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ORIGINAL ARTICLE

Isolation of multidrug-resistant *Escherichia coli*, *Staphylococcus* spp., and *Streptococcus* spp. from dogs in Chattogram Metropolitan Area, Bangladesh

Probir Deb[1†], Tridip Das[1†], Chandan Nath[1], Abdul Ahad[1], Pankaj Chakraborty[2]
[1]Department of Microbiology and Veterinary Public Health, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh
[2]Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh
[†]These authors contributed equally to this work.

ABSTRACT

Objectives: Antibacterial resistance is a great concern in human and food animal medicine, and it poses a significant concern in pet animals like dogs. This cross-sectional study was conducted to evaluate the antimicrobial resistance pattern of *Escherichia coli*, *Staphylococcus* spp., and *Streptococcus* spp. along with the carryover of some resistance genes in *E. coli* from dogs in the Chattogram metropolitan area, Bangladesh.

Materials and Methods: Rectal swab (n = 50), nasal swab (n = 50), and skin swab (n = 50) samples were collected from dogs having respiratory infections, skin infections, and/or enteritis, respectively. Three types of bacteria were identified and isolated by conventional bacteriological techniques and biochemical tests. Antimicrobial susceptibility testing was carried out against 12 antimicrobials by disk diffusion methods. Six resistance genes, namely bla_{TEM}, bla_{CTX-M}, *tet*A, *tet*B, *Sul*-I, and *Sul*-II, were screened for phenotypically resistant *E. coli* isolates by the polymerase chain reaction.

Results: A total of 39 (78%) *E. coli*, 25 (50%) *Staphylococcus* spp., and 24 (48%) *Streptococcus* spp. isolates were isolated from the rectal swab, nasal swab, and skin swab samples, respectively. In the cultural sensitivity test, the *E. coli* isolates showed resistance to ceftriaxone (79%) and sulfamethoxazole/trimethoprim (64%). Doxycycline (80%) demonstrated the highest resistance among *Staphylococcus* isolates, followed by sulfamethoxazole/trimethoprim (60%). *Streptococcus* isolates showed the highest resistance to penicillin (63%), followed by ceftriaxone (54%), while no isolate showed resistance to gentamycin. The prevalence of bla_{TEM}, bla_{CTX-M}, *tet*A, *tet*B, *Sul*-I, and *Sul*-II genes in phenotypically resistant *E. coli* isolates were 100%, 61.29%, 100%, 8.33%, 56%, and 72%, respectively.

Conclusions: Spillover of such multidrug-resistant bacteria and resistance genes from pet dogs pose a serious public health risk.

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Antimicrobial resistance; common pathogens; resistance genes; dog

Introduction

The emergence of antimicrobial resistance (AMR) in companion animals is a matter of global concern, not only as patient factors but also as public health issues [1]. As companion animals, dogs are adopted worldwide, contributing to emotional comfort to the owner, especially children [2]. Several common pathogens are causing upper and lower respiratory tract infections in dogs. Several other diseases, like diarrhea and skin infection, were recorded in dogs [3]. *Escherichia coli* are commensal organisms found in the lower gastrointestinal tract of all warm-blooded animals, e.g., dogs [4]. *Staphylococcus* spp. mainly remain dormant on the healthy skin of dogs. These bacteria are zoologically significant [5]. *Streptococcus* spp. are normally present in the upper respiratory tracts of dogs, which can cause localized infections and even septicemia [6]. Horizontal transfer of such pathogens from companion animals to humans is likely to occur due to close contact between them [7].

Correspondence Tridip Das ✉ das.vet671@gmail.com 🏠 Department of Microbiology and Veterinary Public Health, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

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In some geographical regions, antimicrobial drugs are thoroughly used for animal prophylaxis and therapeutics, where many of these belong to a similar group of antimicrobials used in treating humans [8]. It is noteworthy that the widespread scenario in developing countries like Bangladesh is due to the indiscriminate use of antimicrobials [9]. Nowadays, multidrug-resistant (MDR) bacteria, along with their resistance and transmission pattern within and/or between similar and/or different hosts, are a matter of global concern. As these bacteria have zoonotic significance, carryover of resistance genes and their horizontal transfer to humans from dogs will shorten the treatment phenomenon in both species [10]. Therefore, it is crucial to identify the involved bacterial pathogens and use of the most susceptible antimicrobials, which may direct the antimicrobial selection for those bacteria [11].

Several surveillance reports on AMR of zoonotic bacteria like *E. coli*, *Staphylococcus* spp., and *Streptococcus* spp. from food animals, food, and human have been established and published [12]. Still, a few reports are available on dogs. There are limited data on the AMR pattern of common pathogens of dogs in Bangladesh, despite an increasing number of dogs as pet animals among elite people of the metropolitan cities. The study was designed to evaluate the AMR pattern of *E. coli* from rectal swabs, *Staphylococcus* spp. from skin swabs, and *Streptococcus* spp. from nasal swabs along with the carryover of some resistance genes in *E. coli* from dogs in the Chattogram metropolitan area, Bangladesh.

Methods and Materials

Ethical approval

Animal dealings and experiments were carried out following the guidelines approved by the Chattogram Veterinary and Animal Sciences University (CVASU) ethics committee [Memo No: CVASU/Dir(R&E)EC/2018/105/(03)]. Appropriate measures were taken to minimize pain or discomfort during sample collection from the dogs.

Study area and study population

This study was carried out between January 2018 and September 2018 in S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) of the CVASU. Dogs brought to the hospital for treatment purposes were selected for sampling.

Collection of samples

In total, 150 samples comprising rectal swabs (n = 50), nasal swabs (n = 50), and skin swabs (n = 50) were collected from dogs. Dogs having respiratory infections, skin infection, or enteritis were sampled during the study period. The samples were collected using sterile cotton and immediately transferred to Stuart's transport medium (Oxoid, Basingstoke, UK) and stored at −80°C in the laboratory of the Department of Microbiology and Veterinary Public Health, CVASU, Bangladesh, for further use [13].

Bacteriological investigation

Escherichia coli, *Staphylococcus* spp., and *Streptococcus* spp. were isolated and identified from the collected samples based on their cultural properties, biochemical tests, including pigment production, and hemolytic activities [14,15].

Escherichia coli

The collected rectal swab samples were enriched overnight in buffered peptone water (BPW) (Oxoid Ltd., Basingstoke, UK) [16]. A loopful of the enriched broth was inoculated onto MacConkey agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24 h at 37°C. The suspected large pink color colonies were subcultured onto eosin-methylene blue agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24 h at 37°C. Colonies which produced a typical metallic sheen were subcultured onto the blood agar and further confirmed by the Gram stain properties (Fig. 1C) and biochemical tests (Fig. 1F).

Staphylococcus spp.

The collected skin swabs were enriched overnight in BPW at 37°C. One loopful of the enriched broth was directly streaked onto the Mannitol Salt Agar (MSA) (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37°C. The suspective positive colonies were identified based on the colony characteristics on the MSA. The suspected bright yellow positive colonies were then subcultured onto the blood agar and incubated at 37°C for 24 h to detect characteristics appearance on the blood agar and the hemolytic properties of the organism [5]. Then, the organism was confirmed by the Gram stain properties (Fig. 1B) and several biochemical tests (Fig. 1D and E).

Streptococcus spp.

The nasal swabs were placed in BPW and incubated overnight at 37°C, and a loopful was inoculated onto the blood agar. White to transparent dew drop-like colonies with alpha- and beta-hemolysis were observed on the blood agar after incubation overnight at 37°C. The suspected colonies were then confirmed by the Gram stain properties (Fig. 1A) and catalase test (Fig. 1D).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all positive isolates was carried out using the disk diffusion technique recommended by the Clinical Laboratory and Standards

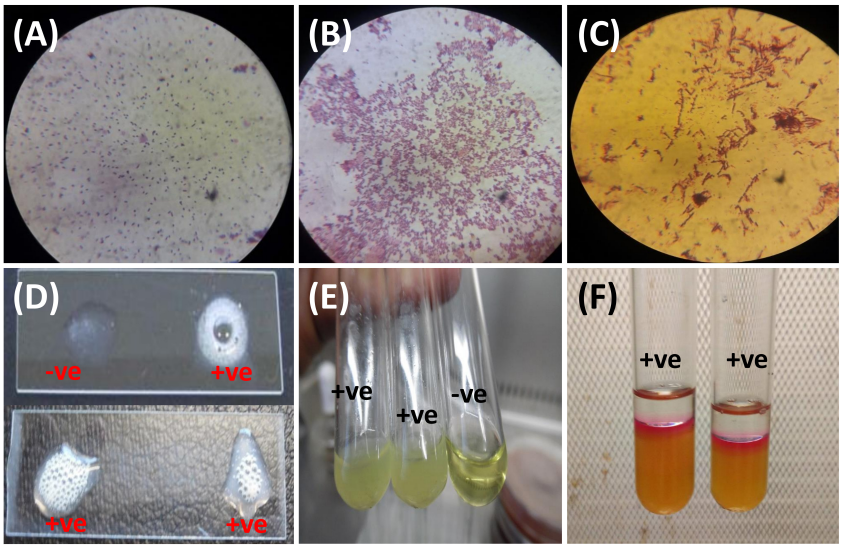

Figure 1. Gram stain and different biochemical tests of the isolated bacteria. (A) *Streptococcus* spp., (B) *Staphylococcus* spp., (C*) E. coli*, (D) catalase test, (E) coagulase test, and (F) indole test.

Institute (CLSI) [17]. The ATCC25922 was used for quality control during the disk diffusion technique. A total of 12 antimicrobials of different groups were used at the given disk content: penicillin (6 µg), ampicillin (10 µg), cephradine (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), azithromycin (15 µg), gentamycin (30 µg), oxytetracycline (30 µg), doxycycline (5 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), and sulfamethoxazole/trimethoprim (25 µg). These antimicrobials were selected based on the history of commonly used antimicrobials at the SAQTVH. The interpretation of the test results was made according to the CLSI guidelines [17]

DNA extraction

The conventional crude boiling method was used to extract the genomic DNA of *E. coli* isolates [18]. In brief, 2–3 fresh colonies were taken in a sterile 1.5-ml microcentrifuge tube containing 200-µl sterile Milli-Q water and vortexed thoroughly. After heating the microcentrifuge tube at 99°C for 10 min, it was rapidly frozen at −20°C and centrifuged at 13,000 rpm for 5 min. Finally, the supernatant (100 µl) was collected and used as the DNA template in polymerase chain reaction (PCR), followed by storing at −20°C for further use.

Screening of AMR genes

The *E. coli* isolates, which were resistant to tetracycline, were tested for the presence of *tet*A and *tet*B genes. Similarly, the *E. coli,* which were resistant to ampicillin and cefotaxime, were tested for the presence of bla_{TEM} and $bla_{CTX\text{-}M}$ genes, respectively. All sulfamethoxazole/trimethoprim-resistant isolates were tested for *Sul-I* and *Sul-II* genes. The oligonucleotide primers used for the amplification of the genes by PCR are mentioned in Table 1. Due to resource limitation, *Staphylococcus* spp. and *Streptococcus* spp. isolates were not tested for any resistance genes. The specific thermal cyclic conditions of each resistance gene are displayed in Table 2. The PCR products were visualized on a gel documentation system (UVP UVsolo touch – Analytik Jena AG) after electrophoresis with 1.5% agarose gel (SeaKem® LE Agarose from Lonza).

Statistical analysis

Field and laboratory data obtained were entered into MS Excel 2010 spreadsheets and were analyzed using the R package [19]. The heatmap of antimicrobial susceptibility testing phenotype was generated by using Graphpad Prism 7.0.

Results

Frequency of samples and organisms

By using the standard bacteriological culture technique, 39 (78%; 95% Confidence Interval 59.84%–89.60%) rectal swabs, 25 (50%; 95% Confidence Interval 36.64%–63.36%) skin swabs, and 24 (48%; 95% Confidence Interval 34.80–59.42) nasal swabs were found positive for *E. coli*, *Staphylococcus* spp., and *Streptococcus* spp., respectively.

AMR patterns

The summary of the AMR profile is outlined in Table 3. In the cases of *E. coli*, the highest resistance was found

Table 1. Oligonucleotide primer sequences used to detect resistance genes in *E. coli* isolates.

Gene	Primer Name	Primer sequence (5′- 3′)	Annealing temperature	Amplicon size (bp)	Reference
bla_{TEM}	bla_{TEM}-F	TACGATACGGGAGGGCTTAC	50°C	716	[39]
	bla_{TEM}-R	TTCCTGTTTTTGCTCACCCA			
bla_{CTX-M}	bla_{CTXM}-F	ACGCTGTTGTTAGGAAGTG	58°C	857	[40]
	bla_{CTXM}-R	TTGAGGCTGGGTGAAGT			
*tet*A	*tet*A-F	GGCGGTCTTCTTCATCATGC	64°C	502	[41]
	*tet*A-R	CGGCAGGCAGAGCAAGTAGA			
*tet*B	tetB-F	CATTAATAGGCGCATCGCTG	64°C	930	[41]
	tetB-R	TGAAGGTCATCGATAGCAGG			
Sul-I	*Sul*-I-F	CGG CGT GGG CTA CCT GAA CG	68°C	779	[41]
	Sul-I- R	GCC GAT CGC GTG AAG TTC CG			
Sul-II	*Sul*-II-F	CCT GTT TCG TCC GAC ACA GA	66°C	721	[41]
	Sul-II-R	GAA GCG CAG CCG CAA TTCAT			

Table 2. Thermal cycling conditions for PCR of tested resistance genes.

Gene name	bla_{TEM}	bla_{CTX-M}	*tet*A, *tet*B	*Sul*-I	*Sul*-II
Initial denaturation	94°C, 3 min	94°C, 3 min	95°C, 4 min	95°C, 5 mins	94°C, 4 min
Cyclic denaturation	94°C, 1 min	94°C, 1 min	95°C, 1 min	95°C, 1 min	94°C, 1 min
Cyclic annealing	50°C, 1 min	58°C, 30 sec	64°C, 1 min	68°C, 1 min	66°C, 1 min
Cyclic extension	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min
Final extension	72°C, 10 min	72°C, 10 min	72°C, 7 min	72°C, 10 min	72°C, 7 min
Cycle number	25	36	35	35	35
References	[39]	[40]	[41]	[41]	[41]

Table 3. Phenotypic antibiogram of *Streptococcus* spp., *Staphylococcus* spp., and *E. coli*.

Antibiotic name (concentration)	*E. coli* (*n* = 39)			*Staphylococcus* spp. (*n* = 25)			*Streptococcus* spp. (*n* = 24)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
PEN (6 µg)	15	5	79	36	16	48	33	4	63
AMP (10 µg)	36	21	44	60	28	12	54	8	38
CEP (30 µg)	41	18	41	76	4	20	58	17	25
CRO (30 µg)	15	5	79	36	16	48	33	13	54
ERY (15 µg)	44	26	31	60	8	32	58	13	29
AZM (15 µg)	95	3	3	96	4	0	92	4	4
GEN (30 µg)	72	13	15	56	36	8	58	42	0
OTC (30 µg)	51	18	31	40	36	24	58	25	17
DOX (5 µg)	92	3	5	100	20	80	79	17	4
CIP (5 µg)	21	26	54	24	44	32	13	46	42
NAL (30 µg)	15	18	67	20	40	40	13	42	46
SXT (25 µg)	23	13	64	8	32	60	21	29	50

n = number of isolates; S = Susceptible; I = Intermediate; R = Resistant; PEN = Penicillin; Ampicillin; CEP = Cephradine; CRO = Ceftriaxone; ERY = Erythromycin; AZM = Azithromycin; GEN = Gentamycin; OTC = Oxytetracyline; DOX = Doxycycline; CIP = Ciprofloxacin; NAL =Nalidixic acid; SXT = Sulfamethoxazole/trimethoprim.

toward ceftriaxone (79%) and penicillin (79%), followed by nalidixic acid (67%), sulfamethoxazole/trimethoprim (64%), ampicillin (44%), and oxytetracycline (31%). Only 3% of the isolates showed resistance to azithromycin. On the other hand, doxycycline (80%) demonstrated the highest resistance for *Staphylococcus* isolates (n = 25), followed by sulfamethoxazole/trimethoprim (60%), ampicillin (12%), and ceftriaxone (48%). From the resistance pattern of *Streptococcus* isolates (n = 24) among the 12 tested antibiotics, penicillin (63%) turned out to have the highest level of resistance, followed by ceftriaxone (54%), while no isolate displayed resistance to gentamycin. The overall AMR profile of all isolates is shown in Figure 3. The MDR isolates were classified based on the

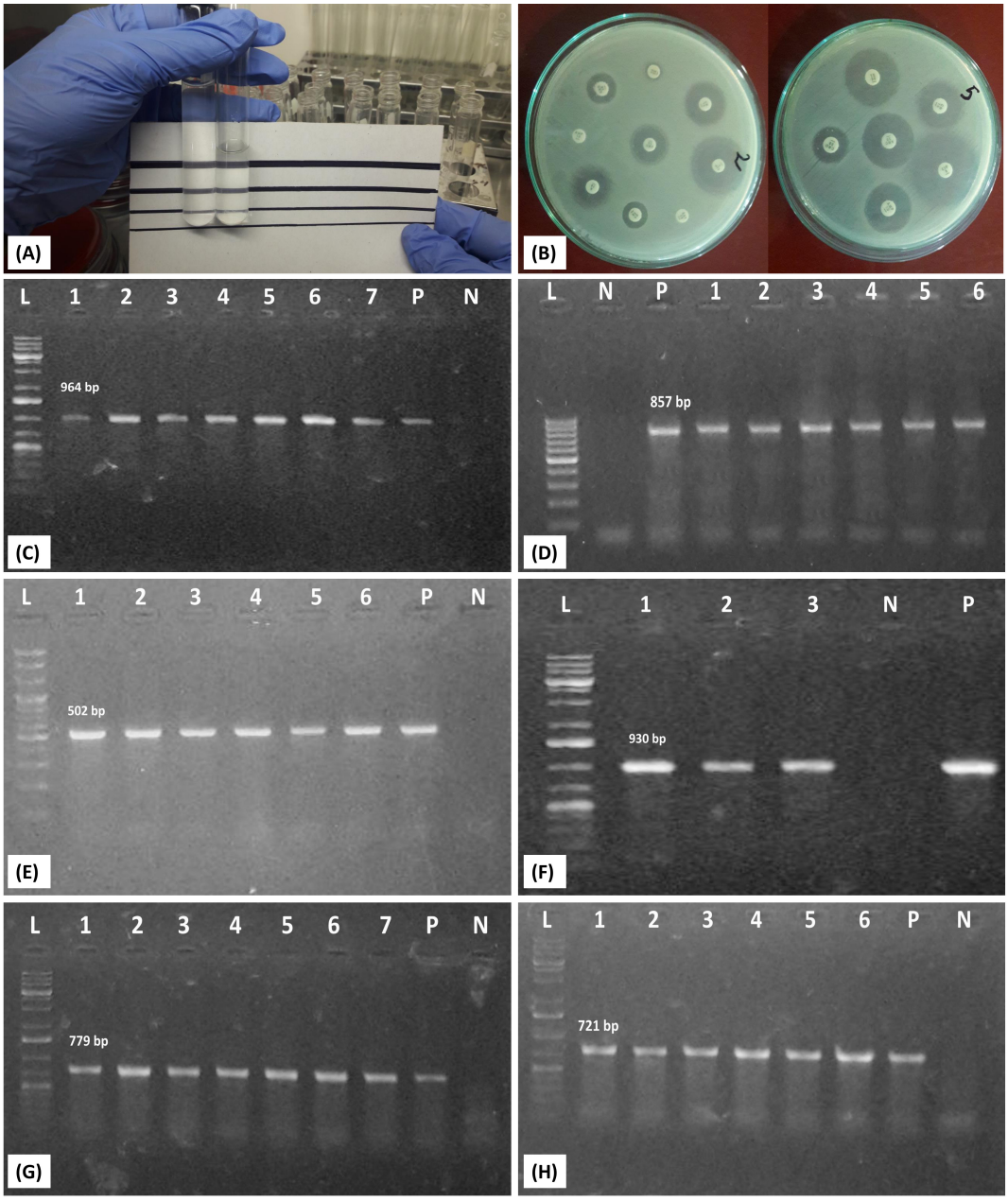

Figure 2. Antibiogram phenotype (disk diffusion) and PCR gel images of resistance genes. (A) Comparing inoculum with 0.5 McFarland standard; (B) antimicrobial susceptibility testing plates of selected *E. coli* isolates after 18 h incubation; (C) bla_{TEM} gene (D) $bla_{\text{CTX-M}}$ gene, (E) *tet*A gene, (F) *tet*B gene, (G) *Sul*-I gene, and (H) *Sul*-II gene. In all the gel images, L, P, N stands for DNA ladders, positive control (previously isolated *E. coli* strain), and negative control (ATCC25922), respectively.

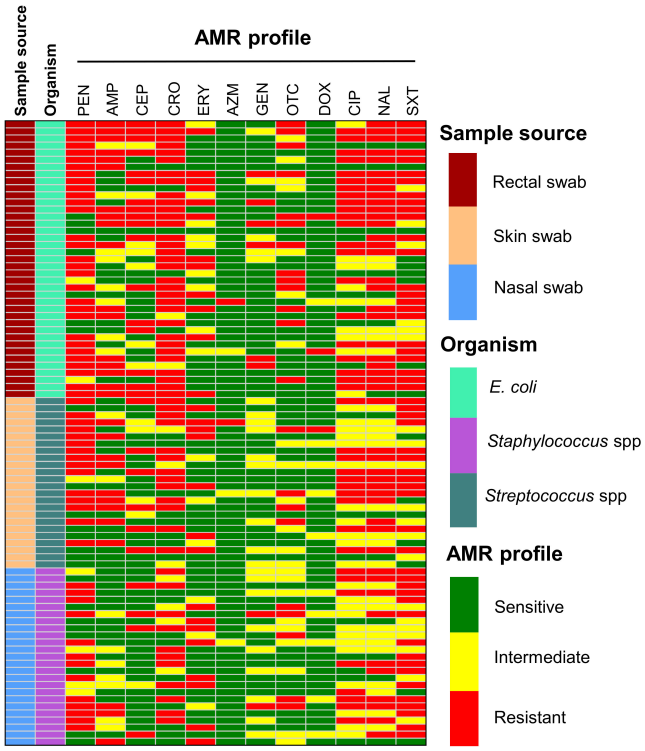

Figure 3. Distribution of AMR phenotype *Streptococcus* spp., *Staphylococcus* spp., and *E. coli* isolates across the samples. Where PEN = Penicillin; AMP = Ampicillin; CEP = Cephradine; CRO = Ceftriaxone; ERY = Erythromycin; AZM = Azithromycin; GEN = Gentamycin; OTC = Oxytetracycline; DOX = Doxycycline; CIP = Ciprofloxacin; NAL = Nalidixic acid; and SXT = Sulfamethoxazole/trimethoprim.

resistance profiles of AMR. If the isolates showed resistance to one antimicrobial agent in three or more antimicrobial classes, they were considered as MDR [20]. Among the three types of the isolated organisms, most of the *E. coli* isolates were MDR, rather than *Staphylococcus* spp. and *Streptococcus* spp. (Fig. 4). About 90% of the *E. coli* isolates were found to be MDR. On the other hand, 56% *Staphylococcus* spp. and 71% *Streptococcus* spp. isolates were MDR.

Distribution of resistance genes in E. coli

Six resistance genes were screened among the phenotypically resistant *E. coli* isolates. The PCR gel images of all tested resistance genes of some selected *E. coli* isolates are shown in Figure 2. All the phenotypically ampicillin-resistant *E. coli* isolates (17) carried the bla_{TEM} gene, and 61.29% of phenotypically ceftriaxone-resistant *E. coli* isolates (31) harbored the bla_{CTX-M} gene. In the phenotypically tetracycline-resistant isolates (12), all carried the *tet*A gene. In the case of phenotypically sulfamethoxazole/trimethoprim-resistant isolates (25), 72% carried the *Sul*-II gene. The distribution of the resistance genes is shown in Table 4.

Discussion

The trend of keeping pet animals has increased dramatically over the last decade in Bangladesh. Pet owners treat them as family members and usually provide first medications at home when they become sick. Antimicrobials are also included in their medication profiles, and the use of unregulated antibiotics in these animals has received

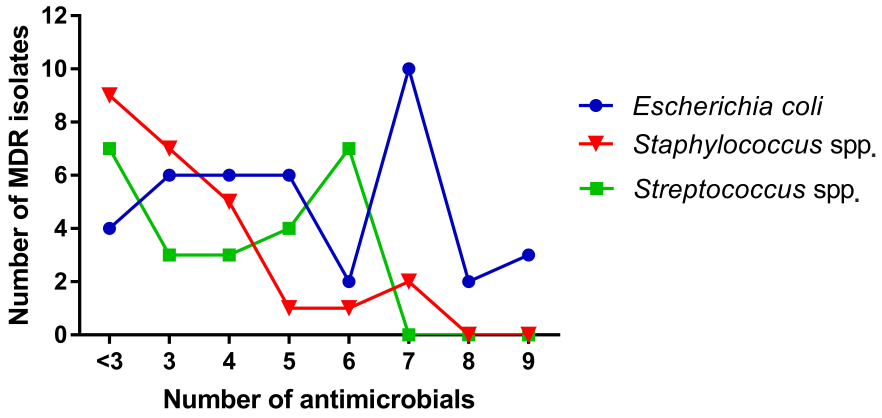

Figure 4. Multidrug-resistance pattern of *E. coli*, *Staphylococcus* spp., and *Streptococcus* spp.

Table 4. Prevalence of resistance genes in phenotypically resistant *E. coli.*

Antimicrobial	Resistance genes	Number of phenotypically resistant isolates	Number of isolates harboring genes	%
Ampicillin	bla_{TEM}	17	17	100
Ceftriaxone	bla_{CTX-M}	31	19	61.29
Tetracycline	*tet*A	12	12	100
Tetracycline	*tet*B	12	1	8.33
Sulfamethoxazole/Trimethoprim	*Sul*-I	25	14	56
Sulfamethoxazole/Trimethoprim	*Sul*-II	25	18	72

little attention. Moreover, like in humans, the indiscriminate use of antibiotics is widespread among dogs in Bangladesh, which is increasing the risk of antibiotic resistance. Studying antibiotic sensitivity patterns might explore the possible MDR bacteria in dogs, and due to living in close contact with dogs, it may cause problems in humans if they are infected with MDR bacteria from dogs. The findings of the study reveal the common bacterial pathogens circulating in dogs, and also show their extended spectrum of resistance to several antibiotics that are commonly used in therapeutic purposes. The categories of the samples, like a rectal swab, skin swab, and nasal swab, selected for isolating *E. coli*, *Staphylococcus* spp., and *Streptococcus* spp., respectively, were based on the expected availability of the common investigated pathogens, either in a healthy or diseased condition. The isolation frequency of *E. coli*, *Staphylococcus* spp., and *Streptococcus* spp. varied in terms of sources [1,21,22]. The frequency was higher in *E. coli* (78%) than that of *Staphylococcus* spp. (50%) and *Streptococcus* spp. (48%). Similar findings were also stated in a previous study [23].

In dogs, a variety of antimicrobials are used to treat common bacterial infections, including respiratory infection, urinary tract infections, wound infections, ear infections, gastroenteritis, and pyoderma. Resistance to these antimicrobials squeezes the treatment options of the companion animals. Multiple studies have reported that the isolation frequency of resistant bacteria from dogs which were treated for infection were higher than untreated dogs [7,24]. In this study, 3 organisms were subjected to 12 antimicrobials to evaluate their resistance patterns against antimicrobials. The *E. coli* isolates from dogs were found to be highly resistant to ceftriaxone (79%). As ceftriaxone is also a widely used antibiotic in humans, it might be risky for humans in the context of AMR if these *E. coli* can somehow pass into humans by direct contact or from the environment. Higher levels of resistance toward sulfamethoxazole/trimethoprim (64%) and nalidixic acid (67%) were also observed in this study, which is higher than that reported by Chang et al. [26]. In this study, phenotypic resistance was 44% to ampicillin, 41% to cephradine, and 31% to erythromycin, whereas Abdi et al. [25] reported that the resistance to ampicillin, cephradine, ceftriaxone, and erythromycin were 100%, 43%, 46%, and 2%, respectively. Oxytetracycline and doxycycline were found to be 31% and 5% resistant, respectively, in the study, which is lower than that reported by Chang et al. [26].

Rantala et al. [24] reported that the level of sulfamethoxazole/trimethoprim resistance among canine *Staphylococcus* infection varied from country to country. Rantala et al. [24] isolated 57% *Staphylococcus* spp. resistant to sulfamethoxazole/trimethoprim, but in our study, it was 60%. Ceftriaxone (a third-generation cephalosporin) is the most used antimicrobial in canine practice, and it showed 48% resistance in our study, which is lower than the values reported by Punia et al. [27] and Saravanan et al. [28]. About 12% of isolates showed resistance to ampicillin, which was partially higher than other studies [29]. Onwubiko and Sadiq [30] reported 31.2% resistance to penicillin and 31.2% resistance to oxytetracycline in their study, but the values were 48% and 24%, respectively, in the present study.

Of the *Streptococcus* spp. isolates, high resistance was found toward penicillin (63%) and ceftriaxone (54%), which was higher than that in the study conducted by Norton et al. [31]. The resistance to ampicillin, erythromycin, and azithromycin was 38%, 29%, and 4%, respectively, which is lower than the results obtained in an earlier study [32]. In this study, we found that *Streptococcus* spp. were resistant to oxytetracycline (17%) and doxycycline (4%), as reported by Norton et al. [31]

The MDR isolates showed resistance against at least three groups or classes of antimicrobials [20,33]. Overall, 77% of the isolates were found to be MDR. Among them, the highest frequency was found in *E. coli* isolates (90%). Dutta et al. [18] also observed a high percentage of MDR *E. coli* isolates, although it was from livestock origin. On the other hand, MDR *Staphylococcus* spp. and *Streptococcus* spp. isolates were also found in higher frequency. This high MDR frequency is alarming for the prophylaxis treatment of pet animals.

Fecal Gram-negative bacteria are considered as good indicator bacteria for humans and animals, which act as

a reservoir of several AMR genes that could be of zoonotic significance [33]. Several resistance genes, namely bla_{TEM}, bla_{CTX-M}, *tet*A, *tet*B, *Sul*-I, and *Sul*-II were detected in fecal *E. coli* in the present study. All the phenotypically ampicillin-resistant isolates were positive for bla_{TEM} gene in the current study, which is lower than that reported by Costa et al. [34]. Among all *β*-lactamases, ceftriaxone (a third-generation cephalosporin) is frequently used in dogs. The frequency of bla_{CTX-M} gene was 61.29% in this study, whereas Seputiene et al. [35] found a frequency of 96% in Lithuania, 76% in Portugal [36], and 83.18% in Turkey [37]. This variation might be due to the presence of multiple genes of the CTXM group, which are responsible for extended-spectrum *β*-lactamase *E. coli.* Among the phenotypically tetracycline-resistant isolates, 100% isolates were positive for *tet*A gene, whereas 8.33% were positive for *tet*B gene. Although there are several genes, like *tet*A, *tet*B, *tet*C, *tet*D, and *tet*E, that are responsible for tetracycline resistance in *E. coli*, the dominant gene is *tet*A, which verifies the findings of the current study with another study [34]. Simultaneously, among the sulfamethoxazole/trimethoprim resistance genes, like *Sul*-I, *Sul*-II, and *Sul*-III, *Sul*-I is predominant over others for such acquisition of resistance by *E. coli* [38]. However, in this study, the prevalence of *Sul*-II gene is a bit higher than *Sul*-1. This might be due to the random selection during the isolation of the bacteria.

Conclusion

Antibiotic-resistant bacteria have been isolated from dogs, indicating the random use of antibiotics or it may be due to cross-infection from the environment. Therefore, awareness against random and excessive use of antimicrobials in companion animals may help in reducing the spread of MDR bacteria.

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Conflict of interest

The authors have no potential conflict of interest.

Authors' contribution

PD, TD, and PC planned the study. PD and TD executed, analyzed, as well as interpreted the data, and drafted the current manuscript. PD, TD, and CN collected the data and also assisted in the preparation of the manuscript. AA and PC supervised in preparing, drafting, and correcting this manuscript.

References

[1] Rakib TM, Islam MS, Nur-E-Azam M, Islam S, Faruq A Al, Das T, et al. Multidrug resistance pattern of *Salmonella* typhimurium isolated from rectal swabs of stray dogs at Chittagong Metropolitan Area (CMA), Bangladesh. Microbiol Res J Int 2018; 25:1–11; https://doi.org/10.9734/MRJI/2018/43939

[2] Powell L, Edwards KM, McGreevy P, Bauman A, Podberscek A, Neilly B, et al. Companion dog acquisition and mental well-being: a community-based three-arm controlled study. BMC Public Health 2019; 19:1428; https://doi.org/10.1186/s12889-019-7770-5

[3] Rheinwald M, Hartmann K, Hähner M, Wolf G, Straubinger RK, Schulz B. Antibiotic susceptibility of bacterial isolates from 502 dogs with respiratory signs. Vet Rec Open 2015; 176:357–7; https://doi.org/10.1136/vr.102694

[4] Schaufler K, Bethe A, Lübke-Becker A, Ewers C, Kohn B, Wieler LH, et al. Putative connection between zoonotic multiresistant extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in dog feces from a veterinary campus and clinical isolates from dogs. Infect Ecol Epidemiol 2015; 5(1):25334; https://doi.org/10.3402/iee.v5.25334

[5] Rana EA, Das T, Dutta A, Rahman M, Bostami MB, Akter N, et al. Coagulase positive methicillin-resistant *Staphylococcus aureus* circulating in clinical mastitic goats in Bangladesh. Vet World 2020; 13(7):1303–10; https://doi.org/10.14202/vetworld.2020.1303-1310

[6] Lamm CG, Ferguson AC, Lehenbauer TW, Love BC. Streptococcal infection in dogs: a retrospective study of 393 cases. Vet Pathol 2010; 47(3):387–95; https://doi.org/10.1177/0300985809359601

[7] Hartantyo SHP, Chau ML, Fillon L, Ariff AZBM, Kang JSL, Aung KT, Gutiérrez RA. Sick pets as potential reservoirs of antibiotic-resistant bacteria in Singapore. Antimicrob Resist Infect Control 2018; 7:106; https://doi.org/10.1186/s13756-018-0399-9

[8] Lekshmi M, Ammini P, Kumar S, Varela MF. The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. Microorganisms 2017; 5:11; https://doi.org/10.3390/microorganisms5010011

[9] Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis 2013; 13:1057–98; https://doi.org/10.1016/S1473-3099(13)70318-9

[10] Cloeckaert A, Zygmun MS, Doublet B. Editorial: genetics of acquired antimicrobial resistance in animal and zoonotic pathogens. Front Microbiol 2017; 8:2428; https://doi.org/10.3389/fmicb.2017.02428

[11] Hindley KE, Groth AD, King M, Graham K, Billson FM. Bacterial isolates, antimicrobial susceptibility, and clinical characteristics of bacterial keratitis in dogs presenting to referral practice in Australia. Vet Ophthalmol 2016; 19:418–26; https://doi.org/10.1111/vop.12325

[12] Ferri M, Ranucci E, Romagnoli P, Giaccone V. Antimicrobial resistance: a global emerging threat to public health systems. Crit Rev Food Sci Nutr 2017; 57:2857–76; https://doi.org/10.1080/10408398.2015.1077192

[13] World Organisation For Animal Health (OIE: Office International Des Epizooties). OIE Terrestrial Manual. OIE, Paris, France, 2008.

[14] Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, et al. Identification of *Staphylococcus aureus*: DNase and mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob 2010; 9:23; https://doi.org/10.1186/1476-0711-9-23

[15] Virpari PK, Nayak JB, Thaker HC, Brahmbhatt MN. Isolation of pathogenic Escherichia coli from stool samples of diarrhoeal patients with history of raw milk consumption. Vet World 2013; 6(9):659–63; https://doi.org/10.14202/vetworld.2013.659-663

[16] Sarker MS, Ahad A, Ghosh SK, Mannan MS, Sen A, Islam S, et al. Antibiotic-resistant *Escherichia coli* in deer and nearby water sources at Safari parks in Bangladesh. Vet World 2019; 12:1578; https://doi.org/10.14202/VETWORLD.2019.1578-1583

[17] Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, VET08, Clinical and Laboratory Standards Institute, Wayne, PA, 2018.

[18] Dutta A, Islam MZ, Barua H, Rana EA, Jalal MS, Dhar PK, et al. Acquisition of plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* of livestock origin in Bangladesh. Microb Drug Resist 2020; 26(9):1058–62; https://doi.org/10.1089/mdr.2019.0304

[19] R Core Team. R : a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2016.

[20] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18(3):268–81; https://doi.org/10.1111/j.1469-0691.2011.03570.x

[21] Yehualaeshet T, Edmonds-Wiggins G, Jones C, Graham M, Dillard K. Bacterial microbiome of nasal swabs from healthy dogs and their owners: bacterial diversity, intra-and interspecies interface. Int J Vet Anim Med 2019; 2:120; https://doi.org/10.31021/ijvam.20192120

[22] Greetham HL, Giffard C, Hutson RA, Collins MD, Gibson GR. Bacteriology of the labrador dog gut: a cultural and genotypic approach. J Appl Microbiol 2002; 93:640–6; https://doi.org/10.1046/j.1365-2672.2002.01724.x

[23] Wong C, Epstein SE, Westropp JL. Antimicrobial susceptibility patterns in urinary tract infections in dogs (2010–2013). J Vet Intern Med 2015; 29:1045–52; https://doi.org/10.1111/jvim.13571

[24] Rantala M, Lahti E, Kuhalampi J, Pesonen S, Järvinen AK, Saijonmaa-Koulumies L, et al. Antimicrobial resistance in *Staphylococcus spp.*, *Escherichia coli* and *Enterococcus spp.* in dogs given antibiotics for chronic dermatological disorders, compared with non-treated control dogs. Acta Vet Scand 2004; 45:37; https://doi.org/10.1186/1751-0147-45-37

[26] Chang SK, Lo DY, Wei HW, Kuo HC. Antimicrobial resistance of *Escherichia coli* isolates fromcanine urinary tract infections. J Vet Med Sci 2015; 77:59; https://doi.org/10.1292/JVMS.13-0281

[25] Abdi S, Ranjbar R, Vala MH, Jonaidi N, Bejestany OB, Bejestany FB. Frequency of bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and qnrA among *Escherichia coli* isolated from urinary tract infection. Arch Clin Infect Dis 2014; 9: e18690; https://doi.org/10.5812/archcid.18690

[27] Punia M, Kumar A, Charaya G, Kumar T. Pathogens isolated from clinical cases of urinary tract infection in dogs and their antibiogram. Vet World 2018; 11:1037; https://doi.org/10.14202/VETWORLD.2018.1037-1042

[28] Saravanan M, Nanda A, Tesfaye T. Antibiotic susceptibility patterns of methicillin resistant *Staphylococcus aureus* from septicaemia suspected children in tertiary hospital in Hosur, South India. Am J Microbiol Res 2013; 1:21–4; https://doi.org/10.12691/ajmr-1-2-2

[29] Tsering DC, Pal R, Kar S. Methicillin-resistant *Staphylococcus aureus*: prevalence and current susceptibility pattern in sikkim. J Glob Infect Dis 2011; 3:9–13; https://doi.org/10.4103/0974-777X.77289

[30] Onwubiko N, Sadiq N. Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. Pan Afr Med J 2011; 8:4; https://doi.org/10.4314/pamj.v8i1.71050

[31] Norton R, Smith HV, Wood N, Siegbrecht E, Ross A, Keethesan N. Invasive group A streptococcal disease in North Queensland (1996–2001). Indian J Med Res 2004; 119:148–51; https://icmr.nic.in/ijmr/ijmr_supp/31.pdf

[32] Pilegi Sfaciotte RA, Bordin JT, Capoia Vignoto VK, Munhoz PM, Pinto AA, Baptista Barbosa MJ, et al. Antimicrobial resistance in bacterial pathogens of canine otitis. Am J Anim Vet Sci 2015; 10:162–9; https://doi.org/10.3844/ajavsp.2015.162.169

[33] Li X, Atwill ER, Antaki E, Applegate O, Bergamaschi B, Bond RF, et al. Fecal indicator and pathogenic bacteria and their antibiotic resistance in alluvial groundwater of an irrigated agricultural region with dairies. J Environ Qual 2015; 44:1435–47; https://doi.org/10.2134/jeq2015.03.0139

[34] Costa D, Poeta P, Sáenz Y, Coelho AC, Matos M, Vinué L, et al. Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. Vet Microbiol 2008; 127:97–105; https://doi.org/10.1016/J.VETMIC.2007.08.004

[35] Seputiene V, Linkevicius M, Bogdaite A, Povilonis J, Planciuniene R, Giedraitiene A, et al. Molecular characterization of extended-spectrum-lactamase-producing *Escherichia coli* and Klebsiella pneumoniae isolates from hospitals in Lithuania. J Med Microbiol 2010; 59:1263–5; https://doi.org/10.1099/jmm.0.021972-0

[36] Mendonça N, Leitão J, Manageiro V, Ferreira E, Caniça M. Spread of extended-spectrum beta-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother 2007; 51:1946–55; https://doi.org/10.1128/AAC.01412-06

[37] Copur Cicek A, Saral A, Ozad Duzgun A, Yasar E, Cizmeci Z, Ozlem Balci P, et al. Nationwide study of *Escherichia coli* producing extended-spectrum β-lactamases TEM, SHV and CTX-M in Turkey. J Antibiot 2013; 66:647–50; https://doi.org/10.1038/ja.2013.72

[38] Phuong Hoa PT, Nonaka L, Hung VP, Suzuki S. Detection of the *Sul1, Sul2*, and *Sul3* genes in sulfonamide-resistant bacteria from wastewater and shrimp ponds of north Vietnam. *Sci Total Environ* 2008; 405:377–84; https://doi.org/10.1016/j.scitotenv.2008.06.023

[39] Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother 2005; 56:115–21; https://doi.org/10.1093/jac/dki190

[40] Feizabadi MM, Delfani S, Raji N, Majnooni A, Aligholi M, Shahcheraghi F, et al. Distribution of bla_{TEM} , bla_{SHV} , bla_{CTX-M} genes among clinical isolates of *Klebsiella pneumoniae* at labbafinejad hospital, Tehran, Iran. Microb. Drug Resist 2010; 16:49–53; https://doi.org/10.1089/mdr.2009.0096

[41] Lanz R, Kuhnert P, Boerlin P. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet Microbiol 2003; 91:73–84; https://doi.org/10.1016/S0378-1135(02)00263-8