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Cross Sectional Study of Nasal Contamination of *Staphylococcus aureus* among Food Handlers in Eateries of the Obafemi Awolowo University, Ile Ife, Osun State Nigeria

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Abstract

Food borne diseases encompass a wide spectrum of illnesses especially in underdeveloped and developing countries and poor personal hygiene resulting in the hands being heavily contaminated not only with the enteric rods but gram positive cocci pathogens.

In this study twenty two (22) nasal sources subjects were taken using sterile swab stick on nasal cavities. Following conventional methods of isolation with gram stain, catalase, coagulase reactions couple with antibiotic resistance procedures were adopted.

About 13.6% Streptococcal and 86.4% Staphylococcal isolates recovered and of which 68.2% confirmed positive for *Staphylococcus aureus*. All isolates were susceptible to pefloxacin, streptomycin and septrin, while there was 100% isolates resistant to ampiclox used. 10 (83.3%) of isolates tested were susceptible to gentamicin with no isolate being an intermediate. 11 (91.7%) showed resistance to the zinacef antibiotic while 1 (9.3%) was of intermediate value. The same trend is seen in amoxicillin which is also a beta-lactam compound, having a frequency 83.3% resistant strain, and both the susceptible and resistant strains with frequencies of 8.3% respectively. The septrin antibiotic used showed similar trend to that observed in amoxicillin and zinacef as it had a frequency of 83.3% resistant strain and 16.7% intermediate strain. For rocephin, the frequency of resistance was 58.3% while the frequencies for the intermediate and susceptible strains were 16.7% and 25% respectively. The higher rate of susceptible strains was observed with erythromycin where a frequency of 66.7% as compared to the susceptible and intermediate strains, both having a frequency of 16.7% each.

In conclusion, pefloxacin, ciprofloxacin and streptomycin could be useful as potent antibiotics in the treatment of nasal Staphylococcal infections. However, effective hygiene modality concepts should be drawn for all food handlers in the study area.

Keywords: Food handlers; Antibiotics; *Staphylococcus aureus*; Infection; Ciprofloxacin; Nasal cavity

Introduction

Food is the basic need for sustenance of human life. Food can be contaminated from the production level to consumption level. Unsafe food and water have been a human health problem since history was first recorded, and many food safety problems encountered today are not new. Health is a dynamic process of adjustment and readjustment to the continuous changing environmental conditions. Among the various factors which play a important role in the maintenance of health of which nutrition is one. In the food industry, contamination from microorganisms can be responsible for infectious disease outbreaks passed from food employees to consumers through food. The origins of microbial contaminants in food surface facilities include the environment, food workers, the source of the food, and the food itself. According to a report by the Centers for Disease Control and Prevention (CDC), hands may be the most important means by which enteric pathogens are transmitted if employees do not wash their hands adequately. Food borne illnesses have a dramatic impact in both developing and developed countries. Food becomes unsafe when it was contaminated with harmful organisms and/or chemical ingredients. Unsafe food generates a vicious cycle causing food borne diseases ranging from self-limiting or mild gastrointestinal

symptoms (such as abdominal pain, mild diarrhea) to severe form of diseases like severe diarrhea, dysentery or cancers.

Many foods borne disease outbreaks are reported every year all over the world. Numerous factors, contribute to this high number of incidents. However, it is important to note that most cases of food borne disease are not reported, so the true extent of the problem is unknown. In most countries of the region, the surveillance infrastructure for food borne diseases of both microbiological and chemical etiology is weak or non-existent. This absence of reliable data on the burden of food borne disease impedes understanding about its public health importance and prevents the development of risk based solutions to its management.

American centers for Disease Control and Prevention (CDC) estimated 48 million people suffer from a foodborne infection, 128,000 persons are hospitalized and 3,000 people die every year in the United States. It was stated that >4,000 food borne outbreaks including water-borne outbreaks in Europe 2016. In Ethiopia, *Staphylococcus aureus* (*S. aureus*) and other bacteria such as *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeruginosa* were the common bacterial contaminants among food handlers.

In this study, we investigated cross sectional study of nasal contamination of *Staphylococcus aureus* among food handlers in eateries of the Obafemi Awolowo university, Ile Ife, Osun state.

Materials and Methods

Collection of samples

The specimens were collected from food handlers by means of sterile cotton swabs moistened in sterile nutrient broth. The swab put into the nasal cavity of the food handlers *via* the nose. Each swab was placed in small tube, labeled and immediately transported to the research laboratory.

Isolation of *Staphylococcus aureus*

The samples collected from the nasal cavity of food handlers were immediately immersed into nutrient broth contained in a McCartney bottle and incubated at 37˚C for 24 hours to encourage the growth of the organisms and to prevent the microorganisms from dying off at inappropriate temperatures if kept too long after which the broth is observed for growth of the microorganisms indicated by turbidity of the liquid medium. The bottles containing turbid liquid medium were then streaked on nutrient agar plates aseptically and incubated at 37˚C for 24-48 hours.

Identification and characterization

Sample culture from nutrient broth was cultured on a solid selective media mannitol salt agar and on differential media; MacConkey agar. All the plates were incubated at 37°C for 24 hr. Discrete colonies were further sub cultured onto freshly prepared plates of nutrient agar. Gram staining, morphological identification, catalase and coagulase tests, sugar fermentation analysis and antimicrobial susceptibility trends of the isolate were conducted.

Results

A total of 19 staphylococcal isolates were recovered from 22 isolates collected from the nasal cavity of food handlers from several eateries within the Obafemi Awolowo university campus, and 16 were confirmed *Staphylococcus aureus* (Tables 1-3 and Figure 1).

Of the 22 isolates, 3 isolates were identified to be of the genus *Streptococci*.

Table 1: Identification of *Staphylococcus aureus* isolates in sample sources.

S/N	Code name	Gram stain	Shape	Catalase	Oxidase	Citrate test	Indole	Coagulase	DNase	Organism
1	X_1	Purple	Cocci	+ve	-ve	+ve	-ve	-ve	-ve	*Staphylococci*
2	X_2	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
3	Y_1	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
4	Y_2	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
5	Z_1	Purple	Cocci	+ve	-ve	+ve	+ve	+ve	+ve	*Staphylococcus aureus*

6	Z_2	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
7	JMS1	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
8	JMS2	Purple	Cocci	-ve	-ve	-ve	+ve	-ve	-ve	*Streptococci*
9	MMS1	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
10	MMS2	Purple	Cocci	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococci*
11	$FH1^{-1}$	Purple	Cocci	+ve	-ve	+ve	+ve	+ve	+ve	*Staphylococcus aureus*
12	$FH1^{-2}$	Purple	Cocci	+ve	-ve	+ve	+ve	+ve	+ve	*Staphylococcus aureus*
13	$FH2^{-1}$	Purple	Cocci	+ve	-ve	+ve	+ve	-ve	-ve	*Staphylococci*
14	$FH2^{-2}$	Purple	Cocci	+ve	-ve	+ve	+ve	+ve	+ve	*Staphyloc occus aureus*
15	$FH3^{-1}$	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
16	$FH3^{-2}$	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
17	$FH4^{-1}$	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
18	$FH4^{-2}$	Purple	Cocci	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococci*
19	$FH5^{-1}$	Purple	Cocci	+ve	-ve	+ve	+ve	-ve	-ve	*Staphylococci*
20	$FH5^{-2}$	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
21	$FH6^{-1}$	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*

22	$FH6^{-2}$	Purple	Cocci	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*

Overall triple sugar iron test results on isolates

Table 2: Triple iron sugar test was carried out on the following samples: X_1, X_2, Y_1, Y_2, Z_1, Z_2, JMS1, JMS1, MMS1, MMS2.

Isolates	Sugar	Gas	H_2S
X_1	Absent	Absent	Present
X_2	Absent	Absent	Present
Y_1	Absent	Absent	Present
Y_2	Absent	Absent	Present
Z_1	Absent	Absent	Present
Z_2	Present	Present	Absent
JMS1	Present	Absent	Absent
JMS2	Present	Absent	Absent
MMS1	Present	Absent	Absent
MMS2	Present	Absent	Absent

Table 3: Overall percentage antibiotic sensitivity patterns of *Staphylococcus aureus* isolates from food handlers.

Antibiotics	N	NR	%	NI	%	NS	%
PEF	12	0	0	0	0	12	100
CN	12	2	16.7	0	0	10	83.3
APX	12	2	100	0	0	0	0
Z	12	11	91.7	1	8.3	0	0
AM	12	10	83.3	1	8.3	1	8.3
R	12	7	58.3	2	16.7	3	25
CPX	12	0	0	0	0	12	100
S	12	0	0	0	0	12	100
SXT	12	10	83.3	2	16.7	0	0
E	12	2	16.7	2	16.7	8	66.7

Note: N: Total number of isolates; NR: Number of Resistant isolates; NI: Number of Intermediate isolates; NS: Number of Susceptible isolates; PF: Pefloxacin (10 mg); CN: Gentamycin (10 mg); APX: Ampiclox (30 mg), Z: Zinnacef (20 mg); AM: Amoxicillin (30 mg); R: Rocephin (25 mg); CPX: Ciprofloxacin (30 mg); S: Streptomycin (30 mg); SXT: Septrin (30 mg); E: Erythromycin (10 mg).

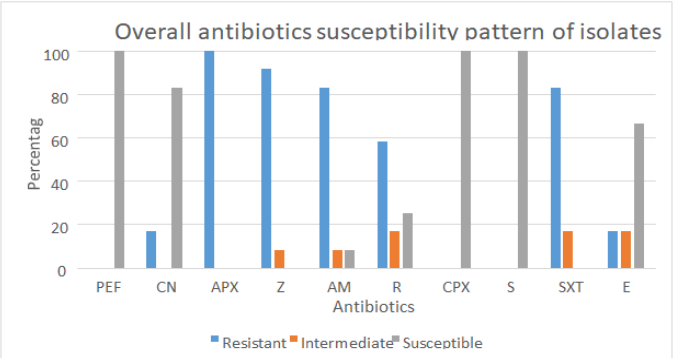

Figure 1: Chart showing the overall antibiotics susceptibility of isolates.

Note: PF=Pefloxacin (10 mg); CN=Gentamycin (10 mg); APX=Ampiclox (30 mg); Z=Zinnacef (20 mg); AM=Amoxicillin (30 mg); R=Rocephin (25 mg); CPX=Ciprofloxacin (30 mg); S=Streptomycin (30 mg); SXT=Septrin (30 mg); E=Erythromycin (10 mg).

Discussion

Food handlers have been implicated in a plethora of foodborne diseases, and it has been reported that one of the important pathogens o ten transmitted *via* food contaminated by infected food handlers is *S. aureus*.

Staphylococcus aureus is an opportunistic pathogen for both human and livestock population, and is one of the most frequent etiological sources of foodborne illnesses. It is a common colonizer of the skin and nose has become one of the most successful adaptable human pathogens. Although food handlers represent a section of the healthy population in the community, their carriage of *S. aureus* reported [1] to have remarkable ability to acquire antibiotic resistance contributing to its emergence as an important pathogen in a variety of setting, is a great cause of public health concern [2].

During the course of this study revealed that although there was no frequent training of food hygiene, yet some had an excellent knowledge related to food safety but they did not translate their knowledge into practice in spite of positive responding of food handlers for food safety related questions [3]. Our results were in coincidence with Kibret and Abera results as they concluded that poor hygienic practices by food handlers coupled with poor sanitary conditions in food and drink establishments can contribute to foodborne illnesses [4-7].

Green et al., documented that improvement of food worker hand washing practices is critical to the reduction of foodborne illness and was dependent upon a clear understanding of current hand washing practices, hand washing and glove use were more likely to occur in conjunction with food preparation than with other activities [8]. He also reported that only 32% of the workers were attempted to wash their hand frequently during food preparation [9].

In this study, out of the total number (22) of isolates, 13.6% Streptococcal and 86.4% Staphylococcal isolates were recovered, of which 68.2% con irmed positive for *Staphylococcus aureus* which was slightly higher in comparison to the 57.7% detected in Gaborone, Botswana. (n=200) by Loeto et al.

In a study in Spain, 27.6% of 300 food handlers were found to be nasal coagulase positive *Staphylococci* carriers. The study in Kuwait city by Al Bustan M.A. et al., revealed that (26.6%) of 500 restaurant workers were nasal *S. aureus* carriers. In Brazil, (24.4%) and (75.6%) of nasal and hand swabs of 82 food handlers were positive for coagulase positive and coagulase negative *staphylococci*. There were (19.8%) and (10.4%) isolates from the nasal cavity of the food handlers respectively. In contrast, the result of the present study is higher than those studies [10].

The result of the antimicrobial sensitivity pattern of 12 isolates from food handlers shows that, all isolates (100%) were susceptible to pefloxacin, streptomycin and septrin, while there was 100% resistance to ampiclox.

For gentamycin, 10 (83.3%) of the 12 isolates tested were susceptible to the antibiotic while 2 (16.7%) of the 12 isolates were resistant to the antibiotic, with no isolate being an intermediate.

For zinacef, 11 (91.7%) showed resistance to the antibiotic while 1 (9.3%) was intermediate. Zinacef is a member of the class of drugs, known as the cephalosporins antibiotics which belong to the group of beta-lactam group of antibiotics. The same trend is seen in amoxicillin which is also a beta-lactam compound, having a frequency 83.3% resistant strain, and both the susceptible and resistant strains with frequencies of 8.3% respectively. The high frequency of resistance to zinacef and amoxicillin in these strains of *Staphylococcus aureus*, is because the bacteria “has developed resistance to the beta-lactam antibiotics due to the production of chromosomal or plasmid mediated beta-lactamase.

Septrin also showed similar trend to that observed in amoxicillin and zinacef as it had a frequency of 83.3% resistant strain and 16.7% intermediate strain.

For rocephin, the frequency of resistance was 58.3% while the frequencies for the intermediate and susceptible strains were 16.7% and 25% respectively.

Finally, for erythromycin, a higher rate of susceptible strains was observed with a frequency of 66.7% as compared to the

susceptible and intermediate strains, both having a frequency of 16.7% each.

From the observations above, pefloxacin, ciprofloxacin and streptomycin could be useful as potent antibiotics in the treatment of Staphylococcal infection from the results of this study. Whereas ampiclox, zinacef, amoxicillin and septrin can be seen as the least effective as they had the higher frequencies of resistant strains, indicating that almost all isolates were resistant to them.

Conclusion

The pathogenic strain of *Staphylococcus aureus* present in the nasal cavity of food handlers, increases the risk of food poisoning. If by chance, a food handler carries, an enterotoxin producer *Staphylococcus aureus* he/she may contaminate the food and causes Staphylococcal food poisoning outbreak in the population.

This study revealed high bacterial contamination and antibacterial resistance to *Staphylococcus aureus* from the food handlers, justifying the screening testing methods to detect carriers and protect people from Staphylococcal food poisoning before appointing and during working as food handlers in eateries. Misuse of antibiotics paves the way for the *Staphylococcus aureus* to cause infections and to develop drug resistance.

These findings indicate that the food handlers may be potential source of food borne disease for the students' population being served in these eateries.

Also *Staphylococci* distributed in the environment and strains present in the nose often contaminate the back of hands, fingers and face, and nasal carriers could therefore easily become skin carriers.

Food handlers in public places are required to go through regular screening for both nasal and skin carriage of *Staphylococcus aureus* for the early detection and treatment of carriers so as to protect the community from staphylococcal food poisoning and the spread of resistant *Staphylococcus aureus* strains among the population.

Resistance to antimicrobials in commonly used antimicrobial such as cloxacillin has shown increasing prevalence in last of 15 years. It is thought that sustained like antibiotics may enhance the ability of microbes to resist the presence of antibiotics or drugs. It is advisable that prudent use of antibiotics and infection control, sanitation and hygiene practices are steps that must be taken now to stem the trend of rising resistance.

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