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Article

Characterization of bacterial pathogens from egg shell, egg yolk, feed and air samples of poultry houses

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Abstract: This study was selected to find out the bacterial pathogens in egg yolk, egg shell, feed and air samples of poultry houses at Dinajpur district in Bangladesh with isolation, identification and characterization of bacterial pathogens present in those samples. For this study, a total of 147 samples comprising egg shell (36), egg yolk (36), feed (45) and air (30) were collected during the period from January to May, 2012 and the collected samples were then examined for the bacteriological study by using cultural, morphological and biochemical techniques. On the basis of their cultural, morphological and biochemical properties the isolated organisms were identified as *Escherichia coli*, *Staphylococcus* spp., *Salmonella serovars* and *Bacillus* spp. In this study it was observed that out of 147 samples a total of 51 were identified as bacterial pathogens in which egg shell containing 10 (27.78%), egg yolk 11 (30.56%), feed 20 (44.44%) and air 10 (33.33%) respectively. In this study it was also observed that the highest prevalence of bacterial pathogens in feed samples (44.44%) in comparison with egg shell (27.78%), egg yolk (30.56%) and air samples (33.33%). In this study it was demonstrated that out of four (04) pathogens *Escherichia coli* was more abundant (39.21%) in the layer house and its environment in comparison with *Staphylococcus* spp. (25.49%), Salmonella (23.52%) and *Bacillus* spp. (11.76%) respectively.

Keywords: poultry house; bacterial pathogens; egg shell; egg yolk; feed; air

1. Introduction

Poultry is emerging as an important agribusiness started practically during eighties (Hoque, 1997). The present population of poultry is estimated to be 262.62 million including 221.39 million of chicken and 41.23 million of ducks (DLS, 2009). The annual rate of ascending agribusiness is chicken 6.2% and duck 2.38% while in commercial bird it is 15% (DLS, 2008). But this sector facing various problems related to production and health management. Outbreaks of several devastating diseases constitute major constraints causing economic loss and discouraging poultry rearing in this country (Das *et al.,* 2005). Various infections are hampering poultry industry and non-infectious disease, which causes 30% mortality of chickens and has been estimated to cost about TK.8000 cores annually in Bangladesh (Ahmed and Hamid, 1991). Infection gains entrance to a flock from various sources. Presence of bacteria in the poultry environment occurs through poultry feed, egg and air of poultry house (Saleh *et al.,* 2003). Salmonellosis is one of the most important bacterial disease in poultry industry that can transmit vertically and cause heavy economic loss through mortality and reduced productivity (Begum *et al.,* 1993; Hoque *et al.,* 1997; Kabir, 2010). It is potentially responsible for various pathogenic processes in man and animals including poultry (Freeman, 1985).*Escherichia coli* causing colibacillosis are of the most important infectious diseases of poultry (Kabir *et al*., 2017). The disease is a worldwide problem of

chickens, which refers to any systemic or localized infection characterized by enteritis, omphalitis, air saculitis, avian cellulites, peritonitis, synovitis, swollen- head syndrome, colisepticemia, coligranuloma and salpingitis. Colibacillosis is responsible for major problem in many poultry farm and there is report on their transmission though contaminated food, egg and air (Rahman *et al.*, 1999). Staphylococcal infections are distributed worldwide and responsible for chronic and acute infection in poultry. Bacillus spp. occurs widely in nature, being found in the air, feed and egg. Poultry environment such as air may act as a source of many bacterial agents. There are also reports indicating that poultry feed and egg may act as a source of various infectious diseases (Rahman *et al.*, 1999). In addition, poultry itself may act as a reservoir or source of infectious agents for other healthy birds. Therefore it is important to know the prevalence and distribution of different bacterial pathogens in poultry itself and in its environment as many of them may be potential pathogen for poultry. Such information is also required to take necessary actions for prevention and control of outbreak of diseases caused by potential avian bacterial pathogens.

2. Materials and Methods

2.1. Selection of study area

The study was conducted during the period of January to May, 2012 at different areas in Dinajpur district. The samples were collected from various farms such as Matasagar poultry farm, (Sadar), Raja-Badsha poultry farm, (Setabgonj Thana), Masud poultry farm, (Rajbari, Sadar) and brought to the Department of Microbiology, HSTU, Dinajpur for laboratory analysis.

2.2. Collection and processing of different samples

2.2.1. Egg yolk

Egg yolk sample was collected with sterile cotton swab after breaking of egg shell. Then sterile swabs poured into the nutrient broth and transport to the laboratory. In laboratory it was stored at 37 °C for 24 hours and further cultured on agar media. Some samples were given in Nutrient agar media and incubated at 37 °C for 24 hours, on next day when colony of suspected bacteria were found, then samples were taken from the nutrient agar media and sub cultured on specific media for specific organism, such as Blood agar for staphylococcus and salmonella-shigella agar for *Salmonella serovars* identification.

2.2.2. Egg shell

Eggs are collected from the farm aseptically and then brought to the laboratory. Egg Shells were collected after removing the egg yolks and grinding with the help of mortar and pastle. Then inoculated on ordinary culture media and incubated at 37 °C temperature for 24 hours and then sub cultured on selective media for identification.

2.2.3. Feed

In this study poultry feed were analyzed on weekly basis for four weeks. Some of them were collected from layer farm and some are purchased from market retailers in Bahadurbazar market and aseptically collected from opened and unopened poultry feed bags using sterile 250ml beakers and transferred into sterile universal bottles. The samples were labeled properly and brought immediately to the laboratory where bacteriological study was carried out within 2 hours of samples collection. Sterile hand gloves were worn during the time of sample collection. Four types of feeds namely Starter (S), Grower (G), Layers (L) and Finishers (F) were collected from each brand of feed thus corresponding 16 samples. The feed samples were processed by homogenizing 5 grams of each sample in 45 ml of sterile physiological saline and bacterial load enumerated using Nutrient Agar; coliform, staphylococcal, and fastidious organisms were assayed using MacConkey, Mannitol salt, and blood agar respectively. Plates were incubated at 37 °C for 24 hours. Alternatively food processing system is, 1 gram of feed was carried out using 9ml sterile distilled water and 0.1 ml of the dilution was cultured by spread plate technique into nutrient agar, MacConkey agar and Blood agar. The inoculated plates were then incubated at 37 °C for 24 hours. Further identification of bacterial isolates were done following a series of biochemical tests which included, tests for methyl red, Voges-Proskauer reactions, indole test and Triple Sugar Iron agar slant preparation.

2.2.4. Air samples

From layer house six sterile open nutrient agar plates were placed in two layer cages (3 in each cage). After 10 minutes the open nutrient agar plate were covered and wrapped with brown paper then brought to the laboratory.

After 24 hrs incubation at 37 ^{0}C, each plate were examined for identification of organisms and stored at refrigerator for further study.

2.3. Isolation and identification of bacterial pathogens

2.3.1. Cultural and morphological characterization

The four samples were then sub-cultured on the selective media for identification of bacteria by observing specific colony characteristics and stained by Gram's staining techniques for morphological study according to the procedure described by Marchant and Packer (1967).

2.3.2. Biochemical tests

A series of biochemical tests such as Methyl-Red (MR), Voges-Proskauer (VP), triple sugar iron (TSI), Indole test, Sugar fermentation, Motility, Indole and Urease (MIU), Catalase, Coagulase were performed according to the procedure described by Buxton and Fraser (1977), Merchant and Packer (1967) and OIE (2004).

3. Results and Discussion

3.1. Isolation and identification of bacterial pathogens

In this study the colony characteristics of *Escherichia coli* was observed on different culture media and the morphology of this bacteria exhibited small rod shape, single or paired in arrangement that were similar to the findings of Mosupye and Holy (2000). The isolated organism was able to produce metallic sheen on EMB agar and bright pink red colonies on Mac conkey agar which also supported by Buxton and Fraser (1977), Merchant and Packer (1967).

In our present study the colony characteristics of Salmonella serovars observed on different media were similar to the findings of Koowatananukul *et al.* (1994) and the morphology of Salmonella exhibited small rod shape single or paired in arrangement which was supported by Schutze *et al*. (1996) and Rusul *et al.* (1996).

In our present findings the colony characteristics of *Staphylococcus* spp. was whitish, opaque, circular, translucent appearance on Nutrient agar media and produce hemolysis on Blood agar and cluster shape, coccci on Gram's staining which was also supported by Terzolo and Shimizu (1979) and Linares and Wigle (2001).

The colony characteristics of *Bacillus* spp. was thick, grayish, of white cream colour in Nutrient agar and abundant growth, creamy yellow colour on Blood agar and rod shape, violet color organism on Gram's staining was similar to Marchant and Packer (1967).

3.2. Prevalence of bacterial pathogens in different samples

3.2.1. Egg shells

Out of 36 samples (egg shells) a total of 10 bacterial pathogens were isolated as *Staphylococcus* spp. (5.55%), *E. coli* (11.11%), *Salmonella serovars* (2.77%) and *Bacillus* spp. (8.33%) respectively (Table 1). In our present findings it was observed that the highest percentage of bacterial pathogens present in egg shell was *Escherichia coli* (11.11%) than other pathogens. This might be due to the several factors like contamination of the eggshell surface related to the hygienic conditions in which the hens are reared, the breeding environment, the breeding practices, the housing system, the geographical area, and the season. Contamination may also occur during egg transport and/or packaging in farms or in the conditioning centre, either through the environment or from one egg to another. Contamination of cracked egg with dirty shell and storage in contaminated surroundings or during formation and laying process. It was also revealed that stored or aged eggs have more possibility to become infected than fresh eggs due to the degradation of natural defense mechanisms in egg over time and unwashed eggs collected from commercial caged layer farms. These findings supported by the earlier observation of Abdullah (2010), Elliott (1954) and Chousalkar *et al.* (2010).

3.2.2. Egg yolk

Out of 36 samples (egg yolk) a total of 11 bacterial pathogens were isolated as *Staphylococcus* spp. (5.55%), *E. coli* (13.88%), *Salmonella serovars* (11.11%) and *Bacillus* spp. (0%) respectively (Table 2). In our present findings it was observed that the highest percentage of bacterial pathogens present in egg yolk was *Escherichia coli* (13.88%) than other pathogens. This might be due to the several factors such as contaminated egg, air and unwashed eggs collected from commercial caged layer farms. Infected ovaries and oviducts of the hen are the major sources of bacterial infection. These findings supported by the earlier observation of Rahman *et al.* (1999), Abdel Karem and Mattar, (2001) and Chousalkar *et al.* (2010).

3.2.3. Poultry feed

Out of 45 feed samples a total of 20 bacterial pathogens were isolated such as *Staphylococcus* spp. (13.33%), *E. coli* (13.33%), *Salmonella serovars* (2.22%) and *Bacillus* spp. (15.55%) respectively (Table 3). In our present findings it was observed that the highest percentage of bacterial pathogens present in feed was *Salmonella serovars* (15.55%) than other pathogens. This might be due to the several factors such as contamination of food borne pathogen during harvesting and eventual marketing of the bagged feed and egg .These findings supported by the earlier observation of Rahman *et al.* (1999) and Chousalkar *et al.* (2010).

3.2.4. Air of poultry houses

Out of 30 samples (air) a total of 10 bacterial pathogens were isolated such as *Staphylococcus* spp. (16.66%), *E. coli* (6.66%), *Salmonella serovars* (0%) and *Bacillus* spp. (10%) respectively (Table 4). In our present findings it was observed that the highest percentage of bacterial pathogens present in air was *Staphylococcus* spp. (16.66%), than other pathogens. This might be due to lack of biosafety, poor hygienic condition and unwashed eggs collected from layer farms. These findings supported by the earlier observation of Rahman *et al.* (1999) and Chousalkar *et al.* (2010).

3.2.5. Determination of overall prevalence of bacterial pathogens

A total of 147 samples comprising egg shell (36), egg yolk (36), feed (45) and air (30) were examined for the bacteriological study by using morphological, cultural (Table 6) and biochemical (Table 7) techniques according to the procedure described by Merchant and Packer (1967). In our present study it was observed that out of 147 samples a total of 51 were identified as bacterial pathogens in which egg shell containing 10 (27.78%), egg yolk 11 (30.56%), feed 20 (44.44%) and air 10 (33.33%) respectively (Table 5). In this study it was also observed that the highest prevalence of bacterial pathogens in feed samples (44.44%) in comparison with egg shell (27.78%), egg yolk (30.56%) and air samples (33.33%). This findings supported by the earlier observation of Rahman *et al.* (1999).

In our present findings it was also observed that out of four (04) pathogens *Escherichia coli* was more abundant (39.21%) in the layer house and its environment in comparison with *Staphylococcus* spp. (25.49%), Salmonella (23.52%) and *Bacillus* spp. (11.76%). This present findings also supported by the earlier observation of Costa *et al.* (2007).

Table 1. Bacterial pathogens isolated from egg shell.

No. of egg shell collected	Name of isolated bacterial pathogens	Number of positive case	Percentage (%)
36	*Staphylococcus* spp.	2	5.55
	Escherichia coli	4	11.11
	Salmonella spp.	1	2.77
	Bacillus spp.	3	8.33

Table 2. Bacterial pathogens isolated from egg yolk.

No. of egg yolk collected	Name of bacteria isolated	Number of positive case	Percentage (%)
36	*Staphylococcus* spp.	2	5.55
	Escherichia coli	5	13.88
	Salmonella spp.	4	11.11
	Bacillus spp.	**0**	**0**

Table 3. Bacterial pathogens isolated from poultry feed.

Total number of samples examined	Name of bacterial isolates	Number of samples found positive	Percentage (%)
45	*Staphylococcus* spp.	6	13.33
	Escherichia coli	6	13.33
	Salmonella spp.	7	15.55
	Bacillus spp.	1	2.22

Table 4. Bacterial pathogens isolated from air.

Total number of samples examined	Name of bacterial isolates	Number of samples found positive	Percentage (%)
30	*Staphylococcus* spp.	3	16.66
	Escherichia coli	5	6.66
	Salmonella spp.	0	0
	Bacillus spp.	2	10

Table 5. Determination of overall prevalence of bacterial pathogens.

Name of samples	Total number of positive isolates				Total no of pathogens isolated from different samples with percentage
	***Staphylococcus* spp.**	***E. coli***	***Salmonella* spp.**	***Bacillus* spp.**	
Egg shell (36)	2	4	1	3	10 (27.78%)
Egg yolk (36)	2	5	4	0	11 (30.56%)
Feed (45)	6	6	7	1	20 (44.44%)
Air (30)	3	5	0	2	10 (33.33%)
Total (147)	**13 (25.49%)**	**20 (39.21%)**	**12 (23.52%)**	**6 (11.76%)**	**51 (34.69%)**

Table 6. Characterization of field isolates by cultural properties.

Bacteriological culture media	Colony characteristics			
	E. coli	***Salmonella* spp.**	***Staphylococcus* spp.**	***Bacillus* spp.**
Nutrient agar	White, translucent, color less colonies	White, translucent, pale color colonies	Whitish, opaque, circular, glistening colonies	Thick, grayish white of cream colour colony
MacConkey agar	Bright pink color smooth transparent raised colonies	Pale, colorless smooth, transparent, raised colonies	Small, pinkish-yellow colonies	No growth
Eosin Methylene Blue agar	Greenish black colony with metallic sheen	-	-	-
Salmonella – Shigella agar	-	Opaque, translucent, colorless, smooth, round with or without black center	-	-
Brilliant Green Agar	-	Pale, pink color colonies against a pinkish back ground	-	-
Blood agar	-	-	Whitish, opaque, circular, translucent appearance and no hemolysis	Abundant growth, creamy yellow colour colony
Mannitol salt agar	-	-	Yellow color, translucent, circular colonies	-
Motility test	Motile	Non motile	Non motile	Non motile
Staining characteristics	Pink short rod, Gram negative bacilli	Gram negative, pink color, small rod shaped appearance arranged in single or paired	Gram positive, grape – like cluster shape cocci	Gram positive, rod shaped, single or pair

Table 7. Biochemical characters of the *E. coli.*

Test performed		Results			
		E. coli	***Salmonellae* spp.**	***Staphylococcus* spp.**	***Bacillus* spp.**
TSI	Glucose	+	+	+	+
	Lactose	+	-	-	-
	Sucrose	+/-	-	-	-
	Mannitol	+	+	+	+
Indole		+	-	-	-
MR		+	+	+	+
VP		-	-	-	+
H_2S		-	+	-	-
MIU		+	-	-	-
Citrate		-	+	-	-

Legends: + = Positive, MR = Methyl Red, - = negative, VP = Voges-Proskauer
H_2S= Hydrogen sulfide, MIU = Motility indol urease

4. Conclusions

The prevalence of bacterial pathogens in poultry houses at Dinajpur district of Bangladesh were 10 (27.78%), 11 (30.57%), 20 (44.44%) and 10 (33.33%) in egg shell, egg yolk, feed and air respectively. In this study it was observed that the highest prevalence of bacterial pathogens in feed samples (44.44%) in comparison with egg shell (27.78%), egg yolk (30.56%) and air samples (33.33%). The highest prevalence of bacterial pathogens in feed samples call for attention in the storage strategies of feed as well as proper hygienic management should bemaintained by the feed producer, seller and poultry house management team.

Conflict of interest

None to declare.

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