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Research article

Assessment of the presence of multidrug-resistant *Escherichia coli*, *Salmonella* and *Staphylococcus* in chicken meat, eggs and faeces in Mymensingh division of Bangladesh

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ABSTRACT

The emergence of bacteria that is resistant to several drugs of clinical importance poses a threat to successful treatment, a phenomenon known as multidrug resistance that affects diverse classes of antibiotics. The purpose of this study was to evaluate the prevalence of multidrug-resistant *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* in chicken egg, meat and faeces from four districts of Bangladesh. A total of 120 chicken samples were collected from different poultry farms. Conventional culture and molecular detection methods were used for identification of bacterial isolates from the collected samples followed by antibiotic susceptibility test through the disc diffusion method, finally antibiotic resistant genes were detected by PCR. *E.* coli, *Salmonella* spp. and *Staphylococcus aureus* were detected in meat, egg and faecal samples. Antimicrobial susceptibility results revealed isolates from faeces were 100 % resistant to amoxicillin, while all *S. aureus* and *Salmonella* sp. from faeces were resistant to doxycycline, tetracycline and erythromycin. *Salmonella* spp. isolates from eggs indicated 100 % resistance to erythromycin, amoxycillin, while *E. coli* were 100 % resistant to erythromycin. *E. coli* and *S. aureus* from meat were 100 % resistant to amoxicillin and erythromycin. However, *Salmonella* spp. from eggs were 100 % susceptible to doxycycline, gentamicin, levofloxacin and tetracycline. The *mecA* and *aac(3)-IV* genes were only found in *S. aureus* and *E. coli*, respectively. The *Sul1, tetB*, and *aadA1* were highest in *Salmonella* spp. and *S. aureus,* while the *sul1, tet*A and bla_{SHV} were higher in *E. coli*. Isolates from all samples were multidrug resistant. These findings indicate a high risk of transmission of resistance genes from microbial contamination to food of animal origin. The study emphasizes the need for effective biosecurity measures, responsible antibiotic use, and strict regulations in poultry production to prevent the spread of antibiotic resistance.

1. Introduction

Large amounts of antimicrobials are used to prevent, treat diseases, and serve as growth promoters. This has led to the

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indiscriminate use of antibiotics in poultry production. Nearly, all the antibiotic classes that are important for human medicine are extensively used in livestock production, not only as therapeutic agents but also for prevention purpose [1]. Foodborne resistant pathogens, particularly those originating from poultry, pose a significant risk to human health as a result of the abuse of antibiotics in the animal production sector [2]. These antibiotics can remain in the food chain and help to develop resistant microbes that provide enabling environment for transmission of resistance factors. These bacteria thrive well in the tissues and proteins we consume, and they easily gain entry into our body from contamination. Antimicrobial residues may not always degrade appreciably, even after conventional cooking procedures [3]. As a result, drug metabolites can cause bone marrow toxicity, allergy and mutagenicity [4]. The potential for drug residues to persist in soil or drainage water, thus contributing to the development of microbial resistance is a cause for concern [5]. More serious is the ability of the drug residues to remain in the soil or drainage water and help to develop microbial resistance in the agricultural chain [6]. These resistant bacteria have become difficult to treat leading to a global health challenge. The different ways that bacteria become resistant are by intrinsic or acquired means, prevention of access to target site, inactivation of the antibiotic and change in target structure [7].

Poultry meat and eggs are a major source of dietary protein and earnings in middle- and low-income countries around the world. Bangladesh is no exception as meat and eggs are cheap sources of protein that are accepted by many faiths and cultures [8]. However, the quest to provide affordable and profitable sources of protein, such as those from poultry, comes with a great risk because of the use of antibiotics in the production chain. The meat, eggs, and faeces from poultry can be a source of multidrug resistant *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*, which are among the bacteria frequently implicated in foodborne illnesses. These three bacteria are known to be associated with the contamination of poultry sourced-proteins and bi products like faeces [9]. They can be transmitted from animal sourced food to human hence zoonotic in nature. The selection pressure to develop resistance in commensal bacteria like *E. coli*, *Salmonella* spp. and *Staphylococcus* from poultry is high, thus increasing the burden of antimicrobial resistance [10,11]. These bacteria are part of the normal human flora that can harbor resistance genes or plasmids which can migrate and affect both human and animals, by causing drug-resistance [12].

E. coli and *Staphylococcus* are present in the alimentary system of man and animals as commensals but they can cause infections [13]. *E. coli* can have detrimental effects on poultry health causing diseases like colibacillosis, meningitis, diarrhoea, septicaemia, etc. that could lead to economic losses [14]. Although most strains do not cause problem, nearly 15 % of them can be pathogenic [15]. The pathogenic *E. coli* is divided into enterohemorrhagic, enterotoxigenic, enteropathogenic, enteroaggregative, and enteroinvasive categories based on the mechanism of illness manifestation [16]. Furthermore, commensals, intraintestinally pathogenic, and extraintestinally pathogenic *E. coli* are the three primary types of *E. coli* based on virulence determinants. Certain virulence factors, such as the synthesis of toxins, hemolysins, siderophores, proteases, and adhesions, are unique to each pathotype and are important in the development of the disease [17]. The primary cause of multidrug resistance (MDR) in *E. coli* is the presence of antimicrobial resistance genes, including bla_{TEM}, $bla_{\text{CTX-M}}$, *tet*A and *tet*B, *qnr*A, *qnr*B, and *qnr*S, and *sul*1 [18].

Staphylococcus can also be pathogenic in both man and animal under favourable conditions [19,20]. Certain strains of *S. aureus* produce enterotoxins that cause food poisoning. Antimicrobial resistance (AMR) is primarily caused by a mutated penicillin-binding protein (PBP2a), which is encoded by the *mecA* gene [21,22]. *S. aureus* has a variety of virulence factors, including extracellular toxins [23]. Among these, enterotoxins are the source of food poisoning, while other toxins can produce a wide range of clinical symptoms in both humans and animals. When humans eat contaminated food, enterotoxins, which are thermostable, result in food poisoning [24]. The third most common cause of foodborne diseases worldwide is *S. aureus* and its enterotoxins [25]. It is capable of colonizing and surviving in various media, inanimate objects, and environments [26]. A major contributor to pathogenicity are virulence factors, which include adhesins and surface proteins including proteins A, β-hemolysin (Hlb), and *Staphylococcal enterotoxins* (SEs) [27].

Salmonellosis is one of the most deadly bacterial illnesses in the poultry industry, causing considerable financial losses from mortality and decreased productivity [28,29]. Almost all known *Salmonellas* serovars can cause illness in animals and man alike [30]. The two most frequent serovars of *Salmonella enterica* that cause *salmonellosis* are Enteritidis and Typhimurium. A serovar known as infantis affects people globally. Each year, *salmonella* infections affect around 20 million people and animals. It results in 150,000 animal and human deaths annually and lowers animal productivity. Infection with typhoid fever is more prevalent in south-central and south-east Asia [31]. Therefore, *Salmonella* is probably the most prevalent food-borne pathogen that is known to cause around 155,500 deaths worldwide annually [32]. Poultry meat is reported to be one of the major sources of this food borne pathogen [33,34].

AMR now poses a serious worldwide threat since it affects every aspect of public health [35]. By 2050, it is predicted that the AMR issue will result in hundreds of millions of human deaths worldwide, a severe financial crisis, and significant harm to the livestock industry [36]. Bangladesh and other low- and middle-income nations will suffer tremendously as a result of the consequences [37]. In addition, several recent investigations reported the emergence of multidrug-resistant bacterial pathogens from different origins that is considered a public health threat which increase the necessity of the proper use of antibiotics. Besides, the routine application of the antimicrobial susceptibility testing to detect the antibiotic of choice as well as the screening of the emerging MDR strains [18,21,22,38–40].

Studies have shown the occurrence of different food borne bacteria in poultry meat from different parts of the chicken [41–44]. However, enough data needs to be gathered with respect to the multiple drug resistance profile of these bacteria because it is of great importance to the global public health. This research was aimed to investigate the occurrence of multidrug resistant *E. coli, Salmonella* spp. and *S. aureus* in chicken egg, meat and faeces from four districts of Bangladesh.The study would help to provide evidence-based policy interventions to address the multidrug resistance epidemic scenario from zoonotic pathogens.

2. Materials and methods

2.1. Sampling

Samples were collected from four districts under Mymensingh division (Mymensingh, Jamalpur, Netrokona, and Sherpur) of Bangladesh. A total of 120 samples comprising 40 faeces (20 each from broiler and layer chickens), 40 meat samples (20 each from broiler and layer chickens) and 40 egg samples (30 from layer chickens and 10 from ducks) (Table 1). The samples were collected using sterile instruments and aseptic with a simple random sampling technique. Immediately after collection, samples were transferred to sterile eppendorf tubes or zipper bags, placed in transport boxes kept at 4 °C, and then transported to the laboratory for microbiological analysis.

2.2. Culture and identification of Escherichia coli, Salmonella spp. and Staphylococcus aureus

Broth containing faeces samples, egg surface washings and processed meat samples were incubated aerobically at 37 °C overnight for the growth of bacteria [41,45–47]. Each broth culture was streaked onto eosin methylene blue agar (EMB) (HI media, Maharashtra, India), *Salmonella Shigella* (SS) agar (HI media, Maharashtra, India) and mannitol salt (MS) agar (HI media, Maharashtra, India) media for the isolation of *E. coli, Salmonella* and *Staphylococcus*, respectively. Initially, freshly grown broth culture of each sample was streaked on EMB, SS and MS agar media using sterile platinum loop. Then the inoculated agar plates were aerobically incubated at 37 °C overnight to obtain pure colonies. Single green-colored metallic-sheen colonies on EMB agar media, black-centered colonies on SS agar media and rounded colonies with metallic yellow colour of MS agar media represented the growth of *E. coli*, *Salmonella* spp. and *Staphylococcus*, respectively [41,46,47]. For further confirmation, selected colonies were subjected to morphological study by Gram staining [48,49]. Pure culture of *E. coli*, *Salmonella* and *Staphylococcus* was obtained by inoculating individual colonies into fresh nutrient broth followed by streaking onto respective selective agar media [41,46,47].

2.3. Genotypic confirmation of E. coli, Salmonella spp. and Staphylococcus aureus

Genomic DNA was extracted from pure cultures of *E. coli, Salmonella* and *Staphylococcus* using the conventional boiling method [45,46,50]. Isolates from pure colonies were inoculated into respective broth and incubated at 37 °C for 8–12 h. After incubation 1 ml of cultured broth was transferred into an Eppendorf tube followed by centrifugation for 5 min at 10,000 rpm. The bottom content was suspended in 100 μl of distilled water after carefully discarding the supernatant and centrifugation repeated. Supernatant was discarded and 100 μl distilled water was added. Then Eppendorf tubes were again vortexed and boiled for 20 min. After boiling the samples in the Eppendorf tubes were immediately maintained in cold shock for 10 min and centrifuged at 10,000 rpm for 10 min. The template DNA was stored at −20 °C for further use.

PCR assay was performed to detect *E. coli, Salmonella* spp. and *Staphylococcus aureus with* primers of *fliC, inv A* and *nuc* genes, respectively using the conditions and methods described by different authors under standard operating procedures [51–53]. The forward primer F'CCCCCTGGACGAAGACTGAC and a reverse primer R′ ACCGCTGGCAACAAAGGATA was used for the amplification of *fliC* gene (401bp) for *E. coli* [51]. Similarly, primers F′ ATCAGTACCAGTCGTCTTATCTTGAT and R'TCTGTTTACCGGGCATACCAT were used to amplify *invA* gene (211bp) for *Salmonella* spp., and primers F′ AGCGGGGGATAACTATTGGA and R'TACGCATTTCACCGCTACAC for the amplification of the *nuc* gene (284bp) *for Staphylococcus aureus* [52,53]. The PCR amplification was carried out in a 25 μl reaction mixture, consisting of 12.5 μl of master mixture (Promega, Madison, WI 53711 USA), 1 μl of forward primer, 1 μl of reverse primer, 3 μl of DNA template and 7.5 μl of nuclease free water. A thermal cycler (Thermo Fisher scientific, Waltham, MA USA) was used for the amplification with different PCR thermal conditions as validated by different authors [51–53] and the PCR products were visualized in gel (1.5 %) by UV-Trans illuminator.

2.4. Antimicrobial susceptibility pattern for E. coli, Salmonella spp. and Staphylococcus aureus

The disc diffusion method was used to determine the antibiotic susceptibility of *E. coli, Salmonella* and *Staphylococcus* isolates [54]. A total of ten antibiotics under six classes namely tetracycline (30 μg) and doxycycline (30 μg) of tetracycline, ciprofloxacin (5 μg), levofloxacin (5 μg) and enrofloxacin (5 μg) of fluroquinolones, amoxicillin (30 μg) of penicillin, erythromycin (25 μg) of macrolide,

Table 1
Distribution of samples collected from poultry in Mymensingh division of Bangladesh.

Name of the district	Sample Type						Total
	Broiler		Layer			Duck	
	Faeces	Meat	Faeces	Meat	Egg	Egg	
Mymensingh	5	5	5	5	8	3	31
Netrokona	5	5	5	5	8	3	31
Sherpur	5	5	5	5	7	2	29
Jamalpur	5	5	5	5	7	2	29
Total	20	20	20	20	30	10	120

gentamicin (25 μg) and neomycin (30 μg) of aminoglycosides, and sulphur (25 μg) of sulphonamide were selected based on findings from a survey we carried out [55]. The McFarland standard (1.5×10^6 CFU/ml) of turbidity was used to measure the colonies' turbidity suspended in saline. All freshly suspended bacteria were gently spread onto Mueller Hinton Agar (HI media, Maharashtra, India). The antibiotic discs were gently placed onto the Mueller Hinton agar plates and incubated for 24 h at 37 °C. The zones of inhibition that were observed were measured to the nearest millimetre and used to determine the susceptibility pattern of the isolates according to the Clinical and Laboratory Standards Institute standard guidelines [56]. Basis on inhibition zone, isolates were categorized to susceptible (S), Intermediate (I), or Resistant (R). Whereas basis on the susceptibility or resistance pattern, the isolates were also categorized to multidrug resistant (MDR), extensively drug resistant (XDR) and pandrug resistant (PDR) bacteria [57].

2.5. Detection of resistance genes

PCR Detection of Antibiotic resistance genes was done using genomic DNA extracted and preserved from the bacterial isolates. Screening for antimicrobial resistance genes in *Salmonella, E. coli* and *Staphylococcus* isolates was performed by validated PCR conditions and methods described by different authors [58–60] (Table 2).

2.6. Statistical analysis

The entry of results was done using Excel 2013 spreadsheet (Microsoft Office 2013, Microsoft, Los Angeles, CA, USA) and the analysis was performed using the Statistical Package for Social Science- SPSS (IBM SPSS 25, IBM, Chicago, IL, USA). Frequencies, and percentage occurrence for each isolate or resistance gene were used to summarize the result and presented using Tables and Figures [61].

3. Results

3.1. Isolation and identification of E. coli, Salmonella spp. and S. aureus

Three bacterial species were isolated from the poultry samples collected in the four districts of Mymensingh division. Out of 120 samples analyzed, 90 (50 %) were suspected as *E. coli*, 31(17 %) as *Salmonella* spp. and 58 (32 %) as *S. aureus* based on their cultural and staining properties (Table 3).

3.2. Phenotypic characteristics of the recovered isolates

The *E. coli* produced black colored colonies with metallic sheen, *Salmonella* spp. produced black centered, smooth, small and round colonies while *Staphylococcus* produced small rounded colonies which changed the color of media to metallic yellow. Both *E. coli* and *Salmonella* spp. were gram-negative rods while *S. aureus* was gram-positive cocci with cluster arrangement.

Table 2
List of primers used to detect antibiotic resistant genes using polymerase chain reaction (PCR).

Antibiotic	Target gene	Primer	Sequence (5′-3′)	Amplicon size (bp)	References
Tetracycline	*tetA*	F	GGT TCA CTC GAA CGA CGT CA	577	[58]
		R	CTGTCCGACAAGTTGCATGA		
Tetracycline	*tetB*	F	CCTCAGCTTCTCAACGCGTG	634	[58]
		R	GCACCTTGCTGATGACTCTT		
Tetracycline	*tetC*	F	AAC AAT GCG CTC ATC GT	1138	[59]
		R	GGA GGC AGA CAA GGT AT		
Erythromycin	*ereA*	F	GCCGGTGCTCATGAACTTGAG	419	[58]
		R	CGACTCTATTCGATCAGAGGC		
Beta lactam	bla_{TEM}	F	ATA AAA TTC TTG AAG AC	1076	[59]
		R	TTA CCA ATG CTT AATCA		
Beta lactam	bla_{SHV}	F	TCGCCTGTGTATTATCTCCC	768	[58]
		R	CGCAGATAAATCACCACAATG		
Beta lactam	bla_{CMY}	F	TGGCCAGAACTGACAGGCAAA	462	[58]
		R	TTTCTCCTGAACGTGGCTGGC		
Sulphur	*sul1*	F	TTCGGCATTCTGAATCTCAC	822	
		R	ATGATCTAACCCTCGGTCTC		
Streptomycin	*aadA1*	F	TATCCAGCTAAGCGCGAACT	447	
		R	ATTTGCCGACTACCTTGGTC		
Methicillin	*mecA*	F	AAAATCGATGGTAAAGGTTGGC	533	[60]
		R	AGTTCTGGAGTACCGGATTTGC		
Gentamicin	*aac(3)-IV*	F	CTTCAGGATGGCAAGTTGGT	286	[58]
		R	TCATCTCGTTCTCCGCTCAT		

Table 3
Bacterial isolation rate from poultry samples based on culture and staining properties.

Sample type	No. of samples	*E. coli*	Bacteria *Salmonella* spp.	*S. aureus*
Broiler faeces	20	19	16	8
Broiler meat	20	17	1	12
Layer faeces	20	18	10	4
Layer meat	20	20	Nil	3
Layer egg	30	10	2	23
Duck egg	10	6	2	8
Total	120	90 (50 %)	31 (17 %)	58 (32 %)

3.3. Genotypic detection of the recovered isolates based on PCR assay

The PCR amplification of *fliC* gene for the detection of *E. coli* confirmed all isolates to be positive by the amplification of *fliC* gene at the 401bp. Similarly, the PCR amplification of *invA* gene for the detection of *Salmonella* genus confirmed the suspected genus isolates at 284 bp. The *S. aureus* isolates were confirmed by the amplification of *nuc* gene at the 155 bp. All the 90 culture positive *E. coli* isolates were confirmed by PCR with the detection rate of 100 %. Sixteen isolates out of 31 suspected *Salmonella* isolates were confirmed by PCR and the isolation rate was 51.6 %. Among the 58 culture positive isolates, 53 (91.3 %) were PCR positive for *Staphylococcus aureus*.

3.4. Overall antimicrobial resistance pattern of E. coli, Salmonella spp. and S. aureus isolated from poultry faeces in Mymensingh division

The result of the antimicrobial resistance pattern of isolates from poultry faeces showed that all (100 %) isolates of the *E. coli, Salmonella* spp. and *S. aureus* were resistant to Amoxicillin. In addition, all (100 %) of the *Salmonella* and *S. aureus* were resistant to Doxycycline, while (100 %) of the *Salmonella* isolates were resistant to Enrofloxacin. It was also observed that (100 %) each of *Salmonella* spp. and *S. aureus* were resistant to erythromycin and tetracycline. The study also revealed that all (100 %) *Salmonella* spp. isolates were susceptible to gentamycin (Fig. 1). Relatively lower susceptibility of *E. coli* to neomycin and gentamicin was observed. *Salmonella* also showed decreased susceptibility to levofloxacin and neomycin. *Staphylococcus* also showed decreased susceptibility to neomycin, levofloxacin and gentamicin. The results of this study revealed that all of the isolates had multiple antibiotic resistance index (MAR) above 0.2, with the exception of one isolate that had an MAR index of 0.2.

3.5. Antimicrobial resistance pattern of E. coli and S. aureus isolated from poultry meat in Mymensingh division

The result of the antimicrobial resistance pattern of isolates from poultry meat showed that all (100 %) *E. coli* isolates were resistant to Amoxycillin, while all (100 %) *S. aureus* isolates were resistant to Amoxicillin, Ciprofloxacin, Doxycycline, Enrofloxacin,

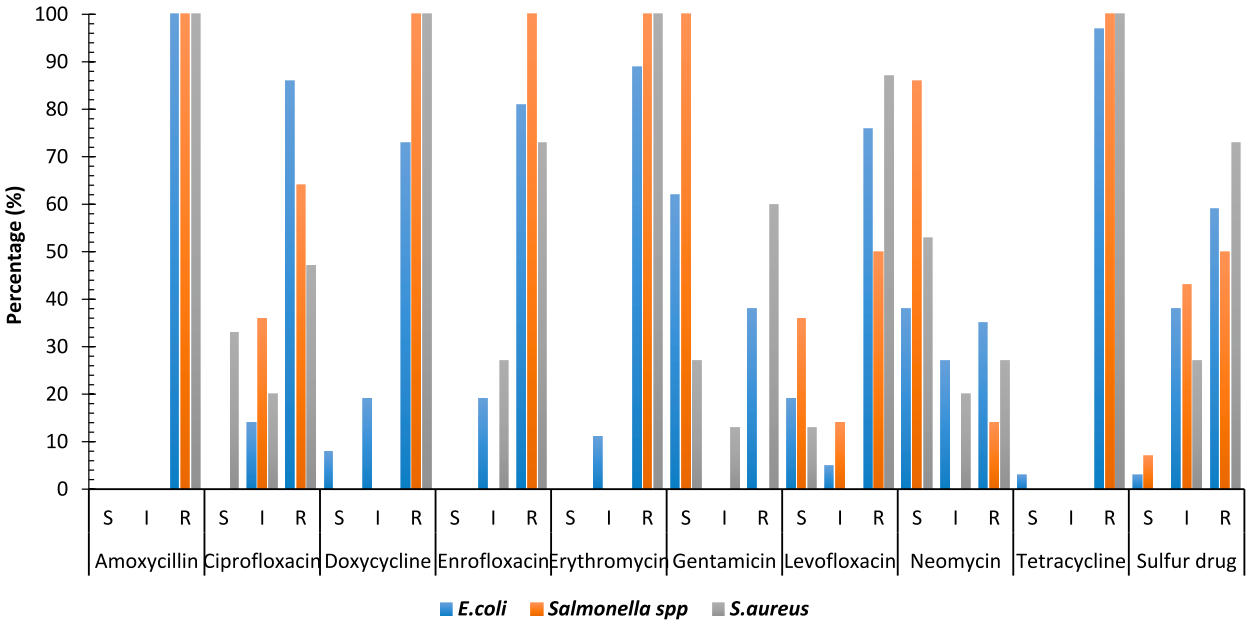

Fig. 1. Antimicrobial resistance pattern of *E. coli, Salmonella* spp. and *S. aureus* isolated from poultry faeces in Mymensingh division. Infectious agents (bacteria) are classified as "susceptible as S," "intermediate as I," or "resistant as R″ to specific antibiotics.

Erythromycin, Levofloxacin, Tetracycline and Sulphur drug (Fig. 2).

3.6. Antimicrobial resistance pattern of *E. coli, Salmonella* spp. and *S. aureus* isolated from poultry eggs in Mymensingh division

The result of the antimicrobial resistance pattern of isolates from poultry egg showed that all (100 %) of *E. coli, Salmonella* spp. and *S. aureus* isolates were resistant to Amoxicillin. While all the *Salmonella* isolates (100 %) were susceptible to Gentamicin, Levofloxacin and Tetracycline (Fig. 3).

3.7. The occurrence of MDR and XDR among the recovered isolates from poultry faeces, meat and eggs in Mymensingh division

A total of 89 isolates were found MDR and 68 isolates were found XDR among the 179 isolates of *E. coli*, *Salmonella* spp. and *S. aureus* isolated from faeces, meat and egg (Table 4). Highest percentage of MDR was observed by the *S. aureus* isolates recovered from poultry faeces and meat.

3.8. Resistance genes detection

For *E. coli* the *sul1* gene was the most abundant followed by *tetA* (13 %) and then *tetB* (10 %) and bla_{SHV} (10 %). The least occurring resistant genes in *E. coli* were the bla_{CMY} and *aac (3)-IV* with (2 %) occurrence each. *Salmonella* isolates also had 55 % and 35 % of *sul1* and *tetB* genes with highest occurrence followed by the *aadA1*(25 %) and bla_{TEM} (24 %) genes. The least occurring was the bla_{SHV} at (3 %). Resistance encoding genes found in *Staphylococcus aureus* were *sul1*(41 %), *tetB* (17 %) while bla_{CMY} and *mecA* had (2 %) occurrence each. The *mecA* and *aac(3)-IV* genes were only found in *Staphylococcus aureus* and *E. coli* respectively. The *sul1, tet*B and *aadA1* were highest in *Salmonella* spp. and *Staphylococcus aureus* while the *sul1, tet*A *and* bla_{SHV} were higher in *E. coli.* The genotypic resistance pattern in *E. coli, Salmonella* spp. and *Staphylococcus aureus* are shown in Figs. 4–6, respectively.

4. Discussion

Antibiotic resistant pathogens and other disease causing organisms can be found in the environment from farm effluence or agricultural manure that eventually find their way into the food chain [62]. Insufficient documented data in the poultry sector of Bangladesh, hinders safe poultry production, despite the importance of the sector to the national economy [63]. In this study *E. coli, Salmonella* spp., and *Staphylococcus* species were isolated from poultry food product and by-product. Many researches have shown multidrug resistant *Salmonella* spp. emanating from overuse of antibiotics [64,65]. In fact many countries have reported the prevalence of extended-spectrum β-lactamases from poultry sourced protein [66–68].

Methicillin-resistant *Staphylococcus* is known to invade food animals of great public health significance [69]. *Staphylococcus aureus* from eggs and faeces of poultry have been reported to cause problems in many instances [20,70]. *Salmonella* spp. from infected poultry faeces may contaminate the egg shells and penetrate the interior of eggs, causing bacteria to grow inside [71]. It has been established that the presence of *Salmonella* spp. in more than 25 % of poultry meat is considered unsafe for human consumption [72]. In this study we found 28.9 % *Salmonella* spp. from eggs samples. However, Fearnley et al. [73], studied the presence of *Salmonella* spp. in chicken meat, eggs and humans, in Adelaide, South Australia and reported the absence of *Salmonella* spp. in all eggs sampled.

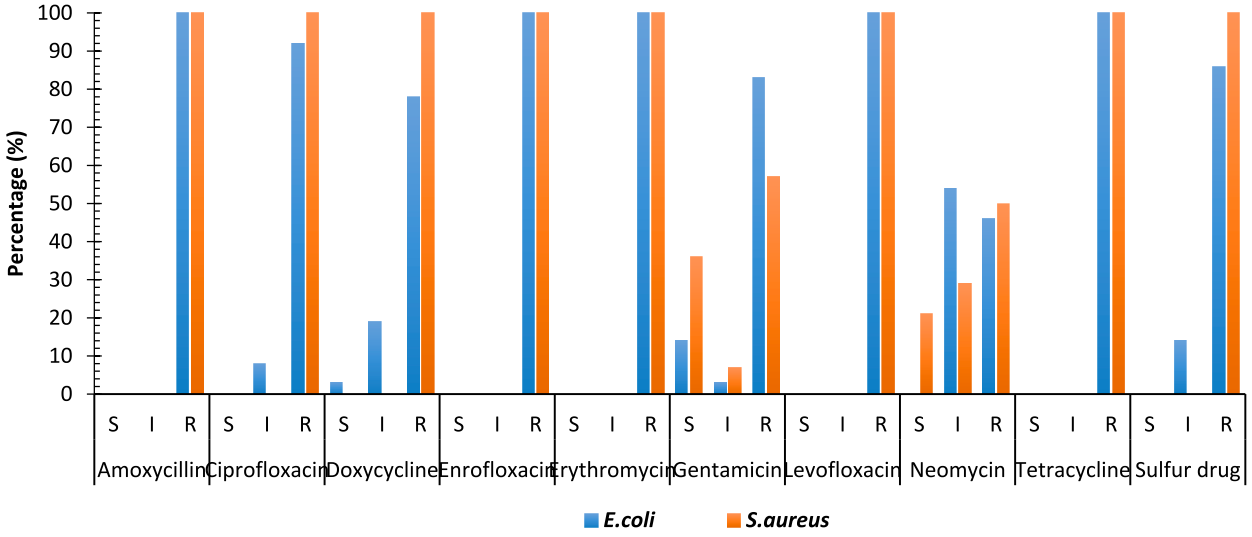

Fig. 2. Antimicrobial resistance pattern of *E. coli* and *S. aureus* isolated from poultry meat in Mymensingh division. Infectious agents (bacteria) are classified as "susceptible as S," "intermediate as I," or "resistant as R″ to specific antibiotics.

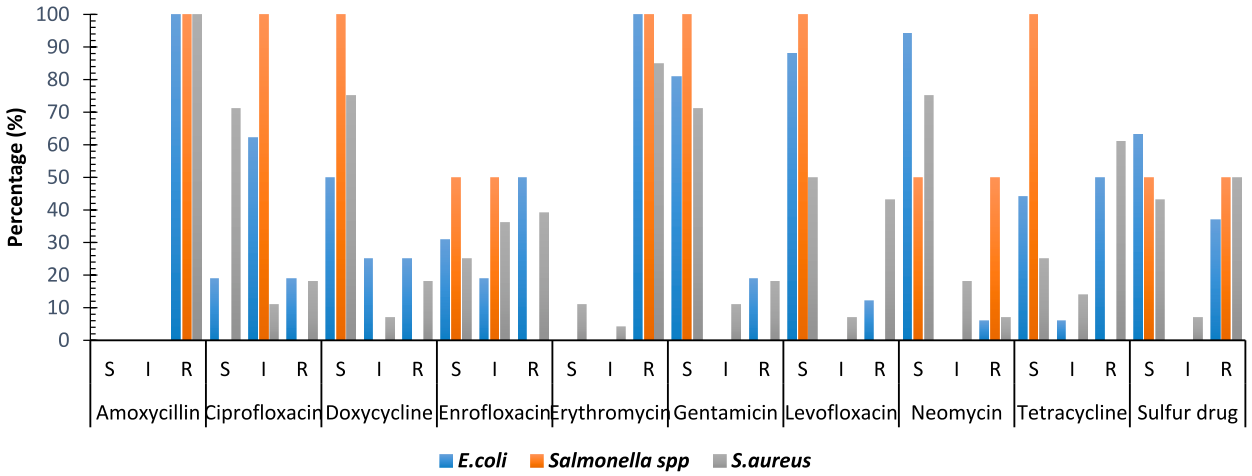

Fig. 3. Resistance pattern of *E. coli, Salmonella* spp. and *S. aureus* isolated from poultry eggs in Mymensingh division. Infectious agents (bacteria) are classified as "susceptible as S," "intermediate as I," or "resistant as R″ to specific antibiotics.

Table 4
The occurrence of MDR and XDR among the recovered isolates from poultry faeces, meat and eggs.

Name of sample	Source of sample	Name of isolate	No. of isolate	No. of drug resistant isolate (%)	
				MDR	XDR
Faeces	Broiler	*E. coli*	19	5 (26.3 %)	14 (73.7 %)
		Salmonella	16	2 (12.5 %)	13 (81.3 %)
		S. aureus	8	8 (100 %)	–
	Layer	*E. coli*	18	9 (50 %)	8 (44.4 %)
		Salmonella	10	1 (10 %)	9 (90 %)
		S. aureus	4	4 (100 %)	–
Meat	Broiler	*E. coli*	17	16 (94.1 %)	1 (5.9 %)
		Salmonella	1	1 (100 %)	–
		S. aureus	12	12 (100 %)	–
	Layer	*E. coli*	20	15 (75 %)	5 (255)
		Salmonella	–	–	–
		S. aureus	3	3 (100 %)	–
Egg	Layer	*E. coli*	10	2 (20 %)	6 (60 %)
		Salmonella	2	–	2 (100 %)
		S. aureus	23	16 (69.6 %)	2 (8.7 %)
	Duck	*E. coli*	6	2 (33.3 %)	4 (66.7 %)
		Salmonella	2	–	1 (50 %)
		S. aureus	8	3 (37.5 %)	3 (37.5 %)
Total			179	89 (49.7 %)	68 (38 %)

MDR: Multidrug resistance, XDR: Extensively drug resistance.

Poultry meat should be completely free from *E. coli* before consumption because of the nature and severity of the disease that can be caused by the bacteria in human [72]. *E. coli* is often used as a safety indicator for microbiological significance in food borne pathogens screening [74]. In this research there was an isolation rate of (56.6 %) for *E. coli* which is close to the (63.5 %) detection rate obtained by Rahman et al., [75]. In the study of Rahman et al. [75], the overall prevalence of *E. coli* in chicken meat was 63.5 %, which is similar to the findings of this study which was 41.1 %. In our study 85 % of the meat samples from broiler and all 100 % of the meat samples from layer birds tested positive for *E. coli*. In this regard previous study showed 65.67 % broiler meat and 61.33 % layer meat samples tested positive for *E. coli* [75]. However, Jakaria et al. [76], reported that in Bangladesh, the prevalence rates of *E. coli* in layer and broiler chicken were 78.67 % and 82 % respectively.

S. aureus is among the most common cause of clinical infections globally and has attracted substantial public attention due to the increased mortality associated with the multidrug resistant bacteria. Contrary to this study where the isolation rate for *S. aureus* was 33.3 %, the isolation rate of *S. aureus* was 14 % in the previous study by Al-Humam and Mohamed [77], when they monitored *Escherichia coli*, *Salmonella* spp. and *Staphylococci aureus* found in poultry based fast foods. However, our finding was lower than the isolation rate (100 %) from chicken meat in another study reported by Lika et al., [78].

This variation in isolation rate of the three bacterial organisms in the present study and other previous studies from various locations may be due to differences in study methodology, gene-specific involvement, sample types, sample size and hygiene practices in farms, and geographic locations. The difference in prevalence reported might also be attributed to the collection of samples from

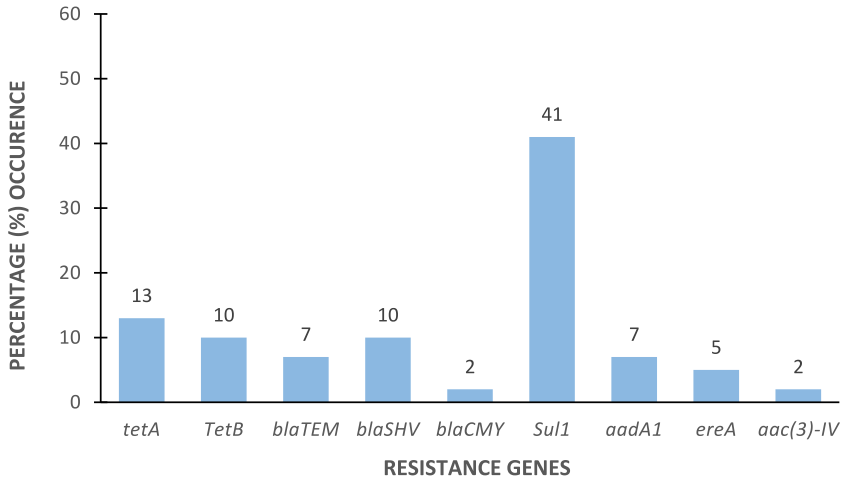

Fig. 4. Genotypic resistance pattern in *E. coli*.

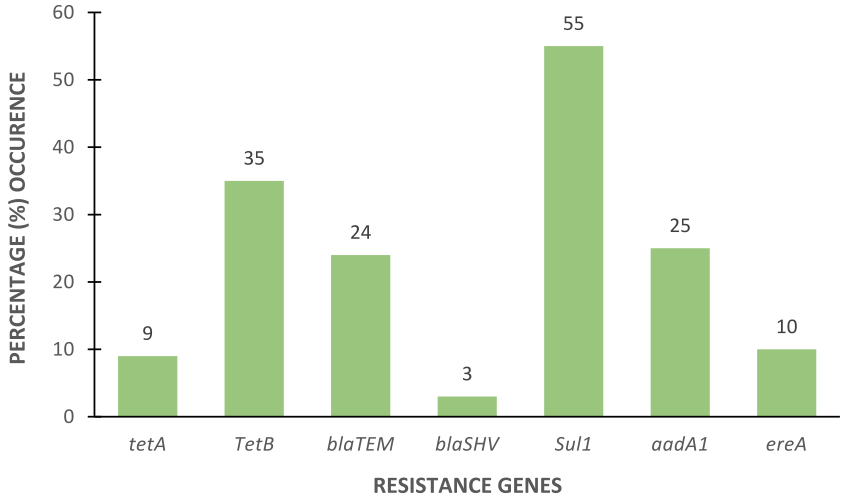

Fig. 5. Genotypic resistance pattern in *Salmonella* spp.

Fig. 6. Genotypic resistance pattern in *Staphylococcus aureus*.

apparently healthy birds [79]. The isolation of three bacterial species in the present study is an indication of the level of contamination in the poultry products.

The detection of *invA* gene in poultry samples as seen in this study, implies that these isolates have the ability to invade cells and survive in macrophages [80]. WHO [81], stated that *Salmonella* spp. has serovars harbouring the virulent *invA* gene causing salmonellosis globally. The isolation of *Salmonella* spp. carrying invasion *invA* gene in this study may indicate the poor sanitation of the poultry farms environment under which birds are kept with an increased burden for foodborne infections.

The detection of *S. aureus* from poultry samples in this current study is similar to the detection rate obtained by Islam et al. [82], in chicken from Bangladesh. In addition, detection of *nuc* gene in this study is similar to the previously published report by Islam et al., [82].

The antibiotic susceptibility pattern of the collected isolates from poultry faeces revealed that all (100 %) *E. coli* isolates were resistant to amoxicillin, while all the (100 %) *Salmonella* isolates were resistant to amoxicillin, doxycycline, Enrofloxacin, erythromycin and tetracycline. The *S. aureus* isolates were all (100 %) resistant to amoxicillin, doxycycline and erythromycin. The *Salmonella* spp. isolates were all (100 %) susceptible to gentamycin and 86 % to neomycin. A previous study conducted by Akond et al. [83], reported a prevalence of 82 % for *E. coli* from poultry samples in Bangladesh, while 67.7 % and 69.8 % from Nigeria in poultry and cloacal swabs, respectively [84,85]. A possible explanation for the difference between the studies carried out in northern Nigeria by Akond et al. [83], and our present study could be due to the sample types collected as our study isolated *E. coli* from freshly dropped chicken faecal samples as opposed to cloacal swabs. The similarity observed between our present study findings and that of other studies may be due to similarities in poultry farming practices. The susceptibility result of the isolates from poultry meat in the current study showed that all (100 %) of the *E. coli* isolates from poultry meat were resistant to amoxicillin, enrofloxacin, erythromycin, levofloxacin and tetracycline, while all (100 %) the *S. aureus* isolates from poultry meat were resistant to amoxicillin, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, levofloxacin, tetracycline and sulphur drugs.

The result of the *E. coli* isolates from poultry eggs in the present study showed that all (100 %) isolates were resistant to amoxicillin and erythromycin, while all (100 %) of the *E. coli* isolates were resistant to ciprofloxacin and erythromycin. All (100 %) *Salmonella* isolates were susceptible to doxycycline, gentamicin, levofloxacin and tetracycline. For the *S. aureus* isolates from poultry egg the susceptibility result revealed that all (100 %) of the isolates were resistant to amoxicillin. In this study all (100 %) *Staphylococcus aureus* isolates were resistant to Amoxycillin, which is contrary to the previous study by Elsohaby et al. [86], who reported that all the *Staphylococcus aureus* isolates were susceptible to Amoxycillin.

Serious human health concerns worldwide have been attributed to multi-drug resistant producing *E. coli* strains from poultry meat [87]. All the bacterial isolates in this study were multidrug resistant showing resistance to between 5 and 6 different classes of antibiotics, is similar to that report by Afayibo et al. [88], from Eastern China where all *E. coli* isolates were multi-drug resistant (MDR). Bamidele et al. [89], reported that the prevalence of MDR was highest in *E. coli* as compared to *Pseudomons, Salmonella or Klebsiella,* even though all were MDR with multiple antibiotic resistant index (MAR) $\geq$ 0.2. Awosan et al. [90], reported high level of MDR *E. coli* to be resistant to aminoglycosides, quinolones, tetracycline, sulfonamides classes of antibiotics. High resistance rates of *E. coli* isolates to beta-lactams, tetracyclines, macrolides, and sulfonamides was also previously reported by Briñas et al., [91]. This finding is not surprising as these antimicrobials are easily accessible and commonly used in poultry production for preventive as well as therapeutic purposes especially in the absence of antimicrobial stewardship programs [91]. Talebiyan et al. [92], also reported multidrug resistance (MDR) of all *E. coli* isolates from diseased poultry. In another study Tadesse et al. [93], reported that *E. coli* isolates from human clinical samples carry the same resistant genes as those found in livestock probably due to indiscriminate discharge of residues into the environment [94].

The emergence of multi drug resistant food borne and environmental pathogens reflects evolutionary process that might have taken place as the animals were being exposed to antibiotics [95,96]. The resistance of bacterial pathogens to antibiotics can also occur due to inheritance and horizontal gene transfer, which is more likely to be happen in locations where antibiotics use is frequent [96]. The implication of a multidrug resistant pathogen is that; it becomes more pathogenic compared to non-multidrug resistant pathogens [97]. They also make treatment difficult in infected patients [98] and there is additional cost of treatment, as well as additional days in hospital stay [99]. Multi drug resistant pathogens have a greater risk of causing death e.g. Methicillin resistant *Staphylococcus aureus* (MRSA) of infected individuals have been estimated to be 64 % more likely to die than Methicillin Susceptible *Staphylococcus aureus* (MSSA) infected individuals. The spread of bacterial resistance in the population can be revealed by measurement of multiple antibiotic resistances (MAR) index [100]. The result of MAR index of the isolates revealed that all the isolates had MAR index above 0.2, with the exception of one isolate that had an MAR index of 0.2, an indication that these isolates originated from a high-risk source of contamination e.g., farm animals that are frequently exposed to antibiotics [101]. Any bacterial strain exhibiting MAR index values lower than 0.2 is thought to originate from sources, in which antibiotics are seldom or never used (lower risk). Multiple antibiotic resistance in bacterial spp. has been attributed to antimicrobial selective pressure and gene transfer mechanisms between and among isolates and close relatives. Infections caused by resistant microorganisms may result in failure to respond to treatment, result in prolonged illness, higher health care expenditure and greater risk of death [102].

In this study, the presence of *tetA* (32 %) and *tetB* (8 %) genes may be attributed to the high resistance rates against tetracycline. In a previous study by Enany et al. [103], from Egypt, high resistance rates were also observed against amoxicillin (100.0 %) and tetracycline (100 %) which is in line with our present study. They stated that the presence of the *tetA* and *tetB* genes may be responsible for this high resistance to tetracycline. The prevalence of *tetA* and *tetB*, suggests that tetracycline is frequently used on these poultry farms from the study area.

We detected beta lactam resistance encoding genes (bla_{TEM}, bla_{SHV}, and bla_{CMY}). The *blaTEM*, a β-lactamase gene, is the most common mechanism of resistance in *E. coli*, and was previously detected in resistant *E. coli* isolates from foods, humans, and healthy

animals [91], as found in the present study. Murray et al. [104], also detected extended spectrum beta lactamase (bla_{TEM}, bla_{SHV}, and bla_{CMY}) in their isolates. Cheng et al. [105], also reported market sourced chicken samples to be potential reservoirs of antibiotic resistant genes (ARGs). The quinolone-resistance genes, *aac* (8.0 %) was reported in this study, which is less frequent than the report of Abdullah et al., [106].

Our study revealed the *Sul1* gene to be the most frequently found resistance gene in the isolates studied (*E. coli*, *Salmonella* spp. and *Staphylococcus aureus*). These results are in agreement with a similar work that reported the presence of the *Sul1* gene in all isolated *E. coli* strains from raw chicken meat sold in supermarkets in the city of Taif of Saudi Arabia by Soufi et al., [107]. Kim and Cho [108] also detected sulphonamide harbouring the resistance gene (*Sul1*) in *E. coli* strains isolated from chicken and turkey meat sampled from a food processing plant in Tunisia.

The low (36 %) and high (100 %) susceptibility of the *Salmonella* isolates from poultry faeces and egg, respectively towards ciprofloxacin in this study provokes questions about the efficiency of this antibiotic in future which is also similar to the result of Kim and Cho [108] where they studied the resistance of *Salmonella* isolates from poultry farms, hatcheries and slaughter houses and found high resistance to ciprofloxacin, levofloxacin, and nalidixic acid.

Indiscriminate use of antimicrobials led to growth and spreading the MDR pathogens causes financial losses not only in the poultry production but also public health sector in Bangladesh; however, the precise data regarding these financial losses are not well documented. Economic losses are due to high treatment and management costs, loss of production and mortality in poultry farm [28].

Based on our current finding, we believed that further research can look into the use of new techniques that have been developed to tackle drug resistance problem in human and animals which may include the use of nanotechnology for effective drug delivery. In this regard, Chitosan, which is a cockle shell sugar derivative that can be used in nanotechnology backed drug delivery has anti-microbial activity, low molecular weight and economically low cost [109]. Therefore, further work can be done to look into the use of chitosan or other low molecular weight particles for cost-effective antibiotics drug delivery aimed at challenging the existing mechanisms of resistance in a wide range of antibiotic agents. Finally, the current study data indicated that there is a high risk of transmitting antibiotic resistance genes or MDR pathogens in foods of poultry origin from microbial contamination. Strict monitoring measures need to be taken to tackle the indiscriminate use of antibiotics in the poultry production cycle, which could mitigate the detrimental effects of antibiotic resistance.

5. Conclusions

The role of food products from poultry for the spread of antibiotic resistant pathogens and genes cannot be underestimated. Based on the result of our study, raw poultry products and by-products (faeces) from farm remain a potential source of transmitting pathogenic and antibiotic resistant bacteria. The isolation rate for *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* were higher in raw poultry products and by-products. All the samples were resistant to most antibiotics tested indicating MDR pathogen regardless of the sample source. The study also revealed that all the isolates had an MAR index above 0.2, with the exception of one isolate. In addition, the collected isolates harboured *tetA, tetB, blaTEM, blaSHV, blaCMY, sul1, aadA1, ereA* and *aac(3)* resistant genes. Although the antibiotics gentamycin, neomycin, levofloxacin, doxycycline and tetracycline remained effective against the isolated isolates. Finally, we conclude that poultry products (meat and eggs) and their by-products (faeces) could be a source and disseminator of antibiotic resistant foodborne pathogens not only in poultry farming environment but also human and environment. This result highlights the importance of performing sensitivity test before prescribing antimicrobials, continued surveillance, policy formulation, implementation and awareness building training of poultry farmers to reduce the detrimental effects of MDR pathogens in humans, animals and the environment.

Additional information

No additional information is available for this manuscript.

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Institutional review board statement

The study protocol was authorized by the Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University, Mymensingh, Bangladesh [approval number: AWEEC/BAU/2018(31); date 30. 12. 2018].

Data availability

Upon reasonable request, the corresponding author will provide the data Grant this work.

CRediT authorship contribution statement

Kazi Rafiq: Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Aminatu Abubakar Sani:** Writing – original draft, Investigation, Formal analysis, Data curation. **Muhammad Tofazzal Hossain:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Md Tarek Hossain:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Md Hadiuzzaman:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mohammad Abdus Sattar Bhuiyan:** Writing – review & editing, Software, Resources, Investigation, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kazi Rafiq reports financial support, administrative support, and equipment, drugs, or supplies were provided by Bangladesh Agricultural Research Council, Dhaka, Bangladesh. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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