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Isolation and identification of bacteria from shell surface of eggs in local market of Sylhet

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ABSTRACT

Two categories of duck and hen eggs (clean and dirty) were selected from wholesalers for this study from January- June, 2013. After collection and transportation to the laboratory bacteriological analysis was performed under two major principles of assessments. At first microbiological quality was evaluated and then Total viable count (TVC), Total coliform count (TCC) and Total Salmonella Count (TSC) were performed. A total of 40 egg (20 egg of hens and 20 eggs of duck) samples were subjected to assessment on microbiological quality. The TVC, TCC, TSC of clean (intact) eggs of the egg shell of duck were log 4.94, log 4.70 and log 4.75, respectively and in case of hen eggs values were log 4.73, log 4.50 and log 4.53, respectively. The mean value of TVC, TCC and TSC of dirty eggs of the egg shell of duck were log 5.42, log 4.87 and log 4.76, respectively and in hen eggs the values were log 5.25, log 4.83 and log 4.67, respectively. The highest significant correlation was found between TVC of clean hen and TSC of dirty hen eggs. The correlation among TVC, TCC and TSC of clean duck and dirty duck eggs ranged from 0.0174 to 0.8055. The highest significant correlation was found between TCC of clean duck and TSC of dirty duck eggs. The correlation among TVC, TCC and TSC of clean hen eggs ranged from 0.0224 to 0.6915. The TVC and TCC showed highly significantly correlation with TSC of clean hen eggs. The correlation among TVC, TCC and TSC of dirty hen eggs ranged from 0.3269 to 0.7837. The TVC and TCC were highly significantly correlated with TSC of dirty hen eggs. The correlation among TVC, TCC and TSC of clean duck eggs ranged from 0.0873 to 0.8390. The TCC showed highly significant correlation with TVC of clean duck eggs. The correlation among TVC, TCC and TSC of dirty duck eggs ranged from 0.3959 to 0.8597. The TVC and TCC showed highly significant correlation with TSC of dirty duck eggs. The highest bacterial load was found in dirty duck eggs and lowest score were found in clean hen eggs. The study indicated that hen eggs are safer than duck eggs.

INTRODUCTION

Most of the eggs produced in the country are of two types- deshi eggs (from native breed) and farm eggs (from hi-breed or commercial breed) which are produced from the household of farmers and small and large scale farms. Eggs collected from these sources are carelessly handled and do not maintain hygienic practices before these arrived to the consumers. Moreover the unpackaging condition, high environmental temperature, poor transportation and storage facilities lead to spoilage of eggs. The consumers are therefore deprived of the nutritional quality of the food and often are deceived by purchasing spoiled eggs. The situation of production and consumption of eggs in Bangladesh is quite different from all developed countries of the world. Eggs collected in local markets are dispatched to markets in town and cities for consumption of the dwellers there. During transportation many eggs go cracked, some always

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remain soiled and some remain dirtied. All these eggs regardless of size and shape arrive at the wholesalers market. The retailers then purchase these eggs from them and sell to consumers in the prevailing condition. This may be health hazardous and constitute risk factors for consumers. Freshly laid eggs are generally of good quality with the exception of meat and blood spots. Most of the loss in quality results from the effect of the environment in which the eggs are held (Romanoff and Romanoff, 1963).

In our country fresh, cracked, dirtied and even addled eggs are sold to buyers without giving any attention to quality (Borhanuddin et al., 1986). In Bangladesh there exists no regulation in respect of egg quality and preservation. Contamination of eggs may be due to bacteria within the hen's ovary or oviduct before the shell forms around the yolk and white, organism doesn't make the hen sick. It is also possible for eggs to become infected by *Salmonella enteritiis* fecal contamination through the pores of the shells after they're laid. Researchers say that, if present, the *S. enteritidis* is usually in the yolk. However, they cannot rule out the bacteria being in egg whites. So everyone is advised against eating raw or undercooked egg yolks and whites or products containing raw or undercooked eggs. People with health problems, the very young, senior citizens, and pregnant women (the risk is to the unborn child) are particularly vulnerable to *S. enteritidis* infections. In Bangladesh eggs are kept in cold storage, rather are exposed to high environmental temperatures until sold. As a consequence the inhibitory factors naturally present in egg contents fail to lyse the cell wall of gram-positive bacteria and the p^H prevent the growth of spoilage bacteria. The members of the genus *Salmonella* were identified by studying cultural properties on different selective media such as selenite broth, Salmonella-Shigella (SS) agar, XLD agar, MacConkey agar, Brilliant green agar (BGA); biochemical tests, and finally by PCR. Antibiogram study, serum agglutination test and PCR (Polymerase chain reaction) are widely being used to identify and characterize *Salmonella* species in the laboratories (Deighan et al., 2000; Veling et al., 2000). *Salmonella* organisms were isolated from various hosts such as from chickens (Begum, 1992), cattle (Islam, 2007), goat (Rahman, 2006), sheep (Karim, 2007) and other animals in Bangladesh and from chicks of Japan by Begum in 2005.

The higher incidence of *Salmonella* sp. in duck eggs calls forth the public health significance.

The available data indicate the highest prevalence of coliforms that reveal the fact that eggs are contaminated with fecal materials. The egg content samples of soiled and fecal contaminated eggs have been found by early investigators to be loaded with appreciably high number of microorganisms. The recovery of salmonella organism in clean duck eggs and in dirtied and cracked eggs calls forth its impact on public health and gives indication for its hygienic handling and processing in the preparation of foods for human consumption. The present study has therefore been undertaken to assess the nature of surface contamination and bacterial load of clean and dirty eggs of both duck and hen. Isolation and identification of spoilage bacteria and associated factors that foster their growth were also determined in this study.

MATERIALS AND METHODS

Sample collection

Eggs (clean and dirty) of duck and hen were collected from the wholesale markets in Sylhet town. The representative eggs for the study were collected aseptically using sterile instruments and transferred carefully to appropriate sterile containers and brought to the laboratory for subsequent studies to determine the bacteriological quality.

Bacteriological analysis were performed under two major principles of assessments: Firstly, microbiological quality were evaluated and then the determination of Total viable count (TVC), Total coliform count (TCC) and Total Salmonella Count (TSC) were performed by using Nutrient agar (NA), MacConkey agar (MCA) medium Nutrient broth (NB), MacConkey broth (MB), Peptone broth, Methyl-Red and Voges-Proskauer broth (MR-VP broth), Selenite broth (SB), Koser's Citrate medium etc and assessed the microbiological quality of egg samples.

The media used for bacteriological analysis were Nutrient agar (NA), Plate Count Agar (PCA), MacConkey agar (MA), Eosin-Methylene-blue agar (EMB), Salmonella-Salmonella agar (SSA), Brilliant Green Agar (BGA), Violet Red Bile Agar (VRB) etc.

Preparation and bacteriological analysis of samples

To find out microbial surface contamination of eggshell, six eggshells from each category were rinsed aseptically after the eggs were dipped in 200 milliliter of distilled water for five minutes. This constitutes the individual sample. One milliliter of this sample was transferred to nine milliliter of PBS solution to make 1:10 dilution. Surface plating method was carried out in accordance with the standard procedure of Harrigan and McCance (1976).

To determine the microbial contamination level of eggshell surface, the procedure of sampling adopted by Collins and Patricia (1976) was followed. According to the technique, contents of six eggs of each kind were aseptically obtained and blended at a low speed for a period of three minutes. One milliliter of this sample, which constituted the representative sample, was transferred to nine milliliter of PBS solution to make 1:10 dilution. Surface plating method was carried out in accordance with the standard procedure described by Harrigan and Mc Cance (1976).

Isolation of bacteria

After collection, the samples of tigers and lions were grown in the recently prepared nutrient broth at 37^0C for 24 hours. Then overnight bacterial broths were streaked on SS agar, Brilliant Green Agar (BGA) (for *Salmonella*), EMB (for *E. coli*), Mannitol salt agar were incubated at 37^0C for 24 hours.

Identification of bacteria

Identification of bacteria was done on the basis of colony morphology, Gram's staining technique, Motility Indole Urease (MIU) test, Carbohydrate fermentation test (e.g. dextrose, sucrose, lactose, maltose and mannitol), Reaction of the organisms in TSI agar slant, Simmons citrate agar utilization test, indole test Voges-Proskauer test, Methyl Red (MR) test, oxidase and catalase.

Cultural and biochemical examination of bacterial isolates

In order to find out different types of microorganisms in eggs different kinds of bacterial colonies were isolated in pure culture. Motility test was performed under microscope.

Gram's staining

Grams method of staining was followed during the experiment for the morphological study of bacteria to provide basic information about the presumptive bacterial identification as per recommendation of Cowan (1985).

Biochemical test for E. coli

For the isolation and identification of coliform organism the samples were first inoculated to MCA agar. The suspected colonies were inoculated on TSIA slants. Acid slant, acid but, no hydrogen sulphide and no gas in butt were indicative of coliform. The organisms are oxidase negative. Lactose fermenting red colonies from the MCA was sub-cultured on EMB agar. Colonies on EMB agar with metallic sheen were suspected as positive for *E. coli* is characterized by positive to indole and MR tests and negative to VP and citrate tests. Enterobacter is negative to indole and MR test, positive to VP and citrate test.

***Biochemical test for Salmonella* spp**

Processed samples were inoculated on MCA and incubated at 37^oC for 24-48 hours. Lactose non fermented colour less colonies form MCA were sub-cultured on SSA. Translucent, round and colour less colonies on SSA were suspected to be *Salmonella* which were later confirmed by biochemical and the motility test.

Maintenance of stock culture

For the maintenance of stock culture, nutrient agar slants were employed. One slant was used for individual isolate and was kept at room temperature. Finally sterile mineral oil was overlaid and the culture was kept at refrigeration temperature.

Statistical analysis

The data on Total Viable Count (TVC), Total Coliform Count (TCC) and Total Salmonella Count (TSC) obtained from the bacteriological examination of egg samples of duck and hen eggs sold by wholesalers were analyzed, by employing factorial experiment in completely randomized design (CRD) and using computer package SUS software (Freed, 1992). Using applicable Duncan's Multiple Range Test was employed, treated product differences. Correlation among TVC, TCC and TSC were also evaluated.

RESULTS

Total Viable Counts (TVC)

The result of total viable bacteria of egg content samples presented in tables 1, 2 and 3 showed the total viable bacterial load of egg samples of two different types (i. e., duck eggs and hen eggs) and each having two categories, such as clean (intact) and dirty eggs from different wholesalers of Sylhet town.

Each category of eggs consists of eggshell washings were subjected to bacteriological examination. The bacterial load found was not uniform and varied quite consistently by at least 2 to 3 log cycles, more in clean eggs in the former.

The average counts of eggshell washings/ml samples belonging to clean (intact) and dirty eggs of duck were found log 4.94; log 5.42, respectively (Table 1).

In case of duck egg shell washing samples the minimum and maximum counts of different categories ranged in clean (intact) from log 3.95 (90 x $l0^2$ CFU/ml) to log 6.15 (140 x 10^4 CFU/ml), in dirty egg log 4.30 (200 x 10^3 CFU/ml) to log 6.14 (140 x 10^4 CFU/ml) (Table 1).

In case of hen egg samples, the results were represented in Table 4.1, 4.2 and 4.3. The mean or average values of TVC of different shell washing samples belonging to clean (intact) eggs were log 4.73 dirty eggs log 5.25, respectively (Table 1).

The range of minimum and maximum counts of hen eggshell washing samples were in clean (intact) eggs 60 x 10^2 CFU/ml (log 3.78) to 120x 10^3 CFU/ml (log 5.08) and in dirty eggs 35 x 10^4 CFU/ml (log 5.54) to 45 x 10^4 CFU/ml (log 5.65), (Table 1). It is clearly evident from the above data that the duck eggs exhibited more microbial load than that of the hen egg samples.

Table 1: Total Viable counts (TVC) of egg samples

	Category of samples (Eggshell Washing/ml)			
Sample No.	Clean eggs		Dirty eggs	
	Duck egg (log10 value)	Hen egg	Duck egg	Hen egg
1	$140×10^4$ (6.15)	90×103 (4.95)	30×104 (5.48)	45×104 (5.65)
2	$100×10^3$ (5.00)	80×103 (4.90)	35×104 (5.54)	200×103 (5.30)
3	$240×10^3$ (5.38)	100×102 (4.00)	36×104 (5.56)	55×103 (4.65)
4	$90×10^2$ (3.95)	50×103 (4.70)	150×104 (5.18)	220×103 (5.34)
5	$120×10^2$ (4.08)	120×103 (5.08)	140×104 (6.14)	150×103 (5.18)
6	$150×10^3$ (5.18)	90×103 (4.95)	55×104 (5.74)	40×104 (5.60)
7	$200×10^3$ (5.30)	60×102 (3.78)	30×103 (4.48)	35×104 (5.54)
8	$200×10^3$ (5.30)	90×103 (4.95)	125×104 (6.10)	200×103 (5.30)
9	$100×10^3$ (5.00)	110×103 (5.04)	200×103 (4.30)	35×103 (4.54)
10	$130×10^2$ (4.11)	100×103 (5.00)	48×104 (5.68)	250×103 (5.40)
Average (SD)	4.94 (0.698)	4.73 (0.460)	5.42 (0.614)	5.25 (0.376)

*All counts are expressed in Colony Forming Units (CFU).

Total Coliform Count (TCC)

The values of the Total Coliform Count of duck egg samples are recorded in Table 2. The average counts of eggshell washings/ml samples of clean (intact) eggs and dirty eggs were log 4.70; log 4.87; respectively (Table 2).

The minimum and maximum ranges of coliform count as revealed in duck eggshell washing samples in clean (intact) eggs were log 3.30 (200 x 10^1CFU/ml) to log 6.00 (100 x 10^4 CFU/ml); in dirty eggs, log 4.25 (180 x 10^2 CFU/ml) to log 5.84 (70 x 10^4 CFU/ml), (Table 2).

The coliform counts of hen egg samples are shown in Table 2. The mean values as obtained in eggshell washing of clean (intact) eggs were log 4.50; dirty eggs log 4.83; respectively (Table 2).

The values of the minimum and maximum range of coliform count as revealed in hen eggshell washing samples were for clean (intact) eggs log 3.90 (80 x 10^2 CFU/ml) to log 5.39 (250 x 10^3 CFU/ml); dirty eggs, log 4.00 (100 x 10^2 CFU/ml) to log 5.47 (30 x 10^4 CFU/ml), (Table 2).

Table 2: Total coliform counts (TCC) of egg samples

	Category of samples (Eggshell Washing/ml)			
Sample No.	Clean eggs		Dirty eggs	
	Duck egg (log10 value)	Hen egg	Duck egg	Hen egg
1	$90×10^2$ (3.95)	$100×10^2$ (4.00)	$200×10^2$ (4.30)	$100×10^2$ (4.00)
2	$200×10^1$ (3.30)	$80×10^2$ (3.90)	$180×10^2$ (4.25)	$150×10^3$ (5.17)
3	$70×10^3$ (4.84)	$75×10^3$ (4.87)	$250×10^2$ (4.39)	$120×10^3$ (5.07)
4	$150×10^2$ (4.17)	$65×10^3$ (4.81)	$150×10^3$ (5.17)	$30×10^3$ (4.47)
5	$100×10^2$ (4.00)	$120×10^2$ (4.07)	$200×10^2$ (4.30)	$150×10^2$ 4.17
6	$250×10^3$ (5.39)	$250×10^2$ (4.39)	$30×10^3$ (4.47)	$70×10^3$ (4.84)
7	$130×10^3$ (5.11)	$200×10^3$ (5.30)	$70×10^4$ (5.84)	$280×10^3$ (5.44)
8	$150×10^{3(}$5.17)	$150×10^2$ (4.17)	$40×10^4$ (5.60)	$30×10^4$ (5.47)
9	$110×10^{3(}$5.04)	$30×10^3$ (4.47)	$75×10^3$ (4.87)	$200×10^3$ (5.30)
10	$100×10^{4(}$6.00)	$95×10^3$ (4.97)	$35×10^4$ (5.54)	$250×10^2$ (4.39)
Average (SD)	4.70 (0.815)	4.50 (0.473)	4.87 (0.618)	4.83 (0.540)

*All counts are expressed in Colony Forming Units (CFU).

Total Salmonella Counts (TSC)

The *Salmonella* count of the eggshell washing of duck egg samples as obtained are presented in Table 3.The mean values of duck eggshell washing of different samples were in clean (intact) eggs log 4.75; dirty eggs, log 4.76; respectively (Table 3).

The duck eggshell washing samples showing minimum and maximum ranges of TSC were in clean (intact) eggs log 4.00 (100 x 10^2 CFU/ml) to log 5.30 (200 x 10^3 CFU/ml) and in dirty eggs log 4.08 (120 x 10^2 CFU/ml) to log 5.60 (40 x 10^4 CFU/ml), (Table 3).

In case of hen egg samples the results are presented in Table 3. The average TSC values of hen eggshell washing samples were in clean (intact) eggs log 4.53; dirty eggs log 4.67, respectively (Table 3).

In hen egg shell washing samples the minimum and maximum TSC ranged respectively in clean (intact) eggs log 3.90 (80 x $l0^2$ CFU/ml) to log 5.40 (250 x 10^3 CFU/ml), dirty eggs log 4.00 ($l00xl0^2$ CFU/ml) to log 6.08 (120 x $l0^4$ CFU/ml), (Table 3).

Table 3: Total *Salmonella* counts (TSC) of egg samples

	Category of samples (Eggshell Washing/ml)			
Sample No.	Clean eggs		Dirty eggs	
	Duck egg (log10 value)	Hen egg	Duck egg	Hen egg
1	200×10^3(5.30)	100×10^2 (4.00)	200×10^2 (4.30)	250×10^2 (4.40)
2	130×10^3 (5.11)	80×10^2 (3.90)	120×10^2 (4.08)	100×10^2 (4.00)
3	100×10^3 (5.00)	150×10^2 (4.18)	100×10^3 (5.00)	120×10^3 (5.08)
4	120×10^2 (4.08)	200×10^2 (4.30)	40×10^4 (5.60)	150×10^2 (4.18)
5	150×10^3 (5.18)	120×10^3 (5.08)	250×10^3 (5.40)	160×10^2 (4.20)
6	90×10^3 (4.95)	100×10^3 (5.00)	150×10^3 (4.18)	190×10^2 (4.30)
7	250×10^2 (4.40)	220×10^3 (5.34)	220×10^2 (4.34)	220×10^3 (4.41)
8	160×10^3 (5.20)	250×10^3 (5.40)	180×10^2 (4.26)	40×10^4 (5.60)
9	220×10^2 (4.34)	90×10^2 (3.95)	200×10^3 (5.30)	35×10^3 (4.54)
10	100×10^2 (4.00)	150×10^2 (4.18)	160×10^3 (5.20)	120×10^4 (6.08)
Average (SD)	4.75 (0.497)	4.53 (0.601)	4.76 (0.586)	4.67 (0.685)

Correlation

The correlation among TVC, TCC and TSC of clean hen and dirty hen eggs were presented in table 4a which ranged from 0.3227 to 0.9323. The correlation among TVC, TCC and TSC of clean duck and dirty duck eggs were provided in table 4b which ranged from 0.0174 to 0.8055. The correlation among TVC, TCC and TSC of clean and dirty hen eggs were set out in table 4c which ranged from 0.0224 to 0.6915 and . 0.0873 to 0.8390, and 0.3269 to 0.7837 respectively. The correlation among TVC, TCC and TSC of dirty duck eggs were set out in table 4d which ranged from 0.3959 to 0.8597.

Table 4a: Correlation among TVC, TCC and TSC of clean hen and dirty hen eggs

	TVC (Dirty hen)	TCC (Dirty hen)	TSC (Dirty hen)
TVC(Clean hen)	0.8043**	0.2314	0.9323**
TCC(Clean hen)	0.8297**	0.5237**	0.3678*
TSC(Clean hen)	0.3227	0.4445*	0.8344**

Table 4b: Correlation among TVC, TCC and TSC of clean duck and dirty duck eggs

	TVC (Dirty duck)	TCC (Dirty duck)	TSC (Dirty duck)
TVC (Clean duck)	0.6955**	0.5755**	0.0174
TCC (Clean duck)	0.7768**	0.0567	0.8055**
TSC (Clean duck)	0.0898	0.0592	0.0917

Table 4c: Correlation among TVC, TCC and TSC of clean hen and dirty hen eggs

	TVC		TCC		TSC	
	clean hen eggs	dirty hen eggs	clean hen eggs	dirty hen eggs	clean hen eggs	dirty hen eggs
TVC					0.5634**	0.7637**
TCC	0.0224	0.3269				
TSC			0.6915**	0.7837**		

Table 4d: Correlation among TVC, TCC and TSC of clean and dirty duck eggs

	TVC		TCC		TSC	
	clean duck eggs	dirty duck eggs	clean duck eggs	dirty duck eggs	clean duck eggs	dirty duck eggs
TVC					0.0873	0.7142**
TCC	0.8390**	0.3959*				
TSC			0.1645	0.8597**		

Growth of various bacteria on different media

Table 5: growth characteristics of isolated organisms into differential media

	Bacterial colony characteristics on different media						
Name of isolated	Mannitol Salt Agar (MSA)	MacConkey Agar (MCA)	Eosin Methylene Blue Agar (EMBA)	Nutrient Agar (NA)	Brilliant Green Agar (BGA)	SS-Agar	VRB Agar
Escherichia coli	No characteristic growth observed.	Large, mucoid, pink colored colonies.	Heavy metallic sheen in the medium.	Circular, low convex, smooth, colorless colonies.	Yellowish colored colonies.	Slight pinkish colonies.	Isolated large pink color colonies.
Salmonella spp.	No characteristic growth observed.	Colorless colonies.	Pale colonies without Metallic sheen.	Round, smooth, Dew drop observed. like colonies	Pink coloration of media, pale colonies.	Colorless colonies with black center.	Colorless colonies.

Isolation and identification of bacteria

Colony characteristics (growth) of *Escherichia coli* and *Salmonella* spp on different media were analysed to identify the different bacteria present in egg shell surface.

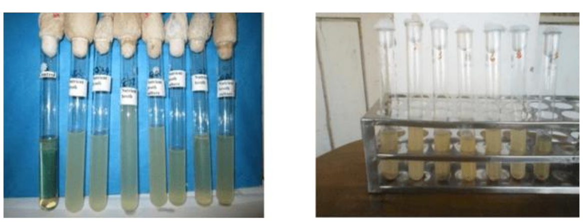

Figure 1: Isolated *Escherichia coli* (left) and *Salmonella spp.* (right) in nutrient broth, organism produced cloudiness, heavy sediment at the bottom of the test tube.

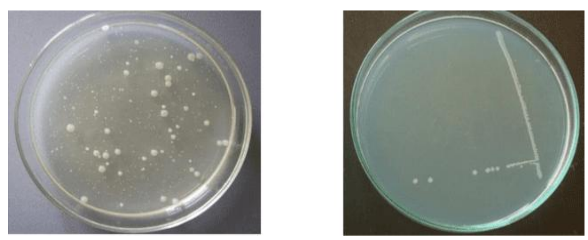

Figure 2: *Escherichia coli* (left) and *Salmonella spp.* (right) in nutrient agar plate, organism produced isolated circular, low convex, smooth, opaque, colorless colonies.

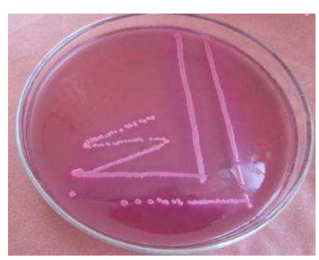

Figure 3: *Escherichia coli* in MacConkey agar plate, organism produced isolated large bright pink colored colonies with lactose fermentation.

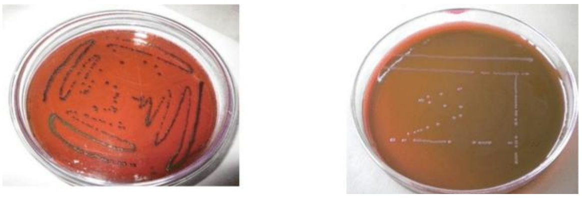

Figure 4: *Escherichia coli* (left) and *Salmonella spp.* (right) organism produced isolated metallic sheen in Eosin Methylene Blue (EMB) agar plate.

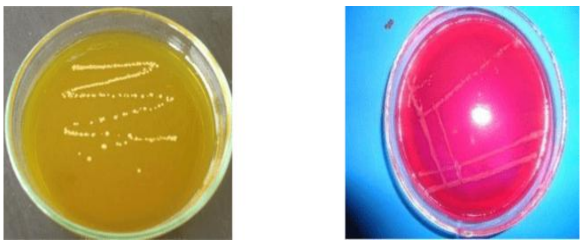

Figure 5: *Escherichia coli* (left) and *Salmonella spp.* (right) organism produced yellowish green colonies in Brilliant green agar and produces red colonies due to peptone hydrolysis.

Figure 6: *Escherichia coli* (left) and *Salmonella spp.* (right) organism produced isolated large pink color colonies produces colour less colonies in Violet Red Bile (VRB) agar.

Salmonella-Salmonella (SS) agar:

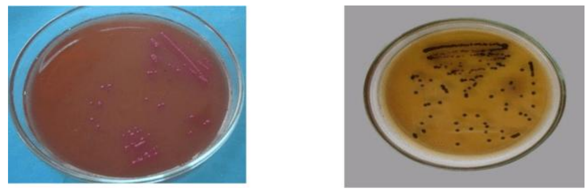

Figure 7: *Escherichia coli* (left) and *Salmonella spp.* (right) organism produced isolated slight pinkish colonies bacteria produced colorless colonies with dark/black center colonies in Salmonella-Salmonella (SS) agar

Biochemical test

Characteristics growth of bacteria into differential media indicated them as *E. coli* and *Salmonella spp.* These bacteria were then subjected to various biochemical tests for more confirmation (Table 6).

Table 6: Different bio-chemical tests

Sl. No.	Name of the Test	*Escherichia coli*	*Salmonella spp*
01	Sugar fermentation test	+	
02.	MR Test	+	+
03.	VP Test	-	-
04.	Catalase test	+	
05.	Indole Test	+	+
06.	Citrate test	-	-
07.	TSI Test	Slant-Red, Butt-Yellow	Butt-Black
09	Motility Test	+	-

N/A = Not applicable, (+) = Growth,(-) = No growth, (+ve) = Positive, (-ve) = Negative.

Sugar fermentation test

Escherichia coli fermented six basic sugars with production of acid and gas by color change.

Table 7: Carbohydrate fermentation test

Parameter	*Escherichia coli*	*Salmonella spp*
Glucose	A & G	A
Maltose	A & G	A
Sucrose	A & G	A
Lactose	A & G	A
Mannitol	A	A
Arabinose	-	A
Other Sugars	-	-

A & G = Acid production and gas production, A = Acid production only and no gas production. (-) = Negative result (No Acid production and No gas production).

Carbohydrate Fermentation Test

The *Escherichia coli* bacteria produced acid and gas in glucose, maltose, and sucrose and lactose fermentation but in mannitol fermentation they produced acid only. *Salmonella spp* produced acid only in carbohydrate fermentation (Figure 8).

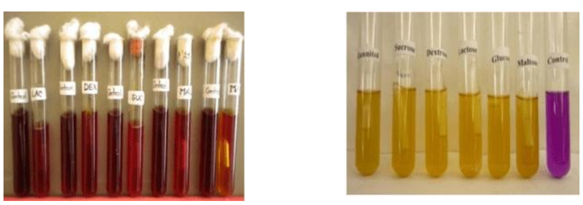

Figure 8: *Escherichia coli* (left) and *Salmonella spp.* (right) fermented glucose, lactose, sucrose, maltose and mannitol that were designated by color change and production of acid and gas in Durham's tube.

Methyl Red test

Figure 9: *Escherichia coli* (left) and *Salmonella spp.* (right) organism were Methyl Red positive (Stable red color).

Voges Proskaure test

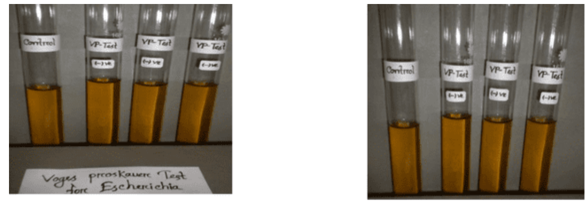

Figure 10: *Escherichia coli* (left) and *Salmonella spp.* (right) organism were Voges Proskauer negative (No color change).

Catalase Test

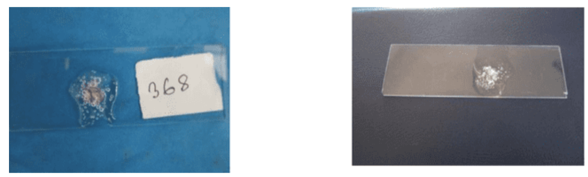

Figure 11: Catalase test showing gas bubbles of *Escherichia coli* (left) and *Salmonella spp.* (right).

Indole test

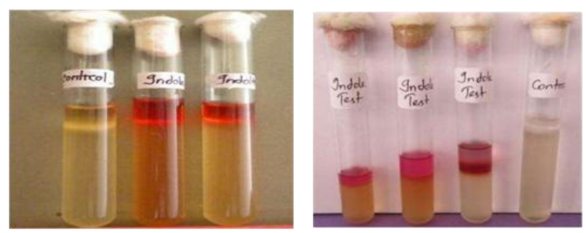

Figure 12: *Escherichia coli* (left) and *Salmonella spp.* (right) organism were indole positive (Pink color ring).

Citrate Utilization test

Figure 13: *Escherichia coli* (left) organisms were negative in Citrate Utilization test (No color change) but *Salmonella spp.* (right) color change from green to blue).

Triple Sugar Iron (TSI) slant Test

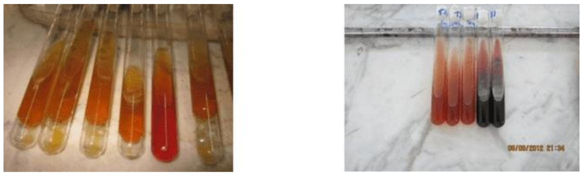

Figure 14: On TSI agar slant, *Escherichia coli* (left) and *Salmonella spp.* (right) showed positive result that was fermented glucose, lactose and sucrose (butt and slant are yellow colored), gas bubbles in butt and media frequently split.

Table 8: Citrate utilization test and Triple Sugar Iron (TSI) agar slant reaction of *Escherichia coli* and *Salmonella spp.*

Name of test	Name of tested bacteria	Test results	Indications
Citrate Utilization Test	*Escherichia coli*	+	No color changed (Negative test result).
	Salmonella spp.		
Triple Sugar Iron (TSI) agar slant reaction	*Escherichia coli*	+	Slant revealed characteristic red color and Butt-Yellow color (Positive test result).
	Salmonella spp.		

Gram's staining technique

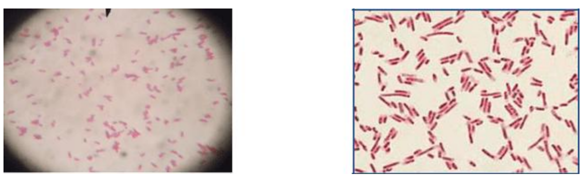

Figure 15: *Escherichia coli* (picture: 1) and *Salmonella spp.* (picture: 2) in Gram's staining under microscope revealed Gram-negative, pink color, small rod shaped and arranged in single or paired characteristics.

Motility test

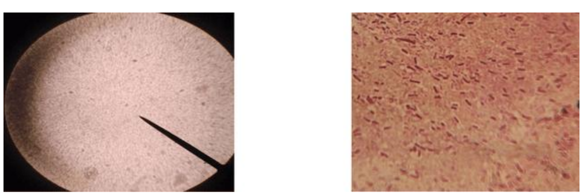

Figure 16: *Escherichia coli* (picture: 1) showing motile (forward movement) with hangingdrop slide and *Salmonella spp.* (picture: 2) showing non motile.

Preservation of isolated *Escherichia coli* and *Salmonella spp.* sample by 20% glycerine

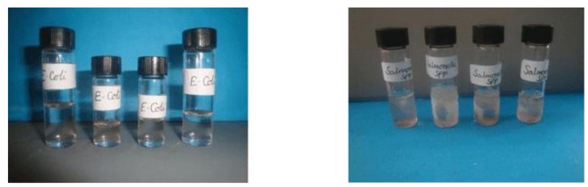

Figure 17: Preservation of *Escherichia coli* (picture: 1) and *Salmonella spp.* (picture: 2) pure samples by 20% glycerine.

DISCUSSION

The discussion presented below focused on the factual information to accommodate useful scenario of microbial quality of fast foods and critical aspects concentrating on major concepts.

Total Viable Count (TVC)

Since the numbers of bacteria in foods are indicative of index of hygienic production, therefore the bacterial content of eggs could be taken as a measure to determine the sanitary quality. In view of the above perspective, the present study reflects an approach to the recognition of potential public health hazard in egg samples obtained from wholesale stores. The study revealed that the content present in eggs did not inhibit the propagation of microbes; rather they multiply at the ambient temperature. In this investigation generally the bacterial content of the duck egg and hen egg samples differed consistently. The former was always higher than the later. This may be thought to be due to unhygienic condition, which favored the growth and propagation of organisms. It is evidenced from the analyses of the above data that the samples of three categories of duck and hen eggs differed among themselves with regard to their microbiological quality. When total counts were taken as an index of quality then samples belonging to hen eggs revealed the lowest counts. On the other hand the samples belonging to duck eggs demonstrated to have the maximum load of organisms. Board (1968) stated that the presence of dirt adds immeasurably 10 the number of contaminating organisms. Recommended standards of 50,000 to 100,000 organisms per gram have been suggested for food sample, shellfish and some milk products (Quartermaster Food and Container Institute of the Armed Forces, MIL-M-13966, 1955). The present study determined the bacteriological quality of eggs sold at wholesale shops. It was found that most of the samples of eggs of two categories of duck and hen eggs met the recommended standard.

Total Coliform Count (TCC)

Many research workers while examining foods of various types believe that a minimum level of coliform contamination will always be present (Lewis and Angelotti, 1964).The exact significance of the association of these organisms although is more or less understood, but the sanitarians consider coliform counts as an indicator of faecal pollution (Hall et al. 1967). Recommended limits for food samples, raw meats and some milk products are in the range of 10 or less coliform organism per gm (Quartermaster Food and Container Institute of the Armed Forces, MIL-M-13 966, 1955).

All egg samples of different categories exceeded the coliform limit of the recommended standard. The lowest count was found in eggshell washing samples of clean hen egg and the value was log 4.50. The source of contamination could be the different dust and other dirty products that adhered to the eggs. During handling and transportation egg may be contaminated with organisms. The presence of coliforms in duck egg samples was found in high density than that of the hen egg samples. Many investigators were of opinions that the increase of coliforms is related to the sanitary and technological procedures employed. The poultry industry finds it a useful tool in maintaining good sanitary condition. Evidence was presented in this study that the eggs held at ambient temperature increase the number of food borne coliforms. Exposure of eggs at room temperature may allow coliforms to multiply.

While the presence of large numbers of coliforms is highly undesirable, it would be virtually impossible to eliminate all of these organisms from fresh and processed eggs. The coliform index as an index of sanitary quality is applicable to at least some foods. It can be seen that low numbers of coliform are permitted ranging from 1 to not over 100/g or ml. Implicit in these standards are answers to questions of feasibility and safely (Slanetz et al., 1962). The present investigation showed that in duck egg samples the presence of coliform were too high and did not meet the microbial standards and limit. Although in hen egg samples there was less in number of coliform, but the counts found were always more than microbial limit. Data available indicated that the highest prevalence of coliforms in eggs was indicative of insanitary conditions, which revealed the fact that the eggs were contaminated with the faceal material as were also reffered by Pennigton (1960).

Total Salmonella Count (TSC)

Salmonella are capable of growing in foods at room temperature has been reported by Phillips et al. (1947) and Hall et al. (1967), but probably would be unable to grow in properly refrigerated foods. Also, as suggested by preliminary trials, growth is unlikely even in foods held at 37°C. Thus, the levels of Salmonella found in the eggs from contamination during handling, transportation or other ways, giving cause to question the quality of the eggs and the sanitation practices. In addition, given that the eggs may be held at ambient temperature for several days there is the potential danger of growth and enterotoxin production in these products as demonstrated by Christiansen and King (1970) Adame et al., (1960) The Staphylococcus count on some of the "dry" type food were greater than the standard plate count. No coagulate positive strains were recovered. However, they indicated that food poisoning types might be able to multiply under the same condition, i.e., at ambient air temperature (23- 30°C) for several days prior to sale. The highest Salmonella count was found in eggshell washing samples of dirty duck eggs and the value was log 4.76 where as the lowest count was found in eggshell washing samples of clean hen eggs and the value was log 4.53. In such a case should the eggs be held at ambient temperature and due to mishandling, the Salmonella would require only a short time to multiply to a level at which they could produce a significant amount of enterotoxin. The occurrence of Salmonella in nearly all the samples evaluated, emphasized the fact that constant vigilance must be maintained over the technological and sanitary procedures used in the transportation storage of eggs. Although it may be difficult to find out eggs, which are constantly free of these organisms; certainly the operation should be such that their number will be low. The data obtained dictated that the eggs, which contain *Salmonella* might have a food poisoning potential. These organisms are capable of growing in eggs at ambient temperature. It can be thought that gross mishandling; transportation of the eggs has resulted in growth of the Salmonella. This may cause possible health hazard, as the Salmonella would require only a short time to multiply to a level at which they could produce a significant amount of enterotoxin. The *Salmonella* count on some eggs was greater than the total viable count. It is fortunate that no coagulase positive strains were recovered. However it is evident that food poisoning types might be able to multiply under the same condition that is at ambient air temperature for several days prior to sale (Miller, 1961).

Correlation among TVC, TCC and TSC in egg samples

The correlation among TVC, TCC and TSC of clean hen and dirty hen eggs ranges from 0.3227 to 0.9323. The highest significant correlation was found between TVC (clean hen) and TSC (dirty hen). Although there were found significant correlation in most of the cases. The correlation among TVC, TCC and TSC of clean duck and dirty duck eggs ranged from 0.0174 to 0.8055. The highest significant correlation was found between TCC (clean duck) and TSC (dirty duck). The TVC, TCC (clean duck) were highly significantly correlated with TVC (dirty duck). The TVC (clean duck) was also highly significantly correlated with TCC (dirty duck). The correlation among TVC, TCC and TSC of clean hen eggs ranged from 0.0224 to 0.6915. The TVC and TCC were highly significantly correlated with TSC of clean hen eggs. The correlation among TVC, TCC and TSC of dirty hen eggs ranged from 0.3269 to 0.7837. The TVC and TCC were highly significantly correlated with TSC of dirty hen eggs. The correlation among TVC, TCC and TSC of clean duck eggs ranged from 0.0873 to 0.8390. The TCC was highly significantly correlated with TVC of clean duck eggs. The correlation among TVC, TCC and TSC of dirty duck eggs ranged from 0.3959 to 0.8597. The TVC and TCC were highly significantly correlated with TSC of dirty duck eggs. The TVC was also significantly correlated with TCC of dirty duck eggs. The result contradicts with the report of Sankaran et al., (1975), where they found no significant correlation between viable count and coliform count of foods. The present study however agrees with one point of Sankaran et al., (1975). It appears from the study that where Salmonella were detected in large numbers, the total plate count was proportionally evidenced high in density.

CONCLUSION

Unwholesome egg has been considered to be responsible for a number of cases of egg borne infections and intoxications. It has been widely accepted that the microbial population that comes in contact with egg during handling and transportation presents a challenging problem to egg industry. The higher incidence of microorganisms may be attributed to unhygienic and improper handling during collection, transportation and storing of the eggs. Constant microbiological monitoring is therefore essential for maintaining the hygienic measures that should be followed during handling, transportation and storage to minimize the contamination of eggs. Although the supply of eggs to communities for providing nutrition is beset with a factor of pronounced economic and hygienic importance, but the situation of egg handling, collection, transportation and storing practices in Bangladesh takes Place in a very unhealthful situation. The following principles may be suggested to ensure the quality assurances and quality control of eggs and egg products:

1. Appropriate cleaning and disinfections of the poultry houses.
2. Proper elimination of the rodent and pest.
3. Egg should be washed properly.
4. Bio-security measures should be maintained.
5. Prevention of contamination during handling, transportation and storage.
6. Proper maintenance of keeping quality of the eggs prior to sale.

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