

Research Article

Antibiotic Resistant Patterns of Bacterial Species Isolated among Hybrid Chicken Meat Sold within Makurdi Metropolis

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ABSTRACT

Antibiotic resistance is a growing global public health concern, with foodborne pathogens posing significant risks through contaminated poultry products. This study assessed the antibiotic-resistant patterns of bacterial species isolated among hybrid chicken meat sold within Makurdi metropolis, Nigeria. A total of 36 chicken meat samples were collected, six (6) each from the major markets (Wurukum, High Level, Wadata, Northbank, Railway, and Modern Markets) and analyzed for bacterial contamination. Samples from different anatomical parts (mouth, blood, lap, gut, and skin) were processed using standard microbiological techniques, including serial dilution, culturing on selective media (Nutrient Agar, MacConkey Agar, EMB, and Salmonella Shigella Agar), and biochemical identification. Antibiotic susceptibility testing was performed using the disc diffusion method, following standard guidelines. Results revealed significant ($P < 0.05$) bacterial contamination, with the gut exhibiting the highest microbial load (2.69×10^7 CFU/mL). The most prevalent isolates were *Enterobacter* spp. (28.15%), followed by *Escherichia coli* (20.74%), while *Salmonella* spp. (14.44%) was the least. Antibiotic susceptibility testing showed that Gram-negative bacteria were highly sensitive to penicillin, pefloxacin, and gentamicin, while Gram-positive *Staphylococcus aureus* were most susceptible to erythromycin and gentamicin. Data analysis confirmed significant differences ($P < 0.05$) in resistance patterns among isolates. The prevalence of antibiotic-resistant bacteria in hybrid chicken meat, may be due to indiscriminate antibiotic use in poultry farming and poor hygiene practices. This study highlights a substantial risk of bacterial contamination in hybrid chicken meat within Makurdi and underscores the need for improved hygiene practices, regular microbial surveillance, and rational antibiotic use.

Keywords: Antibiotics; Resistant; Bacterial Contamination; Hygiene; Hybrid Chicken

Citation: Hemen, T.J. & Orpin J.B. (2025). Antibiotic Resistant Patterns of Bacterial Species Isolated among Hybrid Chicken Meat Sold within Makurdi Metropolis. *Sahel Journal of Life Sciences FUDMA*, 3(2): 222-227. DOI: <https://doi.org/10.33003/sajols-2025-030222-27>

INTRODUCTION

Antibiotics are natural, semisynthetic or synthetic substances, which interfere with the growth or survival of bacterial microorganisms, and are used to prevent or treat the associated infections (Galgano *et al.*, 2025). Although traditional antimicrobial compounds have been recognized for thousands of years since their discovery by ancient civilizations, it was only in 1928 that the first antibiotic, penicillin, was developed by Alexander Fleming (Alfatlawi *et al.* 2021 and Qadri *et al.*, 2022).

Poultry farming is amongst the most extensive food sectors across the world, and chickens are often the most farmed poultry species, with extensive production of chicken meat accounted for more than 90 billion tons annually (Food and Agriculture Organization of the United Nations, 2017). When these poultry products are consumed by humans, they pose a risk of infections that are difficult to treat with conventional antibiotics. There are some evidence of human health concerns regarding the existence of antibiotic components in eggs and meat (Khan *et al.*, 2019).

The advent of antibiotics revolutionized medicine due to their ability to combat bacterial infections, allowing an increase in the average life expectancy of humans and animals, the control of infectious diseases and the reduction in morbidity and mortality, while also contributing to food safety (Wang *et al.*, 2022 and Vikal *et al.*, 2024). Unfortunately, due to the extensive use of these conventional antibiotics, multidrug resistant (MDR) microorganisms have emerged and disseminated, which is currently a global health concern (Abreu *et al.*, 2023).

Antibiotic-resistant bacteria in food pose a serious risk to human health. Consumption of bacterial contaminated chicken meat can lead to infections that are difficult to treat, increasing morbidity, healthcare costs, and mortality rates. Understanding antibiotics resistant patterns may help assess the potential risk to consumers within Makurdi. Antibiotic resistance (AR) is one of the biggest challenges to the contemporary healthcare community. A total of 1.27 million global deaths that occurred in 2019 were attributed to AR, which is almost twice the annual estimates made in 2016 (Murray *et al.*, 2022). In Nigeria and other developing countries, poultry farmers often misuse or overuse antibiotics as growth promoters or for disease prevention. Hybrid chickens, being commercially raised, are especially prone to such practices (Onyeuka *et al.*, 2022). Makurdi, a growing urban center, has a high demand for poultry meat, especially hybrid chicken, which is considered more profitable and productive. Studying the resistance patterns in this specific locality helps provide relevant data for regional public health policies, food safety regulations, and awareness campaigns. By identifying specific bacterial species and their resistance profiles, this study may guide the rational use of antibiotics in veterinary practice and inform strategies to minimize the spread of resistance through the food chain. The study is, therefore, aimed at determining the antibiotic-resistant patterns of bacterial species isolated among hybrid chicken meat sold within Makurdi metropolis in Benue State, Nigeria.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Makurdi the capital of Benue State, a town that lies between Latitude 7°44'N and Longitude 8° 32'E covering an area of 820 km² with an estimated population of 472,000 people. Makurdi lies on the south bank of the Benue River. The climatic condition in Makurdi is influenced by two air masses: The warm, moist South-Westerly air mass, and the warm, dry North-Easterly air mass. The mean annual rainfall in

Makurdi is about 1,290 mm. The temperature in Makurdi is, however, generally high throughout the year, with February and March as the hottest months. The temperature in Makurdi varies daily from 40°C and a maximum of 22.5°C.

Collection of Meat sample and Preservation

A total of thirty-six (36) solid indigenously bred meat (chickens) samples were purchased from the 6 locations; Wurukum, High Level, Wadata, Northbank, Railway and Modern Markets, with two (2) samples purchased randomly from each of the mentioned locations weekly for three (3) weeks. These samples were further divided into six (6) portions namely; mouth, water, blood, lap, gut and skin, and were transported without delay in a sterile container to Microbiology Laboratory of the Department of Biological Sciences, Benue State University, Makurdi, for microbial analysis

Media Presentation and Preparation

The culture media that were used for this study are Nutrient Agar (NA), MacConkey Agar (MA), Eosine Methylene Blue (EMB), Salmonella Shigella agar (SSA) and Mueller Hinton Agar (MHA). The media were prepared according to manufacturer's instructions.

Microbiological Analysis

For each sample, a swap stick was used to take a swab of the sample and 10ml of distilled water was added into the swap stick container and homogenised to obtain a 1/10 serial dilutions. Serial dilutions were done by transferring 1ml of the original dilution into 9ml of subsequent dilutions. This was done for 5 different dilutions (10^{-1} to 10^{-7}) and inoculated on sterile Nutrient Agar medium to support the growth and for isolation of gram-positive bacteria on MacConkey agar, (Anihouvi *et al.*, 2024), and Eosine Methylene Blue agar and *Salmonella shigella* agar as selective and differential media respectively at 37°C for 24hrs to get pure isolates of Enterobacteriaceae. Biochemical tests such as gram stain, catalase test, coagulase test, Simmons' citrate and indole test were conducted to identify and differentiate bacterial species based on their cell wall characteristics (Gram stain), enzyme production (catalase, coagulase), metabolic capabilities (Simmons citrate), and biochemical reactions (indole test) according to the procedures of Mir *et al.* (2022).

Antibiotic Susceptibility

The isolated bacteria were tested for susceptibility to a panel of antimicrobial agents. Susceptibility was determined on Mueller Hinton Agar using standard disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI). After incubation at 37°C for 24hrs, the diameter in (mm) of clear zones around each antibiotic disc was

measured using a ruler. Measurements were translated accordingly to the CLSI guidelines and the tested isolates were scored as sensitive, intermediate, and resistant (Asif *et al.*, 2021).

Data Analysis

The data (means) obtained during the study were subjected to Analysis of Variance (ANOVA) in order to compare the multiple antibiotics effect across different bacterial species and chicken parts at 5% level of probability, using statistical Package for social Science (SPSS) version 23.

RESULTS

The result of the bacterial load of isolates from various parts of the hybrid chicken meat obtained during the study as presented in Table 1 showed

that, the gut (intestine) had significant ($P < 0.05$) highest bacterial load (2.69×10^7 CFU/ml), indicating high microbial contamination. Blood and thigh had relatively lower loads (2.6×10^6 and 2.7×10^6 CFU/ml respectively). Water used in washing and mouth also showed notable contamination. Skin had a moderate load (4.5×10^6 CFU/ml).

The result in Table 2 reveals that *Enterobacter spp.* was the most prevalent in the mouth and gut. Meanwhile, *E. coli* and *Staphylococcus spp.* were also frequent across multiple sites, especially in gut and water samples. However, *Proteus spp.* and *Salmonella spp.* were detected in varying but lower frequencies. No statistically significant differences ($P > 0.05$) were found in the distribution of these bacteria across parts, suggesting uniform contamination levels.

Table 1: Mean Bacterial Load of isolates from various parts of Hybrid Chicken Meat

| Chicken part examined | Mean Bacterial Load (Cfu/ml) |
|-------------------------------|------------------------------|
| Mouth | 1.69×10^6 |
| Water from washing body parts | 14.0×10^5 |
| Blood | 26.0×10^5 |
| Thigh | 27.0×10^5 |
| Gut (intestine) | 26.9×10^6 |
| Skin | 45.0×10^5 |
| P-value | 0.011 |

Table 2: Distribution of the isolates in relation to different anatomical parts of the Chicken examined

| Part | <i>Staphylococcus aureus</i> | <i>E. coli</i> | <i>Enterobacter spp</i> | <i>Salmonella spp</i> | <i>Proteus spp</i> |
|----------------|------------------------------|-----------------|-------------------------|-----------------------|--------------------|
| Mouth | 3.00 ± 0.57 | 2.67 ± 0.88 | 6.33 ± 1.45 | 3.67 ± 1.76 | 3.00 ± 0.57 |
| Water | 4.33 ± 1.76 | 4.33 ± 0.88 | 3.67 ± 2.19 | 1.67 ± 0.67 | 0.67 ± 0.67 |
| Blood | 1.00 ± 0.57 | 3.33 ± 0.88 | 4.00 ± 0.57 | 2.00 ± 0.57 | 2.67 ± 0.88 |
| Lap | 1.33 ± 0.33 | 1.00 ± 0.00 | 3.00 ± 0.57 | 2.33 ± 0.88 | 2.33 ± 1.20 |
| Gut | 4.33 ± 0.88 | 4.67 ± 1.45 | 5.00 ± 2.31 | 3.67 ± 1.20 | 2.67 ± 1.20 |
| Skin | 2.33 ± 0.88 | 2.67 ± 1.45 | 2.33 ± 0.88 | 3.33 ± 1.45 | 1.67 ± 1.20 |
| p-value | 0.113 | 0.23 | 0.509 | 0.72 | 0.596 |

The bar chart (Figure 1) visually represents the percentage occurrence of bacterial isolates from hybrid chicken meat sold in Makurdi. The result showed that, *Enterobacter spp* was the most (28.15%) frequently isolated bacterium, while *Escherichia coli* (20.74%), *Proteus spp* (18.52%) and *Staphylococcus spp* (18.15%) followed closely. Meanwhile, *Salmonella spp* had the lowest (14.44%) occurrence among the listed bacteria. This distribution suggests a notable presence of multiple potential pathogens in chicken meat, which may highlight possible public health concerns and the need for improved hygienic handling.

The result presented in Table 3 showed that all isolates (Gram-negative and Gram-positive) showed high sensitivity to multiple antibiotics most especially gram-negative bacteria (like *E. coli*, *Salmonella*, *Proteus*) which had significantly ($P < 0.05$) highest (44mm, 41mm, and 39mm respectively) zones of inhibition with penicillin (PN), pefloxacin (PEF), and gentamicin (CN). While, gram-positive (*Staphylococcus spp*) was most (56mm) sensitive to erythromycin (E). Although, statistically significant differences ($P < 0.01$) in antibiotic sensitivity were observed, indicating varying degrees of effectiveness among antibiotics.

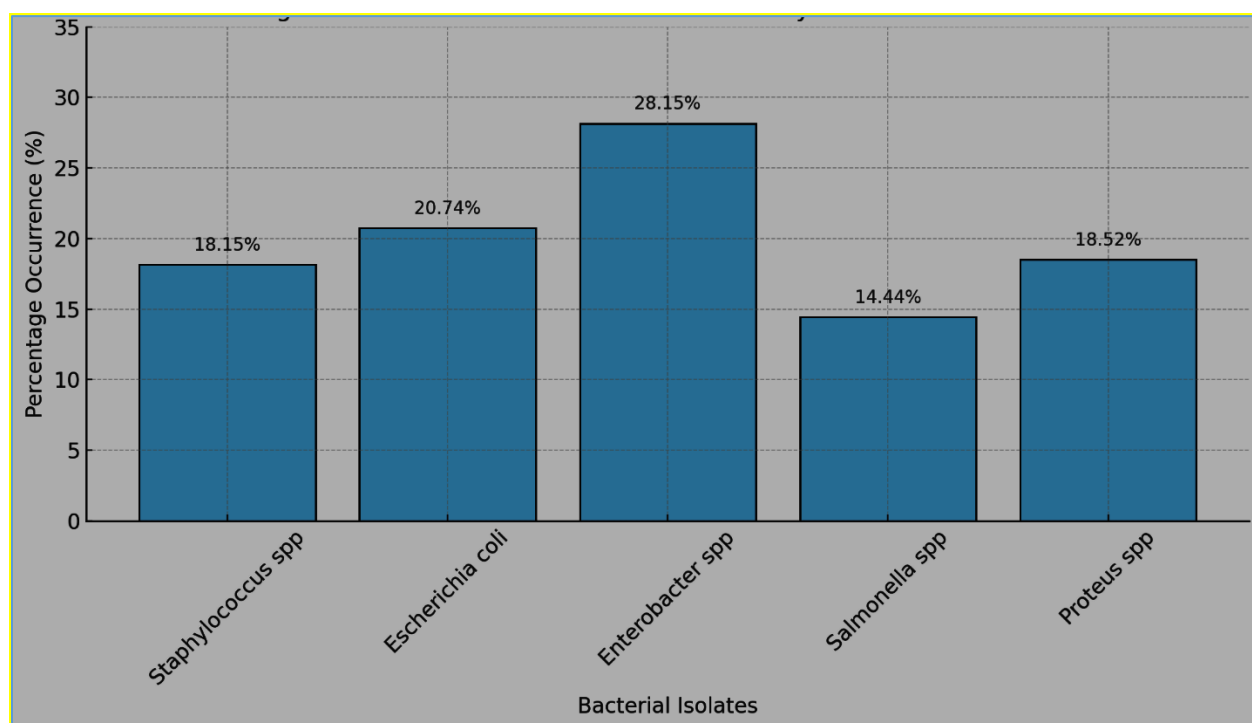


Figure 1: Percentage occurrence of bacterial isolates from hybrid chicken meat sold in Makurdi metropolis

Table 3: Antibiotic Sensitivity of Bacterial isolates (gram negative and gram positive) from Hybrid Chicken Meat sold in Makurdi Metropolis

| Bacterial isolate | Antibiotics zone of inhibition (mm) | | | | | | | | | |
|---------------------------|-------------------------------------|-------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|
| <i>Gram negative</i> | CPX | SXT | AU | S | PN | CEP | OFX | NA | PEF | CN |
| <i>Escherichia coli</i> | 37 | 31 | 34 | 36 | 44 | 30 | 38 | 34 | 41 | 42 |
| <i>Enterobacter spp</i> | 42 | 36 | 35 | 31 | 29 | 29 | 39 | 28 | 41 | 41 |
| <i>Salmonella spp</i> | 24 | 33 | 32 | 34 | 32 | 32 | 35 | 33 | 37 | 41 |
| <i>Proteus spp</i> | 39 | 35 | 34 | 33 | 34 | 32 | 36 | 33 | 39 | 38 |
| p-value | 0.004 | 0.00 | 0.00 | 0.00 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>Gram Positive</i> | E | CT | AP | CF | LV | NB | CPX | CN | OFX | CD |
| <i>Staphylococcus spp</i> | 56 | 22 | 29 | 35 | 29 | 42 | 39 | 45 | 42 | 22 |

KEY: CPX=Ciprofloxacin; SXT=SulfamethoxazoleTrimethoprim; AU= Augmentin; S=Sulfamethoxazole; PN= Penicillin; CEF= Ceftriaxone; OFX=Ofloxacin; NA= Nalidixic acid; PEF= Pefloxacin; CN= Gentamicin; E= Erythromycin; CT= Ceftriaxone; AP= Ampicillin; CF= Cefixime; LV= Levofloxacin; NB=Norfloxacin; CD=Clindamycin

DISCUSSION

The study on antibiotic resistant patterns of bacterial species isolated from hybrid chicken meat sold within Makurdi metropolis reveals significant microbial contamination and alarming antibiotic resistance trends. The findings align with regional and global reports, highlighting a growing public health concern. This prevalence is consistent with previous studies that highlighted *Enterobacter* as a dominant contaminant in poultry due to its ability to thrive in a variety of environmental conditions, including water, soil, and animal intestines (Smith *et al.*, 2020). The

presence of *Enterobacter* spp. in such high proportion suggests poor hygienic conditions during processing and storage, potentially from cross-contamination or unsanitary slaughtering environments Sabrine *et al.*, (2022).

Globally, Mir *et al.* (2018) documented that poultry meat is a major reservoir of antibiotic-resistant bacteria due to indiscriminate antibiotic use in farming. The high bacterial load in Makurdi's chicken meat suggests inadequate adherence to food safety regulations, mirroring trends in low- and middle-income countries (LMICs) where regulatory enforcement is weak (WHO, 2020).

The study identified *Staphylococcus aureus*, *E. coli*, *Enterobacter* spp., *Salmonella* spp., and *Proteus* spp. as common isolates. This distribution aligns with Adesiji *et al.* (2017) in Nigeria, who reported *E. coli* and *Salmonella* spp. as the most prevalent pathogens in poultry. Similarly, Van *et al.* (2020) in Vietnam found *Staphylococcus aureus* and *E. coli* as dominant contaminants, linking them to cross-contamination during slaughter and handling. *Escherichia coli* was the second most isolated organism, a finding that also underscores fecal contamination either during evisceration or through contact with contaminated surfaces or water. *E. coli* is widely used as an indicator organism for fecal contamination and its presence in chicken meat is a red flag for unsafe handling practices (Oladipo *et al.*, 2019). Similarly, the presence of *Proteus* spp. and *Staphylococcus* spp., further demonstrate the polymicrobial nature of contamination in poultry meat. *Staphylococcus* spp., particularly *S. aureus*, is a known contributor to food poisoning due to its ability to produce enterotoxins, while *Proteus* spp. is associated with spoilage and opportunistic infections (Iroha *et al.*, 2018).

Salmonella spp. had the lowest occurrence among the isolates, which might suggest it's relatively lower prevalence or possibly the effectiveness of some control measures in place. However, even at this level, *Salmonella* remains a critical concern due to its pathogenic potential and frequent involvement in poultry-associated outbreaks (WHO, 2022). The cumulative presence of these bacteria may highlights a significant public health concern, pointing toward systemic lapses in poultry hygiene management in Makurdi.

Further insight was provided in the result, which reports bacterial loads across different anatomical parts of the chicken. The gut harbored the highest microbial load, a result that was statistically significant ($P < 0.05$). This was expected given the gut's role in housing diverse microbial communities, including pathogens. This high bacterial burden underscores the importance of strict sanitary procedures during gutting and processing to prevent cross-contamination. Conversely, the blood and thigh muscle, though were less contaminated, and were not exempted from microbial infiltration, due to systemic spread or contamination during butchering. The skin, mouth, and water samples also showed microbial presence, further emphasizing the pervasive nature of contamination and the inadequacy of hygienic measures during processing and storage.

E. coli and *Staphylococcus* spp. were also observed across multiple locations, especially in the gut and

water samples, indicating the likelihood of contamination through shared water sources or improper cleaning practices (Abdul-Rahman *et al.*, (2021) and Sabrine *et al.*, (2022)). Interestingly, although *Proteus* spp. and *Salmonella* spp. were present, their detection was limited in scope and frequency, suggesting either localized contamination or more transient colonization patterns (Ridlon *et al.*, 2024).

The findings also highlight the antibiotic susceptibility patterns of the bacterial isolates, and also revealing how all the isolates, both Gram-positive and Gram-negative, showed considerable susceptibility to multiple antibiotics. Particularly, Gram-negative bacteria (*E. coli*, *Salmonella*, and *Proteus* spp.) which exhibited a significantly higher zones of inhibition when treated with penicillin (PN), pefloxacin (PEF), and gentamicin (CN) ($P < 0.05$). These results were encouraging, suggesting that these antibiotics remain effective options for managing infections arising from consumption of contaminated poultry meat (Onyeka *et al.*, 2022).

CONCLUSION

The findings from this study call for urgent improvements in hygiene practices across the poultry value chain in Makurdi. Regular microbial monitoring, public health education, and stricter enforcement of food safety regulations are imperative to mitigate the risks posed by bacterial contamination in poultry meat. Moreover, rational use of antibiotics in poultry farming and enhanced surveillance of antimicrobial resistance trends are crucial to preserving the efficacy of available treatments.

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