Exploring Antibiotic Resistance Pattern of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp. Isolated from Eggs in Rajshahi

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ABSTRACT

Chicken eggs are a major component of people's diets, with an average yearly consumption of approximately 103 eggs per person in Bangladesh. Eggs act as an important carrier of food-borne pathogen worldwide. The study was conducted to identify the prevalence and antibiotic resistance pattern of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp., in eggs isolated from farms and different markets of Rajshahi, Bangladesh. A total of 60 eggs were collected randomly between April to December 2019. The isolation and identification of bacterial pathogen was done in accordance with standard procedures. The bacterial isolates were subjected to antibiotic susceptibility testing against seven commonly used antibiotics using Kirby-Bauer disk diffusion method. An overall prevalence of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. were found to be 35.0%, 28.33%, and 23.33%, respectively. *E. coli* were found highly resistant to penicillin (100%), tetracycline (80.95%), ampicillin (100%), and erythromycin (85.71%) and were sensitive to amoxicillin (71.42%), ciprofloxacin (85.71%), and gentamicin (95.23%). *Salmonella* spp. was highly resistant to penicillin (100%), erythromycin (82.35%) and tetracycline (82.35%), and was sensitive to gentamicin (94.11%), amoxicillin (76.47%) and ciprofloxacin (70.58%). *Staphylococcus* spp. was resistant to penicillin (100%), erythromycin (78.57%), tetracycline (85.71%), amoxicillin (100%), and ampicillin (100%) but sensitive to ciprofloxacin (85.71%), and gentamicin (92.85%). The higher prevalence of multidrug-resistant (MDR) bacteria can easily enter the food chain, which poses a public health threat.

Keywords: Antibiotic resistance, *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., Eggs.

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I. INTRODUCTION

The global poultry business has grown tremendously to meet the growing demand for animal proteins for human consumption. According to the Food and Agriculture Organization of the United Nations, annual egg production will reach 100 million tonnes by 2050 due to world

population growth [1]. One of the most expensive and ideal consumables is the egg. Eggs contain all of the nutrients required for organisms to develop and survive, including humans. It includes many vitamins A, D, E, K, B12, riboflavin, folic acid, pantothenic acid, zinc, phosphorus, choline, and selenium. At the same time, many of the nutrients included in eggs provide an ideal environment for

the growth of bacterial microflora, including pathogenic bacteria. Eggs can be contaminated or infected in two ways: horizontally (through the shell) or vertically (transovarially), making them a possible source of pathogens involved in the genesis of food-borne illness in humans [2]. Freshly deposited eggs are generally sterile; however, they can become a public health threat if infected, resulting in economic losses due to spoiling. With the rising popularity of eggs and egg products, it is more important than ever to look into the various variables that can lead to egg contamination. Deposition of faeces on the shell, ovarian or oviduct and gut flora, waste material, egg crates, packing and storage, poultry workers' clothes and hands, dust, the environment, weather conditions, transportation, and marketing can all cause eggshell contamination [3]. As a result, additional study on table eggs is urgently needed to protect poultry, the people, and animal health.

In the last few decades, antibiotics have considerably increased cattle and poultry output worldwide for the prevention and control of a number of bacterial diseases. Antibiotic resistance (ABR) in bacteria has been produced by the overuse and misuse of antibiotics, putting natural microbial systems under a lot of stress [4], [5]. Due to the widespread use of antibiotics as treatments and growth promoters in chicken farms, ABR has emerged as a major concern in clinical practice, representing a significant and global public health threat [6]. Each year, AMR is predicted to kill approximately 700,000 people around the world [7]. Antibiotic-resistant bacteria are now widespread in the environment, posing inversely cabalistic and contradictory effects on all aspects of one health, including animal, human, and environmental health [8], [9]. The uncontrolled and accidental use of antibiotics in poultry and a lack of fundamental information among the general population contribute to the development of antibiotic-resistant microorganisms in the environment [10]. Poultry, on the other hand, is permitted as a key growing source of ABR because to the tremendous selection pressure for ABR in *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. [11]. Furthermore, because antibiotic-resistant bacteria are found in chickens' guts, they can serve as reservoirs for disease transmission from poultry to humans, the environment, and other animals [12]. Antibiotic-resistant *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp. can also infect poultry farm environmental settings like feed, litter, water, air, and human hand washing through chicken droppings and table eggs. These resistant bacteria built up in the farm environment can be transmitted directly to farm workers, posing a significant human health risk [13].

E. coli, a zoonotic commensal pathogen, is a major organism in the global chicken industry, causing massive economic losses [14], [15]. Although most *E. coli* strains are non-pathogenic, only a few strains cause gastrointestinal (GI) illness since they are ubiquitous in the GI tract as part of both humans' and animals' natural microbial flora [16]. Furthermore, pathogenic *E. coli* strains can cause UTIs, abdominal sepsis, meningitis, and septicemia in humans, resulting in zoonotic disease [17]. *Salmonella* spp. is common food-borne pathogens that can also be zoonotic [18]. Poultry can serve as natural reservoirs for *Salmonella* spp., which can then be transmitted to humans and cause

Salmonellosis, including septicemia, enteric fever, and gastroenteritis [19], [20]. Several *Salmonella* serotypes have developed resistance to commonly used antibiotics, raising manufacturing costs [21]. *Staphylococcus* spp. is one of the most common human opportunistic pathogens, producing everything from minor skin infections to infective endocarditis, osteomyelitis, bacteremia, and necrotizing pneumonia [22]. Some strains of *Staphylococcus* spp. have developed a high level of resistance, such as methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to nearly every antibiotic used to treat Staphylococcal infections [23], [24]. In today's medicine, the development of antibiotic resistance in commensal bacteria is a critical and developing concern. Surveillance data on the presence of AMR bacteria in Bangladesh's chicken farming system, particularly table eggs, is crucial for developing AMR-related risk mitigation strategies. In light of the foregoing, the current investigation was conducted to isolate, identify, and profile the antibiotic sensitivity pattern of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. in table eggs from several retail markets in Rajshahi.

II. MATERIALS AND METHODS

A. Sources and Collection of Samples

In sterile polythene bags, 60 egg samples were collected from several retail markets in Rajshahi city. The samples were then immediately sent to the Department of Veterinary and Animal Sciences, Rajshahi University, for bacteriological investigation, all while maintaining sterile and cold chain conditions. Eggshell surface samples were collected in the laboratory using moist cotton swabs and gentle rubbing. The Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee (IAMEBBC) of the University of Rajshahi's Institute of Biological Science (IBSc) granted ethical approval for experiments on animals, humans, microbes, and living natural sources (Memo no:144/320/I.A.M.E.B.B.C./IBSc). All of the procedures were carried out under the applicable rules and regulations.

B. Isolation and Preservation of Bacteria

The samples were initially inoculated onto nutrient broth and immediately incubated at 37 °C for 24 hours following collection. Next, the bacteria were injected onto nutrient agar (NA) plates using the streak plate method after primary growth in nutrient broth and incubated at 37 °C for 24 hours. Finally, single individual colonies of different characteristics were injected into NA plates from the primary culture to obtain pure culture (subculture). The cultural procedure will be continued until pure culture is obtained [25]. Both agar slant and 20 percent pure buffered glycerin were used to maintain stock cultures [26].

C. Identification of Bacteria

Standard laboratory methods were used to identify the isolated organisms, including staining, motility, colony morphology, string test, and several biochemical assays such as sugar fermentation test, Catalase test, Coagulase test, Methyl red test, Voges-Proskauer test, and Indole test [24].

D. Antibiotic Susceptibility Test

Antibiotic sensitivity tests were performed using the disc diffusion test, which was modified somewhat from the Kirby-Bauer approach [27]. In a nutshell, 0.5 McFarland standards concentrations of freshly grown broth culture were put onto Mueller-Hinton agar (Hi-Media, India) plates and evenly distributed. Antibiotic discs were aseptically placed on the surface of the inoculation plates using sterile forceps and incubated for 24 hours at 37 °C. The plates were examined after incubation, and the sizes of the inhibitory zones were measured. The sizes were then classified as sensitive (S), intermediate (I), or resistant (R) using the NCCLS (1999) standard diameters (R). Penicillin (10 µg), erythromycin (15 µg), amoxycillin (10 µg), gentamycin (10 µg), ciprofloxacin (5 µg), and ampicillin (10 µg) were utilized for the disc diffusion test. These antibiotics were chosen at random based on their commercial availability and widespread use in the chicken industry in Bangladesh and worldwide.

III. RESULTS AND DISCUSSION

A. Overall Prevalence of Bacteria Isolated from Eggs

The table eggs play crucial role in the everyday diets of millions of people worldwide as it entails high nutritional value and digestibility as well as safety of use. Also, the presence of pathogenic bacteria in table chicken eggs may pose a serious health problem to human health like food poisoning and food-borne infections [28]. This study revealed that 86.67% of samples (52 out of 60) from four randomly selected retail marketplaces in Rajshahi, Bangladesh, tested positive for microbial contamination. Among them, 22 samples (36.67%) tested positive for *E. coli*, 18 samples (30%) tested positive for *Salmonella* spp., and 12 samples (20%) tested positive for *Staphylococcus* spp. (Table I). *Escherichia coli* are known to contaminate the surface of eggs, while the mechanical process can spread further the bacteria. The present study has demonstrated an overall prevalence (36.67%) of *E. coli* contamination in table eggs, where the highest prevalence was recorded 46.67% at Kazla market and the lowest 20.0% at Binodpur Bazar. The highest prevalence in the Kazla market was probably due to the poor hygienic practice in the market. This finding agrees with the previous study where the prevalence of *E. coli* was reported as 37.0% in table eggs [29]. In the present study, *Salmonella* spp. in table egg from the selective market was 30%. The highest prevalence was 33.33% at Binodpur and Kazla Bazar, and the lowest was 13.33% at Station Bazar, RU. Almost similar prevalence of *Salmonella* spp. (23.52%) was also reported previously [30]. Contamination of egg by *Salmonella* spp. may occur at any production stage like collection, transportation, or marketing either through vertical or horizontal transmission. Importantly, reusable egg tray is a potential source for contaminating eggshell by *Salmonella* spp. in developing country like Bangladesh. *Staphylococcus* spp. isolation rate in table egg from the different markets was 20%, where the highest prevalence was 26.67% at Binodpur and Station Bazar, RU and lowest 13.33% at Kazla and Katakhal Bazar. The above result is lower than the results reported by

Parveen *et al.*, [30]; the referred prevalence of *Staphylococcus* spp. was 25.49% in table egg. This is in agreement with Chaemsanit *et al.* [31], who found isolates from 15 different genera in chicken eggs and their contents, including *Staphylococcus* spp., *Micrococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *Neisseria* spp. [31]. Similarly, Salihu *et al.* [32] found that 100 percent of egg samples were positive for bacterial (nine different genera) contamination, including *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Corynebacterium* spp., *Proteus* spp., *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp. [32]. Hang' Ombe *et al.* [33] revealed microbial contamination of chicken eggs with primary members of the Enterobacteriaceae family, supporting the current findings [33]. Microorganisms can be discovered on both the exterior and interior of the eggshell, according to this study and USDA [34]. Microorganisms inside an uncracked egg or unbroken egg may be due to the pathogen's presence within the hen's ovary or through the oviduct before the shell forms around the yolk and albumin the egg comes from the hen's body through the same channel as feces is ejected. After the shells have been laid, fecal pollutants could enter through the pores. Egg contamination has been linked to several reasons. Bird faeces, litter, incorrect egg handling by retailers, unsanitary circumstances in markets where these eggs are sold, contaminated egg crates, packing, and poor storage methods are only a few of them [35]. Others include poultry employees' clothes and hands, surroundings, dust conveying marketing, improper retail store storage, and weather conditions.

TABLE I: PREVALENCE OF *E. COLI*, *SALMONELLA* SPP. AND *STAPHYLOCOCCUS* SPP. IN EGG SAMPLES

Source	<i>E. coli</i> positive (%)	<i>Salmonella</i> spp. positive (%)	<i>Staphylococcus</i> spp. positive (%)	Prevalence (%)
Binodpur Bazar	20	33.33	26.67	80
Kazla Bazar	46.67	33.33	13.33	93.33
Katakhal Bazar	40	40	13.33	93.33
Station Bazar, RU	40	13.33	26.67	80
Total	36.67	30	20	86.67

B. Antibigram Study

The in-vitro antibiotic sensitivity test results indicated that the isolated *E. coli* showed the highest resistance to penicillin and ampicillin, followed by erythromycin and tetracycline, with 100%, 100%, 85.71%, 88.89%, and 80.95%, respectively. This bacterium, on the other hand, showed the maximum sensitivity to gentamicin (95.23%), followed by ciprofloxacin (85.71%), and amoxicillin (71.42%) (Table II and Fig. 1).

In our study, *Salmonella* spp. had the highest resistance patterns against penicillin (100%), erythromycin (82.35%), and tetracycline (82.35%), while gentamicin, amoxicillin, and ciprofloxacin had the highest sensitivity rates of 94.11%, 76.47%, and 70.58%, respectively (Table III and Fig. 2).

TABLE II: ANTIBACTERIAL SENSITIVITY AND RESISTANT PATTERN OF *E. COLI*

No of isolates tested	Name of Antibiotics	Sensitivity pattern		
		Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
21	Penicillin	0	0	100
	Erythromycin	2 (9.52)	1 (4.77)	18 (85.71)
	Amoxicillin	15 (71.42)	5 (23.81)	1 (4.77)
	Tetracycline	3 (14.28)	1 (4.77)	17 (80.95)
	Gentamicin	20 (95.23)	1 (4.77)	0
	Ciprofloxacin	18 (85.71)	3 (14.29)	0
	Ampicillin	0	0	100

Fig. 1. Antibiotic sensitivity pattern of *E. coli* isolated from egg samples.

In the case of *Staphylococcus* spp., the majority of isolates were resistant to penicillin (100%), erythromycin (78.57%), tetracycline (85.71%), amoxicillin (100%), and ampicillin (100%), but susceptible to ciprofloxacin (85.71%), gentamicin (85.71%), and ampicillin (100%) (Table IV and Fig. 3). The findings of Papadopoulou [36], who found resistant strains of *Staphylococcus aureus* (to penicillin-G, tetracycline, erythromycin, clindamycin, cephalosporins, oxacillin, gentamycin, chloramphenicol, and tobramycin), *Enterococcus faecalis* (to ampicillin, ciprofloxacin, clindamycin).

TABLE III: ANTIBACTERIAL SENSITIVITY AND RESISTANT PATTERN OF *SALMONELLA* SPP.

No of isolates tested	Name of Antibiotics	Sensitivity pattern		
		Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
17	Penicillin	0	0	100
	Erythromycin	2 (11.77)	1 (5.88)	14 (82.35)
	Amoxicillin	13 (76.47)	3 (17.65)	1 (5.88)
	Tetracycline	1 (5.88)	2 (11.77)	14 (82.35)
	Gentamicin	16 (94.11)	1 (5.89)	0
	Ciprofloxacin	12 (70.58)	4 (23.59)	1 (5.88)
	Ampicillin	0	0	100

Fig. 2. Antibiotic sensitivity pattern of *Salmonella* spp. isolated from egg samples.

According to Jain and Yadav [37], isolated bacteria from eggs had the highest resistance rate (86.66%) to Cefixime, but the highest sensitivity rate (100%) to Gentamicin, Levofloxacin, and Ciprofloxacin. In addition, the majority of the isolates (73.3%) were discovered to be multi-drug resistant, meaning they were resistant to three or more antibiotics. According to Eid (2015), 94% of *E. coli* isolates were resistant to five or more antibiotics [38]. Another study by Adesiyun *et al.* [39] found that 46.6% of *E. coli* isolates were resistant to three or more antimicrobial drugs. *Salmonella* isolates were resistant to ampicillin, streptomycin, and tetracycline, according to Kilonzo-Nthenge *et al.* [40], whereas *E. coli* isolates were resistant to ampicillin and nalidixic acid. Ampicillin, tetracycline, and gentamycin resistance have also been documented in *Salmonella* spp. recovered from chickens [41]. *E. coli* isolated from meat and poultry has been found to be resistant to at least one antimicrobial treatment, according to recent research [42]. *S. aureus* strains were shown to be resistant to at least one of the antibiotics evaluated by Pyzik and Marek [28], with some (55.55%) showing resistance to five or more of the 17 therapeutic drugs. Resistance to erythromycin (66.66%), tetracycline (66.66 %), and oxytetracycline (61.11%), penicillin G (50%), and amoxicillin was found in the most significant number of strains (44.44%). The uncontrolled, random, and repeated use of these most resistant bacteria in chicken farms worldwide, including Bangladesh, may have resulted in their growth.

TABLE IV: ANTIBACTERIAL SENSITIVITY AND RESISTANT PATTERN OF *STAPHYLOCOCCUS* SPP.

No of isolates tested	Name of Antibiotics	Sensitivity pattern		
		Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
14	Penicillin	0	0	100
	Erythromycin	2 (14.28)	1 (7.14)	11 (78.58)
	Amoxicillin	1 (7.14)	3 (21.42)	10 (71.43)
	Tetracycline	2 (14.29)	0	12 (85.71)
	Gentamicin	13 (92.86)	1 (7.14)	0
	Ciprofloxacin	12 (85.72)	1 (7.14)	1 (7.14)
	Ampicillin	0	0	100

Fig. 3. Antibiotic sensitivity pattern of *Staphylococcus* spp. isolated from egg samples.

IV. CONCLUSION

The table eggs of commercial layers are infected with antibiotic-resistant *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. These antibiotic-resistant bacteria could potentially be transmitted to humans through tainted eggs.

Antibiotic resistance in microorganisms from chicken eggs should be taken seriously as a public health issue.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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