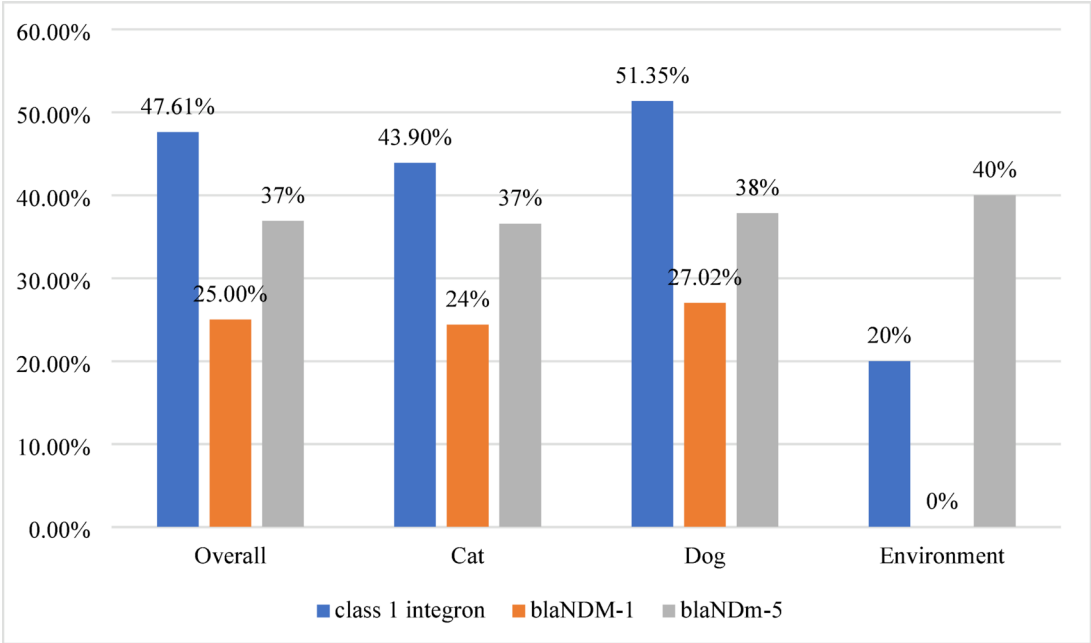

Fig. 5. Prevalence of class 1 integron, blaNDM-1, and blaNDM-5 among isolates.

Combined Disk Test, respectively. In dog samples, the rates were even higher, at 35% and 22%. This indicates a substantial capacity for MDR in *E. coli* strains, especially in dogs.

These findings are consistent with previous studies from other regions. For example, a study conducted in China found that 32% of companion animal *E. coli* isolates were ESBL producers, similar to our results for cat samples[51]. In contrast, a European study reported lower rates of ESBL production in *E. coli* isolates from dogs, around 20%, which is notably lower than the 35% identified in our study[50]. The higher prevalence in Bangladesh may be attributed to differences in antibiotic use practices, particularly the overuse or misuse of antibiotics in veterinary medicine, as well as the lack of stringent regulations governing the sale and prescription of antibiotics[52]. Additionally, frequent human-animal interaction, combined with poor sanitation in certain areas, may facilitate the spread of MDR pathogens between animals and humans[53].

Variables	Categories	Class 1 integron		Bivariate analysis	
		No (n %)	Yes (n %)	χ^2	P value
bla_{ndm-1}	No	48 (70.6%)	20 (29.4%)	11.41	0.001
	Yes	4 (25.0%)	12 (75.0%)		
bla_{ndm-5}	No	47 (81.0%)	11 (19.0%)	29.07	< 0.001
	Yes	5 (19.2%)	21 (80.8%)		

Table 2. Bivariate analysis (Chi square) of class 1 integron with resistance genes.

The study found class 1 integron, bla_{NDM-1}, and bla_{NDM-5} prevalence rates of 47.61%, 25%, and 37% respectively. Globally, bla_{NDM-1} has been reported at varying rates, with some regions showing higher prevalence due to widespread carbapenem use[54,55]. While bla_{NDM-5}, a newer variant, has gained traction in recent years, often associated with more resistant strains[55]. Comparatively, the prevalence of bla_{NDM-5} in this study is higher than what has been reported globally, suggesting regional differences in resistance patterns and the emergence of newer NDM variants[56,57]. Studies from other regions, such as Thiland, and Pakistan, report similar prevalence rates of bla_{NDM} genes in *E. coli* isolates from animals, emphasizing that companion animals are exposed to environments conducive to the transmission of AMR genes[58,59]. The presence of bla_{NDM-5} at a higher prevalence compared to bla_{NDM-1} suggests the recent emergence of this gene variant, which has been linked to broader dissemination and greater adaptability in bacterial populations[60]. The clinical significance of this findings in companion animals in Bangladesh suggests a potentially higher risk of dissemination of *E. coli* to humans, complicating treatment options and increasing the threat of untreatable infections[56]. The probable cause of these findings can be attributed to several factors, including the indiscriminate use of antibiotics in both human and animal healthcare in Bangladesh, as well as insufficient regulation of antibiotic use in veterinary clinics[57].

The significant association between bla_{NDM-1}, bla_{NDM-5}, and class 1 integron positivity observed in this study aligns with findings from previous studies, which have also reported integrons as key contributors to the horizontal transfer and accumulation of antimicrobial resistance genes[61,62]. Biologically, class 1 integrons are highly mobile elements capable of capturing, integrating, and expressing diverse gene cassettes, including potent carbapenemase genes like bla_{NDM} variants. Their mobility and recombination capability allow for the co-selection and co-expression of multiple resistance determinants, particularly under antibiotic selective pressure[61,63]. This association may be driven by the integrons' ability to integrate and express multiple resistance genes under selective pressure, such as exposure to antibiotics, enhancing bacterial survival and resistance[61].

Future research should focus on tracking the progression of antibiotic resistance in companion animal pathogens through longitudinal studies, which could reveal ongoing increases in resistance rates to multiple antibiotics. This will underscore the urgent need for proactive measures to curb the spread of resistance, including promoting prudent antibiotic use, enhancing surveillance efforts, developing novel antimicrobial agents, and implementing robust infection prevention and control strategies in veterinary settings[64].

In essence, while the findings of this study provide valuable insights into the antibiotic resistance landscape among companion animal pathogens in Bangladesh, they underscore the need for a coordinated global response to address the complex challenge of antimicrobial resistance effectively. Collaborative efforts involving veterinarians, healthcare professionals, policymakers, researchers, and the pharmaceutical industry are essential to preserve the efficacy of existing antibiotics and ensure sustainable use of antimicrobial agents in both veterinary and human medicine.

Limitations of the study

This study has several limitations: the sample size and geographic scope may not fully represent Bangladesh's companion animal populations, potentially limiting generalizability. It focused only on *E. coli*, excluding other bacterial pathogens, and lacked data on antibiotic usage and patient histories, which could impact the understanding of resistance. The reliance on phenotypic testing may have missed genetic resistance factors. Additionally, the study did not address the genetic background or clonal relatedness of isolates and the lack of whole-genome sequencing (WGS) means potential resistance determinants may have been overlooked. The study also did not explore plasmid-mediated resistance transmission, which is a critical aspect of AMR. Future research should involve larger, diverse samples, molecular analyses, and contextual factors to better understand antibiotic resistance in companion animals. Additionally, future studies should explore the selective pressures associated with the use of antimicrobials in animals and the environment in the study areas.

Conclusion

The study sheds light on the AMR profiles of pathogenic *E. coli* isolates from companion animals in Bangladesh. The findings underscore the widespread resistance to commonly used antibiotics, highlighting the urgent need for prudent antibiotic prescribing practices and comprehensive antimicrobial stewardship programs in veterinary medicine. The emergence of multidrug-resistant strains, coupled with the presence of clinically significant resistance genes such as bla_{NDM-1} and bla_{NDM-5}, emphasizes the interconnectedness of human and animal health in the context of antimicrobial resistance. Moving forward, concerted efforts are needed to enhance surveillance, promote responsible antibiotic use through robust veterinary antimicrobial stewardship policies, and develop alternative therapeutic strategies to mitigate the spread of antibiotic resistance in companion animal populations, thereby safeguarding animal welfare and public health.

Data availability

Upon reasonable request, the corresponding author will provide access to all the data supporting this article.

Received: 15 September 2024; Accepted: 6 May 2025
Published online: 12 May 2025

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Acknowledgements

The authors express their sincere appreciation to all the staffs and technicians of AMR Reference Laboratory (Research), Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.

Author contributions

Authors' contributionAll listed authors have thoroughly reviewed and approved the manuscript, and no eligible individuals have been excluded from authorship. The specific contributions of each author are detailed below: Md. Hafizur Rahman: Conceptualization, Methodology, Microbiological investigation, Writing - original draft, Writing - critical review & editing, Supervision, Validation. Abu Bakkar Siddique: Conceptualization, Methodology, Microbiological investigation, Writing - original draft, Data curation, Data Collection, Data analyses, Validation. Md Aseif Hossain Zihadi: Microbiological investigation, Data curation, Data analysis and interpretation, Writing - original draft, Validation. SM Soheb Ahmed: Microbiological investigation, Data curation,

Writing - original draft, Validation. Md Sazzad Hossain Sumon: Microbiological investigation, Data curation, Writing - original draft, Validation. Shihab Ahmed: Microbiological investigation, Conceptualization, Methodology, Writing – critical review & editing, Supervision, Validation.

Funding
This research was partially supported by small grants from Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh (GOB fund).

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
This research was conducted in strict accordance with ethical guidelines to ensure the integrity and welfare of all involved. The study protocols were reviewed and approved by the Bangladesh Livestock Research Center's Ethical Review Committee, ensuring compliance with national and international standards for the ethical treatment of biological samples. All procedures, including sample collection, handling, and laboratory analyses, were carried out with the utmost care to avoid harm and ensure the confidentiality of the data. The research team was committed to maintaining the highest ethical standards throughout the study, ensuring that the research was conducted responsibly and transparently.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-01417-0.

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