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(RESEARCH ARTICLE)

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Enterotoxin-encoding genes and antibiogram of *Staphylococcus aureus* isolated from nasal cavity of restaurant workers at the Taraba State University, Jalingo, Taraba State

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Abstract

Staphylococcal enterotoxin is one the main causes of food poisoning. Carriage of *S. aureus* in the nose and hands of food handlers has been reported as a major source of Staphylococcal food poisoning. This investigation was aimed at determining the prevalence of *S. aureus* in the nasal cavity of food handlers in a university community in Nigeria. It also assessed the antibiotic sensitivity profile of *S. aureus* and carriage of classical enterotoxin genes. A total of 198 swab specimens were screened for *S. aureus* using standard microbiological methods. Antibiotic sensitivity testing was performed using disc diffusion method. Staphylococcal enterotoxin (*sea-sed*) genes were detected by PCR. This study recorded 26/198 (13.1%) prevalence of nasal *S. aureus. S. aureus* was mostly isolated among female food workers 19(14.4%) and age-group 46-60 years 16 (17.1%). The least resistance to *S. aureus* was observed in ciprofloxacin (22.0%), while higher resistance was recorded in Penicillin (86.0) and trimethoprim (79.0%). The *sea* gene was the most predominant, and was present in 6/26 (23.1%) of the isolates. No isolate harboured the *see* gene. The study revealed that a significant percentage of food handlers in the university community carry enterotoxigenic *S. aureus* strains in their nostrils. However, hygiene measure and screening programmes should be implemented to prevent food contamination with *S. aureus* during food processing.

Keywords: *S. aureus*; Nasal *S. aureus*; Food handlers; Enterotoxin genes; PCR; Disc diffusion

1. Introduction

Staphylococcus aureus is a significant pathogen recognized for its capacity to induce a diverse range of infections in humans and animals (Uzeh *et al.,* 2023). Carriage of *S. aureus* in the nose and hands of food handlers has been reported as a major source of Staphylococcal food poisoning (Uzeh *et al.,* 2023). Staphylococcal food poisoning is one of the most common food borne diseases caused by ingestion one or more Staphylococcal enterotoxins in food contaminated with bacteria. Food handlers in the eateries and restaurant play an important role in food safety because they can contaminate food during processing and distribution (Asgarpoor *et al.,* 2024). Enterotoxins produced by *S. aureus* are known as Staphylococcal enterotoxins (SEs). SEs are classified into classic enterotoxins (SEA–SEE) and new enterotoxins (SEG–SEQ and SER–SEU) based on their biological and serological characteristics. It was reported that about 95% Staphylococcal food poisoning outbreaks were caused by classical SE (SEA-SEE) and the remaining 5% of outbreaks were associated with other identified SEs (Udo *et al.,* 2009; Xing *et al.,* 2013). Many studies in different parts of the world have reported the carriage of enterotoxin-producing *S. aureus* in the nostrils and hands of food handlers (Udo *et al.,* 1999; Xing *et al.,* 2013). These studies have allowed a better understanding of the type of Staphylococcal enterotoxin harboured by *S. aureus* population in different geographical regions. At present, data on the carriage rate of

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Staphylococcal enterotoxin and the antibiotic sensitivity pattern of isolates from the nostrils of food handlers are lacking in the study area. Therefore, the present study may provide information on the enterotoxins produced by *S. aureus* and the antibiogram among the population of *S. aureus* obtained from food handlers operating within the Taraba State University campus and its environs.

2. Materials and methods

2.1. Study area and population

This study was conducted at the Department of Medical Laboratory Science, Taraba State University, Jalingo, Taraba State, Nigeria. Jalingo is a city located in the North East Nigeria and it is the administrative capital of Taraba State. The study population consist a total of 198 restaurants workers, of which 66 were males and 132 females operating within the university and environs. The age range of workers were 15 to 60 years.

2.2. Consent to participate

Sample collection commenced after verbal informed consent was obtained from each participant. The sample collection procedures were non-invasive and all data were analyzed with no reference to participant's identity. Only those who voluntarily gave their consent were screened.

2.3. Centres of study

The study conducted in two centres; Bacteria isolation, identification and antibiotic sensitivity testing were performed at the Department of Medical Laboratory Science, Taraba State University, Jalingo. Molecular analysis was carried out at Molecular Biology Laboratory of Inqaba Biotec Laboratory, Ibadan, Oyo State, Nigeria.

2.4. Sampling, isolation and identification of *S. aureus*

In this cross-sectional study, 198 nasal swabs were collected from individuals working in restaurant. The restaurant workers include those who were involved in cutting, washing, cooking and serving food. Nasal swabs were taken from the anterior part of the nostrils using sterile moistened cotton-wool swabs, they were cultured on mannitol salt agar (MSA) and incubated at 37^0C for 48 hrs. Cultures were examined for fermentation on MSA after 48hrs. Colonies suspected to *S. aureus* were subcultured on Brain Heart infusion agar (BHIA) and incubated at 37^0C for 24hrs. The isolates were identified on the basis of cultural characteristics, Gram's stain reaction and the results of catalase and tube coagulase tests (Lyophilized Rabbit plasma; Becton, Dickinson and Company, Sparks, USA). They were preserved in brain heart infusion broth with 10% (v/v) glycerol (Oxoid, Basingstoke, UK) at −20˚C for further analysis.

2.5. Preparation of standard inoculum

Briefly, the standard inoculum of pure isolates of *S. aureus* was prepared by transferring 2-4 representative isolates from overnight culture plates into a universal bottle containing sterile normal saline. The preparation was shaken to achieve a homogenous suspension. Standard inoculum was achieved by adjusting the turbidity of homogenous suspension to a turbidity equivalent to 0.5 X McFarland standard (1.5×10^8 CFU/ml).

2.6. Antimicrobial sensitivity testing

The suspension was poured on to Mueller-Hinton agar (MHA) (Oxoid, UK) plates and excess fluid was discarded and plates left to air dry. Antibiotic discs were placed on the dried surface and the plates were incubated at 37°C for 24 hours. *S. aureus* strain, ATCC 25923, was used as control for sensitivity testing. The inhibition zone around each disc was measured and interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2017).

2.7. Molecular techniques

2.7.1. DNA extraction

DNA extraction was carried out according to the method described by Udo *et al.,* (1999). Pure *S. aureus* isolates were streaked on Brain Heart Infusion Agar (BHIA) (Oxoid, Basingstoke, Hampshire, England) and incubated overnight at 37°C. Using a sterile applicator stick, three to five identical colonies were added to an Eppendorf tube containing solution of 50vµl lysostaphin (150 µg/ml) and 10 µl RNase (10 µg/ml). The tube was incubated at 37°C in the heat block for 20 minutes (ThermoMixer, Eppendorf, Hamburg, Germany). Thereafter, 50 µL of diluted proteinase K (20 mg/ml) and 150 µL of Tris buffer (0.1 M) were added and mixed by pipetting. The tube was further incubated at 60 °C in the water bath (Grant, England) for 10 minutes. The tube was transferred to a heating block at 95°C for 10 minutes in order

to inactivate proteinase K activity. The tube was centrifuged at 13000 rpm for five minutes. The extracted DNA (supernatant) was stored at 4°C till used for PCR.

2.7.2. Preparation of agarose gel and gel electrophoresis

Gel electrophoresis was used to separate DNA on the basis of their sizes by applying an electric field to move the DNA through an agarose matrix. Two percent (2%) (w/v) concentration of agarose gel used in this study was prepared by weighing and dissolving 4 grams of agarose powder (Promega, Madison, USA) in 200 ml Tris-Borate-EDTA (1XTBE buffer) (Gibco, UK) using microwave oven. The mixture was heated in a microwave oven until it became clear and transparent. The molten agarose was allowed to cool to about 45°C and 300 µl (1 mg/ml) of ethidium bromide was added to the gel. The molten gel was gently poured in a mould (casting) fitted with comb, the gel was allowed to solidify after which the comb was removed to create wells for DNA sample application. It was transferred from the mould into an electrophoretic chamber (Bio-Rad, USA) filled with (1x TBE) buffer. The gel was totally submerged in the buffer. Prior to loading the gel with PCR products (amplicons), tracking dye (bromophenol blue) was mixed with PCR products to enable tracing of migrating DNA across the gel during electrophoresis. The quantity of PCR product to be loaded in the gel varies with the assay. The electrophoretic chamber (Bio-Rad, USA) was connected to the power source (Bio-Rad, USA) and maintained averagely at 90-120V for 30-45 min, the time-voltage combination depends on the assay. Each amplified DNA was visualized by illumination with UV light in a transilluminator connected to a computer. DNA bands (images) were recorded by photography using computer system (SynGene Bioimaging System).

2.7.3. Detection of sea to sed in S. aureus isolates by PCR

The detection of Staphylococcal enterotoxin genes *sea, seb, sec, sed, see* was performed using the primers listed in Table 1. Each PCR tube contain a total volume of 25 µL. This volume contained 2µl of genomic DNA, 12.5 µL of Hot Star Red Taq Master mix, 8.5µl PCR H_2O and 2µl each of se primers (Qiagen, Hilden, Germany). DNA amplification was carried out for 40 cycles according to the following protocol: denaturation at 94° C for 30 s, annealing at 55° C for 30 seconds, and extension at 72° C for 1 min with a final extension at 72° C for 5 mins.

Eight microliters (8 µL) of each PCR products were mixed with tracking dye and transferred accordingly into the wells of 2% (w/v) agarose gels. DNA sample was included as control in every batch of the agarose run. The electrophoresis chamber was connected to the power source and the DNA was run at 120V for 30 minutes. The separated DNA fragments in the gel were transferred into a transilluminator and were visualized by illumination of UV light. The DNA bands were recorded by photography using computer system.

Table 1 Primer sequences to be used for amplification of Staphylococcal enterotoxin gene

Genes	Forward primer (5^1–3^1)	Product size (bp)
sea	Forward: CCTTTGGAAACGGTTAAAACG Reverse: TCTGAACCTTCCCATCAAAAAC	127
seb	Forward: TCGCATCAAACTGACAAACG Reverse: GCAGGTACTCTATAAGTGCC	477
sec	Forward: CTCAAGAACTAGACATAAAAGCTAGG Reverse: TCAAAATCGGATTAACATTATCC	271
sed	Forward: CTAGTTTGGTAATATCTCCTTTAAACG Reverse: TTAATGCTATATCTTATAGGGTAAACATC	219
see	Forward: CAGTACCTATAGATAAAGTTAAAACAAGC Reverse: TAACTTACCGTGGACCCTTC	178

Source: Omoe *et al.,* 2005

Table 2 Distribution of *S. aureus* according to gender and age group of restaurant workers

Gender	**No. Examined**	**No. positive for *S. aureus* (%)**
Male	66	7 (10.6)
Female	132	19 (14.4)
Age-group		
15-30	102	12 (11.8)
31-45	61	8 (13.1)
46-60	35	6 (17.1)
Total	198	26 (13.1)

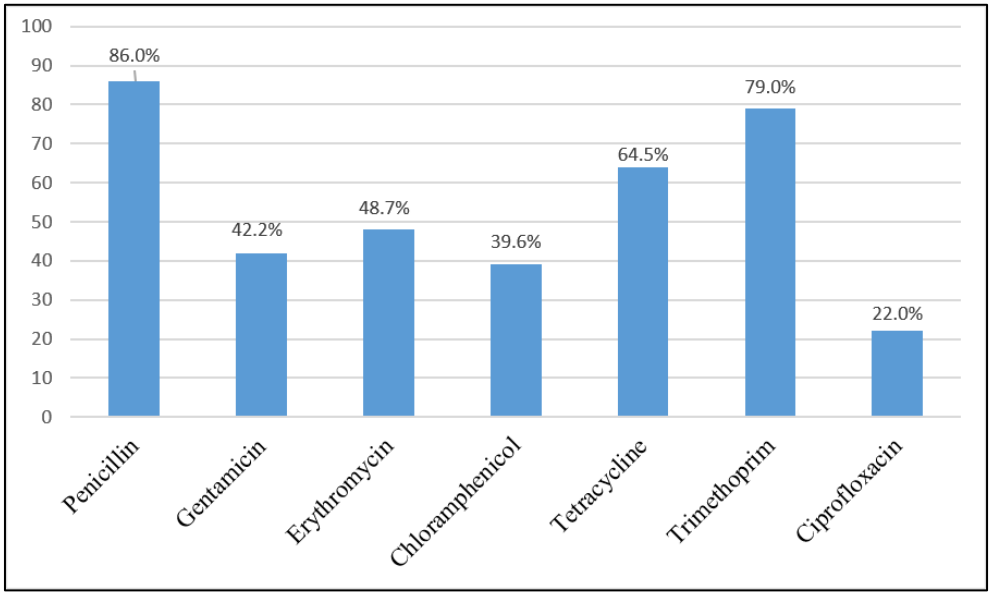

Figure 1 Antibiotic resistance profile of *S. aureus* isolated from nasal cavity of food handlers.

Table 3 Frequency of Staphylococcal enterotoxin (SE) among *S. aureus* isolates

Enterotoxin genes	**Frequency of *S. aureus* (n=26)**	**Percentage**
sea	6	23.1
seb	3	11.5
sec	1	3.8
sea+seb	1	3.8
sea+sed	1	3.8
Total	12	46.2

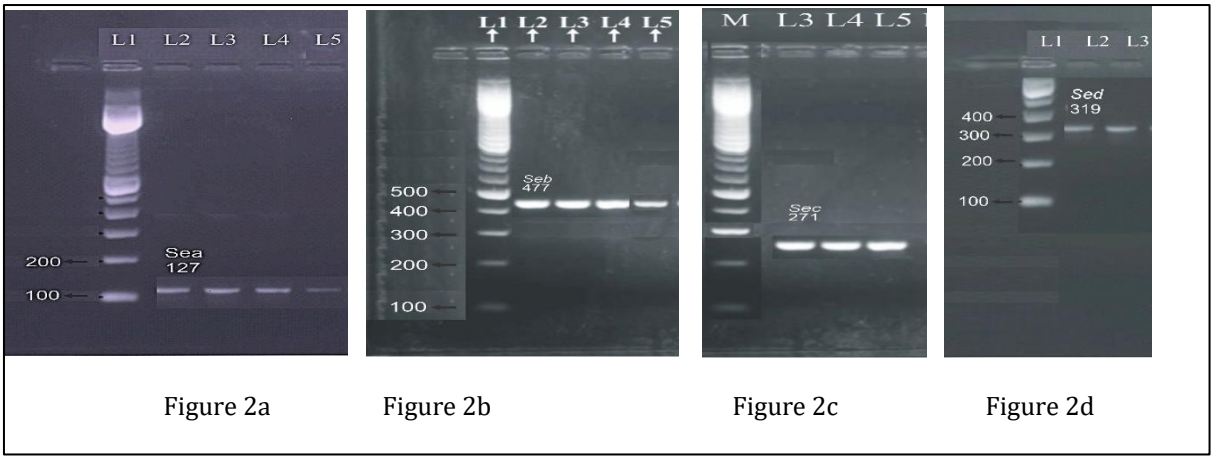

Figure 2a Figure 2b Figure 2c Figure 2d

Figure 2 (a-d) Agarose gel electrophoresis used for detection of amplified *sea, seb, sec,* and *sed* by PCR.

Lanes 1 and M are 100bp DNA molecular size ladder used for sizing DNA bands of samples. The ladder consists of 100bp DNA bands ranging from 100 to 1500bp. Line 2,3,4 represents DNA bands from test samples. Line 5 represents positive control samples. The *sea* (Figure 2a) *seb* (Figure 2b) *sec* (Figure 2c), and *sed* (Figure 2d) are the PCR appearance of the enterotoxin genes.

3. Results and discussions

A total of 26 *S. aureus* were isolated from 198 nasal swab specimens, yielding a prevalence rate of 13.1%. Majority of the isolates 19 (14.4%) were recovered from female subjects, while the male subject produced 7 (10.6%) of the isolates. The distribution of *S. aureus* according to age-group indicates that *S. aureus* was predominant 6 (17.1%) within the age-group 46-60 years, this was followed by age-group 31-45 year 8 (13.1%), and 12 (11.8%) for age-group 15-30 years.

In this study, the frequency of *S. aureus* (13.1%) in the nostrils of the food handlers analyzed was consistent with the nasal colonization rate (12.5%) of those evaluated in a study in Skopje, North Macedonia (Bukovetz *et al.,* 2026). Also, similar colonization rate (14%) was reported in study conducted by Cakici *et al.,* (2023) among food workers in Turkey. Higher carriage rates were found in study reports in Nigeria (32%) (Akinnola *et al.,* 2022), (60%) (Eke *et al.,* 2015), (57%) (Lawani-Luwaji *et al.,* 2024). However, lower colonization rate (10%) has been reported among food handlers in a university community in Lagos, Nigeria (Uzeh, *et al.,* 2023)

The antibiotic resistance profiles of the 26 nasal *S aureus* isolates are presented in figure1.The rate of resistance was high for benzyl penicillin (86.0%) and trimethoprim (79.0%). This was followed by resistance to tetracycline (64.5%), erythromycin (48.7%), gentamicin (42.2%), and chloramphenicol (39.6%). The least resistance to isolates was observed in ciprofloxacin (22.0%). The high resistance to penicillin (86.0%) observed in this study was similar to previous study conducted on nasal *S. aureus* isolated by Beyene *et al.,* (2019) and Akinnola *et al.,* (2022) that yield a prevalence of 90.7% and 93.75% respectively at Jimma town in Ethiopia and Covenant university. Contrary to this study report, Akinnola *et al.,* (2022) observed a high (84.3%) sensitivity to trimethoprim among nasal *S. aureus* in food handlers operating in covenant university. Our study result recorded least resistance to ciprofloxacin (22.0%). Also, studies that revealed higher sensitivity 96.5% and 100.0% have been previously reported by Beyene *et al.,* (2019) in Ethiopia and Eke *et al.,* (2015) among *S. aureus* isolated from nasal cavity of restaurant workers in Ekpoma, Edo State, Nigeria.

The overall enterotoxin carriage rate in *S. aureus* was (46.2%), and the most prevalent SE gene among food handler was *sea* 6 (23.1%%), followed by *seb* 3 (11.5%), and *sec* 1 (3.8%), one (3.8%) isolate each possessed the combination of *sea+seb* and *sea+sed* genes. None of the isolates had the *see* gene. It was reported that (63.1%) of *S. aureus* from nasal swab of food handlers in Iran carried at least one enterotoxin gene (Asgarpoor *et al.,* 2024). In another study conducted among food vendors in a university community in Nigeria, (35.3%) of *S. aureus* strains harboured *sea* gene and *sec* was the predominant SE gene and account for (26.5%) of SE genes. No isolate harboured *seb* gene (Uzeh *et al.,* 2023). Similar studies conducted among food handler in Kuwait City restaurant revealed enterotoxin carriage rate of (86.6%) and their distribution was 28% SEA, 28.5% SEB, 16.4% SEC, and 3.5% SED (Al-Bustan *et al.,* 1996). Another investigation on enterotoxin carriage in Kuwait City restaurant recorded a carriage rate of (71.0%) (Udo *et al.,* 2009).

4. Conclusion

This study found that *S. aureus* significantly colonized the nasal cavity of food handlers. This investigation further revealed that food handlers harboured enterotoxigenic *S. aureus*. In Taraba State there has been no study on detection of enterotoxin genes of *S. aureus* among food handlers. However, this study will provide the basis for the establishment of guidelines for food safety hygiene and screening programme to prevent food contamination with *S. aureus* during food processing.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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