

Study on Isolation and Identification of *Salmonella* and *Escherichia coli* from Different Poultry Feeds of Savar Region of Dhaka, Bangladesh

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Abstract

The study was conducted aiming at the isolation and identification of *Salmonella* and *Escherichia coli* (*E. coli*) from different brands of poultry feeds sold in Savar, Dhaka, Bangladesh. Seven different poultry feeds were subjected to microbiological analysis. All these samples were analyzed by culturing in different media such as nutrient broth (NB), nutrient Agar (NA), SS Agar (Salmonella-Shigella Agar), BGA (brilliant-green Agar), Mac Conkey, DHL and EMB (eosin methylene blue) media. Total bacterial colonies of all the samples were counted separately on the nutrient Agar media. Hence, bacteria were counted to be 9.5×10^5 in the feed sample C (Layer) which was found to be the highest in number among the poultry feeds. Total viable count (TVC) of *Salmonella* and *E. coli* in the feed samples were as 0 to 6.75×10^4 and 0 to 3.05×10^4 respectively. Both organisms were found in 71.43% and 57.14% of the analyzed feed samples, respectively. The highest number of *Salmonella* was found in sample C (Layer) feeds and that of *E. coli* was found in sample B (Grower) feeds. The widespread occurrence of *Salmonella* and *E. coli* in poultry feeds reinforces the need for effective control measures, hygiene in processing and handling of feeds.

Keywords: *Salmonella*; *Escherichia coli*; Poultry feeds; Total viable count; Contamination; Hygiene.

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1. Introduction

Poultry feeds are food materials used in raising poultry birds. Poultry feeds are referred to as complete feeds as they are designed to contain all the nutritional materials needed for proper growth, meat and egg production in birds. Various brands of poultry feeds are in existence depending on the functions they perform in the birds. Thus, there are growers, finishers, layers, starters among others. Poultry feeds can potentially become contaminated with food borne pathogenic microorganisms during harvesting and eventual

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marketing of the bagged feeds. Poultry feeds contaminated with bacteria pathogenic to humans can contribute to human food borne illness through the feed-poultry-food-human chain. The production of poultry feeds requires microbiological safety regulations to escape microbial contamination of the product. Prominent among these microorganisms, the bacteria *Salmonella* and *E. coli* infections of poultry have been shown to be of critical importance in Bangladesh.

Salmonella are spread from poultry to humans, often through foods such as eggs and meat. *Salmonella spp.* causes an intestinal infection in humans known as Salmonellosis [1]. The investigation of survey of *Salmonella* serovars in broilers and laying breeding reproducers in Eastern Algeria was conducted [2]. One egg colonized with *Salmonella* could contaminate all eggs and chicks during hatching [3]. *Salmonella* contamination of food products can significantly reduce consumer demand and affect producer profits [4]. *E. coli* are one of other common microbial floras of gastrointestinal tract of poultry [5, 6]. Among the diseases some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhoea of adults and children [7] and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma and colibacillosis [8]. Enteritis caused by *E. coli* (colibacilliosis) is an important disease in the poultry industry because of increased mortality and decreased performance [9, 10].

Therefore, the objectives of the present study is to investigate (i) the isolation and identification *E. coli* and *Salmonella* strains from different sources of poultry feeds, (ii) the prevalence and enumeration of *E. coli* and *Salmonella* from different sources of poultry feeds and (iii) the causes of contamination of bacterial load in the poultry feeds. The current study was conducted at Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh aiming at the microbial assessment of the various brands of poultry feeds available at Savar market, Dhaka.

2. Materials and Methods

2.1. Study area

The present study was carried out at the Animal Health Research Division (AHRD), BLRI, Savar, Dhaka during the period from April 2010 to June 2010.

2.2. Collection of samples

Seven different brand types of poultry feed samples were aseptically collected from the different poultry farm and poultry market places at Savar, Dhaka, Bangladesh. These samples of different brands were labeled by Sample A (Grower), Sample B (Grower), Sample C (Layer), Sample D (Starter), Sample E (Starter), Sample F (Layer), Sample G (Starter) and taken immediately to the laboratory of BLRI, Savar, Dhaka.

2.3. Bacteriological analysis

The samples were analyzed within 2-6 hours of collection. The different media such as nutrient Agar (NA), nutrient Broth (NB), SS Agar (Salmonella-Shigella Agar), BGA (Brilliant Green Agar), EMB (eosin methylene blue), Mc Conkey and DHL were prepared separately. The last five media are called selective media. The above media were prepared separately by the following method:

2.3.1. Preparation of nutrient Agar (NA) media

The Nutrient Agar media were prepared by suspending 14gm nutrient Agar in 500ml distilled water in a beaker and boiled to dissolve completely. The media and some Petridis were sterilized by autoclaving at 121°C for 20 minutes at 15lbs. Then media were kept on the Petridis sterilizing by laminar air flow.

2.3.2. Preparation of nutrient broth (NB) media

The Nutrient Broth media were prepared by suspending 6.5 gm nutrient broth in 500ml distilled water. The media were heated to dissolve completely. The media were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Then media were kept on the Petridis sterilizing by laminar air flow.

2.3.3. Preparation of SS Agar media

The SS Agar media were prepared by suspending 31.5 gm SS Agar in 500ml distilled water. The media were heated to boiling with frequent agitation to dissolve completely but not autoclaved or overheated, because overheating may destroy the selectivity of the medium. The media were cooled to about 50°C. The media were mixed well and poured into sterile Petridis sterilizing by laminar air flow.

2.3.4. Preparation of BGA media

The BGA Agar media were prepared by suspending 29gm BGA Agar in 500ml distilled water. The media were heated to dissolve completely and sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Overheating was avoided for more selectivity; aseptically added rehydrated contents of 1 vial of sulphur supplement (FDO68). The media were mixed well before pouring into sterile Petridis.

2.3.5. Preparation of Mc Conkey media

The media were prepared by suspending 27.75 gm Mac Conkey Agar in 500ml distilled water. The media were heated to boiling with gentle swirling to dissolve completely. The

media were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Overheating was avoided. Then media were cooled to 45-50°C and poured into sterile Petridis. The surface of the medium was dried when inoculated.

2.3.6. Preparation of EMB Agar media

The media were prepared by suspending 18 gm EMB Agar in 500ml distilled water. The media were heated to boiling to dissolve completely. The media were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Overheating was avoided and was cooled to 50°C. Then the medium was shaken in order to oxidize the methylene blue (i.e., to restore its blue color) and to suspend the flocculent precipitate.

The samples were first cultured into the nonselective media such as nutrient Agar and nutrient broth media for total bacterial count. Then these samples were subcultured into the selective media for identification of the bacteria by their colony morphology. Again the samples were direct cultured to the selective media for enumeration of the total identified bacteria.

For culturing, 10 gm feed samples were taken and then ground. Then 90 ml peptone water was poured into the bicker and mixed with the samples. Then 900µl PBS was taken to each of the small bottles accordingly. Then 100µl mix sample from bicker was taken to the one of the small bottles for serial dilution. Thus the serial dilution was made up to 10⁻⁴. These appropriate dilutions were cultured by spread plate technique using sterile bent glass rod on the NA media. These inoculated NA media were then incubated overnight at 37°C in the incubator. Thus serial dilution of other samples were done by the same way and incubated overnight at 37°C. Then the bacteria of different samples were grown and formed many colonies to the NA media. Then these colonies were counted which is called Total viable count (TVC). On the other hand, all the feed samples were measured in 1 gm and taken to the NB media in the test tube separately and kept in the incubator at 37°C overnight.

For sub culturing, the colonies of the NA media were inoculated in the selective media by looping for the identification of *Salmonella* and *E. coli* bacteria from the different feed samples and were incubated at 37°C overnight. On the other hand, the samples of the NB media were inoculated to all the selective media by looping from the different feed samples. After inoculation of all of the samples to all the selective media, the samples were incubated at 37°C overnight.

For direct culture of samples on the selective media, 1gm sample were taken from each feed. Then 9ml PBS was taken to the bicker and mixed with the samples respectively. Then these samples were inoculated to these selective media. Then these media were incubated overnight at 37°C. After incubation, the bacteria were grown in the selective media. *Salmonella* and *E. coli* were identified by the color of the colony morphology in the selective media. Then *Salmonella* and *E. coli* were counted in the particular media. *Salmonella* and *E. coli* were counted by the mean of their colony of the different particular media and multiplied by 1000µl (i.e, 1 gm sample).

Count of *Salmonella* = Mean of their colony \times 1000 μ l

Count of *E. coli* = Mean of their colony \times 1000 μ l

3. Results and Discussion

The present study was conducted to investigate the isolation and identification of *Salmonella* and *E. coli* from different poultry feed samples analyzed at BLRI laboratory, Savar, Dhaka. Seven different feed samples were examined using different media. Total bacterial colonies of all of the samples were counted separately on the Nutrient Agar media. Bacterial colonies were counted up to 10^{-4} serial dilution of the Petridish. The formula of the Total viable count (TVC) is as follows:

$$\text{TVC} = \text{Mean of the colony amount of sample} \times 0.1\text{ml} \times \text{dilution factor}$$

Here, mean of the colony shows the average no. of bacterial colony from 10^{-1} to 10^{-4} of the Petri dish of the Nutrient Agar, amount of sample is 10gm, 0.1ml is 100ul and dilution factor is 10^{-4} .

Table 1. Total viable count (TVC) of different feed samples.

Different samples	Mean of the colony from 10^{-1} to 10^{-4}	TVC
Sample A (Grower)	67.9	6.79×10^5
Sample B (Grower)	81.5	8.15×10^5
Sample C (Layer)	95	9.5×10^5
Sample D (Starter)	50	5.0×10^5
Sample E (Starter)	76	7.6×10^5
Sample F (Layer)	82.5	8.25×10^5
Sample G (Starter)	90	9.0×10^5

Table 1 represents the total viable count of bacteria of all of the samples on the nutrient Agar media. Here, the highest no. of total bacteria is present in the feed sample C (Layer) and the lowest no. of total bacteria is present in the feed sample D (Starter).

Presence or absence, enumeration and percentage of Salmonella and E. coli bacteria in the different feed samples: The bacteria were identified by determining colony morphology in the selective media. Among the bacteria, *Salmonella* and *E. coli* strains were isolated from the poultry feeds. Colony morphologies in these selective media determine the identified bacteria. SS Agar, BGA and Mac Conkey determine *Salmonella* and EMB, DHL detect *E. coli*.

Table 2. Presence or absence of *Salmonella* and *E. coli* bacteria isolates from different feed samples.

Samples	<i>Salmonella</i>	<i>E.coli</i>
Sample A (Grower)	+	-
Sample B (Grower)	-	+
Sample C (Layer)	+	+
Sample D (Starter)	+	-
Sample E (Starter)	+	-
Sample F (Layer)	+	+
Sample G (Starter)	-	+

Table 2 shows that *Salmonella* were present in sample A (Grower), sample C (Layer), sample D (Starter), sample E (Starter) and sample F (Layer) and *E. coli* were present in sample B (Grower), sample C (Layer), sample F (Layer) and sample G (Starter). These samples determine the presence of *Salmonella* and *E. coli*, because they show the positive results of *Salmonella* and *E. coli* in the different selective media by their colony morphology. Sample C (Layer) and sample F (Layer) contain both *Salmonella* and *E. coli*.

Table 3. Bacterial load of different feed samples.

Samples	<i>Salmonella</i>	<i>E. coli</i>
Sample A (Grower)	3×10^4	No
Sample B (Grower)	No	3.05×10^4
Sample C (Layer)	6.75×10^4	1.15×10^4
Sample D (Starter)	2.85×10^4	No
Sample E (Starter)	3.05×10^4	No
Sample F (Layer)	4.95×10^4	2×10^4
Sample G (Starter)	No	1.0×10^4

Table 3 shows the enumeration of *Salmonella* and *E. coli* in the different feed samples with their content. The total viable count (TVC) of *Salmonella* in the feed samples was found to be within the range from 0 to 6.75×10^4 and that of *E. coli* ranged from 0 to 3.05×10^1 . The highest number of *Salmonella* was found in Sample C (Layer) and that of *E. coli* contamination was found in Sample B (Grower) feeds.

Table 4. Percentage and range of *Salmonella* and *E. coli* from different feed samples.

Bacteria	Percentage	Range
<i>Salmonella</i>	71.43	0 to 6.75×10^4
<i>E. coli</i>	57.14	0 to 3.05×10^4

From Table 4 out of 7 type samples, *Salmonella* were found in 5 samples and *E. coli* were found in 4 samples. The percentage of *Salmonella* of the feed samples was 71.43%. On the other hand, the percentage of *E. coli* of the feed samples was 57.14%. The incidence of *Salmonella* was higher than that of *E. coli*. The highest no. of *Salmonella* contamination was given from sample C (Layer) poultry feeds and the highest no. of *Escherichia coli* contamination was given from sample B (Grower) poultry feeds.

Similarly, the antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh were studied and found positive for *Escherichia coli* 145 samples (58%), out of total 250 [11]. The study was conducted to determine the isolation and antibiotic resistance of *Enterococcus* spp. and *E. coli* from poultry feed and feed ingredients and *Enterococcus* spp in 66% of samples and *E. coli* in 50% and 32% of feed and raw feeding materials were detected respectively [12]. *E. coli* isolates from broiler and layer poultry in Bangladesh were found resistant to chloramphenicol, ampicillin, ciprofloxacin, tetracycline and streptomycin etc [13].

The transmission of *salmonella* spp through the environment has been shown too cyclic, and poultry feeds have historically been viewed as important links for contamination in poultry [14, 15]. Similarly, the occurrence of *Salmonella* spp 2.33% in poultry feed in Jordan, out of 1546 feed samples taken from north, middle and south regions, 36 suspected *Salmonella* were isolated [16]. The occurrence of *Salmonella* in poultry feed samples was 2.33% and are comparable to levels of 2% in Egypt and 4.4% in Brazil [17, 18]. Six isolates of *Salmonella* from 37 (16.2%) imported broiler fish meal were recovered [19] and mash feed contains fish and meat and bone meals mostly used for layer breeder were far more frequent 21% and 4% respectively [20] contaminated with *Salmonella*. Broiler and layer feed is one of the important sources of chicken farm contaminated with *Salmonella* [21].

The present higher prevalence of *Salmonella* in the feed sample C (Layer) and higher prevalence of *E. coli* in the feed sample B (Grower) poultry feeds sold in Savar market, Bangladesh is of economic and public health significance. The presence of the above bacteria in all the feed samples calls for attention in the storage strategies employed by the poultry feed manufacturers, the ware house condition, distributors and the sellers. From preliminary survey of the setting and operation of the market, it was discovered that market is not organized into specific commodity sections for better access by customers to article for purchase. Similar microbiologically unhealthy settings abound in the market. In fact, 77–80% of salmonellosis out breaks have been associated with grade A shell eggs, or egg-containing foods [22]. 82 *Salmonella* serotypes were found in both production animals and humans, 45 of these were isolated in feed [23].

4. Conclusion

The presence of the above bacteria in all the feed samples calls for attention in the storage strategies employed by the poultry feed manufacturers, the ware house condition, distributors and the sellers. The absence of *Salmonella* and *E. coli* from other poultry

feeds suggests that the food processing is well handled. In recent years, with the increasing in density of poultry and infectious diseases in poultry caused by pathogenic bacteria, the healthy development of the poultry industry is facing serious threat. These result in lower feed conversion rates, the declining in egg laying rates of hens as well as fertilization rates, slower growth, the increasing rejection rates of dead chicken and even a large number of deaths. Poor management of farming, overcrowding, dirty sanitation environment, bad ventilation, poor feed quality and stress can cause chicken to infect with diseases. Therefore, how to take effective measures to prevent and control infectious diseases from chicken is the most important task.

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