



Determination of Bacterial Species Associated with Contamination of Poultry Feeds Produced by Three Different Companies in Abuja, Nigeria

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Abstract

*Poultry farming is an efficient source of income and nutrition. Feeds used for poultry are vulnerable to contamination by microorganisms. This study aimed to assess bacterial contaminants of the poultry produced from three different companies in Abuja, Nigeria. Samples of poultry feeds produced by three different companies were collected from three different batches and were processed and inoculated in nutrient agar, Eosin methylene blue agar (EMB), and Mannitol salt agar (MSA), incubated at 37 °C for 24 hours. Colonies were counted, characterized, and subcultured to obtain pure cultures. Pure isolates were identified using Gram reaction, cellular morphology, and biochemical characteristics. Antibiotic susceptibility profiles of the isolated bacterial species were determined using Kirby-Bauer disc diffusion. The total bacterial counts of samples analyzed showed that poultry feed from sample C had the highest mean bacterial counts of 2.49×10^5 CFU/g. The lowest was poultry feed sample A, with a mean count of 1.06×10^5 CFU/g. Sample B had a mean bacterial count of 2.14×10^5 CFU/g. The bacterial species isolated in the present study are *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., and *Proteus mirabilis*. *Staphylococcus aureus* was the most occurring isolate with a 47% occurrence rate and was present in all poultry feed samples. *Escherichia coli* is the second most occurring with a 35% occurrence rate, then *Salmonella* with a 10% occurrence rate. The least occurring was *Proteus mirabilis*, with an 8% occurrence rate. *S. aureus* showed 100% susceptibility to all the antibiotics used, while *P. mirabilis* recorded the highest resistance of 40 %, showing resistance to four of the ten antibiotics used. This study reveals the presence and high microbial count in the different poultry feeds investigated. This usually reflects the degree of hygienic procedures and biosecurity used in the feeds' handling, production, and storage.*

Keywords: Poultry feeds, contamination, *Proteus mirabilis*, *Escherichia coli*, Susceptibility

INTRODUCTION

In animal husbandry, poultry refers to domestic or commercial birds raised for their meat, eggs, and feathers. Poultry farming, especially on a small scale, is renewable and efficient and can provide a source of income and nutrition. Birds raised for meat, eggs, and feathers, commercially or domestically, are referred to as poultry in animal husbandry. A reliable source of income and nutrition, poultry farming is efficient and renewable, particularly when done on a small scale (Wong et al. 2017). With farming becoming more specialized, many farms kept flocks that were too big to feed, leading to the development of nutritionally complete feeds. Grain, vitamin and mineral supplements and protein supplements like soybean oil meal comprise most modern poultry feeds (Alshelmani et al., 2021).

Microbial contamination can occur naturally from feed and ingredients from plant and animal sources or exposure during manufacturing, such

as poultry farm workers, vectors, processing plants, the farmhouse itself, storage temperature, etc. It can affect poultry feed and feed ingredients in various ways, ranging from bacteria and viruses to molds and mycotoxins. Buying feed or feed ingredients carries some risk of contamination, but some ingredients are more prone to contamination than others. All feedstuffs, however, are susceptible to spoilage and contamination. Certain ingredients are more likely to become contaminated than others; examples include high-protein materials that can harbor bacteria like *Salmonella* or corn that grows mold (Mikesell, 2021).

Due to the zoonotic nature of some pathogens and the sensitivity of humans to some toxins, contaminants in the feed also pose a risk to human health, as some of these toxins are said to be teratogenic and carcinogenic, in addition to the health, welfare, and productivity of livestock (Mikesell, 2021).

It can result in nutrient depletion by microbes, decreased productivity and bird performance, potential sources of antimicrobial resistance (AMR), etc. Animals and humans may both be at risk for illness if they consume commonly monitored bacteria, such as *Salmonella* species, *Listeria monocytogenes*, and pathogenic *Escherichia coli* (Nemser *et al.*, 2014; Delgado *et al.*, 2023).

The quality of the feed provided to the poultry birds determines their proper growth and health, and the pathogenic bacteria from the poultry products can infect humans and cause various acute and chronic diseases (Marjan *et al.*, 2014). Furthermore, understanding the occurrence and diversity of bacterial contaminants in poultry feeds will provide information for designing effective monitoring and hazard control strategies. Therefore, this study aims to assess the bacterial species in poultry feeds produced by three (3) companies in Abuja, Nigeria.

MATERIALS AND METHODS

Sample collection

Samples of poultry feeds were purchased in three batches from three different commercial feed-producing companies, A, B, and C, in Abuja, Nigeria, and were taken to the Microbiology laboratory of Nile University of Nigeria for analyses.

Microbiological Analyses

Serial Dilution

One gram (1g) of each sample was dissolved in 9 ml of sterile distilled water and used to prepare a five-fold serial dilution, according to Matthew *et al.* (2017).

Enumeration and Isolation of Bacteria

Using the pour plate method, the media (Eosin Methylene Blue agar (EMB), Nutrient Agar (NA), and Mannitol Salt Agar (MSA) were inoculated in duplicates and incubated at 37 °C for 24 hours. Distinct bacterial colonies were counted, characterized, and subcultured to obtain pure cultures.

Identification of isolates

The conventional processes for identifying bacterial isolates were followed, according to Cheesbrough (2009). Bacterial colonies were identified using morphological features, Gram reactions, and biochemical tests, according to Baker and Breach (1980). The identities of the bacterial isolates were further confirmed using Bergey's Manual of Determinative Bacteriology (Bruchanan & Gibbons, 1994).

Hydrogen sulphide production test

Bacterial isolate was streaked on a triple sugar iron agar slant and incubated at 37°C overnight. A positive result was indicated when the agar turned black.

Lactose test

Bacterial isolate was transferred into a tube containing phenol red lactose broth and was incubated at 37 °C for 24 hours. Media colour change was observed for positive or negative results.

Catalase test

Small inocula of the bacterial isolate were mixed into hydrogen peroxide solution (3%), and oxygen bubbles rapidly emerged (positive). The lack of catalase is evident by a lack of or weak bubble production.

Coagulase test

Smear of isolates was mixed with citrate plasma and checked for agglutination. Another procedure served as control.

Oxidase test

To test for the production of oxidase, the bacteria isolates were placed on a filter paper impregnated with 2 drops of 1% oxidase reagent. It was observed for change in colour. Purple coloration indicates positive. No color change indicates negative (Vashist *et al.*, 2013).

Methyl red test

To determine the ability of a microorganism to oxidize glucose with the production and stabilization of high concentrations of acid end products. Isolates were inoculated on methyl red broth at 37 °C for 48 hours, after which 5ml of the methyl indicator was added. Red coloration indicates positive. Yellow indicates negative

Vogues Proskauer test

To determine the ability of bacteria to produce non-acid end product. Isolates were inoculated on MR-VP broth and incubated at 37 °C for 24 hours, after which equal volumes of Barrit's reagent A and B were added to the culture and observed for colour change. A red colour indicates a positive result.

Indole test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole, pynivic acid, and ammonia after incubating at 35 °C for 48 hours in peptone water. The test organism was inoculated into tryptophan broth, which contains tryptophan. KOVAC's reagent was used to detect the indole produced. Cherry red coloration indicates positive.

Christensen’s urea agar slant, incubated at 37 °C for 24 hours, and observed for colour change. Media that turns pink indicates a positive result.

Citrate test

This determines the ability of a microorganism to utilize citrate as the sole source of carbon and as an energy source for growth and ammonium salt as a sole source of nitrogen. Simmon citrate agar was inoculated with isolates and incubated at 35 °C for 48 hours. Bluish coloration indicates positive.

Urease test

This test determines the ability of bacteria to degrade urea. Bacterial isolate was streaked on

Antibiotic Susceptibility test

The susceptibility testing of antibacterial agents was done using the Kirby Bauer disk diffusion method outlined by Clinical Laboratory Standards Institute guidelines (CLSI, 2018). The antibiotic discs were selected based on their availability in the study area. Sterilized forceps

placed the antibiotic discs on the media and gently pressed them onto its surface. The MHA plates were then incubated at 37 °C for 24 hours. After incubation, the bacterial growths around each disc were observed, and a metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used. The measurements were done in millimeters, and the zones were compared and confirmed with the CLSI standards for interpretation.

RESULTS

Total Bacterial Counts of the Samples

The total bacterial count is presented in Table 1. The results showed varying bacterial loads in the samples analyzed from poultry feed companies. The total bacterial counts of samples showed that poultry feed sample C had the highest mean bacterial count of 2.49 x 10⁵cfu/g, and the recorded lowest was poultry feed samples A, with a mean colony forming unit of 1.06 x 10⁵cfu/g, while sample B had a mean bacterial count of 2.14 x 10⁵cfu/g.

Table 1: Total bacterial counts

Feed (Batch)	A	B	C
1	1.01x10 ⁵	1.86x10 ⁵	2.73x10 ⁵
2	1.04x10 ⁵	2.05x10 ⁵	2.23x10 ⁵
3	1.13x10 ⁵	2.5x10 ⁵	2.5x10 ⁵
Mean	1.06x10 ⁵	2.14x10 ⁵	2.49x10 ⁵

Staphylococcus aureus, *Escherichia coli*, *Salmonella* sp., and *Proteus mirabilis* were the four bacterial species isolated and identified in this study (Table 2). *Staphylococcus aureus* has the highest frequency of 47% and was isolated from all the feed samples. *Escherichia coli* followed with a 35% occurrence, while *Salmonella* species had 10% occurrence. The least recorded was *Proteus mirabilis*, with an 8% occurrence (Figure 1).

Table 2: Morphological and Biochemical Characteristics of Bacterial Isolates

Iso	Cultural characteristics	Gram	Shape	Mot	H ₂ S	Lac	Cat	Coa	Oxi	MR	VP	Ind	Cit	Ure	Organism
1	Smooth, creamy	+	Cocci	-	-	-	+	+	-	-	+	-	+	+	<i>S. aureus</i>
2	Most white/gray	-	Rod	+	-	+	+	-	-	+	-	+	-	-	<i>Escherichia. coli</i>
3	Gray/white, opaque	-	Rod	+	+	-	+	+	-	+	-	-	+	-	<i>Salmonella sp.</i>
4	Colourless, glistening	-	Rod	+	+	-	+	-	-	+	-	-	+	+	<i>Proteus mirabilis</i>

Key: Iso= Isolates; Mot = Motility Test; Lac= Lactase Test; Cat= Catalase Test; CoA= Coagulase Test; Oxi= Oxidase Test; MR= Methyl Red Test; VP= Voges-Proskauer; Ind= Indole Test; Citr= Citrate Test; and Ure= Urease Test.

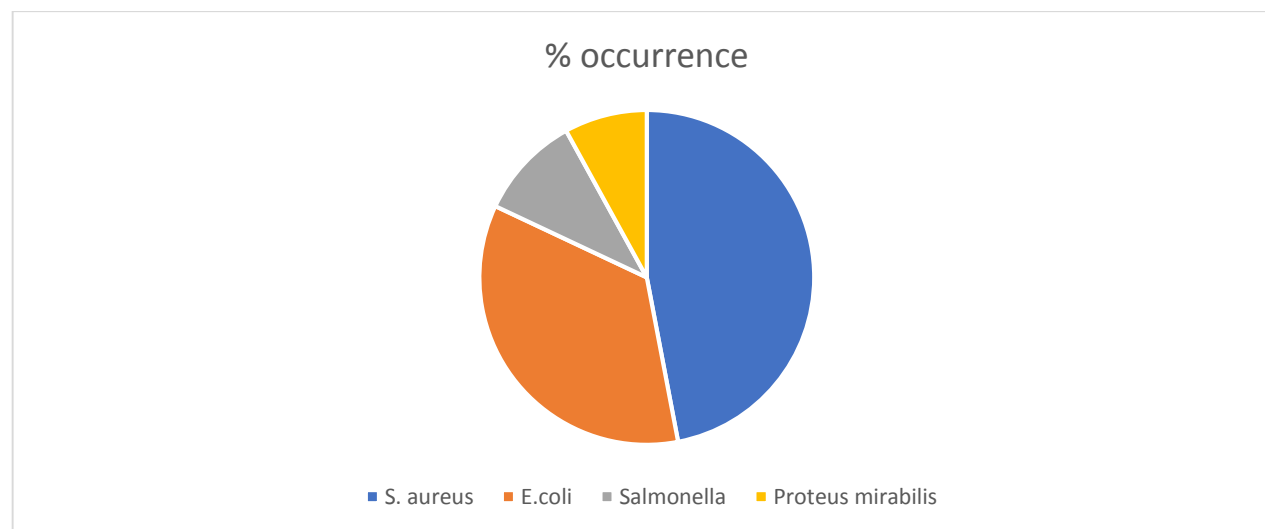


Figure 1. Frequency of occurrences of bacterial isolates

The antibiotic susceptibility results showed that *S. aureus* was 100 % susceptible to all the antibiotics used, which include Ceftriaxone, Gentamycin, Erythromycin, Amoxicillin, Ampicillin/Cloxacillin, Cefuroxime, Doxycycline, Levofloxacin, Tetracycline, and Ciprofloxacin. *Salmonella* sp. was resistant to Amoxicillin and Levofloxacin, having 20 % resistance. *E. coli* showed resistance to only gentamycin, while *P. mirabilis* recorded the highest resistance of 40 %, showing resistance to four of the ten antibiotics used. The degree of sensitivity is presented in Table 3.

Table 3: Antibiotics Susceptibility Profiles of bacterial isolates

Antibiotics	<i>Staphylococcus aureus</i>	<i>Salmonella</i> sp.	<i>Proteus mirabilis</i>	<i>Escherichia coli</i>
Ceftriaxone	-	-	+	-
Gentamycin	-	-	-	+
Erythromycin	-	-	-	-
Amoxicillin	-	+	-	-
Ampicillin/Cloxacillin	-	-	-	-
Cefuroxime	-	-	-	-
Doxycycline	-	-	+	-
Levofloxacin	-	+	+	-
Tetracycline	-	-	+	-
Ciprofloxacin	-	-	-	-
Total	0(0%)	2(20%)	4(40%)	1(10%)

DISCUSSION

The results of this study are quite lower than those of Roy *et al.* (2017) and Opara *et al.* (2018), whose recorded bacterial counts were as high as 5.0×10^6 CFU/mL and 7.4×10^5 CFU/mL, respectively, from poultry feeds. Marija *et al.* (2019) and Sule and Ilori (2017) reported similar bacterial counts to this present study. The results from this study showed that the total bacterial counts were slightly above the microbiology standard for poultry feeds by the Nigerian Industrialist Standard (approved by SON, 2018) with the recommended reference value, which states that the total viable count of poultry feeds should be 1.0×10^5 cfu/g or below. All feed samples for this study were slightly higher than the recommended value, which may mean a higher chicken infection risk.

Concerns regarding public health are raised by the high abundance of bacterial species, which may indicate a serious health danger if farm animals ingest contaminated feed or come into contact with the toxins released by the bacteria (Aliyu *et al.*, 2016). The levels of microbial contamination of the different feeds in this study could reflect the level of exposure and the handling processes among the sellers or workers in the farms. The observed counts could be ascribed to various environmental factors, such as the feeds' exposure to air, the equipment utilized during preparation, the cleanliness of the storage facilities, and the retailers' hygiene. Due to extensive handling and mixing, some pathogens may have been introduced during the selling process. According to Marija *et al.* (2019), a variety of factors, such as temperature, moisture content, feed type, aerobic and anaerobic conditions, raw materials' chemical and physical properties, feed pH, feed additive presence, storage

conditions, and feed decomposition products, can affect how many microorganisms proliferate in feeds.

The bacteria species isolated in this work include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Proteus mirabilis*. Matthew *et al.* (2017), Roy *et al.* (2017), and Munoz *et al.* (2021) reported similar isolates on works with poultry feeds. Most isolated species in this study have been associated with diseases of the poultry farm. The majority of these species have been linked to illnesses that affect chickens. *Salmonella* is responsible for causing salmonellosis, which two-week-old chicks and ducklings commonly contract. *Salmonella* gastroenteritis in humans has also been associated with consuming infected poultry, which may have contracted the infection through tainted feeds (Mathew *et al.*, 2017). *E. coli* in the feeds indicates contamination with fecal materials, which depicts a poor hygienic condition (Li *et al.*, 2021). Gram-negative *P. mirabilis* is widely dispersed throughout the environment and the gut microbiota of numerous animal species that act as reservoirs. *P. mirabilis* is classified as an opportunistic pathogen because it can potentially cause severe and enduring infections in people, such as eye infections, wounds, gastrointestinal system infections, and urinary tract infections. There are few studies linking *P. mirabilis* to illnesses in animals, although reports of urinary tract infections in fish and dogs have led to a high death rate (Sanches *et al.*, 2020).

Mathew *et al.* (2017) reported that *Staphylococcus aureus* was associated with microbial disease outbreaks in poultry farming. *S. aureus* was the most prevalent in the present study, which agrees with Arotupin *et al.* (2007) and Mahmudullah *et al.* (2015).

This bacterium is a pathogenic organism that seriously threatens public health. As is widely known, most *Staphylococcus aureus* strains are considered pathogenic primarily because of the heat-stable enterotoxins they generate in proportion to the amount of inoculum they contain (Adebayo *et al.*, 2010). The antibiotic susceptibility pattern demonstrated by the bacterial species in this study showed a high degree of susceptibility. However, the 40% resistance rate of *P. mirabilis* to the antibiotics used, including tetracycline, calls for concern. This is because, according to WHO, only tetracyclines are permitted for use as growth promoters and in preventing poultry diseases. The report of Mbegbu and Onyemelukwe (2023) stated that there was strong resistance demonstrated by several bacterial isolates from the poultry feeds of up to 92% to tetracycline. This contrasts with the present study's result, where *S. aureus* showed zero percent resistance to the antibiotics used. The 20% resistance of *Salmonella* sp. to the antibiotics, specifically Amoxicillin and Levofloxacin, as well as the 10% resistance demonstrated by *E. coli* to

Gentamycin, could be attributed to possible frequent exposure of those bacterial species to the antibiotics. *Proteus* sp. demonstrated 66.7 % resistance to tetracycline in a report on poultry feeds by Gyang *et al.* (2019). The high resistance of *Proteus* to tetracycline could be associated with overusing this antibiotic or the expression of tetracycline-resistant genes.

CONCLUSION

The total bacterial counts recorded in this study, higher than the Nigerian Industrialist Standard for total bacterial counts in poultry feeds is of great health concern. Moreover, some bacterial species associated with the feeds demonstrated some resistance to common antibiotics. The implication is that the health of chicken and humans who work on the chicken and those who consume them may be threatened. Therefore, necessary actions need to be taken to help prevent the likely resultant infection and death of poultry birds, which would lead to great economic loss, and most importantly, help prevent the transmission of these pathogenic organisms to humans.

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