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# Bacterial Contamination of Poultry Feeds, Molecular Studies and Antibacterial Resistance Profiles of Isolates in Keffi Metropolis, Nigeria

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**----------ABSTRACT ----------**

A study to investigate the presence of bacteria and their molecular characterization from poultry feeds sold in Keffi Metropolis was conducted. A total of 50 samples from three different brands involving four feed types namely Broilers, Layers, Quail and sussex were aseptically collected and analysed using standard microbiological techniques. Preliminary characterization of the bacterial isolates revealed the presence of the following contaminants: Escherichiacoli, Salmonella sp., Bacillus sp., Klebsiella sp., Enterococcus sp., Staphylococcusaureus and Proteus sp. The bacterial load of the feeds were in the range of 0.49–7.08cfu/g with Brand A having the highest number of bacterial contamination. Bacillus sp. and Salmonella sp. had the highest occurrence rate of 34% each, while Proteus sp. and Klebsiella sp. had the lowest frequency of occurrence of 6% each. Staphylococcus sp., Enterococcus sp. and Escherichiacoli had 14%, 12% and 24 % occurrence rates respectively. Nonetheless, the isolates were highly resistant to Tetracycline (65.5%), Ceftazidime (51.9%), Amoxicillin (49.9%), Septrin (49.4%) and Ampicillin (47.9%), moderately resistant to Chloramphenicol (39.5%) and Cefoxitin (35.8%). However, the isolates showed varying degree of sensitivity to the following antibiotics: Cefoxitin (35.8%), Ciprofloxacin (30.4%) and Streptomycin (27.8%), but was highly susceptible to Gentamicin with a point resistant levels of 22.9%. Molecular studies of the isolates with multiple drug resistance showed presence of at least one Plasmid DNA with band weights between 1330bp to 1900bp. Thus all the poultry feeds were found to be contaminated with resistant bacteria which may pose a public health risk humans. It therefore becomes imperative to for routine bacteriological quality assessment to be conducted on poultry feeds in other to improve production performances in poultry management..

**Keywords**: Bacteria, Poultry Feeds; Resistance, Molecular, Nigeria.

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## I. INTRODUCTION

Poultry refers to all birds that have been domesticated by man. The birds include domestic fowl, duck, geese, turkey, guinea fowl, pigeons and ostriches.Common constituents' of feeds include whole cereals, soya beans, vitamins and vegetables such as water leaf, tridax and Amaranthus spp. The protein contents of the feed vary depending on the purpose of keeping the birds. The protein contents of 13%,16%,18%, 21% and 23% is usually a standard measurement for chicks marsh, layers marsh, growers marsh, broiler starter and broiler finish respectively (Barakat, 2004).Various brands of poultry feeds are in existence depending on the functions they perform in the birds. Thus, there are growers, finishers, layers, starters among others (Yusuf et al., 2016). Materials for formulation of feeds are sourced from different origin both animals and plants and are mostly agro wastes (Obi and Ozugbo, 2007; Afolayan and Afolayan, 2008).Nonetheless, the safety and quality of poultry feeds are great subject in developed countries, that feed safety is an essential requirement for all animals. Unsafe feed may also causes great economic losses because of destroying an infected flock of birds and there is a proof that poultry feed is often infected with food-borne pathogenic bacteria (Al-Musawiet al., 2016).

Accordingly, poultry feed can serve as a carrier for a range of microbial contaminants such as moulds, mycotoxins and bacteria (Maciorowkiet al., 2007). In fact, many bacteria are associated with environmental contamination of feed ingredients from the family enterobacteriaceae. This family comprises of many established genera including Escherichia, Enterobacter, Salmonella,etc (Okogunetal., 2016). Also, several microorganisms such as Bacillus spp., Staphylococcus spp., E.coli, Campylobacter spp. and Clostridium perfringenscan contaminate poultry feeds either from feed ingredients, through farms' workers, equipment, air, handling, used bags or raw materials (Nahid, 2010). There is a considerable evidence that poultry feed is

frequently contaminated with food-borne bacterial pathogens.Animal feed has been shown to be a major vector for transmission of salmonella and other bacteria to the farm and processing plants(Nahid, 2010). Humans become infected when they ingest contaminated meat or poultry products (Crump et al., 2002). Aflatoxins are known to be present in poultry eggs and human diseases like diarrhoea, Salmonellaparatyhi-fever have been associated with consumption of poultry birds that contracted the infections from contaminated poultry feeds (Obi and Ozugbo, 2007).

In modern poultry production, antibiotics are used for treatment and prevention of infectious diseases in farm animals intended for food production and to protect public health from food borne diseases. Antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic resistant microorganisms in both veterinary and human medicine (Akondet al., 2009).Common antibiotics used are bacitracin, chlrotetracycline, erythromycin, and penicillin. The fluoroquinolones are important members of the quinolone group of antibiotics used to treat diseases in humans and their use in livestock animals. This can contribute to increased resistance in food borne bacteria (Campylobacter and Salmonella) which may infect humans (Onyezeet al., 2013).As such the transmission of plasmid mediated resistance between different bacterial species and genera have now widely occurred (Davies, 1994).The emergence and continuing presence of multidrug resistant isolates has roused so much concern in terms of horizontal transfer of the resistant gene (multi-drug resistant) when such poultry are consumed. As such there has been a ban placed on use of antibiotics in poultry formulation in United States, Europe and Australia (Ezekiel et al., 2011).

In Nigeria,Obi and Ozugbo(2007), Ezekiel et al. (2011), Onyezeet al. (2013) and Okogunet al. (2016) independently isolated pathogenic bacteria genera and species from the poultry feed samples sold in different parts of the country. Lateefet al. (2014) had observed that the production of poultry feeds for local and commercial farmers in the developing countries including Nigeria requires adequate microbiological safety regulations to escape microbial contamination of the product. Interestingly, molecular sub-typing and antibiotic resistant profiles are phenotypic properties that have been used worldwide, usually in association with a number of nucleic acid-based typing methods that have been proposed for typing bacteria. Another approach is the analysis of fragment patterns obtained by macro-restriction and separated using the pulsed field gel electrophoresis (PFGE) technique (Ammariet al., 2009; Abatchaet al., 2014). Consequently, this study was designed to determine the bacterial contamination of poultry feeds in North Central Nigeria, subject the isolates to molecular studies and determine their antibacterial resistance to commonly dispensed antibiotics.

## II. MATERIALS AND METHODS

**Study Area**

The study was carried out in Keffi Nasarawa State. Keffi is approximately 68km from Abuja, the Federal Capital Territory of Nigeria and 128km from Lafia, the Capital of Nasarawa state Keffi is located between latitude $8^{o}5$ N of the equator and longitude $7^{o}8$ E and situated on an altitude of 850m above sea level (Akwaet al., 2007).

**Sample Collection**

A total of fifty (50) samples were collected from three brands of poultry feeds from markets in Keffi Metropolis, Nasarawa State, Nigeria. Each brand had four types namely: Broilers, Layers, Quails and Sussex. The samples were collected aseptically by wearing gloves and using sterile spatula to fetch out the feed sample from the bag into sterile white polythene bags and labelled appropriately. The samples collected were transported to the Microbiology Laboratory of Nasarawa State University, Keffi for analyses.

**Determination of Bacterial Load**

The feed samples were processed according to Matthewet al. (2017). 1g of each poultry feed was homogenized in 9ml of sterile water in a test tube. Serial dilution was carried out to $10^{-5}$ dilution by transferring 1ml of the homogenized sample into another test tube containing 9ml of sterile distilled water. 1ml was picked from the first test tube using a pipette and transferred to a second test tube containing 9ml sterile distilled water to get $10^{-1}$. One (1ml) was picked from the test tube containing $10^{-1}$ dilution by the pipette and transferred to a third tube containing 9ml sterile distilled water to get $10^{-2}$.The steps were repeated till $10^{-5}$. 0.5ml aliquot of $10^{-5}$ diluted sample was inoculated onto already prepared and solidified agar. Nutrient agar for viable count, MacConkey agar for coliform, Mannitol salt agar, for Staphylococcus aureus, Eosine Methylene Blue for faecal count using spread plate method. The dilution was spread with a bent glass rod to ensure even distribution of the sample and incubated for 18 hours at $37^{o}$C. Then the grown bacteria were counted and expressed as colony forming unit (cfu) using colony counter.

The grown colonies were observed and the colonies were picked individually from the initial culture plate and streaked onto already prepared Nutrient Agar plates. The isolation of pure culture was performed bycontinuous sub-culturing until pure culture was obtained. The pure cultures were then stocked in agar slants and labelled appropriately and stored in a refrigerator at 4-8$^{o}$C for subsequent characterization.

**Characterization and Identification of Isolates**

The isolates were characterised and identified mainly on the basis of their colony appearance, cellular morphology and biochemical reactions. The colony characteristics of all the isolates (shape, size, consistency, pigments and type of growth on media) were observed and recorded. A small portion of the pure culture was transferred onto a clean grease-free glass slide, and emulsified in a drop of distilled water until a thin homogeneous film was obtained, then the wire loop was re-sterilized and the thin homogeneous film was allowed to air-dry, and heat-fixed by passing through Bunsen burner flame. It was viewed under the microscope at ×40 and × 100 lenses and the cellular morphology recorded.The Gram staining technique was carried out as described by Cheesbrough (2006). The following biochemical tests were carried out on the suspected isolates: Catalase test, Indole, Methyl red, Vorges-Proskauer tests, Nitrate reduction, Urease production, Oxidase test, Citrate utilisation, and glucose fermentation tests.

**Antibiotics Susceptibility Testing**

The antibiotics susceptibility test of the isolates were carried out using Kirby-Bauer disk diffusion method with some modifications as described in Clinical Laboratory Standard Institute manual (CLSI, 2012). The antibiotic disks were firmly placed on the sterile Mueller Hinton Agar (MHA) plates seeded with test organisms, standardized to 0.5 McFarland's standard (equivalent to $10^{5}$cfu) and incubated at 37$^{o}$C for 24 hours. Diameter of zones of inhibition was then measured to the nearest millimetre and reported.

**Determination of Number and Sizes of Bacterial Plasmids**

Molecular studies of bacterial isolates with very high multidrug resistance were carried out. The pure culture of these selected isolates were stored in a refrigerator of 4-8$^{o}$C. The trans-conjugant strains and resistant isolates were subjected to plasmid DNA isolation following the protocol of Carattoliet al. (2003). Each isolate was inoculated into 10ml Luria-Bertani (LB) broth incorporated with appropriate selection antibiotic and incubated for 16hours at 37°C while shaking at 200-250rpm. The bacterial culture was harvested by centrifugation at 8000rpm in a micro centrifuge for two minutes at room temperature. The supernatant was decanted and all remaining medium removed. The pelleted cells were re-suspended in 250µl of re-suspension solution and transferred to microcentrifuge tube.

Thereafter, exactly 250 µl of lysis solution was added and mixed thoroughly by inverting the tube 4-6 times until solution was viscous and slightly clear. This were followed by adding 350 µl of neutralization solution and mixed by inverting the tube. Centrifugation was carried out at 10000rpm for five minutes to pellet cell debris and chromosomal DNA. The supernatant was transferred to GeneJET spin column by decanting.Centrifugation was also carried out for one minute and the flow-through was discarded. Wash solution of 500 µl was added to the column and centrifuged for 30 to 60 seconds, flow-through was discarded and column placed back into collection tube. The wash procedure was repeated to avoid residual ethanol in plasmid preparations. The Gene JET spin column was transferred into fresh 1.5 ml micro centrifuge tube and 50µl of elution buffer was added to the centre of the column to elute plasmid DNA. This was incubated for 2 minutes at room temperature and centrifuged for 2 minutes. The purified plasmid DNA was stored at -20°C for further studies.

Agarose gel electrophoresis was carried out by using one per cent (1.0%) agarose gel to resolve DNA fragment. This were prepared by combining 1g agarose in ten times concentration of tris-borate ethylene diaminetetraacetate (10ml 10XTB-EDTA) buffer and 90ml sterile distilled water in 250ml beaker flask and heating in a microwave for 2 minutes until the agarose is dissolved (Moore et al., 2002).Exactly 0.5 µl of Ethidium bromide were added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and casting combs were inserted. The gel was allowed to gel for 30 minutes. The casting combs were carefully removed after the gel had solidified completely. One times concentration (1X) TBE buffer was added to the reservoir until it covered the agarose gel. Precisely 0.5 µl of gel tracking dye (bromophenol blue) was added to 20 µl of each sample with gentle mixing. The samples were loaded onto the wells of the gel at a concentration of 20µl, the mini horizontal electrophoresis gel setup will be covered and electrodes connected. Electrophoresis was carried out at 100-200mA for one hour. At the completion of electrophoresis, the gel was removed from the buffer and viewed under UV-trans-illuminator. The band pattern of DNA fragments were photographed with a Polaroid camera and documented using electrophoresis gel documentation system.

**Statistical Analyses**

The poultry feed samples were collected randomly across all three brandsensuring representation of each feed type.Statistical analyses package SPSS 15.0 was used to determine the mean, the differences within the means were expressed using one way analysis of variance. Data obtained were presented in tablesand percentages.

## III. RESULTS

The bacterial load of the poultry feeds for three brands produced mean counts of the total viable counts, coliforms, faecal and Staphylococcus spp. accordingly and was presented in Table 1. The total viable counts (cfu/g) within the range of 5.66-10.83, 3.83-6.50, 4.33-6.66 for brands A, B and C respectively; while the total coliform count (cfu/g) ranged between 1.50-4.83, 0.83-2.66 and 1.00-4.50 for brands A, B and C respectively. Similarly, the total faecal coliform counts were within the range of 1.16-3.33, 0.01-1.16 and 0.50-2.00 for brands A, B and C respectively, while the total staphylococcal count (cfu/g) is 0.42-1.80, 0.16-0.66 and 0.33-1.66 respectively.Preliminary culture and biochemical characterization of the isolates indicates that all the plates had visible growth indicating bacterial contamination in the three poultry feed brands without any exception.The characterization and identification of the bacterial isolateswas shown in Table 2, while the pattern and frequency of isolation of the bacteria was presented in Tables 3 and 4. The following bacteria were isolated: Salmonella sp.(34%), Bacillus sp.(34%), Escherichiacoli (24%), Staphylococcusaureus(14%), Enterococcus sp. (12%), and finally Klebsiella sp.andProteussp.(6% each).

Nevertheless, the antimicrobial resistance showed that the bacteria isolated from the poultry feeds showed varying degree of resistance, while a few drugs are sensitive against the bacterial contaminants. Accordingly, Escherichiacoli had 100% resistance each to ceftazidime and tetracycline, but were sensitive to gentamicin at 8.33%. Salmonella specie was most resistant at 41.2% to ceftazidime, tetracycline, amoxicillin and ampicillin, but displayed sensitivity to gentamicin at 11.8%. Similarly, Klebsiella sp. had a value of 100% resistance to chloramphenicol, gentamicin, cefoxitin, ceftazidime, tetracycline, amoxicillin and ampicillin. Klebsiella sp. was observed to be less resistant to streptomycin at 33.3%. However, the highest resistance value for Bacillus sp. was 29.4% for tetracycline and septrin respectively, but was less resistant at 11.8% to gentamicin, cefoxitin, and streptomycin. Meanwhile, Staphylococcusaureus had 71.4% as highest resistance value for ceftazidime and tetracycline and lowest resistance value of 14.3% each was recorded for gentamicin and septrin accordingly. Interestingly, Proteus sp. were completely susceptible to chloramphenicol, gentamicin, ciprofloxacin ceftazidime, cefoxitin and streptomycin, while the bacteria showed high resistance of 2(66.7%) to tetracycline and Septrin. Enterococcus sp. on the other hand was most resistant to tetracycline and septrin at 50% of the isolates tested, although Enterococcus sp. displayed considerable sensitivity to a rate of 16.7% to the following antibiotics: chloramphenicol, ciprofloxacin, ceftazidime, amoxicillin, streptomycin and ampicillin, and moderately susceptible to gentamicin and ceftazidime at 33.3% (Table 5).The molecular studies of the isolates with high multidrug resistance values, showsthe number of plasmids and their respective sizes obtained and recorded are shown on Table 6. Five isolates had two plasmidsDNA,hence five isolates had a single plasmid DNA each. There was no plasmid seen for isolate 2 and isolate 8 which isolates are Bacillus spp. in both cases.

**Table 1:Bacterial Load Determination from Poultry Feed Samples in Keffi Metropolis**

| Brands | Total viable count(cfu/g) | Total coliform count(cfu/g) | Total fecal count(cfu/g) | Total staphylococcal count(cfu/g) |
|---|---|---|---|---|
| A | 10.83 | 4.83 | 3.33 | 1.80 |
| | 6.00 | 2.83 | 1.83 | 0.83 |
| | 5.66 | 2.84 | 1.83 | 1.00 |
| | 5.83 | 1.50 | 1.16 | 0.42 |
| Total mean sum | 7.08±2.50 | 2.99±1.37 | 2.03±0.91 | 1.01±0.57 |
| B | 6.50 | 2.66 | 1.16 | 0.51 |
| | 5.00 | 2.00 | 0.83 | 0.66 |
| | 4.66 | 1.50 | 0.83 | 0.66 |
| | 3.83 | 0.83 | 0.01 | 0.16 |
| Total mean sum | 4.99±1.11 | 1.74±0.77 | 0.70±0.49 | 0.49±0.23 |
| C | 5.83 | 4.50 | 2.00 | 1.66 |
| | 6.16 | 2.50 | 1.66 | 0.66 |
| | 6.66 | 2.16 | 1.33 | 0.83 |
| | 4.33 | 1.00 | 0.50 | 0.33 |
| Total mean sum | 5.74±1.00 | 2.54±1.45 | 1.37±0.64 | 0.87±0.58 |

**Table 2:Characterisation and Identification ofBacterialIsolates fromPoultry Feeds in Keffi Metropolis**

| Cultural characteristics Shape size colour | Morphological characteristics | | Biochemical Characteristics | | | | | | Sugar fermentation | | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram Reaction | Morphology | IND | MR | VP | CT | OXI | UR | NT | Glucose | Sucrose | Maltose | Lactose | CAT | |
| Smooth, Pale on Mac | - | Rod | - | + | - | - | - | - | + | + | - | + | - | + | Salmonella sp. |
| Golden-yellow on MSA | + | Cocci (cluster) | - | + | + | + | - | + | + | + | + | + | + | + | S. aureus |
| Purple on Mac | - | Rod | - | - | + | + | - | + | + | + | + | + | + | + | Klebsiellasp. Bacillus |
| Large, cream on NA | + | Rod | - | - | - | + | - | - | + | + | + | + | + | + | sp. |
| Greenish on EMB | - | Rod | + | + | - | - | - | - | + | + | + | - | + | + | Escherichia coli |
| Whitish-cream | + | Cocci | - | - | + | - | - | - | + | + | + | + | + | - | Enterococcusp |
| Colourless on EMB | - | Rod | + | + | - | + | - | + | + | + | - | + | - | + | Proteusp. |

Keys: + = Positive, - = Negative, IND = Indole, MR = Methyl red, VP = Voges-Proskauer, CT = Citrate, CAT= Catalase, NIT=Nitrate, OXI= Oxidase UR = Urease, NT = Nitrate Mac= MacConkey agar, MSA=Mannitol Salt Agar, NA= Nutrient Agar, EMB=Eosine Methylene Blue Agar.

**Table 4: Percentage Occurrence of bacteria isolated frompoultry feeds in Keffi metropolis.**

| Bacteria | No sample | No isolated (%) |
|---|---|---|
| Bacillussp. | 50 | 17 (34) |
| Staphylococcussp. | 50 | 7 (14) |
| Salmonellasp. | 50 | 17 (34) |
| Klebsiellasp. | 50 | 3 (6) |
| Escherichia coli | 50 | 12 (24) |
| Enterococcussp. | 50 | 6 (12) |
| Proteussp. | 50 | 3 (6) |

**Table 3: Occurrence of Bacteria from Poultry Feed Samples in Keffi Metropolis**

| Isolate Sample | E.coli (n=12) | Salmonella (n=17) | Klebsiella (n=3) | Bacillus (n=17) | S. aureus (n=7) | Proteus (n=3) | Enterococcus (n=6) |
|---|---|---|---|---|---|---|---|
| **Brand A** | | | | | | | |
| Q | 2 | 0 | 0 | 1 | 1 | 0 | 0 |
| L | 0 | 2 | 0 | 1 | 0 | 0 | 1 |
| B | 3 | 0 | 0 | 2 | 1 | 0 | 0 |
| S | 0 | 0 | 2 | 2 | 1 | 0 | 0 |
| **Brand B** | | | | | | | |
| Q | 0 | 2 | 0 | 0 | 3 | 0 | 1 |
| L | 2 | 2 | 0 | 0 | 0 | 2 | 1 |
| B | 1 | 3 | 0 | 2 | 0 | 0 | 0 |
| S | 0 | 1 | 0 | 2 | 0 | 0 | 1 |
| **Brand C** | | | | | | | |
| Q | 1 | 3 | 0 | 2 | 0 | 0 | 0 |
| L | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| B | 1 | 2 | 0 | 3 | 0 | 1 | 1 |
| S | 1 | 2 | 1 | 2 | 1 | 0 | 0 |

Key: Q=Quail, L=Layers, B=Broilers, S=Sussex

**Table 5:Percentage Antimicrobial Susceptibility of Bacterial Isolates from Poultry Feeds in Keffi Metropolis**

| Antibiotics | Disc Content µg | E.coli (n=12) | Salmonella (n=17) | Klebsiella (n=3) | Bacillus (n=17) | S.aureus (n=7) | Proteus sp. (n=3) | Enterococcus sp (n=6) |
|---|---|---|---|---|---|---|---|---|
| Chloramphenicol | 30 | 6(50) | 5(29.4) | 3(100) | 4(23.5) | 4(57.1) | 0(0) | 1(16.7) |
| Gentamicin | 15 | 1(8.33) | 2(11.8) | 3(100) | 2(11.8) | 1(14.3) | 0(0) | 2(33.3) |
| Ciprofloxacin | 30 | 5(41.7) | 6(35.3) | 2(66.7) | 4(23.5) | 2(28.6) | 0(0) | 1(16.7) |
| Cefitioxon | 30 | 6(50) | 5(29.4) | 3(100) | 2(11.8) | 3(42.9) | 0(0) | 1(16.7) |
| Cefitdezione | 30 | 12(100) | 7(41.2) | 3(100) | 3(17.6) | 5(71.4) | 0(0) | 2(33.3) |
| Tetracycline | 15 | 12(100) | 7(41.2) | 3(100) | 5(29.4) | 5(71.4) | 2(66.7) | 3(50) |
| Amoxicillin | 10 | 11(91.7) | 7(41.2) | 3(100) | 4(23.5) | 3(42.9) | 1(33.3) | 1(16.7) |
| Streptomycin | 30 | 8(66.7) | 4(23.5) | 1(33.3) | 2(11.8) | 3(42.9) | 0(0) | 1(16.7) |
| Septrin | 30 | 10(83.3) | 6(35.3) | 2(66.7) | 5(29.4) | 1(14.3) | 2(66.7) | 3(50) |
| Ampicillin | 30 | 11(91.7) | 7(41.2) | 3(100) | 4(23.5) | 2(28.6) | 1(33.3) | 1(16.7) |

**Table 6:Numbers and Sizes ofBacterial PlasmidsIsolated from Poultry Feeds in Keffi Metropolis**

| Isolates | Number of Plasmids | Estimated Molecular sizes (base pairs bp) |
|---|---|---|
| 1 | 2 | 1450-1850 |
| 2 | 0 | - |
| 3 | 2 | 1749-1768 |
| 4 | 2 | 1400-1598 |
| 5 | 1 | 1610 |
| 6 | 2 | 1500-1650 |
| 7 | 2 | 1330-1580 |
| 8 | 0 | - |
| 9 | 1 | 1770 |
| 10 | 1 | 1850 |
| 11 | 1 | 1900 |
| 12 | 1 | 1800 |

Lines 1 to 12 represents the plasmid isolates, M represent a molecular Ladder

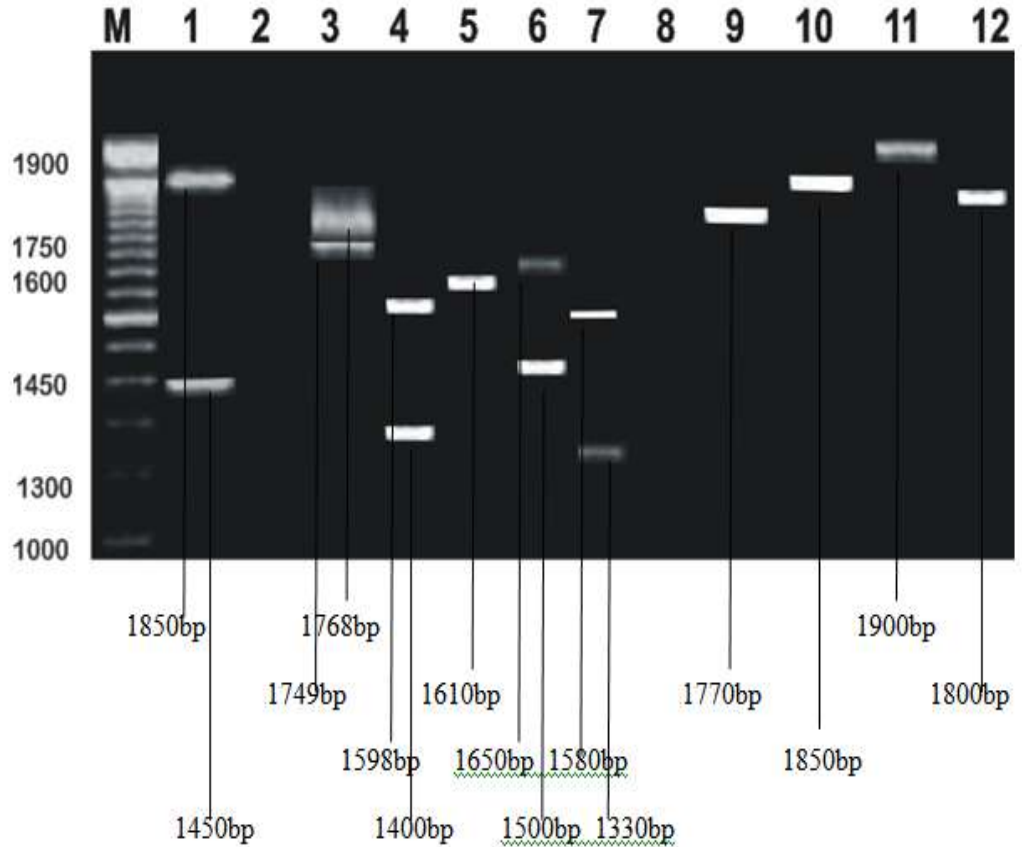


**Plate 1: Agarose gel electrophoresis of plasmids from resistant isolates obtained from poultry feeds in Keffi metropolis**

**Key**: Lane M supercoil plasmid DNA marker: Lane 1 – 12 Isolates E1, B2, ST3, E4, E6, SA5, SA8, B5, K2, EN4, SA11 and ST7. E=E.coli, B= Bacillussp., ST= Staphylococcussp., SA=Salmonellasp., K=Klebsiellasp.andEN=Enterococcussp.

## IV. DISCUSSION

All the poultry feed samples examined in this study showed the presence of bacterial contaminants which were Escherichiacoli, 24%; Staphylococcus aureus, 14%; Salmonella sp.,34%; Bacillus sp.,34%; Klebsiella sp., 6%; Proteus sp.,6% and Enterococcus sp. 12%. The presence of E. coli a coliform and Salmonella sp in the poultry feeds suggests faecal contamination either at factory or by retailers which can be associated with poor hygiene (Matthew et al., 2017). Earlier, White and Collins (2003) had isolated Salmonella spp. from many commercial poultry mills. This coincides with the findings of this present study. The presence of these microorganisms in the animal feeds suggest that the feeds contain sufficient nutrients for the growth of these organisms and the activities of these microorganisms on the feeds under study may cause degradation,

thereby reducing the nutrients that would have been wholly available for the livestock to feed on. This is in consonance with the reports of Aganagaet al. (2000) and Arotupinet al. (2007) on animal feeds and their pattern of sensitivity and association with microorganisms. Also, these microorganisms may probably have originated from the raw materials from which the feeds are being produced. In addition, most of the isolated microorganisms owned their origin from air and soils (Arotupin and Akinyosoye, 2001). Most of the bacterial isolates are highly pathogenic in poultry industry. Dhandet al. (1998) and Hancock et al. (1998) separately implicated Bacillus cereus and Staphylococcus aureus in the microbial infection outbreak of poultry farming. Even so, the presence of Staphylococcus aureus and Proteus sp. in the poultry feeds suggests recent contamination, most probably from market sellers. These organisms are non-spore formers and their presence in a sample like feeds with very low water activity suggests recent contact with the sample, hence the contamination (Jawetzet al., 1995; Onyezeet al., 2013).

The high prevalence of bacterial species of public health importance in such foods indicate a potential hazard to human health in terms of direct consumption of bacteriological contaminated feed or their toxins by farmed animals (Obiekezieet al., 2012). Nevertheless, Staphylococcus aureus is known to be a major foodborne pathogen which produces staphylococcal toxin (Argudetal., 2010). Enterococcus sp. and Bacillus sp. are ubiquitous which makes them a frequent contaminant in feeds as demonstrated by Maciorowskiet al. (2007). Klebsiella sp. is pathogen of concern especially because of its evolution of high antibiotic resistance and difficulty to treat in hospitals.In this present study, the antimicrobial susceptibility test showed the isolates to be strongly resistant to tetracycline (65.5%), ceftazidime (51.9%), amoxicillin (49.9%), Septrin (49.4%) and ampicillin (47.9%); moderately resistant to chloramphenicol (39.5% and cefoxitin (35.8%). However, the following antibiotics displayed low resistance and are thus comparably sensitive against the isolates, they are: cefoxitin (35.8%), ciprofloxacin (30.4%), streptomycin (27.8%) and gentamicin (22.9%). The susceptibility pattern observed for this present study were comparable to those reported by Obiekezieet al. (2013) and Onyezeet al. (2013). Recently, many of the bacterial pathogens associated with epidemics of human disease have evolved into multidrug-resistant forms subsequent to antibiotic use.Accordingly, the antibiotic susceptibility results observed for this study is a cause for concern since the WHO had earlier recommended that antibiotics such as aminoglycosides, 3$^{rd}$ and 4$^{th}$ generation cephalosporins and macrolides as well as penicillins and Sulphonamides should not be used in animal disease prevention or as growth promoters (WHO, 2011).
In the same reports of the World Health Organization, only Tetracyclines are allowed for use in poultry disease prevention and as growth promoters because they are not classified as important to humans. This is worrisome because only Tetracycline displayed positive antimicrobial action against the isolates in this present study. Typically, the high sensitivity of the isolates gentamicin might be due to their requirement for paranteral administration as demonstrated by Makutet al. (2013) and Makutet al. (2014). Furthermore, this present study shows multiple drug resistance to even the important human antibiotics by the bacteria isolated. Further analyses of the multidrug resistant isolates at molecular level presented five isolates with single plasmids, five with double plasmids having molecular weight ranging from 1330bp to 1900bp. Escherichiacoli,Salmonella sp., Staphylococcus sp., Enterococcus sp. and Klebsiella sp. had at least one plasmid DNA. Bacillus sp. had no visible plasmid DNA, while Proteus sp. was not a multidrug resistant isolate in this study. This corresponds with an earlier report by Awogbemiet al. (2018). This is understandable since recent metagenomics and functional genomics studies have provided compelling evidence that antibiotic resistance genes are widespread and the natural reservoirs of potential antibiotic resistance include many ecosystems such as in agriculture (e.g., animal manure, soil, water, wastewater lagoons), the gut of humans and food animals, and even ancient soils (Lin et al., 2015).

## V. CONCLUSION

The poultry feeds analysed in this study contained high presence of bacteria. Specific pathogenic bacteria test revealed the presence of Escherichiacoli, Salmonella sp., Bacillus sp., Klebsiella sp., Enterococcus sp., Staphylococcusaureus and Proteus sp. Most of the isolates were determined to be multidrug resistant to commonly dispensed antibiotics.The molecular studies showed a correlation of multidrug resistance to plasmid DNA presence in the majority of bacterial isolates. Poultry feed manufacturers should be encouraged to invest in sterilization of feed additives to curb contamination of the feed and also have food safety department to help monitor standards of production as well as train the personnel on good manufacturing practices and proper hygiene.

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