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Staphylococcus aureus in Food and Nares of Food Handlers in Kuala Pilah, Malaysia

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ABSTRACT

The outbreak of food poisoning due to *Staphylococcus aureus* has been reported with a significant level of morbidity worldwide. In this study, 128 samples taken from nasal swabs of 64 food handlers and 64 food items were collected and analyzed for the presence of *S. aureus*. The antibiotic susceptibility profiles of the isolates were also determined. Cross-sectional was used as a study design in this research. The isolates were identified as *S. aureus* based on colonial morphology, gram stain, mannitol salt agar fermentation, catalase and coagulase test. Fifteen (23.4%) of food handlers were positive for *S. aureus* nasal carriage and 24 (37.5%) of food items were contaminated with an average of 8.4×10^6 CFU/g of *S. aureus*. All isolates were susceptible to oxacillin and mupirocin respectively. However, 74.4% and 5.1% isolates were resistant to penicillin and erythromycin. Our findings suggest that the prevalence of *S. aureus* carriage is high among our food handlers and food items. Thus, we believed that there is a need for proper training on food safety among food handlers and quality improvement in the food premises.

Keywords: Food poisoning, food handlers, nasal carriers, *Staphylococcus aureus*

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INTRODUCTION

Food poisoning is one of the food borne diseases affecting many people worldwide. It occurs through the ingestion of certain microorganisms or their toxins (Campos *et al*, 2009). It is important to note that

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a wide variety of etiological agents have been associated with food poisoning such as viruses, bacteria and parasites (Gunduz *et al*., 2011). In general, *Staphylococcus aureus* has been reported to be a significant source of food borne infection worldwide due to its ability to produce several enterotoxins such as enterotoxin A, B, C1, C2, C3, D and E (Shimizu *et al*., 2000). Since these enterotoxins are heat-resistant (Reiser *et al*., 1984), high temperature can only eliminate *S. aureus* but not its toxins. However, the proliferation of this microorganism can be controlled by keeping the food items hot or cold to ensure its multiplication does not exceed 10^3 cfu/g, which is considered as harmless to humans (Loir *et al*., 2003).

Several control strategies have been implemented to prevent food poisoning, but so far the number of reported cases has not reduced. According to the Ministry of Health Malaysia (2009) there has been an increase of food poisoning cases from 2005 to 2008, and death cases due to food poisoning have also been reported. It is shown that 10% to 20% of food borne diseases is due to contamination by the food handlers (WHO, 1998). Inappropriate ways of handling the food and unhygienic practices by food handlers could potentially enhance the transmission of the disease to susceptible consumers.

S. aureus can colonize human interior nares (Zahoor & Bhatia, 2007), skin (Noble, 1998), oropharynx (Smith *et al*., 2001) and faeces (Arvola *et al*., 2006). This microorganism can also be transferred from food handlers to food items via unhygienic practices such as sneezing or coughing, touching the food without washing hands, neglecting the importance of a clean work area and exposing the food items at room temperature for too long.

Food handlers' knowledge, attitude and practices (KAP) influence the outcome of food preparation (Ansari-Lari *et al*., 2010; Baş *et al*., 2006; Zain & Naing, 2002). Therefore, having a good KAP can reduce the risk of contamination that leads to food poisoning. The aims of this study were to determine the prevalence *S. aureus* nasal carriers among 64 food handlers in the district of Kuala Pilah, Malaysia and the rate of 64 food samples contaminated with *S. aureus*. The antibiotic susceptibility profile of the isolates was also determined.

MATERIALS AND METHODS

The study area was Kuala Pilah, Negeri Sembilan which is located 77 km from Kuala Lumpur, Malaysia. It was selected due to the differences of geographical area, culture, as well as the backgrounds of food handlers.

A total of 64 food handlers were selected through a purposive sampling method with the help of district health officers in charge of inspecting the food premises. Sixty four food samples were also collected during this study.

Data Collection

Sociodemographic characteristics of the respondents, such as gender, age, educational level, work duration and premise grades were collected during the study. The respondents'

age groups were classified as "youth" (17 to 35 years old) and "adult" (36 years old and above), and the educational background was categorized as "low educational level" (received education up to secondary level) and "high educational level" (received education after their secondary level). The working experience was grouped into "experienced" (working for one year and more) and "inexperienced" (working for less than one year), and the cleanliness of working area was chosen as high grade premises (grades A and B) and low grade premises (grade C and no grade).

Isolation and identification of Staphylococcus aureus

The method used for collecting nasal swabs and food samples were modified from Kozioł-Montewka *et al*. (2006) and Huong *et al*. (2010). The nasal swab samples were taken using sterile cotton swabs with Stuart transport medium. The specimens were obtained from approximately 1cm inside nostrils and rotated five times (Askarin *et al*., 2009). The swabs were streaked on Mannitol Salt Agar (MSA) and incubated at 37°C for 24 to 48h.

Ten grams of the food samples were homogenized in 90 ml sterile saline water (0.85%), and inoculated on the MSA plate and incubated at 37°C for 24 to 48h. The identification of *S. aureus* was based on colonial morphology, gram stain, mannitol salt agar fermentation, catalase and coagulase tests (Acco *et al*., 2003; Lues & Tonder, 2007). American type culture collection (ATCC) 25923 *S. aureus* was used as the positive control and *S. aureus* ATCC 12228 was used as the negative control.

Antibiotic Susceptibility Testing (AST)

The sensitivity patterns of the isolates were performed using disk diffusion method according to the Clinical and Laboratory Standards Institutes (CLSI, 2010). Antibiotics disc used in this study were erythromycin 15 µg, oxacilin 1 µg, mupirocin 5 µg, vancomycin 30 µg, and penicillin G 10 units. The turbidity of the tested microorganisms was set according to 0.5 McFarland Turbidity Standard. The entire Mueller-Hinton agar was streaked with overnight culture and antibiotic discs were applied on the agar and incubated at 37°C for 24 h. The zones of inhibition were interpreted according to CLSI (2010).

Statistical Analysis

The data were analyzed using computer software Statistical Packages for Social Sciences (SPSS) version 16.0. The chi-square test was used to determine the significant relationship between the variables and the risk factors. The level of significance was set at $p<0.05$.

Ethical consideration

The Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) had approved the study. The respondents were informed verbally as well as in written form about the research. Consent from the respondents was taken prior to the collection of nasal swabs and food samples.

RESULTS AND DISCUSSION

Subjects

Sixty four respondents were involved in this study. A total of 75% of the respondents were female and 25% were males. Forty six of the respondents (71.9%) were youth whereas 18 (28.1%) were adult respondents. Majority of the respondents (68.8%) had lower educational level and the remaining (31.2%) had higher educational level. The inexperienced food handlers comprised 36%, while 64% were experienced food handlers. Only 29 (45.3%) respondents can be classified as food handlers working in high grade food premises and 35 (54.7%) of the respondents were food handlers working in low grade food premises (Table 1).

TABLE 1
Socio-demographic characteristics of the respondents (N = 64)

Variables	n (%)
Gender	
Male	16 (25)
Female	48 (75)
Age group	
Youth	46 (71.9)
Adult	18 (28.1)
Educational level	
Lower educational level	44 (68.8)
Higher educational level	20 (31.2)
Experienced level	
Inexperienced	23 (35.9)
Experienced	41 (64.1)
Food premise grade	
High grade food premises	29 (45.3)
Low grade food premises	35 (54.7)

Microorganism

Growth of yellow colonies was observed microscopically. All isolates that grew on the MSA were gram positive micro-organisms and appeared as cocci in clusters with a few of them appeared singly or in pairs. Identity of the *S. aureus* isolates on the MSA was confirmed through positive coagulase and catalase tests (Ahmad, 2010).

Staphylococcus aureus *Nasal Carriers Identified in Food Handlers and Food Contamination*

Based on Table 2, out of 64 food handlers tested, 23.4% were found to be positive *S. aureus* nasal carriers. The prevalence of *S. aureus* nasal carriage among the respondents was almost equal to the prevalence rate as reported by Neela *et al*. (2008), which was 20.9%. The prevalence rate was lower compared to other studies by Souza and Santos, 2009 (29.5%), Acco *et al*., 2003 (30%), and Oteri *et al*. 1989 (24%). However, this study reported a higher prevalence rate than the study by Gündüz *et al*. (2008), which was 0.77%.

Isolates were recovered from 24 (37.5%) of the 64 food samples. The number of *S. aureus* isolated from the samples ranging from less than 1.0×10^1 to 8.7×10^7 CFU/g with an average of 8.4×10^6 CFU/g. Fifty eight percent of the contaminated food samples contain more than 10^4 organisms/gram and this could potentially cause food poisoning (Loir *et al*., 2003). Food samples that are contaminated by this amount of *S. aureus* is considered unsafe to be consumed because the organism can produce sufficient

TABLE 2
Antibiotic susceptibility profiles of *S. aureus* isolated from the nasal swabs of food handlers and food samples

Source	No. of samples	No. of isolates (%)	Mean (range) CFU/g	Resistance to antibiotic (%)			
				OX	P	MUP	E
Food handlers' nasal swab	64	15 (23.4)	ND[a]	0 (0.0)	11 (73.3)	0 (0.0)	1 (6.7)
Food samples	64	24 (37.5)	8.4×10^6 (< 1.0×10^1 – 8.7×10^7)	0 (0.0)	8 (33.3)	0 (0.0)	1 (4.2)
Total	128	39 (30.5)	-	0 (0.0)	29 (74.4)	0 (0.0)	2 (5.1)

[a] ND – not determined
OX: oxacillin, P: penicillin G, MUP: mupirocin, E: erythromycin

amount of enterotoxins. A study conducted by Normanno *et al*. (2005), showed a lower prevalence (17.3%) of *S. aureus* in food and food contact surface swab. In addition, Aydin *et al*. (2011) and Huong *et al*. (2010) reported 13.8% and 21.2% of food contamination by *S. aureus*, respectively. The high number of food contamination suggests a need for improving hygiene practices among food handlers to avoid an outbreak of food poisoning.

Antibiotic Susceptibility Test (AST)

S. aureus was isolated from 39 out of 128 of total samples (Table 2) of which 23.4% and 37.5% were isolated from nasal swabs of food handlers' and food samples, respectively. All isolates were sensitive to oxacillin and mupirocin. Most of the isolates were resistant to penicillin (74.4%), whereas only a few of the isolates were resistant to erythromycin (5.1%). Oxacillin was used in this study as a preliminary test to detect methicillin resistant *S. aureus* (MRSA) (Swenson *et al*., 2007). In addition, all isolates were sensitive to mupirocin. These findings are similar to the study done by Askarin *et al*. (2009) whereby *S. aureus* in this study was also sensitive to oxacillin and mupirocin.

The result of this study showed that 29 (74.4%) of the isolates were resistant to penicillin, while Vaez *et al*. (2011) reported 95.6% penicillim resistant in their *S. aureus*. These results supported the fact that 80% to 90% of *S. aureus* isolates are β-lactamase producers (Livermore, 1995; Smith & Jarvis 1999). In addition, only 5.1% of the isolates were resistant to erythromycin, and this result is in concordance with Vaez *et al*. (2011) who have shown only 6.7% of erythromycin resistance.

Relationship between Staphylococcus aureus *Nasal carriers and Sociodemographic Characteristics Food Handlers (Table 3)*

No significant relationship was observed between male and female respondents ($p = 0.505$), youth and adult respondents ($p = 0.336$), respondents from lower and higher

TABLE 3
S. aureus carriage pattern in the relationship with the socio-demographic characteristic of food handlers (N=64)

Variables	Non-carrier [n (%)]	Carrier [n (%)]	n (%)	χ^2	p	Prevalence ratio (C.I)
Gender						
Male	13 (81.2)	3 (18.8)	16 (25)	0.261	0.609	0.692 (0.168-2.850)
Female	36 (75)	12 (25)	48 (75)			
Age group						
Adult	15 (83.3)	3 (16.7)	18 (28.1)	0.640	0.427	0.567 (0.139-2.306)
Youth	34 (73.9)	12 (26.1)	46 (71.9)			
Educational level						
Higher	14 (70.0)	6 (30.0)	20 (31.2)	0.698	0.403	1.667 (0.500-5.559)
Lower	35 (73.9)	9 (20.5)	44 (68.8)			
Experience level						
Experienced	34 (82.9)	7 (17.1)	41 (64.1)	2.575	0.109	0.386 (0.118-1.259)
Inexperienced	15 (65.2)	8 (34.8)	23 (35.9)			
Food premise grade						
High grade	24 (82.8)	5 (17.2)	29 (45.3)	1.135	0.287	0.521 (.155-1.748)
Low grade	25 (71.4)	10 (28.6)	35 (54.7)			

educational levels (p = 0.533), inexperienced and experienced respondents (p = 0.051), as well as respondents from high and low grade food premises (p = 0.469) (Table 5). There was no variable that can predict the carriage pattern among the food handlers because the percentage of the carriers is small compared to the non-carriers. VandenBergh and Verbrugh (1999) stated that the nasal carrier status of *S. aureus* can be determined by genetic make-ups, sex, age, hormonal status in women, and anatomic alterations of the nares. This study found that gender and age were not significantly associated with *S. aureus* nasal carriers (p>0.05). Peacock *et al*. (2001) demonstrated that, the status of nasal carriers can also be determined by the receptors for bacterial adherence found in the nares, an immune response that can either eliminate or tolerate the *S. aureus* and also anti-staphylococcal constituents that are found in the nasal secretions. Hence, the determination of *S. aureus* nasal carriers involves many factors and it is still not well understood. However, food contamination can be prevented by good personal hygiene practices especially during food preparation and handling.

CONCLUSION

This study indicated that the prevalence of *S. aureus* among food handlers was high. Contamination of food was also high indicating lack of hygienic practices

among the food handlers. All the isolates were sensitive to oxacillin and mupirocin. However, none of the variables studied can significantly be associated with *S. aureus* nasal carriers among the respondents.

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