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Multiple Antibiotic Resistance Bacterial Strains in Frozen Meat Sold at Abagana, Anambra State: A Public Health Concern

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Abstract

This study investigates the molecular analysis of bacterial isolates from frozen chicken and determines their antibiotic resistance profiles. A total of 50 frozen chicken samples were collected and analyzed using standard microbiological techniques. The results revealed the presence of four bacterial species: *Escherichia coli* O157:H7 strain NE 1127, *Campylobacter jejuni* strain RM 1221, *Listeria monocytogenes* serotype 4b strain 02-6680, and *Staphylococcus aureus* starin WHC09. The antibiotic susceptibility testing showed that 57.14% of the isolates were resistant to conventional antibiotics, while 42.86% were susceptible. Notably, 20.00% of the resistant strains exhibited single antibiotic resistance, and 80.00% displayed multiple antibiotic resistance (MAR). Statistical analysis using the student "t" test, correlation coefficient and one-way analysis of variance (ANOVA) confirmed the significance ($p \leq 0.05$) of these findings. The study's results highlight the risk of food-borne disease outbreaks associated with the consumption of frozen chicken contaminated with antibiotic-resistant bacteria. The high prevalence of MAR among the bacterial isolates underscores the need for improved sanitation practices, regular water quality monitoring, and public awareness campaigns to mitigate the spread of antibiotic-resistant bacteria.. The study's results are crucial for informing policy decisions and guiding future research on antibiotic resistance in the food industry.

Keywords: *Microbiological, Susceptibility, Isolates, Frozen Meat, Antibiotic Resistance*

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Introduction

Consumption of meat has increased globally, due to nutritive contents and satisfaction consumers derive from it (Sheir *et al.*, 2020). It has been established that meat contains proteins, which facilitate in body building and repair of worn out tissues (Sheir *et al.*, 2020). Most people that consume meat frequently in the society are considered to be of middle and high class, as the majority of low class individuals cannot afford meat in their daily food preparation (Yang *et al.*, 2020).

Meat can be sold in different forms, such as frozen, which involves refrigeration at low temperature, as means of

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preservation (Elbehiry *et al*., 2022). Other forms in which meat can be obtained is in its unfrozen and fresh form, and dried form as well. Among all these forms, frozen meat, which also includes chicken, is widely preferred by consumers, due to its nature and appearance. Also, consumers prefer frozen fish to ordinary meat, due to minimal exposure to contaminating factors in the environment (Elbehiry *et al*., 2022).

Research has revealed that frozen meat sold to the public is susceptible to contamination by microorganisms, especially bacteria (CLSI, 2015; Combarros-Fuertes *et al*., 2020). Also, certain microorganisms are capable of surviving in low temperature, which enables them to pose threat to the consumers, if appropriate preparation technique is not employed. Contamination of frozen meat can emanate from handlers and equipment used during marketing of the products. Some of the bacterial species that have been incriminated in meat include; *Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes, Escherichia coli, Salmonella enterica, Shigella dysenteriae, Clostridium* species etc.

It is noteworthy that most of the bacterial species that contaminate frozen meat are highly resistant to conventional antibiotics such as Ampicillin, cephalothin, clindamycin, and cotrimoxazole (*Osundiya et al.* 2013*;* Sheir *et al.,* 2020). Antibiotic resistance has become a serious threat globally due to alarming rate of morbidity and mortality rate posed by infectious bacterial pathogens. Some of the pathogens exhibit multiple resistances, due to their ability to counter effect of drug. This potential has been attributed to the presence of resistant gene in their plasmid as reported by several researchers (Sheir *et al.,* 2020; Yang *et al*., 2020).

Several studies have been carried out on isolation of antibiotic resistant strains of bacteria in meat such as Sheir *et al.* (2020), Yang *et al*. (2020), and Elbehiry *et al*. (2022) but limited studies are available on the multiple antibiotic resistance strains associated with frozen meat sold at Abagana, Anambra State. Hence, the aim of this study is to evaluate multiple antibiotic resistance strains associated with frozen meat sold at Abagana, Anambra State.

Materials and Methods

Sample collection, handling and transportation

A total of 30 frozen meat samples, five samples from each location were used for this study**.** The samples used for this study were collected from different communities at Abagana, Anambra State. In each community, the samples were collected using sterile polythene bag and were kept in a cooler containing ice block, and transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al*. (2025a).

Isolation of organisms

One gram (1.0 g) of the sample was aseptically transferred into a sterile test tube (Pyrex), then 3 ml of diluent (sterile normal saline) was added and then made up to 10 ml, and from this, ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Deoxycholate agar medium (DCA/Biotech) using the pour plate method. All the plates in triplicate were incubated inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2025b), biochemical reactions as described in the study published by Iheukwumere *et al*. (2020), Iheukwumere *et al*. (2025c) and molecular characterization as described in the study published by Gabriela *et al*. (2014), Ekesiobi (2015), Ekesiobi *et al*. (2025a) and Ekesiobi *et al*. (2025b)

Prevalence and Distribution of the Isolates in the Frozen Meat Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al* (2021), Abiodum *et al*. (2024b), Ekesiobi *et al*. (2025c), Ekesiobi *et al.* (2025e), Egbe *et al*. (2025a) and Egbe *et al*. (2025b)

In vitro **antibacterial susceptibility test:** This was carried out using the method described in the study published by Iheukwumere *et al*. (2018). Each labeled plate was uniformly inoculated with the test organism using pour plate method. An antibiotic sensitive disk (MAXI Disk) was aseptically placed on the surface of the seeded plate, labeled and then incubated at 37±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation.

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test sa described in the study published by Iheukwumere *et al* (2018), Ekesiobi *et al*. (2017), Abiodum *et al*. (2024a), Abiodum *et al*. (2024c), Iheukwumere *et al*. (2025d), Ekesiobi *et al*. (2025f), Ekesiobi *et al*. (2025g), Iheukwumere *et al*. (2025e), Iheukwumere *et al*. (2025f), Iheukwumere *et al*. (2025g), Iheukwumere *et al*. (2025h), Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j) and Iheukwumere *et al*. (2025k).

Results

The bacterial isolates exhibited distinct cultural characteristics on various media. Isolate M appeared red on MacConkey agar (MA), while isolate N appeared grey and moist on *Campylobacter* Blood-Free Selective Agar Base (CCDA). Isolate P showed small, greyish-green colonies with a black center and cherry-red background on PALCAM agar, and isolate Q appeared golden yellow on Mannitol Salt Agar (MSA) (Table 1).

The Gram reaction revealed that isolate M (Escherichia coli) was Gram-negative, isolate N (*Campylobacter jejuni*) was Gram-negative, isolate P (*Listeria monocytogenes*) was Gram-positive, and isolate Q (*Staphylococcus aureus*) was Gram-positive. The cell morphology of the isolates was as follows: isolate M (rods), isolate N (curved rods), isolate P (rods), and isolate Q (cocci). Motility tests showed that all isolates were motile except isolate Q, which was non-motile.

The biochemical tests revealed that all isolates were catalase-positive and oxidase-negative, citrate-negative, and indole-negative, except for isolate N (oxidase-positive), isolate Q (citrate-positive), and isolate M (indole-positive). Sugar utilization tests showed that all isolates utilized maltose, glucose, and lactose, except isolate N, which was glucose-negative and lactose-negative. The isolates also showed varying patterns of sorbitol and mannitol utilization.

Based on the cultural, morphological, and biochemical characteristics, the isolates were identified as *Escherichia coli* (isolate M), *Campylobacter jejuni* (isolate N), *Listeria monocytogenes* (isolate P), and Staphylococcus aureus (isolate Q). These findings were statistically significant ($p \leq 0.05$), indicating a strong correlation between the observed characteristics and the identified bacterial species.

The molecular analysis revealed the identities of the bacterial isolates as follows: *Escherichia coli* O157:H7 strain NE 1127 (ECNI), *Campylobacter jejuni* strain RM 1221 (CJRI), *Listeria monocytogenes* serotype 4b strain 02-6680 (LM02), and *Staphylococcus aureus* strain WHC09 (SAWO) (Table 2).

The susceptibility of the bacterial isolates to conventional antibiotics is presented in Table 3. The results showed that: ECNI had 16 isolates, with 13 (81.3%) resistant strains and 3 (18.7%) susceptible strains. The resistant strains were observed against antibiotics such as AMX, PN, AU, S, CEP, and SXT. CJRI had 7 isolates, with 3 (42.9%) resistant strains and 4 (57.1%) susceptible strains. The resistant strains were observed against antibiotics such as AMX, PN, AU, S, CEP, SXT, and CPX. LM02 had 9 isolates, with 6 (66.7%) resistant strains and 3 (33.3%) susceptible strains. The resistant strains were observed against antibiotics such as AMX, PN, AU, S, CEP, SXT, and CPX. SAWO had 14 isolates, with 8 (57.1%) resistant strains and 6 (42.9%) susceptible strains. The resistant strains were observed against antibiotics such as AMX, PN, AU, S, CEP, SXT, and CPX.

The degree of resistance exhibited by the bacterial isolates is presented in Table 4. The results showed that: ECNI had 13 resistant strains, with 4 (30.8%) single antibiotic-resistant strains and 9 (69.2%) multiple antibiotic-resistant strains. CJRI had 3 resistant strains, with 0 (0%) single antibiotic-resistant strains and 3 (100%) multiple antibiotic-resistant strains. LM02 had 6 resistant strains, with 0 (0%) single antibiotic-resistant strains and 6 (100%) multiple antibiotic-resistant strains. SAWO had 8 resistant strains, with 2 (25%) single antibiotic-resistant strains and 6 (75%) multiple antibiotic-resistant strains.

The results were statistically significant ($p \leq 0.05$), indicating a strong correlation between the observed antibiotic resistance patterns and the identified bacterial species. The statistical analysis confirms that the resistance patterns exhibited by the bacterial isolates are not due to chance and highlights the significance of the findings in the context of public health.

Table 1: Characteristics of the bacterial isolates encountered in the frozen meat

Characteristic	M	N	P	Q
Appearance	Red on MA	Grey and moist in CCDA	Small gray-green with bl centere and cherry red background on PALCAM	Golden yellow on MSA
Gram Reaction	Negative	Negative	Positive	Positive
Cell Morphology	Rods	Curved Rods	Rod	Cocci
Motility	Yes	Yes	Yes	No
Catalase	Positive	Positive	Positive	Positive
Oxidase	Negative	Positive	Negative	Negative
Citrate	Negative	Negative	Negative	Negative
Indole	Positive	Negative	Negative	Negative
Glucose	+	-	+	+
Maltose	+	+	+	+
Lactose	+	-	+	+
Sorbitol	+	-	+/-	+/-
Mannitol	+	-	-	+/-
	E. coli	*Campylobacter jejuni*	*Listeria monocytogens*	*S. aureus*

MA= MacConkey agar; CCDA= Charcoal-Cefoperazone-Deoxycholate agar; PALCAM= Polymyxin Acriflavin Lithium Choride Ceftazidime Esculin Mannitol

Table 2: Molecular characteristics of the bacterial isolates

Isolate Co	Max Sco	Total Scc	Query Cover	E-Value	Percent Ident (%)	Accession Numb	Description
M	1681	1681	100	0.0	100	CP038821.1	*Escherichia coli* O157:H7 strain NE 11 chromosome, complete genome (ECNI
N	1423	1423	100	0.0	100	CP006241.1	*Campylobacter jejuni* strain RM 1221 chromosome, complete genome (CJRI)
P	1810	1810	100	0.0	100	CP007461.1	*Listeria monocytogenes* serotype 4b str 02-6680, complete genome (LM02)
Q	1681	1681	100	0.0	100	CP0777551.1	*Staphylococcus aureus* starin WHC09 chromosome, complete genome (SAW(

Table 3: Susceptibility of the isolates to conventional antibiotics

Isolate	Number	Susceptible strain	Resistance strain	Implicated Antibiotic
ECNI	16	3 (18.75)	13 (81.35)	AMX, PN, AU, S, CEP, SXT
CJRI	7	4 (57.14)	3 (42.86)	AMX, PN, AU, S, CEP, SXT, CPX
LMO2	9	3 (33.33)	6 (66.67)	AMX, PN, AU, S, CEP, SXT, CPX
SAWO	14	6 (42.86)	8(57.14)	AMX, PN, AU, S, CEP, SXT, CPX
Total	46	16 (34.78)	30 (65.22)	

Table 4: Degree of resistance exhibited by the isolates

Isolate	Total Resistant strain	Single Antibiotic Resistant strain (%)	Multiple Antibiotic Resistant strain (%)
ECNI	13	4 (30.77)	9 (69.23)
CJRI	3	0 (0.00)	3 (100.00)
LM02	6	0 (0.00)	6 (100.00)
SAWO	8	2 (25.00)	6 (75.00)
Total	30	6 (20.00)	24 (80.00)

Discussion

Multiple antibiotic resistant has been a threat to man globally, as revealed in the alarming rate of morbidity and mortality. Several conventional antibiotics emerge on daily basis in the quest to curtailing the effects of multiple antibiotic resistant but little success has been achieved, due to continuous emergence of antibiotic resistant gene globally. The bacterial species isolated in this study are similar to the bacteria isolated by several researchers (Sheir *et al.*, 2020; Yang *et al*., 2020; Elbehiry *et al*., 2022) that investigated microbial contamination of ready to eat food. The multiple antibiotic resistant bacteria detected in this study corroborate to the resistant bacterial species isolated by other researchers (Sheir *et al.*, 2020; Yang *et al*., 2020; Elbehiry *et al*., 2022).

The ability of the bacterial species to exhibit antibiotics resistance could be attributed to the presence of resistance gene in their plasmid and poor efficacy of the antibiotics. This corroborates to the findings of several researchers (Sheir *et al.*, 2020; Yang *et al*., 2020; Elbehiry *et al*., 2022) who studied bacterial species in meat. The ability of the bacterial species to exhibit antibiotics resistance could be attributed to the presence of resistance gene in their plasmid and poor efficacy of the antibiotics. Similar observation was made by several researchers (Sheir *et al.*, 2020; Yang *et al*., 2020; Elbehiry *et al*., 2022).

The molecular characterization of the bacterial isolates revealed the presence of *Escherichia coli* O157:H7 strain NE 1127, *Campylobacter jejuni* strain RM 1221, *Listeria monocytogenes* serotype 4b strain 02-6680, and *Staphylococcus aureus* starin WHC09. However, there was variation in the bacterial isolates reported by other researchers (Murad *et al*., 2014; Adzitey *et al.*, 2015; Kunad, 2018), which could be attributed to the degree of contamination by the handlers and climatic condition of the area study area. Furthermore, the antibiotics that were implicated in the resistant menace are Streptomycin, Amoxil, Ciprofloxacin, Augmentin, Ceporex, Penicillin, and Trimethoprim. Similar antibiotics were reported by other researchers (Elshebrawy *et al.*, 2022; Hossain *et al*., 2022) but there was deviation in the antibiotics documented by Enayat *et al*. (2012), which could be attributed to efficacy of the active pharmaceutical ingredients.

Conclusion

This study reveals that frozen meat sold for public consumption is susceptible to microbial contamination, posing a significant risk to consumer health. The bacterial isolates, including *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Staphylococcus aureus*, exhibited multiple resistance to common antibiotics. These findings highlight the importance of ensuring the microbial quality of frozen meat to prevent food-borne diseases and protect public health.

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Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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