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Detection of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* in chickens

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ABSTRACT

Objective: Indiscriminate use of antibiotics in poultry farms increases the chance of antibiotic resistant and extended-spectrum β-lactamase (ESBL) producing bacteria in Bangladesh. Therefore, the study was under taken to detect ESBL producing *Escherichia coli* (*E. coli*) in chickens.

Materials and methods: A total of 60 cloacal swab samples (20 from commercial layer, 20 from commercial broiler and 20 from commercial sonali chickens) were collected from Rajshahi district of Bangladesh. The *E. coli* was isolated from these samples and identified based on cultural, staining, and biochemical characteristics. The disk diffusion method was used to assay the antibiotic resistant/sensitivity patterns of the isolated *E. coli*. Phenotypc detection of ESBL producing *E. coli* was also done.

Results: The prevalence of *E. coli* in chickens was 61.67% in Rajshahi district of Bangladesh, where the prevalence was 60%, 60%, and 65% in commercial layer, commercial broiler, and commercial sonali chickens, respectively. The antibiotic sensitivity assay of *E. coli* isolated from commercial layer chickens showed 100%, 80%, 50%, 40%, and 40% resistant to amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively. *E. coli* isolated from commercial broiler chickens showed 100%, 100%, 60%, 50%, and 40% resistant to amoxicillin, tetracycline, cefotaxime, ceftazidime, and ciprofloxacin, respectively. *E. coli* isolated from commercial sonali chickens showed 90%, 70%, 50%, 50%, and 40% resistant to amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively. In phenotypic detection, the overall prevalence of ESBL producing *E. coli* was 43.33%, where 40%, 50%, and 40% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively in Rajshahi district of Bangladesh.

Conclusion: These results indicated that chickens are a potential reservoir for ESBL producing *E. coli* and their antibiotic resistances are obviously significant. These findings will help us to make proper guideline for the treatment, prevention and control of *E. coli* prevalent in chickens in Bangladesh.

Keywords: *Antibiotic resistance, Chickens, E. coli, ESBL, Prevalence*

INTRODUCTION

The β-lactamases are bacterial enzymes that hydrolyze β-lactam ring of antibiotics which results in ineffective compounds. Extended-spectrum β-lactamases (ESBLs), have the capability of hydrolyzing and causing resistance to various types of β-lactam antibiotics, including the penicillins, 1st, 2nd and 3rd generation cephalosporins and aztreonam. They are not active against the cephamycins (cefoxitin and cefotetan), but are susceptible to β- lactamase inhibitors (clavulanic acid) (Mohanty et al., 2010). The main ESBL types are TEM (trimethylamine), SHV (sulfhydryl variable), and CTX-M (cefotaximase). These

enzymes confer resistance to β-lactam antibacterial drugs, particularly cephalosporin, and may be accompanied by co-resistance to drugs of other classes (Paterson and Bonomo, 2005; Canton and Coque, 2006). Specifically, the ESBL enzymes are increasingly expressed by pathogenic bacteria like *Escherichia coli* (*E. coli*) with potential for dissemination. These enzymes have also been identified in several other members of the family *Enterobacteriaceae* and in certain non-fomenters (Jacoby et al., 2005). The *E. coli*, ESBLs has increased the resistance traits and the evolution of different genes worldwide (Paterson and Bonomo, 2005). Indiscriminate uses of antibiotics for the treatment of poultry diseases increase the chance of antimicrobial exposure to microorganisms resulting in resistant and ESBL producing bacteria in poultry (Hasan et al., 2012). With the misuse and overuse of antibiotics to treat diseases, resistance to the drugs has begun to appear and has become more serious because of selective pressure. In Bangladesh, there is limited data on this perspective. Therefore, the aim of this study was to detect ESBL producing *E. coli* in chickens as well as antibiogram assay of these isolates.

MATERIALS and METHODS

The study area and period

The study was conducted in the commercial layer, commercial broiler, and sonali chicken's farms in Rajshahi district of Bangladesh. Cloacal samples were collected with the sterile swab and brought to the Microbiology Lab., Department of Veterinary and Animal Sciences, University of Rajshahi for bacteriological analysis. The study was conducted during the period from July to December, 2020, with the ethical number 144/320/IBSc.

Sample collection

A total of 60 cloacal swab samples were collected from randomly selected chicken farms. Out of 60 swab samples, 20 were collected from commercial layer farms, 20 were collected from commercial broiler farms, and 20 were collected from commercial sonali farms. These samples were collected from four upazila (Charghat, Durgapur, Godagari and Paba) and metropolitan area of Rajshahi district of Bangladesh. From each study area (4 upazila and 1 metropolitan area) 4 swab samples from commercial layer chickens, 4 swab samples from commercial broiler chickens, and 4 swab samples from commercial sonali chickens were collected.

Isolation and identification of E. coli

The collected samples were separately inoculated into freshly prepared nutrient broth and incubated at 37°C for 24 hours in the bacteriological incubator for enrichment. The incubated tubes were examined for the growth of bacteria. Then the broth culture of bacteria was inoculated on nutrient agar by streak plate techniques and inoculated 37°C for 24 hours for the development of colonies. The colonies on primary culture was repeatedly sub-cultured on different selective culture media (EMB agar, MacConkey agar and SS agar) by the streak plate method until the pure culture with homogenous colonies was obtained.

Colony morphology: The colony morphology of the isolated *E. coli* was studied as mentioned by Merchant and Packer (1967). Colony morphology such as shape, size, surface texture, edge and elevation, color and opacity developed after 24 hours of incubation were carefully studied and recorded.

Gram's staining: Gram's staining was performed according to the method described by Cheesbrough (1985).

Biochemical identification of isolated bacteria: Pure culture of bacteria was subjected to different biochemical tests like sugar fermentation test (with five basic sugars for the production of acid with or without H_2S gas), catalase test, indole test, MR test, VP test and TSI agar slant reaction. Standard methods were followed to conduct these tests and interpretation (Cowan, 1985).

Antibiogram assay of the isolated E. coli

The disk diffusion method (Bauer et al., 1966; Jorgensen and Turnidge, 2015) was used to test the susceptibility of the *E. coli* isolates. In brief, pure colonies of the *E. coli* isolates were inoculated in nutrient broth and incubated at 37°C for overnight. Then 100 µl of broth culture (OD 0.5) was taken and placed onto Mueller Hinton agar plate and spread evenly with a sterile glass rod spreader. The antibiotic discs were dispensed onto the surface of the inoculated agar plates keeping about 1 cm apart. After 18 to 20 hours of incubation at 37°C, each plate was examined. The susceptibility test of the *E. coli* was done against nine antibiotic disks including; penicillin (10 IU), amoxicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), cefotaxime (10 µg), ceftazidime (30 µg), gentamicin (10 µg), meropenem (10 µg), and imipenem (10 µg). Using a ruler, the susceptibility zones were measured and interpreted according to criteria (Table 1) set by the

Clinical and Laboratory Standards Institute document M100-S17 (CLSI, 2016).

Table 1. Used antibiotics with their disc concentration and standard zone of inhibition.

Antimicrobial agent	Disc concentration (µg/disc))	Interpretation of results (zone diameter in mm)		
		R	I	S
Penicillin	50 µg	≤11	12-21	≥22
Gentamicin	10 µg	≤12	13-14	≥15
Tetracycline	30 µg	≤14	15-18	≥19
Amoxycillin	30 µg	≤13	14-17	≥18
Ciprofloxacin	5 µg	≤15	16-20	≥21
Cefotaxime	30 µg	≤13	14-17	≥18
Ceftrazidine	30 µg	≤16	18-20	≥19
Imipenem	10 µg	≤13	14-15	≥16
Meropenem	10 µg	≤15	16-22	≥23

µg: Microgram, SL: Serial, mm: millimetre. S: Sensitive, I:Intermediately sensitive, R: Resistant, ≥: Greater than or equal to, ≤: Less than or equal to

ESBL screening and confirmatory tests

To identify potential ESBL producer's disk diffusion breakpoints were used for cefotaxime (10 µg) and ceftazidime (30 µg) according to (CLSI, 2016) guidelines. An ESBL-producing strain might hydrolyze one or more of these agents. Results were interpreted based on the CLSI guidelines as follows: zones of inhibition of ≤22 mm for ceftazidime and ≤27 mm cefotaxime or combination of two. The less susceptible or resistant isolates were subjected to confirmatory test using double discs diffusion method according to (CLSI, 2016). The intermediate and resistant *E. coli* isolates were tested with both cefotaxime and ceftazidime alone and in combination with clavulanic acid (10µg). The zone diameter increased ≥5 mm compared to when tested without clavulanic acid, confirms ESBL production (CLSI, 2016).

RESULTS

Cultural characteristics

The growth of *E. coli* on nutrient agar was indicated by the development of smooth, circular, white to grayish white colony and on EMB by the development of smooth, circular, black color colonies with metallic sheen (Figure 1). The growth of *E. coli* on MacConkey agar was indicated by the development of bright pink colored colony and on Salmonella-Shigela (SS) agar by the development of pink to rose red colonies.

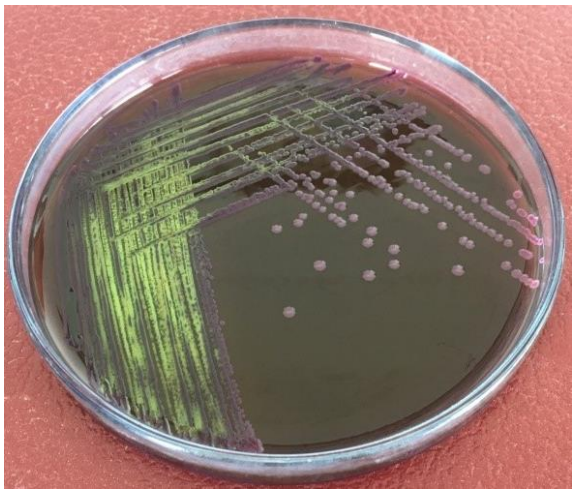

Figure 1. Growth of *E. coli* on EMB agar (produced greenish-black colonies with metallic sheen)

Biochemical properties

Isolated *E. coli* fermented dextrose, lactose, sucrose, maltose and mannitol with the production of acid and gas (Figure 2). Isolated *E. coli* showed positive results in catalase test, indole test and MR test but showed negative result in VP test. Isolated *E. coli* produced acidic slant and acidic butt (yellow slant, yellow butt) with gas production in TSI agar slant reaction (Table 2).

The overall prevalence of *E. coli* in the present study was 61.67% in chickens in Rajshahi district of Bangladesh, where the prevalence was 60%, 60%, and 65% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively (Table 3).

Figure 2. Fermentation activity of isolated *E. coli* with five basic sugars (fermented dextrose, lactose, sucrose, maltose and mannitol with the production of acid and gas).

The overall prevalence of *E. coli* in the present study was 61.67% in chickens in Rajshahi district of

Bangladesh, where the prevalence was 60%, 60%, and 65% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively (Table 4).

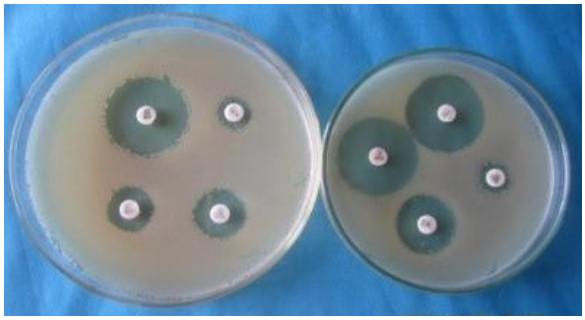

Figure 3. Antibiotic sensitivity patterns of isolated *E. coli* on Muller Hinton agar media (showed sensitive to meropenem, imipenem and gentamicin but resistant to penicillin).

The antibiotic sensitivity assay of isolated *E. coli* from commercial layer chickens showed 100%, 90%, 90%, 50%, 40%, and 40% sensitive to meropenem, imipenem, gentamicin, ciprofloxacin, ceftazidime, and cefotaxime, respectively. *E. coli* isolated from commercial broiler chickens showed 100%, 90%, 80%, 30%, 30%, and 20% sensitive to meropenem, imipenem, gentamicin, cefotaxime, ceftazidime, and ciprofloxacin, respectively. *E. coli* isolated from commercial sonali chickens showed 100%, 90%, 60%, 30%, 30%, and 20% sensitive to meropenem, imipenem, gentamicin, cefotaxime, ceftazidime, ciprofloxacin, respectively. Whereas, *E. coli* isolated from commercial layer chickens showed 100%, 100%, 80%, 50%, 40%, and 40% resistant to penicillin, amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively. *E. coli* isolated from commercial broiler chickens showed 100%, 100%, 100%, 60%, 50%, and 40% resistant to penicillin, amoxicillin, tetracycline, cefotaxime, ceftazidime, and ciprofloxacin, respectively. *E. coli* isolated from commercial sonali chickens showed 100%, 90%, 70%, 50%, 50%, and 40% resistant to penicillin, amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively (Figure 3, Table 4).

Table 2. Biochemical properties of isolated *E. coli.*

Tests	Used sugars	Acid production	Gas production	Results
Fermentation reaction with five basic sugars	Dextrose	+	+	+
	Maltose	+	+	+
	Lactose	+	+	+
	Sucrose	+	+	+
	Mannitol	+	+	+
Indole test				+
Catalase test				+
MR test				+
VP test				-
TSI agar slant reaction	Acidic slant and acidic butt (Yellow slant, yellow butt) with gas production			+

Table 3. Prevalence of *E. coli* in chickens.

Types of chickens	No. of the samples tested	Prevalence of *E. coli* (%)	Overall prevalence of *E. coli* (%)
Commercial layer chickens	20	12 (60)	61.67
Commercial broiler chickens	20	12 (60)	
Commercial sonali chickens	20	13 (65)	
Total	60	37	

Table 4. Antibiotic sensitivity and resistant pattern of isolated *E. coli*.

Name of antibiotics used	Sensitivity patterns (%)								
	E. coli from commercial layer chickens			*E. coli* from commercial broiler chickens			*E. coli* from commercial sonali chickens		
	S	I	R	S	I	R	S	I	R
Penicillin	0	0	100	0	0	100	0	0	100
Amoxicillin	0	0	100	0	0	100	0	10	90
Cefotaxime	40	10	50	30	10	60	30	20	50
Tetracycline	0	20	80	0	0	100	0	30	70
Ciprofloxacin	50	10	40	20	40	40	20	30	50
Ceftazidime	40	20	40	30	20	50	30	30	40
Gentamicin	90	10	0	80	10	10	60	30	10
Meropenem	100	0	0	100	0	0	100	0	0
Imipenem	90	10	0	90	10	0	90	10	0

S: sensitive, I: intermediate sensitive, R: resistant.

Table 5. Prevalence of extended-spectrum β-lactamase producing *E. coli* in chickens.

Types of chickens	Antibiotics used in combination with CVA	I	R	Increased Zone diameter*	ESBL producing *E. coli* (%)	Overall ESBL producing *E. coli* (%)
Commercial layer chickens	CTX with CVA	1	5	4	40	13 (43.33)
	CAZ with CVA	3	3			
Commercial broiler chickens	CTX with CVA	1	5	5	50	
	CAZ with CVA	2	5			
Commercial sonali chickens	CTX with CVA	2	5	4	40	
	CAZ with CVA	3	4			

*Increased zone diameter (≥5 mm than the previous zone) in a combination with CVA, CTX: cefotaxime; CAZ:ceftazidime; CVA: clavulanic acid.

The overall prevalence of ESBL producing *E. coli* was 43.33% in chickens in Rajshahi district of Bangladesh, where 40%, 50%, and 40% were in commercial layer, commercial broiler, and commercial sonali chickens, respectively (Table 5).

DISCUSSION

In the current study, the results of cultural, staining and biochemical tests of isolated *E. coli*, was successfully done. Our results are similar to the findings of Freeman (1985), Buxton and Fraser (1977) and Merchant and Packer (1967). In the present study the overall prevalence of *E. coli* in commercial chickens was 61.67%; where 60%, 60%, and 65% were in commercial layer, commercial broiler and commercial sonali chickens, respectively in Rajshahi district of Bangladesh. Previously the prevalence of *E. coli* was reported in commercial chickens as 64%, 71%, and 65% at Bogura, Gazipur and Joypurhat districts, respectively in Bangladesh (Hadiujjaman et al., 2016). They also reported that the prevalence of *E. coli* was 56%, 80%, and 68% in the commercial broiler, commercial layer and commercial sonali chickens, respectively. The present study also showed that *E. coli* isolated from commercial layer, commercial broiler and commercial sonali chickens were 90%, 80%, and 60% sensitive, respectively to gentamicin. Almost similarly result was reported previously (Hadiujjaman et al., 2016). They

reported that all *E. coli* isolates (100%) from commercial layer chickens were sensitive to gentamycin. The current study revealed that 20% *E. coli* isolates from commercial sonali chickens were sensitive to ciprofloxacin. It was previously reported that 12.5% *E. coli* from commercial sonali chickens were sensitive to ciprofloxacin (Hadiujjaman et al., 2016). The sensitivity patterns of our study showed that *E. coli* isolated from commercial chickens were sensitive to meropenem and imipenem in 100% and 90%, respectively. This finding is more likely because meropenem and imipenem are not commonly practiced in chickens in Bangladesh. More or less similarly result was reported previously (Ahoyo et al., 2007; Muvunyi et al., 2011). They reported that 96.4% (Ahoyo et al., 2007) and 93% (Muvunyi et al., 2011) *E. coli* isolates from commercial chickens were sensitive to imipenem. The results of our study showed that *E. coli* isolates from commercial layer chickens were resistant to penicillin, amoxicillin, tetracycline, ciprofloxacin, and ceftazidime in 100%, 100%, 80%, 40%, and 40%, respectively. Similarly, the high resistance rates of *E. coli* isolates were reported to commonly used antibiotics such as ampicillin (97.6%) and amoxicillin (95.2%) in Benin (Anago et al., 2015). The overall ESBL producing *E. coli* in the present study was 43.33%. This finding is agreed with other studies (Costa et al., 2009; Moyo et al., 2010; Kashyap et al., 2013). In this study, the prevalence of ESBL producing *E. coli* was 40%, 50%, and 40% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively. Similarly, result was reported previously (Charles et al., 2017). They reported that ESBL-positive *E. coli* were 87% in the commercial broiler, 42% in commercial layer and 49% in commercial layer chickens.

CONCLUSION

The overall prevalence and the prevalence of ESBL producing *E. coli* was 61.67% and 43.33%, respectively in chickens in Rajshahi district of Bangladesh. Out of 60%, 60% and 65% prevalence the ESBL producing *E. coli* was 40%, 50%, 40%, respectively in commercial layer, in commercial broiler, and commercial sonali chickensin this study area. The prevalence of ESBL producing *E. coli* in chickens and their antibiotic resistance is obviously significant. These resistance genes are transmitting to the human body through the food chain. Therefore, the poultry sector should be provided with immediate attention by the government to control the indiscriminate use of antibiotics.

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