Prevalence and Antimicrobial Resistance of Major Pathogenic Bacteria Isolated from Table Egg Shell in the Retail Market of Dhaka City, Bangladesh

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Abstract
The current research was conducted to isolate and identify pathogenic bacteria from table eggs, with special emphasis on E. coli, Salmonella spp., and Staphylococcus spp., which are responsible for foodborne illnesses. This investigation aims to understand better the specific types of harmful bacteria that can contaminate food, thereby posing a risk to public health. By isolating these microorganisms, the study seeks to provide valuable insights into their characteristics and behaviors, which could ultimately help in developing strategies to prevent foodborne diseases. Approach forward to isolate, identify and characterize the bacterial species based on the results of cultural, morphological, biochemical tests and staining. The antimicrobial susceptibility patterns of isolates were scrutinized by the disc diffusion method. A total number of 40 eggs were collected from different markets of Dhaka city, such as Krishi market, Bihari camp market, Agargaon market and SAU market. *E. coli* from 20 samples, *Salmonella* spp. from 11 samples and *Staphylococcus* spp. from 7 samples were isolated from total of 40 samples where prevalence was 50%, 27.5% and 17.5%, respectively Isolated bacteria were found resistance to some of commonly used antibiotics in in veterinary and human practices like- neomycin, penicillin, amoxicillin and erythromycin and sensitive to gentamycin, ciprofloxacin and tetracycline. The risk of contamination in eggs can be minimized by proper hygiene practice, cleaning of the egg before storage and shorter storage time.

Keywords: Pathogenic bacteria, prevalence, antibiogram, table egg

1. Introduction

Food-borne illness is a major public health concern and the main cause of diarrheal diseases in people worldwide (Akbar *et al*. 2014; Ejo *et. al.,* 2016). A large number of people from both developing and developed countries suffer from food-borne diseases every year, but the trend is comparatively higher in developing countries like Bangladesh (Ejo *et. al*., 2016), and around 600 million (10 people in the world) become sick due to the consumption of contaminated food (Guerra *et al*., 2016). According to the WHO, 30% of the population suffers from foodborne infection every year in developed countries and up to 2 million people are estimated to die each year in developing countries (Abunna *et al.,* 2016). Consuming undercooked or contaminated eggs has been linked to egg-borne infectious illnesses, which are a major global public health concern (Ema et al., 2018).

According to Stępień-Pyśniak (2010), eggs can get contaminated either vertically (trans-ovarian) or horizontally (through the shell), making them a possible source of harmful germs. According to Al-Bahry et al. (2012), fresh eggs are usually sterile, but contamination of the egg shell can occur from a variety of sources, including dust, ovarium or oviduct and gut flora, debris, egg crates, packing and storage, poultry workers' hands and clothing, the environment, weather, transportation, and marketing. Gram-positive bacteria like S. aureus and Gram-negative bacteria like Salmonella and E. coli can cause food poisoning episodes when eggs are contaminated at both

the egg shell and the egg contents (Ricke et al., 2011; Ashraf et al., 2018; Ema et al., 2018; Wang et al., 2020). The yolk, albumen, and shell membrane or egg shell are the three primary areas of the egg where these bacterial contaminations might occur (Bahrouz, 2005). According to Mead et al. (1999), salmonellosis is the most common foodborne bacterial disease in many countries worldwide, with an estimated 1.4 million cases per year in the USA. From 2005 to 2008, a number of Salmonella spp. outbreaks were documented in Tasmania, Australia (Hawkey et al., 2013). The most significant cause of diarrhea in children and a significant public health issue in underdeveloped nations is strains of Escherichia coli (Toma et al., 2003). In contrast, enterotoxemia in humans and other animals is caused by Escherichia coli. Furthermore, the food sector depends on S. aureus in human consumption items because certain strains can result in food-borne poisoning (Tsepo et al., 2016). According to Beyene et al. (2017), they are the source of food-borne illness, food deterioration, and a decrease in food safety and shelf life. As these are zoonotic food-borne bacteria, isolation, identification and antibiogram study are needed to establish the pathogenic reasons and therapeutic targets that are effective against these bacteria. Antibiogram research would identify any cross-resistance and pave the way for antimicrobial resistance and successful antibiotic treatment.

So, this study has an importance for understanding risks of egg-borne diseases on human health in Dhaka city and will help to take necessary measures to reduce the risk by creating public awareness, improving knowledge among consumers. Considering the mentioned facts, the study was designed with the objectives of (i) isolating the pathogenic bacteria from eggs available at different markets in Dhaka city by cultural, morphological, and biochemical properties, (ii) to determine the prevalence of isolated bacteria, and (iii) to know the antibiogram profile of isolated bacteria.

2. Materials and Methods

2.1 Sample collection and preparation

The research work was conducted in the Microbiology & Parasitology laboratory, Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period from January to July 2018. Samples of approximately 40 table eggs were collected from different selective markets of Dhaka city by using an ice box. Collected samples were immediately transported on ice to the laboratory of Microbiology and Parasitology, SAU, for bacteriological cultural analysis.

2.2 Isolation of pathogenic bacteria from egg sample

Samples were cultured primarily in nutrient broth at 37ºC for 24 hours for enrichment and then it was streaked onto Nutrient agar. A loopful colony from nutrient agar was streaked on Mannitol salt (MS) agar, MacConkey agar and Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar and incubated at 37°C for 24 hours. Colonies of bacteria were sub-cultured onto the MS (for *Staphylococcus* spp.), EMB (for *E. coli)*, and SS (for *Salmonella* spp.) agar to get a pure culture.

2.3 Identification of isolated bacteria

Identification of isolated bacteria was done based on the observation of colony morphology, like shape, size, surface texture, edge and elevation, color and opacity developed on various selective media; Gram's staining reaction and different biochemical tests (Cheesbrough, 1985).

2.4 Gram's staining reaction

All suspected cultures of *Staphylococcus* spp., *E. coli and Salmonella* spp. were subjected to Gram's staining reaction and observed under an electronic light microscope (100X) using immersion oil. Gram's staining reaction was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria.

2.5 Biochemical tests

The bacterial isolates were characterized biochemically to evaluate their chemical nature. We conducted sugar fermentation test, catalase test, coagulase test, Voges-Proskauer (V-P) test, Methyl-Red (M-R) test and indole production test according to the standard protocols (Cheesbrough, 1985).

2.6 Antibiotic sensitivity test

Antibiotic sensitivity tests were performed against eight different types of antibiotic disc (table 1) for all isolates (*Staphylococcus* spp., *E. coli and Salmonella* spp.) to determine their antibiotic-resistance profiles by disc diffusion or Kirby–Bauer method (Bauer *et al.*, 1966). Results of antibiotic sensitivity tests were then classified as resistant, intermediately resistant or susceptible to a particular antibiotic based on the standard interpretation table (Table 2), and updated according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). Multiple drug-resistant phenotypes were recorded for isolates showing resistance to three and more antibiotics.

Table 1: Antibiotic discs used in this study with the concentrations of antibiotics

Name of the antibiotics	Symbol	Dose (µg/disc)
Penicillin	P	10
Erythromycin	E	15
Tetracyclin	TE	30
Amoxicillin	AML	10
Gentamycin	GM	10
Ciprofloxacin	CIP	5
Neomycin	N	30

3. Results and Discussion

3.1 Occurrence of *Staphylococcus spp., E. coli and Salmonella spp.*

Out of 40 samples of table egg shells in the study area, 7 (17.5%) were positive for pathogenic *Staphylococcus spp.,* 20 (50%) were positive for *E. coli* while 11 (27.5%) yielded *Salmonella* spp. (table 2)

Table 2: Occurrence of Staphylococcus spp., E. coli and Salmonella spp. isolated from table eggs

Pathogens	Number of samples tested	Positive samples	Occurrence (%)
Staphylococcus spp.	40	7	17.5
E. coli	40	20	50
Salmonella spp.	40	11	27.5

3.2 Market-wise occurrence of *Staphylococcus* spp., *E. coli* and *Salmonella* spp.

Among *E. coli* positive samples, the highest occurrence was recorded in the Bihari Camp market, whereas the highest occurrence of *Salmonella* spp. and *Staphylococcus* spp. was recorded in the Krishi market and Agargaon market, respectively (Figure 1).

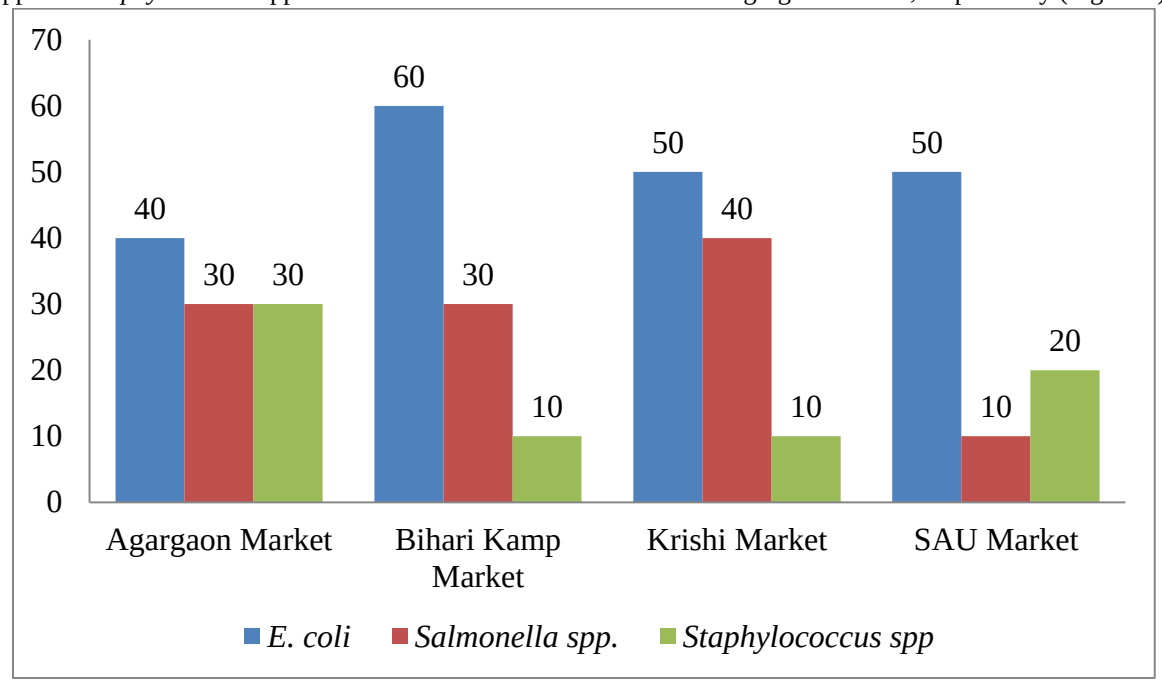

Figure 1: Occurrence of *Staphylococcus* spp., *E. coli* and *Salmonella* spp. isolated from table eggs in the different markets of Dhaka city

3.3 Antibiotic sensitivity test

Three isolates in total, such as *Staphylococcus* spp., *Escherichia coli* and *Salmonella* spp., were subjected to an antibiotic sensitivity test. The antibiotic sensitivity test (figures 2,3,4) revealed that the isolated *Staphylococcus* spp. was only sensitive to erythromycin, gentamicin, ciprofloxacin and tetracycline while resistant to neomycin and penicillin. *E. coli* was found sensitive to gentamicin, ciprofloxacin and tetracycline while resistant to penicillin, erythromycin and amoxicillin. *Salmonella* spp. exhibited sensitivity to gentamicin, ciprofloxacin and tetracycline, while intermediate sensitive to erythromycin, resistant to penicillin and amoxicillin. The results of the antibiotic sensitivity testing are presented in Table 3.

Table 3: Resistance pattern of antibiotics against isolated *Staphylococcus* spp. *E. coli* and *Salmonella* spp. from table egg shells

Name of antibiotic disc	Staphylococcus spp.		*E. coli*		*Salmonella spp.*	
	Diameter of zone of inhibition (mm)	Interpretation	Diameter of zone of inhibition (mm)	Interpretation	Diameter of zone of inhibition (mm)	Interpretation
Neomycin	0	R				
Amoxicillin			0	R	0	R
Penicillin	0	R	0	R	0	R
Erythromycin	19	S	0	R	0	IS
Gentamicin	17	S	15	S	14	S
Ciprofloxacin	23	S	24	S	22	S
Tetracycline	22	S	16	S	17	S

R= Resistant, S= Sensitive, IS= Intermediate sensitive

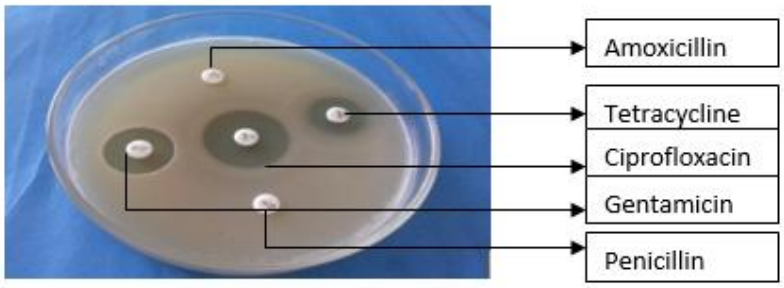

Figure 2: Results of the antimicrobial profile of *E. coli* against antibiotics

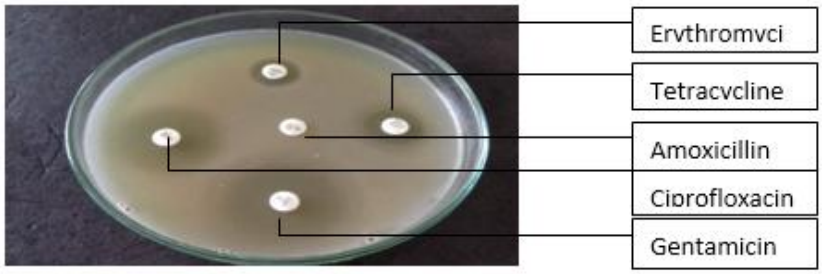

Figure 3: Results of the antimicrobial profile of *Salmonella* spp.

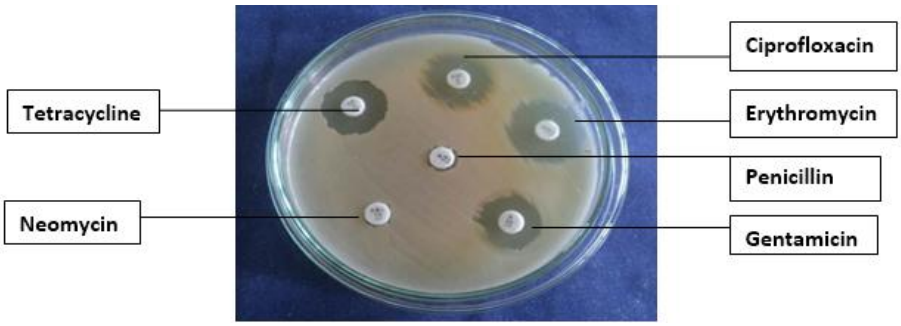

Figure 4: Results of antimicrobial profile of *Staphylococcus* spp.

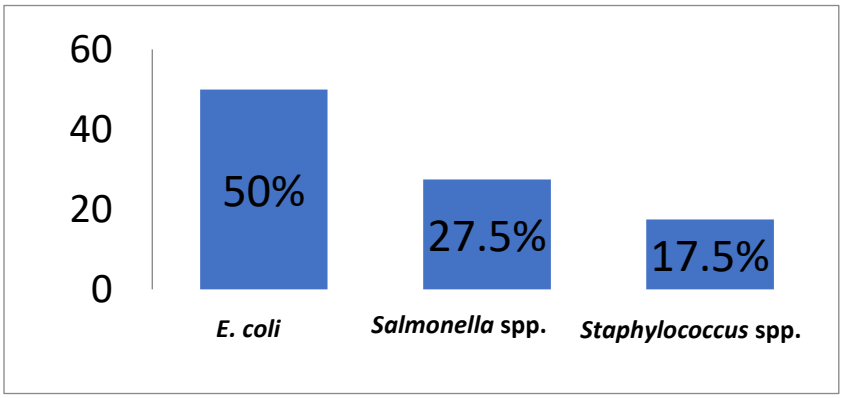

Figure 5: of E. coli, Salmonella spp. and Staphylococcus spp.

Egg-borne bacterial contamination is a common outcome from poultry and has a serious public health importance. These pathogenic bacteria can transmit vertically and can cause egg-borne disease like Salmonellosis, *Colibacillosis* and Staphylococcal infection (Gantois *et al.*, 2009; Parveen *et al.*, 2017). The present study showed that there is high contamination of table egg shells with *E. coli* (50%) and *Salmonella* spp. (27.5%) in Dhaka city and the result supported by USDA, 2011 and Okorie-Kanu *et al.*, 2016. The egg and the feces share the common route and environment within and outside the laying bird; this could be the reason for the high prevalence of bacteria. The present study has demonstrated that eggs from the Biharikamp market (60%) have significantly higher contamination with *E. coli*, whereas the lowest rate was found in the Agargaon market (40%). This finding is in agreement with the previous study where the prevalence of *E. coli* was reported as 37.0% in table egg shells (Aur-Angzeb *et. al.*, 2015). The highest prevalence in the Bihari Kamp market was probably due to the poor hygienic practices in the market and overcrowding.

The highest contamination rate of *Salmonella* spp. in egg shells was detected in the Krishi market (40%) and the lowest was recorded in the SAU market (10%). Similar prevalence of *Salmonella* spp. (23.5%) was also reported previously (Parveen *et al.* 2012). Krishi Market is one of the biggest retail markets in Dhaka city, where eggs are stored and displayed for a long period. This could be the reason for the high prevalence of *Salmonella* spp. in the Krishi market. On the other hand, the SAU market is a small out-of-market where products are sold daily, and therefore, there is less chance of contamination with microbes.

Staphylococcus spp. is the organism that might cause human infection with the production of toxins. Contamination with *Staphylococcus* spp. in table egg shells was found to be high in Agargaon market (30%) and low in Biharikamp market (10%). The above result is lower than the results reported by Parveen *et al.* (2012) where the prevalence of *Staphylococcus* spp. was reported as 25.49% in table egg shells

Colony morphology of *Escherichia coli* in MacConkey Agar and EMB agar showed large, pink color colonies and greenish with metallic-sheen-like sheen like colonies, respectively. These results were similar to the findings of Hossain *et al.* (2008), Dey *et al.* (2013) and Norhan *et al.*, (2014. The colonies of *Staphylococcus* spp. observed in MSA and produced golden yellow colonies, which have

morphological similarity with Konuku *et al*. (2012). On SS agar, *Salmonella* spp. showed to be non-lactose fermenting with black centers.

In Gram stain, *Salmonella* spp. showed Gram-negative, short rod with single arrangement, as recorded by Samad (2005), Freeman (1985), *Escherichia coli* revealed Gram-negative, pink color, small rod-shaped appearance, arranged in single or paired short colonies, which is similar to the finding of Khaton *et al*. (2008), Joshi *et al*. (2012) and Maha and Al-Ashmawy (2013). Microscopically, *Staphylococcus* spp. were Gram-positive cocci arranged in grape-like clusters, as reported by Brooks *et al.* (2002) and Habib *et al.* (2015).

The isolated and identified bacteria were reconfirmed using different biochemical tests. Isolated *Escherichia coli* was positive in sugar fermentation tests, and fermented all sugars with acid and gas production. *Escherichia coli* was positive to the MR and Indole test, whereas negative to the VP test. *Salmonella* spp. was positive to sugar fermentation tests and fermented all sugars with acid and gas production except sucrose. *Staphylococcus* spp. was positive to the MR, VP test, but negative to the Indole test. *Staphylococcus* spp. was positive for to catalase test and the coagulase test. All these results were found similar to the findings of Khaton *et al.* (2008), Dey *et al*. (2013) and Adeyanju and Ishola (2014).

The potential spread of multi-drug resistant (MDR) bacteria from birds to humans has recently attracted a lot of attention to antibiogram profile studies of bacterial isolates from different chicken sources. The resistance of E. to antibiotics. When coli obtained from table egg shells were examined, it was discovered that they were extremely resistant to amoxicillin, erythromycin, and penicillin, but sensitive to gentamycin, ciprofloxacin, and tetracycline. Escherichia coli from poultry sources were found to be resistant to penicillin, rifampicin, kanamycin, streptomycin, cefixime, erythromycin, ampicillin, chloramphenicol, and neomycin in 88%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of cases, according to Akond et al. (2009). For norfloxacin, gentamicin and chloramphenicol, neomycin, tetracycline, streptomycin, and ampicillin, the corresponding sensitivity values were 86%, 80%, 60%, 36%, 30%, and 26%. Because of their structural differences, Escherichia coli was resistant to erythromycin, amoxicillin, and penicillin. This antibiotic cell wall has a peptidoglycan layer, which makes the antibiotic resistant to Escherichia coli. The identified Salmonella species were susceptible to gentamycin, tetracycline, and ciprofloxacin, but extremely resistant to penicillin, erythromycin, and amoxicillin. Salmonella spp. was reported to be 100% sensitive to gentamicin, 91.67% sensitive to cefixime, ciprofloxacin, amikacin, and levofloxacin, and 83.33% sensitive to cefpodoxime and amoxicillin by Rather et al. (2012). As a result, Salmonella species exhibited resistance to rifampicin (91.7%), erythromycin (100%), and nalidixic acid (100%). The identified Staphylococcus species were susceptible to ciprofloxacin, erythromycin, tetracycline, and gentamicin, but resistant to neomycin and vancomycin. These results are nearly consistent with those of Brintya et al. (2014). They discovered that S. aureus was extremely vulnerable to ciprofloxacin (94.4%), streptomycin (86.1%), and gentamycin (78.6-91.7%). Staphylococcus species were susceptible to ciprofloxacin, gentamicin, erythromycin, and tetracycline, but resistant to penicillin and amoxicillin.

Conclusions

Based on the results, it can be concluded that food-borne pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp. *and Staphylococcus* spp. were found in egg shells with significant occurrence. This contamination might be due to prolonged storage of eggs and unhygienic conditions in the market. Forever isolated bacteria were found resistant to some of the commonly used antibiotics (neomycin, penicillin, amoxicillin and erythromycin) that are used in veterinary and human practices. It has a significant public health importance because it could be transmitted to the human food chain.

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Authors contributions

AAM and TS designed the experiment. AAM and TS performed experiments. AAM analyzed the data. AAM drafted the manuscript. MMR and AAM critically revised the manuscript.

Conflict of interest

The authors declare no conflict of interest; all authors contributed equally. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

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