Antibiotic Susceptibility Profile of Staphylococcus Aureus Isolated From Food Handlers in Abuja, Nigeria Review

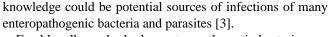
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Abstract— The antibiotic susceptibility profile of S. aureusisolated from food handlers was evaluated in Abuja using standard epidemiological and agar disc diffusion methods. The result showed that out of 180samples collected from the six area councils in Abuja, nasal swabs had 18.2% of S. aureus isolates and hand swabs had 12.3%. The prevalence rate of S. aureus was 15.5% (28) out of 180 food handlers sampled, the highest occurrence (68.8%) was observed in age group 20 - 39 years, while there was no significant difference in the distribution of S. aureus in the area sampled. Male food handlers (64.3%) had higher infection rate compared to their female counterparts (35.7%) except in AMAC and Kwali area councils. The S. aureus isolates were highly resistant to Cefoxitin and Oxacillin (82.1%) and Tetracycline (71.4%); mildly resistant to Ceftazidime (64.3%), Amoxicillin (53.6%), Erythromycin (50.0%) and Clindamycin (46.4%), but 100% susceptible to Vancomycin, Linezolid (85.7%) and Ciprofloxacin (67.9%). Significant percentages of the isolates (35.7%) weremultidrug resistant and 60.7% had MARI index > 0.2. The most common resistance pattern observed wasFOX,OX,CIP, and it occurred in 18.8% of the populace. This study showed the possibility of food handlers serving as route of transmission of antibiotic resistance pathogens and more so showcase the need to monitor the antimicrobial susceptibility profile of pathogenic bacteria in our environment as this will enable clinicians, in selecting empiric antimicrobial therapy,formulate rational public healthcare policies, and provide useful information on the global surveillance of this pathogen.

Index Terms—S. aureus, antibiotic resistance, food handlers, Abuja.

I. INTRODUCTION

The presence of microorganism of public health importance in food products for human consumption is indeed important to both clinicians and the food industry as these organisms account for significant mortality and morbidity associated with foodborne diseases [1]. Among the organisms, which are frequently isolated in unhygienic food, are norovirus, nontyphoidalSalmonella spp., Clostrodiumperfringens, Campylobacter spp., E. coli, Klebsiella spp., Proteus spp.andStaphylococcus species. These organisms are majorly transferred from the environment and the food handlers, which serve as bacteria reservoirin ready to eat food. Reference [2] showed that food handlers with poor personal hygiene and inadequate



Food handlers who harbor enteropathogenic bacteria may contaminate foods with their faeces via their fingers, which could in turn lead to food poison, and finally to infection of consumers [4]. Annually, an estimated 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths are caused by food-borne diseases in the United States [5]. Among these cases, 31 known pathogens cause 9.4 million illnesses, 56,000 hospitalizations, and 1300 deaths [6]. Among the organisms of concern areover 30 species of Staphylococcus. These species encode coagulase, heat stable nuclease or enterotoxins gene [7]. Reference [6] stated that S. aureus is a significant cause of food borne disease, causing an estimated 241,000 illnesses per year in the United States. In most foodssampled, the prevalence of S. aureusvaries based on different geographical location, type of food sampled, population size, and sources of food. For example, as in [7] in Iran had 5.8% isolation rate with the highest prevalence found in traditional cheese (11.1%), followed by traditional ice-cream (5.9%), cream (5.6%), and butter (5.3%). Reference [8] reported 26% prevalence rate of S. aureus from sushi and sashimi food outlets in the Klang Valley of Malaysia. Reference [9] reported 23.9% from raw meat (poultry, pork and beef) during a one-yearsurvey in Italy. These reports justified that Staphylococcus aureus is a foodborne pathogen that is considered one of the worlds' leading causes of disease outbreak.

The coagulase positive species of *S. aureus*have been linked tothe most common enterotoxins producing specie and this specie have been isolated from various food products such as beef, poultry, eggs, dairy, seafood, as well as bread and bakery products [10], [11]. Many virulence factors have also been characterized among coagulase positive species of *S. aureus*, and the most notable are the five major classical types of staphylococcal enterotoxins (SEs: SEA to SEE), the non-classical SE-like toxins (SEI: SEG to SEU), and other virulence genes such as toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins and cytolytic toxins (leukocidin and hemolysins). Staphylococcal enterotoxins (SEs) are heat stable proteins that are mainly associated with food poisoning outbreaks [12], [13], while TSST-1 is a superantigenic exotoxin that causes toxic shock syndrome [14].

The exfoliative toxins are responsible for staphylococcal scalded skin syndrome that typically affects infants and young children[15]. lukPVcytotoxin causes leukocytosis with necrotic lesions in the skin or mucosa as in[16] while



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hemolysins involve epithelial barrier disruption[17].Among coagulase positive species of *S. aureus*isolated from food handlers, varying degrees of multidrug resistance have been reported in different environment and strains with reduced sensibility to vancomycinare emerging worldwide. This could be attributed to the extended use and misuse of antibiotics (e.g. in agriculture, stock farming and in the treatment of human diseases), the number of bacteria that are resistant to antimicrobial agents is rapidly increasing [18]. This has necessitated the evaluation of the prevalence and antimicrobial susceptibility patterns of *S. aureusfrom food handlers in Abuja, Nigeria.*

II. MATERIALS AND METHODS

A. Study Location

The study was carried out in the six Area Councils in Abuja, FCT, consisting of Abuja Municipal, Gwagwalada, Abaji, Kuje, Kwali, and Bwari area councils. Abuja is the capital city of Nigeria. It is located in the centre of Nigeria, within the Federal Capital Territory (FCT). It has longitude and latitude of $9^{\circ}4'_{.0''N}$ 7°29'0''E. It has an area of City 713 km² Elevation 840 m (2,760 ft).

B. Ethical Approval

Ethical clearance with assertion number FHREC/2014/01/65/05-11-14 was obtained from Health Research Ethics Committee of Federal Capital Territory, Health and Human Services Secretariat, Area 11, Garki Abuja. Individual consent wasobtained using the information leaflet and consent form during sampling survey.

C. SampleCollection

A total of 180 samples from nasal, and hand swabs of food handlers in the six area councils of Abuja were collected from

road side food handlers for the period of 10 months (February 2015 to November 2015). These samples were collected using a labeled sterile swab sticks and transported in an ice park to the laboratory within two hours of collection for inoculation in nutrient broth.

D. Isolation, Identification and Biochemical Characterization of *S. aureus*

The definitive diagnosis of *Staphylococcus aureus* was made by isolation and identification. This involves microscopy, culture, coagulase, catalase, Dnase and serology. *Staphylococcus aureus* isolate were preserved on Mueller Hinton agar slant for characterization and susceptibility. The samples were subcultured onMannitol salt agar (MSA) and incubated at 37°C for 24-48 hours.*S. aureus* was identified and differentiated from related organisms based on the colony morphology (production ofgolden yellow coloured colonies as a result of mannitol fermentation and subsequent drop in the medium's pH),Gram stain reaction (Gram positive cocci in chains or clusters), and biochemical characteristics (plasma coagulase positive, positive to catalase and ability to produce deoxyribonuclease (Dnase) enzymes) as described by Cheesbrough, [19] for identification of *S. aureus*.

E. Antibiotic Susceptibility Testing

Antibiotics Susceptibilityprofiles of the isolates were performed as in [19] agar disc diffusion method using tencommonly available antibiotics. Colonies from an overnight culture of *S. aureus* isolateswere inoculated into 5mL normal saline suspension to match 0.5McFarland standard. *S aureus* ATCC 25923 was used as the control strain. Antibiotics usedand concentrations in µg are shown in interpretativechats according to CLSI [20] inTable 1.

Antibiotics	Abbreviation	Sensitivity (S)	Intermediate (I)	Resistant
Cefoxitin (30 µg)	FOX	≥22	-	≤21
Oxacillin (1µg)	OX	≥22	-	≤21
Amoxycillin (30µg)	AML	≥17	14-16	≤13
Ceftazidime (30 µg)	CAZ	≥21	18-20	≤17
Erythromycin (15µg)	E	≥23	14-22	≤13
Ciprofloxacin (5 µg)	CIP	≥21	16-20	≤15
Clindamycin (2 µg)	DA	\geq	15-20	≤14
Tetracycline (30 µg)	TE	\geq	15-18	≤14
Vancomycin (30 µg)	VA	_	-	_

 Table 1: Antibiotics Used and their Interpretative Criteria

III. RESULTS

Based on samples evaluated, nasal swab (18.2%)had the highest isolation rate of *S. aureus* followed by hand swab (12.3%) Table 2.A total of 28 *S. aureus* were isolated from six area councils sampled in Abuja from food handlers, the highest occurrence (68.8%) was observed in age group 20 - 39 years, while there was no significant difference in the distribution of *S. aureus* in the area sampled.In the areas sampled *S. aureus* was isolated more in male (64.3%) compared to their female counterparts (35.7%) except in

AMAC and Kwali area councils. High isolation rates were observed in Gwagwalada, and AMAC area councils followed by Bwari, and Kuje while the list isolation rate was observed in Abaji.The isolates were highly resistant to cefoxitin and oxacillin (82.1%) and tetracycline (71.4%), mildly resistant to linezolid (14.3%), ciprofloxacin (32%). ceftazidime (64.3%), and 100% susceptible to vancomycin.Significant percentage of the isolates (35.7%) were multidrug resistant and 60.7% had MARI index > 0.2 while the most common resistance pattern was FOX, OX, CIP, which was observed in 18.8% of the populace



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Table 2: Distribution of S. aureus by Samples Sources in Food Handlers					
Source	No sampled	No of isolate	% of Positive isolate		
Hand	81	10	12.3		
Nasal	99	18	18.2		
Total	180	28			

Table 3: Distribution of S. aureus in different Age Groups of Food Handlers

AGES	FOOD HANDLERS						
	AMA	BWAR	KUJ	KWAL	ABAJ	GWA/LA	ТОТА
	С	Ι	Ε	Ι	Ι	DA	L
< 10	0	0	0	0	0	0	0
10 - 19	0	0	0	3	0	0	3
20 - 29	4	2	2	1	0	3	12
30 - 39	2	1	2	0	0	2	7
40 - 49	0	0	1	0	2	1	4
50 - 59	0	0	0	0	0	0	0
60 - 69	0	2	0	0	0	0	2
TOTAL	6	5	5	4	2	6	28

Table 4: Gender Distribution of S. aureus among Food Handlers

GENDER	FOOD HANDLERS						
	AMA BWAR KUJ KWAL ABAJ GWA/LA						
	С	Ι	Ε	Ι	Ι	DA	TOTAL
Male	2	5	4	0	2	5	18
Female	4	0	1	4	0	1	10
TOTAL	6	5	5	4	2	6	28

Table 5: Distribution of Staphylococcus aureus According to Studied Area

	FOOD HANDLERS					
STUDY AREA	Nasal	Hand	TOTAL			
AMAC	16(3)	14(3)	30(6)			
BWARI	15(3)	15(2)	30(5)			
ABAJI	17(1)	13(1)	30(2)			
KUJE	17(4)	13(1)	30(5)			
KWALI	18(3)	12(1)	30(4)			
GWA/LADA	16(4)	14(2)	30(6)			
TOTAL	99(18)	81(10)	180(28)			

NB: Figures outside the brackets are number of samples collected while those inside are number of S. aureus isolated.



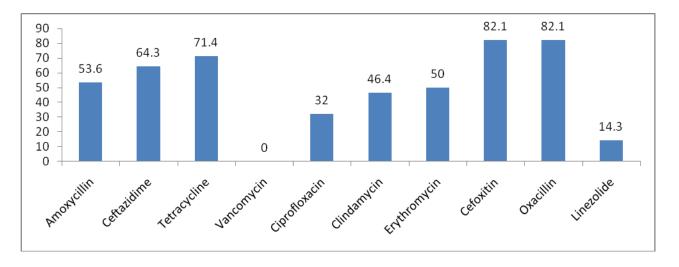


Figure 1: Antibiotics susceptibility profile of *Staphylococcus aureus* isolated from food handlers in Abuja, Nigeria.

S/N	Isolate		NART	MARI	CR
	Code	Resistant Pattern			
1.	AMFHS	AML,CAZ,E	3	0.3	NMDR
2.	AMFHS	FOX,OX,CIP	3	0.3	NMDR
3.	AMFHS	E,AML,OX	3	0.3	MDR
4.	AMFNS	FOX,	1	0.1	NMDR
5.	AMFNS	FOX,OX,CIP	3	0.3	NMDR
6.	AMFNS	FOX	1	0.1	NMDR
7.	BFHS	FOX	1	0.1	NMDR
8.	BFHS	E,TE,FOX	3	0.3	MDR
9.	BFNS	DA,	1	0.1	NMDR
10	BFNS	OX,CIP	2	0.2	NMDR
11	· BFNS	OX,E,FOX	3	0.3	NMDR
12	GFHS	FOX,CAZ	2	0.2	NMDR
13	GFHS	E,DA,OX	3	0.3	MDR
14	GFNS	FOX, AML,	2	0.2	NMDR
15	GFNS	FOX,DA,OX,CAZ	4	0.4	NMDR
16	GFNS	OX,E,DA,TE	4	0.4	MDR
17	GFNS	TE,AML	2	0.2	NMDR
18	· KJHS	CAZ,E,CIP,OX,FOX	4	0.4	MDR
19	· KJNS	CIP,TE	2	0.2	NMDR
20	· KJNS	AML,FOX	2	0.2	NMDR
21	· KJNS	TE,FOX,E,OX	4	0.4	MDR
22	· KJNS	AML,OX,FOX	3	0.3	NMDR
23	· AFHS	OX,FOX,AML,TE	4	0.4	NMDR
24	· AFNS	FOX,OX,DA,CAZ	4	0.4	NMDR
25	· KWFHS	CIP,OX,FOX,TE	4	0.4	MDR
26	. KWFNS	FOX,TE	2	0.2	NMDR
27	· KWFNS	OX,E,TE,DA	4	0.4	MDR
28	· KWFNS	OX,FOX,AML,CAZ	4	0.4	NMDR

Table 6: Antibiotics Resistant Pattern, Classification of MARI and Level of Multidrug Resistant

Key words: MARI-Multiple antibiotics resistance index,MDR-Multiple drug resistance, NMDR-Not multiple drug resistance index.



IV. DISCUSSION

Food borne diseases continue to be a major global health problem and are the leading causes of morbidity and mortality in developing countries. Food handlers play a major role in the transmission of food borne pathogens via hands, especially *Staphylococcus aureus* that is a normal flora of the human body. *S. aureus* is an important human pathogen and is implicated in a wide variety of infections [21]. Nigeria is the most densely populated African country, and Abuja is the Federal Capital Territory, with a population range of **3.1**million, and is the largest urbanized area in North-Central, Nigeria.

This study evaluated the prevalence and antibiotics susceptibility profile of S. aureus in food handlers in Abuja. The result showed a 15.6% (28/180) prevalence rate of S. aureus among the samples evaluated. The findings from this study showed that 18.2% of S. aureusisolated was from nasal swab and 12.3% from hand swab Table 2. The isolation of S. aureus from various samples collected from the food handlers is in agreement with the finding of other studies that foodborne disease outbreaks are influenced by infected food handlers, contaminated raw ingredients, cross-contamination and inadequate heat treatment. The result obtained in this study concurs with the report in [22] in Abuja who observed a prevalent rate of 40% S. aureus. The findings as in[23], noted that S. aureus is a common human commensal organism but carriage varies between geographic location, age, gender, ethnicity and body niche, this justifies the variation in percentage difference between our study and that obtained by [22].

These findings are in agreement with some other studies. Reference[24], reported that the isolation of S. aureusfrom nasal cavities, could vary from 20 to 55% in a healthy adult population. Reference [25]showed an overall prevalence of 33.3% of S. aureus in the nostrils of the healthy inhabitants of Amassoma in Niger Delta region of Nigeria which is in support with previous findings as in [26] in U.S.A and also in [27] in Japan who reported a nasal colonization in healthy adults of 32.4% and 36.0% respectively. In contrary, as in[28] findings revealed S. aureus nasal colonization rate of 43.8% in staffs of teaching hospital in Nepal. In addition, in Abia State, Nigeria; as in [29] reported 50% nasal colonization in hospital both non-hospital and subjects. Reference[30]reported a much lower (14.0%) nasal colonization in medical students in Lagos, Nigeria. The percentage of S. aureus in hand swabs of food handlers reported in this study is higher than 5.6% reported as in [31] in Jordan but lower than the report of [32] andas in[33], who observed 38.3% and 100% isolation rate of S. aureus in hand swab of food vendors in Benin city, Nigeria and India respectively. These findings showed that hand swab and nasal samples of food handlers may account for approximately high percentage of food borne illnesses in food service establishments and homes.

The variations observed in this study may be attributed to the characteristics of the population under study. Based on age, sex and area sampled, the distribution of *S. aureus* was observed high 12(68.75%) within the age group 20-39 years



Table 3. In the areas, sampled S. aureus was isolated more in male food handlers (64.3%) compared to their female counterparts (35.7%) except in Bwari and Kwali area councils Table 4. High isolation rate was observed in Gwagwalada, and AMAC area councils followed by Bwari, and Kuje while the list isolation rate was detected in Abaji Table 5. The findings of this study could be attributed to the fact that food handlers of this age group are the most active in the society, with high interaction/contact rate with one another during pleasantries; than children and elderly who might sometime be in isolation from the general populace. The isolation of S. aureus from food handlers in different sampled area showed that S. aureus infection affect both those in the rural areas and urban areas; hence, infection of patients with S. aureus is not dependent on the location, as S. aureus is a normal floral of human skin and nasal [34]; [35].

The presence of *S. aureus* in large percentage of the food handlers (male and female) reflect improper hygiene practice such as pocking nose with fingers. This study correlated with the report in[36] in Jessy, Bangladeshi and that of[37] in Benin city, who observed that food handlers hands are common source of food contamination, food spoilage, food poisoning, and causation of diseases in consumers, due to lack of training in food hygiene and safety; inadequate personal hygiene and/or cross contamination [38].A population that is on antibiotics as at the time of sampling may yield a much lower prevalence of *S. aureus* while a population from hospital settings may yield a much higher prevalence because of the high prevalence of infectious patients in that environment. Other factors that can cause variations may be sampling and culture techniques [30].

The antibiotics susceptibility profile showed that the isolates were highly resistant to cefoxitin, and oxacillin (82.1%) and tetracycline (71.4%), mildly resistant to ceftazidime (64.3%), amoxicillin (53.6%), erythromycin (50.0%) and clindamycin (46.4%), but susceptible to vancomycin (100%), linezolid (85.7%) and ciprofloxacin (67.9%) Figure 1.The findings observed in this study correlate with other studies that percentage resistance of S. aureus varies by different antibiotics and geographical location [39]. High resistance to cefoxitin and oxacillin might be associated to the presence of MRSA genes, which has been reported to code for resistance against all beta lactamases. This trait has always been an obstacle for antimicrobial chemotherapy, and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen [40];[41].Reference[42]has however noted that high resistance observed in community-associated infections might be because of the use of sub-dose of these drugs in poultry and misuse of these drugs in the hospitals.

Susceptibility of these isolates to Vancomycin and Linezolid might be linked to the findings of other studies that Vancomycin remains the reference standard for the treatment of systemic infection caused by MRSA as a result of its relatively clean safety profile, its durability against the development of resistance, and, for many years, the lack of other approved alternatives [43]. The work of as in[44] supported the claim that linezolid could be used as alternative drug to Vancomycin as it has been found to showcasepromising therapeutic option in an era of rapidly growing antibiotic resistance [45]. In an event of failure, new treatment options for invasive MRSA infections have been reported to include linezolid, daptomycin, tigecycline, and quinupristin/dalfopristin [43].

Significant percentage (35.7%) of the isolates were also multidrug resistant, 60.7% had MARI index > 0.2 while the most common resistance pattern was FOX, OX, CIP, which was observed in 18.8% of the populace Table 6. This indicates that some of the isolates have been exposed to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms [42].

V. CONCLUSION

This study observed high prevalence of S. aureus among food handlers sampled in Abuja, Nigeria. It also showed that the isolates could harbor multidrug resistant genes of public health importance. It also emphasized the importance of food handler's hands, and nasal as a potential vector for food borne bacteriacontaminants that could constitute a possible risk to food borne outbreaks. Hence, new employees, young and inexperienced food handlers should be well trained on personal hygienic practices; pointing out on the importance of hand hygiene and appropriate hand washing techniques. This study also suggests the need to carry out periodic surveillance of the antimicrobial susceptibility patterns of S. aureus in our environment, as this is important in understanding new and emerging resistance trends, and could however, help in the management of both hospital- and community-acquired infections.

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