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PATHOGENIC BACTERIAL CONTAMINATION IN SMOKED FISH AND CHICKEN: A STUDY ON PREVALENCE AND PUBLIC HEALTH IMPLICATION

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ABSTRACT

Smoked fish and chicken are popular food products globally, but their contamination with pathogenic bacteria poses significant public health risks, emphasizing the need for investigation and mitigation strategies. This study investigated the prevalence of pathogenic bacterial contamination in smoked fish and chicken and explored its public health implications providing insights for improving food safety practices and protecting consumer health. A total of 280 samples that comprises 40 samples each of native chicken meat, layers chicken meat, broiler chicken meat, *Chupea havengus* (Herring/sawa), *Truchurus trachurus* (Horse Mackerel/Kote), *Scomber Scombrus* (Atlantic Mackerel/Titus) and *Sphyraema barracuda* (panla). *In vitro,* the pathogenicity study of the isolates was carried out using haemolytic assay and congo red assimilation test. The data obtained were analyzed using a one-way analysis of variance (ANOVA) and turkey's test as post hoc analysis. The pathogenic bacteria majorly encountered in the studied samples were *Escherichia coli* 0157:H7 strain ECP19-598 (ECEC1), S*taphylococcus aureus* strain JP18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* serovar Enteritidis EC20110358 (SEEC2). These pathogens were encountered more in chicken meat than smoked fish, and SAJP1 was most significantly ($p \leq 0.05$) seen in the studied samples. Therefore, ECEC1, SAJP1, LMLM1 and SEEC2 were encountered in the studied smoked fish and chicken meat samples, and these were detected more in chicken meat, and SAJP1 was mostly detected

Keywords: Smoked-fish, Chicken-meat, *In vitro*, Haemolytic, Pathogenic

INTRODUCTION

Globally, smoked fish and chicken are essential foods that provide nutrients to consumers, especially when wholesome ones are consumed (Chatreman *et al*., 2020, Farouk and Montossi, 2022). Several individuals depend on smoked fish and chicken as their major meal in combination with alcoholic or soft drinks because they are readily available, cost-effective, and ready to be consumed at any time (Ezemonye *et al.,* 2019). These types of food are majorly prepared and sold by the roadsides by men and women, who serve as vendors to earn their living (Babatunde *et al.,* 2018).

The preparation of smoked fish and roasted chicken is carried out using local firewood and wire gauze, which allows smoke to penetrate the raw fish and chicken until the desired form is actualized (Adeyeye *et al.,* 2017). Materials that are used in this preparation are not sterile but most of the bacterial pathogens are eliminated during preparation as open fire destroys the vegetative cells while spores survive (Adzitey *et al.,* 2013b). It is worth noting that most pathogenic bacteria that pose a threat to consumers are introduced into smoked fish and roasted chicken after preparation. This could be through contaminated hands, contaminated containers, and contaminated environment, as some vendors market their products close to a polluted environment (Amadi *et al*., 2016).

Research has shown that most of foodborne diseases are contracted through the consumption of contaminated ready-to-eat food in the environment (Font-j-Furnols and Guerre, 2014; Adeloja *et al*., 2018; Farouk and Montossi, 2022). Pathogenic bacteria that are frequently found in ready-to-eat food include *Salmonella* species, *Listeria* species, *Shigella, Klebsiella* species, *Clostridium* species, *Escherichia coli*, *Vibrio cholerae* etc. (Adzitey *et al.,* 2013b; Amadi *et al*., 2018). The impact of these pathogens to individuals in the society has been described as endemic and several species have developed resistance to known conventional antibacterial agents (Dike-Ndudim *et al.,* 2014). There was a case emanated due to usage of contaminated water by the handlers of ready to eat smoked fish, which led to introduction of pathogenic bacteria (Fasina *et al.,* 2018).

It is worthy to note that the effect of foodborne pathogens has been described as pandemic (Ibrahim *et al.,* 2019;

Karisma *et al*., 2021). According to the World Health Organization (WHO, 2022), 420,000 deaths occur annually due to effect of pathogenic bacteria in ready to eat food and about 33 million people deviate from their normal healthy lives due to consumption of unwholesome food in the environment. The greatest burden of this ugly menace is against children below 5 years, which had recorded about 125,000 deaths annually. The occurrence of this disease mostly affects developing countries due to socioeconomic development, poverty, and negligence of environmental health laws by the government authorities and individuals (Ogunsanya *et al.*,2018; Ogunshe *et al.,* 2018).

Several researchers have studied the occurrence of pathogenic bacteria in ready to eat meat and food sold in the environment such as Amadi *et al*. (2016), Adeyey *et al*. (2017), Ezemonye *et al.* (2019) and Babatunde *et al*. (2018) but few studies are available on the pathogenic bacteria in smoked fish and chicken in respect to prevalence and public health implication. Hence, the aim of this study is to evaluate the prevalence and public health implication of pathogenic bacteria in smoked fish and chicken.

MATERIALS AND METHODS

Sample Collection: A total of 280 samples which comprises 120 roasted chicken meat samples and 160 smoked fish were used for this study (Iheukwumere *et al*., 2018a). The roasted chicken meat samples included 40 samples of each of Native chicken meat samples, old layer meat samples and broiler chicken meat samples. The smoked fish samples included 40 samples of each of *Clupea harengus* (Sawa/Herring), *Truchurus trachurus* (Kote/Horse Mackerel), *Scomber scombrus* (Titus/Atlantic Mackerel) and *Sphyraema barracuda* (Panla). Ready-to-eat samples were aseptically separated using a sterile stainless spoon (Hamada) and collected into a sterile aluminium foil through hand picking (Iheukwumere *et al*., 2018b). Before the sampling, the hands were washed thoroughly with soap and cleaned water and then rinsed with 70 % ethanol. Sampling was done at different selling locations in different towns in Anambra State. The samples were placed into a cooler containing ice blocks wrapped in a sterile polythene bag and were used for sample transportation. The temperature of the cooler was checked and adjusted to 28^{o}C-30^{o}C by reducing the quantity of ice inside the cooler to reduce or

prevent microbial shock. The samples were carefully and aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was then covered and the drain plug was securely taped with packing tape to prevent accidental opening of the cooler. The cooler was then sagely carried to the Laboratory for analysis within 2 hours of sample collection. The same procedure was repeated for other collection times (Iheukwumere *et al*., 2018c).

Sample Preparation: The samples were prepared using the routine laboratory technique. The meat samples were ground using a sterile blender (LXB 242). Then 1.0 g of each of the ground samples was aseptically weighed into a 10 mL test tube (Pyrex) each respectively. Three milliliter of sterile peptone water was aseptically added into each test tube and these were shaken thoroughly and then made up to 10.0 mL using the sterile peptone water for each test tube as described in Chesbrough (2010), Ekesiobi *et al*. (2025a), Ekesiobi *et al*. (2025b), Ekesiobi *et al*. (2025c), Ekesiobi *et al*. (2025d) and Ekesiobi *et al*. (2025e).

Screening the Bacterial Isolates for Pathogenic Potentials

***In vitro* technique**: The *in vitro* pathogenic potentials of the bacterial isolates was carried out by testing for the ability of the bacterial isolates to produce haemolysis on blood agar and took up Congo red dye as described in the study published by Zahid *et al*. (2016), Iheukwumere *et al*. (2017), Ejike *et al*. (2017), Iheukwumere and Ejike, (2016) Ekesiobi *et al*. (2025f)

Haemolysis on blood agar: Blood agar was prepared following the manufacturer's instruction, the medium was allowed to solidified and aseptically streaked with the test isolates. This was incubated in inverted position at 35±2℃ for 24 h. The presence of clear zones around the colonies was an indication of β haemolysis, partial zones of inhibition which was detected by greenish to gray zones was an indication of y-haemolysis whereas absence of clear zones as described by Zahid *et al*. (2016)

Reaction with Congo red dye: Nutrient agar was prepared following the manufacturer's instruction, this mixed with Congo red dye, and the medium was sterilized and allowed to solidified. The medium was aseptically streaked with the test isolates. This was incubated in inverted position at 35±2℃ for 24 h. The presence of colonies with tiny red with wrinkled surface was an indication

of positive test whereas colonies with pink colour and smooth surface was an indication of negative colour as described by Ugwu *et al*. (2020)

Characterization of the Bacterial Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described in the study published by Cheesbrough (2010) Iheukwumere *et al*. (2018a) and Iheukwumere *et al*. (2018d). The isolates' cultural descriptions (size, appearance, edge, elevation, and colour) were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015) and Ekesiobi *et al*. (2025g).

Determination of Prevalence of the Isolates in the Studied Samples

The occurrences of different strains of the pathogenic bacterial isolates associated with the smoked fish and roasted chicken meat samples were counted and recorded according to the method described in the study published by Kukleci *et al.* (2019), Iheukwumere *et al*. (2017b) and Uzoh *et al*. (2015). The number of occurrences of the predominate pathogenic bacteria were counted, and their percentages of occurrences were appropriately calculated and recorded.

Statistical Analysis

The data obtained from this study were represented in Tables, Figures and as mean ± standard deviation. The significance of the study was carried out using a one-way Analysis of Variance (ANOVA) at 95 % confidence level. Pair wise comparison was done using Turkey test as described by Iheukwumere *et al*. (2021), Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d), and Iheukwumere *et al*. (2025e).

RESULTS

In vitro Pathogenic Screening of the Bacterial Isolates

The studied isolates showed that 25.00% of the bacterial isolates from roasted chicken meat samples were pathogenic and 36.36% of the bacterial isolates from the smoked fish samples were pathogenic as shown in Table 1. Also, 12.50% of the bacterial isolates from roasted chicken meat samples were slightly pathogenic whereas no isolate

from smoked fish samples showed slight pathogenic characteristics as shown in the hemolytic test in Table 1.

Characteristics of the Bacterial Isolates

The bacterial isolates showed varying appearances in their respective growth medium. Isolate P showed red colonies on MacConkey agar (MA), Isolate Q showed golden yellow colonies on Mannitol salt agar (MSA), isolate R showed grey-green colonies with dark centre on Polymyxin Acriflavin Lithium-Chloride Ceftazidime Esculin Mannitol (PALCAM) agar and isolate S showed colourless with dark centre on *Salmonella-Shigella* Agar (SSA), as shown in Table 2. They had smooth surfaces, and entire/smooth edges with convex and raised elevation. They were non-spore formers and non-encapsulated. Isolates P and S were Gram-negative rods. Isolates Q and R were Gram-positive, Q was coccus in shape whereas R was a rod in shape. Biochemically, the bacterial isolates were catalase and methyl red positive, oxidase and Urease negative as shown in Table 3. Isolate P was indole positive, citrate, H_2S negative and VP positive. Isolate R was VP positive but indole, citrate and H_2S negative. Isolate S was citrate, H_2S and VP positive but indole negative. The isolates utilized glucose and trehalose, but showed varying utilization rates to lactose, sucrose, D – Mannitol, sorbitol, Xylose and ribose.

The nucleic acid confirmation as shown in Table 4 revealed that the nucleic acids were Deoxyribo nucleic acid (DNA) as the ratio of 260nm / 280nm ranged from 1.8 – 1.9. The molecular characteristics revealed the presence of *Escherichia coli* O157:H7 strain ECP19-598 (ECEC1), *Staphylococcus aureus* strain JP 18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain EC20110358 (SEEC2) as shown in Table 5.

Prevalence of the pathogenic Bacterial Isolates in the Studied Samples.

The occurrences of the pathogenic bacterial isolates are shown in Table 9. The study revealed that ECEC 1 was seen most in layer's chicken meat whereas the least occurrence was seen in SB. SAJP 1 was seen most in native chicken meat samples which *Truchurus trachurus* (TT) recorded the least occurrence. LMLM 1 recorded the highest occurrence in broiler chicken meat samples, no LMLM 1 was detected in *Scomber scombrus* (SS) and

Sphyraema barracuda (SB) samples. SEEC 2 was seen most in native chicken meat samples whereas TT and SB were not able to record the presence of SEEC 2. The study further revealed that SAJP 1 significantly ($P < 0.05$) occurred most in the studied samples whereas LMLM 1 recorded the least occurrence in the studied samples. Also, the study highlighted that the load of these pathogenic bacteria isolates was most in roasted chicken meat samples, mostly in the native roasted chicken meat samples as represented in Table 6.

Table 1: *In vitro* pathogenic screening of the isolates.

Test	Chicken Meat (%) (n = 10 + 16)	Smoked fish (%) (n = 11)
Congo Red		
• Tiny Red with a wrinkled surface	4 (25.00)	4 (36.36)
• Pink with a smooth surface	12 (75.00)	7 (63.64)
Hemolysis		
• B-hemolysis	4 (25.00)	4 (36.36)
• α- hemolysis	2 (12.50)	0 (0.00)
• γ- hemolysis	10 (62.50)	7 (63.64)

Table 2: Cultural and Morphological Characteristics of the bacterial isolates

Parameter	Isolate P	Isolate Q	Isolate R	Isolate S
Appearance	Red colonies on MA	Golden yellow on MSA	Gray-green with dark center on PALCAM	Colourless with dark center on SSA
Surface	Smooth	Smooth	Smooth	Smooth
Edge	Entire	Entire	Smooth	Smooth
Elevation	Convex	Raised	Raised	Raised
Size	Moderate	Moderate	Small	Moderate
Spore	-	-	-	-
Capsule	-	-	-	-
Motility	+	-	+	+
Gram reaction	-	+	+	-
Shape	Rods	Cocci	Rods	Rods
Bacterium	*Escherichia coli*	*Staphylococcus aureus*	*Listeria*	*Salmonella*

+ = positive; - = Negative; MA = MacConkey agar; MSA = Mannitol Salt Agar; PALCAM = Polymyxin Acriflavin Lithium-Chloride Ceftazidime Esculine Mannitol.

Table 3: Biochemical Characteristics of the bacterial isolates

Parameter	P	Q	R	S
Catalase	+	+	+	+
Oxidase	-	-	-	-
Indole	+	-	-	+
		-	-	
Citrate	-	+	-	+
H_2S	-	-	-	+
Urease	-	-	-	-
MR	+	+	+	+
VP	-	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	-
Trehalose	+	+	+	+
Sucrose	+/-	+/-_	+	-
D-Mannitol	+/-	+	-	-
Sorbitol	+/-	+/-	+/-	+
Xylose	+	+/-	-	+/-
Ribose	+/-	+/-	-	-

Table 4: Quality of nucleic acid (DNA) used for the study

Sample	Concentration of Nucleic acid (ng/µL)	A260	A280	260/280
P	109.80	3.098	1.702	1.82
Q	119.70	3.279	1.782	1.84
R	128.40	3.355	1.804	1.86
S	117.10	3.109	1.708	1.82

Table 5: Molecular characteristics of the bacterial isolates

Parameter	Isolate P	Isolate Q	Isolate R	Isolate S
Max score	38284	34485	25078	26613
Total Score	38284	34485	60734	26613
Query cover (%)	100	100	100	100
E-value	0.0	0.0	0.0	0.0

Identity (%)	100	100	100	100
Accession Number	CP066753.1	CP097114.1	CP027029.1	CP007260.1
Description	*Escherichia coli* 0157:H7 strain ECP19-598 C.G (ECEC1)	*Staphylococcus aureus* strain JP18269 complete genome (SAJP1)	*Listeria monocytogenes* strain LM16 chromosome (LMLM16)	*Salmonella enterica* subsp *enterica* serovar Enteritidis strain EC 20110358 complete genome (SEEC2)

Table 6 Prevalence of the Pathogenic Bacterial Isolates in the Studied Samples

Sample	ECEC 1	SAJP 1	LMLM 1	SEEC 2
	(%)	(%)	(%)	(%)
NM	5 (17.24)	19 (25.00)	2 (10.53)	11 (36.67)
LM	9 (31.03)	11 (14.47)	5 (26.32)	10 (33.33)
BM	6 (20.69)	15 (19.74)	9 (47.37)	4 (13.33)
CH	2 (6.90)	11 (14.47)	2 (10.53)	3 (10.00)

TT	2 (6.90)	6 (7.89)	1 (5.26)	0 (0.00)
SS	4 (13.79)	7 (9.21)	0 (0.00)	2 (6.67)
SB	1 (3.49)	7 (7.21)	0 (0.00)	0 (0.00)
Total	29 (18.83)	76 (49.35)	19 (12.34)	30 (19.48)

DISCUSSION

The significant mean bacterial counts, majorly total heterotrophic bacterial counts (THBC), total coliform counts (TCC), total faecal coliform counts (TFCC), total *Salmonella* counts (TSC), total *Shigella* counts (TSHC), total *Staphylococcus aureus* counts (TSAC) and total *Listeria monocytogenes* counts (TLMC) supported The findings of many researchers (Adeyanju and Ishola, 2014; Mashak, 2018; Ishihava *et al.,* 2020; Karisma *et al.,* 2021).

The occurrences of pathogenic bacterial isolates in smoked fish more than the roasted chicken meat in the studied samples could be attributed to the handling nature of bacteria encountered in the samples and the growth condition that can induce the production of virulent factors. Karisma *et al*. (2021) were able to encounter *E. coli* 0157:H7, *Staphylococcus aureus* and *Salmonella* species in their studied samples. Rortana *et al*. (2021) encountered *Salmonella* species and *Staphylococcus aureus* in their studied chicken meat samples. Ishihava *et al*. (2020) encountered food-borne bacterial pathogens in their studied samples. The presence of *Escherichia coli* O157:H7 strain ECP19-598 (ECEC1), *Staphylococcus aureus* strain JP18269 (SAJPI), *Listeria monocytogenes* strain LM 16 (LMLM1) and *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain EC20110358 (SEEC2) in the studied roasted chicken meat and smoked fish samples agrees with the findings of many researchers (Kulasooriya *et al.,* 2019; Camargo *et al.,* 2017; Daramola *et al.,* 2020; Ishihava *et al.,* 2020; Savariraj *et al.,* 2020; Sobby and Shaltout, 2020; Karisma *et al.,* 2021; Gupta and Adhikari, 2022; Kayode and Okoh, 2022;Shaltout, 2024). Several researchers such as Ishihava *et al*. (2020) detected *E. coli* and *Salmonella* species in their studied samples, Karisma *et al*. (2021) detected *E. coli* O157:H7,

Salmonella species and *Staphylococcus aureus* in their studied samples, and other researchers who detected varying pathogenic bacterial isolates in their samples.

The highest occurrences of ECEC1 in the studied samples could be attributed to human activities such as handling, transportation, storage and poor hygienic conditions. Similar deductions were made by many researchers (Mashak, 2018; Ishihava *et al.,* 2020; Karisma *et al.,* 2021; Shaltout, 2024). The study further highlighted that roasted chicken meat samples harboured the highest number of LMLM1, and this could be attributed to the conditions that favoured the growth of LMLM1 in the studied samples. The study also highlighted that many researchers (Harper, 2012; Goh *et al.,* 2014; Camargo *et al.,* 2017; Ayeloja *et al.,* 2018; Daramola *et al.,* 2020; Musa *et al.,* 2020), detected LMLM1 in their studied samples. The absence of LMLM1 in *Scomber scombus* (SS) and *Sphyraema barracuda* (SB) samples could be attributed to the fact that the conditions did not support the growth of the organism. SEEC2 was detected most in native roasted chicken meat samples, and this could be attributed to the fact that salmonella species multiply rapidly in the reticuloendothelial system (RES) of birds. Similar findings were reported by Iheukwumere *et al*. (2018a). The absence of SEEC2 in *Trachuras trachurus* (TT) and SB could be attributed to low moisture content and unfavourable growth conditions. A similar deduction was made by Kamruzzaman *et al*. (2016). The occurrences of other bacterial isolates such as SAJP1 in the studied samples agrees with the findings of other researchers (Nwachukwu and Madubuko, 2013; Dike-Ndunim *et al.,* 2014; Ikutegbe and Sikoki, 2014; Pires *et al.,* 2014; Udochukwu *et al.,* 2016; Edris *et al.,* 2017).

CONCLUSION

Therefore, *Escherichia coli* 0157:H7 strain ECP19-598 (ECEC1), S*taphylococcus aureus* strain JP18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* serovar Enteritidis EC20110358 (SEEC2) were encountered in the studied smoked fish and chicken meat samples, and these were detected more in chicken meat, and SAJP1 was mostly detected

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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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