Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(4): 918-926
© 2017 JEZS
Received: 29-05-2017
Accepted: 30-06-2017

M Saiful Islam
Professor (Dr.); Genetics & Molecular Biology Laboratory
Department of Zoology
University of Rajshahi
Rajshahi 6205, Bangladesh

Suraiya Khanam
MS student; Genetics & Molecular Biology Laboratory
Department of Zoology
University of Rajshahi
Rajshahi 6205, Bangladesh

Moni Krishna Mohanta
Assistant Professor (Dr.); Genetics & Molecular Biology Laboratory, Department of Zoology, University of Rajshahi
Rajshahi 6205, Bangladesh

Correspondence
M Saiful Islam
Professor (Dr.); Genetics & Molecular Biology Laboratory
Department of Zoology
University of Rajshahi
Rajshahi 6205, Bangladesh

Isolation, characterization and identification of bacterial isolates from the poultry environment at Rajshahi Metropolis, Bangladesh

M Saiful Islam, Suraiya Khanam and Moni Krishna Mohanta

Abstract
Bacterial isolates from commercial poultry feeds and from eggs, ovaries and oviducts of the broiler hens (*Gallus domesticus* L.) were used for characterization, bacterial load estimation, antibiogram profile analyses and identification. The presence of both Gram-positive and Gram-negative bacteria in the environment was indicated and bacterial loads in terms of total viable counts (TVCs) of the isolates were much higher in the reproductive organs than in the poultry feeds. Majority (33.33%) of the isolates was sensitive, 28.33% hypersensitive, 25.0% resistant and 13.33% intermediate against eight antibiotics. Three bacteria *Bacillus cereus*, *Sphingobacterium daejeonens* and *Bacillus* sp. were confirmed by 16S rDNA gene sequencing. Pathogenic bacterial contamination in the poultry feeds and reproductive organs of broiler chickens reinforces the need for proper hygiene in the processing of the poultry products for human consumption and a need for implementing measures that guard against the misuse of antimicrobial drugs in chicken feeds is therefore emphasized.

Keywords: *Gallus domesticus*, Bacterial isolates, poultry feed, reproductive organs, antibiogram profiles, pathogenic bacteria

1. Introduction

Feeds are good and nourishing food supplements with varying constituents of, among others, animal and vegetable proteins, cereals, essential amino acids, minerals, salts, antibiotics, vitamin pre-mix and antioxidants. Since commercial feed and feed ingredients are usually sourced from various locations, they remain the major vehicles for the introduction of both commensal and pathogenic microbes to the farm environment [1]. During the past decades, poultry industry in Bangladesh grew at the rate of 20% per annum [2] and nowadays, it is one of the fastest growing agro-based enterprises in the country [3]. About 18.6% of the GDP comes from the agricultural sector, and one third of which is from the poultry industry, where a large number of private-owned companies produce poultry feeds of varying standards [3-4].

All four basic types of poultry feeds *viz.*, starters, growers, finishers and layers, may potentially become contaminated with food borne pathogenic microbes during harvesting, processing, handling, and marketing of the bagged feeds [5]. Prominent bacterial species in the poultry feeds include *Bacillus*, *Escherichia, Salmonella, Enterococcus, Campylobacter, Clostridium* and *Lactobacillus* that have been shown to be of critical importance in tropical countries like Bangladesh [5-6] and elsewhere in the world [7-8]. Several studies have demonstrated that the sources of microbial infections in poultry include contaminated feeds, drinking water, utensils, personnel, human wastes, rodents and hatchery related unhygienic activities [9]. Recently, poultry feeds have been implicated in several poultry diseases of viral, bacterial and fungal origin, suggesting that such feeds can potentially act as carriers for human as well as animal pathogens [10]. Moreover, poultry environment like soil and drinking water [11], faeces, litters and wastes [12-13], live, moribund and dead chickens [14], meat, carcass, viscera, eggs, and poultry by-products [15-16] could also carry microbes of public and veterinary health importance.

Antibiogram profiles of several bacterial isolates from poultry sources have been reported in the past and recent times. Antibiotic resistance patterns of *Enterococcus* sp. [8], *Salmonella* spp. [10-11], *Aeromonas* sp. [12], *Escherichia coli* [15], *Bacillus* spp. [17], *Clostridium* spp. [18] and *Lactobacillus* spp. [19] are worth mentioning. Keeping the aforesaid literature review in mind,

the present study was aimed to investigate the following aspects: (a) Isolation and identification of the bacteria from two vital sources of poultry environment, for example, poultry feeds and diseased hens (*Gallus domesticus* L.); (b) Assessment of the total viability counts (TVCs) of bacteria in the bacterial isolates; (c) Estimation of the bacterial load in chicken reproductive tracts and eggs; (d) Characterization of the antibiotic profiles of the isolated bacteria; and (e) Identify the bacterial strains using 16S rDNA sequencing. The study will enrich our understanding for better, healthy and emerging poultry enterprise in Rajshahi Metropolis and adjacent areas.

2. Materials and Methods

2.1 Collection of samples

A total of 30 bacterial isolates, comprising 15 from three branded poultry feeds *viz*., broiler starter (BS), broiler grower (BG) and layer layer (LL) 15 from eggs, ovaries and oviducts of the diseased broiler hens, *G. domesticus* (breed: Cobb 500) were used in the present study. The isolates were collected aseptically from five randomly chosen commercial poultry farms located in Rajshahi City Corporation areas. After collection, all the samples were transported in insulating foam boxes with ice immediately to the Laboratory of Genetics and Molecular Biology, Department of Zoology, University of Rajshahi, Bangladesh, for bacteriological studies.

2.2 Isolation of bacteria from collected samples

Feed samples (1g each) were taken separately into nutrient broth media (Hi-Media, India), and incubated at 37 ºC for 48h with shaking at 120 rpm. The diseased hens were dissected and eggs, ovaries and oviducts were removed carefully. Specimens were then cut into small pieces and suspended in physiological saline. One loopful of each suspension was separately inoculated into nutrient broth media. Control flasks without inocula were also prepared and incubated at 37 ºC with an orbital shaker. After a period of 0-48h, cultures that were found turbid were used as inocula for further experiments.

2.3 Identification of bacterial isolates

The bacterial isolates were characterized and identified by observing their gross colony morphology grown in MacConkey's, Salmonella-Shigella (SS) and Voges-Proskauer (VP) agar media. Pure cultures of the bacteria were isolated and maintained using MacConkey's agar medium (Plate 1). The identities of the microbes were confirmed employing an approved technique [20]. In addition, the isolates were subjected to conventional biochemical tests (Plates 2 and 3) such as indole productivity, methyl red, motility, Simon citrate, sulphur reduction and sugar utilization, following the standard methods [21].

2.4 Determination of bacterial load of the isolates

Enumeration of total viable counts (TVC) of the bacterial isolates was made as per the ISO recommendation [22]. In brief, 1g of the feed or chicken sample each was taken in a test tube and 10 mL distilled water or saline solution was added. The sample was then serially diluted 12 times by adding sterile distilled or saline water to make the volume of each preparation 10mL. The preparation was homogenized in every step of the serial dilution. A 0.1mL aliquot of the tenth (10^{10}), eleventh (10^{11}) and twelfth (10^{12}) dilutions were each inoculated in triplicate by the spread plate technique on a nutrient agar plate. Then the inoculated Petri plates were incubated at 37 °C for 24h, after which the bacteria of different samples were grown to form many colonies on the nutrient agar media. Finally, the number of colony forming units per millilitre (cfumL^{-1}) was calculated as follows: total number of bacteria per mL= number of colonies counted × dilution factor.

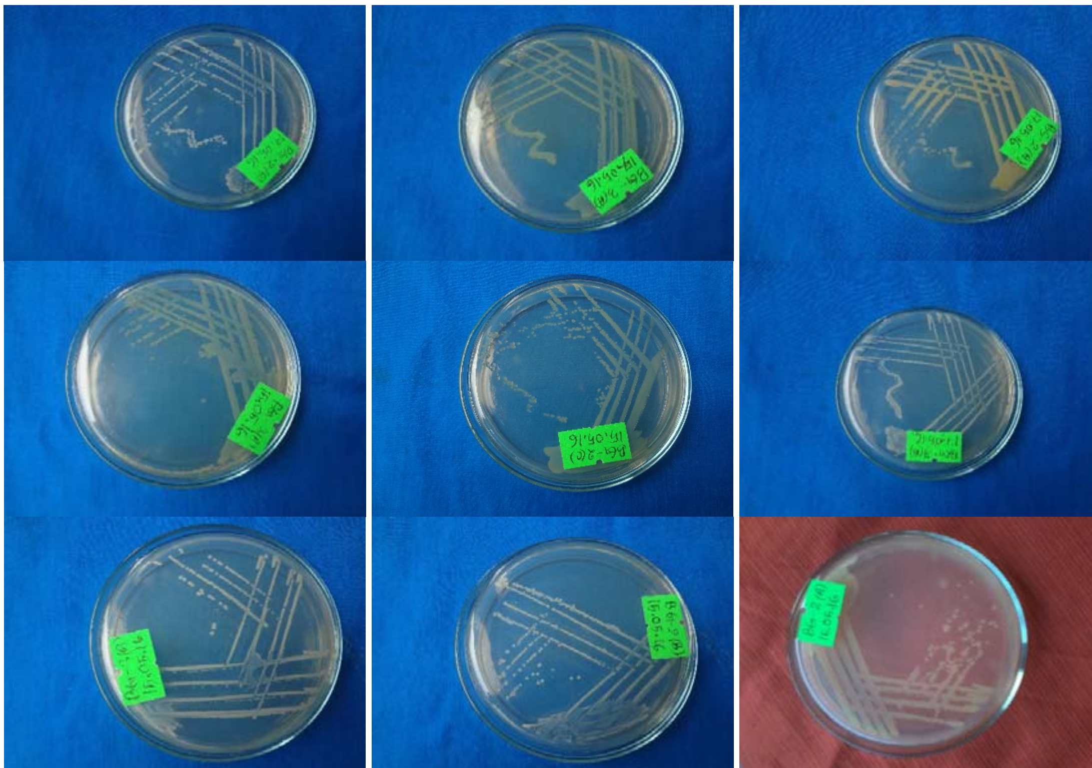


Plate 1: Cultures for bacterial isolates

Plate 2: Indole productivity test

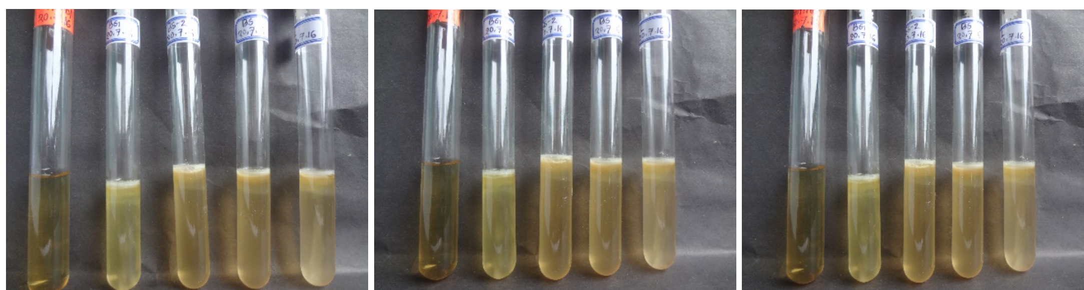


Plate 3: Indole mobility test

2.5 Determination of antibiotic susceptibility pattern

Susceptibility pattern of the bacterial isolates to eight commonly used antibiotics was determined *in vitro* employing standard disk diffusion method [23-24]. The antibiotic susceptibility pattern of the isolates was interpreted using manufacturer's guidelines, where disc distance of 5-9 mm was considered resistant (R), 10-14 mm intermediate (I) and 15 mm and above sensitive (S). Antibiotics and their concentrations used were as follows: ampicillin (AMP, 10µg/disc), bacitracin (BAC, 10µg/disc), ceftriaxone (CEF, 30µg/disc), ciprofloxacin (CIP 5µg/disc), doxycycline (DOX, 30µg/disc), gentamicin (GEN, 10µg/disc), sulphamethoxazole (SUL, 25µg/disc) and tetracycline (TET, 10µg/disc).

2.6 Molecular characterization of the bacterial isolates

Standard techniques such as agarose gel electrophoresis were used to elude 16S rDNA fragments and to observe variations between the experimental samples [25]. Further, extraction of plasmid DNA was made by the Pure Yield™ Miniprep System and then genomic DNA isolation protocol (Tiangen, China) was maintained. Finally, PCR amplification of the products was made using 16S-Forward (8f) and 16S-Reverse (805r) primers by the Invent Technologies® at Dhaka University. The purified PCR products were sequenced in single direction by big dye cycle sequence method. The sequences obtained were checked using Chromas. The 16S rDNA sequences were then compared with sequences deposited in the GenBank and EMBL. The sequences used for the multiple alignments were retrieved from GenBank and EMBL. Further details of the protocols are described recently [26].

2.7 Statistical analyses

Data were analyzed using SPSS for Windows (version 19.0). Prevalence of the bacterial isolates was expressed in simple descriptive statistics such as means and standard deviations. For cfumL^{-1} values, one-way analysis of variance (ANOVA) was used, where the levels of significance were set at $P<0.05$, and the means between the samples were separated using Fisher's least significant difference (LSD) tests [27]. Antimicrobial susceptibility profile of the bacterial isolates in response to antibiotics has been presented in a histogram.

3. Results

3.1 Characterization of the bacteria isolates

Three bacterial isolates from poultry feeds (samples BS, BG and LL) were subjected to various culture media and biochemical tests (Tables 1 and 2). This was accomplished simultaneously by gross colony morphology and a number of biochemical tests on the basis of presence (+) or absence (-) criteria. Poultry feeds BS and BG had *Bacillus cereus* strain CP133 only, LS contained all three bacteria *viz*., *B. cereus* strain CP133, *Sphingobacterium daejeonense* strain TR6-04 and *Bacillus* sp. strain EPG3, whereas LL lacked *Bacillus* sp. strain ERG3. On the other hand, both eggs and ovaries carried *Bacillus cereus* strain CP133 only, while oviducts bore *S. daejeonense* strain TR6-04 and *Bacillus* sp. strain EPG3.

3.2 Gram staining tests

The technique was used for differentiating Gram-positive from Gram-negative bacteria which was usually the first step in bacterial identification. The cell walls of Gram-positive bacteria are different from those of their Gram-negative counterparts. Gram-positive walls have a thick layer of peptidoglycan associated with teichoic acids and in Gram-negative walls lipoprotein lipopolysaccharide are associated with thin peptidoglycan layer. The structural difference between cell walls results in a different ability to retain certain dyes and a different ability to resist de-colourization.

The Gram-positive cells were not discoloured but remained purple. The Gram-negative cells lost their purple colour. After applying safranine, Gram-negative bacteria became pink or red. Here, the isolated bacteria were Gram-negative and showed pink colour but the remaining three bacteria were Gram-positive which showed purple colour (Table 3; Plate 4).

Table 1: Culture media and biochemical tests for the isolated bacteria from poultry feed samples

Test parameters	BS	BG	LL
Culture media			
MacConkey's agar	+	+	+
Salmonella-Shigella (SS) agar	-	-	+
Voges-Proskauer (VP) agar	-	-	-
Biochemical tests			
Indole production	+	-	-
Methyl red	+	+	+
Motility	+	+	+
Simon citrate	-	+	+
Sulphur reduction	+	+	+
Sugar utilization			
(i) Cellulose	+	+	+
(ii) Fructose	+	+	+
(iii) Galactose	+	+	+
(iv) Lactose	+	-	-
(v) Maltose	+	+	+
(vi) Sucrose	+	-	-
(vii) Xylose	+	+	+

BS=Broiler Starter; BG=Broiler Brower; LL= Layer Layer; += Presence; - = Absence.

Table 2: Bacterial species isolated and identified from poultry feeds and chicken samples

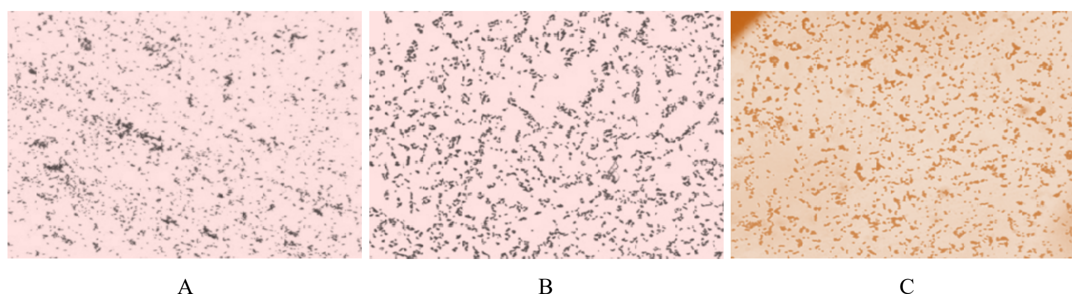
Isolates	***B. cereus* strain CP133**	***S. daejeonense* strain TR6-04**	***Bacillus* sp. strain EPG3**
Feed samples			
BS	-	-	+
BG	-	-	+
LL	+	-	+
Chicken samples			
Eggs	-	-	+
Ovaries	-	-	+
Oviducts	+	-	+

BS= Broiler starter; BG= Broiler grower; LL= Layer layer.

Table 3: Gram characteristics of the isolated bacteria from three feed samples

Name of samples	Characteristics
Sample 1 (BS)	+ve
Sample 2 (BG)	-ve
Sample 3 (LL)	+ve

BS= Broiler starter; BG= Broiler grower; LL= Layer Layer.



A B C

Plate 4: Gram staining tests for isolated bacteria from Sample 1, BS (A), Sample 2, BG (B) and Sample 3, LL (C); BS= Broiler starter; BG= Broiler grower; LL= Layer Layer.

3.3 Bacterial load of the isolates

Total viable counts (TVC) of the bacterial isolates ranged from 19 to 27×10^{12}, 27 to 31×10^{12} and 32 to 35 $\times10^{12}$, respectively for BS, BG and LL poultry feeds, and 92 to 112×10^{12}, 68 to 76×10^{12} and 65 to 75×10^{12}, respectively for egg, ovary and oviduct samples (Table 4). Results revealed that the bacterial loads differed significantly between both poultry feed types as well as diseased chicken samples ($P<0.001$). However, the cfumL^{-1} values between feeds BS and BG and those between ovaries and oviducts did not exceed statistical significance levels (P>0.05).

Table 4: Total viable counts (TVCs) of bacteria from poultry feeds and chicken samples

Isolates	TVC (cfumL^{-1})*
Feed samples (n=15)	
BS	$^{a}23.40\pm2.88\times10^{12}$
BG	$^{a}28.60\pm1.52\times10^{12}$
LL	$^{c}33.20\pm1.30\times10^{12}$
F-value at 2, 29	228.89***
Chicken samples (n=15)	
Eggs	$^{a}100.40\pm7.54\times10^{12}$
Ovaries	$^{b}72.20\pm3.35\times10^{12}$
Oviducts	$^{b}70.20\pm3.96\times10^{12}$
F-value at 2, 29	51.11***

BS= Broiler starter; BG= Broiler grower; LL= Layer layer; TVC= Total viable counts;* mean ±SD; dissimilar superscripts in each group of samples differ significantly by LSD tests at P<0.05.

3.4 Antibiotic susceptibility patterns of the isolates

Results on the antibiogram profiles of the bacterial isolates (Table 5) demonstrate that *E. coli* showed susceptibility towards all antibiotics except BAC and SUL, *Enterococcus* sp. was highly resistant to SUL only, but *Salmonella* sp. showed high to moderate resistance to three such antibiotics as AMP, GEN and SUL. The overall antibiogram profiles are of indicative that the bacteria are susceptible to the majority of the antibiotics used.

Table 5: Antibiotic susceptibility pattern of the bacterial isolated from three poultry feed samples.

Antibiotics	BS	BG	LL
	Sensitivity (DD)	Sensitivity (DD)	Sensitivity (DD)
AMP (10µg)	S (25)	S (22)	R (6)
BAC (10µg)	R (9)	S (25)	I (11)
CEF (30µg)	S (25)	S (26)	S (15)
CIP (5µg)	S (30)	S (25)	S (25)
DOX (30µg)	S (28)	S (25)	I (12)
GEN (10µg)	S (35)	S (25)	R (9)
SUL (25µg)	R (9)	R (6)	R (6)
TET (30µg)	S (30)	S (22)	S (20)

BS= Broiler starter; BG= Broiler grower; LL= Layer layer; DD= disc distance in mm; R= resistant (DD= 5-9); I= intermediate (DD= 10-14); S= sensitive DD= 15 and above); AMP= ampicillin; BAC= bactricin; CEF= ceftriaxone; CIP= ciprofloxacin; DOX= doxycycline; GEN= gentamicin; SUL= sulphamethoxazole and TET= tetracycline.

3.5 Molecular characterization of the bacterial isolates

Extraction of genomic DNA and PCR amplification products of 16S rDNA: The genomic DNA was extracted from the isolated bacteria and subjected to gel electrophoresis to view the quality and estimate quantity (Plate 5).

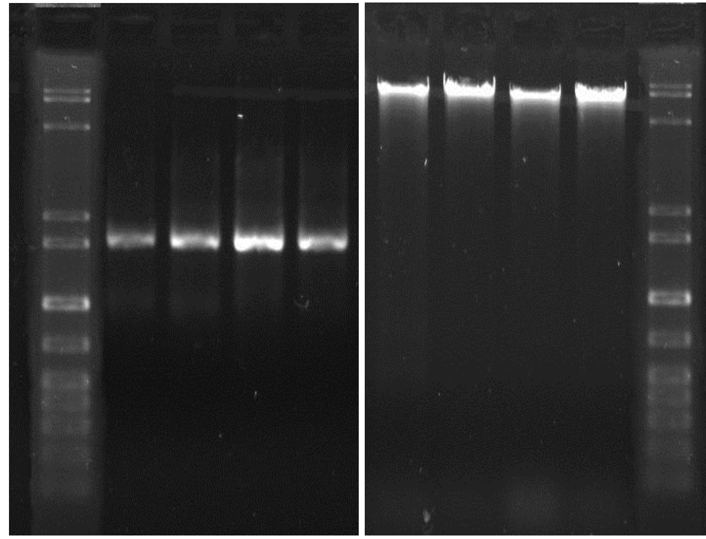


Plate 5: (A) Genomic DNA of the four isolates S_1, S_2, S_3 and S_4**;** (B) PCR amplification of the 16S rDNA of isolates S_1, S_2, S_3 and S_4. The PCR products were run on ethidium bromide stained in 1% agarose gels.

Sequences of 16S rDNA: The amplified 16S rDNA fragments of the three four isolates were sequenced only in one direction by using forward primer (Figs 1, 3 and 5). The sequencing data obtained as Chromas file and the consensus sequence was used to perform blast (Figs 2, 4 and 6).

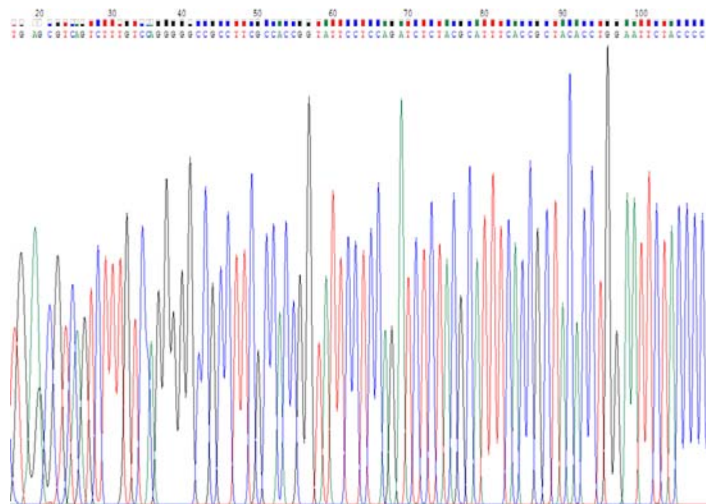


Fig 1: Chromatogram of the bacterial isolate in Sample 1 (Broiler Starter)

ATAAAATTTTTCGCGCCTAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACG
CATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTT
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTG
CCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTT
ATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTC
CGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGT
GTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGAC
GCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTA
GCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCA
TAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCCAGGATC
CAAACTCTAACCCAAAATGCA

Fig 2: Nitrogen bases of the bacterial isolate in Sample 1 (Broiler Starter)

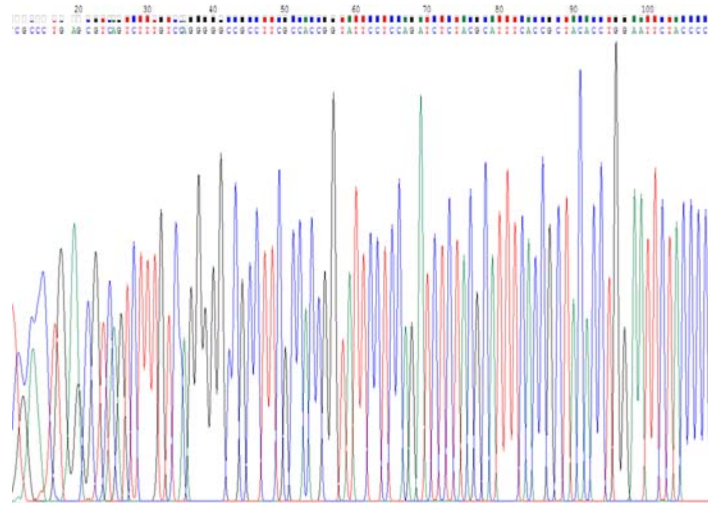


Fig 3: Chromatogram of the bacterial isolate in Sample 2 (Broiler Grower)

TAAATCTTTTCGTGCTTAGCGTCATAACGGATTAGACAGCTGCCTTCGCATCGGTGTTCTGAGACATATCTATGCAT
TTCACCGCTACTTGTCTCATTCCGCCGTCTTCAACCGCATTCAAGCACTTCAGTATCAAGGGCACTGCGACAGTTGA
GCTGCCGTCTTTCACCCCTGACTTAAAGTGCCGCCTACGCACCCTTTAAACCCAATAAATCCGGATAACGCTCGGAT
CCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGATCCTTATTCTCCGGGTACGTTCAGCCCACTACACGTAG
TGGGGTTTATTCCCCGGCAAAAGCAGTTTACAACCCATAAGGCAGTCGTCCTGCACGCGGCATGGCTGGTTCAGGG
TTGCCCCCATTGACCAATATTCCTAACTGCTGCCTCCCGTACGAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGGA
TTCTCATCTCAGAGCCCCTAGACATCGTCGCCTTGGTGGGCCGTTACCCCACCAACTAGCTAATGTCACGCGAGCCC
ATCCATATCCTATGAAGATTTGACGCCGGAACGATGCCGTCCCGGCGTGTCATGCGGTGTTAATCCGGATTTCTCCG
GGCTATCCCCCTGATATGGGCAGGTTGCTCACGCGTTACGCACCCGTGCGCCACTCTCACCGGAAGTAGCAAGCTA
CTCCCGGATCCCGTCCGACTTGCATGTATTAGGCCTGCCGCTAGCGTTCATCCTGAGCCAGATCAAACTCTATTCAA
ATCTACTGGCCAA

Fig 4: Nitrogen bases of bacterial isolate in Sample 2 (Broiler Grower)

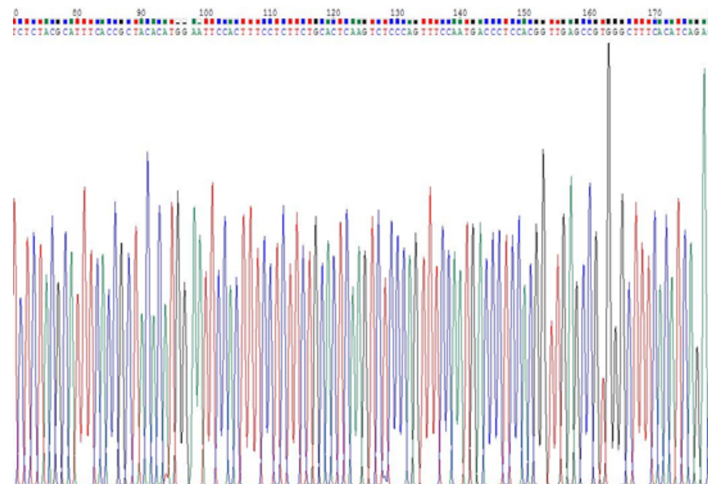


Fig 5: Chromatogram of the bacterial isolate in Sample 3 (Layer Layer)

AAAATCTTTTTGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACG
CATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTT
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTG
CCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTT
ATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTC
CGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGT
GTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGAC
GCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTA
GCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCA
TAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCCAGGATC
AAACTTCTACTCTTAAATTGC

Fig 6: Nitrogen bases of bacterial isolate in Sample 3 (Layer Layer)

3.6 Confirmation of bacterial identity using 16S rDNA sequencing data

Following the molecular characterization of the experimental samples, the poultry feeds BS and BG were found to possess *Bacillus cereus* strain CP133 only, LS contained all three bacteria *viz*., *B. cereus* strain CP133, *Sphingobacterium daejeonense* strain TR6-04 and *Bacillus* sp. strain EPG3, whereas LL lacked *Bacillus* sp. strain ERG3. On the other hand, both eggs and ovaries carried *Bacillus cereus* strain CP133 only, while oviducts bore *S. daejeonense* strain TR6-04 and *Bacillus* sp. strain EPG3.

4. Discussion

4.1 Poultry feeds and their contamination

An earlier investigation revealed that commercial feeds are important vehicles for the introduction of multi-drug resistant *E. coli* into poultry [1]. *Salmonella* sp. and *E. coli* were isolated and identified from seven poultry feeds in Dhaka, Bangladesh, in which TVC values were 6.75×10^4 and 3.05×10^4, respectively [5]. In another report, the bacterial load of 20 samples of poultry feed ranged from 1.03×10^8 cfug^{-1} to 1.232×10^9 cfug^{-1} and the prevalent bacteria identified were *Bacillus*, *E. coli*, *Nocardia*, *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* [9]. Several species of *Salmonella* from 94 poultry feed and waste samples were isolated, in which antibiotics tests showed highest susceptibility of the bacteria to ciprofloxacin but resistant to antibiotics such as tetracycline, norfloxacin, amoxicillin, ampicillin and chloramphenicol [10]. In West Bengal, India, presence of *Clostridium perfringens* in poultry feeds, dead broilers, litters and drinking water was evident, and when subjected to antibiotic sensitivity tests, penicillin G was found the most effective drug and the pathogen was resistant to gentamycin, streptomycin, kenamycin and tetramycin [18]. *Lactobacillus acidophilus* and *L. sporogenes* were reported from poultry feed and faecal samples, where the bacteria were sensitive to penicillin G, amoxicillin, ampicillin and chloramphenicol, but resistant to metronidazole and nalidixic acid [28]. In Mymensingh, Bangladesh, bacterial load in adult layer and its environment such as poultry feed, faeces, litter, drinking water and air were assessed, in which pathogenic *E. coli* and *Pasteurella* spp. and non-pathogenic *Bacillus* spp., *Diplococcus* spp. and *Streptococcus aureus* were identified [29]. In this study, the total viable counts of the feed was $6.5\pm1.87\times10^5$ cfug^{-1} and antibiotic sensitivity tests showed ciprofloxacin most effective against *E. coli* and ampicillin and chloramphenicol against *Pasteurella* spp. In Nigeria, however, species of pathogenic bacteria such as *Streptococcus, Bacillus, E. coli, Salmonella,* and *Pseudomonas* were isolated from commercially available feeds [30]. Bacterial counts in starter, grower, finisher and layer poultry feeds using pour plate technique were studied, where *E. coli* (42.0%), *Salmonella* (24.4%) and *Proteus* (33.6%) were found as the major poultry feed contaminants in Nigeria [31]. Over 90% poultry feed samples had Gram positive bacterial growth where 263 bacterial species or genera including *Corynebacterium, Bacillus, Enterobacter, E. coli, Listeria, Pasteurella, Proteus, Salmonella, Staphylococcus* and *Streptococcus* were identified [32]. In cloacal swab, intestinal fluid, egg surface, faecal material and hand wash of chicken handlers in Dhaka poultry shops, 58% samples were found positive for *E. coli* prevalence [33]. The present findings are somewhat different from those of the aforesaid ones, both in terms of the bacterial species and their load in poultry feeds and moribund chicken specimens, perhaps owing to the difference in manufacturing, handling and distribution of the feed items, coupled with differences in the levels of hygiene, bio-safety measures and management practices in rearing chickens in the study area.

4.2 Antibiotic resistance pattern

Studies on antibiotic resistance pattern of *Salmonella*-like species from poultry soil in Nigeria exacerbated the global problem of antibiotic resistance and a serious health related implication for antibiotic use in poultry [11]. On the other hand, faecal samples from 19 isolates showed 100% sensitivity to ciprofloxacin, gentamicin and tetracycline, 53% resistant to erythromycin and 47% to streptomycin [12]. Recently, poultry meat was found to be contaminated by *Salmonella enterica* and *E. coli* where the former species showed 93% resistance to tetracycline and 100% to augmentin and amoxicillin, but the latter species exhibited 100% resistance to augmentin and amoxicillin [15].

4.3 Antibiogram profile studies

Antibiotic susceptibility patterns of *Lactobacillus, Salmonella* and several species of lactic acid bacteria were studied, respectively from poultry feed, 21 day-old chicks and faeces of broiler chickens [7, 10, 19]. Moreover, antimicrobial sensitivity profiles of seven strains of *Bacillus* species using 12 antibiotics were investigated, where all strains were resistant to bacitracin but were susceptible to gentamycin, neomycin, ormethoprim, triple sulfa and spectinomycin [17], while *Clostridium perfringens* from poultry sources was found resistant to gentamycin, streptomycin, kenamycin and tetramycin [18]. Antibiogram profile studies of bacterial isolates from various poultry sources have recently drawn considerable attention due to the probable dissemination of multi-drug resistant (MDR) bacteria from birds to humans. *E. coli* isolated from moribund poultry birds in Bangalore, India [34], indicated maximum resistance to nitrofurazone (90.77%), followed by tetracycline (83.08%) and cotrimoxazole (76.92%) but the bacterium was highly sensitive to ciprofloxacin and enrofloxacin (83.08%), chloramphenicol (81.54%), pefloxacin (76.92%) and norfloxacin (75.39%). These findings corroborate nicely to the present antibiogram profiles of three pathogenic bacteria under study.

5. Conclusions

To sum up, the following conclusions are derived from the present study and accordingly, a number of recommendations are suggested below. (a) Both poultry feeds and vital parts of the live chickens possess bacterial contaminants; (b) The bacterial isolates showed Gram-positive and Gram-negative staining properties; an average cfumL^{-1} ranged from 28.4×10^{10} to 39.0×10^{10} in the feeds and from 67.0×10^{10} to 103.6×10^{10} in the eggs, ovaries and oviducts; (c) Biochemical tests mostly revealed negative results except for Voges-Proskaur (VP) and carbohydrate utilization tests; the antibiogram profiles of the isolates exhibited sensitive (S) against most antibiotics, some were intermediate (I) and only a few were resistant (R) depending on the zone diameters; and (d) DNA sequence data confirmed the identity of the bacterial isolates.

6. Recommendations

(i) The commercial poultry feeds should be examined periodically for bio-safety, so as to reduce or probably prevent the risk of cross contamination of poultry and poultry products; (ii) The presence of pathogenic bacteria in poultry feed samples calls for attention in the storage, warehouse

condition, distributors and sellers. The findings could be a base line data in setting public health standard for poultry feeds to achieve food security concern issues; (iii) The pathogenic bacteria isolated from this study are of public health importance and their high levels of resistance to commonly used antibiotics in human and vet medicine make them a great risk to human and animals; (iv) Poultry feeds harbour potential pathogenic bacteria including *B. cereus* and their bacterial loads were far above the accepted levels. This constituted a public health hazard and necessitates for the application of the standard measures for the production of feeds by trained manufacturers and health authorities; (v) Personal hygiene is therefore essential in processing and handling of poultry feeds and poultry products; (vi) Results of the present study are to be reconfirmed on the role of poultry as a source of resistant bacteria that may get into human food chain, leading to, among others, food borne intoxications; (vii) There is need to implement measures which guard against misuse of antimicrobial drugs in chicken feeds in order to minimize the emergence and dissemination of antibiotic resistant clones to the humans in close contact with chickens and chicken farms, and so, it is essential to advise farmers about the antibiotic applications in animal farms including poultry; (viii) Potentially pathogenic bacterial strains in chicken feeds and vital body parts suggest potential risk for infection which could be disseminated to humans and other animals; (ix) Hygienic condition of the poultry processing plant therefore should be maintained or monitored regularly in order to reduce or prevent contamination; and (x) Use of regulations to control poultry litter disposal.

7. Acknowledgements

We express our sincere thanks to Research Assistant Mr. Abdullah, an MS non-thesis student and Mr. Tipu Sultan, Laboratory Attendant, whose meticulous collections of field data and sincere laboratory work enriched the contents and findings of this work. Sincere co-operation and assistance received from Government farm officials and private poultry farm owners are also thankfully acknowledged. This work was funded to MSI by the University Grants Commission (UGC) of Bangladesh through the Faculty of Life and Earth Sciences, University of Rajshahi, Bangladesh, during the fiscal year 2015-2016.

8. References

1. Okoli IC, Herbert U, Ozoh P, Udedibie AB. Anti-microbial resistance profile of Escherichia coli isolates from commercial poultry feeds and feed raw materials. Animal Research International. 2005; 2(2):322-328.
2. Boddington M. Bangladesh Poultry Sub-sector Report. Dhaka, Bangladesh, 2007, 160.
3. FEB (The Financial Express Bangladesh). Poultry could help and Bangladesh hunger. Poultry Site News Desk, 2015.
4. Khan MMH, Chowdhury B, Bhuiyan MRH, Uddin MJ, Daulatana M, Rahim M. Nutrient content of poultry feed used in different poultry farms of Bangladesh. Bangladesh Journal of Scientific and Industrial Research. 2008; 43(1):1-12.
5. Chowdhuri A, Iqbal A, Giasuddin M, Bhuiyan AA. Study on isolation and identification of *Salmonella* and *Escherichia coli* from different poultry feeds of Savar region of Dhaka, Bangladesh. Journal of Scientific Research. 2011; 3(2):403-411.
6. Hossain MA, Islam MM, Islam AF, Iji PA. Constraints to use all-vegetable feed ingredients and strategies to improve such diets for poultry birds: A review. Bangladesh Research Publication Journal. 2011; 6(1):120-125.
7. Noohi N, Ebrahimipour G, Rohani M, Talebi M, Pourshafie MR. Phenotypic characteristics and probiotics potential of *Lactobacillus* spp. isolated from poultry. Jundishpur Journal of Microbiology. 2014; 7(9):e17824.
8. Mohanta MK, Islam MS, Saha AK, Khatun S, Khatun Z. Antibiogram profiles of bacterial isolates from poultry feeds and moribund hens in Rajshahi, Bangladesh. Imperial Journal of Interdisciplinary Research. 2016; 2(5):41-46.
9. Okonko IO, Nkang AO, Eyarefe OD, Abubakar MJ, Ojezele MO, Amusan TA. Incidence of multi-drug resistant (MDR) organisms in some poultry feeds sold in Calabar metropolis, Nigeria. British Journal of Pharmacology and Toxicology. 2010; 1(1):15-18.
10. Musa IW, Mansur MS, Saidu L, Mohammed B, Kaltungo BY, Lawan MK et al. Isolation and antibiogram of Salmonella species from waste and poultry feed in selected commercial farms in Zaria, Nigeria. Times Journal of Agriculture and Veterinary Science. 2014; 2(2):75-80.
11. Eja ME, Samuel-Udoekong N, Ikpeme EM, Enyi-Idoh KH, Lennox JA, Etim KD. Antibiogram studies and extended spectrum beta-lactamase activity profile of *Salmonella*-like species isolated from poultry soil of the University of Uyo, Nigeria. Malaysian Journal of Microbiology. 2012; 8(4):280-284.
12. Igbinosa IH. Antibiogram profiling and pathogenic status of *Aeromonas* species recovered from chicken. Saudi Journal of Biological Sciences. 2014; 21:481-485.
13. Khan MS, Akhtar N, Haque ME, Barua A, Chowdhury T, Mullick R et al. Isolation and identification of non-plasmid multidrug resistant *E. coli* from poultry wastes in Chittagong region, Bangladesh. Journal of Bacteriology and Parasitology. 2014; 5(1):1-7.
14. Hossain MT, Siddique MP, Hossain FMA, Zinnah MA, Hossain MM, Alam MK et al. Isolation, identification, toxin profile and antibiogram of *E. coli* isolated from broilers and layers in Mymensingh district of Bangladesh. Bangladesh Journal of Veterinary Medicine. 2008; 6(1):1-5.
15. Adeyanju GT, Ishola O. *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springer Plus. 2014; 3:139. http://www.springerplus.com/ contents /3/1/139.
16. Laban SE, Moustafa GZ, Anwer W, Badawy EM. Microbial load of poultry by-products following rendering process. Global Vet. 2014; 12(6):756-759.
17. Menconi A, Morgan MJ, Pumford NR, Hargis BM, Tellez G. Physiological properties and *Salmonella* growth inhibition of probiotics *Bacillus* strains isolated from environmental and poultry sources. International Journal of Bacteriology. 2013; Article ID 958408, 8 pp; http://dx.doi.org/ 10.1155/2013/958408.
18. Sarkar M, Ray JP, Mukhopadhaya SK, Niyogi D, Ganguly S. Study on *Clostridium perfringens* Type A infection in broilers of West Bengal, India. IIOABJ 2013; 4(4):1-3.
19. Shazali N, Foo HL, Loh TC, Choe DW, Rahim RA. Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia.

Gut Pathology. 2014; 6(1):1-7.

20. Buchannan RE, Gibbons NE. *Bergey's Manual of Determinative Bacteriology* (9th edn). Williams and Wilkins Co., Baltimoe, USA, 1985.
21. Cowan ST. *Cowan and Steel's Manual for Identification of Medical Bacteria* (2nd edn). Cambridge University Press, Cambridge, UK, 1985, 138-139.
22. ISO (International Standardization Organization). Recommendation of meeting of the subcommittee, International Organization for Standardization on meat and meat products. Iso/tc. 1995; 36(Section 6):10-18, The Netherlands.
23. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45(4):493-496.
24. CLSI (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. M100-S16, 2006; 26(3):1-37. http://www.sld.cu/galerias/ pdf.
25. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology. 1981; 145:1365-1373.
26. Khanam MS. Characterization of the bacterial isolates from poultry feeds and reproductive organs of the broiler hens in Rajshahi, Bangladesh. Unpubl. M.S. Thesis, Department of Zoology, Rajshahi University. 2016, 111, 12.
27. Steel RGD, Torrie JH. Principles and Procedures of Statistics: A Biometrical Approach. 1984; McGraw Hill, Tokyo, Japan.
28. Lonkar P, Harne SD, Kalorey DR, Kurkure NV. Isolation *in vitro* antibacterial activity, bacterial sensitivity and plasmid profile of lactobacilli. Asian-Australian Journal of Animal Science. 2005; 18(9):1336-1342.
29. Nasrin MS, Islam MJ, Nazir KHMNH, Choudhury KA, Rahman MT. Identification of bacteria and determination of their load in adult layer and its environment. Journal of Bangladesh Society for Agricultural Science and Technology. 2007; 4(1, 2):69-72.
30. Uwaezuoke JC, Ogbulie JN. Microbiological quality of commercially available feeds in parts of Eastern Nigeria. Journal of Applied Science and Environmental Management. 2008; 12(1):113-117.
31. Onyeze RC, Onah GT, Eluke OC. Bacterial contaminants associated with commercial poultry feeds in Enugu Nigeria. International Journal of Life Science, Botany and Pharmacy Research. 2013; 2(3):432-437.
32. Aliyu RM, Egwu EO, Abubakar MB, Adamu AY, Salihu MD, Dabai AI et al. Bacteriological quality of commercially prepared and self compounded poultry feeds in Sokoto metropolis, Sokoto, Nigeria. International Journal of Applied Biology and Pharmacy Technology. 2012; 3(1):345-350.
33. Akond MA, Alam S, Hassan SMR, Shirin M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Internet Journal of Food Safety. 2009; 11:19-23.
34. Sharada R, Ruban S, Thiyageeswaran M. Antibiotic resistance pattern of *Escherichia coli* isolated from poultry in Bangalore. Internet Journal of Microbiology. 2008; 7(1).