IDENTIFICATION AND ANTIBIOGRAM ASSAY OF *ESCHERICHIA COLI* ISOLATED FROM CHICKEN EGGS

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Abstract

Chicken eggs contain all the essential components such as proteins, lipids, vitamins, minerals, carbohydrates, and growth factors required by the human being. Despite of their nutritional values eggs can cause health problems through consumption of contaminated eggs with pathogenic microorganisms. Therefore, the present study was undertaken to identify *Escherichia coli* isolated from chicken eggs with their antibiogram assay in Rajshahi district of Bangladesh. *E. coli* was isolated from 180 chicken eggs collected from different areas of Rajshahi district and identified based on cultural, staining, and biochemical characteristics. Antibiogram assay of all the isolates were done by disk diffusion method. The overall prevalence of *E. coli* was 38.89% in chicken eggs of which 27.78% was on egg shells and 11.11% was in egg content. The prevalence of *E. coli* was 58.33% in commercial layer farm eggs, 41.66% in whole seller eggs, and 16.67% in retailer eggs. In antibiogram study, isolated *E. coli* showed 64.28% to 92.85% sensitivity to chloramphenicol, gentamycin, and ceftriaxone. The highest sensitivity was found to meropenem (100%). Isolated *E. coli* showed resistance to ciprofloxacin, tetracycline, amoxicillin, ampicillin, and erythromycin ranging from 50% to 71.42%. Judicious use of antibiotics and public awareness will help to reduce the development of antibiotic resistance.

Key words: Antibiotic resistance, Chicken eggs, *E. coli*, Prevalence

Introduction

Poultry industry is a promising sector for poverty elevation in Bangladesh. The Bangladesh poultry industry primarily produces chickens, although a few other species like duck, pigeon, quail, goose, turkey, and guinea fowl are available. In Bangladesh two types of chickens have been reared, one for eggs and another for meat purpose. Currently Bangladesh is producing 15.52 billion chicken eggs against the current annual demand of 17.13 billion. Globally chicken eggs are common food and consume in various dishes as a cheap source of protein which considered as the most nutritious foodstuffs for human (Pasquali et al. 2014). Egg components have been attributed diverse biological activities including antimicrobial activity, protease inhibitory action, vitamin binding properties, anticancer activity, and immunomodulatory activity. Eggs are also an important source for minerals as phosphorus and irons, and a good source of vitamins like A, D, E, K, B1, B2, B9, B12, choline and selenium. On the other hand, nutrient substances present in eggs create an excellent environment for the growth and multiplication of bacteria. Eggs can be contaminated with many bacteria such as *Escherichia coli*, *Salmonella* sp*., Proteus* sp*., Listeria monocytogenes*, *Staphylococcus* sp*., Streptococcus* sp*.,* and *Bacillus* sp*.* (Lee et al. 2016). *E. coli* is one of the common microbial flora of the gastrointestinal tract of poultry and human. *E. coli* contaminations are more likely with a cracked eggs, dirty shells, and storage in contaminated surroundings. Contaminating egg shells increased the changes of egg contents when the shells are broken (Neira et al. 2017). Eggs contaminated with bacteria may lead to

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transmission of pathogens which associated with food-borne illness to consumers has already been established (Osimani et al. 2016, Chousalkar et al. 2018). Although most of the *E. coli* strains are harmless, some can cause food poisoning and diarrhea especially in elderly, infants, and those with impaired immune systems (Begum et al. 2014). Antibiotics can lead to the emergence and dissemination of resistant *E. coli* which can then be passed into human via eggs or direct contact with chickens. The emergence of antibiotic resistance in bacteria has become a serious problem worldwide. Antibiotic resistance is increasing day by day and become a public health hazard globally (Ferri et al. 2017). In Bangladesh, antibiotics are used as growth promoters as well as to control infectious poultry diseases (Hasan et al. 2014). This misuse or overuse of antibiotics in the poultry industry results the development of an increasing number of antibiotic resistant *E. coli* (Islam et al. 2018). So, identification of *E. coli* from chicken eggs and determination of their antibiotic sensitivity patterns is very much essential for the proper treatment and control purposes. The present study was therefore conducted to determine the prevalence of *E. coli* in chicken eggs in Rajshahi district and antimicrobial sensitivity assay of this bacterium that has public health significance.

Materials and Methods

The whole research work was conducted in the Microbiology Laboratory, Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh.

Sample collection

A total of 180 chicken egg samples were aseptically collected from randomly selected commercial layer farms, whole sellers, and retail outlets (small shops and roadside vendors). Out of 180 egg samples, 60 eggs were collected from commercial layer farms, 60 from whole sellers, and 60 from retailers. Among these, 60 were brown shelled, 60 were white shelled, and 60 were indigenous chicken eggs.

Samples preparation

Every 5 eggs represented as one composite sample (Adesiyun et al. 2007, El-kholy et al. 2014). For egg shells, a pool of five eggs was used and one sterile swab was moistened with sterile saline and was applied on the surface of each egg. Each swab applied on five egg shells was submerged in 6 ml of sterile saline as 'shell wash' (Adesiyun et al. 2007). The shell wash was mixed with a mini vortex and inoculated into different culture media. After that, the egg shells were sterilized by swabbed with 70% ethyl alcohol, flamed, and broken with a sterile forceps from the broad ends, and yolk and albumen of five eggs were pooled. The contents thoroughly mixed for nearly one minute using a blender and mixer was used to inoculate into different culture media.

Isolation and identification of *E. coli*

After enrichment in nutrient broth, a loopful of enriched culture was taken on a sterile glass slide and Gram's staining was performed for morphology study. Then the broth culture of bacteria was inoculated on nutrient agar by streak plate technique and inoculated at 37°C for 24 hours for the development of colonies. The colony on primary culture was repeatedly sub-cultured on different selective culture media (EMB agar, MacConkey agar and blood agar) by the streak plate technique until the pure culture with homogenous colonies was obtained. Identification of *E. coli* was done through a series of biochemical tests.

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 hour of incubation were carefully studied and recorded.

Gram's staining

Gram's staining was performed according to the method described by Cheesbrough (1985). In brief, a small colony was picked up with a bacteriological loop, smeared on separate glass slides and fixed by gently heating. Crystal violet was applied on each smear to stain for two minutes and washed with running tap water. Few drops of Gram's iodine was added as mordent for one minute and again washed with running tap water. Acetone alcohol was added for a few seconds as a decolouring agent. After washing with water, safranin was added as a counter stain and allowed to stain for two minutes. The slides were washed with water, blotted and dried in air, and examined under light microscope with high power objective (100X) using immersion oil.

Biochemical studies for the identification of isolated *E. coli*

Pure culture of *E. coli* 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

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 separately inoculated in nutrient broth and incubated at 37°C for overnight. Then 100 µl of broth culture was taken and placed on Mueller Hinton agar plate and spread evenly with a sterile glass rod spreader. The antibiotic disks were placed on the surface of the plates keeping about 1cm 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 ten antibiotic disks namely, gentamycin, ciprofloxacin, erythromycin, doxycycline, chloramphenicol, tetracycline, amoxicillin, ampicillin, ceftriaxone, and meropenem. The susceptibility zones were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI 2017).

Table 1. Zone diameter interpretative standards for antimicrobial resistance

Name of used antibiotics	Disk concentration (µg/disk)	Interpretation of results (zone diameter in mm)		
		R	I	S
Gentamycin	10	≤12	13-14	≥15
Tetracycline	30	≤ 11	12-14	≥ 15
Ampicillin	25	≤13	14-16	≥17
Amoxycillin	30	≤13	14-17	≥18
Ciprofloxacin	5	≤15	16-20	≥21
Ceftriaxone	30	≤ 13	14-20	≥ 21
Doxycycline	30	≤ 10	11-13	≥ 14
Erythromycin	15	≤ 10	11-15	≥ 16
Chloramphenicol	30	≤ 12	13-17	≥ 18
Meropenem	10	≤ 19	20-22	≥ 23

µg = Microgram, mm = Millimeter. S = Sensitive, I = Intermediate sensitive, R = Resistant, ≥ = Greater than or equal to, and ≤ = Less than or equal to.

Results

Cultural characteristics

The growth of *E. coli* on nutrient agar was indicated by the development of smooth, circular, white to grayish white colonies and on EMB by the development of smooth, circular, greenish black color colonies with metallic sheen (Fig. 1). The growth of *E. coli* on MacConkey agar was indicated by the development of bright pink colored colonies and on blood agar by the development of colorless colonies without hemolysis (Table 2).

Staining characteristics

The staining characteristics of the isolated *E. coli* were determined by Gram's staining technique. The microscopic examination of Gram's stained smears of *E. coli* revealed Gram's negative, pink colored, small rod shaped appearance, arranged in single or paired (Fig. 2 and Table 2).

Table 2. Morphology and cultural characteristics of isolated *E. coli* on different agar media

| Cultural characteristics of *E. coli* on different agar media				Staining characteristics
Nutrient agar	MacConkey agar	EMB agar	Blood agar	
Smooth, circular, clear, moist, white to grayish white colonies	Bright pink colored, transparent, smooth, raised colonies	Greenish black colonies with metallic sheen	Colorless colonies without hemolysis	Gram's negative, small rod shaped appearance, arranged in single or paired
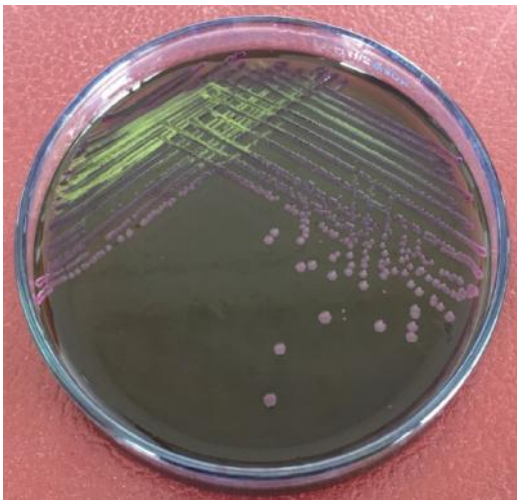
Fig. 1. Growth of *E. coli* on EMB agar (greenish-black colonies with metallic sheen).
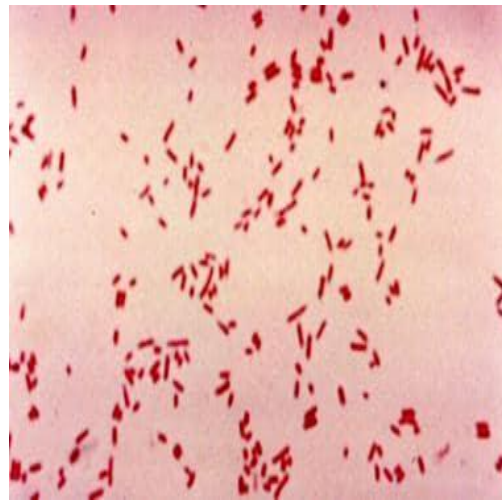

Fig. 2. Gram's stained *E. coli* (Gram's negative, small rod shaped appearance, arranged in single or paired under a light microscope (100X)).

Biochemical properties

Isolated *E. coli* fermented dextrose, lactose, sucrose, maltose, and mannitol with the production of acid and gas in sugar fermentation test (Fig. 3). 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 and yellow butt) with gas production in TSI agar slant reaction (Table 3).

Table 3. 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 and yellow butt) with gas production			+

Fig. 3. Fermentation activity of isolated *E. coli* with five basic sugars (fermented dextrose, lactose, sucrose, maltose, and mannitol with the production of acid and gas).

Prevalence of *E. coli* in chicken eggs in Rajshahi district

The overall prevalence of *E. coli* was 38.89% in chicken eggs in Rajshahi district of Bangladesh of which 27.78% was on egg shells and 11.11% was in egg contents (Table 4 and Fig. 4). The prevalence of *E. coli* was 58.33% in selected commercial layer farm eggs, 41.67% in whole seller eggs, and 16.67% in retailer eggs (Table 4 and Fig. 4).

Table 4. Prevalence of *E. coli* in chicken eggs in Rajshahi district

Source of chicken eggs	No. of samples tested*	Prevalence of *E. coli* on egg shells (%)	Prevalence of *E. coli* in egg contents (%)	Overall prevalence of *E. coli* (%)
Commercial layer farms	12	5 (41.66%)	2 (16.67%)	7 (58.33%)
Whole sellers	12	3 (25.00%)	2 (16.67%)	5 (41.66%)
Retailers	12	2 (16.67%)	0%	2 (16.67%)
Total	36	10 (27.78%)	4 (11.11%)	14 (38.89%)

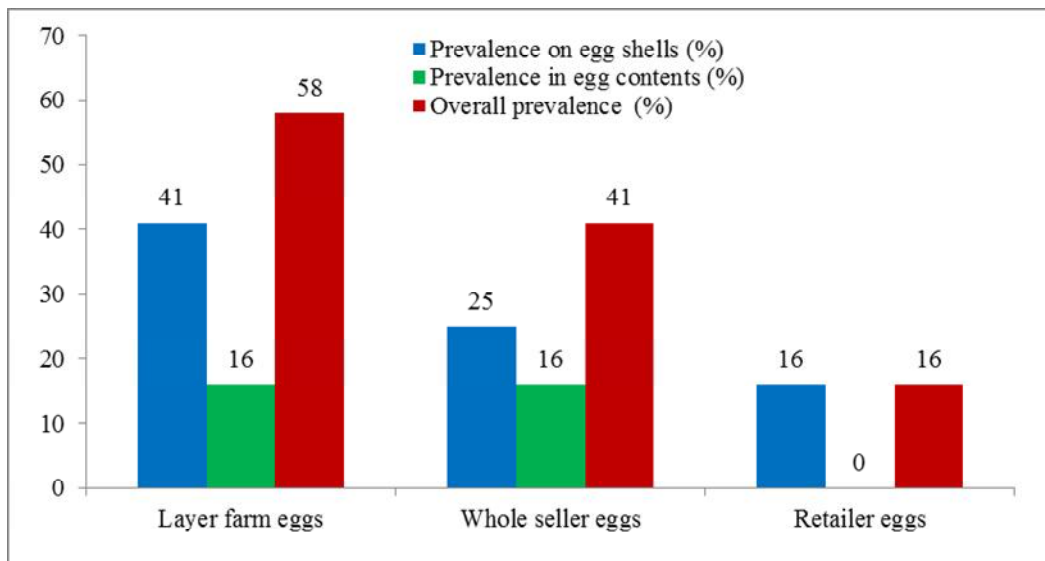*= Pooled egg samples, every 5 eggs represented as one a composite sample.

Fig. 4. Prevalence of *E. coli* in chicken eggs in Rajshahi district.

The prevalence of *E. coli* was 41.67% in indigenous chicken eggs, 25.00% in brown shell chicken eggs, and 50.00% in white shell chicken eggs (Table 5 and Fig. 5).

Table 5. Prevalence of *E. coli* in different type chicken eggs in Rajshahi district

Type of chicken eggs	No. of samples tested*	Prevalence of *E. coli* on egg shells (%)	Prevalence of *E. coli* in egg contents (%)	Overall prevalence of *E. coli* (%)
Indigenous chicken eggs	12	4 (33.33%)	1 (8.33%)	5 (41.67%)
Brown shell chicken eggs	12	2 (16.67%)	1 (8.33%)	3 (25.00%)
White shell chicken eggs	12	4 (33.33%)	2 (16.67%)	6 (50.00%)
Total	36	10 (27.78%)	4 (11.11%)	14 (38.89%)

*= Pooled egg samples, every 5 eggs represented as one composite sample.
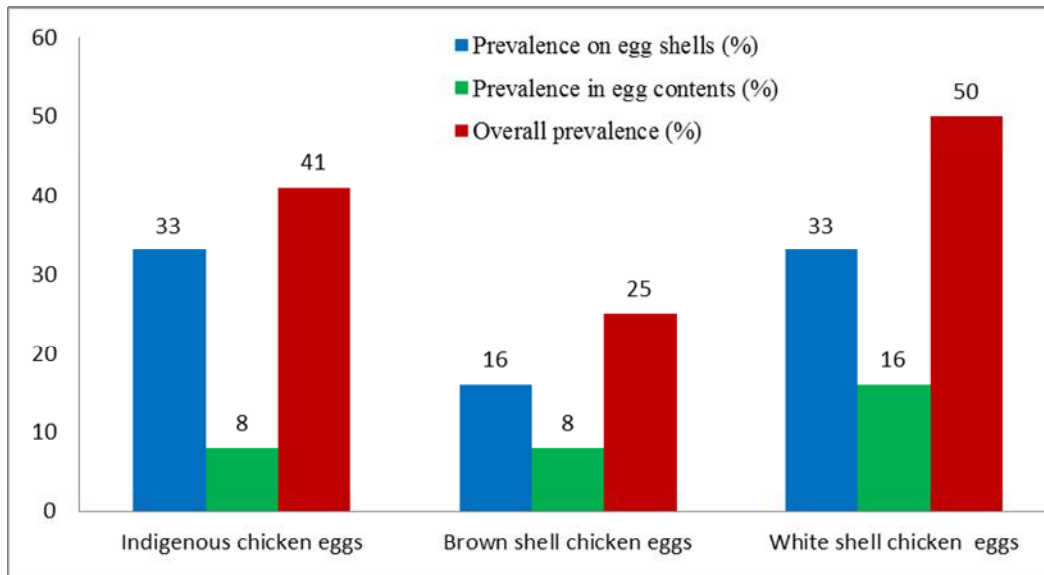
Fig. 5. Prevalence of *E. coli* in different type chicken eggs in Rajshahi district.

Antibiotic sensitivity and resistant patterns of isolated *E. coli*

The results of antibiotic sensitivity patterns of the isolated *E. coli* showed 71.42%, 71.42%, 64.28%, 57.14%, 50.00%, 35.71%, and 21.42% resistant to erythromycin, ampicillin, amoxicillin, tetracycline, ciprofloxacin, doxycycline, and chloramphenicol, respectively (Table 6 and Fig. 7). In sensitivity assay, isolated *E. coli* showed 100%, 92.85%, 78.57%, 64.28%, 35.71%, 21.42%, 14.29%, 14.29%, and 14.29% sensitive to meropenem, ceftriaxone, gentamycin, chloramphenicol, ciprofloxacin, amoxicillin, doxycycline, ampicillin, and tetracycline, respectively (Table 6 and Fig. 7). The isolates also showed intermediate sensitive to doxycycline (50%), erythromycin (28.57%), tetracycline (28.57%), gentamycin (21.42%), ciprofloxacin (14.29%), chloramphenicol (14.29%), amoxicillin (14.29%), ampicillin (14.29%), and ceftriaxone (7.14%).

Fig. 6. Antibiotic sensitivity and resistant patterns of isolated *E. coli* on Mueller Hinton agar.

Table 6. Antibiotic sensitivity and resistant patterns of isolated *E. coli*

| Name of used antibiotics	Sensitivity patterns (n = 14)		
	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
Gentamycin	78.57	21.42	0.0
Ciprofloxacin	35.71	14.29	50.0
Erythromycin	0.0	28.57	71.42
Doxycycline	14.29	50.0	35.71
Chloramphenicol	64.28	14.29	21.42
Tetracycline	14.29	28.57	57.14
Amoxicillin	21.42	14.29	64.28
Ampicillin	14.29	14.29	71.42
Ceftriaxone	92.85	7.14	0.0
Meropenem	100.0	0.0	0.0
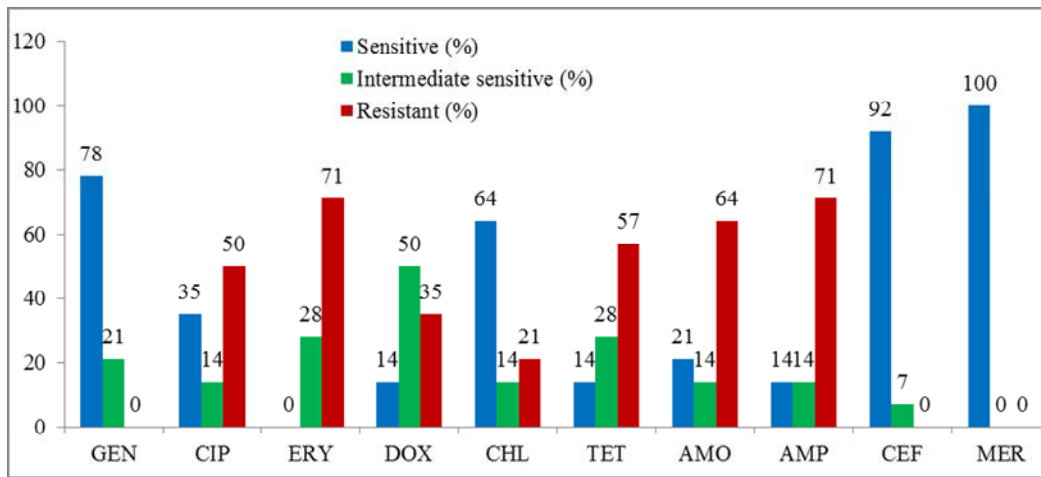
Fig. 7. Antibiotic sensitivity and resistant patterns of isolated *E. coli* (GEN = Gentamycin, CIP = Ciprofloxacin, ERY = Erythromycin, DOX = Doxycycline, CHL = Chloramphenicol, TET = Tetracycline, AMO = Amoxicillin, AMP = Ampicillin, CEF = Ceftriaxone, and MER = Meropenem).

Discussion

The prevalence of *E. coli* was 38.89% in chicken eggs in the present study. This finding is in agreement with the findings of Islam et al. (2018) who reported that 34.64% chicken eggs were contaminated with *E. coli* in Dhaka city of Bangladesh. Our finding is lower than the finding of Gole et al. (2013) who reported that the prevalence of *E. coli* was 60.78% in chicken eggs in Australia. In the current study, the prevalence *E. coli* was 58.33% in commercial layer farm eggs. Similarly, Adesiyun et al. (2005) reported that 58.7% chicken eggs were contaminated with *E. coli* in farms in Trinidad. However, Akond et al. (2009) reported that 42% chicken eggs surfaces were contaminated with *E. coli* in poultry and poultry farms environments in Bangladesh. The prevalence of *E. coli* was 27.78% on chicken egg shells in our study. Similar reports were published previously by Adesiyun et al. (2005) and Eman et al. (2015). Adesiyun et al. (2005) reported that 28.3% chicken egg shells were contaminated with *E. coli* in Trinidad. Eman et al. (2015) stated that 28.58% chicken egg shells were *E. coli* contaminated in Egypt. The prevalence of *E. coli* in egg contents was 11.11% in the present study. Similarly, El-kholy et al. (2014) reported that the prevalence of *E. coli* was 11.76% in chicken egg contents in Beni-suef city, Egypt. However, our finding is higher than the findings of Adesiyun et al. (2005), Sabrinath et al. (2009), and Safaei et al. (2011). Adesiyun et al. (2005) reported that 3.8% chicken egg contents was positive for *E. coli.* Sabrinath et al. (2009) reported that the prevalence of *E. coli* was 13.3% and 45.8% in chicken egg contents collected from large farms and small farms, respectively in Grenada. Safaei et al. (2011) reported that 19% chicken egg contents were contaminated with *E. coli* in Shahrekord, Iran. In the current study, the results of antibiotic sensitivity patterns of the isolated *E. coli* showed 71.42%, 71.42%, 64.28%, 57.14%, 50.00%, 35.71%, and 21.42% resistant to erythromycin, ampicillin, amoxicillin, tetracycline, ciprofloxacin, doxycycline, and chloramphenicol, respectively. The isolated *E. coli* was also showed 100%, 92.85%, 78.57%, 64.28%, 35.71%, 21.42%, 14.29%, 14.29%, and 14.29% sensitive to meropenem, ceftriaxone, gentamycin, chloramphenicol, ciprofloxacin, amoxicillin, doxycycline, ampicillin, and tetracycline, respectively. The antibiotic sensitivity and resistant patterns of *E. coli* are consistent with previous reports of Akond et al. (2009), Islam et al. (2018), Islam et al. (2008), Jain and Yadav (2017), Eid et al. (2015), and Adesiyun et al. (2007). Akond et al. (2009) reported that *E. coli* isolated from chicken eggs was resistant to erythromycin (64%), ampicillin (58%), tetracycline (52%),

ciprofloxacin (82%), and chloramphenicol (20%). Islam et al. (2018) stated that 100%, 88.89% and 77.78% of *E. coli* isolates was resistant to tetracycline, amoxicillin, and ampicillin, respectively prevalent in chicken eggs. Islam et al. (2008) reported that 66-100% *E. coli* strains isolated from chicken eggs showed resistant to tetracycline, erythromycin, and chloramphenicol in Bangladesh. Jain and Yadav (2017) reported that *E. coli* isolated from chicken eggs were 100% sensitive to gentamicin and ciprofloxacin. Eid et al. (2015) reported that 94% of *E. coli* isolated from backyard chicken eggs was resistant to five and more antimicrobial agents. Adesiyun et al. (2007) reported that *E. coli* isolated from chicken eggs was resistant to tetracycline in 35.9% and resistant to gentamicin in 11.5% but 46.6% of isolates were resistant to three or more antimicrobial agents. The variation in the sensitivity and resistant patterns among different studies may due to indiscriminate use of antibiotics.

Conclusion

The overall prevalence of *E. coli* was 38.89% in chicken eggs in Rajshahi district of Bangladesh. The isolated *E. coli* showed 71.42%, 71.42%, 64.28%, 57.14%, 50.00%, 35.71%, and 21.42% resistant to erythromycin, ampicillin, amoxicillin, tetracycline, ciprofloxacin, doxycycline, and chloramphenicol, respectively. In the context of this study, the prevalence of *E. coli* in chicken eggs and their antibiotic resistance is obviously significant. It could be concluded that the antibiotic resistant *E. coli* from chicken eggs may pose a public health hazard to consumers. Thus, the use of antibiotics in the poultry industry should be limited to reduce the development of antibiotic resistant *E. coli* strain.

Acknowledgements

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