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Research Article

Molecular Detection of *Staphylococcus aureus* from Dried Crayfish Sold at Selected Supermarkets in the Federal Capital Territory Abuja, Nigeria

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Abstract

Seafood remains a vital protein source worldwide; however, postharvest contamination poses serious public health concerns. This study investigated the occurrence, antimicrobial resistance profiles, and virulence characteristics of *Staphylococcus aureus* isolated from dried crayfish sold in supermarkets across three locations within the Federal Capital Territory, Abuja, Nigeria. A total of 323 dried crayfish samples were randomly collected and bacteriologically examined using standard microbiological and molecular techniques. *S. aureus* was detected in 40 samples, representing an overall prevalence of 12.3%. The highest occurrence was recorded in Kuje (13.4%), followed by Bwari (12.4%) and Gwagwalada (11.1%), with no statistically significant difference among locations ($p > 0.05$). Antibiotic susceptibility testing revealed high resistance to co-trimoxazole up to 87.5% and erythromycin 81.8%, while isolates remained highly susceptible to gentamicin, levofloxacin, and ciprofloxacin. Multiple Antibiotic Resistance index values ranged from 0.2 to 0.9, with most isolates exhibiting indices ≥0.6, indicating exposure to environments with intensive antibiotic use. Molecular analysis confirmed the presence of enterotoxin genes in some isolates, highlighting their pathogenic potential. The detection of multidrug-resistant and toxigenic *S. aureus* in a widely consumed seafood product underscores critical food safety and one health concerns. Enhanced hygienic handling, regulated antibiotic use in aquaculture, and continuous surveillance are essential to minimize consumer exposure to antimicrobial-resistant foodborne pathogens.

Keywords: *Staphylococcus aureus*; Dried Crayfish; Antibiotic Resistance; Multiple Antibiotic Resistance Index; Food Safety

Introduction

Crayfish (*Astacus leptodactylus*) are a vital source of animal protein in many regions, providing an affordable and nutrient-rich food option for both low- and high-income populations. Beyond their high protein content, crayfish contain essential micronutrients and bioactive compounds such as omega-3 fatty acids, vitamins A, C, and B6, and trace elements like iron, zinc, and manganese [1]. In Nigeria, dried crayfish are widely consumed due to their flavor and long shelf-life, achieved primarily through sun-drying and smoking, which reduce moisture content and inhibit microbial spoilage [2]. However, these traditional preservation methods are prone to post-processing contamination, especially

when products are exposed to dust, insects, and unhygienic handling during packaging and retail display [3]. Among bacterial contaminants of animal-derived foods, *Staphylococcus aureus* stands out as a major foodborne pathogen of veterinary and public-health importance [3,4]. It is a Gram-positive, non-motile, facultatively anaerobic bacterium commonly found on human skin, nasal passages, and mucous membranes [5]. Although often a commensal, *S. aureus* can cause a wide range of opportunistic infections and foodborne illnesses. Its pathogenicity is linked to multiple virulence factors, including staphylococcal enterotoxins (SEs), toxic shock syndrome toxin (TSST-1), hemolysins, and proteases [6]. These toxins are heat-stable and can persist in food even after cooking, causing classical staphylococcal food poisoning characterized by nausea, vomiting, abdominal pain, and diarrhea within a few hours of ingestion [7].

A growing concern in recent years is the emergence of antibiotic-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), which carry the *mecA* gene encoding penicillin-binding protein 2a (PBP2a). This gene confers resistance to beta-lactam antibiotics and limits therapeutic options for both human and veterinary infections [3]. The prevalence of MRSA in animal-origin foods, including seafood, has been increasingly reported across Africa, underscoring the interconnectedness of food safety and antimicrobial resistance [3,8]. Studies have revealed that *S. aureus* isolates from fish, shellfish, and dried seafood frequently exhibit multidrug resistance and harbor enterotoxin genes [9].

Despite the widespread consumption of dried crayfish in Nigeria, limited data exist on the occurrence, virulence determinants, and antimicrobial resistance profiles of *S. aureus* in products sold through formal retail outlets such as supermarkets. Most previous investigations have focused on open markets or fresh seafood, leaving a knowledge gap concerning the microbiological quality of packaged or semi-packaged dried crayfish in urban settings such as the Federal Capital Territory (FCT), Abuja.

Therefore, this study aims to evaluate and identify *Staphylococcus aureus* in dried crayfish sold in supermarkets within Abuja, Nigeria, and to determine the prevalence, antibiotic resistance patterns, multidrug-resistance indices, and staphylococcal enterotoxin gene carriage of the isolates. The findings are expected to provide valuable insights for food-safety monitoring, antimicrobial-resistance surveillance, and public-health risk assessment in the Nigerian seafood value chain.

Materials and Methods

Study area and sample collection

The study was conducted in three randomly selected area councils of the Federal Capital Territory (FCT), Abuja: Kuje, Bwari, and Gwagwalada. The FCT lies between latitudes 7°39′ N and 7°45′ E. According to [10], the estimated population was 3.56 million.

A total of 323 dried crayfish samples were randomly purchased from supermarkets between January, and March 2024. Using the sample size formula by [11] with a 95% confidence level (Z = 1.96), using the prevalence of 30.8% [12] and a desired precision of 0.05, a minimum of 323 samples was determined. Seventy-two samples were obtained from Gwagwalada, 169 from Bwari, and 82 from Kuje. Each sample was aseptically placed in a sterile polyethylene bag, labelled, and transported to the Department of Microbiology Laboratory, Nasarawa State University, Keffi, for analysis.

Isolation and identification of *Staphylococcus aureus*

Each sample (10g) was homogenized in 90mL of sterile peptone water and incubated at 37 °C for 24 h for pre-enrichment. One (1ml) of the homogenised sample was inoculated into 9 ml of tryptic soy broth (TSB) and incubated at 37°C for 24 hrs. After incubation, a loop full of the homogenate was picked using sterile inoculating wire loop and streaked onto Mannitol Salt Agar (MSA, Oxoid) and incubated at 37oC for 24- 48 hrs. Presumptive *S. aureus* colonies (yellow with yellow zones) were sub-cultured and stored on nutrient agar slant (Oxoid) in the refrigerator at 4°C for further identification by standard method. Identification was confirmed by Gram staining (Gram-positive cocci in clusters), and biochemical tests including catalase, coagulase test, DNase test, haemolysis test and sugar fermentation based on standard procedures [13].

Molecular detection of enterotoxin genes

Detection of staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*) was carried out using the primer sequences described by [14]. Each reaction mixture (25 µL) contained 12.5 µL of 2× Master Mix, 1 µL each of forward and reverse primers (10 pmol), 5 µL of template DNA, and 5.5 µL of nuclease-free water. Amplifications were performed under the following conditions: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. PCR products were electrophoresed on 1.5 % agarose gel stained with

ethidium bromide and examined under UV transillumination. A 100 bp DNA ladder was used as a molecular size marker. Positive controls consisted of previously confirmed *S. aureus* enterotoxin gene carriers, and nuclease-free water served as a negative control.

a	Primer Sequence	BP	Reference
SA-Ua-F SEA	TGTATGTATGGAGGTGTAAC R –ATTAACCGAAGGTTCTGT	270	[14]
SEB	R –ATAGTGACGAGTTAGGTA	165	
SEC	R – AAGTACATTTTGTA-AGTTCC	102	
SED	R – TTCGGGAAAATCACCCT-TAA	303	
SEE	R- GCCAAAGCTGTCTGAG	213	

Table 1: List of primers and expected amplicons for amplification of enterotoxin A to E genes of *Staphylococcus aureus*.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines [15]. Bacterial suspensions were standardized to 0.5 McFarland ($\approx 1 \times 10^8$ CFU/mL) and inoculated on Muller Hinton agars plates. Antibiotic discs were placed aseptically and allowed to pre-diffuse for 15 min before incubation at 37 °C for 24 h. Zones of inhibition were measured in millimeters and interpreted as susceptible, intermediate, or resistant according to [15]. The multidrug-resistance (MDR) index was calculated as described by [16]. Values greater than 0.2 indicated exposure to high-risk contamination sources.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 25.0. Descriptive statistics were used to summarize bacterial occurrence and antibiotic resistance patterns. Inferential statistics were applied using the chi-square (χ^2) test to determine associations between sampling locations and *Staphylococcus aureus* occurrence, with significance set at $p < 0.05$. Results were presented in tables and figures for clarity.

Results

A total of 323 dried crayfish samples were examined across three locations in the Federal Capital Territory (FCT), Abuja. Out of these, 40 (12.3%) were positive for *Staphylococcus aureus* (Table 2). The occurrence of *S. aureus* varied slightly by location, with Kuje showing the highest prevalence at 13.4% (11/82), followed by Bwari at 12.4% (21/169), and Gwagwalada at 11.1% (8/72). However, there was no statistically significant association between location and *S. aureus* occurrence ($\chi^2 = 0.08$, $p = 0.18$).

Location	Number of Samples	Number of *S. aureus* isolated	Percentages
Gwagwalada	72	8	11.1
Bwari	169	21	12.4
Kuje	82	11	13.4
Total	323	40	12.3

Table 2: Occurrence of *Staphylococcus aureus* from dried crayfish sold in supermarkets in selected locations in Abuja.

Antibiotic susceptibility profile

The antibiotic susceptibility testing carried out on Staphylococcal enterotoxin isolates reveals that gentamicin demonstrated the highest antimicrobial activity against the isolates. Complete susceptibility (100%) was observed among isolates from Gwagwalada and Kuje, while 90.4% susceptibility was recorded in Bwari, with only 9.5% resistance detected at that site. Similarly, the fluoroquinolones levofloxacin and ciprofloxacin showed strong inhibitory effects, with susceptibility rates ranging from 71.4% to 75.0% for levofloxacin and 62.5% to 81.8% for ciprofloxacin across the three locations. In contrast, co-trimoxazole exhibited the poorest performance, with consistently high resistance levels across all sites. Resistance was most pronounced in Gwagwalada (87.5%) and Bwari (85.7%), and remained high in Kuje (72.7%). Correspondingly, susceptibility to co-trimoxazole ranged from only 14.2% to 37.5%. High resistance rates were also observed for erythromycin, with 75.0% resistance in Gwagwalada, 71.4% in Bwari, and 81.8% in Kuje. Ampicillin resistance was moderate to high, particularly in Bwari (57.1%) and Kuje (54.5%), compared to 37.5% in Gwagwalada. Moderate susceptibility patterns were recorded for oxacillin, rifampicin, clindamycin, and cefoxitin, with variability across locations. Oxacillin susceptibility ranged from 50.0% in Gwagwalada to 63.6% in Kuje. Cefoxitin susceptibility was highest in Kuje (63.6%) but lower in Gwagwalada (37.5%) and Bwari (42.8%). Rifampicin showed nearly equal proportions

of susceptibility and resistance across all sites (50%-54.5%). Collectively, the findings indicate that while gentamicin and fluoroquinolones remain largely effective against *S. aureus* isolates from dried crayfish in the study area, substantial resistance to co-trimoxazole, erythromycin, and ampicillin suggests significant antimicrobial exposure.

Antibiotics	Disc content (µg)	No. (%) sensitive			No.(%) resistance		
		Gwags (n = 8)	Bwari (n = 21)	Kuje (n = 11)	Gwags (n = 8)	Bwari (n = 21)	Kuje (n = 11)
Rifampicin	10	4 (50.0)	11(52.3)	6(54.5)	4 (50.0)	11(52.3)	5(45.4)
Levofloxacin	30	6 (75.0)	15(71.4)	8(72.0)	2 (25.0)	6(28.5)	3(27.2)
Ampicillin	30	5 (62.5)	9(42.8)	5(45.4)	3 (37.5)	12(57.1)	6(54.5)
Clindamycin	10	3(37.5)	12(57.1)	6(54.5)	5(62.5)	9(42.8)	5(45.4)
Erythromycin	30	2(25.0)	6(28.5)	2(18.1)	6(75.0)	15(71.4)	9(81.8)
Oxacillin	5	4(50.0)	11(52.3)	7(63.6)	4(50.0)	11(52.3)	4(36.3)
co-trimoxazole	25	3 (37.5)	3(14.2)	3(27.2)	7(87.5)	18(85.7)	8(72.7)
Cefoxitin	30	3(37.5)	12(42.8)	7(63.6)	5(62.5)	9(42.8)	4(36.3)
Gentamicin	10	8(100)	19(90.4)	11(100)	0(0.0)	2(9.5)	0(0.0)
Ciprofloxacin	5	5(62.5)	14(66.6)	9(81.8)	3(37.5)	7(33.3)	2(18.1)

Table 3: Antibiotic Susceptibility Profile of Staphylococcus aureus Isolates Recovered from Dried Crayfish Sold in Selected Supermarkets in Abuja, Nigeria.

Key: Gwags = Gwagwalada, µg = microgram.

Multiple antibiotic resistance (MAR) index

The MAR index of *S. aureus* isolates ranged from 0.4 to 0.9 (Figure 1). The highest frequency of isolates with a MAR index ≥0.6 was recorded in Gwagwalada (62.5%), Bwari (23.8%), and Kuje (45.4%), suggesting exposure to multiple antibiotic sources and potential contamination from high-risk environments.

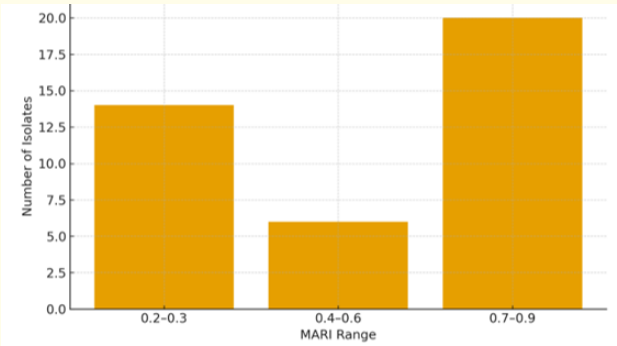

Figure 1: Bar chart showing multiple antibiotic resistance (MAR) index of the isolates.

Molecular detection of enterotoxin genes By PCR

PCR analysis targeting the classical enterotoxin genes (*sea, seb, sec, sed,* and *see*) was conducted on ten *Staphylococcus aureus* isolates recovered from dried crayfish samples. Amplicons were resolved on agarose gel electrophoresis and sized using a 100 bp DNA molecular weight marker. A discrete 213 bp amplification product, corresponding to the expected size of the *see* gene, was detected in nine of the ten isolates (90%). The amplified fragments co-migrated with the positive control, confirming assay specificity. No amplification products corresponding to *sea* (270 bp), *seb* (165 bp), *sec* (102 bp), or *sed* (303 bp) were observed in any of the isolates. The negative control showed no detectable amplification. These findings demonstrate a high prevalence of the *see* gene among the tested isolates.

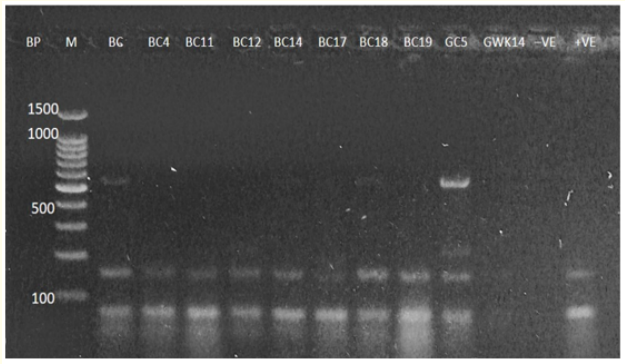

Plate 1: Agarose gel electrophoresis of amplified enterotoxin genes from Staphylococcus aureus isolates obtained from dried crayfish in selected locations in Abuja.

Key: M = DNA ladder (100 bp), +VE = positive control, –VE = negative control.

Discussion

The detection of *Staphylococcus aureus* in dried crayfish sold in Abuja underscores significant public health concerns regarding the microbiological safety of processed seafood. The relatively high overall prevalence (12.3%) observed in this study indicates that dried crayfish can serve as a potential vehicle for foodborne intoxications if consumed without adequate cooking. Similar contamination levels have been reported in seafood and fishery products from other regions of Nigeria and sub-Saharan Africa, suggesting a persistent challenge in post-harvest hygiene and handling practices [12,18]. The presence of *S. aureus* in crayfish likely originates from cross-contamination during processing, packaging, and storage, reflecting lapses in sanitary conditions, personal hygiene, and environmental exposure [2].

The antimicrobial resistance patterns observed among the isolates raises additional concern. A substantial proportion of *S. aureus* isolates displayed multidrug resistance (MDR), particularly to co-trimoxazole and erythromycin, while maintaining high susceptibility to gentamicin, levofloxacin, and ciprofloxacin. Comparable findings have been reported by [19,20], who documented elevated resistance rates to commonly used antimicrobials among *S. aureus* isolates from seafood and meat products. The persistence of MDR *S. aureus* in food chains reflects the broader issue of antibiotic misuse in aquaculture and livestock production systems, a challenge widely recognized in developing countries [21,22]. Continuous exposure of bacterial populations to sub-therapeutic levels of antibiotics promotes the selection and persistence of resistant strains, posing risks to consumers, the environment, and food security [23].

Further insight into antimicrobial resistance intensity was provided by the Multiple Antibiotic Resistance (MAR) index results. The MAR indices ranged from 0.2 to 0.9, with most isolates exhibiting values ≥0.6, indicating exposure of *S. aureus* to environments characterized by frequent or unregulated antibiotic use. High MAR indices were particularly evident among isolates from Bwari and Kuje, suggesting greater antimicrobial pressure or contamination from high-risk sources. According to [16], a MAR index greater than 0.2 signifies that bacteria likely originated from environments where antibiotics are routinely or indiscriminately used. These findings are consistent with previous reports on seafood and ready-to-eat products across Nigeria and Asia [20,22,24]. The observed multiple resistance patterns involving β-lactams, macrolides, and sulfonamides emphasize the adaptive capacity of *S. aureus* and its potential role as a reservoir for resistance genes. This highlights the urgent need for coordinated surveillance programs, prudent antimicrobial use, and strict hygiene measures throughout the seafood production and retail chain to mitigate the spread of MDR *S. aureus*. Although variations in *S. aureus* occurrence among sampling locations (Kuje, Bwari, and Gwagwalada) were not statistically significant, the uniform distribution of contamination likely reflects similar handling and environmental conditions across sites. Factors such as dust exposure, drying conditions, water quality, and packaging materials may all contribute to contamination risk, irrespective of geography. Similar patterns were reported by [25], who observed minimal spatial variation in seafood contamination across Nigerian retail markets.

Polymerase chain reaction revealed that 90% of the *Staphylococcus aureus* isolates harbored the *see* enterotoxin gene, while *sea, seb, sec,* and *sed* were not detected. The predominance of *see* in this study contrasts with several reports in which *sea* and *seb* were more frequently identified among food-associated *S. aureus* isolates [26,27]. For example, studies on seafood and ready-to-eat products in Nigeria and other regions have commonly reported *sea* as the dominant enterotoxin gene, with occasional detection of *seb* and *sec* [19,24].

The absence of *sea, seb, sec,* and *sed* in the present study contrasts with reports where multiple enterotoxin genes were detected in seafood-associated isolates [26,28]. This discrepancy may reflect regional strain variation, differences in ecological niches, or variations in contamination sources and handling practices. Nonetheless, the high occurrence of *see* alone remains significant, as the presence of even a single enterotoxin gene in food-associated *S. aureus* strains represents a potential risk for foodborne intoxication. Enterotoxin gene distribution is known to vary geographically and is influenced by circulating clonal lineages of *S. aureus* [28]. The high prevalence of *see* observed here may therefore indicate the predominance of specific local strains within the crayfish production and retail chain.

Staphylococcal enterotoxins are heat-stable proteins capable of inducing rapid-onset gastrointestinal symptoms following ingestion of contaminated food [27]. Although *see* is less frequently reported than *sea* in global outbreak data, its presence in food products remains clinically significant. Given that dried crayfish is frequently incorporated into meals without extensive reheating, the detection of enterotoxigenic *S. aureus* underscores the need for improved hygiene practices during post-harvest processing, drying, packaging, and retail handling. Strengthened surveillance and food safety education are essential to mitigate the risk of staphylococcal food poisoning in the study area.

Conclusion

This study demonstrated that dried crayfish sold in supermarkets across selected areas of Abuja were contaminated with *Staphylococcus aureus*, with an overall prevalence of 12.3%. The isolates exhibited varying degrees of antibiotic resistance, with notable resistance to co-trimoxazole and erythromycin, but retained high susceptibility to gentamicin, levofloxacin, and ciprofloxacin. The detection of enterotoxin genes among the isolates underscores the potential public health risk posed by the consumption of inadequately cooked dried crayfish. These findings highlight the urgent need for continuous surveillance of enterotoxin-producing *S. aureus* in seafood, stricter enforcement of hygiene and manufacturing standards, and enhanced public awareness on proper seafood handling and preparation. Additionally, prudent regulation of antimicrobial use in aquaculture and animal production systems remains essential to curb the spread of resistant and virulent *S. aureus* strains in the food chain.

Recommendations

Based on the findings of this study, it is strongly recommended that dried crayfish be thoroughly cooked before consumption to eliminate potential *Staphylococcus aureus* contamination. Gentamicin, levofloxacin, and ciprofloxacin may serve as effective therapeutic options for *S. aureus* infections associated with seafood consumption in the study area, pending antimicrobial susceptibility confirmation. Regulatory authorities should intensify the monitoring and control of antibiotic use in aquaculture and livestock production to mitigate the emergence and spread of resistant strains. Additionally, public education on hygienic handling, proper storage, and safe preparation of seafood products is crucial to reduce post-harvest contamination. Further research should focus on molecular characterization of resistance and virulence genes in *S. aureus* isolates to support risk assessment, improve surveillance, and inform evidence-based food safety policies.

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Data Availability

Data is available on reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for Publication

All the authors have approved the submission of this work.

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