J. Bio-Sci. 28: 59-68, 2020 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index
DOI: https://doi.org/10.3329/jbs.v28i0.44711

EVALUATION OF MICROBIAL QUALITY AND PATHOGENIC POTENTIALITY OF ENTEROBACTERIA IN POULTRY FEEDS

S Islam, N Tanjila and MF Begum*

Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract

The present investigation has been carried out to assess the microbial safety and pathogenic potentialities of enterobacteria in poultry feeds. From the results it was observed that total aerobic plate count of poultry feeds samples were recorded as 2.8×10^5 to 5.8×10^9 cfu/g and 100% samples contained $\geq 10^6$ cfu/g while the highest mean of cfu was counted as $\log_{10}$ 8.797/gm. Large number of coliforms were recorded in different poultry feed samples and the ranges of cfu were counted as 1.2×10^4 to 5.2×10^7/g while average 75% samples were contaminated with coliform bacteria with $\geq 10^4$ cfu/g and the highest mean of cfu was counted as $\log_{10}$ 6.10^3/g. The ranges of cfu of *Escherichia coli* were 1.03×10^2 to 1.09×10^5/g and 70% samples contained $\geq 10^2$ cfu/g while the highest mean of cfu was counted as $\log_{10}$ 4.493/gm. But the ranges of cfu of total *Salmonella* sp. were recorded as 1.02×10^1 to 5.25×10^4/g and 50% samples contained $\geq 10^2$ cfu/g and the highest mean of cfu was counted as $\log_{10}$ 3.665/g. Total 29 enterobacterial isolates were isolated from the feed by using selected media. On the basis of morphological characteristics and biochemical test results the isolates were identified as *Salmonella* sp., *Shigella* sp., *Klebsiella* sp., *Citrobacter* sp., *Proteus* sp., *Enterobacter* sp. and *Escherichia coli.* These isolates were tested on blood agar medium and only seven isolates showed positive β-hemolytic activity. In virulence efficacy test, only hemolytic positive isolates were ingested to chicken and observed that *E. coli* (SGE-1), *Klebsiella* sp. (SSE-6) and *Salmonella* sp. (JSS-9) isolates were highly toxic because the experimental chickens were died after 3 days of ingestion of the bacteria, two isolates showed loose motion symptom after 15 days while other isolates showed little sickness. All the selected isolates showed positive hem-agglutination reactivity in poultry RBC. The results indicate that the poultry feeds were highly contaminated with pathogenic enterobacteria which are risk to public health.

Key words: Enterobacteria, Hemagglutination, Hemolytic activity, Microbial quality, Poultry feeds

Introduction

Poultry is now a very important and widespread agricultural industry in the tropics. It is one of the major among livestock sub-sector that committed to supply cheap sources of good quality nutritious animal protein (20%) to the nation. In Bangladesh, there are 49.825 different types of poultry farms and out of them Rajshahi division belongs 20% and the expenditure for feed items was 41,091 million (BBS 2010). But the poultry disease remains one of the major threats to boosting poultry production. Enterobacteriaceae are a large group of related bacteria living in soil, water and decaying matter, and are also common occupants of both human and animal's large bowel. They are acquired through contaminated food or water and are the major cause of enteric illnesses (Talaro and Talaro 2002). Microbiological risk factors can be found in all poultry production systems. The increasing problem of *Salmonella* infection is not necessarily attributable entirely to the growth and intensification of poultry production; changing consumption patterns may also be a

*Author for correspondence: ferdrita@yahoo.com

factor. Other bacteria, such as *Clostridium perfringens, C. botulinum, Listeria monocytogenes* (Rorviket al. 2006) and *E. coli* O157:H7 can also be found in poultry products (WHO 2007) and these organisms cause food-borne illnesses. Most of the pathogenic bacteria found in poultry meat are non –host-specific and are considered capable of causing human food poisoning. Besides pathogens associated with the animals themselves, organisms associated with humans, such as members of the enterobacteriaceae and *Staphylococcus* are major hygiene concerns in the handling of food products. Poultry and poultry meat are often found contaminated with potentially pathogenic microorganisms such as *Salmonella*, *Campylobacter*, *S. aureus*, *E. coli* and *Listeria*. In some occasions *Yersinia enterocolitica*, *Aeromonas* and *C. perfringens* have the potential to be important pathogens in poultry products. However, *Salmonella*, *Campylobacter* and to a lesser extent *Listeria*, are considered to be the major food-borne pathogens in the poultry industry (Hood et al. 1988). To meet the food security and public health, poultry production is increasing day by day and along with this the risk of food borne infection also occur frequently due to lack of unhygienic practices. In this situation Bangladesh is not out of risk for this problem but there is a very few research has done on hygienic practices and microbial safety in poultry feeds. Therefore, the present investigation has been undertaken to assess the microbial quality of poultry feeds through conventional culture method and virulence of some selected enterobacteria were also determined.

Materials and Methods

Sample collection

Poultry feed samples were collected from different commercial poultry farms of Rajshahi Metropolis and surrounding areas. The samples were collected into sterilized poly bags and transported to the Microbiology Laboratory, Department of Botany, and University of Rajshahi, Bangladesh and stored at 4°C for further processing. The samples were also coded properly according to the sources.

Composition of the feed

Only layer grower ration was used for microbial quality analysis and the feed components were as maize (48-52%), rice bran (16-22%), sesame oil cake (5-11%), soybean oilcake (9-11%), oyster shell (2-3%), wheat husk (5-10%), fishmil (8-12%), salt (50 g), premix L (500 g), lysene (250 g), metheonine (500 g), kolin (100 g) and larvadox (50 g).

Sample processing and microbial analysis

For microbial analysis, 1gm of poultry feed was vortex (VM-2000, rpm 300, Taiwan) with 90ml sterile distilled water to prepare homogenous mixture. Further tenfold serial dilution of the resultant homogenates was made upto10^{-6} dilution. From these dilutions, aliquots of 0.5 ml was inoculated in replicate plates of different media using the spread plate technique. Nutrient agar medium was used for total aerobic bacteria count, MacConkey agar for total coliform count, Eosine Methylene Blue (EMB) agar for total *Escherichia coli* count and SS- agar for total *Salmonella* and *Shigella* count. All the plates were incubated under aerobic conditions at 37ºC for 24-72 hrs. The mean number of colonies counted was expressed as log colony forming units (cfu)/per gram.

Isolation and identification of enterobacteria

MacConkey, EMB and SS agar media were used for isolation of enterobacteria. Distinct colonies were isolated from the media on the basis of morphological variability. Pure culture of the isolates was obtained by streaking of a portion from the distinct isolated colonies on culture plate. For identification, morphological characteristics of the isolates on selective media were studied. Biochemical test of the isolates were done according to Bergey's Manual of Determine Bacteriology. Motality, gram staining, indole, triple sugar iron,

methyl red, voges-proskauer (VP), citrate and catalase tests were done. Further identification was confirmed by using Micro-Rao online software.

Hemolytic activity test

Tryptose blood agar medium was used (5% beef blood) for determining the hemolytic reactions of the selected enterobacteria. The medium was poured into Petridis and loop full of each bacterial broth were streaked on blood agar separately and incubated at 37ºC for 48 hrs. Then the plates were examined for growth and hemolytic reactions.

Virulence test through direct ingestion

Virulence test of selected isolates was carried according to Rat Pyometra Model (Mikamo et al. 1998) with slide modification. Each isolate was ingested with constant dose (MacForland OD 0.5) in poultry with three replications while sterile saline was ingested in control and observed for 15 days. Sickness and mortality of the poultry were monitored for each treatment. Percentages (%) of weight losses were determined following the under mentioned formula:

$$\text{Percentage (\%) of weight loss} = \frac{\text{Initial weight } (W_1) - \text{last weight } (W_2)}{\text{Initial weight } (W_1)} \times 100$$

Hemagglutination test

Hemagglutination test of the selected isolates were performed following the method of Costabile (2010). Red blood cells from poultry were collected in 0.20 mM Tris-HCl buffer. 50 µl of 0.20 mM Tris-HCl buffer solution and 50 µl of each bacterial suspension was added in micro-titer plate according to A_1-A_4, B_1-B_4, C_1-C_4, D_1-D_4, and E1-E_4, F_1-F_4 and G_1-G_4 no well and mixed properly. Then one-fold serial dilution of RBC was carried out as 1:1 dilution from wells no A_1 to G_1 down to A_4 to G_4. In case of control 50 µl of 20 mM Tris buffer were taken instead of bacterial suspension and 50 µl blood cell suspensions were mixed in H_1 to H_4 no well. The mixture in the titers plate was mixed well by gentle shaking with shaker continuously for 15 min and wait up to 30 min. After that, one drop of this suspension was examined under microscope for visible agglutination.

Statistical analysis

The experiment was conducted by using a completely randomized design with three replications. Statistical analysis (ANOVA) was performed using MS excel software (version-16.0.4266.1001). All data were reported as means with standard deviations.

Results

Microbial quality of poultry feed in different poultry farms of Rajshahi Metropolis were analysis on different media and the results are presented in Table 1 and Fig 1. In NA medium the ranges of cfu were counted as 2.8×10^5 to 5.8×10^9/g and 100% samples contained $\geq 10^6$ cfu/g in total aerobic bacteria, while the ranges of total coliform were counted as1.2×10^4 to 5.2×10^7/g in MacConkey agar and 50 to 100% samples contained $\geq 10^4$ cfu/g. The ranges of cfu 1.03×10^2 to 1.09×10^5/g of *E. coli* were counted in EMB agar and 50 to 100% samples were contaminated with $<10^2$ cfu/gm. On the other hand, the ranges of cfu/g were counted as 1.02×10^1 to 5.25×10^4/g for total *Salmonella* in SS agar medium and 17 to 67% samples were contaminated with $<10^2$ cfu/g. The maximum mean bacteria were obtained as 8.797, 6.103, 4.493 and 3.665 $\log_{10}$ cfu/g from Iffat, Masum, Rakib and Raton poultry farm in NA, MacConkey, EMB and SS agar medium, respectively.

Table 1. Ranges of cfu of poultry feed samples on Nutrient agar (NA), MacConkey agar, Eosine Methylene Blue (EMB) agar and *Salmonella* and *Shigella* (SS) agar

Name of poultry farm and location	NA		MacConkey		EMB		SS	
	Ranges of cfu/g	% of sample >10^6 cfu/g (n = 6)	Ranges of cfu/g	% of sample >10^4 cfu/g (n = 6)	Ranges of cfu/g	% of sample >10^2cfu/g (n = 6)	Ranges of cfu/g	% of sample >10^2cfu/g (n = 6)
Ismail Poultry Farm, Court	$2.8\times10^5$to 1.4×10^7	83.33	$1.2\times10^2$to 4.4×10^5	66.66	1.03×10^1 to 1.48×10^3	50	1.02×10^1 to 4.85×10^2	16.66
Mahbub Poultry Farm, Meherchondi	1.1×10^6 to 2.3×10^8	100	1.4×10^4 to 3.2×10^6	83.33	1.04×10^3 to 3.96×10^4	66.66	2.26×10^2 to 4.13×10^3	50
Juwel Poultry Farm, Chokpara	2.6×10^6 to 0.8×10^8	100	$1.8\times10^3$to 4.5×10^5	83.33	1.16×10^3 to 5.4×10^4	66.66	5.03×10^1 to 4.85×10^3	50
Khokon Poultry Farm, Chormaajardiar	1.8×10^6 to 1.2×10^8	100	2.3×10^4 to 2.5×10^5	100	3.17×10^3 to 5.12×10^4	66.66	6.09×10^2 to 1.54×10^4	66.66
Mostak Poultry Farm,D asmari	1.7×10^6 to 1.8×10^8	100	1.1×10^2 to 0.7×10^5	66.66	1.09×10^3 to 3.65×10^4	66.66	5.01×10^2 to 4.25×10^3	50
Belal Poultry Farm, Noudapara	1.6×10^6 to 1.1×10^8	100	1.6×10^4 to 6.5×10^5	50	4.30×10^3 to 5.23×10^4	66.66	3.11×10^2 to 1.20×10^3	66.66
Raton Poultry Farm, Maherchondi	0.45×10^6 to 2.3×10^9	100	2.2×10^4 to 2.9×10^6	83.33	5.05×10^3 to 4.65×10^4	83.33	5.03×10^2 to 5.25×10^4	50
Iffat Poultry Farm, Dangipara	1.9×10^6 to 3.8×10^{10}	100	$1.3\times10^6$to 2.5×10^7	100	1.08×10^3 to 3.22×10^4	83.33	1.05×10^2 to 1.20×10^3	50
Sakil Poultry Farm, Parisal	1.7×10^6 to 1.8×10^8	100	1.4×10^4 to 4.5×10^5	50	1.09×10^2 to 1.70×10^4	83.33	5.02×10^2 to 5.11×10^3	50
Rakib Poultry Farm, Daingpara	1.4×10^6 to 2.3×10^9	100	2.1×10^4 to 3.9×10^5	83.33	4.8×10^3 to 5.60×10^5	100	3.22×10^2 to 3.54×10^3	50
Sohidul Poultry Farm, Katakhali	1.9×10^6 to 1.8×10^8	100	1.7×10^4 to 6.4×10^5	83.33	1.06×10^3 to 2.56×10^4	83.33	2.20×10^2 to 4.19×10^3	50
Sojib Poultry Farm, Horipur	1.45×10^6 to 2.3×10^8	100	1.3×10^2 to 3.1×10^4	66.66	1.25×10^3 to 1.28×10^4	83.33	1.11×10^3 to 1.27×10^4	50
Bulbul Poultry Farm, Haragram	1.8×10^6 to 1.2×10^8	100	4.3×10^4 to 2.5×10^5	100	1.12×10^2 to 2.35×10^4	66.66	1.14×10^3 to 1.21×10^4	50
Masum Poultry Farm, Darusa	1.9×10^6 to 1.4×10^9	100	$2.5\times10^5$to 5.2×10^7	100	3.45×10^3 to 4.18×10^4	66.66	5.08×10^2 to 5.15×10^3	66.66

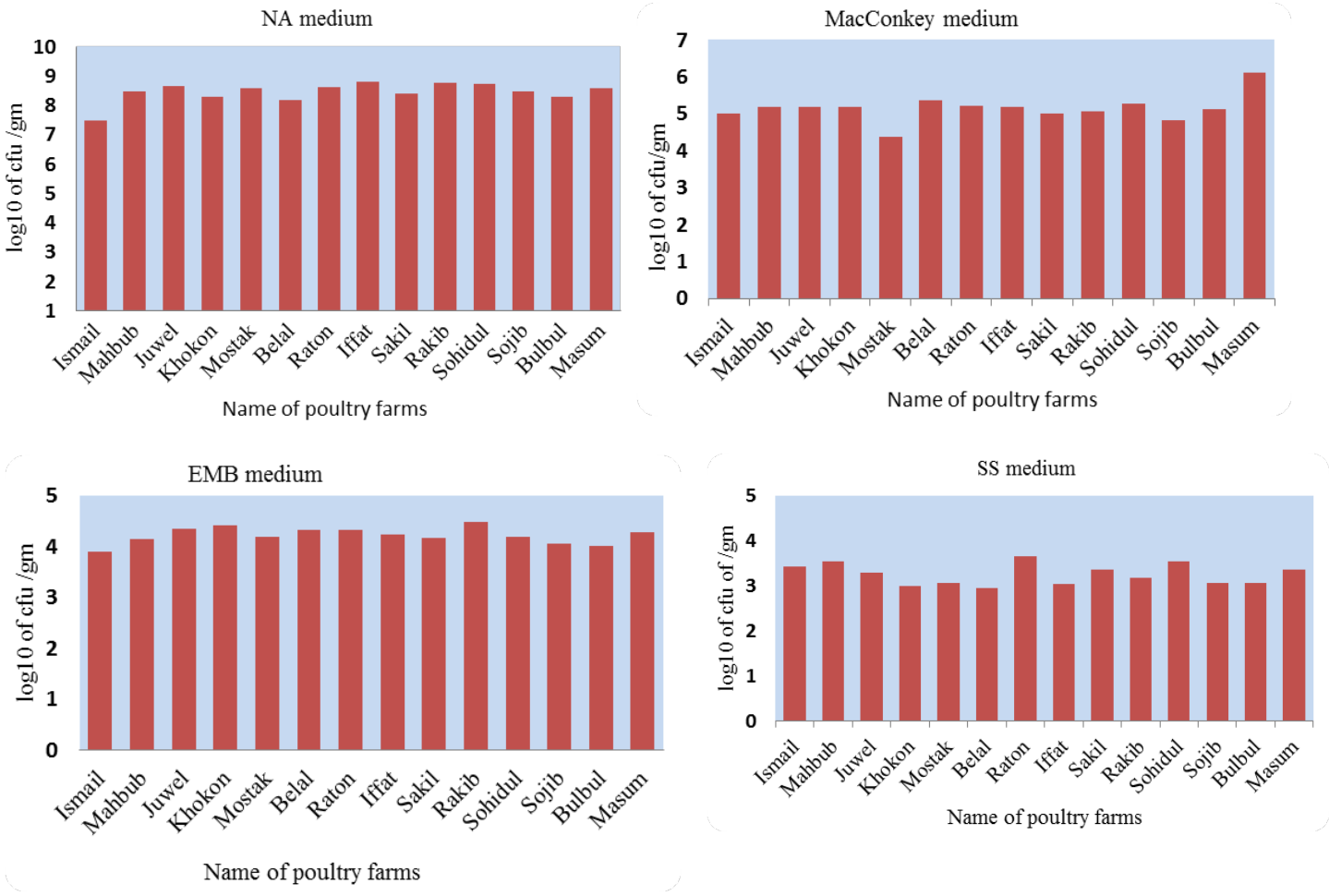

Fig. 1. Mean bacterial load of poultry feeds on different media of different poultry farms.

For isolation of enterobacteria, feed samples were placed on three selective media i.e. MacConkey agar, EMB agar and SS agar medium. Total 29 bacterial isolates were isolated from different media. The conformity level were detected as 99.9% for *Escherichia coli*, 98.41% for *Salmonella* sp., 91.81% for *Shigella* sp., 88.05% for *Citrobacter* sp., 100% for *Proteus* sp., 99,62% for *Enterobacter* sp., and 100% for *Klebsiella* sp. (Table 2). Further the isolates were subjected to pathogenicity test. In hemolytic test, out of 29 isolates, only 7 isolates showed β-hemolytic reactivity (Table 3 and Fig. 1). The virulence effect of these isolates was observed by ingestion of the selected bacterial isolates with standard dose in poultry model. Out of 7 Isolates, SGE-1, SSE-6 and JSS-9 no. isolates were more virulent and caused death of chicken after 3 days of ingestion while isolates KSM-14 and AGM-22 showed loose motion symptom after 15 days and isolates ASS-12 and CSM-20 showed mild sickness (Table 4). All the isolates showed visual agglutination in red blood cell of poultry (Table 5 and Fig. 2).

Table 2. Biochemical test results of the selected isolates of enterobacteria

Isolate codes	Biochemical test results														Suspected bacteria (% of conformity)
	OX	IN	MO	CA	CI	PH	MR	VP	GL	LC	SC	TSI			
												Slant	Butt	H_2S	
JSS-9, SGS-11, ASS-12, MLS-13	-	-	+	+	+	-	+	-	-	+	+	R	Y	+	*Salmonella* sp. (98.41%)
RGS-8, MGS-10, SGN-25 ,MLN-27	-	-	-	+	-	-	+	-	+	+	+	R	Y	-	*Shigella* sp. (91.81%)
SSE-6, IGM-15, BSN-24, SSM-18, ISM-19, RSM-21	-	+	-	+	+	-	-	+	+	+	+	Y	Y	-	*Klebsiella* sp. (100%)
AGE-7, KSM-14,	+	+	+	+	+	-	+	-	-	+	+	R	Y	-	*Citrobacter* sp. (88.05%)
ASN-26, MGN-28, AGM-22	-	-	+	+	-	+	-	+	+	+	+	R	Y	+	*Proteus* sp. (100%)
ISE-3, RGN-23, CSM-20,ISN-29	-	-	+	+	-	-	-	+	+	+	+	Y	Y	-	*Enterobacter*sp. (99.62%)
SGE-1, JSE-2, MLE-4, MSE-5, BGM-16, MGM-17	-	+	+	+	-	-	+	-	+	+	+	Y	Y	-	*Escherichia coli* (99.9%)

OX = Oxidase, IN = Indole, MO = Motility, CA = Catalase, SH = Starch hydrolysis, CI= Citrate, PH = Phenylalanine, MR = Methyle red, VP = Voges-Proskauer, TSI = Triple Sugar Iron, H_2S = Hydrogen sulphide, GL = glucose, LC = Lactose, SC = Sucrose, R = Red, Y = Yellow, + = Positive,- = Negative

Table 3. Hemolytic activity test results of isolated bacteria

Sl. No.	Code of isolates	Hemolytic activity	Sl. No.	Code of isolates	Hemolytic activity
1	SGE-1	+++	16	BGM-16	++
2	JSE-2	++	17	MGM-17	++
3	ISE-3	++	18	SSM-18	+
4	MLE-4	++	19	LSM-19	-
5	MSE-5	++	20	CSM-20	+++
6	SSE-6	+++	21	RSM-21	++
7	AGE-7	+	22	AGM-22	+++
8	RGS-8	-	23	RGM-23	+
9	JSS-9	+++	24	BSN-24	+
10	MGS-10	++	25	SGN-25	-
11	SGS-11	+	26	ASN-26	++
12	ASS-12	+++	27	MLN-27	+
13	MLS-13	+	28	MGN-28	+
14	KSM-14	+++	29	ISN-29	++
15	IGM-15	+			

+++ = β hemolysis, ++ = smaller clear zone, + = very small clear zone, - = no hemolysis.

Table 4. Toxicity test results of selected enterobacteria on chicken

Isolates code	Constant dose 1.5 $\times 10^8$/ml	Initial weight (g)	Weight loss (g) after different days (d)					% of Weight loss	Symptom
			3 d	6 d	9 d	12 d	15 d		
SGE-1	+	645±2.8	632±1.4	-	-	-	-	2.02	Dead
SSE-6	+	635±1.1	619±1.8	-	-	-	-	2.52	Dead
JSS-9	+	475±4.5	461±3.2	-	-	-	-	2.94	Dead
ASS-12	+	605±1.4	590±2.8	570±1.1	545±1.2	530±0.7	520±1.41	8.93	Little sick
KSM-14	+	480±4.9	472±3.3	457±2.8	446±4.9	418±1.06	395±2.83	17.70	Loose motion
CSM-20	+	520±0.8	500±3.3	480±1.1	465±4.5	450±1.25	440±1.41	10.19	Little sick
AGM-22	+	658±2.2	655±3.8	648±1.4	641±2.8	636±4.95	632±2.83	3.95	Loose motion
Control	-	635±3.8	654±1.7	665±2.8	672±4.1	683±0.7	690±1.41	Nill	Healthy

Table 5. Hemagglutination test results in chicken blood

Organisms	Hemagglutination activity
E. coli (SGE-1)	+
Klebsiella sp. (SSE-6)	+
Salmonella sp. (JSS-9)	+
Salmonella sp. (ASS-12)	+
Citrobacter sp. (KSM-14)	+
Enterobactor sp. (CSM-20)	+
Proteus (AGM-22)	+

+ = positive agglutination.

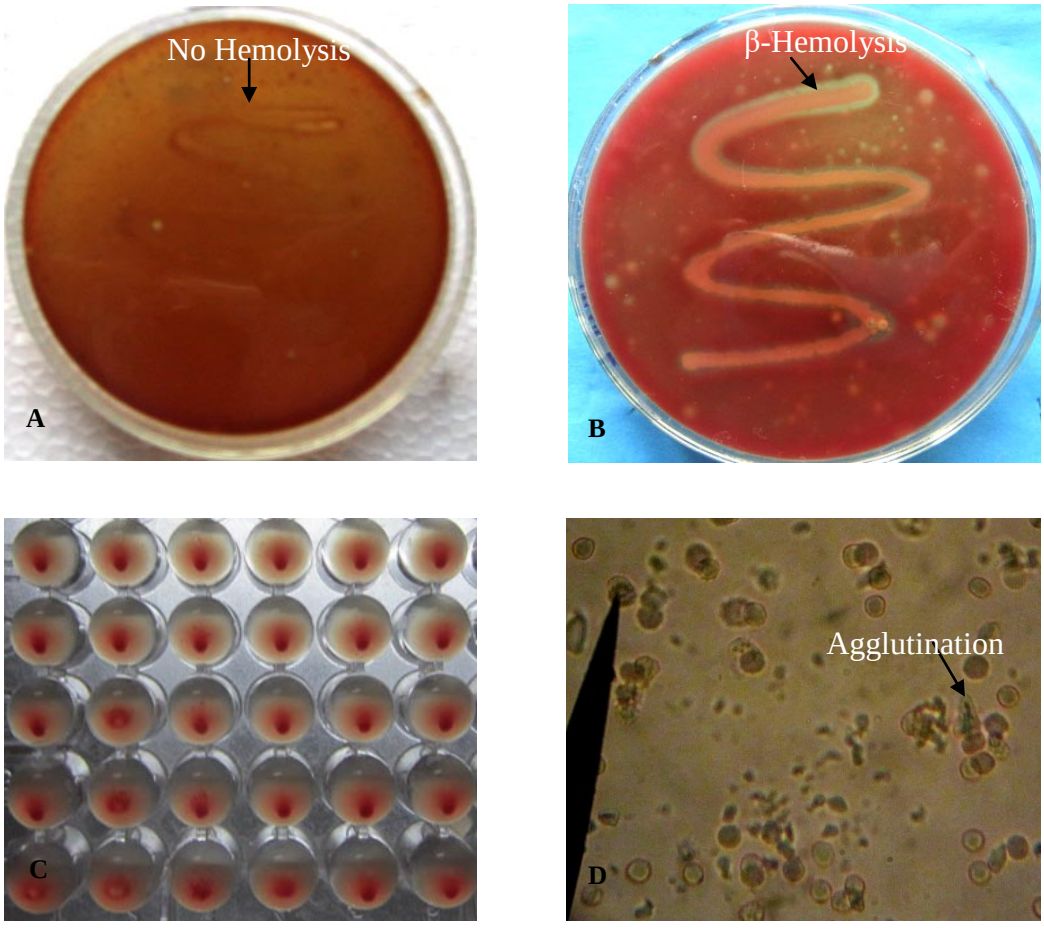

Fig. 2. Photographs showing pathogenicity test results: No hemolysis (A), β-Hemolysis (B), agglutination occurred in micro-titer plate (C), and microscopic view of positive hemagglutination (D).

Discussion

Poultry feed are the media where microorganisms can grow easily during production, processing and storage. This study was performed to assess the bacterial load of poultry feeds samples for detection of sanitary level of the feeds. From the results it reveals that poultry feeds in Rajshahi were highly contaminated with aerobic bacteria and hundred percent samples crossed the limit of international standard for microbial safety (Hood et al. 1988). Large number of coliforms were recorded in poultry feed samples and seventy five percent samples remained in microbial hazard condition. The moderate number of *E. coli* was recorded in the feed samples and about seventy percent samples were exceeding the international standard. The small number of *Salmonella* sp. were also observed in poultry feed. From the results it was exhibited that all the feed samples of the poultry farms of Rajshahi Metropolis were highly contaminated with coliforms and others bacteria. In earlier study De-Shalom (1999) investigated the bacterial contaminants associated with commercial poultry feeds and reported *Staphylococcus aureus* as the most predominant bacterial organism with 52 cfu/g, followed by *Salmonella typhi* with 48 cfu/g, *Bacillus cereus* 40 cfu/g and *Pseudomonas aeruginosa* 18 cfu/g. The findings also concurred with other study which gave total plate count, total coliforms and *E. coli* as $\log_{10}$ 4.99/g, 4.49 $\log_{10}$ cfu/g and <3.85 $\log_{10}$cfu/g (Higenyi et al. 2014).

Enterobacteria are bacteria from the family Enterobacteriaceae, which are primarily known for their ability to cause intestinal upset. Enterobacteria are responsible for a variety of human illnesses, including urinary tract infections, wound infections, gastroenteritis, meningitis, septicemia, and pneumonia. Some are true intestinal pathogens; whereas others are merely opportunistic pests which attack weakened victims. Total 29 enterobacterial isolates were isolated which were identified as *Salmonella* sp.*, Shigella* sp.*, Klebsiella* sp.*, Citrobacter* sp.*, Proteus* sp.*, Enterobacter* sp. and *Escherichia coli*. In earlier study Ahmed (2010) identified *E. coli*, *Klebsiella* sp, *Proteus vulgaris*, *Hafnia alive*, *Salmonella* sp. from poultry feed in Khartoum state which supports the findings of present research.

A potential and more deadly hazard has been associated with the consumption of microbial toxins of bacterial and fungal origin in feed (Gilbert 1995). On the other hand, presence of *E. coli* and *Salmonella* spp. may suggest fecal as well as environmental contamination (Uwaezuoke and Ogbulie 2008). For instance *E. coli* known as coliform bacteria are normal inhabitants of the digestive tract and are abundant in the poultry environment, some of them is implicated in disease conditions such as colibacillosis occurring various forms such as enteric and septicemic colibacillosis that cause increased mortality and performance of birds. *Salmonella* spp. also a serious threat to consumer health due to its ability to adapt to many different environments and broad range of transmission routes producing acute and chronic infections in all or most types of birds and animals (Barnes et al. 2003, Maciorowski et al. 2004). Pathogenic potentiality of the selected isolates were tested and out of twenty-nine isolates, seven isolates showed β-hemolytic activity on blood agar because these isolates break down red blood cells and resulting a clear zone were formed surrounding the colony. Isenberg (1992) reported the similar findings. For virulence test seven hemolytic isolates were selected and standard dose of these isolates were directly ingested to healthy chicken. Out of seven isolates, *E. coli* (SGE-1), *Klebsiella* sp. (SSE-6) and *Salmonella* sp. (JSS-9) isolates showed highly toxic reactivity and the chickens were died after 3 days while two isolates showed loose motion symptom after 15 days and other isolates showed mild sickness compare to control. Similar experiment was conducted by Rahman (2009) while observed positive results of hemolytic test and pathogenecity test for *Escherichia coli, Samonella* sp., *Klebsiella* sp., *Staphylococcus* sp. *and Bacillus* sp. in mice. In another study *Salmonella* produced clinical sign in poultry due to lower dose (3.15×10^4) of ingestion of the organism (Wray et al. 1996). Hemagglutination assay was performed using chicken RBC and all the isolates showed visual positive agglutination results. From the results it may concluded that the commercial poultry feed used in the farms of Rajshahi Metropolis where not in safe condition. Not only that the feed also highly

contaminated with potential pathogenic bacteria like *Escherichia coli*, *Salmonella* sp., *Klebsiella* sp. and *Proteus* sp. which may play adverse effect on poultry farming and public health.

Acknowledgement

The authors are thankful to the University Grant Commission (5/52/UGC/LifeScience-03/16-17/60, date-18/12/16) for the financial support of this research.

References

Ahmed NTH (2010). Bacterial contamination in poultry feed in Khatoum State. M Sc Thesis, Dept. of Microbiology. University of Khartoum, Sudan.

Rahman A (2009). Evaluation of three spices extracts against pathogenic bacteria in Kachagolla: An indigenous sweetmeat of Bangladesh. M. Sc Thesis, Department of Botany, University of Rajshahi, Bangladesh.

Bains BS (1979). A Manual of Poultry Diseases. F. Hoffmann-La Roche & Co. Limited Comp., Basle, Switzerland. pp. 81.

Barnes HJ, Vaillan CJP and Gross WB (2003). Colibacillosis, *In*: Diseases of Poultry, 11th edition, Iowa State University Press, Ames, IA, USA.

BBS (2010). Statistical year of Bangladesh 24th Ed, Bangladesh Bureau of Statistics p. 838.

Costabile M (2010). Determining the reactivity and titre of serum using a haemagglutination assay. Journal of Visualized Experiments (35): 1752.

Gilbert J (1995). Analysis of mycotoxins in food and feed; Certification of DIN in wheat and maize. American Pathology, 3(4): 263-268.

Isenberg HD (1992). Clinical Microbiology Procedures Handbook. American Society for Microbiology, Washington DC.

Higenyi J, Kabasa JD and Muyanja C (2014). Social factors and quality attributes influencing preference for production of local poultry in Butaleja and Tororo, Eastern Uganda. Journal of Animal and Veterinary Sciences, 2(1): 10-17.

Hood AM, Pearson AD and Sahamat M (1988). The extent of surface contamination of retailed chickens with Campylobacter jejuni serogroups. Epidemiology and Infection, 100: 17-25.

Maciorowski KG, Jones FT, Pillai SD and Ricke SC (2004). Incidence, sources and control of food-borne *Salmonella* spp. in poultry feeds, Journal of World's Poultry Sciences, 60: 446-457.

Mallinson ET (1984). Infectious Diseases. *In*: Animal Health. Jack Hayes (ed). Bureau of Animal Industry (Publisher), Maryland, USA.

Mikamo H, Kawazoe K, Izumi K, Watanabe K, Ueno K and Tamaya T (1998). Studies on the pathogenicity of anaerobes, especially *Prevotellabivia*, in a rat pyometra model, Infectious Diseases and Obstetacle Gynecology, 6(2): 61-65.

Rorvik LM, Aase B, Alvestad T andCaugant DA (2006). Molecular epidemiological survey of *Listeria monocytogenes* in broilers and poultry products. Journal of Applied Microbiology, 94(4): 633-640.

Stelma GN, Reyes AL, Peeler JT, Francis DW, Hunt JM, Spaulding PL, Johnson CH and Lovett J (1987). Pathogenicity test for *Listeria monocytogenes* using immune compromised mice. Journal of Clinical Microbiol., 25(11): 2085-2089.

Talaro KP and Talaro A (2002). Foundations in Microbiology, 4th Ed. The McGraw-Hill. Companies, NY, pp. 398-400.

Uwaezuoke JC and Ogbulie JN (2008). Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. Journal of Applied Sciences and Environment Management, 12(1): 113-117.

WHO (2007). Fact sheet No. 237: Food safety and food borne illnesses. Geneva. http://www.who.int/mediacentre/en/print.html.

Wray C, Davies RH and Corkish JD (1996). Enterobacteriaceae. *In*: Poultry Diseases, 4th ed.; Jordan TW and Pattison M (Eds), WB Saunders: Philadelphia, PA, USA, pp. 9-43.

(Manuscript received on August 13, 2019 and revised on October 17, 2019)