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ENTEROTOXIN PRODUCTION AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* AMONG FOOD HANDLERS IN A SUB-URBAN SETTING IN RIVERS STATE, NIGERIA

Augustina Akum Okoro[*1], Smart Enoch Amala[2] and Easter Godwin Nwokah[2]

[1]Department of Medical Microbiology and Parasitology, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

[2]Department of Medical Laboratory Science, Rivers State University, Nkpolu- Oroworukwo, Port Harcourt, Nigeria.

***Corresponding Author: Augustina Akum Okoro**
Department of Medical Microbiology and Parasitology, College of Medical Sciences, Rivers State University, Nkpolu- Oroworukwo, Port Harcourt, Nigeria.

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ABSTRACT

Food handlers have been incriminated in the transmission of food borne diseases, contributing significantly to the global incidence and burden of diseases. This study investigated enterotoxin production and antimicrobial resistance of *Staphyloccocus aureus* among food handlers in a sub-urban setting in Rivers State, Nigeria. A total of 360 hand and nasal swab samples were collected from food handlers in Eleme (n=180) and Oyigbo (n=180) local government areas using a simple random sampling technique. Organisms were isolated using standard microbiological techniques. Prolex™ Staph Xtra Latex kit was used for confirmation of enterotoxin production, while Cefoxitin discs (30 µg) was used for methicillin resistance screening. Disk diffusion method was used to test susceptibility patterns of *Staphylococcus aureus* against panel of antimicrobial agents. Statistical analysis was performed using SPSS version 21 for prevalence, frequency, chi square and correlation tested at 0.05 level of significance. The prevalence of *S. aureus* obtained from this study was 28.1%, out of which Enterotoxin-Producing *Staphylococcus aureus* was 37.6%. The prevalence of *S. aureus* among street food handlers was higher in Oyigbo (34.4%) compared to Eleme (21.7%), while enterotoxin producers occurred at a higher rate (43.6%) in Eleme than in Oyigbo (33.9%). Toxin production and socio-demographic variables of location, sex and education were significantly associated ($p<0.05$), whereas age showed no significant association ($p>0.05$) with enterotoxin production. Correlation analysis for education showed an indirect association with enterotoxin production (0.000); whereas others demonstrated direct association except age ($p=0.088$). *S. aureus* isolates showed maximum resistance to penicillin antibiotic (92.1%) and the highest susceptibility to vancomycin (96.0%). Also, 21.8% of the isolates was resistant to cefoxitin (Methicillin-Resistant *S. aureus*), whereas 78.1% was sensitive (MSSA). The present study considered prevalence rates of *S. aureus*, enterotoxin producing *S. aureus*, MRSA and multidrug resistant which have empirically proven high rate of occurrences among food handlers within the study locations. The public health implications of these findings and appropriate response cannot be overemphasized.

KEYWORDS: Enterotoxin, Antimicrobial Resistance, *S. aureus*, Food Handlers.

1 INTRODUCTION

The battle against bacterial food-borne diseases is facing new challenges due to rapidly changing patterns of human consumption, the globalization of the food market and climate change.[1] Food processing remains an important industry in Nigeria and the World at large. One of the major problems threatening food industry is contamination with food-borne microbes of human origin, resulting from improper handling and processing. In recent times, consumers want more natural food products that are less processed, without preservatives, with low salt, sugar or fat contents, but with an extended shelf-life and high quality. The demand for convenient, ready-to-eat food has also increased, and the food industry has developed new food processing techniques such as semi-prepared, minimally processed, chilled food in response to these demands.[2] Most of these foods create enabling growth environment for toxin-producing bacteria such as *Staphylococcus aureus*, which is able to grow and express toxins in a wide variety of foods such as milk products, mixed foods, meat and meat products, egg and egg products, cakes and ice cream.[3]

Staphylococcus aureus has been considered a food-borne hazard for a long time. In 2013, three hundred and eighty-six (386) Staphylococcal outbreaks were reported by the European Food Safety Authority (EFSA), representing 7.4% of all outbreaks reported in the European Union.[4] It is found in the nostrils and on the skin of warm-blooded animals, and the primary source of

food contamination is the hands of food handlers.[5] *S. aureus* has the ability to grow, and produce staphylococcal enterotoxins (SE), the causative agent of staphylococcal food poisoning (SFP), over an extensive range of temperature, pH, sodium chloride concentration and water activity. The robustness of the organism permits its growth in many types of food, producing heat-resistant enterotoxins that causes food poisoning.

The safety of food remains an important issue throughout the world, and was one of the WHO's 13 strategic objectives for 2013–2018. Due to a number of serious incidents of food-borne cases, the Food and Drug Administration (FDA) stepped up efforts to improve the traceability of contaminated products.[6] In Europe, the EFSA reported a total of 5,550 outbreaks of foodborne illness in 2009, affecting almost 49,000 people and causing 46 deaths. Among these, 293 outbreaks were caused by *Staphylococcus* sp and bacterial toxins (produced by *Bacillus, Clostridium* and *Staphylococcus*) were the fourth most common causative agent in foodborne outbreaks.[4]

Previously, reports on food contamination by *S. aureus* were mainly limited to occasional detections in the environment, the source of food and food itself. However, it was reported that human carriers are the most important source for transmission and the association between food handlers and the transmission of food borne disease frequently presents an investigative challenge.[7]

The aim of this study was to isolate and identify enterotoxin-producing *S. aureus* and to assess the antimicrobial susceptibility of *S. aureus* among food handlers in Oyigbo and Eleme Local Government Areas of Rivers State, Nigeria.

2 MATERIALS AND METHODS

2.1 Area of the Study

The study was conducted in Oyigbo and Eleme Local Government Areas of Rivers State, Nigeria. These are sub-urban settings in the State.

2.2 Study Design

This was a cross sectional study involving 360 subjects. The target population was consenting adult male and female individuals handling food in Oyigbo and Eleme regions of Rivers State. Simple unrestricted randomization, analogous to repeated fair 'coin-tossing' was used in picking subjects for this study, as they all satisfied the inclusion and exclusion criteria for the study. Subjects were 17 years and above, and were residents of Eleme and Oyigbo within the period of study.

2.4 Ethical Consideration

Ethical approval was sought and obtained from the Rivers State Hospital Management Board, Port Harcourt. Hence, permission to collect samples from volunteers.

2.5 Sample Collection and Preparation

The specimens were collected using cotton-tipped swab previously moistened with sterile normal saline. The anterior nares were sampled by rotating the swab tip in both nostrils. Hand swabs were also obtained.

Sample Processing

2.5.1 Media Preparation

The various media used (Blood Agar, Mannitol Salt Agar, MacConkey Agar, Muellar Hinton Agar) were aseptically prepared in the laboratory following the manufactures' instructions and also as described by Cheesbrough.[8]

2.5.3 Culture

The swabs were spread onto Mannitol Salt Agar (MSA) and incubated aerobically at 37^{o}C for 24 hours. Suspect colonies were subjected to Gram-staining, coagulase and other biochemical tests for identification of *S. aureus* as described by Ochei and Kholhatkar.[9]

2.5.4 Detection of Enterotoxin Production

S. aureus isolates were further analyzed for their virulence factors in relation to their Staphylococcus enterotoxin (SE) production following the standard bacteriological methods. The isolates were screened for enterotoxin production following the Prolex™ Staph Xtra Latex kit method as described by Schleifer and Kloos[10] and was reliable for detection of enterotoxin production in *S. aureus.* The Prolex™ Staph Latex Kit was used following manufacturer's instructions.

2.5.5 Identification of MRSA and MSSA

Staphylococcus aureus isolates were screened for methicillin resistance by the modified Kirby-Bauer disk diffusion method described by Cheesbrough.[8] Pure colonies of isolates were seeded unto peptone water and turbidity matched with 0.5 McFarland turbidity standard. Sterile cotton buds were used to lawn the organism on pre-dried Muella Hinton Agar, while Cefoxitin(30µg) and Oxacillin (1µg) discs were placed at the center of the agar and subsequently incubated at 37^{o}C for 24 hours. Diameters of zones of inhibition were measured and results interpreted based on criteria as recommended by CLSI.[11]

2.5.6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed using modified Kirby-Bauer's disc diffusion method and results interpreted according to performance standards of CLSI.[11] The panel of antimicrobial agents tested included, among others, those that are commonly used locally in empirical treatment of *Staphylococcus aureus* infections. The susceptibility discs (Oxoid Ltd., Basingstoke, England) and their disc strengths are as follows: Vancomycin(30µg), Erythromycin (15µg), Penicillin G (10 Units), Tetracycline (30µg), Levofloxacin (5µg), Ciprofloxacin (5µg), Gentamicin (10µg), Cefuroxime (30µg), Chloramphenicol (30µg), Amoxicillin/clavulanic acid (Augmentin) (30µg).

Statistical Analysis

Data obtained were analysed using Statistical Package for Social Science (SPSS) version 21. Frequency, prevalence, mean and standard deviation were performed. Pearson correlation was used to check for interrelationships between variables and p-values less than 0.05 were considered significant.

3 RESULTS

Table 3.1 presents the socio-demographic data of study participants for location, sex, age and education. Table 3.2 shows the overall Prevalence of *S. aureus* colonization, as well as the occurrence of toxin-producing *S. aureus* in the study population. One hundred and one (28.1%) of the 360 subjects were colonized. From a total number of 101 *S. aureus*, 38 (37.6%) were positive for enterotoxin production while 63 of the organisms were negative for toxin production. Frequency distribution of Toxin-Producing *S. aureus* with respect to the various locations showed that Eleme had a higher rate of 43.6% as against Oyibo (33.9%). There were more female carriers 28(41.8%) than their male counterparts 10(29.4%). Participants aged 40 and above recorded the highest prevalence both to *S. aureus* colonization (37.5%) and enterotoxin-producing *S. aureus* (66.7%). Participants that had only primary education recorded the highest rate in terms of toxin-producing *S. aureus* (42.3%). Table 3.3 shows antimicrobial resistance profile of *S. aureus* isolates. Organisms showed highest resistance to penicillin (92.1%) and the least to vancomycin (3.9%). A total of 22 (21.8%) *S. aureus* Isolates were Methicillin Resistant *S. aureus (*MRSA*).*

Table 3.1: Distribution of Socio-demographics of Study Participants.

Variable	Classification	No tested (n = 360)	Percent (%)
Location	Eleme	180	50.0
	Oyibo	180	50.0
Sex	Female	283	78.6
	Male	77	21.4
Age	Less than 20 Years	40	11.1
	20 – 29 Years	192	53.3
	30 – 39 Years	120	33.3
	40 Years & Above	8	2.2
Education	Non-Formal	50	13.9
	Primary	88	24.4
	Secondary	191	53.1
	Tertiary	31	8.6

Table 3.2: Distribution of *S. aureus and* Enterotoxin-Producing *S. aureus among Food Handlers.*

Variable	Classification	No. Tested (n)	Number Positive for *S. aureus* (%)	Number of *S. aureus* Positive for Toxin Production (%)	p- value
	Total population	**360**	**101 (28.1)**	**38(37.6)**	
Location	Eleme	180	39 (21.7)	17(43.6)	0.006
	Oyibo	180	62 (34.4)	21(33.9)	
Sex	Female	283	67 (23.7)	28(41.8)	0.000
	Male	77	34 (44.4)	10(29.4)	
Age	Less than 20Years	40	12 (30.0)	2(16.7)	
	20 - 29Years	192	56 (29.2)	20(35.7)	0.880
	30 - 39Years	120	30 (25.0)	14(46.7)	
	40Years & Above	8	3 (37.5)	2(66.7)	
Education	Non-Formal	50	29 (58.0)	9(31.0)	
	Primary	88	26 (29.6)	11(42.3)	0.000
	Secondary	191	46 (24.1)	18(39.1)	
	Tertiary	31	0 (0.0)	0 (0.0)	

Table 3.3: Antimicrobial susceptibility profile of 101 *S. aureus* Isolates.

Antimicrobial Agent (conc)	Resistant (%)	Sensitive (%)
Augmentin	68(67.3)	33 (32.7)
Erythromycin	62(61.3)	39 (38.6)
Ciprofloxacin	41 (40.6)	60 (59.4)
Levofloxacin	48(47.5)	53 (52.5)
Gentamicin	62(61.4)	39 (38.6)
Penicillin	93(92.1)	8 (7.9)
Cefotaxime	47(46.5)	54 (53.5)
Chloramphenicol	68(67.3)	33 (32.7)
Tetracycline	73(72.3)	28 (27.7)
Vancomycin	4(3.9)	97 (96.0)
Oxacillin	31 (30.7)	70 (69.3)
Cefoxitin	22 (21.8)	79 (78.1)

MRSA=22 (21.8%); MSSA=79 (78.2%)'

4 DISCUSSIONS

Food contamination, occasioned by food handlers, continues to be a major challenge in our sub-urban localities. This study revealed a 28.1% prevalence of *S. aureus* among asymptomatic Food handlers from our setting. The rate is higher than a prior Iran-based study, which reported a prevalence of 20.1% *S. aureus* among food handlers,[12] but about twice lower than those reported by Eke *et al.*,[13] and Emeakaroha *et al.*,[14] which had prevalence rates of 56% and 51.9% respectively. Vanderbergh and colleagues in a study at Netherlands reported that the isolation of *S. aureus* could vary from 20 to 55% in apparently health adult population,[15] while Al-Bahry *et al.*[16] found high asymptomatic *S. aureus* carriers ranging from 40-70% more than what was reported in this study.

From Table 3.2, the prevalence of *S aureus* among street food handlers was higher in Oyigbo with frequency of 34.4% than in Eleme (21.7%), while enterotoxin producers occurred at a higher rate (43.6%) in Eleme than in Oyigbo (33.9%). Although the reasons for these differences is not fully elucidated, it may be attributed to the socioeconomic status of foodhandlers, geographic location, background and microbial strain amongst others.

Enterotoxin-producing *S. aureus* accounted for 37.6% of the isolates in this study, a rate twice as high as 70.1% findings of Fooladvand *et al.*,[12] and the 80% reported by Jeffery *et al.*[17]

Enterotoxin produced by *S. aureus* is a vital arsenal for the organism in disease causation. The enterotoxin production recorded in this study is a pointer and calls for more awareness and hygienic best practices that should be imbibed by food handlers, as toxin-producing S. *aureus* poses threats to humans, hence a concern for public health. Results from some earlier studies had indicated that enterotoxigenicity of *S. aureus* was found to be highly linked with their possible production of DNase, hyluronidase and haemolysis.[16,18]

Gender-prevalence of *S. aureus* as obtained in this study was higher in the male (44.2%) than the female (23.7%). This is in tandem with the study of Eke *et al.*,[13] as male had higher prevalence rate of 34.8% compared to their female counterpart with 24.6%. Curiously and conversely, carriage rate of enterotoxin-producers was significantly higher in the female (41.8%) than the male (29.4%). The reason, though not resolved, could be due to their levels of hygiene.

As pertains age distribution, young aged food handlers tended to harbour the organisms more than the older aged group. This age-specific prevalence is in consonance with Omololu-Aso *et al.*[19] Again, hygiene and immunological status may also play some roles.

Antimicrobial-resistant staphylococci are a major public health concern since the bacteria can be easily spread in the environment. *Staphylococcus aureus*, particularly MRSA is well-known for its notable resistance against all β-lactams.[20] This study observed 21.8% Methicillin-Resistant *S. aureus* carriage (Table 3.3), higher than the 16.5% and 16% reported by Fooladvand *et al.*[12] and Al-Bahry *et al.*,[16] respectively. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as an important nosocomial pathogen and has reportedly been associated with foodborne illnesses.[21] Food handlers have been incriminated as likely sources of contamination.[18,22-23] Although, much still have to be known about the transmission of MRSA infections through food and food contact surfaces, however their possible roles in the dissemination of specific MRSA lineages cannot be ruled out as opined by Kyaw *et al.*[18]

About 97.0% of the isolates in this study, were susceptible to vancomycin, which corroborates with an earlier in-vitro study reported by Nwokah *et al.*,[24] and in support of vancomycin as a very potent antimicrobial against multi-drug resistant Staphylococcal infections.[25] Our finding is however, in contrast to the work of Ateba *et al.*,[26] who detected a 100% resistance of *S. aureus* to vancomycin. Remarkably, some strains that exhibited resistance to vancomycin may likely be because MRSA

strains that are resistant to beta-lactam drugs may develop induced resistance to vancomycin.[26]

Resistances to the aminoglycoside, Gentamycin and the macrolide, erythromycin were 61.4% and 61.3% respectively. Although lower than those reported in a similar study done in South-West Nigeria[19] which revealed resistant rates of 84.61% for Gentamicin and 46.18% for Erythromycin, the gradual erosion of the efficacy of these relatively cost-effective therapies is of great concern.

In general, isolates exhibited varying degrees of resistance to various commonly used antimicrobials. It is noteworthy that the challenge of antimicrobial resistance is prominent at our urban centers, especially as a result of unrestricted access and availability of antimicrobials in both clinical and other uses. However, the high degree of multi-drug resistant community-acquired *Staphylococcus aureus* carriage observed in these sub-urban study locations paints an ugly picture and this calls for serious attention.

Food handlers play significant role in food safety, particularly in the transmission of food-borne *S. aureus*[27] via involuntary act of sneezing and poking of nose. The risk of contamination increases when food is being processed without proper inspection and training of personnel on best practices with respect to food handling.

5 CONCLUSIONS

The prevalence of *S. aureus* carriage among foodhandlers in Eleme and Oyigbo was 28.1%, while a significantly higher rate was recorded at Oyigbo (34.4%) than in Eleme. Percentage Occurrence of Toxin Producing *S. aureus* was 37.6%. There were significant positive correlations between sex and age with enterotoxin production, while education was negatively correlated. *S. aureus* isolates showed maximum resistant to Penicillin antimicrobial and maximum susceptibility to Vancomycin antimicrobial. 21.8% of *S. aureus* isolates were Methicillin-Resistant and Ninety-five (95%) isolates exhibited multidrug resistance status. The public health implications of these findings and appropriate response cannot be overemphasized.

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