IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 5 issue 3 Sept 2026 Research Article

Molecular Characterization and Antibiogram of Bacteria Associated with Poultry Management

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ABSTRACT

Poultry management involves administration of dietary formulations to enhance growth performance. These feed sources may however expose poultry birds to antibiotic resistant bacteria flora, thereby posing serious public health concerns. This study investigated molecular characterization and antibiogram of bacterial isolates associated with poultry management. Seven treatment Groups that consisted of poultry birds fed with commercial poultry feed (B+F), poultry feed + mushroom (B+M), combined fruit wastes (B+FW), pawpaw peel (B+P), orange fruit waste (B+O), poultry feed + combined fruit wastes (B+F+FW) and banana peel (B+B). The poultry droppings and cloacal swab samples were subjected to standard bacteriological molecular procedures for bacteria isolation and characterization. Antimicrobial susceptibility pattern was determined using the Kirby-Bauer Disc diffusion technique. The data from the molecular analysis indicated the bacterial species shared sequence similarities with *Escherichia coli* strain BGS18 (Accession number MN209843.1), *Lactobacillus fermentum* GCUF-BNB-3 (Accession number KX388385.1), *Salmonella enterica* strain S2 (Accession number OL850007.1), and *Salmonella enterica* strain H1-3S (Accession number OQ092753.1). Antibiotic Resistance Patterns varied within isolates, with the multiple antibiotic resistance (MAR) index of isolates from poultry feed and fruit wastes ranging from 0.1-1.0 for Gram-positive bacteria and 0.2 to 0.3 for Gram-negative bacteria. Isolates from birds had a MAR index that ranged from 0.1 to 1.0 for Gram-positive isolates, and 0.2 to 0.9 for Gram-negative isolates. The results therefore indicated the presence of different bacterial communities associated with the poultry feed, fruit wastes and the birds. The study has shown the molecular identity of bacteria associated with poultry management, and the associated antibiotics resistance patterns of the isolates thereby providing critical data with relevance in public health and agricultural sector.

Keywords: Poultry Birds, Fruit Wastes, Gut Bacteria, Antibiogram, 16S rRNA, Antimicrobial Resistance.

Introduction

Poultry farming is one of the most essential parts of Nigeria's agricultural systems. It supplies a low-cost, readily available source of animal protein in the form of both meat and eggs. As such, poultry accounts for a significant proportion of the nutritional requirements of households at all levels of Nigerian society (Ajala *et al.,* 2021).

The poultry gut microbiome is made up of a wide variety of microbes distributed throughout the tract of the gastrointestinal tract and they play vital roles in various processes including digestion, metabolism of nutrients, immune modulation and resistance against disease (Aruwa et al., 2021). Microbes are made up of a substantial proportion of bacteria including genera like *Bacteroides*, *Escherichia*, *Clostridium* and *Lactobacillus* are microbiological communities that are linked with metabolic characteristics of importance to poultry (Wei *et al.,* 2013; Fathima *et al.,* 2022). The composition of the intestinal microbiota can be affected by factors such as age, diet, environment and administration methods (Kers *et al.,* 2018). Microbial populations are associated with poultry feed and other dietary materials and these depend on the source and how they are handled and processed.

The types of bacterial species related to feed ingredients, dietary components, and the gastrointestinal system include *Bacillus*, *Klebsiella*, *Escherichia coli, Pseudomonas*, *Staphylococcus* and Lactobacillus (Fathima *et al.,* 2022; Kassa *et al.,* 2024).

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The identification of bacterial species with poultry is a key component because constituent species in the intestinal microbiota could be significant for to maintaining gut health and function. The gut microbiota supports immune homeostasis and physiological normality in poultry through immune signaling, preservation of intestinal barrier function, and suppression of invading pathogens. A healthy intestinal microbiota has an essential role in the absorption of nutrients and the development of the intestine (Qamar *et al.,* 2021). Lactobacillus species are an important component of the gut microbiota of poultry, known for promoting intestinal health, improving immune function and enhancing feed efficiency. They can limit populations of pathogenic microorganisms through organic acids, including lactic acid, and their presence can promote nutrient efficiency (Jha *et al.*, 2020).

A number of genomic techniques, such as 16s rRNA gene sequencing, are now employed as adjuncts to the conventional culture-based methodology. The genomic procedures can confirm the identity of the microorganism species as well as detect those that cannot be cultured on artificial media (Chen *et al.,* 2023). In one case, a genomic technique has been used to investigate the gut microbial diversity among three native chicken breeds and to select candidate strains of probiotics (Chen *et al.,* 2023). Kassa et al (2024) also used sequencing to confirm the species of culture-based isolates. The 16S rRNA gene, with its conserved and variable regions, allows comparison of isolates to molecular databases.

Researchers (Nima et al 2024; Shang *et al.,* (2018) have described 16S rRNA molecular techniques as useful tools to characterize bacterial communities associated with poultry. Moote *et al.,* (2020) also utilized molecular and culture-based approaches to characterise bacteria in the gastrointestinal tract of broiler chickens. Molecular techniques such as 16S rRNA gene sequencing can further authenticate the bacterial isolates recovered from typical culture-based methods and supply data on the bacterial species found in poultry (Chen *et al.,* 2023; Kassa *et al.,* 2024).

Antimicrobial resistance has been observed among bacteria associated with poultry production. Olonitola *et al.,* (2015) observed antibiotic resistant mesophilic aerobic bacteria in Nigerian chicken litter and assessed their relatedness to different antibiotic resistance genes.

Mbegbu and Onyemelukwe, (2023) observed antibiotic resistant bacteria in the poultry feeds obtained from poultry farms in the Enugu Metropolis Nigeria. Perewari *et al.,* (2023) also observed multidrug resistant *Escherichia coli* in poultry birds from a poultry farm in Rivers State Nigeria.

The multiple antibiotic resistance (MAR) index is used to determine the resistance profile of isolates to the tested antibiotics. The pattern of resistance may be different for different bacterial species and isolates from poultry sources. As such, the antibiogram of bacteria isolates from poultry feeds, fruit wastes, and poultry birds may differ in resistance to the tested antibiotics.

Bacterial disease of poultry and associated antibiotic resistance has been well reported as one of the major challenges of poultry management in tropical nations, including Nigeria (Nima et al 2023). The sources of the bacterial flora may be connected to the bacteriological quality of the feed sources and formulation.

This study was carried out to investigate the molecular characteristics and antibiogram of bacteria associated with poultry management.

The study therefore provides useful data for improved poultry management, feed safety, and antibiotic stewardship and public health surveillance.

Materials and Methods

Study area

The experiment was conducted at the Biology Animal House of Rivers State University, Port Harcourt, Rivers State Nigeria with co-ordinates indicated as 4.824167° N Latitude and 7.033611° E Longitude.

Experimental design

A completely randomized design with seven treatment groups was used. The birds were randomly assigned into the different dietary treatments as shown in Table 1.

The feeding trial lasted for 60 days with each treatment group receiving 150g of feed and 500 ml of sterilized water daily (Forte *et al.,* 2018).

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Table 1: Experimental Design and Treatment Groups

Treatment Codes	Treatment	Dietary Composition per Day
B+F	Bird + poultry feed	150g Commercial poultry feed
B+M	Bird + poultry feed + mushroom	150g poultry feed + 75 g dried mushroom stem granules
B+FW	Bird + combined fruit waste	50 g pawpaw peel + 50 g banana peel + 50 g orange fruit waste
B+P	Bird + pawpaw peel	75 g poultry feed + 75 g pawpaw peel powder
B+O	Bird + orange fruit waste	75 g poultry feed + 75 g orange fruit waste powder
B+F+FW	Bird + poultry feed + combined fruit waste	37.5 g poultry feed + 37.5 g pawpaw peel + 37.5 g banana peel + 37.5 g orange fruit waste
B+B	Bird + banana peel	75 g poultry feed + 75 g banana peel powder

Sources and Preparation of Dietary Materials

The study involved Twenty-eight (28) 1-day old poultry birds purchased from Rumuokoro Market, Port Harcourt for the study. Commercial poultry feed, Fruit wastes such as pawpaw and banana peels as well as spent orange pulp were obtained from Rumuokoro Market, Port Harcourt, while the Oyster mushrooms were obtained from the Rivers State University Mushroom Farm.

Fruit waste and mushroom samples were washed thoroughly with sterilized water and dried over a period of seven (7) weeks. The dried samples were further granulated and stored as feed additives for the birds in the different experimental groups.

Collection of faecal samples

Faecal samples were obtained at a 15-day interval during the experiment. One gram (1g) of the droppings was collected from each cage using a sterile spatula. Additionally cloacal swabs were obtained by using a sterile swab stick to swab the anal region of the poultry birds and sent and the sticks were returned back into the sterile swab stick tube and placed in an ice pack before they were immediately transportedto the microbiology laboratory for bacteriological examination.

Isolation of Bacterial species

The bacteria isolates were recovered from the commercial feeds and additives as well as poultry droppings using the spread plate techniques, while the bacteria from the anal region were recovered using the streak plate method.

The cultured plates were incubated at 37°C for 24hrs and for 48hrs in cases where growth was not obtained (Nima et al 2023).

After incubation, the morphological characteristics of the colonies were identified and different colonies were picked out for isolation using the streak plate technique and grown on Nutrient agar. Using a sterile wire loop, a colony was picked from the mixed culture plate and smeared on a freshly prepared Nutrient agar medium. The wire loop was sterilized by flaming in the Bunsen burner till it was red hot and allowed to cool, lines were streaked from the smear to one end of the medium to make the first quadrant. The same procedure was followed to get the second and third quadrant after which it was closed with a zig zag. These plates were incubated for 48 hours after which pure cultures were visible on the lines of streak.

The phenotypic characterization and identification of the pure isolates was based on Gram staining, motility test and biochemical tests such as methyl red, indole, Voges Proskauer, citrate, oxidase, oxidase, catalase and sugar fermentation, carried out on the isolates (Cheesbrough, 2006).

Preparation of 0.5M McFarland Turbidity Standard

About 1% v/v solution of Sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and properly mixed. About 0.5g of dehydrated Barium Chloride ($BaCl_2$.$2H_2O$) was dissolved in 50ml of distilled water to prepare 1% w/v of Barium Chloride Solution. About 0.6ml of the Barium Chloride solution was added to 99.4ml of the sulphuric acid solution and properly mixed.

A prepared turbid solution was transferred to a capped tube and kept in well-sealed container in the dark at room temperature (25-28ºC) (Barinua et al, 2023).

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Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed using the Kirby-Bauer Disc diffusion method on Mueller-hinton agar, using the standardized bacterial suspensions equivalent to 0.5 McFarland standard. The standard suspension was transferred uniformly on the surface of a Mueller-Hinton agar plate before the selected antibiotics were introduced using discs as reported in previous studies (Nima et al, 2023; Sampson et al, 2021; Sampson et al, 2026). The inhibition zones were measured and the isolates were classified as sensitive, resistant or intermediate based on the CLSI (2017) break point. Multiple antibiotic resistance indices were estimated from the resistance profile of the isolates (Ssemakadde *et al.,* 2025).

Molecular Characterization

The isolates recovered from the culture plates were were subjected to molecular characterization and the bacterial DNA was obtained using the boiling method involving the Bacterial DNA Miniprep Kit (Zymo Research). The 16S rRNA gene was amplified with the primers 27 F primer (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525 R primer (5′-AAGGAGGTGWTCCARCCGC-3′) and the product obtained through polymerase chain reaction was of the size 1500bp.

The band was sequenced with Genetic Analyzer 3500 using BigDyeTM Terminator v3.1 Cycle Sequencing Kit. The samples were analyzed and compared with the reference sequence (Jeyagowri *et al.,* 2023; Kassa *et al.,* 2024).

Results

The amplification of the bacterial 16S rRNA genes yielded fragments of approximately 1500 base pair conforming to the genomic size of bacteria (Plate 1). The Molecular Profile of 16S rRNA genes of selected isolates indicated that isolate B+0; had a 95.56% pairwise similarity with *Escherichia coli* strain BGS18 (Accession number MN209843.1), while pairwise identity of isolate B+01 with *Lactobacillus fermentum* GCUF-BNB-3 (Accession number KX388385.1), was 97.29%.

On the other hand, the pairwise identity of isolate B+FW2 with *Salmonella enterica* S2 (Accession number OL850007.1) was 90.50% , and pairwise identity of isolate B+M2 with *Salmonella enterica* H1-3S (Accession number OQ092753.1) was 99.22% as shown in Table 2.

The evolutionary relatedness of the bacterial via the BLAST output is as indicated in Figure 1.

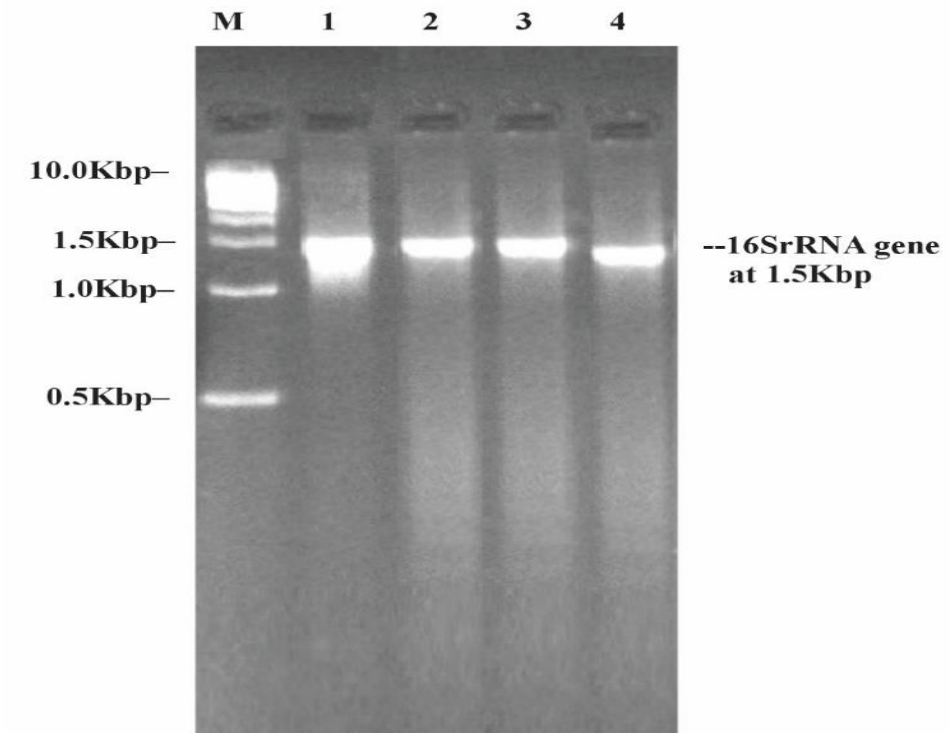


Plate 1: Amplified 16S rRNA Genes at 1,500 base pair.

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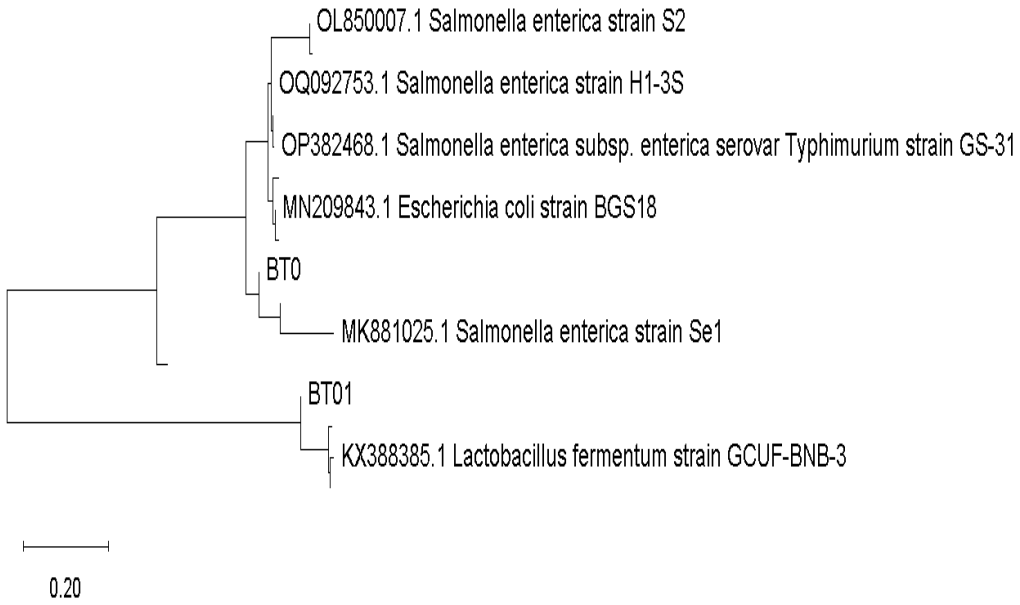


Figure 1: Evolutionary relationship of the isolates

Table 2: Molecular profile of the 16S rRNA genes

Isolate Codes	Pairwise Identity (%)	Accession number (Gene Bank)	Description
B+0	95.56%	MN209843.1	*Escherichia coli* strain BGS18
B+01	97.29%	KX388385.1	*Lactobacillus fermentum* strain GCUF-BNB-3
B+FW2	90.50%	OL850007.1	*Salmonella enterica* strain S2
B+M2	99.22%	OQ092753.1	*Salmonella enterica* strain H1-3S

The data on the antibiotic susceptibility pattern of the isolates revealed that for the poultry feeds and fruit wastes, the only isolate of *Staphylococcus* species was resistant to only Azithromycin (AZM) antibiotic. *Bacillus* species had the highest resistance associated with Erythromycin (E), where all the isolates (100%) of *Bacillus* was resistant to the antibiotic. *Lactobacillus* species displayed similar pattern where all the isolates (100%) recovered from the fruit waste and feed were resistant to only one of the drugs tested (Tale 3). The *Lactobacillus* spp. had the MAR index that ranged from 0.1 to 1.0. The lowest MAR index value value (0.1) was obtained for the different isolates of *Staphylococcus* spp., *Bacillus* spp. and *Lactobacillus* spp. (Table 3).

The study reported *Klebsiella* spp as the only Gram-negative isolates from poultry feed and fruit wastes, and indicated that *Klebsiella* spp was sensitive to most of the antibiotics that was tested albeit 100 % of the isolates showed Resistance was observed to Augmentin and cefuroxime, with a MAR index that ranged from 0.2 to 0.3 (Table 4). The antibiogram of Gram-positive isolates associated with the birds studied showed that all the isolates (100%) of *Bacillus* spp. were resistant Ceftazidime (CTZ) and Erythromycin (E), with majority of the isolates displaying resistance to the other drugs. Isolates of *Lactobacillus s*pp had 100% of the isolates resistant to Amoxicillin (AMX) as shown in Table 5. The MAR index for the Gram positive isolates from the birds however ranged from 0.1 to 1.0 (Table 5).

For the Gram-negative isolates obtained from the poultry birds, all (100%) of *Escherichia* coli isolates were resistant to Augmentin (AU) and Cefuroxine (CEF), while all (100%) of *Klebsiella* spp displayed resistance to Ceftazidime (CTZ), Gentamycin (CN), Ciprofloxacin (CPX) and Cefuroxine (Table 6). The gram-negative isolates from the e poultry birds had multiple antibiotic resistance (MAR) index that ranged from 0.2 to 0.9.

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Table 3: Antibiotic Susceptibility Pattern of Gram-Positive Bacterial Isolates from Poultry feed and Fruit Wastes

Organism	RD	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF	MAR index? range
***Staphylococcus* spp.**	19^{s}	14^{I}	20^{s}	10^{R}	34^{s}	32^{s}	13^{I}	30^{s}	30^{s}	18^{s}	0.1
***Bacillus* spp.**											0.1 – 0.**3**
S(%)	5 (100)	3(60)	1(100)	4(80)	5 (100)	5 (100)	0(0)	5 (100)	4(80)	1(20)	
I(%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(20)	2(40)	
R(%)	0(0)	2(40)	0(0)	1(20)	0(0)	0(0)	5(100)	0(0)	0(0)	2(40)	
***Lactobacillus* spp.**											**0.1 – 1.0**
S(%)	0(0)	0(0)	2(40)	2(40)	0(0)	2(40)	0(0)	2(40)	2(40)	0(0)	
I(%)	1(20)	1(20)	0(0)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	1(20)	
R(%)	4(80)	4(80)	3(60)	3(60)	5(100)	3(60)	4(80)	3(60)	3(60)	4(80)	

Key: Rifampicin (RD), Ceftazidime (CTZ), Streptomycin (S), Azithromycin (AZM), Amoxicillin (AMX), Ciprofloxacin (CPX), Erythromycin (E), Levofloxacin (LEV), Gentamycin (CN), and Cefuroxime (CEF), S (Susceptible), I (Intermediate), and R (Resistant; MARi = Multiple Antibiotic Resistance index.

Table 4: Antibiotic Susceptibility Pattern of Gram-Negative Bacterial Isolates from Poultry feed and Fruit Wastes

Organisms	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF	MARi
Klebsiella spp.											0.2 - 0.3
S(%)	3(100)	0(0)	3(100)	1(33.33)	3(100)	3(100)	3(100)	2(66.67)	3(100)	0(0)	
I(%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.33)	0(0)	0(0)	
R(%)	0(0)	3(100)	0(0)	2(66.67)	0(0)	0(0)	0(0)	0(0)	0(0)	3(100)	

Key: Ofloxacin (OFX), Augmentin (AU), Peflacine (PEF), Ceftazidime (CTZ), Gentamycin (CN), Ciprofloxacin (CPX), Ceporex (CEP), Ceftriaxone (TRX), Streptomycin (S), and Cefuroxine (CEF), S (Susceptible), I (Intermediate), and R (Resistant); MARi = Multiple Antibiotic Resistance index.

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Table 5: Antibiotic Susceptibility Pattern of Gram-Positive Bacterial Isolates from the Poultry Birds

Organism	RD	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF	MARi
***Bacillus* spp**											**0.3 –0.6**
S(%)	4(57.14)	0(0)	5(71.43)	5(71.43)	4(57.14)	4(57.14)	0(0)	7(100)	4(57.14)	0(0)	
I(%)	2(28.57)	0(0)	1(14.29)	1(14.29)	0(0)	2(28.57)	0(0)	0(0)	2(28.57)	1(14.29)	
R(%)	1(14.29)	7(100)	1(14.29)	1(14.29)	3(42.86)	1(14.29)	7(100)	0(0)	1(14.29)	6(85.71)	
***Staphylococcus* spp.**	12^{R}	10^{R}	20^{S}	22^{S}	0^{R}	17^{S}	$0^{(R)}$	10^{R}	10^{R}	0^{R}	0.7
***Lactobacillus* spp**											0.3 – 1.0
S(%)	4(57.14)	0(0)	4(57.14)	5(71.43)	2(28.57)	5(71.43)	1(14.29)	5(71.43)	2(28.57)	0(0)	
I(%)	2(28.57)	0(0)	0(0)	1(14.29)	1(14.29)	1(14.29)	0(0)	1(14.29)	2(28.57)	1(14.29)	
R(%)	1(14.29)	7(100)	3(42.86)	1(14.29)	4(57.14)	1(14.29)	6(85.71)	1(14.29)	3(42.86)	6(85.71)	

Key: Rifampicin (RD), Ceftazidime (CTZ), Streptomycin (S), Azithromycin (AZM), Amoxicillin (AMX), Ciprofloxacin (CPX), Erythromycin (E), Levofloxacin (LEV), Gentamycin (CN), and Cefuroxime (CEF), S (Susceptible), I (Intermediate), and R (Resistant)

Table 6: Antibiotic Susceptibility Pattern of Gram-negative Bacteria Isolated from the Poultry Birds

Organisms	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF	MARi
***Pseudomonas* spp.**	28^{S}	0^{R}	28^{S}	10^{R}	22^{S}	25^{S}	30^{S}	12^{R}	10^{R}	7^{R}	0.6
***Klebsiella* spp**											**0.2- 0.6**
S(%)	2(66.67)	2(66.67)	1(33.33)	0(0)	2(66.67)	3(100)	1(33.33)	3(100)	3(100)	0(0)	
I(%)	0(0)	0(0)	1(33.33)	0(0)	1(33.33)	0(0)	1(33.33)	0(0)	0(0)	0(0)	
R(%)	1(33.33)	1(33.33)	1(33.33)	3(100)	0(0)	0(0)	1(33.33)	0(0)	0(0)	3(100)	
E. coli											**0.3-0.9**
S(%)	8(88.89)	0(0)	2(22.22)	1(11.11)	3(33.33)	8(88.89)	1(11.11)	6(66.67)	4(44.44)	0(0)	
I(%)	0(0)	0(0)	3(33.33)	3(33.33)	1(11.11)	0(0)	2(22.22)	2(22.22)	3(33.33)	0(0)	
R(%)	1(11.11)	9(100)	4(44.44)	5(55.56)	5(55.56)	1(11.11)	6(66.67)	1(11.11)	2(22.22)	9(100)	

Key: Ofloxacin (OFX), Augmentin (AU), Peflacine (PEF), Ceftazidime (CTZ), Gentamycin (CN), Ciprofloxacin (CPX), Ceporex (CEP), Ceftriaxone (TRX), Streptomycin (S), and Cefuroxine (CEF), S (Susceptible), I (Intermediate), and R (Resistant); MARi = Multiple Antibiotic Resistance index.

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Discussion

The molecular characterization of the bacterial isolates included PCR amplificon of the 16S rRNA gene that resulted in an amplicon of about 1,500 base pairs. The 16S rRNA gene is frequently used to identify bacteria because it contains conserved and variable regions that can be used to compare bacterial isolates with other bacteria in molecular databases. Shang et al. (2018) indicate that molecular techniques like 16S rRNA are useful tools for detection and characterization of bacteria communities associated with poultry.

Sequence analysis was similar for some isolates to *Escherichia coli, Lactobacillus fermentum* and *Salmonella enterica* strains. As all isolates were similar to one or more of these bacteria, this could indicate that the isolates are affiliated with these groups of bacteria.

The detection of bacterial species in fruit wastes and poultry feed could potentially have significance for the microbiological safety of poultry production. Aruwa et al. (2021) described interactions that may exist between feed, environment, microbes and the host whereby factors involved in maintaining healthy gut function in the poultry could be affected by fruit wastes introduced into the feed, depending on the source of the waste and hygienic handling of the waste prior to feeding.

Wei et al. (2013) also described the intestinal microbiome of poultry as including a vast bacterial community which plays an essential role in digestion, nutrient processing and developing the immune system of the host. The bacterial groups they identified may therefore have a role in maintaining a stable intestinal microbial community, in contamination of the environment or, particularly for some of the bacterial species identified, opportunistic infection.

These results demonstrate that poultry feed, fruit wastes and the birds themselves were host to a diverse range of bacteria. Some of the bacteria may be significant for their role in maintaining the digestive function of the birds, while others may be useful as indicators of environmental contamination or pose a health risk to the birds. It is therefore important to consider processing, storage and microbiological testing of the fruit wastes used in preparation of feed.

The antimicrobial susceptibility test results showed high variation among the isolates. Some *Lactobacillus* isolates had MARi value as high as 1.0 and some poultry bird isolates of *E. coli* had MARi values as high as 0.9. The presence of isolates with high resistance indices indicates the need for continued surveillance of antimicrobial susceptibility among samples isolated from sites related to poultry production. Similar note on the concern of antimicrobial resistance has been reported among bacteria isolated from feeds used in poultry production in Nigeria (Olonitola *et al.,* 2015; Mbegbu & Onyemelukwe, 2023; Perewari *et al.,* 2023). The pattern of antibiotic susceptibility among the isolates revealed a significant variation in the percentage of susceptible (S), intermediate (I), and resistant (R). The percentage of resistant isolates was higher among the isolates from poultry feeds and fruit wastes in both Gram-positive and Gram-negative isolates. The isolates of gram-positive in poultry feed and fruit wastes showed 100% susceptibility to rifampicin, streptomycin, amoxicillin, ciprofloxacin, levofloxacin, gentamicin, and cefuroxime while on the other hand 100% resistance was recorded in azithromycin. Some of the isolates of *Lactobacillus* showed 100% resistance to all the tested antibiotics with MARi values of 1.0. This finding is similar to Mbegbu and Onyemelukwe (2023), who found a high level of resistance among bacteria isolated from poultry feeds with 92% tetracycline resistant, 90% streptomycin resistant, 90% neomycin resistant, and 80% ampicillin resistant.

Among the isolates of gram-negative from poultry feed and fruit wastes were isolates of *Klebsiella* species with 100% susceptibility to ofloxacin, pefloxacin, gentamicin, ciprofloxacin, ceporex, and streptomycin while on the other hand they had 100% resistance to Augmentin and cefuroxime. This pattern of resistance among poultry-feed bacteria was reported by Mbegbu and Onyemelukwe (2023) from poultry feeds with *Klebsiella pneumoniae* being resistant. The isolates of gram-positive from the experimental poultry birds indicated that they showed a high percentage of resistance especially in the *Bacillus*, *Staphylococcus*, and *Lactobacillus* species. Ceftazidime and erythromycin were 100% resistant among the isolates of *Bacillus* while some isolates of *Lactobacillus* had MARi ranging from 0.5 to 1.0.

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A similar report of the occurrence of multiple resistances was reported by Olonitola et al. (2015) among 67 isolates of bacteria from Nigerian chicken litter with 65.4% resistant to multiple antibiotics and MAR of 0.2. Isolates of Gram-negative from the experimental birds had particularly high resistance with *Escherichia coli* having MARi values of 0.3–0.9 with 0.9 indicating that it was resistant to most of the antibiotics. They had 100% resistant to cefuroxime and 84.62% to Augmentin while showing 92.31% sensitivity to ciprofloxacin.

The high level of resistance among isolates of *Escherichia coli* in the experimental poultry similar to that reported by Perewari et al. (2023) who reported that 90.9% of poultry *Escherichia coli* isolates were multidrug-resistant with 100% resistant to Augmentin, 95.5% resistant to cefuroxime, and 90.91% resistant to tetracycline.

The presence of antimicrobial-resistant bacteria among isolates obtained from sample sites or materials used in or associated with poultry production presents the need to carry out microbiological screening of feed ingredients and alternative feed resources.

Conclusion

The study detected the presence of diverse bacterial populations in the poultry feed, fruit waste, and experimental poultry birds. Bacteria that were commonly isolated were, among others, *Bacillus*, *Klebsiella* and *Lactobacillus* as well as *Staphylococcus*, *Pseudomonas* and *Escherichia coli.* Molecular characterization demonstrated sequence relatedness with *Escherichia coli*, *Lactobacillus fermentum,* and *Salmonella enterica.*

The antibiotics sensitivity test indicated different bacterial isolates to be generally susceptible to most of the antibiotics tested albeit some of the isolates showed multiple antibiotics resistance and underscores the crucial role of microbiological surveillance and anti-microbial resistance monitoring in poultry.

Regular antimicrobial susceptibility testing should therefore be preserved as part of microbiological monitoring in poultry production systems to facilitate the early detection of resistant isolates.

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Citation: Udesike *et al.* (2026). Molecular Characterization and Antibiogram of Bacteria Associated with Poultry Management. *International Journal of Microbiology and Applied Sciences*, ***5(3)***, xx – xx.

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