Research Article

Molecular Characterization of Virulence and Toxigenic Genes Associated with *Staphylococcus aureus* on Ready-to-Eat Meat and Fish Sold in Dutsin-Ma Market, Katsina State

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ABSTRACT

Foodborne pathogens such as *Staphylococcus aureus* pose significant public health risks due to their ability to produce virulent and toxigenic factors. Ready-to-eat (RTE) foods, including meat and fish, are particularly susceptible to microbial contamination during processing, handling, and storage. This study aimed to molecularly characterize the virulence and toxigenic genes associated with *S. aureus* in RTE meat and fish samples sold in Dutsin-ma Wednesday market, Katsina State, Nigeria. A total of 128 samples of RTE meat and fish were randomly purchased from different vendors. Standard microbiological methods were used for the isolation and identification of *S. aureus*. Genomic DNA was extracted from confirmed isolates, and polymerase chain reaction (PCR) was performed to detect *virulence* (*Sea*) and toxigenic (*tsst*-1) genes in *S. aureus*. The prevalence of *S. aureus* in the samples was determined, with a notable percentage (29.69%) for *S. aureus*. The most frequently detected genes in *S. aureus* were *tsst*-1 (100%) and *Sea* gene (67%). No significant differences in contamination level were observed between meat and fish samples. The presence of virulent and toxigenic genes in *S. aureus* isolates from RTE meat and fish underscores potential food safety risks in Dutsin-Ma Market. These findings highlight the need for improved hygiene practices, proper food handling, and regulatory measures to ensure microbial safety of RTE foods in the region.

Keywords: Food safety; Ready-to-eat food; *Staphylococcus aureus*; Toxigenic genes; Virulent genes

INTRODUCTION

Products made from ready-to-eat meat and fish make up a sizable portion of the world's food supply, contributing to consumers' dietary intake and offering vital nutrients. However, several bacterial pathogens, such as *Staphylococcus aureus* can contaminate these food products, posing serious health risks to the public (Naas *et al*., 2019; Noroozi *et al*., 2022). Food safety and food-borne illnesses have emerged as major global concerns in the twenty-first century (Kalwaniya *et al*., 2020). Significant morbidity and mortality can result from

foodborne illnesses brought on by these pathogens, which can cause serious health issues like food poisoning, diarrhoea, and vomiting (Nass *et al*., 2019). According to Huang and Hwang (2010), ready-to-eat (RTE) foods are a category of food items that have been cleaned, cooked, and packaged and are ready to be eaten without any further preparation or cooking. To eliminate microorganisms of public health concern, foods in this category must be cooked to allow the lowest internal temperature to reach a minimum temperature for a minimum holding time during manufacturing. These foods typically contain

raw materials of animal origin, such as eggs, fish, meat, poultry, and ratites (Huang and Hwang, 2010). Due to its accessibility, popularity, profitability, and economic significance, ready-to-eat food is a major dependency for people living in cities and peri-urban areas worldwide (Dela *et al*., 2023). In addition to being valued for their distinct flavours, appropriateness, and expression of social and cultural traditions, ready-to-eat foods also meet a sizable amount of the population's nutritional needs, particularly those of low-income earners (Tshipamba *et al*., 2018). Due to high unemployment and few job opportunities, street food is a common feature of urban life in developing nations like South Africa (Tshipamba *et al*., 2018). In developing countries such as South Africa, street food is a common part of city lifestyle due to high unemployment and limited work opportunities (Tshipamba *et al*., 2018).

Traditional methods of preserving perishable animal flesh through fermentation, sun drying, smoking, and salting result in meat products that are acceptable both culturally and organoleptically (Bhutia *et al*., 2021). Because of their low pH and low water activity (aw), which inhibit the growth of harmful organisms during fermentation, fermented meat products are typically regarded as safe for ingestion (Bhutia *et al*., 2021).

However, due to its many health advantages, including being a rich source of animal protein, omega-3 fatty acids, vitamin D, selenium, and iodine, fish has been a staple of the human diet for many generations (Mumbo *et al*., 2023). Fish is traditionally smoked over a fire in kilns made of clay, cement blocks, drums, or iron sheets to remove moisture, enabling the product to be kept in market stalls for an extended amount of time (Adesoji *et al*., 2019).

Therefore, since ready-to-eat meat and fish products are potential sources of foodborne pathogens, including *Staphylococcus aureus*, which pose a significant risk to public health, it is essential to conduct a comprehensive molecular characterization and antibiogram analysis of these pathogens to understand their prevalence, genetic characteristics, and antibiotic resistance profiles in these food products sold in markets.

MATERIALS AND METHODS

Study Type

This research is a cross-sectional study that involves the collection of ready-to-eat (RTE) meat and fish samples from various vendors in Dutsin-ma Wednesday market, Katsina State. The study focused on the molecular characterization of virulent and toxigenic genes in *Staphylococcus aureus*.

Study Area

The study was conducted at Dutsin-Ma Wednesday market in Dutsin-Ma Local Government Area, Katsina State, Nigeria. Dutsin-Ma is a Town that lies on the latitude 12°26N and longitude 07°29E. According to Abaje *et al*. (2012), Dutsin-Ma is surrounded by Kurfi and Charanchi LGAs to the North, Kankia LGA to the East, Safana and Dan-Musa LGA to the West and Matazu LGA to the Southeast. It has an area size of 552.323 km^2 with a population of 169, 829 as of the 2006 National Census (Jidauna *et al.*, 2017) and 174,245 in 2021 after taking into consideration the annual growth rate of 2.6% (World Bank Data, 2020).

Sample Size Determination

The number of samples was determined using statistical formula: $n = Z^2P(1-P)/d^2$

Where:

n = required sample size

z = Z- score (Standard normal deviate corresponding to the confidence level); 1.96 for 95% confidence level.

P = estimated prevalence of contamination 9% (0.09)

d = margin of error (0.05)

$n = (1.96)^2 \times 0.09 \times (1- 0.09)/ (0.05)^2$

n= 3.8416 x 0.09 x (0.91)/ (0.0025)

n = 0.31462704/0.0025

n = 126 samples

Sampling Technique

Vendors were randomly selected, and samples were purchased from different locations within the market to ensure representation (Abdulahi, 2020).
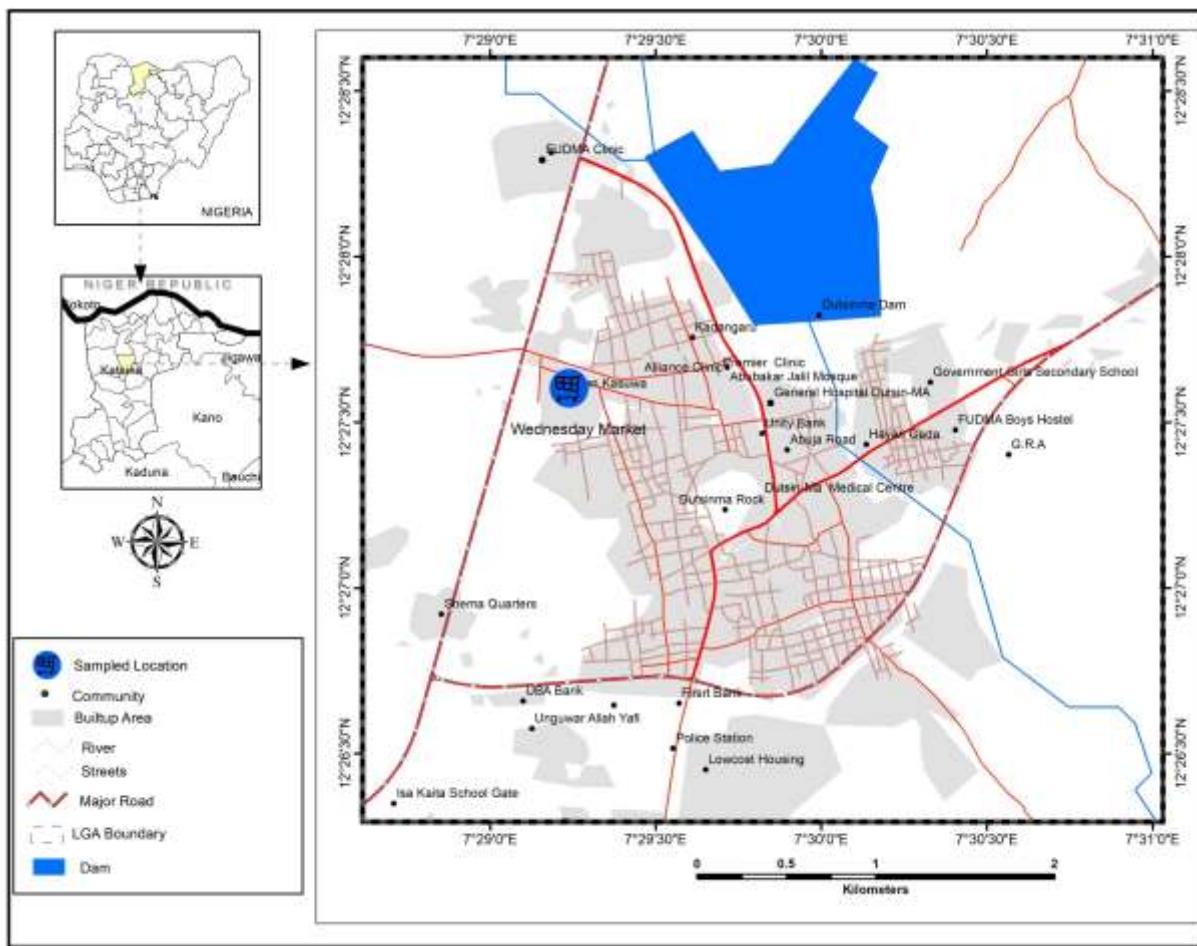
Fig 1: Map Showing Dutsin-ma Wednesday Market

Collection of Sample

In this research, samples of "Balangu" or Barbecue (a traditional ready-to-eat meat dish commonly enjoyed by the Hausa people of West Africa). It is typically made from beef or ram meat, which is thinly sliced and cooked over on an open flame or in hot oil without heavy seasoning. The meat is usually lightly salted and sometimes spiced with local seasonings to enhance its flavours. Balangu is known for its tenderness and rich, natural taste. (Gambo, 2019), "Ragadada" or Meat in spiced broth (A traditional Hausa ready-to-eat meat dish, distinct for being cooked and served in a flavorful broth rather than fried or grilled. It is usually made from beef or ram, which is boiled with salt, spices, and sometimes onions until tender. Ragadada is commonly eaten on its own or with light accompaniments like bread or tuwo. It is popular in northern Nigerian households and street food settings, especially during festive periods or early in the day as hearty starter (Abdulahi, 2020) and fish (Ready-to-eat fried fish, commonly sold in markets across many parts of West Africa, is prepared through a straightforward yet effective process that enhances flavor and helps preserve the fish. The preparation typically begins with selecting fresh fish such as catfish, tilapia, or mackerel. The fish is thoroughly cleaned-scales, guts, and gills are removed, and it is washed with water, sometimes using salt or lime to eliminate odor. After cleaning, the fish is seasoned with a blend of local spices, including salt, pepper, garlic, and occasionally bouillon cubes or ginger. It may be left to marinate for a few hours. The seasoned fish is then deep-fried in hot oil until its golden brown and crispy on the outside. This method not only enhances the taste but also extends the shelf life, allowing it to be sold throughout the day without refrigeration. Once fried, the fish is allowed to cool, drained of excess oil, and

either displayed openly or wrapped in paper or leaves for sale. This convenient and flavourful food is staple in many open-air markets and is often eaten on its own or with local staples like fried yam, rice, or pap (Olayemi, 2018). The samples were randomly collected weekly in sterile poly-ethene bags and placed in an ice-box container from Dutsin-ma Wednesday market and transferred into Microbiology laboratory for microbiological analysis. The duration for sample collection was five (5) weeks which made a total of 128 samples.

Microbiological Analysis

Twenty grams (20g) of each sample was added to 200 milliliters of buffered peptone water as the first enrichment, and the inoculum was incubated aerobically at $37^{o}C$ for 24hours. From the homogenized inoculum, 1ml was pipetted and added to a test tube containing 9mls of sterile buffered peptone water and homogenized by shaking. The diluted inoculum was used as stock solution to make serial dilutions (10^{-2}, 10^{-3}, and 10^{-4}). Zero point five (0.5ml) of the 10^{-4} dilution was pipetted from test tubes and plated on sterile Mannitol Salt agar using a sterile disposable plastic spreader for the Surface spread method (Tshipamba *et al*., 2018).

Determination of Total Viable Count (TVC):

After incubation, plates that contain 30-300 colonies were selected, and the colonies were counted using colony counter (Jay *et al*., 2005). The formula used was TVC (CFU/g) = (Number of colonies x Dilution factor) ÷ Volume plated (ml). The bacterial isolates were stored in refrigerator as stock cultures for further analysis (Tshipamba *et al*., 2018).

Isolation of Bacteria

The positive cultured plates were sub-cultured on fresh Nutient agar plates at $37^{o}C$ for 24hours Isolated bacterial colonies were sub-cultured on fresh Nutrient agar slant and aerobically incubated at $37^{o}C$ for 24hours. The bacterial isolates were sub - cultured three times to obtain pure cultures of the isolates (Tshipamba *et al*., 2018).

Isolates Identification

Identification of *Staphylococcus aureus*

Identification of *S. aureus* was carried out based on morphological structures, Gram reaction, biochemical characteristics (coagulase, catalase and haemolysis test) and molecular characterization of the isolates. *Staphylococcus aureus* forms large golden- yellow colonies when grown on nutrient agar,

making it different from other *Staphylococci* (Keim *et al.,* 2023). Whereas it usually forms grey to deep golden yellow colonies on Mannitol Salt agar (Aryal, 2022). The isolates were gram stained and view using 100x magnification. They were further subjected to biochemical tests, which included catalase, coagulase, and haemolysis tests following standard methods described by Nas *et al.* (2019).

Gram stain

Gram staining was carried out on each isolate using standard methods. A single colony was picked up from a pure culture with a sterile wire loop and smeared on the slide with few drops of normal saline, and the colony was heat fixed on the slide by gentle heating for 20 seconds. After this, the slide was flooded with crystal violet stain for 1 minute, and the stain washed off with sterile water. This was followed by addition of few drops of iodine solution to the slide; the iodine solution was left for a minute before it was washed with sterile water. Next, a few drops of 95% ethanol were applied on the smear to decolorize the slide, and then the slide was washed again with sterile water. Finally, a few drops of safranin solution were placed on the slide as a counterstain for 1 minute; the slide was then washed with sterile water, blotted, and air dried, it was then examined under a bright-field compound light microscope at 100x using oil immersion (Nas *et al.,* 2019).

Antimicrobial Susceptibility Profile

The antibiotic sensitivity profile of each bacterial isolate was determined using the agar disc diffusion (Kirby-Bauer) method. Fresh culture of each bacterial isolate was suspended in 0.85% sterilized physiological saline solution and adjusted to 0.5 McFarland turbidity standard, equivalents to $1.5 \times 10^{8}CFU/ml$. Mŭeller-Hinton agar plates were seeded with 0.1ml of bacterial suspension by spread plate method with the aid of sterilized cotton swab and left to dry for 15 minutes at room temperature. Commercially available antibiotic disc (MASTDISCS®AST, Reinfield, Germany) was aseptically placed on each seeded agar plates. The antibiotic disc includes Amoxicillin/Clavulanate (AUG) 30ug, Gentamicin (GN) 10ug, Ciprofloxacin (CIP) 5ug, Cefoxitin (Fox) 10ug, and Ertapenem (E)15ug for both Gram-positive bacteria. The plates were incubated for 24 hours at $37^{o}C$. The resultant diameter of visible zones of inhibition was measured in millimeters (mm) and classified as resistant (R), intermediate (I) or

sensitive (S) in accordance with the guidelines of the Clinical and Laboratory Standard Institute (CLSI M100-Ed 35; 2025).

PCR Conditions for Different Genes (*Sea*, *Tsst*-1)

Genomic DNA samples of the isolates were amplified in the Thermal cycler PTC 100, MJ Research® following the protocol: Pre-denaturation at 95°C for 5minutes, Denaturation at 94°C for 40seconds, Annealing at 54°C 40seconds, with extension at 72°C for 40seconds and final extension 72°C for 5minutes. The PCR products were subjected to by electrophoresis on 1% agarose gels using 100bp DNA Ladders (Thermal cycler PTC 100, MJ Research). Agarose gels were stained with ethidium bromide (3g/5µl) and visualized using gel imaging system (Biorad) (Wakil and Isa, 2021).

DNA Extraction (Accu prep Genomic DNA Extraction kit from Bioneer)

Genomic DNA from fresh (24hr) culture of the bacteria isolates was extracted using the manufacturer's instructions. 20µl of Proteinase K was added to a clean tube of 1.5ml volume. 200µl of cultured cells was added to the tube containing proteinase K (The sample volume was ensured to be a total of 200µl by adding PBS. 200µl of binding buffer (GC) was added to the sample to achieve maximum lysis efficiency. It was then incubated at 60°c for 10 minutes. 100µl of Isopropanol was added and mixed well by pipetting.

After this step, it was briefly spin down in order to get the drops clinging under the lid so as to have maximum DNA yield. The lysate was carefully transferred into upper reservoir of the Binding column tube (fit in a 2ml tube) without wetting the rim. The tube was closed and centrifuged at 8,000 rpm for 1minute. Each binding column tube was closed in order to avoid aerosol formation during centrifugation. When the lysate has not completely passed through the column after centrifugation, the lysate was centrifuge again at a higher speed (>10,000

rpm) until the binding column tube was empty. The tube was opened and the binding column tube transferred to a new 2ml tube for filtration (Supplied). 500µl of washing buffer 1 (W1) was added without wetting the rim, the tube was closed, and centrifuged at 8,000 rpm for 1 minute.

The tube was opened and the solution was poured from the 2ml tube into a disposal bottle. 500µl of washing buffer 2 (W2) was carefully added without wetting the rim, the tube was closed and centrifuged at 8,000 rpm for 1minute. It was then centrifuged once more at ca. 12,000 rpm for 1 minute to completely remove ethanol, and checked that there is no droplet clinging to the bottom of Binding column tube. This is because, residual W2 in Binding column tube may cause problems in later applications. The Binding column tube was transferred into a new 1.5 ml tube for elution (Supplied), 200µl of Elution buffer was added (EL, or nuclease-free water) onto Binding column tube, It was waited for at least 1 minute at RT (15~25°c) until EL is completely absorbed into the glass fiber of Binding column tube. In order to increase DNA, yield it was waited for 5 minutes after the addition of Elution buffer (EL). The volume of EL added was adjusted from 50µl to 100µl. A smaller volume resulted in a more concentrated solution, but total yield was reduced. Centrifuged at 8,000 rpm for 1 minute to elute. About 180µl ~ 200µl of eluent was obtained when using 200µl of Elution buffer (or nuclease-free water). For an improved yield, the sample was eluted twice and used after concentration process. The eluted genomic DNA was stable and stored at 4°c for later analysis (Wakil and Isa, 2021).

PCR (AccupowerHotstart PCR premix, Bioneer)

for reaction set-up, the following was added, the templates, Specific primers and water to the premix. The reaction was 20µl. PCR set-up items includes dH2O 16µl, Primer 1(1µl), Primer 2 (1µl), and Template (2µl).

Table 1: Genes and Primers used for PCR

Gene	Primers	Primer's sequence	Amplicon size (base pairs)	Reference
Staphylococcal enterotoxin	*Sea*	F: 5'-TGTATGTATGGAGGTGTAAC-3' R: 5'-ATTAACCGAAGGTTCTGT-3'	200-300	Mehrotra, *et al.*, (2000)
Toxic shock syndrome – 1	*Tsst-1*	F: 5'-GATGTTGACAGGCTGACG-3' R: 5'-CAGTACCTGTTGTTGTTGCTG-3'	350-450	Mehrotra, *et al.*, (2000)

Data Analysis

Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) and post-hoc Scheffe tests was used to analyse the level of contamination according to the type and source of Ready-to-eat meats and fish at $P \leq 0.05$ level of significance using Statistical package for social sciences (SPSS). The obtained results for was compared with the microbial contaminant limits (MCLs) which are recommended by codex Alimentarius Commission (CAC) and European Union (EU). This was intended to determine the samples that were within the recommended limits and those that exceeded the limits (Bagumire and Karumuna, 2017).

RESULTS

Table 2 shows mean total viable count, balangu had the highest ($3.7x10^4$ CFU/ml), while ragadada had the least ($2.3x10^4$ CFU/ml).

Table 3; Out of 128 samples, Highest occurrence of *S. aureus* was in balangu (38.33%), followed by ragadada (26.92%) and fried fish with the least (23.81%). Also, the higher standard deviation (7.64) indicates greater variability in the prevalence rates among different food samples tested.

Table 4; The positive cultured isolates were subjected to Microscopy, Gram reaction, and Biochemical test, the presumptive organisms were found to be *S. aureus.*

Table 5 shows antibiotic susceptibility profile against three different ready-to-eat meat and fish samples for *S. aureus*, The result was presented According to Clinical Laboratory Standard Institute (CLSI) guidelines (M100-Ed 35; 2025) as Resistant, Intermediate and Sensitive. The results reveal significant antibiotic susceptibility in *Staphylococcus aureus* isolated from ready-to-eat meat and fish samples.

Table 6; The Isolates showed varying degrees of resistance to the tested antibiotics. Notably, *S. aureus* exhibits high resistant to β-lactam and carbapenem class of antibiotics (Amoxicilin/Clavulanate 85.7%, Ertapenem 42.9%).

Table 7; The result shows diverse and widespread antibiotic resistance patterns among *Staphylococcus aureus* isolates from ready-to-eat meat and fish. Many isolates (9/14) displayed multi-drug resistance, particularly to Amoxicillin/clavulante, Cefoxitin, and Ertapenem. A few isolates (5/14) were resistant to only one or two antibiotics, but most showed resistance to three or more.

Table 8; From the three isolates of *S. aureus* investigated, all encodes a Toxic-shock-syndrome toxin-1 (*tsst*-1) (100%), and two encodes Staphylococcal enterotoxin A *(SEA)* gene (66%) and one (33%) does not encodes for the *SEA* gene.

Table 9; The contamination levels observed in all samples were significantly below the recommended limits for *S. aureus*. This indicates that the tested products meet international food safety standards regarding these specific microbial contaminants.

Table 2: Mean Total Viable Counts and Staphylococcal Counts of Different ready-to-eat Meat Sold in Dutsin-ma Wednesday market

Sample Source	TVC (CFU/ml)	*S. aureus* (CFU/ml)
Balangu	$5.6x10^4$	$3.7x10^4$
Ragadada	$3.5x10^4$	$2.3x10^4$
Fried Fish	$2.8x10^4$	$2.6x10^4$
Total	$11.9x10^4$	$8.6x10^4$

Keys: CFU = Colony forming units, TVC = Total viable count

Table 3: Occurrence of *Staphylococcus aureus* on Ready-to-eat Meat and Fish

Sample Source	Number of samples (n =128)	Number of Positive cultured samples	Percentage Prevalence (%)	Standard Deviation
Balangu	60	23	38.33%	
Ragadada	26	07	26.92%	7.64
Fried-fish	42	10	23.81%	
Total	**128**			

Table 4: Phenotypic Characteristics of the Isolates

Growth on MSA	Growth on NA	Growth on BA	Gram reaction	Coa.	Cat.	Presumptive organism
Ferment Mannitol, colonies appeared opaque and often pigmented golden yellow	Turned medium into grey to deep golden yellow with smooth and shiny surface.	Alpha-haemolytic	Gram positive, small, cocci	+	+	*Staphylococcus aureus*

Keys: NA= Nutrient agar, MSA=Mannitol salt agar

Table 5: Antibiotic Susceptibility Test of *Staphylococcus aureus* Isolated from ready-to-eat Meat and Fish Using MASTDISCS®AST

Sample Code S/N n=14	GEN10mg (mm) R: ≤12 I: 13-14 S: ≥ 15	E15mg (mm) R: ≤14 I: 15-17 S: ≥ 18	Aug30mg (mm) R: ≤13 I: 14-17 S: ≥ 18	Fox15mg (mm) R: ≤ 14 I: 15-17 S: ≥ 18	Cip5mg (mm) R: ≤ 15 I: 16-20 S: ≥21
Bs1	10	14	13	07	20
Bs2	18	21	10	12	15
Bs3	20	07	07	10	12
Bs4	13	18	13	18	19
Bs5	20	12	20	22	20
Bs6	22	20	14	07	14
Rs1	19	10	11	20	13
Rs2	20	20	10	10	18
Rs3	19	07	21	14	10
Fs1	18	13	10	14	04
Fs2	20	22	14	10	18
Fs3	12	10	10	17	22
Fs4	9	07	13	20	20
Fs5	22	14	20	12	12

Keys: Bs= Balangu, Rs= Ragadada, Fs= Fried – fish, GEN= Gentamicin, E= Ertapenem, AUG= Amoxicillin/Clavulanate, FOX= Cefoxitin, CIP= Ciprofloxacin, R= Resistance, I= Intermediate, S= Sensitive, mm= Millimeter in diameter.

Table 6: Antibiotics Susceptibility Profiles of *S. aureus* isolates from RTE Meat and Fish

Antibiotic Potency	Number (%) isolates resistance
	S. aureus (n=14)
Gentamicin (10µg)	4(28.6)
Ertapenem (15µg)	6(42.9)
Amoxicillin/Clavulanate (30µg)	12(85.7)
Cefoxitin (10µg)	8(57.1)
Ciprofloxacin (5µg)	8(57.1)

Keys: GEN = Gentamicin, E= Ertapenem, AUG=Amoxycillin/Clavulanate, Fox= Cefoxitin, CIP= Ciprofloxacin

Table 7: Antibiotic Resistance Patterns for *S. aureus* isolated from RTE Meat and Fish

S/N	Number of isolates	Resistance Pattern
1	1	GEN, E, AUG, FOX
2	1	AUG, FOX, CIP
3	2	E, AUG, FOX, CIP.
4	1	AUG,
5	1	E,
6	1	FOX, CIP
7	1	E, AUG, CIP
8	1	AUG, FOX,
9	2	E, FOX, CIP
10	1	FOX
11	2	GEN, E, AUG,

Keys: GEN = Gentamicin, E= Ertapenem, AUG=Amoxycillin/Clavulanate, Fox= Cefoxitin, CIP= Ciprofloxacin

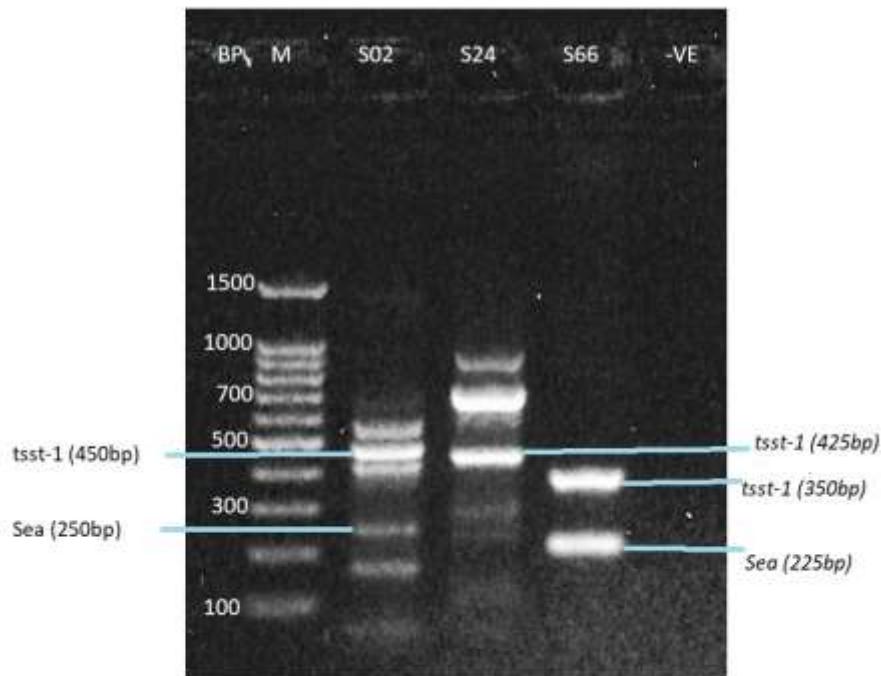**Table 8: Confirmation of *S. aureus* and its Targeted Resistant Genes**

Codes	Genes	
	tsst-1	*Sea*
Bs02	+	+
Rs24	+	-
Rs66	+	+

Keys: Positive (+), Negative (-), *tsst-1*, toxic-shock-syndrome-1 toxin, *sea*, *Staphylococcal* enterotoxin A, Bs, Balangu, Rs: Ragadada

Table 9: Comparison of the Results Obtained with the Recommended Microbial Contaminant (CFU/g)

Microorganism	Recommended Limit (CFU/g)	Balangu (CFU/g)	Ragadada (CFU/g)	Fried -fish (CFU/g)	ANOVA	
S. aureus	≤ 100	2.89×10^{-4}	1.79×10^{-4}	2.03×10^{-4}	p (0.05) = ≥ (0.522)	f = 1.533

Keys: CFU/g = Colony forming unit in grams

Plate 2: Gel Electrophoregram showing *Sea* (200-300bp) and *tsst-1* (350-450bp) genes of *S. aureus,* amplified from *S. aureus* isolates
Lane M-100bp DNA ladder; Lanes 2-4- *S. aureus* isolates; Lane 1-Positive control; Lane 5 – Negative control

DISCUSSION

This Study demonstrates a notable prevalence of *Staphylococcus aureus* in ready-to-eat (RTE) foods sold in Dutsin-ma market, with Balangu showing the highest contamination rate. This finding is consistent with global data indicating a pooled prevalence of *S. aureus* prevalence of approximately 30.2% in RTE foods (Liang *et al*., 2023) and with Nigerian studies reporting similar contamination levels in meat products (Bersamin, 2024). In contrast, much lower prevalence figures reported by Foxcroft *et al*., (2024) highlight substantial regional differences, like influenced by variations in hygiene practices, environmental sanitation, and vendor handling procedures, as also noted in earlier research (Whong *et al*., 2009). The elevated contamination observed in this study may be attributed to repeated handling, exposure of food to the environment, and inadequate temperature control during processing and display. Antimicrobial susceptibility patterns revealed significant resistance to Cefoxitin and Ciprofloxacin, with widespread multidrug resistance among isolates, suggesting the potential presence of methicillin-resistant *S. aureus* (MRSA) strains. These patterns correspond with reports from similar studies conducted in Africa and other regions (Abdeen *et al*., 2021). The detection of virulence – associated genes further underscore the public health risks posed by RTE foods, which are consumed without further heating. Overall, the findings highlight the urgent need for improved hygiene among food vendors, stricter regulatory oversight, and enhanced food safety education to reduce the risk of *S. aureus* related foodborne illnesses.

CONCLUSION

This study revealed that balangu had the highest bacterial load (3.7×10^4 CFU/g), while fried-fish had the lowest (2.3×10^4 CFU/g). Phenotypic and biochemical characterization confirmed the presence of *S. aureus* in the samples. Antibiotic susceptibility pattern demonstrated a high level of resistance, with *S. aureus* exhibiting resistance to Amoxicillin/Clavulanate.
Molecular analysis further confirmed the virulence potential of the bacterial isolates. *S. aureus* strains encoded the tsst-1 gene (100%) and the *SEA* gene (66%). Despite the presence of these bacterial

contaminants, the microbial levels observed were below the Codex Alimentarius Commission and European Union recommended limits for ready-to-eat meat and fish, indicating compliance with international food safety standards.

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