Research Article

Alarming Prevalence of Multidrug-Resistant *Escherichia coli* and *Salmonella* spp. in Diarrheic and Healthy Pet Dogs and Cats in Dhaka, Bangladesh

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Abstract | Diarrhea caused by *Escherichia coli* and *Salmonella* spp. are common in pets; however, difficult to treat because of antimicrobial resistance (AMR). This study aimed to explore the AMR of the diarrhea associated *E. coli* and *Salmonella* spp. of pets in Dhaka city, Bangladesh. Fecal samples from 30 cats and 20 dogs, each with an equal amount of apparently healthy (AH) and diarrheal pets, were used to isolate the above-mentioned bacteria. Faecal microbial load was measured initially. Molecular identification of *E. coli* and *Salmonella* spp. was performed by polymerase chain reaction (PCR) using the 16s rRNA and InvA gene primer sets, respectively along with the standard bacteriological methods. Disc diffusion method was followed to conduct antibiotic susceptibility of the identified bacteria. Diarrheic pets exhibited significantly elevated total viable count (TVC) and total coliform count (TCC) in comparison to AH individuals (P<0.01). Of the 52 isolates that were found, 21 were *Salmonella* species and 31 were *E. coli*. Diarrheic dogs showed a substantially higher occurrence of *Salmonella* spp. (90% vs. 10%) (P<0.01) and a non-significantly higher occurrence of *E. coli* (70% vs. 30%) (P=0.07) than AH dogs. *E. coli* (93.33% vs. 46.67%) and *Salmonella* spp. (66.67% vs. 6.67%) were more common in cats with diarrhea than in AH cats (P<0.01). *E. coli* showed highest resistance against amoxicillin while *Salmonella* spp. against amoxicillin and ampicillin. Notably, a high prevalence of multidrug resistant (MDR) bacteria was detected among the isolates, reaching 93.55% for *E. coli* and 80.00% for *Salmonella* spp. In contrast, both the isolates showed various sensitivity to ciprofloxacin, nalidixic acid, and gentamicin. These findings of AMR is crucial to guide appropriate therapeutic choice and to prevent the emergence of AMR among pets.

Keywords | AMR, Cats, Diarrhea, Dogs, *E. coli*, *Salmonella* spp.

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INTRODUCTION

Pet ownership in Bangladesh has seen a recent surge, particularly in urban areas, as pets are increasingly valued for their contributions to physical, emotional, and social well-being, especially among children (Dohoo *et al.*, 1998; Robertson *et al.*, 2000). Cats are widely recognized as affectionate companions (Gino and Flynn, 2012). Despite the assumption that pet animals are well-cared for, there remains limited empirical evidence to support

this, especially regarding health monitoring and disease prevention (Howell *et al*., 2016). Proper pet ownership involves responsibilities such as adequate housing, disease control, and health management, failure of which may pose risks to public health (William *et al*., 2002).

Companion animals are known reservoirs of numerous zoonotic bacteria, including *Escherichia coli* and *Salmonella* spp., both of which are significant agents of human foodborne diseases (Al-Nasiry, 2011; Guardabassi et al., 2004). Antimicrobial resistance (AMR) among these pathogens is escalating globally, partly due to increased veterinary antibiotic use (Gruel et al., 2021; Cui et al., 2022). In Bangladesh, AMR in animal-derived *E. coli* and *Salmonella* has been demonstrated extensively in poultry and livestock with high resistance rates to tetracycline, penicillin, and fluoroquinolones (Hossain et al., 2021; Khan et al., 2020). A recent review highlights prevalence of AMR *E. coli* genes across domestic animals, wildlife, and their environments in Bangladesh (Khan et al., 2020).

Despite these findings on food animals and environmental sources, data on companion animals remain limited. However, emerging studies are filling this gap: Rahman et al. (2025) reported that 70% of companion animals in Dhaka were colonized with *E. coli*, with 38.1% classified as multidrug-resistant (MDR) and 29% as extended-spectrum β-lactamase (ESBL) producers. Alarming resistance gene presence including bla_{NDM-1} and bla_{NDM-5} and integron-mediated gene spread were also documented Moreover, Das *et al*. (2023) identified Shiga-toxin and ESBL-producing *E. coli* in asymptomatic pet cats in Bangladesh, suggesting pets may act as silent reservoirs of zoonotic and resistant strains.

Salmonella carriage in pets also poses risks. Although less documented in Bangladesh's companion animals, international research shows that pets fed raw diets often shed *Salmonella* and resistant *E. coli*, contaminating households (Joffe and Schlesinger, 2002). Furthermore, the zoonotic significance of companion-animal-borne *Salmonella* is well-established (Sato et al., 2000; Hoelzer *et al*., 2011).

Pets, especially dogs, are recognized as potential sources of zoonotic *Salmonella*. Studies have documented the transmission of *Salmonella* from dogs to humans (Sato *et al*., 2000), with dogs capable of shedding high bacterial loads (10^2 to 10^6 CFU/100 g of feces) over several months (Tanaka *et al*., 1976; Ojo and Adetosoye, 2009). Given their close contact with humans, such carriage poses a significant public health risk (Hoelzer *et al*., 2011).

While previous studies established the presence of antimicrobial resistance in livestock and poultry, this study is explored MDR *E. coli* and *Salmonella* spp. in urban pets. We specifically addressed the gap in knowledge regarding asymptomatic carriage by comparing apparently healthy and diarrheic animals, revealing that even healthy pets serve as silent reservoirs for zoonotic pathogens. Furthermore, our research quantifies the significant escalation of total viable and coliform counts during diarrheal episodes, providing a direct link between clinical status and increased environmental shedding of pathogens. By documenting resistance to critical "last-resort" drugs like meropenem, this study highlights an urgent, unaddressed public health risk at the pet-human interface in Bangladesh.

Diarrheal illnesses in pets represent a direct zoonotic threat. Molecular characterization of *E. coli* and *Salmonella* spp. can help identify specific pathogenic strains and detect resistance genes. With AMR on the rise globally, monitoring AMR profiles in companion animals is essential. This study aimed to investigate the molecular detection and antimicrobial resistance profiles of *E. coli* and *Salmonella* spp. isolated from apparently healthy and diarrheic pets in Dhaka, Bangladesh, to inform treatment strategies and assess potential public health risks.

MATERIALS AND METHODS

Study area and sample collection

This study was conducted in pet clinics and private households across Dhaka City, Bangladesh. Figure 1 represented the clear flowchart of the study design. A total of 50 fecal samples were collected from pet animals 30 cats and 20 dogs. For each species, 50% of the samples were obtained from clinically healthy individuals and the remaining from diarrheic animals. The sample size (N=50) was a pilot-scale investigation conducted across various pet clinics and private households in Dhaka to capture urban diversity. Animals were defined as "apparently healthy" based on the absence of gastrointestinal symptoms (diarrhea, vomiting) and a history of no antibiotic use in the preceding 15–30 days.

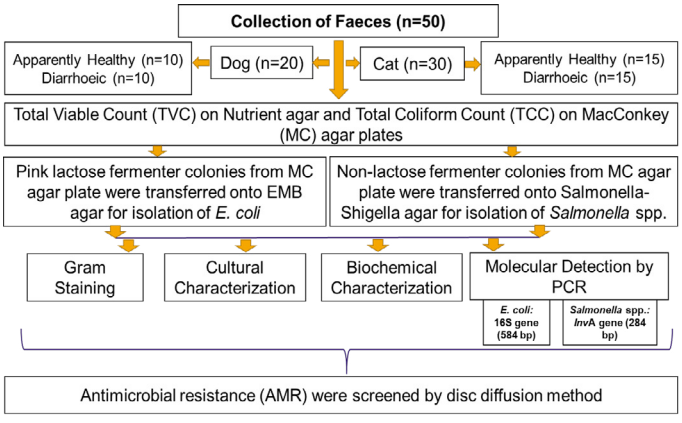

Figure 1: Schematic presentation of the experimental design.

All samples were aseptically collected using sterile tools, transferred into sterile zipper-lock bags, and immediately stored at 4°C in transport containers. Samples were processed at the Microbiology Laboratory, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

Enumeration, isolation, and tentative identification of bacteria

One gram of fecal sample was homogenized in 9 mL of sterile distilled water and made 10-fold serial dilutions. Then, each of 0.1 ml aliquots were inoculated onto Nutrient Agar (for total viable count) and MacConkey Agar (for total coliform count) following standard methods (Cappuccino and Sherman, 2014). Plates were incubated at 37 °C for 24 hours and enumerated the cfu/g for TVC and TCC. Pink lactose fermenter colonies from MC agar were sub-cultured on to Eosin Methylene Blue (EMB), and Xylose Lysine Deoxycholate (XLD) agars to evaluate the cultural characteristics of *E coli*. In contrast, non-lactose fermenter colonies from MC agar were sub-cultured on to *Salmonella*-Shigella (SS), and XLD agars for *Salmonella* spp. (Cheesbrough, 2005). All the isolates were preserved for further characterization by agar slant and 20% buffered glycerin (Cheesbrough, 2005). Isolates were then subjected to gram staining and a series of biochemical tests, including catalase, Sugar fermentation tests (dextrose, sucrose, lactose, maltose, and mannitol), methyl red (MR), Voges-Proskauer (VP), and indole tests (Quinn *et al*., 2002; Holt *et al*., 1994).

Molecular detection of *E. coli* and *Salmonella* spp. by PCR

Genomic DNA was extracted from bacterial isolates using a streamlined boiling–centrifugation protocol validated for its simplicity, cost-effectiveness, and robust performance across diverse matrices (e.g., food, clinical, environmental) (Liu *et al*., 2022; Dimitrakopoulou *et al*., 2020). Colonies were suspended in 200 µL sterile nuclease-free water or 0.1 M KOH, boiled at 100 °C for 10 minutes, then cooled on ice (Liu *et al*., 2022). Lysates were centrifuged at 12,000 × g for 10 minutes, and the supernatant containing genomic DNA was collected and stored at −20 °C (Dimitrakopoulou *et al*., 2020). DNA yield and purity from the boiling method have been shown to match or exceed those from commercial kits for Gram-negative bacteria such as *E. coli* (Ahmed and Dablool, 2017). PCR screening targeted the *E. coli* 16S rRNA gene and the *Salmonella* spp. *invA* gene using published primers (Esquivel-Hernández et al., 2018; Sunar et al., 2014), ensuring high specificity and accuracy. Standard 25 µL reactions contained 12.5 µL Master Mix, 0.5 µL of each primer (10 µM), 2 µL template DNA, and nuclease-free water. Thermocycling conditions were: for 16S rRNA, 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s; final extension at 72 °C for 5 min (Sunar et al., 2014); for invA, 94 °C for 1 min; 30 cycles of 94 °C for 1 min, 64 °C for 30 s, 72 °C for 30 s; and a final extension at 72 °C for 7 min (Esquivel-Hernández et al., 2018). Amplicons were resolved on 1.5% agarose gels in 1× TAE buffer at 100 V for ~45 minutes, stained with ethidium bromide, and visualized using a UV gel documentation system with a 100 bp ladder for size confirmation (Hossain *et al*., 2020). This combined boiling–centrifugation method thus offers a reliable, rapid approach for routine molecular detection of *E. coli* and *Salmonella* spp. in microbiological surveillance studies.

Antibiotic susceptibility testing

Antibiotic susceptibility of the isolates was assessed using the Kirby-Bauer disk dffusion method on Mueller-Hinton Agar, following CLSI guidelines (Bauer *et al*., 1966; CLSI, 2021). Commercial antibiotic discs commonly used in veterinary medicine were applied. Inhibition zones were measured and interpreted as sensitive, intermediate, or resistant according to CLSI standards. The following substances are among the antibiotic classes and antibiotics used in this investigation: Ampicillin (AMP), 10 µg; amoxicillin (AMX), 30 µg; amoxicillin–clavulanic acid (AMC), 20/10 µg; ceftriaxone (CTR), 30µg; ciprofloxacin (CIP), 5 µg; nalidixic acid (NA), 30 µg; erythromycin (E), 15 µg; gentamicin (GEN), 10 µg; tetracycline (TE), 30 µg; and meropenem (MEM), 10 µg. "Multidrug Resistance" (MDR) refers to the resistance of bacteria to at least one agent from three or more of classes of antibiotics (Magiorakos *et al*., 2012).

Statistical analysis

Data were tabulated in Microsoft Excel and analyzed using R (R Core Team, 2021). Prevalence rates were expressed in percentages, and statistical significance was evaluated via chi-square (χ^2) and t-tests (PROC TTEST in SAS). R packages including tidyverse, dplyr, data Table 1, and

Table 1: PCR primers with sequence of *E. coli* and *Salmonella* spp.

Target Gene	Sequence (5'-3')	Amplicon size (bp)	Reference
E. coli 16E1	F: GGGAGTAAAGTTAATCCTTTGCTC	584	(Tsen *et al*., 1998)
E. coli 16E2	R: TTCCCGAAGGCACATTCT		
*Inv*A	F: GTGAAATTATCGCCACGTTCGGGCAA	284	(Kaushik *et al*., 2014)
	R: TCATCGCACCGTCAAAGGAACC		

BSDA facilitated data manipulation and hypothesis testing. Visualization was performed using ggplot2 (Wickham, 2016). Subgroup analyses, such as canine *Salmonella* (n=9), have limited statistical power. The non-significant association between *E. coli* and clinical status (P=0.168) may indeed be subject to a Type II error due to the modest sample size. However, the primary aim was to document the presence of high-level MDR rather than establish definitive prevalence across the entire city.

RESULTS AND DISCUSSION

Enumeration, isolation and identification of *E. coli* and *Salmonella* spp.

The enumeration, isolation, and identification of *E. coli* and *Salmonella* spp. from AH and D dogs and cats are summarized in Tables 2–3, Figures 2–3, and Supplementary Figure S1. As shown in Table 2, both total viable count (TVC) and total coliform count (TCC) were significantly higher in diarrheic animals than in their healthy counterparts ($p < 0.01$). In dogs, TVC increased from 1.053×10^8 CFU/g in AH animals to 2.827×10^8 CFU/g in diarrheic animals, while TCC rose from 5.380×10^7 to 2.237×10^8 CFU/g. A similar trend was observed in cats, where TVC increased from 9.113×10^7 to 2.561×10^8 CFU/g and TCC from 5.387×10^7 CFU/g in healthy animals. These findings agree with earlier reports describing elevated bacterial loads in diarrheic dogs and cats, likely reflecting gastrointestinal disturbances associated with diarrhea (Rahman *et al*., 2020; Islam *et al*., 2016). Such increases may be attributed to intestinal dysbiosis, impaired gut barrier function, and enhanced shedding of enteric bacteria during diarrheal episodes (Cummings *et al*., 2015). *Escherichia coli* was identified as a Gram-negative organism forming smooth, pink colonies on MacConkey agar and exhibiting a characteristic metallic sheen on EMB agar. It produced slightly pink colonies on SS agar and yellow colonies on XLD agar. Biochemical characterization showed that *E. coli* was catalase-positive, methyl red-positive, indole-positive, and Voges–Proskauer-negative, with the ability to ferment dextrose, maltose, mannitol, and lactose with acid and gas production (Cheesbrough, 2005). *Salmonella* spp. were also Gram-negative and produced smooth, colorless colonies on MacConkey agar and gray colonies on EMB agar. On SS and XLD agar, colonies displayed black centers due to hydrogen sulfide production. Biochemical tests confirmed that *Salmonella* spp. were catalase-positive, methyl red-positive, indole-negative, and Voges–Proskauer-negative, fermenting dextrose, maltose, and mannitol but not lactose and sucrose, thereby clearly distinguishing them from *E. coli* (Khan *et al*., 2017).

Table 2: Total viable count (TVC) and total coliform count (TCC) of the isolated samples.

Species	Parameters	Health status	CFU/g (Scientific Notation)	P-value (t test)
Dog	TVC	AH	1.053×10^8	<0 .01
		D	2.827×10^8	
	TCC	AH	5.380×10^7	< 0.01
		D	2.237×10^8	
Cat	TVC	AH	9.113×10^7	< 0.01
		D	2.561×10^8	
	TCC	AH	5.387×10^7	< 0.01
		D	1.988×10^8	

TVC=Total Viable Count; TCC = Total Coliform Count; AH = Apparently healthy, D = Diarrhoeic.

Occurrence of *E. coli* and *Salmonella* spp. isolated from dogs and cats

Table 3 summarizes the occurrence of *E. coli* and *Salmonella* spp. in apparently healthy (AH) and diarrheic (D) dogs and cats. The detection rate of *E. coli* was significantly higher in diarrheic animals, with 70% positivity in dogs and 93.33% in cats, compared to 30% and 46.67% in their healthy counterparts, respectively ($p<0.01$). A similar pattern was observed for *Salmonella* spp., which was detected in 90% of diarrheic dogs and 66.67% of diarrheic cats, whereas only 10% of AH dogs and 6.67% of AH cats tested positive ($p<0.01$). In the present study, all 50 rectal swab samples (30 cats and 20 dogs) showed microbial presence, indicating

Table 3: Occurrence of *E. coli* and *Salmonella* spp. in dogs and cats.

Species	Health status	Number tested	*E. coli* positive No. (%)	*Salmonella* spp. positive No. (%)	P-value for *E. coli*	P-value for *Salmonella* spp.
Dog	AH	10	3 (30.0%)	1 (10.0%)	<0.01	<0.01
	D	10	7 (70.0%)	9 (90.0%)		
Sub-Total		20	10 (50.0%)	10 (50.0%)		
Cat	AH	15	7 (46.67%)	1 (6.67%)		
	D	15	14 (93.33%)	10 (66.67%)		
Sub-Total		30	21 (70.0%)	11 (36.67%)		
Total		50	31 (62.0%)	21 (42.0%)	-	-

AH = apparently healthy, D = diarrhoeic

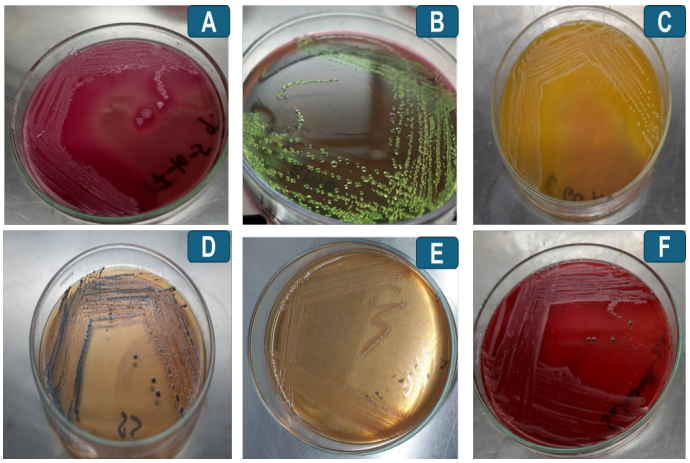

Figure 2: Cultural characteristics of *E. coli*. (A, MacConkey agar; B, EMB agar; C, XLD agar) and *Salmonella* spp. (D, SS agar; E, MacConkey agar; F, XLD agar).

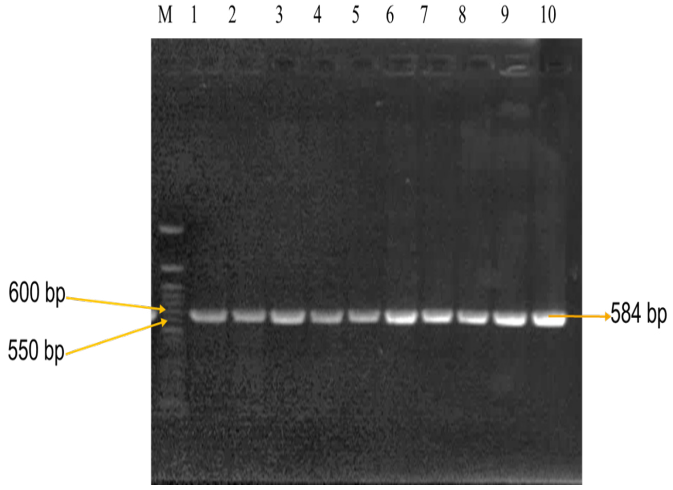

Figure 3: Amplification of 584bp DNA from 16S rRNA gene of *E coli* (Lane M: 50bp DNA Marker, Lane 1: Positive control, Lane 2-10: Test samples).

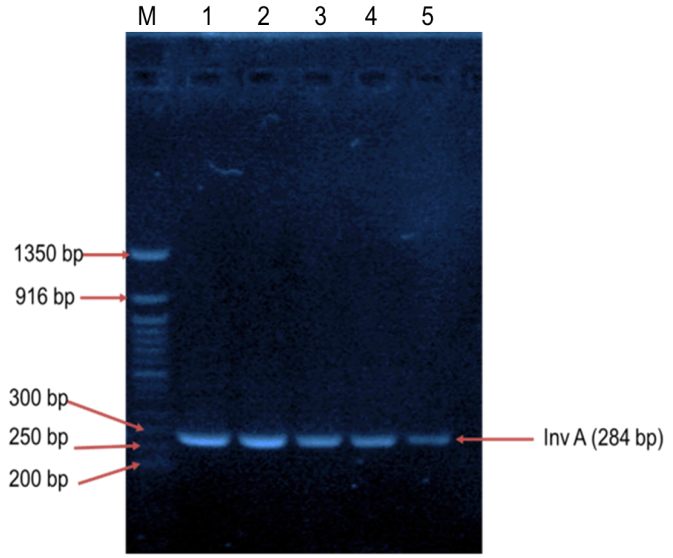

Figure 4: Amplification of 284bp DNA from *inv*A gene of *Salmonella* spp. (Lane L: 50bpDNA Ladder, Lane 1: Positive control, Lane 2-5: Test samples).

a 100% overall occurrence. Among feline samples, *E. coli* was found in 21/30 (70%), notably higher in diarrheic cats (14/15; 93%) than healthy ones (7/15; 46.7%). In dogs, *E. coli* was detected in 10/20 (50%), with 7/10 diarrhea and 3/10 healthy dogs testing positive. These rates exceed those reported by Das *et al.* (2012), who observed *E. coli* in 46.87% of pet cats and 54.58% of pet dogs. *Salmonella* spp. was detected in 11 of 30 (36.7%) feline samples and 10 of 20 (50.0%) canine samples. Among cats, 10 of 15 (66.7%) diarrheic animals tested positive, compared with 1 of 15 (6.7%) apparently healthy animals. In dogs, *Salmonella* spp. was isolated from 9 of 10 (90.0%) diarrheic animals and 1 of 10 (10.0%) apparently healthy animals. These findings align with those reported by Rahman *et al.* (2020), who noted higher isolation rates of enteric pathogens in diarrheic than in non-diarrheic pets in Bangladesh. Islam *et al.* (2016) also reported a significantly higher occurrence of *Salmonella* in diarrheic dogs and cats, attributing the infection in diarrheic animals. Furthermore, the prevalence of *Salmonella* in diarrheic dogs (90.0%) was substantially higher than that reported by Ting *et al.* (2020), who observed a prevalence of 19.2%, indicating potential differences in geographic location, management practices, or exposure risks. The elevated occurrence of both bacteria in diarrheic animals may be due to compromised gut barriers, altered intestinal microbiota, and increased pathogen shedding associated with enteric infections (Cummings *et al.*, 2015; Ahmed *et al.*, 2019).

Antibiotic sensitivity and resistance pattern of the isolated *E. coli* and *Salmonella* spp.

In the present study, *E. coli* and *Salmonella* spp. isolates from diarrheic cats and dogs showed varying levels of resistance (Figures 5-8). Canine *E. coli* isolates (n= 10) showed 60% resistance to amoxicillin and 40% to ceftriaxone and erythromycin, consistent with (Ahmed *et al.*, 2019), who reported growing resistance to broad-spectrum antibiotics among pet isolates in urban Bangladesh. These findings raise concern over the therapeutic challenges posed by MDR *E. coli*, which has been increasingly reported in veterinary and human medicine alike (Morrison and Rubin, 2015). Among feline *E. coli* isolates (n= 21), only 61.9% were sensitive to ceftriaxone, and 76.2% were resistant to erythromycin highlighting the limited efficacy of commonly prescribed antibiotics. Ampicillin and amoxicillin exhibited moderate resistance, mirroring findings from Das *et al.* (2012) and Deb *et al.* (2020), who noted increasing resistance to β-lactams among feline isolates. Among canine isolates (n= 9), resistance to ampicillin and amoxicillin was 66.7%, whereas meropenem and amoxicillin-clavulanic acid (AMC) were most effective, showing 77.8% sensitivity. In cats (n= 11), only 54.5% were sensitive to ceftriaxone and erythromycin, while amoxicillin resistance was highest (54.5%). Similar resistance trends

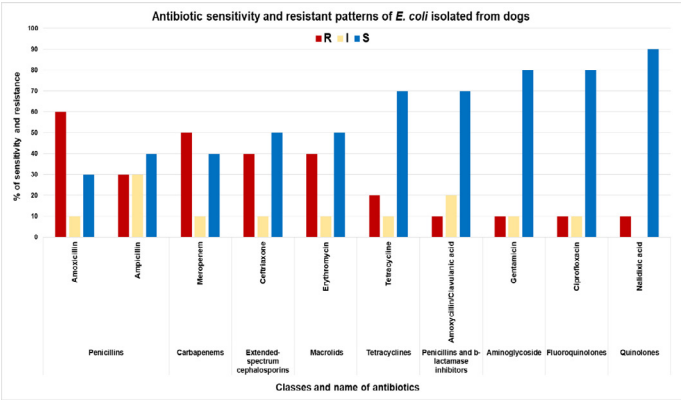

Figure 5: Antimicrobial Resistance of *E. coli* isolates from dogs (n=10).

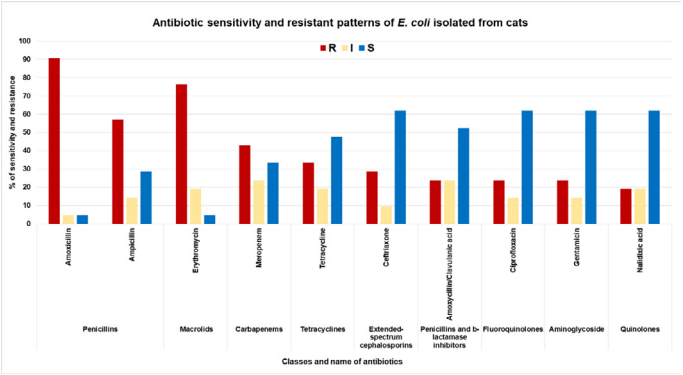

Figure 6: Antimicrobial Resistance of *E. coli* isolates from cats (n=21).

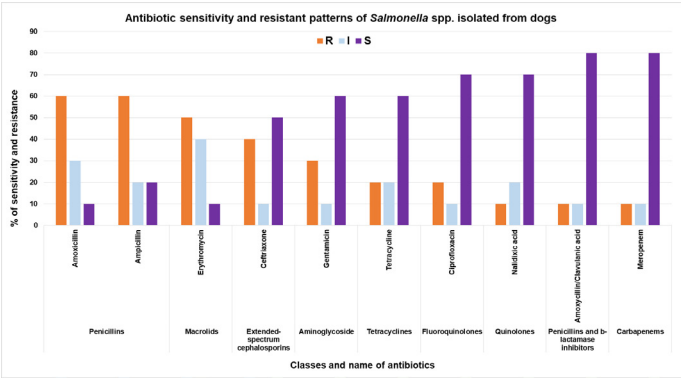

Figure 7: Antimicrobial Resistance of *Salmonella* isolates from dogs (n=10).

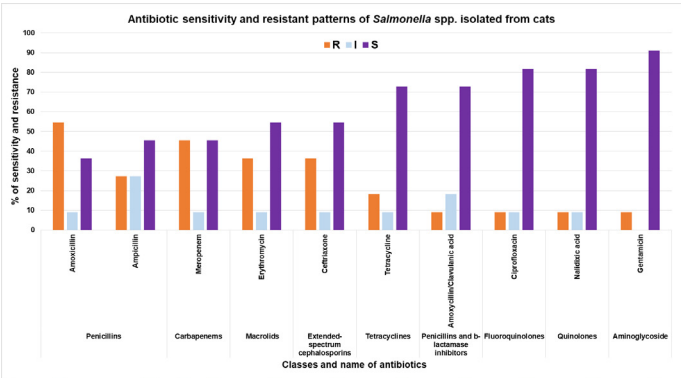

Figure 8: Antimicrobial Resistance of *Salmonella* isolates from cats (n=11).

were observed by Ojo and Adetosoye (2009), and Sringam and Angkititrakul (2015), who reported poor susceptibility of *Salmonella* isolates to penicillins and improved response to carbapenems and fluoroquinolones. A recent regional survey by (Hoque *et al*., 2020) also emphasized the emergence of MDR *Salmonella* strains in pet animals in South Asia, often linked to the unregulated use of antibiotics and the lack of targeted therapy. AMR in companion animals has become a pressing One Health concern, given the increasing evidence of resistant pathogens being shared between pets and humans through direct contact or environmental routes (Lloyd, 2007; Guardabassi *et al*., 2004). Pets can serve as asymptomatic carriers of MDR organisms, including *E. coli* and *Salmonella* spp., thereby acting as reservoirs for resistance genes. This underscores the vital importance of routine antimicrobial susceptibility testing (AST) in veterinary diagnostics, not only for ensuring effective treatment of infections but also for informing antimicrobial stewardship policies and interrupting zoonotic transmission cycles (World Health Organization (WHO, 2017)). Without such testing, empirical or inappropriate antibiotic use in pets may exacerbate the selection and spread of resistance particularly concerning given the widespread use of critically important antibiotics like third-generation cephalosporins in small animal practice (Ghosh *et al*., 2021).

Table 4: Overall MDR pattern among the antibiotics for *E. coli* and *Salmonella* spp.

Bacterial species	No. of positive isolates	No. of MDR isolates	% of MDR isolates
E. coli	31	29	93.55
Salmonella spp.	21	16	76.19

MDR= Multidrug resistant

Table 4 and Supplementary Table S2 stated a markedly high prevalence of MDR *E. coli* (93.55%) and *Salmonella* spp. (76.19%) isolated from companion animals, indicating extensive antimicrobial pressure in pet populations. The MDR patterns in *E. coli* involved resistance to multiple critically important antimicrobial classes, including penicillins, β-lactam/β-lactamase inhibitor combinations, tetracyclines, cephalosporins, fluoroquinolones, quinolones, macrolides, aminoglycosides, and carbapenems, with the most frequent pattern comprising resistance to eight antimicrobial classes. Similar MDR profiles in *E. coli* from dogs and cats have been reported previously, suggesting widespread dissemination of plasmid-mediated resistance determinants and extended-spectrum β-lactamase–producing strains in companion animals (Guardabassi *et al*., 2004; Ahmed *et al*., 2019). The exceptionally high MDR rate observed among *E. coli* isolates exceeds that reported in several previous Bangladeshi studies and may

be influenced by urban pet-keeping practices, including frequent antibiotic exposure, close contact with humans, and environmental contamination. Pets in Dhaka often share living spaces with owners and may be exposed to antimicrobial residues or resistant bacteria through contaminated food, water, soil, or household waste. Although this study did not directly assess antibiotic usage patterns in households or clinics, informal observations during sampling indicated prior antibiotic treatment in several diarrheic animals, supporting the possibility that unregulated or inappropriate antibiotic use contributed to the high resistance burden.

The detection of carbapenem resistance in *E. coli* and *Salmonella* spp. is particularly concerning, as carbapenems are not routinely used in veterinary practice in Bangladesh. This finding suggests that resistance may not arise from direct veterinary antimicrobial pressure but rather from horizontal transmission of resistance genes originating from human sources. Potential pathways include close human–pet contact, environmental contamination with human sewage, or exposure to carbapenem-resistant bacteria in densely populated urban environments. Similar concerns have been raised in other One Health studies, where companion animals were found to harbor carbapenem-resistant organisms linked to human clinical settings (WHO, 2019; Damborg *et al*., 2016; Lloyd, 2012). This highlights the role of pets as potential sentinels and reservoirs of resistance genes of critical public health importance.

In *Salmonella* spp., MDR patterns commonly included resistance to penicillins, tetracyclines, fluoroquinolones, quinolones, macrolides, aminoglycosides, and cephalosporins, with uniform distribution across resistance profiles, suggesting circulation of multiple resistant strains rather than clonal expansion. Resistance to fluoroquinolones and third-generation cephalosporins in *Salmonella* is of major concern due to their importance in the treatment of invasive human infections (Shaheen *et al*., 2013; O'Neill, 2016).

The high levels of AMR observed among *E. coli* and *Salmonella* spp. isolated from diarrheic dogs and cats in this study likely reflect a combination of behavioral, clinical, and environmental drivers prevalent in urban settings such as Dhaka. In Bangladesh, antibiotics are widely available over the country, and pet owners may administer human antibiotics or leftover prescriptions to animals without veterinary consultation. In addition, empirical treatment without prior AST remains common in small animal practice, often involving repeated or prolonged use of broad-spectrum antibiotics. Together, these practices may create substantial selective pressure, contributing to the emergence and persistence of MDR enteric bacteria in companion animals.

While the public health implications of AMR in companion animals are widely recognized, the present findings underscore the need for specific, actionable interventions. First, routine AST should be strongly encouraged if not mandated for the treatment of bacterial infections in veterinary clinics, particularly in cases of recurrent or diarrheal disease. Second, the empirical use of critically important antibiotics, such as third-generation cephalosporins and fluoroquinolones, should be restricted in small animal practice and reserved for cases with laboratory confirmation. Third, targeted education programs for pet owners are essential to discourage self-medication, improper dosing, and the use of human antibiotics in animals. At the policy level, the establishment of a national AMR surveillance system that includes companion animals would allow early detection of emerging resistance trends and inform evidence-based antimicrobial stewardship.

Limitations of the study:

The sample size was relatively small and based on convenience sampling, which may limit the generalizability of the findings to the wider pet population in Bangladesh. Additionally, the lack of quantitative data on prior antibiotic exposure prevented a direct assessment of the relationship between usage patterns and resistance outcomes. Despite these limitations, the consistently high resistance rates across species and bacterial genera suggest that AMR in companion animals in Dhaka represents a substantial and growing concern rather than an isolated observation.

CONCLUSION

This study conducted in Dhaka, Bangladesh, investigated the occurrence and antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. isolated from fecal samples of both diarrheic and apparently healthy dogs and cats. The findings revealed a higher occurrence of these pathogens in both symptomatic and asymptomatic animals. Antimicrobial susceptibility testing indicated significant resistance to commonly used antibiotics, particularly ampicillin and amoxicillin, while lower resistance rates were observed for ciprofloxacin and nalidixic acid. These results underscore the potential role of companion animals as reservoirs for multidrug-resistant bacteria, posing a risk to public health. The study highlights the necessity for prudent antibiotic use, regular monitoring of antimicrobial resistance, and the implementation of biosecurity measures to mitigate the spread of resistant pathogens.

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NOVELTY STATEMENT

The novelty of this study lies in the comparative investigation of diarrheic and apparently healthy pet dogs and cats in Dhaka, Bangladesh, focusing on the molecular detection and antimicrobial resistance patterns of Escherichia coli and Salmonella spp. This study provides the first evidence from this urban setting of an exceptionally high prevalence of multidrug resistance, including resistance to critically important antimicrobials, in both clinically affected and asymptomatic pets. The findings highlight the role of companion animals as silent reservoirs of multidrug-resistant zoonotic bacteria and demonstrate the significant increase in bacterial load associated with diarrheal conditions, emphasizing a critical One Health concern at the pet–human interface.

AUTHOR'S CONTRIBUTION

MRA: Conceptualization, methodology, data curation, formal analysis, writing original draft, writing review and editing. SMA: Data curation, formal analysis, supervision, writing review and editing. MKH and MSS: Data curation; writing review and editing. MAM and MRI: Formal analysis, writing review and editing. AAB, MAHS and MHL: Methodology, writing review and editing. MI: Conceptualization, data curation, formal analysis, validation, supervision, writing review and editing.

Generative AI and AI-assisted technology statement

The authors affirm that the scientific content, data interpretation, and findings in this work were not produced using generative AI technologies. All text was thoroughly examined and verified by the authors, and AI-assisted technologies were only utilized for language editing and clarity enhancement.

Conflict of interest

The authors have declared no conflict of interest.

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Supplementary Table S1: Staining and biochemical characteristics of *E. coli* and *Salmonella* spp. isolated from dogs and cats.

Parameters		*E. coli*	*Salmonella* spp.
Gram staining		–ve	–ve
Catalase test		+ve	+ve
Sugar fermentation tests	Dextrose	AG	AG
	Lactose	AG	NF
	Sucrose	AG	NF
	Maltose	AG	AG
	Mannitol	AG	AG
Methyl red test		+ve	+ve
Voges-Proskauer test		–ve	–ve
Indole test		+ve	–ve

+ve: positive; –ve: negative; AG: acid and gas is produced after fermentation; NF: no fermentation occurred

Supplementary Table S2: Multidrug resistance pattern for *E. coli* and *Salmonella* spp. according to their antibiotic classes.

Bacterial species	MDR pattern (antibiotics)	Antimicrobial classes involved	No. of MDR isolates (%)
E. coli (n=31)	AMP–AMX–TE–AMC–CTR–CIP–NA–E–GEN	Pen–Tet–PenB–Cef–Flu–Qui–Mac–Ami	10 (32.26)
	AMP–AMX–CTR–CIP–NA–E–GEN	Pen–Cef–Flu–Qui–Mac–Ami	2 (6.45)
	AMP–AMX–TE–MEM	Pen–Tet–Carb	3 (9.68)
	AMP–AMX–AMC–MEM	Pen–PenB–Carb	2 (6.45)
	AMP–AMX–TE–CTR–CIP–NA–E–GEN	Pen–Tet–Cef–Flu–Qui–Mac–Ami	5 (16.13)
	AMP–AMX–TE–AMC–MEM	Pen–Tet–PenB–Carb	2 (6.45)
	AMP–AMX–TE–AMC–NA–E–GEN	Pen–Tet–PenB–Qui–Mac–Ami	1 (3.23)
	AMP–AMX–TE–CTR–CIP–NA–E–GEN–MEM	Pen–Tet–Cef–Flu–Qui–Mac–Ami–Carb	2 (6.45)
	AMP–AMX–TE–GEN–MEM	Pen–Tet–Ami–Carb	1 (3.23)
	AMP–AMX–TE–NA–E–GEN–MEM	Pen–Tet–Qui–Mac–Ami–Carb	1 (3.23)
	Subtotal		29 (93.55)
Salmonella spp. (n=21)	AMP–AMX–TE–CIP–NA	Pen–Tet–Flu–Qui	2 (9.52)
	AMP–AMX–AMC–CTR–E	Pen–PenB–Cef–Mac	2 (9.52)
	AMP–AMX–TE–NA–E	Pen–Tet–Qui–Mac	2 (9.52)
	AMP–AMX–TE–NA–GEN	Pen–Tet–Qui–Ami	2 (9.52)
	AMP–AMX–TE–AMC–CTR–E	Pen–Tet–PenB–Cef–Mac	2 (9.52)
	AMP–AMX–TE–AMC–CTR	Pen–Tet–PenB–Cef	2 (9.52)
	AMP–AMX–TE–AMC–NA	Pen–Tet–PenB–Qui	2 (9.52)
	AMP–AMX–TE–AMC–CTR–NA–GEN	Pen–Tet–PenB–Cef–Qui–Ami	2 (9.52)
	Subtotal		16 (76.19)