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Nasal Carriage of Enterotoxigenic *Staphylococcus aureus* and Risk Factors among Food Handlers-Egypt

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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 carriage of *Staphylococcus aureus* among 200 food handlers working in 3 different food processing plants in Egypt. Sixty-one (31%) persons were found to be carriers of *Staphylococcus aureus* of which twenty-one (34.4%) harboured enterotoxigenic staphylococcal strains. The most frequently found enterotoxins were Types A and C (12 isolates each), followed by Type B and Type D (7 isolates, 5 isolates) respectively. A combination of two or three enterotoxins was produced by some of the isolates. The three processing plants recorded statistically significant differences amongst examined personnel in nasal carriage, however neither of the risk factors assessed including age, gender, marital status, education, duration in work, frequency and method of hand wash, incidence of chronic sinusitis and smoking were significant risk determinants of *S. aureus* nasal carriage. The obtained results should be of public health concern and highlights the need for primary health care and hygiene among food handlers.

Keywords Enterotoxigenic *S. Aureus*, Nasal Carriage, Risk Factors, Food Handlers

1. Introduction

According to the World Health Organization (WHO), up to 30% of the populations of developed countries are affected by food-borne illness each year. The problem is more widespread in developing countries where most of these cases are not reported, and thus the true dimension of the problem is not known[1]. In these countries, errors made in food processing plants and settlements emerge as important causes of foodborne disease[2].

Food handlers are potential causes of infections by many food borne pathogens since many diseases are communicable and caused originally by microorganisms carried into their bodies[3]. It is estimated that 30-50% of the human population carries *Staphylococcus aureus* and its main habitat is the nasopharynx, a site where strains can persist as transitory or persistent members of the normal microbiota without causing any symptomatology. Accordingly, body carriage of *S. aureus* by food handlers can be an important source of contamination of the food product via respiratory secretions or direct contact via hands during manufacture and handling of the food product[4].

Staphylococcus aureus is an extraordinary versatile pathogen and the major causative agent of numerous infectious diseases. The disease spectrum includes abscesses, septicemia, endocarditis, osteomyeltis, 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 dependant on the numerous virulence factors produced by each strain[5]. The ingestion of the preformed toxins produced by enterotoxigenic strains in food often leads to the development of food poisoning. The symptoms typically have a rapid onset (2–6 h) and may include nausea, vomiting, diarrhea and abdominal pain[6].

Consequently, the rising concerns over food safety and the lack of research into the proportion of Egyptian food handlers colonized by *S. aureus* have formed the impetus for investigation of the incidence and potential risk factors associated with nasal carriage of *S. aureus* amongst personnel working in three different food processing plants.

2. Materials and Methods

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This work was done over a period of 10 months (Jan. 2012-Nov.2012). Two-hundred food handlers, working in three different food processing plants, participated in this work. These include 42 personnel working in milk and dairy products plant, 67 working in meat and poultry products plants and 91 working in frozen/canned juices and vegetables processing plant. Prior to sample collection a demographic data sheet was collected from each participant.

The *Staphylococcus* spp. nasal carriage study was carried out using sterile swabs, which were inserted in both anterior nares and transferred to a tube containing sterile brain heart infusion broth. Tubes were incubated at 37℃ for 24 h, streaked onto the surface of Baird-Parker Agar Base plates (Oxoid CM0275, Basinggstoke, Hants, UK) supplemented with egg-yolk tellurite emulsion (Oxoid SR 0054) and aerobically incubated at 37℃ for 24-48 h. Three colonies per sample, showing typical colonial morphology of coagulase- positive *Staphylococci*, were subjected to Gram staining, coagulase and catalyse tests. Gram, coagulase and catalyse positive strains were confirmed with an agglutination Staphyloccci Plus test (Oxoid) thereafter biochemically identified with API staph identification System for *Staphylococcus aureus* (bioMerieux Marcy – I'Etoile, France). Definitive identification was based on the presence of DNA using DN-ase test, the presence of protein A and clumping factor using SLIDEX Staph plus (bioMerieux Marcy – I'Etoile, France). A strain of *S. aureus* NCTC 10788 (obtained from food science department, University of Wisconsin-Madison, US) was used as a reference strain.

Only one identified isolate per positive samples was investigated using an immunological technique to verify its ability to synthesize staphylococcal enterotoxins. The strains were grown in 10 ml of tryptone soya broth (CM0219B, Oxoid) by shaking aerobically for 16-18 h at 37℃. After centrifugation at 9000 x g for 20 min at 4℃, the supernatant was tested for presence of staphylococcal enterotoxins and typed using Staphylococcal Enterotoxin Test Reversed Passive Latex Agglutination (SET-RPLA) (TD900 Oxoid Basinkstoke, Hampshire, UK) as recommended by the manufacturer's protocol. Negative controls were used with all the tested samples.

Data collected were statistically analysed using SAS version 8 (SAS Institute, Cary, NC, US). Proportions were compared using Pearson Chi-Square tests. The level of statistical significance was set at a P-value ≤ 0.05.

3. Results and Discussion

Table (1) illustrates the number of persons screened for nasal carriage of *S. aureus* in the investigated food processing plants. The overall prevalence of the pathogen in the two hundred examined personnel was about one third (31%). Subjects working in the milk and dairy processing plant recorded the highest prevalence rate (36%) of colonization by *S. aureus* followed by the other two processing plants; meat and meat products (31%) and frozen/canned vegetables and juices (27%) plants respectively. The difference in prevalence rates amongst the three study plants were significant (P = -0.028), however this prevalence cannot be exactly compared due to differences in sample size, habits and other environmental conditions that may exist in these plants.

Table 1. Incidence of nasal *S. aureus* among food-handlers working in food processing plants

Study plant	No. of examined subjects	No. of positive subjects	No. of enterotoxigenic *S. aureus*	Type of toxin(s) produced
Milk and dairy products	42	15(36%)	5(12%)	A*, C*, D*, AB* and ABC*
Meat and poultry products	91	28(31%)	10(11%)	A**, C**, AB*,AC*,BC*,BD*, ABC* and ACD*
Frozen/ canned vegetables and juices	67	18 (27%)	6(9%)	A*, C*, AC*,BC*, BD* and ACD*
Total	200	61(31%)	21(11%)	

*=one strain **=two strains

The overall prevalence of nasal *S. aureus* amongst the study population, in the current study, recorded values higher than those registered in other studies conducted at US (23%)[7] and UK (25%)[8] and among food handlers working in Ethiopia (20.5%)[9], Turkey (23.1%)[10] and Brazil (29%)[11] and even hospital personnel in Cameroon (23.7%)[12]. Meanwhile, higher prevalence rates were reported among food handlers of a Chilean (65%)[13] and Botswana (57.5%)[14] studies, as well as, amongst healthy subjects working in an Iranian (35.7%), Indian (37.3%) and Nigerian (50%) hospitals[15]. A study conducted in Sudan for food handlers working in restaurants, bakeries, butcheries and groceries demonstrated that 30.1% of the examined handlers harboured *S. aureus* in their anterior nares[16]. Results of the current investigation recorded an *S. aureus* nasal carriage incidence rates that are approximately the same as that of the Sudanese study and within the ranges reported for health personnel (15-56%)[15]. A number of studies estimate the prevalence of *S. aureus* colonization in the general population to be approximately 20%–32%[17]. In the present study, we found a prevalence of 61 carriers in 200 individuals, corresponding to 31%, which shows good accordance with these general findings. Generally, there exists considerable variation of nasal carriage of *S. aureus* prevalence within regions, countries and even inside each country. Global trends of staphylococcal nasal carriage demonstrated that nasal carriage is high in developed countries, as compared to underdeveloped and developing countries[18]. However comparison amongst these studies are difficult since most of these studies identify a particular target population (encompassing students, hospital workers,

infants in neonatal ward, geriatric patients, food handlers ….etc) and study carriage in that cohort with respect to certain standard variables (viz., age, sex, health status, antibiotic intervention in a given time frame and others) within that group.

Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions. The majority of reported Staphylococcal food poisoning outbreaks are associated with classical enterotoxins, SEA-SEE; however enterotoxin A was the most incriminated[19]. Results of the present investigation indicated that 10.5% of the examined persons were found to be carriers of enterotoxigenic *S. aureus*. Enterotoxin type A being the most prevalent type detected in 12 of the 21 isolates (57%) followed by enterotoxin C (52%), SEB (38%) and SED respectively. Results also indicated that some isolates produced more than one type of toxin as shown in Table 1. Similar findings was observed in other studies, where food handlers in a Botswana's study[14] recorded a 21% of occurrence of enterotoxigenic strains among *S. aureus* isolates, the most prevalent being type A which accounted for 34.9% of the toxigenic strains. Similarly a Chilean study[20] reported that out of the 34% of *S. aureus* harbouring individuals, 54% were enterotoxin producers with SEA being the most frequently type detected. On the contrary, another Chilean study reported that out of the 36 food handlers investigated, the most frequently found enterotoxin was type B[13].

Table 2. Risk factors associated with S.aureus nasal carriage amongst study population

Characteristic	Variable	Total	Positive (%)	P-value
Gender	Male	115	35 (30%)	0.631
	Female	85	26 (31%)	
Age	20-40 years old	103	31 (30%)	0.532
	>40-60 years old	97	30 (31%)	
Education	High school	104	33 (31%)	0.611
	Elementary	96	28 (29%)	
Marital Status	Married	140	41 (29%)	0.423
	Single	60	20 (33%)	
Duration in work	5 years	136	45 (33%)	0.611
	≥5 years	64	20 (31%)	
Frequency of hand wash	an hour	63	19 (30%)	0.613
	> 1 hour	137	42 (31%)	
Method of hand wash	Water	116	37 (32%)	0.412
	Soap & water	84	24 (29%)	
Incidence of chronic sinusitis	Yes	19	6 (32%)	0.542
	No/not known	181	60 (33%)	
Smoking	Yes	108	33 (31%)	0.395
	No	92	28 (30%)	

Demographic characteristics of respondents are presented in table (2). About half of the respondents (57.5%) were females and nearly half were below the age of forty. About half of the investigated food handlers had education above primary school. One third of the respondents was single and has spent more than 5 years in their current employment in the studied food processing plants. Findings related to analysis of demographic variables revealed that neither gender, age, marital status nor level of education had a significant effect with respect to the nasal carriage of *S. aureus*. Similarly other demographic characteristics as the work experiences and incidence of chronic sinusitis were statistically insignificant in relation to harbouring *S. aureus* in the anterior nares of the investigated subjects. Surprisingly, personal habits as smoking and hand wash did not significantly seem to affect the colonization of the bacterium in the anterior nares of the respondents.

The current study evaluated the potential risk factors for nasal carriage of *S. aureus*. The investigated variables included fixed factors as gender, age, education in addition to modifiable elements as smoking, hand wash, incidence of chronic sinusitis. Age was identified in earlier studies as a factor influencing nasal colonization with *S. aureus*[21]. Newborns have higher rates of *S. aureus* colonization ranging from 59-100% within a few days of birth and these rates decline to adult carrier rates (10-35%) by 5-6 years of age[22]. This trend towards a decline in *S. aureus* carriage with advancing age is not the case in this study, as the two age groups, cited almost the same incidence of nasal *S. aureus* carriage. This difference among the current study and others may be attributed to the differences in age ranges investigated amongst the study populations. Male gender has also been reported in several studies as a risk factor for nasal carriage of *S. aureus*[21,23]. Unfortunately, the reasons for this association remain unclear. Regarding modifiable characteristics, chronic sinusitis was shown to be a risk factor for nasal carriage of *S. aureus*[24]. Anatomic alterations of the nasopharynx, long-term antibiotic exposure, or other factors might lead to a predisposition of *S. aureus* carriage. However, a recent comparison of the bacteriologic flora of patients with and without chronic sinusitis did not reveal a significant difference in colonization rates regarding *S. aureus* which is in agreement with our present findings where the carriage rate did not significantly differ between both groups with/without a history of chronic sinusitis[25]. The correlation between smoking and nasal carriage of *S. aureus* in previous studies presented biased results where a cross sectional study involving 346 adults conducted in Malaysia revealed that smokers had higher nasal carriage rates than non-smokers[26]. An earlier study had already revealed that passive smoking is associated with an increased risk of *S. aureus* colonization, with active smoking being protective against colonization[27]. In the current investigation, smoking seemed to be an insignificant determinant of *S. aureus* nasal carriage. Food handlers in the current study had the habit of hand wash during their working hours but with a difference in frequency and method of hand wash employed. In a study among 200 food handlers

working at Gondar University, Ethiopia[9] an insignificant association was found between different hand washing practices (hand wash after toilet with water, hand wash after toilet with soap and hand wash before preparing food) and nasal carriage of *S. aureus*, which correlates with the findings in our current investigation.

The discrepancies amongst the findings related to the several factors that are independent risk determinants of nasal carriage of *S. aureus* are attributed to multiple reasons. One of those applies in our study; the self-administered questionnaire may have led to inaccuracies in the assessment of risk factors. Because the study population had different educational backgrounds, socioeconomic status, perceptions, chronic or more severe diseases were underrepresented and, therefore, might have lacked the statistical power to be detected as risk factors for *S. aureus* carriage. Since this study targeted host-related factors, somewhat neglecting the role of the microorganism itself, further coherent research is needed into the complex background underlying staphylococcal nasal carriage.

4. Conclusions

In conclusion, a relatively high prevalence rate of *S. aureus* nasal carriage was recorded among the investigated food handlers. Moreover, 11% of the investigated carriers harboured enterotoxigenic strains 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 nasal carriage are clearly warranted to reduce the high risk of subsequent infection.

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