Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-5, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

Antibiogram Profiles of Bacterial Isolates from Poultry Feeds and Moribund Hens in Rajshahi, Bangladesh

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Abstract: *In a series of experiments, pathogenic bacteria from poultry feeds and moribund hens' reproductive organs were isolated and identified, and susceptibility of the bacteria to a number of antibiotics was assessed. Four types of branded poultry feeds available in Rajshahi City Corporation areas viz., broiler starter (BS), broiler grower (BG), layer starter (LS) and layer layer (LL) were collected from five randomly chosen shops. The feed samples were cultured in nutrient agar media and the bacterial specimens were isolated and examined under microscope for their gross morphological and biochemical characteristics. Results showed that the bacterial isolates belonged to Escherichia coli, Enterococcus sp. and Salmonella sp. Total viable counts (TVC) of the bacteria were replicated five times per feed and the BS, BG, LS and LL showed* $23.40\pm2.88\times10^{12}$, $28.60\pm1.52\times10^{12}$, $83.20\pm7.40\times10^{12}$, *and* $33.20\pm1.30\times10^{12}$ $cfumL^{-1}$ *(colony forming units per millilitre), respectively. The highest TVC was recorded in LS where all three genera of the bacteria were found. In LL, on the other hand, E. coli and Salmonella sp. and in BS and BG, only Salmonella sp. was diagnosed. Sonali moribund hens were collected from five randomly selected poultry farms situated in Rajshahi metropolis. Samples of eggs, ovary and oviduct were suspended in physiological saline, one loopful of each suspension was inoculated separately into nutrient agar medium and the bacterial specimens were isolated and identified to be E. coli and Salmonella sp. TVC of bacteria in the eggs, ovary and oviduct samples yielded* $100.40\pm3.96\times10^{12}$, $72.20\pm3.35\times10^{12}$ *and* $70.20\pm3.96\times10^{12}$ $cfumL^{-1}$, *respectively. The highest TVC was recorded in the eggs, eggs and ovary specimens only had Salmonella sp. whereas oviduct contained E. coli and Salmonella sp. Finally, antibiotic sensitivity patterns of the bacterial isolates were tested against eight commonly used antibiotics viz., ampicillin (AMP), bacitracin (BAC), ceftriaxone (CEF), ciprofloxacin (CIP), doxycycline (DOX), gentamicin (GEN), sulphamethoxazole (SUL) and tetracycline (TET). E. coli showed resistance to BAC and SUL, Enterococcus sp. was highly resistant only to SUL while Salmonella sp. showed high to moderate resistance to AMP, GEN and SUL. The presence of three pathogenic bacteria in poultry feeds in Rajshahi, coupled with the diagnosis of E. coli and Salmonella in the reproductive organs of the moribund hens were indicative of bacterial contamination which reinforces the need for effective control measures against the pathogenic microbes, due care in handling of the poultry feeds and proper hygiene in the processing of the poultry meat and eggs for human consumption.*

Key Words Poultry feeds, bacterial isolates, pathogenic bacteria, moribund hens, antibiotics

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 farm environment [1-2]. During the past decades, poultry industry in Bangladesh grew at the rate of 20% per annum [3] and nowadays, it is one of the fastest growing agro-based enterprises in Bangladesh [4], where a large number of private-owned companies produce poultry feeds of varying standards [5]. All four basic types of poultry feeds *viz*., starters, growers, finishers and layers, however, may potentially become contaminated with

foodborne pathogenic microbes during harvesting, processing, handling, and marketing of the bagged feeds [6]. 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 and elsewhere [7-12].

Several studies have demonstrated that sources of microbial infections in poultry include contaminated feeds, drinking water, utensils, personnel, human wastes, rodents and hatchery related unhygienic activities [13]. 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 [14-15]. Apart from poultry feeds, however, poultry environment like soil and drinking water [16-17], faeces, litters and wastes [18-20], live, moribund and dead chickens (Hossain et al., 2008), meat, carcass, viscera, eggs, and poultry by-products [21-24] 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 *Escherichia coli* [23, 25], *Salmonella* spp. [15, 17, 23], *Bacillus* spp. [26], *Clostridium* spp. [27], *Aeromonas* sp. [19] and *Lactobacillus* spp. [12, 28] are worth mentioning. Considering the health hazard posed to poultry birds and human consumers of microbe-contaminated feeds and live and/or dead poultry birds, the present investigation was designed to isolate, identify and characterize the antibiogram profiles of the bacterial isolates from commercially available poultry feeds and moribund chickens from Rajshahi City Corporation, Bangladesh.

2. Materials and Methods

Collection of samples

A total of 35 bacterial isolates, comprising 20 from four branded poultry feeds *viz*., layer starter [LS], layer layer [LL], broiler starter [BS] and broiler grower [BG], and 15 from eggs, ovaries and oviducts of the crossbred *Sonali* (derived from Fayoumi♀ × RIR♂) moribund hens 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 study.

Isolation of bacteria from poultry feeds and moribund hens

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 moribund 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.

Identification of bacterial isolates

The bacterial isolates were identified by observing their gross colony morphology grown on MacConkey's, Salmonella-Shigella (SS) and Voges-Proskauer (VP) agar media. The identities of the microbes were confirmed employing [29]. In addition, the isolates were subjected to conventional biochemical tests such as indole production, methyl red, motility, Simon citrate, sulphur reduction and sugar utilization, following the standard methods [30]. Pure cultures of the bacteria were isolated and maintained using MacConkey's agar medium.

Determination of bacterial load of the isolates

Enumeration of total viable counts (TVC) of the bacterial isolates was made as per the ISO recommendation [31]. 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 nutrient agar plate. Then the inoculated Petri plates were incubated at 37°C for 24h, after which incubation of the bacteria of different samples were grown, forming many colonies on the nutrient agar media. Finally, colony forming units per millilitre ($cfumL^{-1}$) were calculated as follows: total number of bacteria per mL= number of colonies counted × dilution factor.

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 [32-33]. 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).

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 [34]. Antimicrobial susceptibility profile of the bacterial isolates in response to antibiotics has been presented in a histogram.

3. Results

Identification of the bacteria isolates

Three bacterial species *viz*., *E. coli*, *Enterococcus* sp. and *Salmonella* sp. were isolated and identified from the isolates of poultry feeds and chicken specimens (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 *Salmonella* sp. only, LS contained all three bacteria, whereas LL lacked *Enterococcus* sp. On the other hand, both eggs and ovaries carried *Salmonella* sp. only, while oviducts bore *E. coli* and *Salmonella* sp.

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}, 75 to 94×10^{12} and 32 to 35×10^{12}, respectively for BS, BG, LS 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 3). Results revealed that the bacterial loads differed significantly between both poultry feed types as well as moribund 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 ($P>0.05$).

Antibiotic susceptibility patterns of the isolates

Results on the antibiogram profiles of the bacterial isolates (Table 4) 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 majority of the antibiotics used.

4. Discussion

An earlier investigation revealed that commercial feeds are important vehicles for the introduction of multi-drug resistant *E. coli* into poultry [1]. *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 [2]. *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 [6]. 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 [7]. 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 [9]. 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 [11]. 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* [13]. 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 [14]. 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 [15]. 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 [16]. 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 [27]. 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.

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 [17]. 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 [19]. 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 [23].

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 [25], indicated maximum resistance to nitrofurazone (90.77%), followed by tetracycline (83.08%) and cotrimoxazole (76.92%) but the bacterium was highly sensitivity to ciprofloxacin and enrofloxacin (83.08%), chloramphenicol (81.54%), pefloxacin (76.92%) and norfloxacin (75.39%). Subsequently, 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 [26], while *Clostridium perfringens* from poultry sources was found resistant to gentamycin, streptomycin, kenamycin and tetramycin [27]. 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 [12, 15, 28]. These findings corroborate nicely to the present antibiogram profiles of three pathogenic bacteria under study.

5. Conclusion

The presence of appreciably high bacterial loads of $23\text{-}100\times10^{12}$ cfumL^{-1} in poultry feeds and moribund chicken samples calls for attention to those concerned with rearing, storage, distribution and selling of poultry items in the country. The present data also show that commercial feeds and live poultry birds could be important vehicles for the introduction of multi-drug resistant (MDR) genes from *E. coli*, *Enterococcus* or *Salmonella* into humans through poultry. In addition, these pathogenic bacteria pose threat to health by food poisoning and/or infection to animals and humans. Presence of pathogenic bacteria in the poultry samples also implies that eggs and meat should not be consumed half-cooked or raw. The need therefore for effective control measures for hygiene in processing and handling of poultry feeds as well as poultry birds is emphasized. The present findings could be a base line data in setting public health standard for poultry feeds and meat to achieve food security concern issues. Further study is solicited on the role of poultry environment as a source of resistant and pathogenic bacteria that may get into human food chain leading to, among others, diseases like foodborne intoxication in consumers.

6. Acknowledgements

This forms part of MS research by Salma Khatun and Zakia Khatun. Co-operations offered by the poultry farm owners and technical assistance by the Laboratory Attendants are thankfully acknowledged. The Chairman, Department of Zoology, University of Rajshahi, Bangladesh, deserves special thanks for providing laboratory facilities.

7. References

1. Okoli, I.C., Herbert, U., Ozoh, P., and Udedibie, A.B. Anti-microbial resistance profile of Escherichia coli isolates from commercial poultry feeds and feed raw materials. Anim. Res. Int., 2005; 2(2), 322-328.
2. Lonkar, P., Harne, S.D., Kalorey, D.R., and Kurkure, N.V. Isolation in vitro antibacterial activity, bacterial sensitivity and plasmid profile of lactobacilli. Asian-Aust. J. Anim. Sci., 2005; 18(9), 1336-1342.
3. Boddington, M. Bangladesh Poultry Sub-sector Report. Dhaka, Bangladesh, 2007; pp.160.
4. FEB, (The Financial Express Bangladesh). Poultry could help and Bangladesh hunger. 21 January 2015; PoultrySite News Desk.
5. Khan, M.M.H., Chowdhury, B., Bhuiyan, M.R.H., Uddin, M.J., Daulatana, M., and Rahim, M. Nutrient content of poultry feed used in different poultry farms of Bangladesh. Bangladesh J. Sci. Ind. Res., 2008; 43(1), 1-12.
6. Chowdhuri, A., Iqbal, A., Giasuddin, M., and Bhuiyan, A.A. Study on isolation and identification of *Salmonella* and *Escherichia coli* from different

poultry feeds of Savar region of Dhaka, Bangladesh. J. Sci. Res., 2011; 3(2), 403-411.
7. Nasrin, M.S., Islam, M.J., Nazir, K.H.M.N.H., Choudhury, K.A., and Rahman, M.T. Identification of bacteria and determination of their load in adult layer and its environment. J. Bangladesh. Soc. Agri. Sci. Technol., 2007; 4(1&2), 69-72.
8. Hossain, M.A., Islam, M.M., Islam, A.F., and Iji, P.A. Constraints to use all-vegetable feed ingredients and strategies to improve such diets for poultry birds: A review. Bangladesh Res. Pub. J., 2011; 6(1), 120-125.
9. Uwaezuoke, J.C., and Ogbulie, J.N. Microbiological quality of commercially available feeds in parts of Eastern Nigeria. J. Appl. Sci. Environ. Manage., 2008; 12(1), 113-117.
10. Cegielska-Radziejewska, R., Stuper, K., and Szablewski, T. Microflora and microtoxin contamination in poultry feed mixtures from western Poland. Ann. Agric. Environ. Med., 2013; 20(1), 30-35.
11. Onyeze, R.C., Onah, G.T., and Eluke, O.C. Bacterial contaminants associated with commercial poultry feeds in Enugu Nigeria. Int. J. Life. Sc. Bt. & Pharm. Res., 2013; 2(3), 432-437.
12. Noohi, N., Ebrahimipour, G., Rohani, M., Talebi, M., and Pourshafie, M.R. Phenotypic characteristics and probiotics potential of *Lactobacillus* spp. isolated from poultry. Jundishpur J. Microbiol., 2014; 7(9), e17824.
13. Okonko, I.O., Nkang, A.O., Eyarefe, O.D., Abubakar, M.J., Ojezele, M.O., and Amusan, T.A. Incidence of multi-drug resistant (MDR) organisms in some poultry feeds sold in Calabar metropolis, Nigeria. British J. Pharmacol. Toxicol., 2010; 1(1), 15-18.
14. Aliyu, R.M., Egwu, E.O., Abubakar, M.B., Adamu, A.Y., Salihu, M.D., Dabai, A.I., and Tambuwal, F.M. Bacteriological quality of commercially prepared and self compounded poultry feeds in Sokoto metropolis, Sokoto, Nigeria. Int. J. Appl. Biol. Pharm. Technol., 2012; 3(1), 345-350.
15. Musa, I.W., Mansur, M.S., Saidu, L., Mohammed, B., Kaltungo, B.Y., Lawan, M.K., and Talba, A.M. Isolation and antibiogram of Salmonella species from waste and poultry feed in selected commercial farms in Zaria, Nigeria. Times J. Agric. Vet. Sci., 2014; 2(2), 75-80.
16. Akond, M.A., Alam, S., Hassan, S.M.R., and Shirin, M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Internet J. Food Safety, 2009; 11, 19-23.
17. Eja, M.E., Samuel-Udoekong, N., Ikpeme, E.M., Enyi-Idoh, K.H., Lennox, J.A., and Etim, K.D. Antibiogram studies and extended spectrum beta-lactamase activity profile of *Salmonella*-like species isolated from poultry soil of the University of Uyo, Nigeria. Malasian J. Microbiol., 2012; 8(4), 280-284.
18. Hemen, J.T., Johnson, J.T., Ambo, E.E., Ekam, V.S., Odey, M.O., and Fila, W.A. Multi-antibiotic resistance of some Gram negative bacterial isolates from poultry litters of selected farms in Benue State. Int. J. Sci. Technol., 2012; 2(8), 543-547.
19. Igbinosa, I.H. Antibiogram profiling and pathogenic status of *Aeromonas* species recovered from chicken. Saudi J. Biol. Sci., 2014; 21, 481-485.
20. Khan, M.S., Akhtar, N., Haque, M.E., Barua, A., Chowdhury, T., Mullick, R., and Mahmud, A.S.M. Isolation and identification of non-plasmid multidrug resistant E. coli from poultry wastes in Chittagong region, Bangladesh. J. Bacteriol. Parasitol., 2014; 5(1), 1-7.
21. Haziyamin, T.A.H., and Ezureen, E. Isolation of lactic acid bacteria from Malaysian non-broiler chicken (*Gallus gallus*) intestine with potential probiotics for broiler feeding. IIUM Eng. J., 2011; 12(4), 133-139.
22. Bhandari, N., Nepali, D.B., and Paudayal, S. Assessment of bacterial load in broiler chicken meat from local the retail meat shops in Chitwan, Nepal. Int. J. Infect. Microbiol., 2013; 2(3), 99-104.
23. Adeyanju, G.T., and 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.
24. Laban, S.E., Moustafa, G.Z., Anwer, W., and Badawy, E.M. Microbial load of poultry by-products following rendering process. Global Vet., 2014; 12(6), 756-759.
25. Sharada, R., Ruban, S., and Thiyageeswaran, M. Antibiotic resistance pattern of *Escherichia coli* isolated from poultry in Bangalore. Internet J. Microbiol., 2008; 7(1).
26. Menconi, A., Morgan, M.J., Pumford, N.R., Hargis, B.M., and Tellez, G. Physiological properties and *Salmonella* growth inhibition of probiotics *Bacillus* strains isolated from environmental and poultry sources. Int. J. Bacteriol., 2013; Article ID 958408, 8 pages. http://dx.doi.org/10.1155/2013/958408.
27. Sarkar, M., Ray, J.P., Mukhopadhaya, S.K., Niyogi, D., and Ganguly, S. Study on *Clostridium perfringens* Type A infection in broilers of West Bengal, India. IIOABJ, 2013; 4(4), 1-3.
28. Shazali, N., Foo, H.L., Loh, T.C., Choe, D.W., and Rahim, R.A. Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. Gut Path., 2014; 6(1), 1-7.
29. Buchannan, R.E., and Gibbons, N.E. Bergey's Manual of Determinative Bacteriology, 9th ed., 1985; Williams and Wilkins Co., Baltimoe, USA.
30. Cowan, S.T. Cowan and Steel's Manual for Identification of Medical Bacteria, 2nd ed., Cambridge University Press, Cambridge, UK, 1985; pp. 138-139.
31. ISO, (International Standardization Organization). Recommendation of meeting of the subcommittee, International Organization for Standardization on meat and meat products. Iso/tc. 36, section 6, 1995; pp. 10-18, The Netherlands.
32. Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 1966; 45(4), 493-496.

33. 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.
34. Steel, R.G.D., and Torrie, J.H. Principles and Procedures of Statistics: A Biometrical Approach. 1984; McGraw Hill, Tokyo, Japan.

Table 1 Culture media and biochemical tests for the isolated bacteria from poultry feeds and chicken samples

Test parameters	*E. coli*	*Enterococcus* sp.	*Salmonella* sp.
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	+	+	+

+= Presence; -= Absence

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

Isolates	*E. coli*	*Enterococcus* sp.	*Salmonella* sp.
Feed samples			
BS	-	-	+
BG	-	-	+
LS	+	+	+
LL	+	-	+
Chicken samples			
Eggs	-	-	+
Ovaries	-	-	+
Oviducts	+	-	+

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

Table 3 Total viable counts of bacteria from poultry feeds and chicken samples

Isolates	TVC ($cfumL^{-1}$)*
Feed samples (n=12)	
BS	$^{a}23.40\pm2.88 \times10^{12}$
BG	$^{a}28.60\pm1.52 \times10^{12}$
LS	$^{b}83.20\pm7.40 \times10^{12}$
LL	$^{c}33.20\pm1.30 \times10^{12}$
F-value at 3, 44	228.89***
Chicken samples (n=9)	
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, 24	51.11***

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

Table 4 Antibiotic susceptibility pattern of the bacterial isolated from poultry feeds and chickens samples.

Antibiotics	*E. coli*	*Enterococcus* sp.	*Salmonella* sp.
	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)

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.