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Frozen Fish Pathogens: Antimicrobial Resistance and Public Health Implications

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Abstract

The growing concern of antibiotic-resistant bacteria in the food chain poses a significant threat to public health, particularly with frozen chicken being a potential source of contamination. This study investigates the molecular characterization of bacterial isolates from frozen chicken and their antibiotic resistance profiles. Fifty frozen chicken samples were analyzed using standard microbiological techniques, revealing four bacterial species: *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). The antibiotic susceptibility testing showed that 42.76% of the isolates were resistant to conventional antibiotics, while 57.24% were susceptible. Notably, 33.33% of the resistant strains exhibited single antibiotic resistance, and 66.67% displayed multiple antibiotic resistance (MAR). Statistical analysis confirmed the significance of these findings, with a p-value of ≤ 0.05 using the student "t" test and one-way analysis of variance (ANOVA). The study's results highlight the risk of food-borne disease outbreaks associated with consuming frozen chicken contaminated with antibiotic-resistant bacteria. The high prevalence of MAR among bacterial isolates underscores the need for improved sanitation practices, regular water quality monitoring, and public awareness campaigns. These findings have significant implications for public health and food safety in Awka Metropolis, emphasizing the importance of effective control measures to prevent the spread of antibiotic-resistant bacteria in the food chain. Implementing these measures can help mitigate the risk of antibiotic-resistant infections and protect public health.

Keywords: *Strain, Antibiotic, Microbiological, Species, Susceptibility*

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Introduction

Fish consumption has increased globally due to nutritive contents and satisfaction consumers derive from it (Austin and Austin, 2016). It has been established that fish contains proteins, which aid in body building and repair of worn out tissues. Most people in the society prefer fish to meat, due to taste and health reasons (Wanja *et al*., 2020).

The presence of fish in a meal gives extraordinary flavour, which promotes food consumption such as rice, fufu, yam etc. Fish can be sold in different forms such as frozen form, which involves refrigeration at low temperature, as means of preservation. Other forms in which fish can be obtained is in its unfrozen and fresh form, a dried form, roasted, and smoked form. All these forms could also undergo second preparation stage, though dried, roasted, and smoked fishes can be consumed without further preparation (Elbehiry *et al*., 2022).

Research has revealed that frozen fish 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 method is not critically adhered to. Contamination of frozen fish can emanate from handlers and equipment used during selling of the products. Some of the bacterial species that have been isolatedin frozen fish include; *Klebsiella pneumoniae*, *Campylobacter* species, *Streptococcus* species, *Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes, Escherichia coli, Salmonella enterica, Shigella dysenteriae,* and *Clostridium* species.

It is worthy to note that most of the bacterial species that contaminate frozen fish are highly susceptible to some conventional antibiotics such as Penicillin G, Erythromycin, Ampicillin, Cephalothin, and Clindamycin (Sheir *et al*., 2020; Yang *et al*., 2020). The antibiotics interfere with the synthesis of cell wall, especially the crosslinking of the components of peptidoglycan and synthesis of protein. The broad spectrum activity of the antibiotics also facilitates in the inhibiting and killing of pathogenic bacterial species as reported by several researchers (Sheir *et al*., 2020; Yang *et al*., 2020).

Several researchers (Austin and Austin, 2016; Gufe *et al*., 2019; Wanja *et al*., 2020) have worked on susceptibility pattern of bacterial species isolated from fish but little studies are available on susceptibility patterns of major pathogens encountered in frozen fish against conventional antibiotics. Hence, the aim of this study is to determine the susceptibility patterns of major pathogens encountered in frozen fish against conventional antibiotics.

Materials and Methods

Sample collection and Transportation

A total of 50 random samples of frozen chicken meat cuts represented by wings, drumsticks, thigh and breast, weighing about 15g for wing samples and 100-250 g for the other samples, were purchased from different supermarkets and cold rooms. Each sample was placed in a sterile plastic bag and transported immediately to the laboratory in a disinfected thermos flask. Frozen chicken was allowed to thaw at refrigeration temperatures before microbiological testing.

Sample preparation

As described by Khalafallah *et al*. (2020) with some modification under complete aseptic conditions, 10 g of the sample was weighed, cut into small pieces and then transferred into a sterile flask containing 100 mL of sterile peptone water (0.1%) and the content of the flask was evenly homogenized. One mL from the homogenate was transferred into a separate tube containing nine mL of sterile peptone water (0.1%) from which tenfold serial dilutions were prepared. The prepared samples were subjected to the following examinations.

Culture and Isolation of Bacteria from Frozen Chicken Samples: This was carried out using the modified method of Cheesbrough (2010). The frozen chicken samples were sliced and 1.0 g sample macerated in sterile peptone water, and further diluted to 1:10. One milliliter of the prepared sample was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Deoxycholate citrate agar medium (DCA/Biotech), Thiosulphate Citrate Bile Sucrose (TCBS) agar (BIOTECH), Cetrimide agar (BIOTECH) and Mannitol Salt agr (MSA). All the plates in triplicates were incubated in inverted at 37±2˚C for 24-48 h. (Cheesbrough, 2010; Ekesiobi *et al*., 2025a; Ekesiobi *et al*., 2025b; and Ekesiobi *et al*., 2025c)

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 (Cheesbrough, 2010), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018; and Ekesiobi *et al*., 2025d). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010), Ekesiobi *et al*. (2025e), Ekesiobi *et al*. (2025f), Egbe *et al*. (2025a) and Egbe *et al*. (2025b). The isolates were aseptically subcultured into a broth culture and incubated at 35 + 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

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), Ekesiobi *et al*. (2025g), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d), 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).

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Results

The enteric bacterial isolates exhibited varying characteristics on MacConkey agar, with distinct appearances, elevations, and biochemical reactions (Table 1). All isolates were Gram-negative rods and catalase-positive. However, differences were observed in citrate utilization, indole production, and methyl red tests.

Molecular analysis revealed the presence of *Escherichia coli* O157:H7 strain NE1127, *Escherichia coli* strain JKHS016, *Klebsiella pneumoniae* strain 2014C06-125, and *Klebsiella pneumoniae* strain KP2092 (Table 2). The isolates showed varying susceptibility to conventional antibiotics, with isolate D2 being the most susceptible (63.64%) and isolate D1 being the most resistant (0.00% susceptible) (Table 3). The degree of resistance exhibited among the isolates was significant, with isolate D1 showing 100.00% resistance (Table 4). Statistical analysis confirmed the significance of these findings ($p \leq 0.05$).

Table 1: Characteristics of the enteric bacterial isolates

Parameter	C1	C2	D1	D2
Appearance on MacConkey agar	Pink	Red	Red and Mucoid	Red and Mucoid
Elevation	Convex	Convex	Slightly raised	Slightly raised
Motility	+	+	-	-
Gram reaction	-	-	-	-
Cell morphology	Rods	Rods	Rods	Rods
Catalase	+	+	+	+
Citrate	-	-	+	+
Indole	+	+	-	-
MR	+	+	-	-
VP	-	-	+	+
Glucose	+	+	+	+
Maltose	+	+	+	+
Xylose	+	+	+/-	+/-
Sorbitol	-	+	+/-	-
Inositol	+/-	+/-	+	+/-
Dulcitol	+/-	+	+/-	+/-

Table 2: Molecular characteristics of the enteric bacterial isolates

Isolate code	Max score	Toal score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
C1	1681	1681	100	0.0	100	CP038321.1	*Escherichia coli* 0157:H7 strain NE1127 chromosome complete genome (ECNE11)
C2	1936	1936	100	0.0	100	CP147059.1	*Escherichia coli* strain JKHS016 (ECJ6)
D1	1552	1552	100	0.0	100	CP170972.1	*Klebsiella pneumoniae* strain 2014C06-125 (KP2)
D2	1552	1552	100	0.0	100	CP141801.1	*Klebsiella pneumoniae* strain Kp2092 (KPK2)

Table 3: Susceptibility of the bacterial isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
C1	13	8 (61.54)	5 (38.46)	S, PN, CEP, SXT, AU, CN
C2	12	9 (40.91)	13 (59.09)	AMX, AU, CEP, S, PN, SXT, CN
D1	7	0 (0.00)	7 (100.00)	PN, S, CEP, SXT, AU
D2	11	7 (63.64)	4 (36.36)	AU, PN, S, CEP, SXT, CN
Total	53	24 (45.28)	29 (54.72)	

Table 4: Degree of resistance among the isolates

Isolates	NR	Single resistant strain (%)	Multiple resistant strain (%)
C1	5	1 (20.00)	4 (80.00)
C2	13	8 (61.54)	5 (38.46)
D1	7	0 (0.00)	7 (100.00)
D2	4	1 (25.00)	3 (75.00)
Total	29	10 (34.48)	19 (65.52)

Discussion

Globally, antimicrobial resistant has been a threat to man, as revealed in the high 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 bacteria species isolated in this study corroborate to the bacterial species isolated by other researchers (Austin and Austin, 2016; Gufe *et al*., 2019; Wanja *et al*., 2020).

The pathogenic bacteria isolated in this study correspond to the pathogenic bacteria isolated by several researchers (Austin and Austin, 2016; Gufe *et al*., 2019; Wanja *et al*., 2020) who evaluated bacterial species found in fish. The pathogenic activity exhibited by the bacterial isolates in this study is in conjunction with the pathogenic profile of bacterial species isolated by several researchers (Austin and Austin, 2016; Gufe *et al*., 2019; Wanja *et al*., 2020) who isolated pathogenic bacteria from fish. The bacterial resistance observed in this study corresponds to the bacterial resistance reported by other researchers (Austin and Austin, 2016; Gufe *et al*., 2019; Wanja *et al*., 2020) who investigated bacterial species found in different forms of fishes. Molecular characterization of the bacterial isolates revealed certain bacterial strains such as *Escherichia coli* 0157:H7 strain NE1127, *Escherichia coli* strain JKHS016, *Klebsiella pneumoniae* strain 2014C06-125, and *Klebsiella pneumoniae* strain Kp2092. 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, with bacterial isolates exhibiting multiple resistance to common antibiotics. Molecular characterization identified pathogens like *Escherichia coli* O157:H7 and *Klebsiella pneumoniae*, posing a significant risk to consumer health. Assessing microbial quality of frozen meat is crucial to preventing the spread of infectious diseases.

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