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 hen (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, 25.00% resistant and 13.33% intermediate against eight antibiotics. Three bacteria Bacillus cereus, Sphingobacterium daejeonense 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 °C 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 °C 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 (cfuL\(^{-1}\)) was calculated as follows: total number of bacteria per mL = number of colonies counted × dilution factor.

Plate 1: Cultures for bacterial isolates
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 \cite{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 \cite{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. Further details of the protocols are described recently \cite{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 cfu/mL 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 \cite{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 \textit{Bacillus cereus} strain CP133 only, LS contained all three bacteria viz., \textit{B. cereus} strain CP133, \textit{Sphingobacterium daejeonense} strain TR6-04 and \textit{Bacillus} sp. strain EPG3, whereas LL lacked \textit{Bacillus} sp. strain ERG3. On the other hand, both eggs and ovaries carried \textit{Bacillus cereus} strain CP133 only, while oviducts bore \textit{S. daejeonense} strain TR6-04 and \textit{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

<table>
<thead>
<tr>
<th>Test parameters</th>
<th>BS</th>
<th>BG</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture media</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacConkey’s agar</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella-Shigella (SS) agar</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Voges-Proskauer (VP) agar</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Biochemical tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl red</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Simon citrate</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sulphur reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sugar utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Cellulose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(ii) Fructose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(iii) Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(iv) Lactose</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(v) Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(vi) Sucrose</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(vii) Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>B. cereus strain CP133</th>
<th>S. daejeonense strain TR6-04</th>
<th>Bacillus sp. strain EPG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BG</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LL</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chicken samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ovaries</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oviducts</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Name of samples</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (BS)</td>
<td>+ve</td>
</tr>
<tr>
<td>Sample 2 (BG)</td>
<td>-ve</td>
</tr>
<tr>
<td>Sample 3 (LL)</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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 ×10^{12}, respectively for BS, BG and LL poultry feeds, and 92 to 112×10^{12}, 69 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 cfu\text{L}^{-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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>TVC (cfu\text{L}^{-1})*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feed samples (n=15)</strong></td>
<td></td>
</tr>
<tr>
<td>BS</td>
<td>23.40±2.88 ×10^{12}</td>
</tr>
<tr>
<td>BG</td>
<td>28.60±1.52 ×10^{12}</td>
</tr>
<tr>
<td>LL</td>
<td>33.20±1.30 ×10^{12}</td>
</tr>
<tr>
<td>F-value at 2, 29</td>
<td>228.89***</td>
</tr>
<tr>
<td><strong>Chicken samples (n=15)</strong></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>100.40±7.54 ×10^{12}</td>
</tr>
<tr>
<td>Ovaries</td>
<td>72.20±3.35 ×10^{12}</td>
</tr>
<tr>
<td>Oviducts</td>
<td>70.20±3.96 ×10^{12}</td>
</tr>
<tr>
<td>F-value at 2, 29</td>
<td>51.11***</td>
</tr>
</tbody>
</table>

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 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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>BS Sensitivity (DD)</th>
<th>BG Sensitivity (DD)</th>
<th>LL Sensitivity (DD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP (10µg)</td>
<td>S (25)</td>
<td>S (22)</td>
<td>R (6)</td>
</tr>
<tr>
<td>BAC (10µg)</td>
<td>R (9)</td>
<td>S (25)</td>
<td>I (11)</td>
</tr>
<tr>
<td>CEF (30µg)</td>
<td>S (25)</td>
<td>S (26)</td>
<td>S (15)</td>
</tr>
<tr>
<td>CIP (5µg)</td>
<td>S (30)</td>
<td>S (25)</td>
<td>S (25)</td>
</tr>
<tr>
<td>DOX (30µg)</td>
<td>S (28)</td>
<td>S (25)</td>
<td>I (12)</td>
</tr>
<tr>
<td>GEN (10µg)</td>
<td>S (35)</td>
<td>S (25)</td>
<td>R (9)</td>
</tr>
<tr>
<td>SUL (25µg)</td>
<td>R (9)</td>
<td>R (6)</td>
<td>R (6)</td>
</tr>
<tr>
<td>TET (30µg)</td>
<td>S (30)</td>
<td>S (22)</td>
<td>S (20)</td>
</tr>
</tbody>
</table>

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= bacitracin; 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 S1, S2, S3 and S4; (B) PCR amplification of the 16S rDNA of isolates S1, S2, S3 and S4. 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)

ATAAAATTTTTCGCGCCTAGTGTCAGTTACAGACCAGAAAGTCGCCCTGCCACCTGACTGTTCTCTCCATATTCTTACGCA
ATTTCACCCGGCTACATGGAATTCACCTTCCTTTCTTCTTGCACTAAAGTCTCCAGTTCTCCATATGACCTCCTACGGTT
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCC
ACCTACGTATTACCGGCGGTACGTGCAGTTTTCTGTTAGTAGTACCAGTGCAAGTGCGCTGCTCCTAGGCGTGGCTTTG
ATTCAACTAGCACTTGCTTTTCCGATTGAAAAAGCTTTACACACCTCCATACCGGCGTGTTACGTCCAGTTCCAGCT
ATCTGAGATTCTCGCCGACTCCGCACTCGGCTGTCTCTTGGTGAGCCGTACCTCACCACTAGCTAATGCGACGCGGGTCC
AATAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTGAAAAATGTTATCCGGTATTA

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)

```
TAAATCTTTTTGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACG
CATTTCACCGCTACACATGGAATTCCACTTTCCTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTG
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTG
CCACCTACGTATTACCGCGGCTGCTGGACGTAGTTAGGTTACCGTCACCGGCAAGTTGCGCGAGCTT
```

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

```
AAAATCTTTTTGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACG
CATTTTCACCGCTACACATGGAATTCCACTTTCCTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTG
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTG
CCACCTACGTATTACCGCGGCTGCTGGACGTAGTTAGGTTACCGTCACCGGCAAGTTGCGCGAGCTT
```

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

```
AAAATCTTTTTGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACG
CATTTTCACCGCTACACATGGAATTCCACTTTCCTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTG
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTG
CCACCTACGTATTACCGCGGCTGCTGGACGTAGTTAGGTTACCGTCACCGGCAAGTTGCGCGAGCTT
```

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

```
AAAATCTTTTTGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACG
CATTTTCACCGCTACACATGGAATTCCACTTTCCTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTG
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTG
CCACCTACGTATTACCGCGGCTGCTGGACGTAGTTAGGTTACCGTCACCGGCAAGTTGCGCGAGCTT
```
3.6 Confirmation of bacterial identity using 16S rDNA sequencing data
Following the molecular characteristic 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* [1]. An earlier investigation revealed that commercial feeds are important vehicles for the introduction of multi-drug resistant *E. coli* whereas LL lacked *E. coli* CP133 only, while oviducts bore *S. daejeonense* strain TR6-04 and *Bacillus* sp. strain EPG3. 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.

4.2 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 [13].

4.3 Antiibiogram 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, 9]. 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, ornithoprim, triple sulfa and spectinomycin [17], while *Clostridium perfringens* from poultry sources was found resistant to gentamycin, streptomycin, kanamycin and tetracymin [18]. Antiibiogram 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 cfu/mL ranged from 28.4 × 10^6 to 39.0 × 10^5 in the feeds and from 67.0 × 10^8 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.

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8. References

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