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SHORT COMMUNICATION

Isolation and identification of multidrug-resistant *Escherichia coli* from cattle, sheep, poultry and human in Cumilla, Bangladesh

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ABSTRACT

Aims: Antimicrobial resistance (AMR) is a significant public health concern of modern civilization. The potential risk of AMR is significant in terms of both human and animal health. This study aims to assess the antimicrobial resistance pattern of selected antimicrobials against *Escherichia coli* of animal, poultry and human origin in the Cumilla district of Bangladesh.
Methodology and results: A total of 200 samples were collected from different sources. Isolation and identification of commensal *E. coli* were performed following standard bacteriological and molecular techniques. Antimicrobial susceptibility testing was performed following the Kirby-Bauer disc diffusion technique. Ampicillin, tetracycline and sulfamethoxazole-trimethoprim resistance genes were detected by polymerase chain reactions (PCR). A total of 152 (76%; 95% confidence interval (CI) 70-81%) *E. coli* were isolated from cattle, sheep, chicken and human, where 37.5% of isolates were found to be multidrug-resistant (MDR). In the cultural sensitivity test, *E. coli* showed the highest resistance to sulfamethoxazole-trimethoprim (71%), tetracycline (63%), ampicillin (62%), where gentamicin (23%) showed the lowest resistance, followed by ceftriaxone (26%). The prevalence of resistance genes like *bla*$_{TEM}$, *tetA*, *tetB*, *tetC*, *sul1* and *sul2* were 100%, 95%, 11%, 8%, 58% and 52%, respectively.
Conclusion, significance and impact of study: The emergence of multidrug-resistant commensal *E. coli* and resistance genes circulating in animals, poultry and humans limit the treatment options for serious infections.

Keywords: Commensal *E. coli*, multidrug-resistant, resistance gene, animal, human

INTRODUCTION

Antimicrobial resistance (AMR) in humans and animals is a global public health burden. The emergence and dissemination of multidrug-resistant bacteria is a major concern for human and veterinary medicine. *Escherichia coli* (*E. coli*) is a commensal organism that resides in the gastrointestinal tract of warm-blooded animals (Gupta *et al.*, 2017). It is worrying when commensal organisms develop resistance against important antimicrobial agents used in humans and animals. This resistance is caused by the extensive usage of antimicrobials (Okeke *et al.*, 1999). Bacteria are developing resistance against antimicrobials through horizontal gene transfer mechanisms (Hughes and Andersson, 2015). Several investigations have found that antimicrobials used in human medicine for treatment are being misapplied in animal production for therapeutic and prophylactic purposes (Abbassi *et al.*, 2017). The most common antimicrobials such as β-lactams, fluoroquinolones,

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aminoglycosides and sulfamethoxazole-trimethoprim are used in food-producing animals as well as in human medicine in Bangladesh (Chowdhury *et al.*, 2009; Marshall and Levy, 2011). The widespread scenario of antimicrobial resistance is due to the indiscriminate use of antimicrobials in food-producing animals in developing countries like Bangladesh (Deb *et al.*, 2020). Bangladesh is a densely populated country with diverse livestock populations such as cattle, sheep, goats, poultry, etc. Marginal farmers generally live close to animals. Farmers rely on unqualified individuals (known as quack) to treat their animals and use antimicrobials indiscriminately due to a lack of understanding and the absence of qualified veterinarians, resulting in antimicrobial resistance (Al Amin *et al.*, 2020). In Bangladesh, about 94.16% of poultry farmers use antibiotics in their farms for treatment, prevention of diseases and growth promotion, collected from direct feed dealers or suppliers (Hosain *et al.*, 2021). It is noteworthy that more than 60% of farmers use antibiotics collected from feed dealers or suppliers without veterinarians' prescription (Al Masud *et al.*, 2020).

Multidrug-resistant commensal *E. coli* may not directly cause diseases but serve as a reservoir for several drug resistance genes that may be disseminated to human and zoonotic pathogens (Aworh *et al.*, 2019). The production of β-lactamase enzymes, which hydrolyze β-lactam antibiotics, is one of the most common resistance mechanisms identified in members of the Enterobacteriaceae family (Parvin *et al.*, 2020). β-lactam producing *E. coli* were reported in chicken (Hasan *et al.*, 2011; Parvin *et al.*, 2020), cattle (Enne *et al.*, 2008), sheep (Enne *et al.*, 2008) and human (Briñas *et al.*, 2002). Besides that, tetracycline and sulfamethoxazole-trimethoprim are commonly used in Bangladesh for food-producing animals (Hosain *et al.*, 2021). Target-mediated resistance represents the most common and clinically most significant form (Aldred *et al.*, 2014). Among the known forty tetracycline resistance genes, five genes, namely *tetA*, *tetB*, *tetC*, *tetD* and *tetE* are responsible for tetracycline resistance in *E. coli* (Koo and Woo, 2011). Resistance can be acquired through the efflux protein mechanism, ribosomal protection protein mechanism or enzyme inactivation (Koo and Woo, 2011). In addition, sulfamethoxazole is used with a combination of trimethoprim to treat urinary tract infections in humans. Three resistance genes such as *sul1*, *sul2* and *sul3* were discovered to detect sulfamethoxazole-trimethoprim resistant *E. coli* (Teichmann *et al.*, 2014). These resistance genes could be incorporated into members of the multidrug-resistant enterobacteriaceae family by genetic recombination, severely limiting the current treatment options.

In this study, we have isolated and identified *E. coli* from cattle, sheep, poultry and humans in the Cumilla district of Bangladesh. Our study aimed to assess the antimicrobial resistance phenotype with their resistance genes of commonly used antimicrobials harbored in multidrug-resistant *E. coli* strains circulating in animals, poultry and humans in the Cumilla district of Bangladesh. This study will assist the local physicians, dentists and veterinarians to have information on circulating AMR in the Cumilla region which will lead to better choice of suitable antibiotics for treating diseases.

MATERIALS AND METHODS

Study population and sampling

A cross-sectional study on the proportion of AMR among different animal populations was performed between October 2020 and January 2021 in the Cumilla district of Bangladesh. A total of 200 samples were collected from cattle (n=50), sheep (n=50), chicken (n=50) and human (n=50). Animal and chicken samples were collected from randomly selected farms of Cumilla district and human samples were collected from a hospital namely Eastern Medical college and Hospital, Cumilla. Rectal/cloacal samples from humans and animals were taken by gently stroking the mucosal lining with a sterile cotton swab inserted into the anus/cloaca. Sample was taken in Stuart's transport medium (Oxoid, Basingstoke, UK) and immediately transferred to the Department of Microbiology and Veterinary Public Health Laboratory, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, and stored at –80 °C for further use. All the experiments were conducted following the rules outlined by the ethical committee of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Registered veterinarians and physicians performed all assessments and sample collections from each sampling location. Before sampling, study participants (patients and farmers) were informed about the aim of the research; participation was voluntary.

Isolation and identification of *E. coli*

For isolation of *E. coli*, samples were pre-enriched with buffered peptone water overnight at 37 °C. A loopful sample was inoculated onto MacConkey agar (Oxoid, Basingstoke, UK) plate and incubated at 37 °C for 24 h. An isolated large pink color colony was inoculated onto eosin-methylene blue agar (EMB) (Oxoid, Basingstoke, UK) for biochemical confirmation. Typical metallic sheen colonies were subcultured into blood agar and preserved at –80 °C in brain heart infusion broth (Oxoid, Basingstoke, UK) with 50% glycerin for further use.

Genomic DNA extraction

The genomic DNA was extracted following the crude boiling method. In brief, 2-3 fresh colonies were suspended in a sterile 1.5 mL microcentrifuge tube containing 200 µL sterile Milli-Q water and vortexed thoroughly. Heating the microcentrifuge tube at 99 °C for 10 min, followed by rapid freezing at –20 °C for 5 min and centrifuged at 12,000 rpm for 5 min. Finally, 100 µL of the supernatant was collected and used as the DNA template followed by storing at –20 °C for further use.

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Table 1: Oligonucleotide primer sequences used to detect resistance genes.

Gene	Primer Name	Primer sequence (5′- 3′)	Amplicon size (bp)	Reference
bla$_{TEM}$	*bla*$_{TEM}$-F	GCGGAACCCCTATTTG	964	Hasman *et al.* (2005)
	bla$_{TEM}$-R	TCTAAAGTATATATGAGTAAACTTGGTCTGAC		
sul1	*sul1*-F	GTGACGGTGTTCGGCATTCT	779	Lanz *et al.* (2003)
	sul1-R	TCCGAGAAGGTGATTGCGCT		
sul2	*sul2*-F	CGGCATCGTCAACATAACCT	721	
	sul2-R	TGTGCGGATGAAGTCAGCTC		
tetA	*tetA*-F	CGCCTTTCCTTTGGGTTCTCTATATC	182	Koo and Woo (2011)
	tetA-R	CAGCCCACCGAGCACAGG		
tetB	*tetB*-F	GCCAGTCTTGCCAACGTTAT	975	
	tetB-R	ATAACACCGGTTGCATTGGT		
tetC	*tetC*-F	TTCAACCCAGTCAGCTCCTT	560	
	tetC-R	GGGAGGCAGACAAGGTATAGG		
tetD	*tetD*-F	GAGCGTACCGCCTGGTTC	780	
	tetD-R	TCTGATCAGCAGACAGATTGC		

Table 2: Thermal cyclic conditions used to detect resistance genes.

Gene name	*bla*$_{TEM}$	*tetA*, *tetB*, *tetC*, *tetD*	*sul1*	*sul2*
Initial denaturation	94 °C for 3 min	94 °C for 5 min	95 °C for 5 min	94 °C for 4 min
Cyclic denaturation	94 °C for 1 min	94 °C for 30 sec	95 °C for 1 min	94 °C for 1 min
Cyclic annealing	50 °C for 1 min	55 °C for 30 sec	68 °C for 1 min	66 °C for 1 min
Cyclic extension	72 °C for 1 min	72 °C for 30 sec	72 °C for 1 min	72 °C for 1 min
Final extension	72 °C for 10 min	72 °C for 5 min	72 °C for 10 min	72 °C for 7 min
Cycle number	25	35	35	35
References	Hasman *et al.* (2005)	Koo and Woo (2011)	Lanz *et al.* (2003)	Lanz *et al.* (2003)

Molecular identification of *E. coli*

The molecular identification of *E. coli* was performed using primers for the *uidA* gene (F: TATGGAATTTCGCCGATTTT; R: TGTTTGCCTCCCTGCTGCGG) and flanking region of the *uspA* gene (F: CCGATACGCTGCCAATCAGT; R: ACGCAGACCGTAGGCCAGAT) by species-specific multiplex PCR (Godambe *et al.*, 2017). Amplification was done with 15 µL reaction volume with characteristic 884 bp for *uspA*, 164 bp for *uidA* and maintaining initial denaturation at 94 °C for 5 min and final extension at 72 °C for 10 min with the 35 cycles of denaturation at 94 °C for 10 sec, annealing at 55.2 °C for 10 sec and extension at 72 °C for 1 min (Godambe *et al.*, 2017).

Antimicrobial susceptibility testing of *E. coli* isolates

The Kirby-Bauer disk diffusion technique was performed to assess the antimicrobial susceptibility of all positive isolates against 8 antimicrobial substances from seven different groups (Bauer *et al.*, 1966). The antimicrobials used were ampicillin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), sulfamethoxazole-trimethoprim (23.75 + 1.25 µg), tetracycline (30 µg) and enrofloxacin (5 µg) (Oxoid, Basingstoke, UK). The *E. coli* ATCC 25922 was used for quality control during the disk diffusion technique. The results of susceptibility testing were interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2020).

Detection antimicrobial resistance genes

All positive *E. coli* isolates which resistant to tetracycline were tested, for the presence of *tetA*, *tetB*, *tetC* and *tetD* genes, those resistant to ampicillin for *bla*$_{TEM}$ gene and sulfamethoxazole-trimethoprim resistant ones for *sul1* and *sul2* genes by PCR using the set of specific oligonucleotide primers for each gene described in Table 1. The specific thermal cyclic conditions for all genes were illustrated in Table 2. The reaction volume (15 µL) consisted of 7.5 µL one Taq master mix (New England Biolab Inc., USA), forward and reverse primer (0.5 µL), DNA (1 µL) and nuclease-free water was added in required amount. After amplification in a thermocycler (DLAB scientific, USA), the PCR product was loaded in 1.5% agarose gel (SeaKem® LE Agarose from Lonza) containing ethidium bromide (Sigma-Aldrich, USA) and visualized in a gel documentation system (UVP UVsolo touch-Analytik Jena AG) after gel electrophoresis. The pan-susceptible *E. coli* (ATCC 25922) was used as a negative control during each PCR.

Statistical analysis

The prevalence and 95% confidence interval were analyzed using the modified Wald method in Graph Pad software QuickCalcs (www.graphpad.com/quickcalcs/). Considering the presence of *E. coli* as the target outcome, univariable logistic regression analysis was performed using the “R” program (version 3.5.1) ($P \leq 0.05$ was considered as statistically significant).

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RESULTS

Prevalence of *E. coli*

Out of 200 samples investigated, 152 (76%; 95% confidence interval (CI) 70-81%) samples were identified as *E. coli*. The prevalence of *E. coli* in cattle, sheep, poultry and humans were 72%, 80%, 78% and 74%, respectively (Table 3). There were no significant differences in the distribution *E. coli* isolated from diverse sources (p=0.78).

Antimicrobial resistance pattern of the isolates

The AMR profiles are summarized in Table 4. Overall, the isolates showed highest resistance to sulfamethoxazole-trimethoprim (71%) followed by tetracycline (63%) where ceftriaxone (26%) and gentamicin (23%) showed lower resistance compared to other antimicrobials tested in the study. In cattle, highest resistance were found toward sulfamethoxazole-trimethoprim (72%), tetracycline (64%) and ampicillin (61%) followed by ceftriaxone (39%) and gentamicin (19%) where ciprofloxacin and enrofloxacin (17%) showed lowest resistance. On the other hand, ciprofloxacin and enrofloxacin (85%) showed highest resistance in chicken followed by ampicillin (77%), tetracycline (74%) and sulfamethoxazole-trimethoprim (69%). Besides, in sheep and human, 73% and 70% isolates showed resistance to sulfamethoxazole-trimethoprim, respectively. Furthermore, 37.5% of isolates were found to be multi-drug resistant (MDR) (resistance to ≥3 antimicrobial groups), with 30% showed resistance to three antimicrobial groups, 21% to four antimicrobial groups, and 6% to five antimicrobial groups (Figure 1). Six antimicrobial groups exhibited no resistance in any of the isolates as depicted in Figure 1.

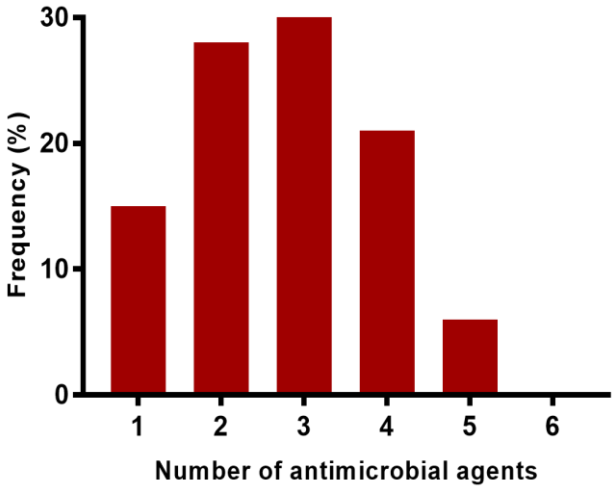

Figure 1: Multidrug resistance (MDR) pattern of *E. coli* isolates from different sources.

Detection of antimicrobial-resistant genes

The amplicons showing the resistance genes in the *E. coli* isolates were illustrated in Figure 2. *Bla*$_{TEM}$ gene was found in all phenotypically ampicillin-resistant isolates (n=94). In the phenotypically tetracycline-resistant isolates (n=95), 95% isolates carried the *tetA* gene followed by *tetB* (11%) and *tetC* (8%). None of the isolates carried the *tetD* gene. In addition, 58% of isolates were harboring *sul1* gene followed by *sul2* gene (52%) among all phenotypically sulfamethoxazole-trimethoprim resistant isolates (n=104). The overview of resistance genes are shown in Table 3.

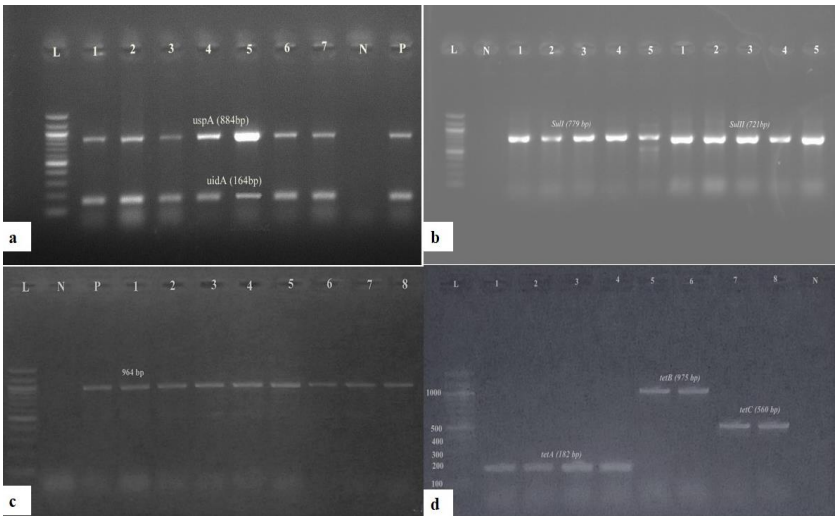

Figure 2: Result of PCR assay for the detection of species specific gene and resistance genes of *E. coli* isolates. a) species specific *uidA* and *uspA* gene, b) *sul 1* and *sul 2* genes, c) *bla*$_{TEM}$ gene, d) *tetA*, *tetB* and *tetC* genes. L: DNA ladder (100 bp); P: Positive control (previously isolated *E. coli* strain); N: Negative control (*E. coli* ATCC 25922). But in case of picture a, *E. coli* ATCC 25922 used as a positive control and nuclease free water used as negative control.

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Table 3: Prevalence of antimicrobial resistance with their resistance genes from different sources.

Source sample (N)	*E. coli* (%)	*P*-value	Ampicillin resistant (%)	Ampicillin resistant gene	Sulphar drug resistant (%)	Sulphar drug resistant gene		Tetracycline resistant (%)	Tetracycline resistant genes		
				*bla*TEM (%)		*sul1* (%)	*sul2* (%)		*tetA* (%)	*tetB* (%)	*tetC* (%)
Cattle (50)	36(72)	0.78	22(61)	22(100)	26(72)	14(54)	13(50)	23(64)	21(91)	3(13)	2(9)
Sheep (50)	40(80)		24(60)	24(100)	29(73)	17(59)	15(52)	24(60)	22(92)	2(8)	1(5)
Broiler (50)	39(78)		30(77)	30(100)	27(69)	16(59)	15(52)	29(74)	29(100)	5(17)	5(17)
Human (50)	37(74)		18(49)	18(100)	26(70)	13(50)	11(42)	19(51)	18(95)	0	0
Total (200)	152(76)		94(61.8)	94(100)	104(68)	60(58)	54(52)	95(63)	90(95)	10(11)	8(8)

Table 4: Antibiogram profiles of *E. coli* isolated from cattle, sheep, broiler and human.

Antimicrobials	Cattle (n=36)			Sheep (n=40)			Broiler (n=39)			Human (n=37)			Total (n=152)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin	9 (25)	5 (14)	22 (61)	10 (25)	6 (15)	24 (60)	7 (18)	2 (5)	30 (77)	15 (40)	4 (11)	18 (49)	41 (27)	17 (11)	94 (62)
Ciprofloxacin	23 (64)	7 (19)	6 (17)	31 (78)	4 (10)	5 (12)	6 (15)	0 (0)	33 (85)	33 (89)	0 (0)	4 (11)	93 (61)	11 (7)	48 (32)
Enrofloxacin	23 (64)	7 (19)	6 (17)	31 (78)	4 (10)	5 (12)	6 (15)	0 (0)	33 (85)	33 (89)	0 (0)	4 (11)	93 (61)	11 (7)	48 (32)
Sulfamethoxazole-trimethoprim	6 (17)	4 (11)	26 (72)	11 (27)	0 (0)	29 (73)	12 (31)	0 (0)	27 (69)	10 (27)	1 (3)	26 (70)	39 (26)	5 (3)	108 (71)
Ceftriaxone	22 (61)	0 (0)	14 (39)	35 (88)	0 (0)	5 (12)	27 (69)	0 (0)	12 (31)	29 (78)	0 (0)	8 (22)	113 (74)	0 (0)	39 (26)
Gentamicin	29 (81)	0 (0)	7 (19)	32 (80)	0 (0)	8 (20)	29 (74)	0 (0)	10 (26)	27 (73)	0 (0)	10 (27)	117 (77)	0 (0)	35 (23)
Tetracycline	9 (25)	4 (11)	23 (64)	12 (30)	4 (10)	24 (60)	5 (13)	5 (13)	29 (74)	18 (49)	0 (0)	19 (51)	44 (29)	9 (13)	95 (63)

DISCUSSION

Antimicrobial resistance is a global public health concern and livestock plays a vital role in this regard. It is alarming when commensal bacteria develop resistance against important antimicrobial agents and disseminate through the microbial community. Our study was designed to assess the antimicrobial resistance spectrum in commensal *E. coli* isolated from livestock and humans in the Cumilla region, Bangladesh. In this study, the prevalence of *E. coli* was 76% higher than the previous records in Bangladesh (Akond *et al.*, 2009; Dutta *et al.*, 2020), where 37.5% of *E. coli* were found to be MDR. However, Dutta *et al.* (2020) reported 70.9% MDR *E. coli* in chicken, animals and humans from the Chattogram district of Bangladesh. This emergence of MDR in the study area might be due to extensive use of antimicrobials in animals and as well as in humans. Here,

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we recovered higher *E. coli* from sheep (80%, n=40) than chicken (78%, n=39). A similar study from Qatar reported a higher recovery rate of *E. coli* from sheep (84.2%, n=144) than poultry (Eltai *et al.*, 2020). Besides, the prevalence of *E. coli* was higher in cattle (72%, n=36) and human (74%, n=37), but a previous study from Bangladesh reported lower prevalence in cattle, human and poultry (Gupta *et al.*, 2017; Mamun *et al.*, 2017; Dutta *et al.*, 2020). Our study aimed to show the resistance patterns of three important antimicrobials such as ampicillin, tetracycline and sulfamethoxazole-trimethoprim in *E. coli*.

In this study, all the isolates showed resistance to routinely used antimicrobials. Chicken isolates showed the highest resistance to ampicillin (77%), followed by isolates from cattle (61%), sheep (60%) and humans (49%), respectively. However, sheep isolates showed high resistance to sulfamethoxazole-trimethoprim (73%), followed by isolates from cattle (72%), chicken (69%) and humans (70%). Besides, tetracycline is a vital drug that usually used alone or in combination with other drugs for the treatment and prophylaxis of many infectious diseases of humans. Alarmingly, this antimicrobial is developed resistance against organisms. In this study, the prevalence of tetracycline-resistant *E. coli* was higher in chicken (74%) followed by cattle (64%), sheep (60%) and humans (51%), respectively. Similar studies reported lower resistance of ampicillin, sulfamethoxazole-trimethoprim and tetracycline in cattle, sheep and poultry (Roug *et al.*, 2013; Bessalah *et al.*, 2020). Fluoroquinolones are a drug of choice for humans against Gram-negative bacterial infection. The prevalence of ciprofloxacin-resistant *E. coli* was higher in chicken (85%) followed by cattle (17%), sheep (13%) and human (11%) in our study. However, Sahm *et al.* (2001) reported that the prevalence was slightly higher (38.8%) in human isolates.

Ceftriaxone (74%) and gentamicin (77%) were exhibited the highest sensitivity against *E. coli* in this study, but a similar study reported 100% sensitivity to ceftriaxone and gentamicin (Bessalah *et al.*, 2020). The differences in *E. coli* distribution and resistance patterns could be attributed to exposure to different antimicrobial agents as a result of differences in the husbandry practices or to other factors such animal feed, different environmental conditions might be the possible contributors (Sayah *et al.*, 2005). Fecal of Gram-negative bacteria is a good indicator bacterium that act as reservoirs of several antimicrobial resistance genes (Li *et al.*, 2015).

Several resistance genes for ampicillin (bla_{TEM}), tetracycline (*tetA*, *tetB*, *tetC*) and sulfamethoxazole-trimethoprim (*sul1*, *sul2*) were detected in *E. coli* isolated from cattle, sheep, chicken and human (Table 3). All ampicillin-resistant isolates (100%) harbor bla_{TEM} gene in our study, but Adelowo *et al.* (2014) reported a prevalence of 85% from poultry, 64.5% from cattle, sheep and pig (Enne *et al.*, 2008), 91% from cattle, poultry and pig (Olesen *et al.*, 2004), 83% from broiler, pig and human (Briñas *et al.*, 2002). Among all phenotypically tetracycline-resistant *E. coli* isolates, 95% harbored *tetA* genes, followed by *tetB* (11%) and *tetC* (8%). In contrast, Bryan *et al.* (2004) reported 63% *tetB* gene followed by *tetA* gene (35%) from animals, poultry and human. Several *tet* genes are responsible for tetracycline resistance, the tetA gene was found to be dominant (Deb *et al.*, 2020) which supports the findings of this study. Simultaneously, among sulfamethoxazole-trimethoprim resistant genes, 58% of isolates were positive for *sul1* gene followed by *sul2* gene (52%). Despite the fact that most study indicated the *sul* gene frequency pattern in *E. coli* as *sul2>sul1* (Lanz *et al*., 2003; Deb *et al.*, 2020), *sul 1* was more frequent in this study. It could be due to the bacteria were chosen at random during isolation.

There are some limitations to this study. First and foremost, this research was carried out in a specific geographical region of Bangladesh. A large-scale study covering the entire country would provide a more detailed picture of AMR circulating in one health environment. Furthermore, whole-genome sequencing would allow us to study the genetic diversity of *E. coli*.

CONCLUSION

In this study, 76% commensal *E. coli* was isolated from animal, poultry and human, where 37.5% of isolates were found to be MDR. Our findings showed high resistance rate to tetracycline, sulfamethoxazole-trimethoprim and ampicillin in *E. coli* strains, indicating the uncontrolled use of antimicrobials in animals and humans. One health approach should be considered to tackle the issue and necessary steps have been taken to disseminate the findings among different stakeholders including veterinarians, physicians and dentists as well as planners from both the Department of Livestock and the Ministry of Health. Therefore, it is essential to develop awareness against the extensive use of antimicrobials in poultry and humans to reduce dissemination of MDR bacteria.

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