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Prevalence and Antimicrobial Susceptibility of Enteric Bacteria from Poultry Farms in Kano State, Nigeria

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Abstract

With poultry being the most abundant domestic animals worldwide, poultry farms have emerged as a prospective and widely distributed business industry in Nigeria. The outbreak of several deadly diseases that cause economic loss and discourage poultry keeping is a major challenge to poultry farming. The main goal of this study is to isolate and identify different enteric bacteria and to find the antimicrobial sensitivity profile against the pathogens isolated from specific poultry farms in Kano State. A total of 50 samples, including both poultry feed and droppings, were collected from five different poultry farms for analysis to detect the presence of enteric bacteria. The results revealed that all bacterial isolates displayed varying levels of resistance to the tested antibiotics, but they were completely susceptible to Sulfamethoxazole and Cephalexin. In general, the results of this study indicate that these samples serve as sources of E. coli, Salmonella spp., Shigella spp., and Proteus mirabilis in poultry. These pathogenic bacteria pose a health threat, potentially leading to food poisoning and infections in both animals and humans. Consequently, efficient control measures such as proper management and handling of poultry birds, and sensitization of farmers on the abuse of antibiotics are crucial to prevent cross-contamination within poultry houses and ensure the provision of high quality poultry products.

INTRODUCTION

In agriculture, the term 'poultry' typically encompasses all domestically raised birds, which are primarily utilized for egg production, meat harvesting, or the procurement of their feathers (Getu and Tadese, 2015; Wilson, 2021). Poultry originates from the French term "poul," which itself has its roots in the Latin word "pullus," signifying "small creatures" (Chat *et al.*, 2019). Poultry is recognized as the swiftest expanding food source globally. However, a significant concern within the industry revolves around the substantial accumulation of waste, including manure and litter. This accumulation could lead to disposal challenges and pollution unless environmentally and economically viable management systems are implemented (Bolan *et al.*, 2010).

Poultry farming (PF) has become a thriving and prevalent industry in Nigeria, but it frequently faces contamination by harmful microorganisms when proper hygiene practices are not adhered to. Poultry farming remains the dominant sector in global animal husbandry when considering the sheer quantity of animals. In Nigeria, the extensive use of poultry droppings as a valuable fertilizer for cultivating crops is widespread. (Ajibola *et al.*, 2022; Adekiya *et al.*, 2020). Utilizing poultry waste as a fertilizer in agricultural fields enhances soil fertility by introducing essential nutrients for crop development and incorporating organic material

to enhance soil quality. Nonetheless, this approach carries potential public health risks, particularly if the crops are consumed without undergoing any form of cooking or processing (Chat *et al.*, 2019; Singh *et al.*, 2018). The poultry sector in Nigeria has experienced significant growth, both in the commercial and traditional household domains. With over three million individuals employed directly in this sector, it plays a pivotal role in supplying the majority of meat and eggs, serving as the primary source of protein for the entire population of the country (Nandi *et al.*, 2013; and Nkukwana, 2018). According to Adeleke and Omafuvbe (2011), poultry has emerged as a rapidly expanding meat source worldwide, accounting for a significant 25% share of the total meat production.

The prevalence of foodborne illnesses among humans has significantly risen on a global scale over the past few years. Poultry products have consistently been associated with cases of foodborne infections. Poultry can serve as a reservoir for various foodborne pathogens. Numerous studies in recent years have demonstrated that Salmonella and Campylobacter species are the primary culprits behind human foodborne bacterial illnesses associated with poultry (Ifeanyichukwu *et al.*, 2016). Foodborne infections and intoxications are responsible for approximately one billion cases of acute diarrhea every year among children under the age of five in regions such as Africa, Asia, Latin America, and other developing nations (Mulata *et al.*, 2014).

Poultry feeds can become contaminated during various stages of processing, including handling, ingredient mixing, and exposure to airborne microorganisms, both for raw materials and finished products (Chat *et al.*, 2019). Hence, a significant incidence of poultry illnesses and fatalities arises due to the consumption of tainted feeds. Food-borne Salmonellosis is primarily transmitted through common sources such as meat, meat-derived products, eggs, and egg-related items, all of which can become contaminated either due to direct animal infection or fecal contamination during the processing stage (Abebe *et al.*, 2020; Rahman *et al.*, 2018). The aim of this study was to isolate and identify different enteric bacteria from poultry farms in Kano State, Nigeria and the objectives are to determine the bacteria load of the samples, to isolate and identify enteric bacteria from poultry feeds and droppings sample, and to carry out antibiotic susceptibility test against the bacteria isolated.

MATERIALS AND METHODS

Sample Collection

Fifty samples were randomly collected from five different poultry farms in Kano State, the Northern Western region of Nigeria namely Hajia (Tarauni), Alhaji Sani (Tarauni), Abba (Kumbotso), Abee (Kumbotso) and Nasara One (Gwale) farms. The collection process entailed acquiring 25 samples of both feed and droppings from specified poultry farms. These samples were meticulously gathered in a sterile environment, utilizing sterile sampling tools, polythene bags, and disposable gloves.

Preparation of Samples

Approximately one gram (1g) of both droppings and feeds was measured and then mixed together in a test tube that contained 9ml of sterile peptone water (Okafor and Ugwuegbulem, 2022). Nine milliliters of peptone water were distributed evenly into four additional test tubes. Using a pipette, one milliliter of the stock solution was aseptically transferred and sequentially diluted into each of the four test tubes. 0.1ml of the 10^{-3} dilution was placed into a sterile petri-dish prior to pouring the prepared nutrient agar.

Enumeration of bacterial count in samples

One gram of the poultry dropping was serially diluted up to 10^{-7}. Aliquots (0.1ml) were plated from different dilutions. The pour plate technique was employed for the enumeration of bacteria using nutrient agar. **Isolation and**

Identification of Bacteria from Poultry Feeds and Droppings

The techniques and protocols suggested by Begum *et al.* (2023) were utilized to isolate and identify *E. coli*, *Salmonella*, *Shigella*, and *Proteus*. Initially, a primary enrichment medium, Selenite feces (SF) broth, was employed for all organisms. Subsequently, distinct selective media were used for each organism: Eosin Methylene Blue (EMB) agar for *E. coli*, *Salmonella-Shigella* (SS) agar for *Salmonella* and *Shigella*, and Blood agar for the isolation of *Proteus*.

To isolate *E. coli*, the inoculated media were first incubated at a temperature of 37°C for a duration of 24 hours. During this incubation period, close attention was given to the distinct colony characteristics, particularly the presence of a metallic green sheen on EMB agar, which is indicative of *E. coli*. Colonies that showed these suspected features were then transferred to nutrient agar and incubated at 37°C for an additional 24 hours for further subculturing. For the initial identification, a set of tests were carried out, commencing with the gram staining and oxidase test. Subsequent to these preliminary evaluations, supplementary biochemical examinations were conducted, encompassing tests for indole production, methyl red, Voges-Proskauer (VP), and the citrate utilization test.

To isolate and detect *Salmonella* and *Shigella*, SS agar plates were used to culture enriched samples, which were then incubated at 37°C for 24 hours. The presence of Salmonella was suspected when a dark center developed within the colony. To confirm the identity of Salmonella species, several biochemical tests were performed, including the Triple Sugar Iron agar (TSI) test, the indole test, the citrate test, and the urease test. Colonies exhibiting specific characteristics were identified as Salmonella: they displayed an alkaline (red) slant with an acid (yellow) butt and produced hydrogen sulfide (H_2S) resulting in blackening on the TSI agar, showed positive results for citrate utilization (indicated by a blue color change), tested negative for tryptophan utilization (indicated by a yellow-brown ring in the indole test), and were negative for urea utilization.

Finally, for the purpose of isolating and identifying *Proteus mirabilis*, enriched samples were used to inoculate Blood agar plates. These plated cultures were subsequently incubated at 37°C for duration of 24 hours. The identification of isolates was carried out in accordance with standard microbiological protocols, relying on observations of morphology and assessment of biochemical characteristics as described by Chessbrough, (2006).

Standardization of Inoculum for Susceptibility Testing

The described process adhered to the protocols outlined by the Clinical and Laboratory Standards Institute (CLSI) in (Dargatz *et al.*, 2017). After identifying the isolates, we selected pure cultures from an 18-hour plate culture. Using a sterile wire loop, we gathered 2 to 3 colonies from each isolate, combining them in 5 ml of normal saline. We adjusted the mixture, adding more inoculum or diluent as necessary, to attain a 0.5 McFarland standard.

Antimicrobial Susceptibility Testing

The bacterial isolated were subjected to in-vitro susceptibility testing using the standard disc diffusion technique. After sub-culturing on Mueller Hinton agar, antibiotic discs were placed on the agar plates using a disc dispenser and gently pressed to ensure complete contact with the agar. The plates were subsequently inverted and incubated at 37°C for 24 hours to evaluate their sensitivity. Ten different antibiotic discs were employed: Gentamycin (10µg), Augmentin (30µg), Ciprofloxacin (5µg), Sulfamethoxazole (25µg), Streptomycin (30µg), Ampicillin (10µg), Cephalexin (30µg), Ofloxacin (5µg), Nalidixic acid (30µg), and Pefloxacin (5µg). Following incubation, the diameter of the inhibition zones was measured using a ruler and interpreted according to CLSI guidelines (Dargatz *et al.*, 2017).

RESULTS

A total of 50 samples (25 feeds, and 25 droppings) were collected from five different poultry farms. The mean number of colonies obtained from poultry droppings and feeds are 1.6 $x10^5$ (CFU/g) and 1.3 $x10^5$ (CFU/g) respectively with the droppings having the highest number of colonies (Table 1).

Furthermore, *E. coli*, *Salmonella*, *Shigella* and *P. mirabilis* was detected in various samples of feed and droppings and isolates were distributed according to sample type (Figure 1). In this study, *Escherichia coli* was found to be present in 76% of the samples collected, indicating its widespread occurrence, while the percentage prevalence of *E. coli* in the feeds and droppings were 36% and 40% respectively. In these samples, the overall prevalence of *Salmonella* spp, and *Shigella* spp. were both 44% while *Proteus mirabilis* was 36% (Figure 1).

The antibiotic susceptibility test showed that *E. coli* was susceptible to all antibiotics except Ampicillin (PN), *Salmonella* and *Shigella* were highly resistant to Augmentin (AU), Ciprofloxacin (CPX), Streptomycin (S), Ampicillin (PN), Cephalexin (CEP), Nalidixic acid (NA), and Pefloxacin (PEF). *Proteus mirabilis* showed high to moderate resistance to five antibiotics such as Gentamicin (CN), Ciprofloxacin (CPX), Streptomycin (S), Cephalexin (CEP), and Pefloxacin (PEF) (Table 2).

Table 1: The average bacterial counts (CFU/g) in the poultry feeds and droppings

SAMPLE	BACTERIAL COUNT (CFU/g)
F1	1.2 X 10^5
F2	1.8 X 10^5
F3	TNTC
F4	8.7 X 10^4
F5	TNTC
MEAN VALUE	1.3 X 10^5
D1	1.8 X 10^5
D2	1.3 X 10^5
D3	TNTC
D4	TNTC
D5	TNTC
MEAN VALUE	1.6 X 10^5

Key: F=Feed, D=Droppings, TNTC=Too Numerous to Count

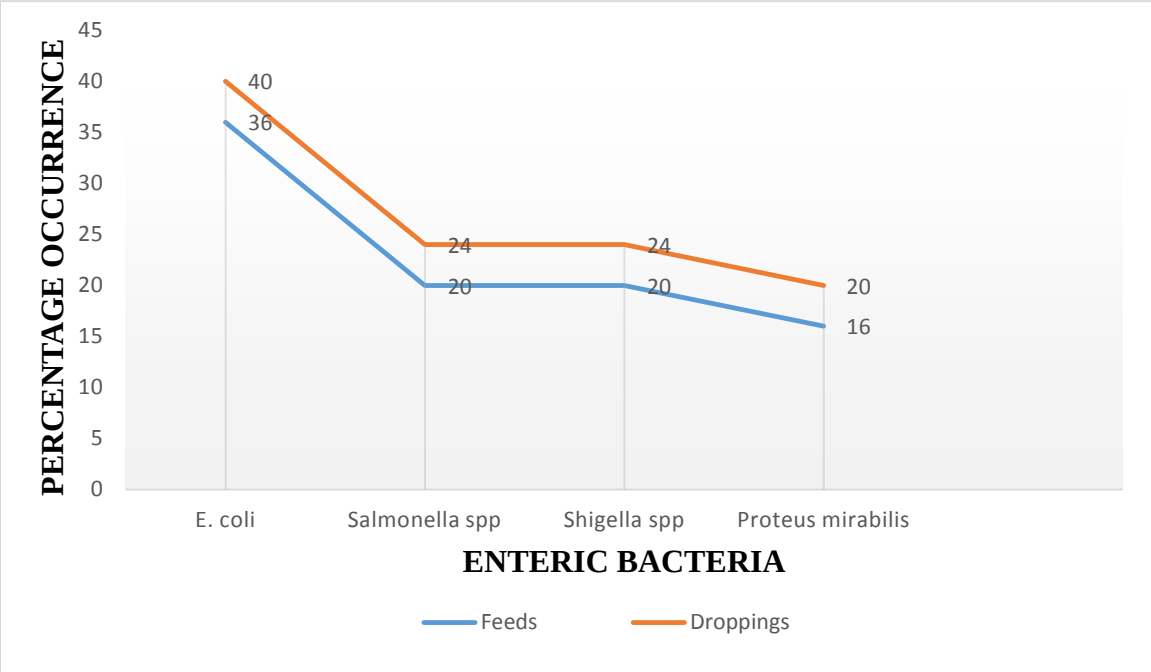

Figure 1: The percentage occurrence of the enteric bacterial in feeds and droppings collected from different poultry farms in Kano state

Table 2 Antibiotic susceptibility pattern of the Enteric bacterial isolates from poultry feeds and droppings

Antibiotics	Diameter of Zone of Inhibition (mm)			
	Escherichia coli	*Salmonella spp.*	*Shigella spp.*	*Proteus mirabilis*
CN	16	21	19	-
AU	21	-	-	-
CPX	-	-	-	-
SXT	21	18	19	16
S	15	-	-	-
PN	-	-	-	-
CEP	19	-	-	-
OFX	-	19	16	22
NA	-	-	-	-
PEF	-	-	-	-

Key: -, No Inhibition; CN, Gentamycin 10µg; AU, Augmentin 30µg; CPX, Ciprofloxacin 5µg; SXT, Sulfamethoxazole 25µg; S, Streptomycin 30µg; PN, Ampicillin 10µg; CEP, Cephalexin 30µg; OFX, Ofloxacin 5µg; NA, Nalidixic acid 30µg; PEF, Pefloxacin 5µg

DISCUSSION

The mean colony counts obtained in this study indicate that the bacterial load in droppings and feeds was 1.6 x 10^5 and 1.3 x 10^5 CFU/g respectively, which was in line with work done by Roy *et al*. (2017). The elevated bacterial load observed could stem from feed contamination during processing or cross-contamination from other sources. It may also result from improper droppings disposal and inadequate hygiene practices within the poultry house. Therefore, it is imperative to maintain proper storage conditions, packaging, and handling practices, as advocated by Roy *et al*. (2017), in addition to minimizing the risk of cross-contamination.

Animal feeds have been identified as a potential source of microorganisms in farmed animals and poultry, as reported by Uwaezuoke and Ogbulie (2008). This investigation found the presence of four bacteria (*Salmonella* spp., *Shigella* spp., *E. coli*, and *Proteus mirabilis*) in both feed and droppings samples, suggesting possible threat to the well-being of the animals. The detection of these bacterial species, particularly those with implications for public health, raises significant concerns regarding the potential direct consumption of feed contaminated with these bacteria or their associated toxins by livestock. This concern was highlighted by (Ramos *et al.*, 2020). In a manner consistent with our own

research, Uwaezuoke and Ogbulie (2008) identified the presence of *Pseudomonas*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella*. Our study has validated these findings, confirming the existence of *Salmonella*, *Shigella* species, *E. coli*, and *P. mirabilis* in the examined samples. This underscores the urgent necessity to address these possible health hazards.

In the current investigation, the combined occurrence rates of *Salmonella* spp. and *Shigella* spp. were 44%, while *E. coli* and *Proteus mirabilis* exhibited rates of 76% and 36%, respectively. The prevalence of *Salmonella* species observed in this study closely matched the findings of Chowdhury *et al*. (2011) and Abu El Hammed *et al*. (2022). However, it's important to highlight that the prevalence of The presence of enteric organisms, such as *E. coli*, *Salmonella*, *Shigella*, and *Proteus mirabilis*, in poultry feed and droppings was verified based on the biochemical characteristics of the isolated bacteria. All the microorganisms identified in this investigation belong to the coliform group, signifying their importance in this context. The identification of *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* species suggests potential contamination from both fecal and environmental sources. It's worth noting that some of these microorganisms are recognized pathogens in birds and livestock. For example, *E. coli* has been linked to various disease conditions, including colibacillosis, which can manifest as enteric and septicaemic colibacillosis (Park *et al*., 2010). It was noted in this study that all the bacterial isolates were totally sensitive to Sulfamethoxazole, whereas all the bacterial isolates were 100% resistant to Ciprofloxacin, Ampicillin, Nalidixic acid and Pefloxacin. *E. coli* was the only bacterial isolate susceptible to Gentamycin, Augmentin, Sulfamethoxazole, Streptomycin and Cephalexin, with the other bacterial isolates showing varying levels of resistance to the other antibiotics. *Proteus mirabilis* displayed the highest level of resistance to all the antibiotics tested, followed by *Salmonella spp.*, *Shigella spp.*, and *E. coli*.

Salihu *et al*. (2014) elaborated on the overuse of antibiotics in poultry, pointing to the widespread availability and affordability of these drugs. Ejeh *et al*. (2017) stressed that antibiotic resistance is a global issue affecting both human and veterinary medicine. Numerous factors have been identified as contributors to bacterial resistance, with Van den Bogaard *et al*. (2001) underscoring antibiotic usage, overcrowding, *Salmonella* species in this research surpassed the results reported by Rodriguez *et al*. (2015), who documented a 17.4% prevalence of *Salmonella* species in broilers in Ibague, Colombia, and Ifeanyichukwu *et al*. (2016), who identified a 9% prevalence during the antibiogram of Salmonella species isolated from poultry products in Ebonyi State, Nigeria. This elevated prevalence of these microorganisms could potentially be attributed to environmental conditions, hygiene practices, and the possibility of cross-infection with other pathogens, given their capacity to cause both sudden and persistent infections in avian species. The detection of *Salmonella* and *Shigella* species in the food source raises public health concerns, as their repeated transmission in the environment has been well-documented (Chat *et al*., 2019).

and inadequate sanitation as the most significant factors. Therefore, the rise of antibiotic resistance among poultry in Nigeria, as highlighted by Ejeh *et al*. (2017), can be reasonably explained by the uncontrolled and excessive use of antibiotics in poultry and livestock in the country, as noted by Olonitola *et al*. (2015).

CONCLUSION

In conclusion, the study revealed a significant bacterial presence in poultry feeds and droppings. Notably, the prevalent enteric bacteria in poultry feeds and droppings across surveyed farms include *Proteus mirabilis*, *Salmonella*, *Shigella*, and *E. coli*. Their presence carries significant implications for both the economy and public health.

Recommendations

It is recommended that:

1. The stock of birds should be maintained at an average level to prevent over-crowding which could facilitate disease transmission in birds.
2. Feed and water bowls should be cleaned daily and fresh feed and water should be supplied.
3. Effective control measures to maintain hygiene should be practiced so as to minimize microbial contamination in feed and droppings.
4. Farmers' understanding of appropriate methods for disposing of poultry waste should be enhanced so as to avoid cross-contamination in poultry facilities and maintain the quality of poultry feeds.
5. Advocating and monitoring the use of antibiotics to mitigate the emergence of antibiotic resistance in poultry farms.

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