PREVALENCE OF STAPHYLOCOCCUS AUREUS IN NASAL AND SKIN OF APPARENTLY HEALTHY FOOD HANDLERS AND ATTENDANTS IN RESTAURANTS

*Iyevhobu K.O, 2Momoh, A.R.M, 3Etafo, J, 4Airefetalor A.I, 5Osagiede, E.K.

1National Open University of Nigeria, Uromi Community Study Centre, Uromi, Edo State, Nigeria.
2Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
3Department of Medical Laboratory Science, Federal Medical Centre, Owo, Ondo State, Nigeria
4CEPI/ISTH Lassa Fever Epidemiology Study, Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo State, Nigeria.
5Lily Hospitals Limited, Benin City, Edo State, Nigeria.

Abstract

Food handlers play a major role in the transmission of food borne diseases which represents a global health burden. Carriage of Staphylococcus aureus, in general, and enterotoxigenic strains, in particular, is an important risk factor for the contamination of food. This study was undertaken to determine the prevalence and risk factors associated with nasal and skin carriage of Staphylococcus aureus among 150 food handlers working in different restaurant in Ekpoma. Thirty (10%) persons were found to be significant (P<0.05) carriers of Staphylococcus aureus of which highest occurrence of 24 (16%) from anterior nasal nares and 6 (7.5%) from skin of food handlers and restaurant workers. Prevalence and distribution of Staphylococcus aureus in relation to gender among food handlers and restaurant workers, showed high occurrence in females of 17 (56.6%) than males 13 (43.3%) with no significant difference in comparison of variability (P>0.05). Susceptibility pattern of Staphylococcus aureus isolated from this study had high sensitivity pattern of 93% to Zennacef, 80% to Rocephin, 93% to Ciprofloxacin, 70% to Gentamycin, intermediate sensitivity to Septrin 53%, Streptomycin 50%, and resistant to Erythromycin 40%, Amoxicilin 36% and Ampiclox 17%. From this study, Staphylococcus aureus is the most prevalent among them isolates that colonizes the skin and mucosal surfaces of healthy food handlers and restaurant workers. These findings resurges the imperative need for protective measures including increased public awareness programs, regular monitoring of food handlers for food borne pathogens and intensive training on primary health care and hygiene and future research addressing effective methods for sustained eradication of Staphylococcal skin and nasal carriage are clearly warranted to reduce the high risk of subsequent infection. It is our opinion that concerted efforts need to be made to educate food handlers and restaurant workers on the importance of personal hygiene and the use of protective gadgets like nose masks while handling food products; since they serve as potential sources of staphylococcal food poisoning.

Keywords: Food, Handler, Staphylococcus, Restaurant, Nasal, Skin.
INTRODUCTION

Staphylococcus aureus avoidable medical and economic burden, the true incidence and prevalence of pathogenic strain of foodborne diseases is difficult to quantify. Risk factors implicated in foodborne diseases as identified by Centers for Disease Control and Prevention included unsafe sources, inadequate cooking, improper holding, contaminated equipment and poor personal hygiene implicating that the food handler dimension is crucially important (FDA, 2009). Food handlers have been implicated in a plethora of foodborne diseases. It has been reported that one of the important pathogens often transmitted via food contaminated by infected food handlers is Staphylococcus aureus (Verkaik et al., 2011). Bacteria of the genus Staphylococcus are Gram-positive cocci that are microscopically observed as individual organisms (Francois and Schrenzelg, 2008). Staphylococcus aureus is pathogenic ubiquitous species and may be a part of human flora found in the axillae, the inguinal and perineal areas, and the anterior nares (Bayer et al., 1998). Von Eiff et al., (2001) described 3 patterns of carriage: those who always carry a strain, those who carry the organism intermittently with changing strains, and a minority of people who never carry Staphylococcus aureus (Bayer et al., 1998). Persistent carriage is more common in children than in adults (Iwase et al., 2010). Nasal carriers may be divided into persistent carriers with high risk of infection and intermittent or non-carriers with low risk of infection (Blot et al., 2002). Direct invasion through breaks in the skin or mucus membrane leads into the production of superficial local infections such as folliculitis, furuncles and abscesses (Wertheim et al., 2005). This versatile pathogen is very well adapted to colonize the human skin and the human body provides some major ecological niches for this species. The anterior nares is the most frequent carriage site for Staphylococcus aureus, nonetheless extranasal sites typically harbor the organism including the skin, perineum and pharynx (Wertheim et al., 2005; Verkaik et al., 2011).

Until recently, reports on food contamination by Staphylococcus aureus, were mainly limited to occasional detections in the environment, the source of food and food itself. However, it is 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 (Jordá et al., 2012). Consideration into risk factors, transmission routes and many aspects of prevalence of carriage of foodborne pathogens among food handlers to eliminate carriage is necessary. Bodies concerned with food safety are left to consider whether interventions such as decolonization, continued monitoring or restrictions in the occupational activities are required (National Disease Surveillance Centre, 2004). Although skin carriage of Staphylococcus aureus, is less reported than nasal carriage, little is known about the prevalence and risk of skin carriage of enterotoxigenic strains of Staphylococcus aureus, among food handlers. Accordingly, the current study investigated the prevalence and risk factors associated with anterior nasal nares and skin carriage of Staphylococcus aureus, amongst food handlers working in different restaurant in Ekpoma Edo State.

Healthy carriers are potential source of Staphylococcus aureus infection and spread to other body sites as well as to other individuals. Staphylococcus aureus have been found frequently as aetiological of a variety of human infections. Centre for disease control (CDC) reported Staphylococcus aureus as primary source of infections, which could be transferred from individual to another, The organism also elaborates toxins that can cause specific diseases or syndromes and likely participate in the pathogenesis of staphylococcal infection. Enterotoxin-producing strains of S aureus cause one of the most common food-borne illnesses (food poisoning). The most common presentation is acute onset of vomiting and watery diarrhea 2-6 hours after ingestion. The symptoms are usually self-limited. The cause is the proliferation of toxin-producing organisms in uncooked or partially cooked food that an individual carrying the staphylococci has contaminated (Matthews et al., 1997). This study is set determines the prevalence of Staphylococcus aureus from skin and nasal nares of apparently healthy food handlers in restaurant which could be the source of Staphylococcus aureus food contamination resulting to food born infection in Ekpoma.

MATERIALS AND METHODS

This project work from its inception, sample collection, sample analysis and compilation was carried out within a period of four months with a total of hundred Fifty Skin and nasal swab samples from different restaurant workers and food handlers in Ekpoma. A total of one hundred fifty (150) nasal and skin swab were randomly collected from male and female food handlers and restaurant workers grant consent.

Informed consent was requested and granted by the food handlers and restaurant workers under investigation. The concept of the study was explained to them and having understood its dimensions, granted their informed consent. Sample Collection: One hundred and fifty specimen were collected randomly from males and females food handlers and restaurant workers within Ekpoma metropolis. The samples (150 nasal swabs and 150 skin swab). Nasal swab were collected in good light vision from subjects by bending

their heads backward to collect the specimens deep down the anterior passages using a sterile swab stick. Both right and left nostrils were swabbed bearing labels as nasal swabs, sex, code number and date of collection. The swabs sticks were carefully returned to their sterile containers, sealed with adhesive tape and labelled accordingly. Skin swab was collected by swabbing their skin (especially their fore arm) with a swab moist with physiological saline aseptically and the swabs sticks were carefully returned to their sterile containers. Collected specimen was taken to the laboratory where bacteriological analysis was carried out immediately.

**Procedure for Culture:** The swab stick were used to make a primary inoculum on each agar surface (blood agar and chocolates agar plate). Spreading was done by streaking from the primary inoculum using a sterile inoculating wire loop to obtain discrete bacterial colonies. The plates were then incubated at 370C for 24 hours. Growth was observed after incubation, and the colonial morphology was studied carefully, noting the size, shape, edge, colour, consistency, haemolysis, elevation and opacity of the colonies. This was followed by Gram staining (Ochei and Kolhatkar, 2000).

**Method for Detection of Staphylococcus aureus:** The colonies that were yellow pigmented or cream white (Cheesbrough, 2000) were sub-cultured onto mannitol salt agar and selected for catalase (using H2O2) and coagulase tests (using plasma). Mannitol fermenting and slide coagulase positive isolates were identified as Staphylococcus aureus.

**Antibiotic Sensitivity Test:** Antibiotic disc such as Erythromycin, Gentamycin, Streptomycin, Ciprofloxacin, Ampicillin, Septrine, Zinnacef, Amoxicillin and Rocephin (manufactured by Abtek Biologicals Ltd) were used to test the susceptibility of Staphylococci aureus isolates obtained. The test isolates were inoculated into sterile peptone water broth. The antibiotic discs were placed aseptically on the seeded plate. They were incubated at 370C for 24hours and examined for zones of inhibition. The zones of inhibition were measured in millimetres and recorded. Antibiotic zones less than 10mm in diameter were recorded as been resistant (R) by the organism while those with diameters of 10mm and above were recorded as sensitive (S).

**Statistical Analysis:** The collected data was expressed as Frequency and percentage. Comparison of qualitative variables was made using chi-square test. In all cases studied, the difference having p<0.05 were considered statistically significant using interactive calculation Chi square tool software (version 18).

**RESULTS**

Based on standard bacteriological analytical methods, from investigation of 300 samples of [nasal swab (150), skin swab (150)] from food handlers and restaurant workers in Ekpoma, revealed 30(10%) distribution of Staphylococcus aureus prevalence with the highest occurrence of 24 (16%) from nasal swab and 6 (7.5%) from skin swab. Other growths of non- Staphylococcus aureus were excluded from this study. The significant difference of Staphylococcus aureus isolates distribution among samples in this study was statistically significant (P< 0.05) with X2cal=26.057 p-value 0.000 (Table 1).

Table 2. shows the prevalence of Staphylococcus aureus among food handlers and restaurant workers. Sample collected from anterior nasal nares of subject with high prevalence of 24(16%) Staphylococcus aureus to that of the skin sample of 6(7.5%). From the total of 67 (22%) bacteria infected subject which was comparatively significant (P>0.05) with X2Cal 18.4, P- value 0.000.

Table 4.3 shows the distribution of Staphylococcus aureus in relation to gender among food handlers and restaurant workers, which showed high occurrence of Staphylococcus aureus in female food handlers and workers of 17 (56.6%) from females and 13 (43.3%) from males co-workers with no statistical significant difference (P>0.05) distribution of Staphylococcus aureus (X2cal=1.663, p-value=0.435).

Table 4 shows the sensitivity pattern of Staphylococcus aureus isolated from food handlers and restaurant workers. Staphylococcus aureus had various degree of sensitivity to antibiotics used and was sensitive to Gentamycin (70%), Zennacef, (93%), Rocephin (80%), Ciprofloxacin (93%), intermediate sensitivity to Septrin (53%), Streptomycin, and resistant to Erythromycin (60%), Amoxicilin (36) and Ampicloxx (17%).

**Table 1:** Distribution of Staphylococcus aureus among Food Handlers and Restaurant Workers

<table>
<thead>
<tr>
<th>Samples</th>
<th>(N) Examined</th>
<th>(N) infected (%)</th>
<th>S. aureus (%)</th>
<th>Other bacteria and no Growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal</td>
<td>150</td>
<td>48(32)</td>
<td>24(16)</td>
<td>102(68)</td>
</tr>
<tr>
<td>Skin</td>
<td>150</td>
<td>19(13)</td>
<td>6(7.5)</td>
<td>131(87)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>67(22)</td>
<td>30(10)</td>
<td>233(78)</td>
</tr>
</tbody>
</table>

X2cal=26.057, Degree of freedom=2, p-value=0.0000

Key: N - Number , S. aureus: Staphylococcus aureus
**Table 2:** Prevalence of *Staphylococcus aureus* among Food Handlers and Restaurant Workers

<table>
<thead>
<tr>
<th>Samples</th>
<th>S. aureus (%)</th>
<th>N (N)</th>
<th>(N) infected (%)</th>
<th>Sk</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal</td>
<td>150</td>
<td>48(32)</td>
<td>24(16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>150</td>
<td>19(13)</td>
<td>6(7.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>67(22)</td>
<td>30(10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$X^2{cal}=18.04$, Degree of freedom=2, $p$-value=0.000,
Key: N - Number

DISCUSSION

*Staphylococcus* species are regional flora of the skin and mucus membrane of the body, certain species have been found frequently as aetiological agent of a variety of human and animal infections. The most common among these infections are the superficial supportive infection caused by *Staphylococcus aureus*. Infection can result to life threatening conditions disease spectrum which includes abscesses, septicemia, osteomyelitis, endocarditis and cellulitis, pneumonia, in addition to various toxin mediated diseases as toxic shock syndrome and staphylococcal food poisoning. The variety of such spectrum of clinical manifestations is mostly dependent on the numerous virulence factors produced by each strain (Vasconcelos and da Cunha, 2010). The ingestion of the preformed toxins produced by *Staphylococcus aureus* (enterotoxigenic strains) in food often results to the development of food poisoning. Findings from this investigation indicate a significant ($P<0.05$) distribution of *Staphylococcus aureus* of 30(10%) prevalence with the highest occurrence of 24 (16%) from anterior nasal nares of food handlers and restaurant workers, 6 (7.5%) from skin swab which is in agreement with investigation reported by Mous-tafa et al., (2013) of 10.5% Nasal Carriage of *Staphylococcus aureus* and Risk Factors among Food Handlers-in Egypt. The findings from this studied in relation to area of study, was not in agreement with findings report by Eke et al., (2015), with a wide variation of 60% prevalence from 100 nasal swab analysis of food handlers and restaurant workers in Ekpoma. The reduced significant prevalence from this study is proportionately an improve hygiene of food handlers and workers in restaurant. This study variables revealed that gender, age, marital status nor level of education had no significant effect with respect to the nasal and skin carriage of *Staphylococcus aureus*. This study findings also reveal anterior nasal nares of food handlers and restaurant workers to harbour pathogenic *Staphylococci* species [Staphylococcus aureus 24(16%) to that of their skin 6(7.5%) with significant increase difference of ($P>0.05$) $p$-value 0.000].

Prevalence and distribution of *Staphylococcus aureus* in relation to gender among food handlers and restaurant workers, showed high occurrence in females food handlers and workers of 17 (56.6%) than males 13 (43.3%) with no significant difference in comparison of variability ($P>0.05$) and not in agreements with the findings by Eke et al., (2015), which report males food handlers to have high prevalence than the females in Ekpoma. The disparity of this report may be due to the subject who consent to participate as at time of study in regards to gender present in restaurant.

The sensitivity pattern of *Staphylococcus aureus* isolated from this study had high susceptibility to Gentamycin, Zennacef, Rocephin, CPX- Ciprofloxacin, SXT-Septrin, S-Streptomycin, E-Erythromycin AM- Amoxicillin , APX- Ampiclox

**Table 3:** Distribution of *Staphylococcus aureus* in Relation to Gender among Food Handlers and Restaurant Workers

<table>
<thead>
<tr>
<th>Samples</th>
<th>S. aureus (%)</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal</td>
<td>24(30)</td>
<td>9(37.5)</td>
<td>15(62.5)</td>
</tr>
<tr>
<td>Skin</td>
<td>6(7.5)</td>
<td>4(67)</td>
<td>2(33.3)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>13(43.3)</td>
<td>17(56.6)</td>
</tr>
</tbody>
</table>

$X^2{cal}=1.663$, Degree of freedom=1, $p$-value=0.435, ($p>0.05$).
which can be source of enterotoxigenic stains causing food born infection observed in our restaurant this days.

From all the organisms known to cause food born infection, Staphylococcus aureus is the most prevalent among them that is easily isolated and it colonizes the skin and mucosal surfaces of healthy individuals. The isolation of this organism learned to us that, as a microflora, it has a high percentage of causing infectious disease related to illness. Evidence from the result obtained has show that the skin and nasal nares has carrying capacity of Staphylococcus aureus. In contrast, healthy individuals as worker and food handlers in restaurant are risk factor of food born infection (food poisoning).

In conclusion, a relatively high prevalence rate of Staphylococcus aureus in nasal nares and skin carriage was recorded among the investigated food handlers. Moreover, 10% of the investigated carriers harboured Staphylococcus aureus in their anterior nares increasing the likelihood of transmission of the pathogen to the handled food. These findings resurges the imperative need for protective measures including increased public awareness programs, regular monitoring of food handlers for food borne pathogens and intensive training on primary health care and hygiene. Finally, the current findings clearly highlight the significance of implementation of efficient quality control systems in areas of direct contact with food product as good manufacturing practices and standard operational procedures and future research addressing effective methods for sustained eradication of Staphylococcal skin and nasal carriage are clearly warranted to reduce the high risk of subsequent infection.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

FUNDING

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHORS' CONTRIBUTIONS

Iyevhobu, K.O. and Obodo, B.N., conceptualized the laboratory work and provided scientific guidance, Momoh A.R.M., Airefetalor, A.I. and Okobi, T.J. designed and wrote the manuscript while Etafo, J. and Osagiede, E.K. conducted experiments.

ACKNOWLEDGEMENTS

The authors would like to thank all the Laboratory and technical staffs of the department of Medical Laboratory Science, Ambrose Alli University Ekpoma, Edo State for their excellent assistance and St Kenny Research Consult, Ekpoma, Edo State for providing medical writing support/ editorial support in accordance with Good Publication Practice (GPP3) guidelines.

References


Clinical Infectious Disease, 32 (11), 1643–1647.