EVALUATION AND CHARACTERIZATION OF MULTIDRUG-RESISTANT BACTERIA ISOLATED FROM RAW BEEF AND CHICKEN LOCALLY SOLD AT NNEWI MARKETS IN ANAMBRA STATE, SOUTH-EASTERN, NIGERIA

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ABSTRACT

Background: The emergence and rapid spread of microbes resistant to affordable and effective first-line antibiotics have become a widespread challenge accounting for a significant portion of the global infectious disease burden. In Nigeria, surveillance and documentation of antimicrobial resistance patterns remain inadequate, with only a limited number of studies available on bacterial prevalence and resistance trends. This lack of oversight exacerbates the challenge of combating antimicrobial resistance in the country.

Aim: This study aimed to evaluate and characterize multidrug-resistant bacteria isolated from raw beef and chicken locally sold at Nnewi markets.

Methods: This descriptive, cross-sectional study was conducted between April and September 2024 across four major markets in Nnewi Metropolis. Forty raw meat samples (28 beef and 12 chicken) were aseptically collected and transported to a research laboratory for microbial analysis. Bacterial isolates were cultured, identified, and tested for antibiotic susceptibility using the Kirby-Bauer method. Highly resistant isolates were further characterized using 16S rRNA sequencing.

Results: A total of 65 bacterial isolates were obtained—61.5% from beef and 38.5% from chicken—mainly Gram-negative bacilli. High resistance was recorded for metronidazole (100%), Ceftazidime (87.7%), and Amoxicillin-Clavulanic (84.6%), while none showed resistance to ciprofloxacin and levofloxacin. No significant difference in resistance patterns between beef and chicken was observed ($p = 0.11–0.95$). Twenty-four isolates (36.9%) were multidrug-resistant (MARI > 0.5).

Conclusion: The presence of MDR bacteria in these meat products poses a serious threat to consumers.

Recommendation: Strengthening routine antimicrobial surveillance and enforcing hygienic meat processing standards are urgently needed.

Keywords: Antibiotic resistance, Beef, meat, Nnewi market

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INTRODUCTION

Despite decades of revolutionizing healthcare and saving countless lives with the introduction of antibiotics, bacterial pathogens have developed resistance to almost all available antibiotics on a global scale[1]. This growing resistance poses a serious threat to the advancements made in modern medicine, including life-saving procedures such as surgeries, cancer treatment, and organ transplants[2]. Antimicrobial resistance remains a significant global concern, accounting for numerous fatalities daily. In Nigeria, the prevalence of multidrug-resistant (MDR) bacteria exacerbates this issue, complicating treatment efforts and posing substantial public health challenges.

The emergence and rapid spread of microbes resistant to affordable and effective first-line antibiotics have become a widespread challenge. This issue is particularly pronounced in bacterial infections, which account for a significant portion of the global infectious disease burden including diarrheal diseases, respiratory infections, meningitis, sexually transmitted infections, and tuberculosis[2]. The resistance of

Staphylococcus aureus to penicillin was first observed in 1942, shortly after its clinical introduction. By the late 1960s, more than 80% of both community-acquired and hospital-acquired *S. aureus* isolates had developed resistance to penicillin[3].

Common MDR pathogens in Nigeria include *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Studies have documented high rates of resistance among these bacteria, particularly in healthcare settings. For instance, research from Sokoto, northwest Nigeria, identified a high prevalence of MDR Gram-negative bacterial infections, predominantly caused by *E. coli* and *K. pneumoniae*[4].

Studies have shown that common antimicrobial-resistant bacteria such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae,* and *Salmonella* species, which are part of the normal human microbiota as well as significant human pathogens, are among the predominant bacteria in foodborne diseases[5, 6]. Poultry and livestock serve as reservoirs for these drug-resistant microorganisms, facilitating the spread of pathogens and antimicrobial resistance[7]. Animal-derived food products, which contain a mix of both pathogenic and non-pathogenic bacteria, have become a breeding ground for bacterial interactions that can lead to the development of new drug-resistant and multidrug-resistant (MDR) bacteria through horizontal gene transfer[8, 9].The widespread use of antibiotics in the poultry industry, both for disease prevention and as growth enhancers, has further accelerated the emergence of resistant bacterial strains.

Globally, the poultry farming sector continues to undergo industrialization to meet rising consumer demand. Nigeria ranks 33rd in global meat production, with the sector contributing approximately 17% to the agricultural Gross Domestic Product (GDP) and 5% to the national GDP. Meat production in Nigeria has seen significant growth over the years, with poultry and livestock farming expanding to fulfill increasing consumer needs. To meet this demand, many farmers resort to the excessive use of antimicrobials as an easy and quick solution for disease prevention and productivity enhancement[10]. However, the overuse and misuse of these medications have profound effects on microbial ecosystems, leading to the emergence and evolution of antimicrobial-resistant bacteria[11]. These newly resistant bacteria pose a heightened threat to individuals working in farms, the animal health sector, meat processing units and consumers, as they can spread through direct or indirect contact with contaminated food, infected animals, manure, and other environmental sources.

In Nigeria, a significant portion of the population purchases meat from small-scale vendors, where storage conditions are often inadequate, and handling practices are suboptimal, leading to increased microbial contamination. Studies have indicated that the hygienic conditions of meat shops in major Nigerian cities are unsatisfactory, with a high prevalence of coliform bacteria contamination. Similarly, slaughterhouses in key urban areas do not adhere to proper sanitation standards, further exacerbating the risk of bacterial transmission [12]. Additionally, the sale of veterinary drugs without professional consultation or prescription contributes to the rising problem of antimicrobial resistance. It has been observed that 50% of antibiotics used as feed supplements are inappropriately prescribed, and approximately 71% of veterinary medicines are dispensed without the guidance of a licensed veterinarian[13].

In Nigeria as well as other developing countries like Nepal, surveillance and documentation of antimicrobial resistance patterns remain inadequate, with only a limited number of studies available on bacterial prevalence and resistance trends[13, 14]. Furthermore, there is currently no government-regulated veterinary antimicrobial resistance monitoring system, nor are there comprehensive guidelines for veterinary drug usage and regulation. This lack of oversight exacerbates the challenge of combating antimicrobial resistance in the country. Therefore, this study aimed at evaluating and characterizing multidrug-resistant bacteria isolated from raw beef and chicken locally sold in selected markets in Nnewi.

MATERIALS AND METHOD

Study Design, Study Location, and Sampling

This descriptive, cross-sectional, and prospective study was conducted in Nnewi Metropolis between April and September 2024. A total of 40 fresh raw meat samples were collected from four major markets, each representing one of the autonomous quarters in Nnewi metropolis. The selected markets included Orie Agbo (Nnewichi), Nkwo Nnewi (Uruagu), Okpunegbu (Umudim), and Eke Amobi (Otolo). The samples were categorized into 28 beef samples and 12 chicken samples. Seven beef samples were obtained from butchers from each market, and 3 chicken samples each were collected from poultry breeders. A simple random sampling technique was employed to collect fresh beef from different butcher stalls in the four markets. All samples were immediately placed in sterile, leak-proof containers and transported in a cold chain box to Perfect Glory Research Laboratory, Nnewi, for microbial analysis.

Bacterial Isolation and Identification

A 25-gram portion of each meat sample was thoroughly homogenized for two minutes in 250 ml of 1% buffered peptone water. A 0.1 ml aliquot of the sample was then inoculated onto different culture media, including Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar, and Mannitol Salt Agar (all from TM-Media, India). The culture plates were incubated at 37°C for 24 hours to facilitate bacterial growth. The resulting colonies were then observed based on their morphological features, staining reactions, and biochemical characteristics, following the standard procedures outlined by U.S. Department of Agriculture, Food Safety and Inspection Service[15].

Pathogenicity (Virulence) Test on Blood Agar Plate

The virulence of bacterial isolates was assessed using Blood Agar Plates (BAP), a nutrient-rich medium containing 5% sheep red blood cells. The plates were prepared by sterilizing nutrient agar at 121°C for 15 minutes, cooling it to 50°C, and aseptically adding 5% sheep blood before gently mixing.

The bacterial isolates were inoculated onto the BAP to evaluate hemolysis. Beta hemolysis (β) was identified by a clear zone around the colonies, indicating complete lysis of red blood cells. Alpha hemolysis (α) showed a greenish, opaque zone, indicating partial hemolysis, while gamma hemolysis (γ) exhibited no noticeable changes, signifying the absence of hemolysis[16].

Antimicrobial Susceptibility Test

The antimicrobial susceptibility of the bacterial isolates was evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Hi-Media, India), following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2017). To determine the antibiotic

susceptibility pattern, commercially available antibiotic discs were employed. The antibiotics tested included Ceftazidime (CTZ - 30µg), Streptomycin (S - 30µg), Azithromycin (AZM - 10µg), Amoxil (AMX - 20µg), Ciprofloxacin (CPX - 10µg), Erythromycin (E - 30µg), Levofloxacin (LEV - 20µg), Gentamycin (CN - 10µg), Cefuroxime (CEF - 30µg), Rifampicin (RD - 20µg), Ofloxacin (OFX - 10µg), Augmentin (AU - 30µg), Peflacine (PEF - 10µg), Ceporex (CEP - 10µg), Ceftriaxone (TRX - 30µg), and Metronidazole (MTZ - 50µg). These antibiotics were selected based on their clinical relevance and effectiveness against a broad spectrum of bacterial pathogens. The plates were incubated at 37°C for 24 hours. Zones of inhibition were measured using a meter rule and interpreted using standard recommendations of the Clinical and Laboratory Standard Institute[17].

RESULTS

The microbial load (**Table 1**) of beef and chicken samples was assessed at different dilution levels, with Total Viable Count (TVC), Total Enteric Count (TEC), and Total Staphylococcal Count (TSC) recorded in Colony Forming Units per milliliter (CFU/ml). At lower dilutions (10^{-1} and 10^{-2}), microbial growth in both beef and chicken samples were too numerous to count (TNTC), indicating a high microbial load. As the dilution factor increased, countable microbial colonies were observed. For beef, TVC ranged from 2.2×10^6 CFU/ml at 10^{-3} to 8.5×10^8 CFU/ml at 10^{-6}, while TEC and TSC followed a similar trend, with values reaching 7.6×10^8 CFU/ml and 5.1×10^8 CFU/ml, respectively, at 10^{-6} dilution.

Chicken samples exhibited a slightly different trend, with TNTC counts persisting up to 10^{-3} dilution for TVC, whereas TEC and TSC became countable at 10^{-3} dilution. The highest recorded counts at 10^{-6} dilution for chicken were 5.7×10^8 CFU/ml (TVC), 6.9×10^6 CFU/ml (TEC), and 4.8×10^6 CFU/ml (TSC). Overall, the microbial load in both meat types was significantly high, especially at lower dilutions, with bacterial counts reducing as the dilution factor increased.

Tables 2A and 2B present the identification and biochemical characteristics of bacterial isolates obtained from the meat samples. Table 2A provides details on the Gram staining reactions, morphological characteristics, and biochemical test results, including catalase, oxidase, indole, urease, citrate utilization, motility, and Kligler iron agar tests. These tests were used to classify the isolates into their respective bacterial species. Table 2B illustrates the sugar fermentation profiles of the isolates, indicating their ability to utilize different carbohydrates such as glucose, lactose, xylose, fructose, and sucrose. The combination of these biochemical tests was essential for confirming the identity of the bacterial species present in the samples. Out of the 65 bacterial isolates, twenty-one (21) were Gram-positive (32.3%), while forty-four (67.7%) were Gram-negative, indicating a higher prevalence of Gram-negative bacteria in the analyzed meat samples.

Table 3 presents the virulence test results of the bacterial isolates based on their hemolysis patterns on a blood agar plate. Hemolysis was categorized into gamma (γ), alpha (α), and beta (β) hemolysis, indicating different levels of virulence. Out of the 65 isolates analyzed, 47 isolates (72.3%) exhibited gamma (γ) hemolysis, 14 isolates (21.5%) showed alpha (α) hemolysis, and 4 isolates (6.2%) demonstrated beta (β) hemolysis. The predominance of

gamma hemolysis suggests that most of the isolates had low virulence, while a smaller proportion exhibited medium and high virulence.

Table 1: Total Microbial Count of the Meat Samples (CFU/ml)

Sample	Location	TVC (cfu/ml)	TEC (cfu/ml)	TSC (cfu/ml)
Beef	Orie Agbor	2.1×10^8	2.09×10^6	7.1×10^6
	Okpuno Egbu	8.7×10^7	2.1×10^5	1.5×10^4
	Eke Amobi	2.2×10^6	7.6×10^8	1.2×10^6
	Nkwo Nnewi	1.4×10^7	1.1×10^7	5.8×10^8
Chicken	Ogbo Okuko A	2.08×10^7	2.07×10^6	1.02×10^7
	Ogbo Okuko B	5.7×10^8	1.3×10^7	5.1×10^7
	Ogbo OKuko C	1.4×10^8	6.9×10^6	1.2×10^6

TVC = Total Viable Count; TEC = Total Enteric Count; TSC = Total Staphylococcal Count; TNTC = Too Numerous Too Count; TFTC = Too Few To Count; CFU = Colony Forming Unit.

Table 2A: Gram Staining and Biochemical Test Identification of Isolates

Isolates	Gram Strain	CAT	OX	IND	UR	CIT	MO	KIA	Suspected Organism
BOA1	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
BOA2	-ve bacilli	+	-	-	+	+	+	+	*Citrobacter murliniae*
BOA3	+ve cluster-cocci	+	-	-	+	-	-	-	*S. aureus*
BOA4	+ve cluster-cocci	+	-	-	-	-	-	+	*S. aureus*
BOA5	-ve bacilli	+	-	-	+	+	-	+	*Salmonella spp.*
BOA6	+ve bacilli	+	-	-	+	-	+	+	*Listeria*
BOA7	-ve bacilli	+	-	-	+	+	-	-	*Salmonella spp.*
BOA8	+ve bacilli	+	-	-	-	-	+	+	*Lysinibacillus boronitolerans*
BOE1	+ve cluster-cocci	+	-	-	-	+	-	-	*S. aureus*
BOE2	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
BOE3	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
BOE4	-ve bacilli	+	+	+	+	+	-	+	*Vibrio spp.*
BOE5	-ve bacilli	+	-	-	+	+	+	-	*Salmonella spp.*
BOE6	-ve bacilli	+	+	+	+	+	-	+	*Vibrio spp.*
BOE7	+ve cluster-cocci	+	-	-	-	-	-	+	*S. aureus*
BOE8	-ve bacilli	+	-	+	-	+	+	-	*Morganella spp.*
BOE9	-ve bacilli	-	-	+	-	-	+	-	*Morganella spp.*
BOE10	+ve bacilli	+	-	-	-	+	+	+	*Citrobacter spp.*
BEA1	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
BEA2	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
BEA3	-ve bacilli	+	-	+	-	-	-	-	*Shigella spp.*
BEA4	-ve bacilli	+	-	+	-	+	-	-	*Salmonella spp.*
BEA5	-ve bacilli	+	+	+	+	+	+	-	*Vibrio spp.*
BEA6	+ve cluster-cocci	+	-	-	-	-	-	-	*S. aureus*
BEA7	+ve cluster-cocci	+	-	-	-	-	-	+	*S. aureus*
BEA8	-ve bacilli	+	+	+	+	+	-	+	*Vibrio spp.*
BNN1	-ve bacilli	+	+	+	+	-	+	-	*Vibrio spp.*
BNN2	-ve bacilli	+	-	-	+	+	+	-	*Salmonella spp.*
BNN3	-ve bacilli	+	-	-	+	+	+	+	*Citrobacter murliniae*
BNN4	-ve bacilli	+	-	-	-	+	+	+	*Citrobacter spp.*
BNN5	-ve bacilli	+	-	-	-	+	+	-	*Providencia sneebia*
BNN6	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
BNN7	-ve bacilli	+	-	-	-	+	+	+	*Salmonella spp.*
BNN8	-ve bacilli	+	-	-	-	+	+	-	*Morganella morganii*
BNN9	+ve bacilli	+	+	+	+	+	-	+	*Vibrio*

BNN10	-ve bacilli	+	+	+	+	+	+	-	*Vibrio*
BNN11	-ve bacilli	+	-	+	-	+	+	-	*Pseudocitrobacter vendiensis*
CHA1	+ve cluster-cocci	+	+	-	-	-	-	-	*S. aureus*
CHA2	+ve bacilli	+	-	-	+	+	+	+	*Listeria*
CHA3	+ve cluster-cocci	+	+	-	-	-	-	-	*S. aureus*
CHA4	+ve bacilli	+	-	-	+	+	+	+	*Lysinibacillus boronitolerans*
CHA5	-ve bacilli	+	-	+	-	+	+	-	*Morganella spp.*
CHA6	-ve bacilli	-	-	+	+	-	-	-	*E. coli*
CHA7	-ve bacilli	+	-	-	-	+	+	+	*Salmonella spp.*
CHA8	-ve bacilli	+	-	+	-	-	+	-	*E. coli*
CHA9	-ve bacilli	-	-	-	-	-	+	+	*Salmonella*
CHA10	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
CHA11	+ve cluster-cocci	+	-	+	-	+	+	+	*Lysinibacillus boronitolerans*
CHB1	-ve bacilli	+	-	+	-	-	+	+	*Salmonella spp.*
CHB2	+ve cluster-cocci	+	+	-	-	-	-	-	*S. aureus*
CHB3	-ve bacilli	+	-	+	-	+	+	-	*Morganella spp.*
CHB4	-ve bacilli	+	-	-	-	+	+	+	*Salmonella spp.*
CHB5	-ve bacilli	+	+	+	-	-	-	+	*Shigella spp.*
CHB6	+ve bacilli	+	-	-	+	+	+	-	*Lysinibacillus boronitolerans*
CHB7	-ve bacilli	+	-	-	+	+	+	-	*Salmonella*
CHB8	+ve bacilli	+	-	-	+	-	+	+	*Listeria*
CHC1	+ve cluster-cocci	+	-	-	+	-	-	-	*S. aureus*
CHC2	-ve bacilli	+	-	-	+	-	-	+	*Shigella spp.*
CHC3	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
CHC4	-ve bacilli	+	-	+	-	-	+	+	*E. coli*
CHC5	-ve bacilli	+	-	-	-	-	-	+	*Shigella spp.*
CHC6	-ve bacilli	+	-	-	-	+	+	-	*Providencia sneebia*
CHC7	+ve cluster-cocci	+	-	-	-	-	-	+	*S. aureus*
CHC8	+ve cluster-cocci	+	-	-	-	-	-	+	*S. aureus*
CHC9	-ve bacilli	+	-	+	-	-	+	-	*E. coli*

CAT = Catalase Test; OX = Oxidase Test; IN = Indole Test; UR = Urease Test; CIT = Citrate Utilization Test; MO = Motility Test; KIA = Kliger Iron Agar Test; +ve = positive while –ve = negative results.

Table 2B: Sugar Fermentation Test of Isolates

Isolates	GLU	LAC	XYL	FRU	MAN	GAL	SUC	DEX	MAL	MANN
Citrobacter murliniae	+	-	+	+	+	-	-	+	+	+
S. aureus	+	+	-	+	+	-	+	-	+	+
E. coli	+	+	+	-	-	+	+	-	-	+
Salmonella spp.	+	-	+	+	+	+	-	-	+	+
Shigella spp.	+	-	+	+	-	+	+	-	+	+
Vibrio spp.	+	-	-	+	-	-	-	+	-	-
Citrobacter spp.	+	-	+	+	+	-	-	-	+	-
Providencia sneebia	+	-	-	-	+	-	+	+	-	-
Morganella morganii	+	+	-	-	+	-	+	+	-	-
Listeria spp.	+	-	-	+	+	+	+	-	-	-
Pseudocitrobacter vendiensis	+	-	+	+	+	-	-	+	-	+
Lysinibacillus boronitolerans	+	-	+	+	-	+	+	-	+	-
Morganella spp.	+	-	-	-	+	-	-	+	+	-

GLU = Glucose; LAC = Lactose; XYL = Xylose; FRU = Fructose; MAN = Mannitol; GAL = Galactose; SUC = Sucrose; DEX = Dextrose; MAL = Maltose; MANN = Mannose.

Table 3: Virulence Test on Blood Agar Plate

Isolates	Hemolysis	Virulence
BOA1	**Γ**	Low
BOA2	**Γ**	Low
BOA3	**Γ**	Low
BOA4	**Γ**	Low
BOA5	**Γ**	Low
BOA6	**Γ**	Low
BOA7	**A**	Medium
BOA8	**Γ**	Low
BOE1	**Γ**	Low
BOE2	**A**	Medium
BOE3	**Γ**	Low
BOE4	**Γ**	Low
BOE5	**A**	Medium
BOE6	**Γ**	Low
BOE7	**Γ**	Low
BOE8	**Γ**	Low
BOE9	**A**	Medium
BOE10	**Γ**	Low
BEA1	**α**	Medium
BEA2	**γ**	Low
BEA3	**α**	Medium
BEA4	**α**	Medium
BEA5	**γ**	Low
BEA6	**γ**	Low
BEA7	**γ**	Low
BEA8	**γ**	Low
BNN1	**γ**	Low
BNN2	**γ**	Low
BNN3	**γ**	Low
BNN4	**γ**	Low
BNN5	**γ**	Low
BNN6	**γ**	Low
BNN7	**γ**	Low
BNN8	**γ**	Low
BNN9	**α**	Medium

BNN10	**α**	Medium
BNN11	**α**	Medium
CHA1	**γ**	Low
CHA2	**β**	High
CHA3	**β**	High
CHA4	**β**	High
CHA5	**α**	Medium
CHA6	**γ**	Low
CHA7	**α**	Medium
CHA8	**α**	Medium
CHA9	**γ**	Low
CHA10	**α**	Medium
CHA11	**α**	Medium
CHB1	**γ**	Low
CHB2	**γ**	Low
CHB3	**γ**	Low
CHB4	**γ**	Low
CHB5	**γ**	Low
CHB6	**γ**	Low
CHB7	**γ**	Low
CHB8	**β**	High
CHC1	**γ**	Low
CHC2	**γ**	Low
CHC3	**α**	Medium
CHC4	**γ**	Low
CHC5	**γ**	Low
CHC6	**γ**	Low
CHC7	**γ**	Low
CHC8	**α**	Medium
CHC9	**γ**	Low

γ = Gamma; α = Alpha; β = Beta.

Table 4 presents the antibiotic susceptibility patterns of the bacterial isolates against 16 different antibiotics. The susceptibility of each isolate was determined based on whether it was resistant (R) or susceptible (S) to the tested antibiotics. The table provides insight into the resistant profiles of the isolates, highlighting variations in their response to different antimicrobial agents.

The prevalence of antimicrobial resistance in isolates from beef and chicken samples is represented in table 5. Overall, our results showed that the bacterial isolates exhibited high resistance to metronidazole (100%), Ceftazidime (87.7%), Amoxicillin-Clavulanic (84.6%), Cefuroxime (78.5%), Amoxicillin (69.2%), and Cefalexin (66.2%). However, no resistance was observed against ciprofloxacin and levofloxacin (0%).

The difference in the distribution of resistant isolates between beef and chicken isolates across the antibiotics tested was not statistically significant with p-values ranging from 0.11 to 0.95 (which were much greater than the typical significance level: $p>0.05$) and $X^2 < 3.84$ indicating no strong evidence that the resistance to the antibiotics differs significantly between beef and chicken isolates; thus the observed variations in resistance were likely due to chance rather than a systematic difference between the two sources.

Antibiotic susceptibility of bacterial isolates based on the CLSI interpretative criteria breakpoints, 2020 is presented in Figure 4.1. High resistance was observed for Metronidazole, Ceftazidime, Amoxicillin, and Amoxi-Clav, indicating they are ineffective. In contrast, Ciprofloxacin, Levofloxacin, and Ofloxacin showed high sensitivity, making them the most effective. Some antibiotics like Streptomycin, Gentamicin, Erythromycin had mixed responses, showing intermediate susceptibility, suggesting that combination therapy or alternative dosing strategies might be needed.

Figure 2 shows the Multiple Antibiotic Resistance Index (MARI) of the isolates, a numerical value that assesses bacterial resistance to antibiotics. It serves as an indicator of the extent to which an organism is resistant to multiple antibiotics.

The MARI was calculated and interpreted[18] using the formula: MARI = $\frac{x}{y}$ where 'x' represents the number of antibiotics to which an isolate was resistant, and 'y' represents the total number of antibiotics tested. The tables show isolates with MARI ranging from 0.50 to 0.88 showing BNN5 with the highest MARI of 0.88.

Figure 3 shows agarose gel electrophoresis of the extracted and amplified genes of the 5 most resistant isolates and Table 4.6 shows their molecular identities.

Table 4A: Antibiogram of Gram-Positive Isolates

Isolates	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF	RD	OFX	AMC	PEF	CEP	TRX	MTZ
BOA3	R	R	I	R	S	S	S	R	I	S	S	R	S	R	R	R
BOA4	R	S	S	I	S	S	S	S	R	R	S	I	S	I	I	R
BOA6	R	I	I	R	S	S	S	R	S	S	S	R	S	R	R	R
BOA8	R	S	S	I	S	I	S	R	R	S	R	R	I	R	I	R
BOE1	R	R	S	I	S	I	S	R	R	S	R	R	I	R	R	R
BOE7	R	I	I	S	S	S	S	I	R	I	S	R	S	R	S	R
BOE10	R	S	R	S	S	R	S	R	R	S	S	R	I	I	S	R
BEA6	R	I	R	R	S	R	S	I	R	I	S	R	S	R	I	R
BEA7	R	S	I	R	S	S	S	I	I	S	S	R	S	R	I	R
BNN9	R	I	I	R	S	R	S	S	I	S	S	R	S	I	S	R
CHA1	R	S	I	R	S	S	S	S	R	S	S	R	I	R	R	R
CHA2	I	S	I	R	S	S	S	S	I	S	S	I	S	I	R	R
CHA3	R	S	I	R	S	S	S	I	R	S	S	I	S	I	R	R
CHA4	R	I	R	R	S	I	S	S	R	S	I	R	S	R	R	R
CHA11	R	S	I	I	S	I	S	I	R	S	S	R	S	R	R	R
CHB2	R	I	R	R	S	R	S	I	R	R	S	I	I	I	S	R
CHB6	R	R	R	R	S	R	S	I	R	I	S	R	S	R	R	R
CHB8	I	S	R	R	S	I	S	S	I	I	S	R	S	R	S	R
CHC1	R	R	R	R	S	R	S	I	R	I	S	S	S	R	R	R
CHC7	R	S	R	S	S	R	S	R	R	S	I	I	S	R	S	R
CHC8	R	I	R	R	S	I	S	S	I	S	S	S	S	I	R	R

Table 4B: Antibiogram of Gram-Negative Isolates

Isolates	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF	RD	OFX	AMC	PEF	CEP	TRX	MTZ
BOA1	R	S	R	R	S	S	S	S	R	S	S	R	S	S	I	R
BOA2	R	I	R	R	S	R	S	I	R	R	S	R	R	R	R	R
BOA5	R	S	R	R	S	I	S	I	R	S	S	I	S	I	I	R
BOA7	R	I	S	I	S	I	S	R	R	S	R	R	I	R	I	R
BOE2	R	S	S	I	S	I	S	I	R	S	S	R	S	R	I	R
BOE3	R	I	R	R	S	R	S	R	R	S	I	R	S	R	R	R
BOE4	R	I	I	R	S	I	S	I	R	S	I	R	S	R	S	R
BOE5	R	R	R	R	S	R	S	I	R	I	S	R	S	R	S	R
BOE6	R	I	I	R	S	R	S	S	R	S	S	R	S	I	S	R
BOE8	R	S	I	I	S	I	S	S	R	I	S	R	S	S	S	R
BOE9	I	I	R	S	S	R	S	I	I	S	S	R	S	R	S	R
BEA1	R	S	I	I	S	R	S	I	R	I	S	R	S	R	S	R
BEA2	R	I	R	R	S	R	S	S	R	I	S	R	S	R	S	R
BEA3	R	R	R	R	S	R	S	R	R	I	S	R	I	I	S	R
BEA4	R	S	S	R	S	S	S	I	R	S	S	R	S	R	R	R
BEA5	I	R	R	R	S	R	S	I	R	I	S	R	S	R	R	R
BEA8	R	I	I	R	S	R	S	S	R	S	S	R	S	R	I	R
BNN1	R	I	R	R	S	R	S	I	R	R	S	I	S	S	S	R
BNN2	R	S	I	R	S	S	S	I	R	R	S	R	S	S	S	R
BNN3	R	S	R	I	S	R	S	R	R	S	I	R	S	S	I	R
BNN4	R	I	R	R	S	S	S	R	R	R	I	R	S	R	R	R
BNN5	R	R	R	R	S	R	I	R	R	R	R	R	R	R	R	R
BNN6	I	S	I	R	S	R	S	S	R	I	S	R	S	R	S	R
BNN7	R	I	R	R	S	S	S	I	R	S	S	R	S	I	S	R
BNN8	R	I	R	R	S	I	S	R	R	S	R	R	R	R	R	R
BNN10	R	I	R	R	S	R	S	R	R	S	S	R	S	R	R	R
BNN11	R	R	R	R	S	R	S	I	R	S	S	R	S	R	R	R
CHA5	I	I	S	I	S	R	S	I	R	R	S	R	S	R	I	R
CHA6	R	S	I	R	S	I	S	I	S	S	I	R	S	R	R	R
CHA7	R	R	R	R	S	R	S	R	R	I	S	R	S	R	I	R
CHA8	R	I	I	R	S	R	S	I	R	I	I	R	S	R	R	R
CHA9	R	S	S	I	S	I	S	I	R	R	I	R	S	I	I	R
CHA10	R	R	R	R	S	I	S	R	R	I	S	R	S	R	I	R
CHB1	R	I	I	R	S	S	S	I	R	I	S	R	S	I	S	R
CHB3	R	R	R	R	S	R	S	I	I	I	S	R	S	R	S	R
CHB4	R	I	S	R	S	R	S	I	R	I	S	R	S	R	R	R
CHB5	R	S	I	R	S	R	S	S	I	I	S	R	S	R	R	R

CHB7	R	I	R	I	S	R	S	R	R	R	I	R	S	R	R	R
CHC2	R	I	R	S	S	S	S	I	I	I	S	R	S	S	R	R
CHC3	R	I	R	R	S	R	S	I	R	I	I	R	S	R	S	R
CHC4	I	S	I	I	S	S	S	I	R	I	S	R	S	R	S	R
CHC5	R	I	R	R	S	S	S	R	I	I	S	R	S	S	S	R
CHC6	I	R	I	I	S	S	S	I	R	S	I	I	I	S	I	R
CHC9	R	S	S	I	S	I	S	S	I	I	I	R	S	I	R	R

CTZ = Ceftazidime; S = Streptomycin; AZM = Azithromycin; AMX = Amoxicillin; CPX = Ciprofloxacin; E = Erythromycin; LEV = Levofloxacin; CN = Gentamycin; CEF = Cefuroxime; RD = Rifampicin; OFX = Ofloxacin; AMC = Amoxicillin-Clavulanic; PEF = Pefloxacin; CEP = Cefalexin; TRX = Ceftriaxone; MTZ = Metronidazole.

S means the isolate is susceptible to the given antibiotic while I shows intermediate resistance and R shows resistance.

Table 5: Prevalence of Antimicrobial Resistance in Isolates from Beef and Chicken

Antibiotics	Concentration (µg)	Beef (n=40) N (%)	Chicken (n=25) N (%)	Total (n=65) N (%)	P-value	X^2
Ceftazidime	30	36 (63.2%)	21 (36.8%)	57 (87.7%)	0.27	1.211
Streptomycin	30	5 (38.5%)	8 (61.5%)	13 (20.0%)	0.20	1.608
Azithromycin	10	18 (54.5%)	15 (45.5%)	33 (50.8%)	0.87	0.025
Amoxicillin	20	26 (57.8%)	19 (42.2%)	45 (69.2%)	0.80	0.063
Ciprofloxacin	10	0 (0.0%)	0 (0.0%)	0 (0.0%)	0	0
Erythromycin	30	17 (54.8%)	14 (45.2%)	31 (47.7%)	0.90	0.015
Levofloxacin	20	0 (0.0%)	0 (0.0%)	0 (0.0%)	0	0
Gentamicin	10	8 (44.4%)	10 (55.6%)	18 (27.7%)	0.33	0.962
Cefuroxime	30	29 (56.9%)	22 (43.1%)	51 (78.5%)	0.89	0.018
Rifampicin	20	4 (40.0%)	6 (60.0%)	10 (15.4%)	0.31	1.028
Ofloxacin	10	3 (60.0%)	2 (40.0%)	5 (7.7%)	0.85	0.034
Amoxi-Clav	30	31 (56.4%)	24 (43.6%)	55 (84.6%)	0.95	0.004
Pefloxacin	10	2 (66.7%)	1 (33.3%)	3 (4.6%)	0.71	0.141
Cephalexin	10	25 (58.1%)	18 (41.9%)	43 (66.2%)	0.77	0.086
Ceftriaxone	30	11 (40.7%)	16 (59.3%)	27 (41.5%)	0.11	2.524
Metronidazole	50	40 (61.5%)	25 (38.5%)	65 (100.0%)	0.36	0.832

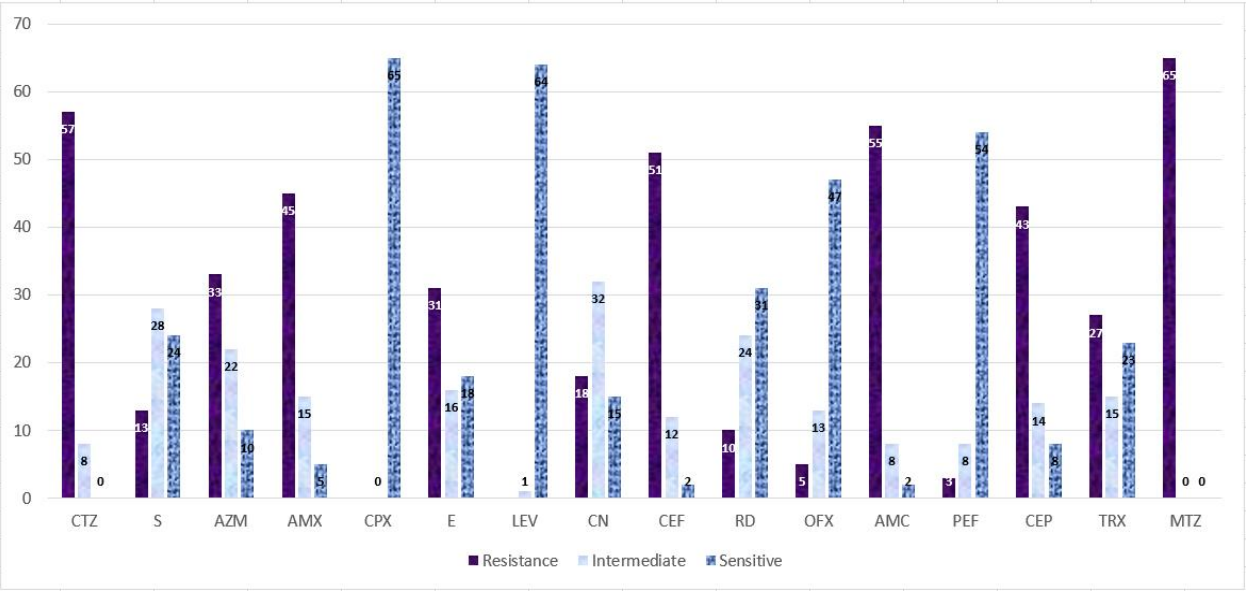

Figure 1: Chart showing the distribution of Antibiotic Susceptibility Test of Isolates

Key:
CTZ = Ceftazidime; S = Streptomycin; AZM = Azithromycin; AMX = Amoxicillin; CPX = Ciprofloxacin; E = Erythromycin; LEV = Levofloxacin; CN = Gentamycin; CEF = Cefuroxime; RD = Rifampicin; OFX = Ofloxacin; AMC = Amoxicillin-Clavulanic; PEF = Pefloxacin; CEP = Cefalexin; TRX = Ceftriaxone; MTZ = Metronidazole.

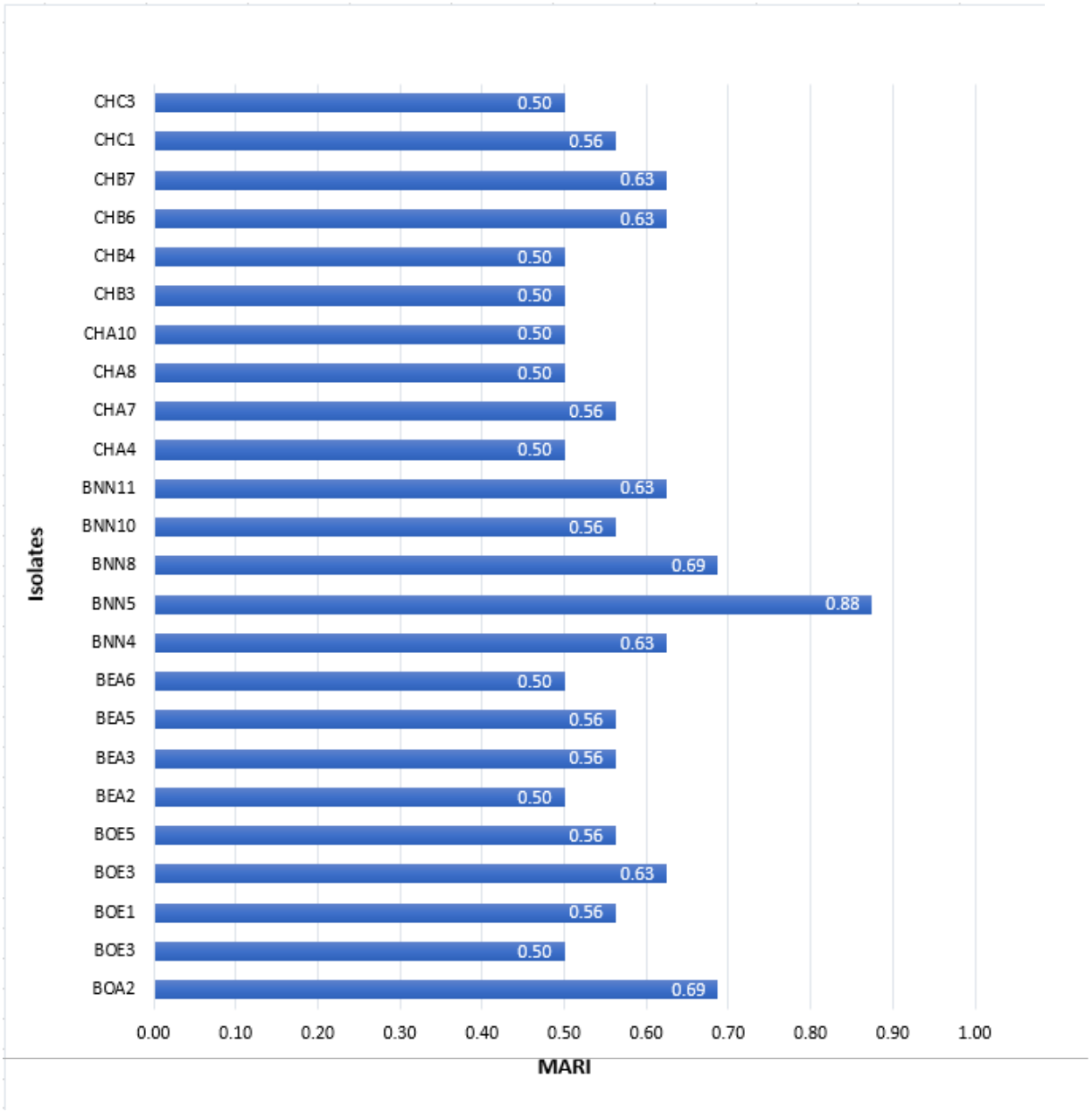

Figure 2: Multiple Antibiotic Resistance Indices of MDR Bacterial Isolates

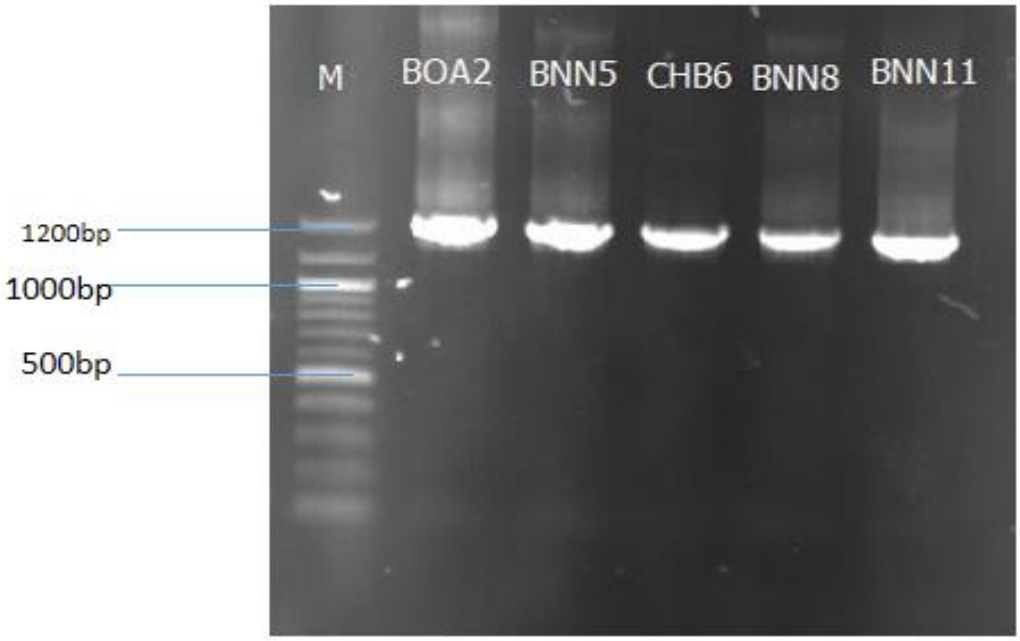

Figure 3: Agarose Gel Electrophoresis Showing Amplicons of Isolates

Table 6: Molecular Identities of Selected Bacterial Isolates

Isolates	Score	Length (bp)	Identity (%)	Sequence ID	Description
BOA2	1095 bits (1214)	1530	99	NR_028688.1	*Citrobacter murliniae* strain CDC 2970-59 16S ribosomal RNA, complete sequence
BNN5	1173 bits (635)	1465	99	NR_104913.1	*Providencia sneebia* DSM 19967 strain A 16S ribosomal RNA, complete sequence
CHB6	891 bits (482)	1478	99	NR_114207.1	*Lysinibacillus boronitolerans* strain NBRC 103108 16S ribosomal RNA, complete sequence
BNN8	1144 bits (619)	1356	99	NR_043751.1	*Morganella morganii* subsp. *sibonii* strain DSM 14850 16S ribosomal RNA, complete sequence
BNN11	1147 bits (621)	1542	99	NR_180316.1	*Pseudocitrobacter vendiensis* strain CPO20170097 16S ribosomal RNA, complete sequence

Figure 4 is a phylogenetic tree used to represent evolutionary relationships among microorganisms based on their genetic data.

The tree shows the genetic distances between the samples labeled BNN11, BOA2, BNN8, BNN5, and CHB6, identified as *Pseudocitrobacter vendiensis, Citrobacter murliniae, Morganella morganii, Providencia sneebia* and *Lysinibacillus boronitolerans* respectively. Branch lengths represent the amount of genetic change. Shorter branches indicate closer genetic relationships, while longer branches suggest greater divergence.

BNN11 and BOA2 share a very close relationship, with a small branch length of 0.01. BNN8 and BNN5 also form a closely related pair, with a similar branch length of 0.02. These two pairs (BNN11/BOA2 and BNN8/BNN5) are part of a larger cluster, sharing a common ancestor with a branch length of 0.02. CHB6 is the most genetically distinct sample in the tree, with a longer branch length of 0.14 separating it from the others.

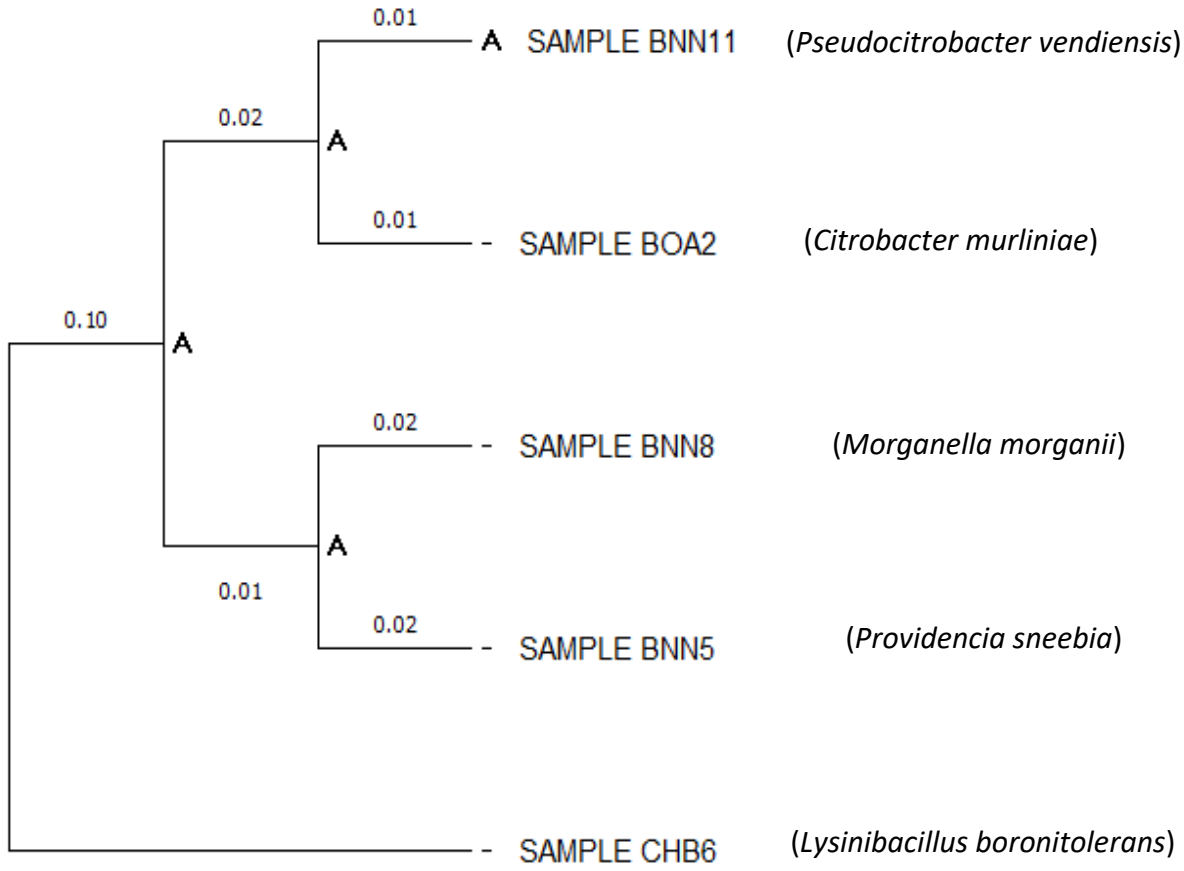

Figure 4.4: Phylogenetic Tree Showing Relationship of Identified Isolates

DISCUSSION

A total of 65 bacterial isolates were obtained, 61.5% from beef and 38.5% from chicken. The microbial isolates identified in this study include; *E. coli, Citrobacter murliniae, Salmonella spp, Providencia sneebia, Staphylococcus aureus, Shigella spp, Lysinibacillus boronitolerans, Morganella morganii* and *Pseudocitrobacter vendiensis,* with a predominance of Gram-negative *bacilli*, particularly members of Enterobacteriaceae and *S. aureus* which is easily carried by human vectors through skin and mucous membranes. The predominance of the Enterobacteriaceae family in this study is similar to a study carried out by Anene *et al.* [11]. and Mir *et al.*[19]. where the predominant organisms were *E. coli* and *Salmonella* amongst a host of other Gram-negative *bacilli* isolated from chicken, beef and mutton. Although the isolates identified

are part of the enteric flora of livestock and poultry birds, they are of public health importance since they are implicated in multi-antibiotic resistance.

The degree of microbial distribution was high with beef (61.5%) showing slightly higher loads than chicken (38.5%). The result of the total microbial count in table 1 highlights the microbial contamination levels in beef and chicken samples, with counts categorized as Total Viable Count (TVC), Total Enteric Count (TEC), and Total Staphylococcal Count (TSC) across the different market locations. TEC ranged from 1.1×10^7 CFU/ml to 7.6×10^8 CFU/ml in beef and 1.3×10^7 CFU/ml to 6.9×10^6 CFU/ml in chicken, while TSC ranged from 1.2×10^6 CFU/ml to 7.1×10^6 CFU/ml in beef and 1.02×10^7 CFU/ml to 5.1×10^7 CFU/ml in chicken. This agrees with the study of Isolates from raw meat samples sold in Ibadan as reported by Afolabi *et al.*[20] where the microbial load ranged from 1.5×10^6 to 9.4×10^8. The high level of total viable count, enteric and staphylococcal load is more than the acceptable level of 10^4 CFU/g count recommended by the Centre for Food Safety, Food and Environmental Hygiene Department[21], a government body in Hong Kong. The acceptable level of *S. aureus* in ready-to-eat food should be below 10^3 colony-forming units per gram (cfu/g) of food. If the number of bacteria is greater than 10^4 cfu/g, the food is unsatisfactory and potentially hazardous for health and/or unfit for human consumption. Thus, it has reached the marginal level where control and critical action are required[22].

The virulence test results (Table 3) showed 47 isolates (72.3%) exhibited gamma (γ) hemolysis, 14 isolates (21.5%) showed alpha (α) hemolysis, and 4 isolates (6.2%) demonstrated beta (β) hemolysis, indicating varying degrees of pathogenic potential among the bacterial isolates. The hemolytic profiles observed in this study predominantly gamma hemolysis (72.3%), followed by alpha (21.5%) and beta (6.2%) suggest a predominance of non-hemolytic bacterial isolates, with a smaller subset exhibiting varying degrees of pathogenic potential. These findings align partially with the study from Nnamdi Azikiwe University Teaching Hospital, where alpha and beta hemolysins were detected in 5.11% and 6.5% of blood donors, respectively[23]. Although their study focused on hemolysin production in human donors, and ours evaluated bacterial hemolytic activity on agar, both point to the clinical relevance of hemolysins in health and disease contexts.

The Multiple Antibiotic Resistance Index (MARI) is an important analysis to check antibiotic resistance and health risk factors. According to Afunwa *et al.*[18], organisms with MARI indices of greater than > 0.2 confirm the presence of multidrug-resistant genes originating from the environment where these drugs are abused. In this study, a high prevalence of multidrug resistance was indicated by an antibiotic susceptibility test that determined the MARI of the different isolates. The analysis of the MARI of isolates (figure 2) showed values ranging from 0.50 to 0.88, indicating moderate to high resistance levels. The isolates BNN5 - *Providencia sneebia* (0.88), BOA2 - *Citrobacter murliniae* (0.69) and BNN8 - *Morganella morganii* (0.69) exhibited the highest MARI, reflecting extreme multidrug resistance. Isolates with 0.50 showed relatively lower resistance but still exceeded the safe threshold of 0.2, indicating significant resistance.

Out of the 65 bacterial isolates analyzed in this study, Twenty-four (36.9%) isolates that demonstrated resistance to at least 8 out of 16 antibiotics tested, with MARI greater

than 0.5 were termed multi drug resistant. This study is similar to Anene *et al.*[11] which showed the MARI of isolates from chicken droppings, *Providencia stuartii*, gave a MARI of <0.20 as it had a MARI of 0.13. The highest MARI index was 0.875 and those above 0.5 were termed multi-drug resistant. Both gram-positive and gram-negative bacteria tested were highly resistant to more than 50% of the used antibiotics. The resistant percentage (%) of some isolates include; BNN5 - Providencia *sneebia* (87.5%), BOA2 - *Citrobacter murliniae* and BNN8 - *Morganella morganii* (69.0%). These findings are in agreement with previous studies done by Kilonzo-Nthenge *et al.*[24] in Tennessee, where members of the *Enterobacteriaceae* including *Citrobacter spp.* and *Morganella morganii* were isolated from beef and chicken meat.

This study revealed that bacterial isolates recovered from meat samples exhibited multidrug resistance, posing a potential risk of transmission to humans through contaminated food. Notably, all poultry and beef isolates were completely resistant to Metronidazole (100%), while Ceftazidime (87.7%), Amoxicillin-Clavulanic Acid (84.6%), and Cefuroxime (78.5%), as well as Amoxicillin (69.2%), also showed widespread resistance, as seen in table 4.5. In contrast, moderate resistance was observed for Ofloxacin (7.7%), and Pefloxacin (4.6%) though still noteworthy. On the other hand, high susceptibility was recorded for Ciprofloxacin and Levofloxacin (100%), while Pefloxacin (95.4%), Ofloxacin (92.3%), and Rifampicin (84.6%) also demonstrated strong effectiveness against the isolates. Out of the 65 isolates analyzed, 65 (100%) were resistant to at least one of the Cephalosporin antibiotics used in the study. 87.7% were resistant to Ceftazidime (3rd gen.), 78.5% were resistant to Cefuroxime (2nd gen.), 66.2% were resistant to Cephalexin (1st gen.), and 41.5% were resistant to Ceftriaxone (3rd gen.). These findings align with the 56.5% Ceftriaxone resistance reported by Gashe *et al.*[25] in clinically isolated *Enterobacteriaceae.* The resistance rates observed in this study are slightly higher than those reported by Akujobi and Ewuru[26] who detected 34.4% Ceftriaxone and 20.8% Ceftazidime resistance. The comparatively lower resistance in their study may be attributed to the inclusion of *Proteeae species,* which may exhibit different resistance mechanisms compared to the isolates in the present study.

Among the Aminoglycosides, 27.7% resistance to Gentamicin and 20.0% in Streptomycin was observed. This is closely related to the 19.6% resistance to Gentamicin reported by Kilonzo-Nthenge *et al.*[24] in the study of the prevalence of drug-resistant *Enterobacteriaceae* from beef and poultry meats but higher than the 43.8% resistance to Streptomycin from the same study. This higher rate might be a result of differences in geographic areas. A slightly close rate of 52.8% and 34.4% for Gentamicin and Streptomycin respectively was observed in Akujobi and Ewuru's[26] study on ESBL gram-negative *bacilli.*

The Macrolides antibiotics detected a 50.8% bacteria resistance to Azithromycin, 47.7% to Erythromycin and a fairly low 15.4% for Rifampicin. Other researchers have also detected resistance to Erythromycin, like Maripandi and Al-Salamah[27] who recorded a 91.3% resistance by *Salmonella* and *Shigella spp.* Similarly, a 100% resistance to Erythromycin was detected in a study by Kilonzo-Nthenge *et al.*[24] and a 69.0% resistance to Azithromycin was detected in porcine and bovine-isolated bacteria by Ivanova *et al.*[28]. Penicillin drugs also

showed high resistance, with 69.2% of isolates showing resistance to Amoxicillin and 84.6% resistance to Amoxicillin-Clavulanic acid (Augmentin). These findings are higher than the 32.6% detected in 2010 by Maripandi and Al-Salamah[27] to Augmentin. The high rate detected in this study means an increase in resistant spread and antibiotic use this day compared to 2010 and 2019, which was a long time.

Metronidazole, a nitroimidazole was observed as the antibiotic with the highest resistance in the study (table 5), with all 65 isolates showing resistance to the drug (100%). This shows how misused this antibiotic has been for human and animal use. It is one of the most commonly available antibiotics used as growth promoter and routine chemoprophylaxis among livestock in Nigeria. They are readily available in different dosage forms and in combination with other antibiotics and vitamins, probably the reason why most organisms have developed resistance to them[20].

Quinolones have been used by clinicians as a "lifesaver" in the treatment of multi-drug resistant bacteria pathogens[29]. In this study, Ciprofloxacin and Levofloxacin showed no resistance, giving us a 100% susceptibility and 0% resistance rate; Pefloxacin and Ofloxacin both showed 4.6% and 7.7% resistance respectively, making them a better choice of antibiotic. These antibiotics may therefore be used as an effective single broad-spectrum antibiotic in treating the infections caused by this type of microorganisms. The higher degree of sensitivity to the quinolones, observed in this study, agrees with the observation done by[30, 20].

CONCLUSION

The challenge posed by multidrug-resistant (MDR) bacteria is becoming increasingly alarming, as these resistant pathogens continue to be isolated from locally sourced raw beef and chicken. This finding underscores the escalating public health crisis driven by the misuse and overuse of antibiotics among local breeders who strive for profit making rather than consumer's overall wellbeing. The widespread presence of MDR bacteria in these commonly consumed meat products signifies a dangerous shift, where antibiotic-resistant pathogens are no longer rare occurrences but are becoming a persistent and pervasive problem.

This research highlights the urgent need for intervention, as the continued emergence of MDR bacteria in raw meat poses a direct threat to food safety, public health, and the effectiveness of current antibiotic stewardship campaign. If left unchecked, this growing resistance could lead to increased treatment failures from hetero resistance, prolonged illnesses, and a surge in untreatable infections, placing immense pressure on healthcare systems. The presence of MDR bacteria in food sources suggests that resistant strains are not only thriving but are also readily transmitted to humans through food consumption, increasing the risk of outbreaks and potentially leading to global health crises.

RECOMMENDATION

Immediate action is essential to halt the alarming rise of antimicrobial resistance. Key strategies include enforcing strict regulations, enhancing surveillance and documentation, improving hygiene during meat processing, regulating antibiotic use in

animal husbandry, and increasing public awareness. Without these measures, antimicrobial resistance will likely intensify, posing a serious threat to global public health and community well-being

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