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Original Article

Isolation and identification of duck egg-borne bacteria and their antibiogram profile

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ABSTRACT

Objective: The present study was aimed to isolate and identify the egg-borne bacteria from different parts of duck eggs such as egg shell (outer and inner), yolk and albumen, and to assess the anti-biogram profile of the isolated bacteria.
Materials and methods: A total of 40 samples were collected randomly from different grocery shops of Bangladesh Agricultural University (BAU) Campus and Kaowatkhali, Mymensingh. Following necessary preparation, the samples were streaked onto various selective media like Salmonella-Shigella (SS) agar (for *Salmonella spp.)*, Eosin Methylene Blue (EMB) (for *E. coli*), and Mannitol Salt (MS) agar (for *Staphylococcus spp.*) respectively for isolation of bacteria. The bacteria were confirmed based on cultural and biochemical characteristics. Antibiotic sensitivity test of the bacterial isolates was performed using seven antibiotics (Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Vancomycin, Kanamycin and Cephalexin) by following disc diffusion method.
Results: *E. coli*, *Staphylococcus spp.* and *Salmonella spp.* were isolated and identified from the duck egg samples. Prevalence of *E coli* in outer egg shell was 80%, whereas in inner egg shell and inner egg content, this prevalence was 20% and 10%, respectively. Similarly, the prevalence of *Staphylococcus spp.* was 75%, 17.5% and 7.5% in outer egg shell, inner egg shell and inner egg content, respectively. The prevalence of *Salmonella spp.* was 82.5% in outer egg shell, 22.5% in inner egg shell and 12.5% in inner content of egg. All these three bacterial isolates were sensitive to Ciprofloxacin and Gentamicin and resistant to Ampicillin and Cephalexin.
Conclusion: The duck eggs harbor multi-drug resistant (MDR) bacteria which may impose public health hazards if these MDR bacteria are transferred to human through food chain.

KEYWORDS

Antibiogram; Duck egg; Egg-borne bacteria; Food safety; Public health

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INTRODUCTION

Poultry is a promising sector for poverty reduction in Bangladesh. Among all of poultry species, chicken and duck are the most popular species that supply egg and meat which provides a unique, well balanced of nutrients for persons of all ages (Layman and Rodriguez, 2009). Egg is an excellent source of choline and selenium and a good source of vitamin B_{12}, riboflavin and phosphorus. The yolk contains different vitamins such as A, D, E and K as well as folic acid and zinc (ENC, 2004). Food-borne illnesses comprises of a variety of diseases which is responsible for causing morbidity and mortality worldwide. Egg-borne infectious diseases are of great public health concern worldwide and many outbreaks of food-borne diseases particularly those of gastro-enteric in nature have been reported due to consumption of undercooked and contaminated eggs. Among the various poultry product-related food-borne pathogens, gastrointestinal infections caused by egg-borne non typhoidal *Salmonella* is a major concern in developed and developing countries (Chousalkar and Gole, 2016). Majority of 166 outbreaks in Australia during a period of 2001 to 2011 were linked to commercial food providers, with raw eggs that resulted in more than 3200 cases, more than 650 hospitalizations, and at least 4 deaths (Moffatt et al., 2016). Since 2010, consumption of duck eggs is identified as the major cause of human salmonellosis outbreaks in the UK (Owen et al., 2016).

In Europe, salmonellosis is considered to be a major cause of food-borne outbreaks, associated with eggs and egg products (Jakociune et al., 2014). An outbreak in New South Wales was confirmed as salmonellosis in eight of 45 residents due to consumption of a dessert containing raw eggs during July to August 2008 (Roberts-Witteveen et al., 2009). A series of *S. typhimurium* outbreaks were reported in Tasmania, Australia during 2005-2008, that were all identified as eggs originating from a single chicken farm (Hawkey et al., 2013). Sixty six cases were identified due to *S. typhimurium*m within 135 cases during March 2007 and January 2008 (Stephens et al., 2008).

The extent of egg spoilage due to effect of microorganisms is very high which result in big economic losses (Saif et al., 2009; Howard et al., 2011). At the beginning, the microbial load is very low but it increases when the shell acquires at oviposition, a few are from the vent and others from the nesting materials and feces. Besides these egg can be contaminated from different stages like during collection, handling, storage and transportation. Among the various microorganisms, the well-known enteric pathogens particularly *Salmonella, Escherichia coli, Campylobacter spp.* and *Listeria spp.* were isolated from table eggs and their contents (Adesiyum et al., 2005). The transmission of the disease from ducks to humans has been suspected. Risk of egg borne disease strongly increases because of unhygienic conditions of egg production and improper practices of egg handling, including also storage times and temperatures. If all the necessary precautions are not taken during the poultry production, marketing and processing chains in that case poultry meat and eggs can be contaminated by infectious agents that are harmful to humans. So, this study holds a great importance to understand the present risks of duck egg borne diseases on human health and will help to take necessary measures to reduce the risk by creating public awareness, improving knowledge in rural women, good hygiene practices, thorough cooking, provision of vaccines and essential medicines and development of linkages with the different agencies. Considering the above facts, the objectives of this research were- (i) to isolate the bacteria from duck egg available at Bangladesh Agricultural University (BAU) campus, (ii) to identify the bacteria by morphological, cultural and biochemical properties, (iii) to determine the prevalence of isolated bacteria, and (iv) to know the antibiogram profile of bacterial isolates.

MATERIALS AND METHODS

Collection of samples: The study was performed during the period from January to June 2015, where eggs were collected for once and examined for the detection of organisms. A total of 40 fresh egg samples were obtained from different parts of the each egg like from outer shell, inner shell, albumin, yolk surface and yolk. For this, the total number of samples became 200. Samples were taken randomly from the different grocery shops situated at BAU campus and Kaowatkhali, Mymensingh.

Isolation of bacteria: Samples were enriched in nutrient broth at 37°C for 24 h and then it was streaked onto nutrient agar at 37°C for 24 h. A loopfull colony from nutrient agar was streaked onto Mannitol salt (MS) agar, Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar and incubated at 37°C for 24 h. Sub culture was performed onto the MS, EMB, and SS agar to obtain pure culture.

Identification of isolated bacteria: The cultural examination of different portions of egg samples for bacteriological analysis was done according to the

standard method (ICMSF, 1985). Identification of bacteria was performed on the basis of colony morphology (shape, size, surface texture, edge and elevation, color and opacity developed on various selective media); Gram's staining reaction; motility test and biochemical tests like sugar fermentation test, catalase, coagulase, Methyl-Red (M-R), Voges Proskauer (V-P), and indole tests (Cheesbrough, 1985).

Antibiotic sensitivity test: Antibiotic sensitivity test against seven commonly used antibiotics (**Table 1**) were done by disc diffusion or Kirby–Bauer method (Bauer et al., 1966). The zones of growth inhibition were compared with the zone-size interpretative standard for *E. coli* and *Staphylococcus spp. Salmonella spp.* provided by Clinical and Laboratory Standards Institute (CLSI, 2007). Antimicrobial testing results were recorded as resistant, intermediate and sensitive.

Table 1: Antimicrobial agents with their disc concentrations

Antimicrobial agents	Symbol	Disc concentration
Ampicillin	AMP	10 µg
Vancomycin	VAN	10 µg
Chloramphenicol	C	30 µg
Ciprofloxacin	CIP	5 µg
Kanamycin	KAN	30 µg
Gentamicin	GEN	10 µg
Cephalexin	CN	30 µg

Source: CLSI (2007).

RESUTS AND DISCUSSION

Salmonella enteritidis (SE) has been considered as the major cause of the food-borne illness in humans and have the ability of contaminating table eggs which may act as most important vehicle of the infection (Gantois et al., 2009). As *Salmonella* has the ability of vertical transmission, it is regarded as egg-borne disease and also there are other pathogens like *E. coli, Staphylococcus spp.* and *Bacillus spp.* (Parveen et al., 2017) which are also associated with human infections through egg transmission, as they can contaminate eggs through the outer shell surface.

In this study, three species of bacteria namely, *E. coli, Staphylococcus spp.* and *Salmonella spp.* were isolated and identified. *E coli* was found as 80% on outer egg shell and as 10% in inner egg content (**Figure 1**). The above result is quite similar with the results reported by Adesiyum et al. (2005) referring a prevalence of *E. coli* as 88.3% in outer egg shell and 7.2% in inner content of egg. Stępień-Pyśniak (2010) found prevalence of *E. coli* as 58.7% in outer shell and 4.3% *E. coli* in inner content. Adesiyum et al. (2005) found prevalence of *E. coli* as 58.7% on shells, 8.3% in egg contents and in all parts at the same time as 12.7% in farm eggs.

In this study, we found 82.5% *Salmonella* in outer egg shell and 12.5% in inner content of egg (**Figure 1**). Stępień-Pyśniak (2010) found prevalence of *Salmonella* as 84% in egg shell and 8.7% in inner content of egg. Mahmud et al. (2015) found 86% *Salmonella* in poultry eggs, of which 83% from outer shell of eggs and 3% from egg contents. Musgrove et al. (2004) showed the prevalence of *Salmonella* ranged from 57 to 94%.

In this study, *Staphylococcus* was found as 75% on outer egg shell and 7.5% in inner content of egg (**Figure 1**). Samah et al. (2015) detected 40% coagulase positive *Staphylococcus* from chicken eggs. Egg is originally designed to create a chick and it has a complete life support system with many natural barriers to prevent bacterial entrance and growth and protecting the developing embryo (Latif et al., 2015). Eggs can be contaminated through the outer shell surface and internally. Penetration through the egg shell or by direct contamination of egg contents before oviposition, originating from infection of the reproductive organs are considered as the main cause of internal contamination (Gantois et al., 2009).

We all know that duck eggs have thicker shells, a heavier and more waxy coating than chicken eggs but duck eggs can be contaminated by the *Salmonella spp.* as it can infect reproductive organs of duck and can transmit by the eggs (Gantois et al., 2009). Eggs may also become susceptible to bacterial growth if the shell membranes are broken or may have cracks (Latif et al., 2015).

Colony characteristics of *E. coli* observed in EMB agar showed metallic sheen (greenish black) colony which was similar to the finding of Hossain et al. (2008) and Norhan et al., (2014), morphological characteristics of *E. coli* observed in the different cultural media was similar to the findings of Mishra et al. (2002), Thomas et al. (2005) and Dey et al. (2013). The colonies of *Staphylococcus aureus* fermented mannitol and produced golden yellow colonies on mannitol salt agar which were characteristically similar to the report of Konuku et al. (2012).

Morphological and staining characteristics of bacteria were recorded from eggs by Gram stain. In Gram stain, *Salmonella spp.* revealed short rod, Gram negative, single or pair in arrangement, as reported by Samad (2005), Freeman (1985) findings; *E. coli* revealed short plump rod, Gram negative, single, paired or in short chain

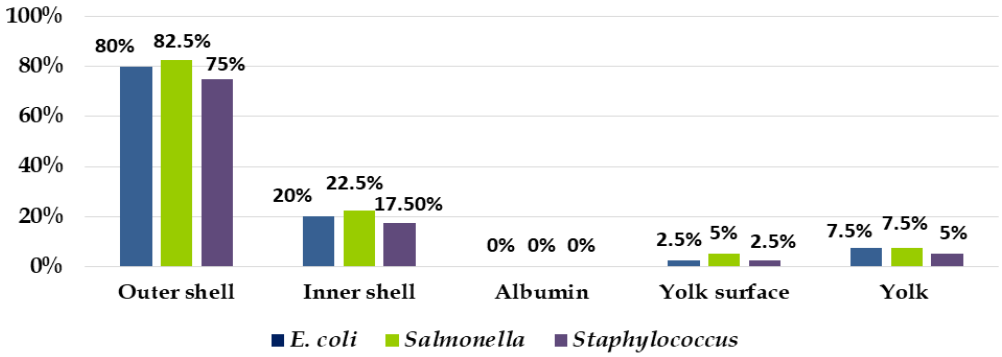

Figure 1: Prevalence of *E. coli, Salmonella spp.* and *Staphylococcus spp.* in the different parts of eggs.

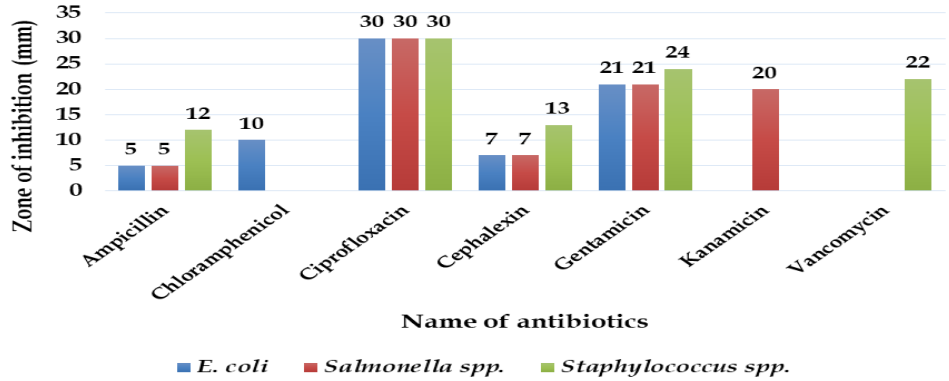

Figure 2: Summary of antibiogram profile of *E. coli, Salmonella spp.* and *Staphylococcus spp.* using different antibiotics.

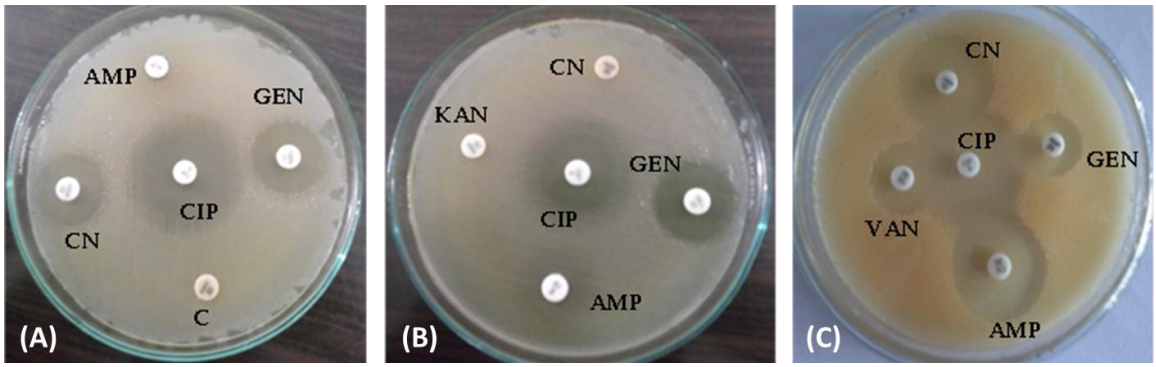

Figure 3: Antimicrobial profile of *E. coli* against Ciprofloxacin (CIP), Cephalexin (CN), Ampicillin (AMP), Chloramphenicol (C) and Gentamicin (GEN) **(A)**. Antimicrobial profile of *Salmonella spp.* against Ampicillin (AMP), Cephalexin (CN), Kanamycin (KAN), Gentamicin (GEN) and Ciprofloxacin (CIP) **(B)**. Antimicrobial profile of *Staphylococcus spp.* against Ciprofloxacin (CIP), Cephalexin (CN), Vancomycin (VAN), Ampicillin (AMP) and Gentamicin (GEN) **(C)**.

in arrangement similar to the characteristics reported by Khaton et al (2008), Joshi et al. (2012) and Maha and Al-Ashmawy (2013). Microscopically, *Staphylococcus spp.* was Gram positive cocci arranged in grape like cluster, as reported by Brooks et al. (2002) and Habib et al. (2015).

E. coli and *Salmonella spp.* were found motile as they caused turbidity of MIU media and *Staphylococcus spp.* were non motile because of inability to show turbidity on MIU media.

The identified bacteria were re-confirmed through the use of different sugar fermentation and other biochemical test. One of the important facts for the isolation of coagulase-positive *Staphylococcus* was that the organism might cause human infection with production of toxin. *E. coli* produces acid-gas and *Salmonella spp.* produce acid in different sugar fermentation tests, whereas *Staphylococcus spp.* produces none. All were positive in M-R test. *Staphylococcus spp.* was positive in V-P test *E.coli* was positive in Indole production test. Both *E. coli* and

Salmonella spp. were catalase negative. *Staphylococcus spp.* was catalase positive. All these were found similar with the finding of Khaton et al. (2008), Dey et al. (2013) and Adeyanju and Ishola (2014).

A total of three isolates such as *Salmonella spp., E. coli* and *Staphylococcus spp.* were subjected to antibiotic sensitivity assay. The antibiotic sensitivity test revealed isolated *Salmonella spp.* was only sensitive to CIP, KAN, GEN and resistant to AMP, CN and this result agree with Cox et al. (2006) and Pyzik and Marek (2013). *E. coli* were found sensitive to CIP and GEN and resistant to AMP, CN and C. The result is in assessment with Pyzik and Marek (2013) who showed resistance to amoxicillin, which was not in agreement with Cox et al. (2006), who reported resistance to GEN and CIP. *Staphylococcus spp.* were found sensitive to VAN, CIP, C and GEN and resistant to CN and AMP, which was differed to the reports of Pyzik and Marek (2013) and Yurdakul et al. (2013), who reported resistant to GEN, AMP and VAN. The results of the antibiotic sensitivity test are presented in **Figure 2-3**.

CONCLUSION

The prevalence of *Salmonella*, *E. coli* and *Staphyloccus spp.* in outer egg shell are 82.5, 80 and 75%, respectively. In inner egg shell, the prevalence are 12.5, 10 and 7.5%, respectively. The presence of MDR bacteria in duck egg particularly in the inner content of egg is alarming as they cause public health hazards. Findings of this study indicate the importance of improving hygienic measures and increasing public awareness of sanitation during egg production, handling, transportation and processing to prevent the spread of resistant bacteria and food-borne illness through consumption of these contaminated eggs.

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CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

FAE, MAI and MMK designed the experiment. FAE collected the samples and conducted an experiment. FAE, MA drafted the first version of the manuscript. MMK and MAI critically reviewed the article and finally approved for publication.

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