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Classical Enterotoxin Genes Carriage among *Staphylococcus aureus* from Food Handlers in a Nigerian University Community

Roseline E. Uzeh, Solayide A. Adesida*, Adesina O. Adedokun

Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria.

Abstract

Enterotoxigenic *Staphylococcus aureus* is one of the major causes of food poisoning due to asymptomatic carriage. This study aimed to determine the presence of *S. aureus* in the nasal cavities and hands of food handlers in a university setting in Nigeria. It also assessed their susceptibility to antibiotics and examined the carriage of classical enterotoxin genes. Swab samples (120) collected from the anterior nares and hands of 40 food handlers were screened for *S. aureus* using standard microbiological methods. Susceptibility testing for cefoxitin, ciprofloxacin, and vancomycin was performed using the disk diffusion method. Staphylococcal enterotoxin (*sea* - *sed*) genes were detected by multiplex PCR. A total of 34 *S. aureus* isolates were identified, with 12 from the nares, 12 from the right hand, and 10 from the left hand. Ciprofloxacin resistance was found in 55.9% of the isolates, cefoxitin resistance was 32.4%, and none was susceptible to vancomycin. The *sec* gene was the most prominent, accounting for 26.5% of all enterotoxin genes identified. No isolates harbored the *seb* gene but a nasal methicillin-susceptible *S. aureus* strain from one of the food handlers harbored three enterotoxin genes (*sec*, *sed*, *see*) simultaneously. Three (3) sec-carrying strains were resistant to the three antibiotics tested. The study revealed a substantial proportion of carriers of classical enterotoxin genes among the food handlers examined. It also showed resistance to antibiotics relevant to the management of staphylococcal infections. These strains could spread through the community.

Keywords: body sites, *Staphylococcus aureus*, enterotoxin genes, antibiotic resistance, food handlers, healthy carriers

Резюме

Ентеротоксигенният *Staphylococcus aureus* е една от основните причини за хранително отравяне поради асимптоматично носителство. Това проучване имаше за цел да определи наличието на *S. aureus* в носните кухини и ръцете на хора, работещи с храна в университетска среда в Нигерия. Той също така оценява тяхната чувствителност към антибиотици и изследва носителството на класически ентеротоксинови гени. Проби от тампони (120), събрани от предната част на носа и ръцете на 40 лица, работещи с храни, са изследвани за *S. aureus*, като се използва стандартен микробиологичен метод. Тестването за чувствителност към цефокситин, ципрофлоксацин и ванкомицин се извършва с помощта на метода на дисковата дифузия. Гените на стафилококов ентеротоксин (*sea* - *sed*) са доказани чрез мултиплексна PCR. Идентифицирани са общо 34 изолата на *S. aureus*, като 12 са от носа, 12 от дясната ръка и 10 от лявата ръка. Резистентност към ципрофлоксацин е открита при 55.9% от изолатите, резистентност към цефокситин е 32.4% и никой не е чувствителен към ванкомицин. Генът *sec* е най-известният, представляващ 26.5% от всички идентифицирани ентеротоксинови гени. Нито един изолат не съдържа гена *seb*, но назален метицилин-чувствителен щам *S. aureus* от един от работещите с храни съдържа едновременно три ентеротоксин гена (*sec*, *sed*). Три щама, носители на *sec* са резистентни към трите тествани антибиотика. Проучването установи значителна част от носители на класически ентеротоксинови гени сред изследваните лица, работещи с храни. Освен това, доказа резистентност към антибиотици, свързани с лечението на стафилококови инфекции. Тези щамове могат да се разпространят в общността.

* Corresponding author: sadesida@unilag.edu.ng; sadesidashittu@gmail.com
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Introduction

Staphylococcus aureus is a prominent organism with a well-known ability to cause various infections both in clinical and community settings. Carriage of the pathogen by food handlers has been regarded as a major source of staphylococcal food poisoning. In Northwest Ethiopia, 200 healthy food handlers at Gondar University were screened for *S. aureus* colonization and the results indicated that the overall occurrence of nasal carriage was 20.5% (Dagnew *et al*., 2012). Another cross-sectional investigation of 300 food handlers in hotels and restaurants in Jimma Town, Ethiopia, found that 28.7% of samples from their hands and nares were positive for *S. aureus* (Beyene *et al*., 2019). Among Sudanese food handlers, the percentage of *S. aureus* strains recovered from 165 swab specimens was 29% (Arwa and Humodi, 2016).

Several authors observed that many *S. aureus* strains could produce a variety of immunologically distinct enterotoxins. Over 20 staphylococcal enterotoxins (SEs) have been described, of which the classical SEs are considered to be the most common causes of food poisoning resulting from the ingestion of preformed toxins in foods (Udo *et al*., 2009; Rajkovic *et al*., 2020; Sankomkai *et al*., 2020). Staphylococcal food poisoning is frequently attributed to *S. aureus* exposure in crowded community settings, unsanitary environments, or insufficient hygiene procedures (Schelin *et al*., 2011; Fetsch and Johler, 2018). It may thus be difficult to avoid staphylococcal food poisoning from infected food handlers since they are asymptomatic as carriers (Al-Bahry *et al*., 2014).

Food handlers with enterotoxin-producing *S. aureus*, according to Udo *et al*. (2009), play a critical role in the transmission of foodborne illnesses, substantially contributing to the global burden of staphylococcal foodborne infections. Alhashimi *et al*. (2017) revealed that food workers in Kerbala Region, Iraq harbored one or more strains of *S. aureus* with enterotoxins. The problem of enterotoxin-producing *S. aureus* carriage is becoming more challenging with the extensive impact of antibiotic-resistant *S. aureus* that continues to threaten the effective management of staphylococcal colonisations and infections. Methicillin-resistant *S. aureus* (MRSA), in particular, has acquired resistance genes that allow it to resist not only beta-lactams (Tibebu *et al*., 2021) but also other antibiotic classes such as aminoglycosides, glycopeptides, and macrolides.

Boucher *et al*. (2010) explained that infections caused by MRSA have more severe outcomes than methicillin-susceptible *S. aureus* (MSSA) infections, even with appropriate chemotherapy. In a cross-sectional investigation of food handlers in Tripoli, Lebanon, *S. aureus* nasal carriage was shown to be common (23.8%), with a high proportion of MRSA carriage (Osman *et al*., 2019). In another study elsewhere, MRSA strains were found to have a greater percentage of SE genes (61.5%) than MSSA strains (14.8%), raising concern about the carriage of enterotoxin-producing MRSA in food handlers (Ahmed, 2020).

Although the study of enterotoxin genes in *S. aureus* is an essential aspect of continuous surveillance systems to ensure food safety in many countries, the existence of enterotoxin-producing *S. aureus* among Nigerian food vendors has received little attention, and only a few reports have looked at the characteristics of these strains in other populations (Kolawole *et al*., 2013; Ayeni *et al*., 2018). Thus, the purpose of this study was to assess antimicrobial susceptibility patterns and detect classical staphylococcal enterotoxin genes among food handlers in a University community in Nigeria.

Materials and Methods

Ethics approval and consent to participate

The research was carried out in accordance with the principles outlined in the Declaration of Helsinki of the World Medical Association. Sampling 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 the participants' identities. Only those who voluntarily gave their consent were screened. The procedure for collecting samples was non-invasive and no identifiable information was involved in the analyses.

Sample collection

A total of 120 swab samples were collected from the right (40 samples) and left hands (40 samples) as well as nasal cavities (40 samples) of food handlers working in 20 cafeterias within the University community. The nasal sample was carefully collected aseptically from the anterior nares of the food handlers using sterile swab sticks as previously described (Adesida *et al*., 2016). For the hands, swab sticks were used to swab the entire surface of the palms, areas in between the fingers, and beneath the fingernails. Samples were transported immediately on ice packs to the Microbiology research laboratory, at the University of Lagos for analysis.

Isolation of S. aureus from hand and nasal swabs

Each swab was used to inoculate Mannitol salt agar (MSA) plates and incubated under aerobic conditions at 37°C for 24-48 hours. Putative colonies of *S. aureus* based on growth characteristics on MSA were sub-cultured to obtain pure isolates. Identification was based on cultural, morphological, and biochemical characteristics following standard procedures (Kateete *et al*., 2010).

Antimicrobial susceptibility test

The *S. aureus* isolates were subjected to an antimicrobial susceptibility test by disk diffusion method and the interpretation of resultant zones of inhibition was based on Clinical and Laboratory Standards Institute (CLSI, 2018) recommendations. The antibiotics used were cefoxitin (30µg), vancomycin (30µg) and ciprofloxacin (5µg). *S. aureus* ATCC 25293 was used for quality control. Isolates with intermediate susceptibility patterns were classified as resistant, while *S. aureus* strains resistant to cefoxitin disk were designated as MRSA (Pourmand *et al*., 2014).

Multiplex PCR amplification of S. aureus enterotoxin genes

DNA extraction was done using the procedure described by Hassanzadeh *et al*. (2016). Multiplex PCR for the detection of *sea-see* genes was performed as outlined by Mehrotra *et al*. (2000). Table 1 depicts the primer sets used for the detection of the genes.

Data analysis

Data was analyzed using Microsoft Excel software version 2013 for descriptive statistics, and the results were expressed as percentages.

Results

Staphylococcus aureus was detected in 34 of the 120 samples collected from the 40 food handlers, resulting in a colonization rate of 28.3%. Most of the isolates originated from the participants' right hand and nasal cavities. Ten subjects had *S. aureus* on their left hands, 12 on their right hands, and 12 had it in their nostrils. In all, 32.5% (13/40) of those tested had the organism on one or both hands, and 30% (12/40) had it in their nostrils. The frequency and site of isolation are depicted in Fig. 1.

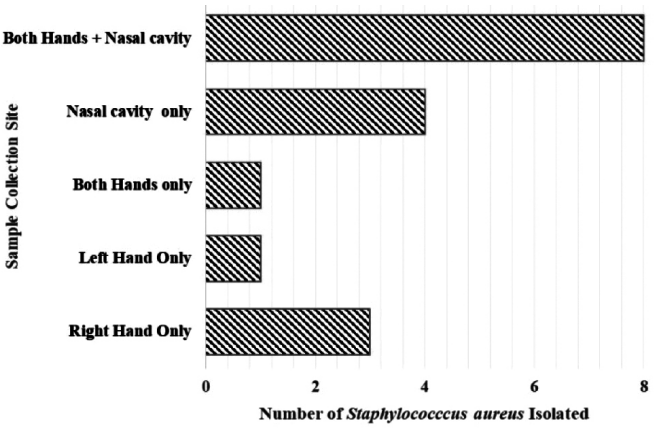

Fig. 1. Frequency of *S. aureus* from hands and nasal cavities of the 40 food handlers investigated

Antimicrobial resistance patterns varied among the *S. aureus* isolates, and none showed concurrent sensitivity to the three antibiotic classes tested (Table 2). Also, different antibiotic susceptibilities were observed among the multiple isolates from some subjects. The overall resistance rates for each antibiotic examined were 55.9% for ciprofloxacin, 32.4% for cefoxitin, and 100% for vancomycin. Eleven (55.9%) *S. aureus* were resistant to two classes of antimicrobial drugs (fluoroquinolones: ciprofloxacin and glycopeptides: vancomycin), 8 were resistant to all the antibiotic classes (ciprofloxacin, cephalosporin, and glycopeptides) and a total of 4 resistance phenotypes were identified

Table 1. Primer sequences and expected amplicon sizes

Gene	Primer	Oligonucleotide (5'-3')	Size of amplified product (bp)	Reference
sea	Forward	GGT TAT CAA TGT GCG GGT GG	102	Bania *et al*., 2005
	Reverse	CGG CAC TTT TTT CTC TTC GG		
seb	Forward	GTA TGG TGG TGT AAC TGA GC	164	
	Reverse	CCA AAT AGT GAC GAG TTA GC		
sec	Forward	ACA CCC AAC GTA TTA GCA GAG AGC	631	Bohach and Schlievert, 1987
	Reverse	CCT GGT GCA GGC ATC ATA TCA TAC		
sed	Forward	CCA ATA ATA GGA GAA AAT AAA AG	278	Bania *et al*., 2005
	Reverse	ATT GGT ATT TTT TTT CGT TC		
see	Forward	AGG TTT TTT CAC AGG TCA TCC	209	
	Reverse	CTT TTT TTT CTT CGG TCA ATC		

Table 2. Antibiogram of the 34 *Staphylococcus aureus* isolates from the nasal and hand carriage of food handlers

Isolate Code (Isolation Site)	Ciprofloxacin (mm)	Cefoxitin (mm)	Vancomycin (mm)	Antibiotic Susceptibility Pattern
1(LH)	R (16)	S (24)	R (8)	cip^R, fox^S, van^R
1(RH)	R (0)	R (0)	R (0)	cip^R, fox^R, van^R
1(NC)	S (24)	S (28)	R (0)	cip^S, fox^S, van^R
3(LH)	S (22)	R (16)	R (0)	cip^S, fox^R, van^R
3(RH)	R (0)	R (0)	R (0)	cip^R, fox^R, van^R
3(NC)	S (26)	S (26)	R (0)	cip^S, fox^S, van^R
5(NC)	S (28)	S (28)	R (10)	cip^S, fox^S, van^R
8(LH)	S (30)	R (18)	R (12)	cip^S, fox^R, van^R
8(RH)	S (30)	S (30)	R (12)	cip^S, fox^S, van^R
8(NC)	R (0)	R (0)	R (0)	cip^R, fox^R, van^R
12(LH)	S (22)	S (28)	R (10)	cip^S, fox^S, van^R
12(RH)	R (0)	S (28)	R (10)	cip^R, fox^S, van^R
12(NC)	R (0)	S (26)	R (10)	cip^R, fox^S, van^R
15(LH)	R (0)	S (28)	R (12)	cip^R, fox^S, van^R
17(LH)	R (18)	S (28)	R (10)	cip^R, fox^S, van^R
17(RH)	S (26)	S (28)	R (12)	cip^S, fox^S, van^R
20(LH)	S (28)	S (28)	R (16)	cip^S, fox^S, van^R
20(RH)	S (30)	S (32)	R (14)	cip^S, fox^S, van^R
20(NC)	R (0)	S (28)	R (12)	cip^R, fox^S, van^R
22(NC)	R (4)	S (28)	R (12)	cip^R, fox^S, van^R
23(RH)	R (0)	R (0)	R (0)	cip^R, fox^R, van^R
25(LH)	R (20)	R (8)	R (0)	cip^R, fox^R, van^R
25(RH)	R (20)	R (10)	R (0)	cip^R, fox^R, van^R
25(NC)	R (20)	R (10)	R (0)	cip^R, fox^R, van^R
28(RH)	S (25)	S (27)	R (15)	cip^S, fox^S, van^R
29(NC)	S (25)	S (29)	R (17)	cip^S, fox^S, van^R
32(LH)	S (30)	R (15)	R (0)	cip^S, fox^R, van^R
32(RH)	R (0)	R (0)	R (0)	cip^R, fox^R, van^R
32(NC)	S (22)	S (28)	R (10)	cip^S, fox^S, van^R
35(RH)	R (0)	S (28)	R (10)	cip^R, fox^S, van^R
38(LH)	R (0)	S (26)	R (10)	cip^R, fox^S, van^R
38(RH)	R (0)	S (28)	R (20)	cip^R, fox^S, van^R
38(NC)	R (18)	S (28)	R (10)	cip^R, fox^S, van^R
40(NC)	S (26)	S (28)	R (12)	cip^S, fox^S, van^R

*Isolate with same code are from same individual. *Isolates with intermediate susceptibility were classified as resistant. Abbreviations: S- Susceptible, R- Resistant, LH- Left hand, RH- Right hand, NC- Nasal cavity.

(Table 3). Cefoxitin resistance (≤14mm) phenotype was recognized in 8 isolates and was termed MRSA (Fig. 2).

Out of the 34 isolates screened for the classical enterotoxin (A-E) genes, 12 (35.3%) of which 5 were MRSA showed positive outcomes for at least one enterotoxin gene while the remaining isolates had none of the genes examined (Table 4).

Nine (26.5%; 9/34) isolates showed positive results for the enterotoxin C gene, followed by 3 (25%) testing positive for *sed* and *sea*. More than one enterotoxin gene was detected among 4 (11.8%) isolates, with the strongest interaction found between *sec-sed and sec-see*. While no isolates had *seb* genes, one nasal MSSA strain possessed three enterotoxin genes simultaneously (*sec*, *sed*, and

see). On the whole, enterotoxin genes were detected in 45.5% (5/11) of MRSA and 30.4% (7/23) of MSSA isolates. Four of the *sec* strains were resistant to the three antibiotics (Table 4).

Table 3. Phenotypic resistance profile of *Staphylococcus aureus* from hands and nose of the food handlers (n=34)

Antimicrobial resistance phenotype*	No. of isolates (%)
CIP-FOX-VAN	8 (23.5)
FOX-VAN	3 (8.8)
CIP-VAN	11 (32.4)
VAN	34 (100)

*Isolates with intermediate susceptibility were classified as resistant.
CIP- Ciprofloxacin (5 µg), FOX- Cefoxitin (30 µg), VAN- Vancomycin (30 µg)

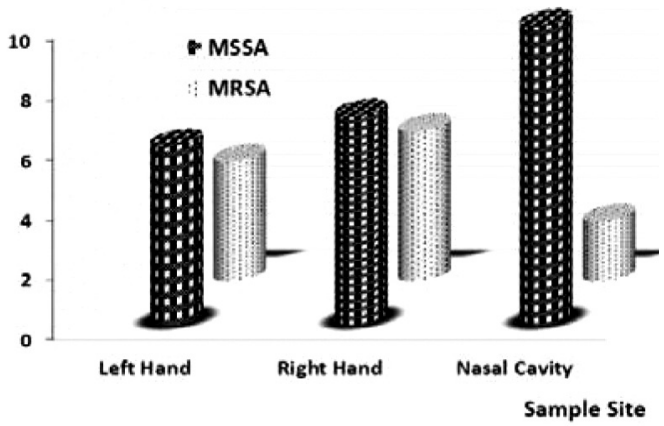

Fig. 2. Cefoxitin Resistance Pattern of *Staphylococcus aureus* from the hands and nasal cavities of 40 food handlers. MRSA: Methicillin resistant *Staphylococcus aureus*, MSSA: Methicillin Susceptible *Staphylococcus aureus*

Discussion

In this study, the frequency of *S. aureus* (30%) in the nostrils of the food handlers analyzed is consistent with the nasal colonization rate of those evaluated in a study in Kerbala City, Iraq (Alhashimi *et al*., 2017). The rates were higher than those found in Portugal (19.8%) (Castro *et al*., 2016), Lebanon (23.8%) (Boucher *et al*., 2010), and Jimma Town in Southwest Ethiopia (9%) (Beyene *et al*., 2019). Our results, on the other hand, were lower than those of Kuwait (40.8%) (Udo *et al.,* 2009) and Nigeria (60%) (Omololu-Aso *et al*., 2017). We identified a higher number of *S. aureus* on both hands (55%) than in the nostrils of the food handlers but the right hands were more colonized than the left.

Some authors have indicated lower hand carriage rates (Ho *et al*., 2015; Vatansever *et al.,* 2016). These discrepancies between our findings and those of other studies could be attributed to the processing and hygiene procedures of the workers. In addition, sanitation variances between study centers as a result of differences in environmental conditions could also be a factor. Lastly, the types of protective gear used by the workers could also have impacted the hand carriage rates. As the organism was found in both the nostrils and hands of some participants, it was likely transmitted from hand to nose and vice versa. In the same way, the presence of *S. aureus* on the hands but not in the nostrils implies that hand contamination could come from sources other than the individual, such as environmental sources.

Antibiotic resistance in *S. aureus* is a well-documented phenomenon with substantial implications in clinical and community settings. In this study,

Table 4. Classical enterotoxin gene profile in relation to antibiotic susceptibility pattern of *S. aureus* from food handlers

Isolate Code*	Site of Collection	Enterotoxin genes	Methicillin Resistance Status	Antibiotic Susceptibility Pattern
1RH	Right Hand	*Sec*	MRSA	van^R, cip^R, fox^R
1N	Nose	*Sec*	MSSA	van^R, cip^S, fox^S
3LH	Left hand	*sec*	MRSA	van^R, cip^S, fox^R
3RH	Right Hand	*sec*	MRSA	van^R, cip^R, fox^R
3N	Nose	*sec, see*	MSSA	van^R, cip^S, fox^S
5N	Nose	*sec, sed, see*	MSSA	van^R, cip^S, fox^S
8LH	Left hand	*sec, sed*	MRSA	van^R, cip^S, fox^S
8RH	Right Hand	*sec*	MSSA	van^R, cip^S, fox^S
8N	Nose	*sec, sed*	MRSA	van^R, cip^R, fox^R
12LH	Left hand	*sea*	MSSA	van^R, cip^S, fox^S
12RH	Right Hand	*sea*	MSSA	van^R, cip^R, fox^S
17LH	Left Hand	*sea*	MSSA	van^R, cip^R, fox^S

*Isolates with the same code originated from the same individual

three antibiotics pertinent to managing staphylococcal infections were examined. Despite the limited number of antibiotics screened, the strains showed four distinct resistance patterns. *S. aureus* resistance to vancomycin and ciprofloxacin is disturbing, and this has embossed food handlers as a potential source of disseminating resistant strains. Although the disk diffusion technique for detecting vancomycin has significant limitations, all of the isolates examined in this investigation exhibited resistance to the antibiotic. However, while we recognize that this conclusion may be overestimated, it is consistent with research undertaken in other parts of the world (Abulreesh and Organji, 2011). In fact, vancomycin is considered the last resort for *S. aureus* infections but resistance to the antibiotic has emerged in a noticeable way especially in hospital settings thereby constituting a severe public health concern (Deyno *et al*., 2017; Gitau *et al*., 2018). This suggests that more research is needed to determine the true incidence of these strains with attention to the molecular mechanisms that underpin the phenotype.

The quinolone, ciprofloxacin, has been considered a viable alternative to vancomycin for the treatment of staphylococcal infections. More than half of our isolates were resistant to this antibiotic. This is in line with a study conducted in Zagazig, Egypt, which found that 55% of *S. aureus* from ready-to-eat meat and food handlers were ciprofloxacin-resistant (Saber et al. 2022). Ciprofloxacin-resistant strains were also discovered among *S. aureus* from food workers in Portugal, though at a lower rate than ours (Castro *et al*., 2016). In contrast, studies in Nigeria (Omololu-Aso *et al*., 2017) and elsewhere (Beyene *et al*., 2019) found that ciprofloxacin was effective in over 90% of cases.

In Nigeria, ciprofloxacin is widely prescribed and readily available over the counter. In all, the simultaneous high occurrence of ciprofloxacin and vancomycin resistance in these strains coincides with findings from a Bangladeshi evaluation of *S. aureus* from burn wound infections (Hasan *et al*., 2016). It is thus opined that ciprofloxacin be used with caution in regions where it is freely accessible without medical prescriptions. Also, as a critical option for treating severe MRSA infections, vancomycin resistance must be continually evaluated.

We also observed that *S. aureus* resistance to cefoxitin was relatively lower than vancomycin and ciprofloxacin. Methicillin resistance was defined using 30 µg cefoxitin disk and 32.4% (11/34) were recognized as MRSA. This contradicts research carried out in Turkey, where methicillin resistance was detected using cefoxitin (30µg), and all the isolates were susceptible (Vatansever *et al*., 2016). Likewise, Castro *et al*. (2016) hypothesized that MRSA isolation from food handlers was uncommon because none of their isolates possessed the *mec*A gene or showed resistance to oxacillin.

However, evidence from other geographical areas with different study designs and periods confirmed the existence of MRSA among food handlers. In central Iran, methicillin resistance was reported to be 16.5% (37/224) among nasally contaminated food handlers (Fooladvand *et al*., 2019). Similarly, Osman *et al*. (2019) discovered a limited number of MRSA among their isolates. The high frequency of MRSA found in the current study underlines its growing problem in community settings. Similar to our findings, *S. aureus* strains resistant to vancomycin and methicillin have been reported in Egypt (Al-Amery *et al*., 2019). Other authors have also discovered that *S. aureus* recovered from food handlers and patient samples exhibited resistance to methicillin and vancomycin (El-Zamkan *et al*., 2019; Thwala *et al*., 2021). Thus, the present findings indicate an increase in resistance to the tested antibiotics. This is possibly an unavoidable consequence of antibiotic exposure or unrestricted access in our country. As a result, future research should focus on a more integrated study that uses a range of molecular techniques. This will ameliorate the detection of antibiotic resistance in *S. aureu*s from food handlers.

In this investigation, 35.3% of the *S. aureus* isolates had one or more of the enterotoxin genes tested. This observation matched that of other researchers, who found enterotoxin genes in 39.4% of their isolates (Jordá *et al*., 2012). We detected enterotoxin genes: *sea*, *sec*, *sed*, *and see* but no *seb*. Almost always, staphylococcal food poisoning is connected to *sea* and *seb*, causing most staphylococcal food poisoning worldwide (Pinchuk *et al*., 2010; Ahmed, 2020). A predominance of *sec* gene was discovered among our *S. aureus* isolates, which is usually not the case in other studies (Alhashimi *et al*., 2017; Ahmed, 2020). Notably, a significant number of the MSSA harbored enterotoxin genes, and a nasal strain (MSSA) of a participant was found to be positive for three enterotoxin genes (*sec*, *sed*, *see*), signifying the pathogenic potential of these strains. Nevertheless, the combination of enterotoxin genes and resistance among isolates from these participants is disquieting and needs to be appropriately addressed.

Conclusions

This work demonstrates that a high percentage of *S. aureus* from the food handlers investigated were highly resistant to vancomycin and ciprofloxacin. This could serve as a basis for establishing appropriate control guidelines for antibiotic usage. The study also discovered that some of the participants had *S. aureus* strains with enterotoxin genes. These strains might easily contaminate foods and pose a serious risk to consumers. While more research into the variables that favor resistance, the transmission of high-risk strains, and the genetic basis of resistance is required, community-based antimicrobial susceptibility monitoring is strongly suggested.

Acknowledgments

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