

Cross Sectional Study of Skin Carriage and Enterotoxigenicity of *Staphylococcus aureus* among Food Handlers

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Received 30 December 2013; revised 29 January 2014; accepted 7 February 2014

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Abstract

The prevalence of enterotoxigenic *Staphylococcus aureus* was investigated among 200 participants working in three different food processing plants in Egypt. Using skin swabs, 75 (38%) of the 200 tested persons were positive for the presence of *S. aureus*. Of the *S. aureus* positive persons, 28 (14%) harboured *S. aureus* produced staphylococcal enterotoxins. The serotypes of these enterotoxins were enterotoxin A (68%), enterotoxin B (36%), enterotoxin C (46%) and enterotoxin D (18%). Some of these isolates produced more than one type of enterotoxins namely AB, AC, BC, BD, ABC and ACD. Analysis of risk factors implicated in skin carriage of *S. aureus* as age, gender, marital status, education, duration in employment, frequency and method of hand wash and incidence of chronic skin infection revealed insignificant association with staphylococcal skin carriage. The obtained results put forth the risk of food contracting contamination with enterotoxigenic strains of *S. aureus* owing to skin colonization of *S. aureus* among food handlers.

Keywords

Enterotoxigenic *S. aureus*; Skin Carriage; Risk Factors; Food Handlers

1. Introduction

Food borne diseases are a major public health concern. They cause personal distress, preventable death and

How to cite this paper: El-Shenawy, M., et al. (2014) Cross Sectional Study of Skin Carriage and Enterotoxigenicity of *Staphylococcus aureus* among Food Handlers. *Open Journal of Medical Microbiology*, 4, 16-22.

<http://dx.doi.org/10.4236/ojmm.2014.41003>

avoidable economic burden. The true incidence of foodborne diseases is difficult to quantify. In industrialized countries, it is estimated that every year more than 30% are exposed to foodborne diseases [1]. 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 [2].

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*. 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 *S. aureus*, nonetheless extranasal sites typically harbor the organism including the skin, perineum and pharynx [3] [4].

Until recently, 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 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 [5]. 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 [6].

Although skin carriage of *S. aureus* is less reported than nasal carriage, little is known about the prevalence and risk of skin carriage of enterotoxigenic strains of *S. aureus* among food handlers. Accordingly, the current study investigated the prevalence and risk factors associated with skin carriage of enterotoxigenic *S. aureus* amongst food handlers working in three different food processing plants in Egypt.

2. Materials and Methods

2.1. Data Collection

Data collection occurred between April 2013 and October 2013. Personnel working in three different processing plants namely milk and dairy products, meat and poultry products as well as frozen/canned vegetable products were eligible to participate. Two hundred participants formally consented to involvement. Participants were verbally surveyed to gather information about age, gender, marital status, education and potential risk factor including duration in work, hand wash habit, presence of inflammatory skin condition and smoking.

2.2. Microbiological Analysis

Participant's hands and faces were well swabbed. Swabs were transferred to brain heart infusion broth, incubated at 37°C for 24 h and streaked onto the surface of Baird-Parker Agar Base plates (Oxoid CM0275, Basingstoke, Hants, UK) supplemented with egg-yolk tellurite emulsion (Oxoid SR 0054) and aerobically incubated at 37°C for 24 - 48 h. Three colonies per sample, showing typical colonial morphology of coagulase-positive *Staphylococci*, were subjected to Gram-staining, coagulase and catalase test. Gram, coagulase and catalase positive strains were confirmed with an agglutination Staphylococci Plus test (Oxoid). Thereafter they were 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 individual, was investigated using an immunological technique to verify its ability to synthesize staphylococcal enterotoxin(s). The strains were grown in 10 ml of tryptone soya broth (CM0219B, Oxoid) by shaking aerobically for 16 - 18 h at 37°C. After centrifugation at 9000 × g for 20 min at 4°C, 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.

2.3. Statistical Analysis

Data collected were statistically analyzed 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

Of the 200 participants, seventy five (38%) were positive for *S. aureus* skin carriage of which 37% harboured enterotoxigenic strains as presented in **Table 1**. *S. aureus* colonization rates in the general population are estimated to be approximately 20% - 32% [4]. The present study recorded a prevalence rate (38%) that is slightly higher than the general population estimates. In spite of this, a 38% carriage rate is within the ranges reported in several studies of food handlers in different food settings. A cross-sectional study conducted among 127 food handlers working in cafeterias in Ethiopia indicated that 16.5% of fingernail contents of the food handlers were cultured positive for *S. aureus* [7]. Another study done in Botswana reported that an even higher proportion (57.5% out of 200 food handlers) tested positive for *S. aureus* [8]. Prevalence of enterotoxigenic *S. aureus* in food handlers is variable between industries and countries. Prevalence estimates from several small studies ranged from 2% of food handlers in Italy (n = 545) [9], 12% of flight-catering staff in Finland (n = 136) [10], 19% of restaurant workers in Chile (n = 102) [11] to 62% of fish processing factory workers in India (n = 87) [12]. In a study of nasal carriage of *S. aureus* amongst food handlers by the same investigators [13], a nasal carriage rate of 31% was reported that is higher than the skin carriage rate recorded in the current investigation. Although nasal carriage is expected to be higher than skin carriage, incompliance to strict Good Manufacturing Practice procedures in food settings may attribute to such finding. There exists a variation among *S. aureus* prevalence within regions, countries and even inside each country. Furthermore, studies target different populations encompassing students, hospital workers, infants, geriatrics, food handlers etc. and study carriage with respect to certain standard variables (viz., age, sex, health status, antibiotic intervention in a given time frame and others) within that group.

As seen from **Table 1**, subjects working in the milk and dairy processing plant recorded the highest prevalence rate (45%) of colonization by *S. aureus* followed by the other two processing plants; meat and poultry products (36%) and frozen/canned vegetables and juices (34%) 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 habits and other environmental conditions that may exist in these plants.

Staphylococcal food poisoning is an intoxication that is caused by the ingestion of food containing pre-formed Staphylococcal Enterotoxin SE [14]. There are several different types of SE; enterotoxin A is most commonly associated with staphylococcal food poisoning. Enterotoxins D, E and H, and to a lesser extent B, G and I, have also been associated with staphylococcal food poisoning [15]. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions [14]. Results of the current investigation demonstrated that 14% of the examined subjects were enterotoxigenic *S. aureus* carriers. Enterotoxin A was the most recorded in 18 of the 28 isolates followed by enterotoxin C, B and D respectively. In surveys conducted on food handlers, *S. aureus* enterotoxins types A, B, C, D, and E have been isolated with varying frequency. In Kuwait, the majority of *S. aureus* isolates from hands of food workers was type B, whereas those isolated from the nose were predominantly of types A and B (28% and 28.5% respectively) followed by types C and D (16.4% and 3.5% respectively) [16]. Enterotoxigenic *S. aureus* isolates from the nose, throat, hands and nails of food handlers in cafeterias of a Chilean restaurant were predominantly enterotoxin B and enterotoxin D producers [17]. Meanwhile, another Chilean study and a Botswana's survey reported prevalence rate and predominance enterotoxin type that are in ac-

Table 1. Prevalence of skin carriage of *S. aureus* among food-handlers working in food processing plants.

Food processing plant	Total subjects (n)	<i>S. aureus</i> + ve n (%)	Enterotoxigenic SA + ve n (%)	Type of toxin(s) produced
Milk and dairy products	42	19(45%)	7(17%)	A**, C*, D*, AB** and ABC*
Meat and poultry products	91	33 (36%)	12 (13%)	A***, C**, AB*, AC*, BC*, BD*, ABC* and ACD*
Frozen/canned vegetables and juices	67	23 (34%)	9 (13%)	A***, C*, AC**, BC*, BD* and ACD*
Total	200	75 (38%)	28 (14%)	

* = one strain; ** = two strains; *** = three strains.

cordance with results reported in the current survey [8] [11].

Recent studies substantiate that the mechanisms leading to *S. aureus* carriage are multi-factorial. Bacterial factors (e.g. staphylococcal toxins and cell wall-associated proteins), environmental factors (e.g. hospitalization and crowding), as well as, host susceptibility factors (e.g. immune suppression or other serious underlying diseases) play an important role. Colonization of *S. aureus* in human beings can be viewed as the net result of repellent and attracting forces that can be imposed by either of the interacting parties [18].

Survey response data in relation to prevalence of *Staphylococcus aureus* among the surveyed risk factors are given in **Table 2**. More than half of the respondents were males, aged between 20 - 40 and high school educated. Meanwhile about two thirds of the respondents had been employed for a period of less than 5 years and use water for hand wash at a frequency of >1 hr period. None of the surveyed factors demonstrated statistically significant association with staphylococcus skin carriage. These findings are in agreement with other studies that demonstrated that gender did not influence skin carriage of staphylococcus [19] [20] however, larger population-based cross-sectional studies have reported higher risk of *S. aureus* nasal carriage among men as compared with women [21]. In literature, the association between gender as a fixed factor and staphylococcal carriage remains unclear. Earlier studies have also reported that age is a modifier for SA carriage, and the correlation between age and carriage was evident for high age groups as those over 70 [19] [22] and in some instances young age as teenagers (13 - 18 years) which was reported in a study investigating staphylococcal carriage on armpits of secondary school students [20]. The association between age and *Staphylococcus aureus* carriage in older age has been attributed to the fact that old participants have increased lifetime exposure to antibiotics which consequently affects prevalence rates. The current results are comparable to those estimated in previous records [20] [23] and the carriage rate cited was nearly the same among the two age groups investigated (20 - 40 and 40 - 60).

Table 2. Correlation between staphylococcal carriage and surveyed risk factors.

Factor	Total (n)	<i>S. aureus</i> + ve n (%)	p value
<u>Gender</u>			
Male	115	42(37%)	0.612
Female	85	33 (39%)	
<u>Age</u>			
20 - 40 years	103	36 (35%)	0.521
>40 - 60 years	97	39 (40%)	
<u>Education</u>			
High school	104	39 (38%)	0.610
elementary	96	36 (37%)	
<u>Marital status</u>			
Single	140	49 (35%)	0.381
Married	60	26 (43%)	
<u>Duration in work</u>			
<5 years	136	45 (33%)	0.623
≥5 years	64	30 (47%)	
<u>Frequency of hand wash</u>			
<an hour	63	25 (40%)	0.613
>1 hour	137	50 (36%)	
<u>Method of hand wash</u>			
Water	116	44 (38%)	0.412
Soap and water	84	31 (37%)	
<u>Incidence of inflammatory skin condition</u>			
Yes	19	7 (37%)	0.520
No	181	68 (38%)	
<u>Smoking</u>			
Smoker	108	39 (36%)	0.393
Non smoker	92	36 (39%)	

p-value < 0.05 was considered significant.

There is evidence from large population-based studies for an inverse association between current smoking and *S. aureus* carriage among both men and women; in the Rotterdam study, smoking was associated with a 36% reduced risk and in the Tromso Staph and Skin Study the *S. aureus* carriage rate was 28% lower in smokers than in non-smokers ($P < 0.01$) [24] [25]. The results in the current investigation were in accordance with previous findings as carriage rates in non smokers (39%) were higher than that recorded in smokers. Possible explanations for the suggested protective effect of smoking include the bactericidal activity of cigarette smoke and the increased immune activity associated with smoking-induced hypoxia [26].

Several modifiable factors related to staphylococcal skin carriage have been investigated, research reported discrepancies among the correlation of these factors with skin harbouring of staphylococcus. It was reported that variables such as the type of soap used, bathing and shaving frequency and use of deodorant had no effect on *S. aureus* skin carriage in the armpits of investigated individuals [20]. In another study on staphylococcal carriage in correctional facilities as jails and prisons, factors associated with MRSA skin harbour were multiple of which antibiotic use in the previous year and lower rate of showering were significant factors influencing skin carriage [27]. The view that hand washing insignificantly affected staphylococcal skin carriage was opposed in a Chilean study which demonstrated that washing hands and skin surfaces has minimal effect on reducing *S. aureus* cell numbers on humans, largely because *S. aureus* is part of the resident flora of skin [17]. Skin diseases that alter the epithelium are reported to be an influential factor on *S. aureus* carriage. Atopic dermatitis (AD) is a pruritic skin disease characterized by barrier dysfunction of the skin and chronic inflammation [28]. Several smaller clinical studies have shown strong associations between AD and *S. aureus* colonisation of the skin and nose. However, others observed no association between *S. aureus* load and disease severity [24]. In the current investigation there was no significant association between the presence of chronic inflammatory skin condition and *S. aureus* carriage. Moreover, the carriage rates among individuals with/without inflammatory skin condition were nearly the same.

From population based studies, there are numerous reports of factors associated with *S. aureus* carriage. Observational studies of *S. aureus* carriage discussed the influence of bacterial, host and environmental/modifiable factors which might have influence on the relationship. However, to extract specific host determinants can be challenging, since variation in carriage rates are likely to be multifactorial and single determinants would be very hard to identify. Still, epidemiological studies can unravel valuable information for formulating hypothesis about complexity that might be involved in carriage.

Acknowledgements

Financial support for this research was provided by the Agencia Española de Cooperación Internacional para el Desarrollo (A/019106/08, A/025113/09 and A1/035779/11).

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