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# Nasal carriage of enterotoxin-producing *Staphylococcus aureus* among restaurant workers in Kuwait City

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## SUMMARY

Enterotoxin-producing *Staphylococcus aureus* is a common cause of staphylococcal food poisoning. To determine the incidence of carriage of enterotoxin-producing *S. aureus* in a sample of the healthy population in Kuwait city, restaurant workers in the city were screened for nasal carriage of *S. aureus*. 26·6% of 500 workers studied carried *S. aureus* and 86·6% of the *S. aureus* produced staphylococcal enterotoxins. 28% produced enterotoxin A, 28·5% produced enterotoxin B, 16·4% produced enterotoxin C and 3·5% produced enterotoxin D. Ten isolates produced both enterotoxins A and B or A and C. 73% of the isolates were untypeable with standard phages. However, 17·1%, 3% and 6% belonged to phage groups I, II and III respectively. The results demonstrated a high level of enterotoxigenic *S. aureus* carriage among restaurant workers which although lower than that reported for the general population and hospital workers may be important in the restaurant industry.

## INTRODUCTION

*Staphylococcus aureus* strains are widespread in nature. They inhabit the skin, mucous membranes, anterior nares, eyes and gastrointestinal tract of asymptomatic individuals [1–6] where they can exist as resident or as transient members of the normal flora without causing disease. However, some strains of *S. aureus* also cause infections in humans including food poisoning [7–10]. *S. aureus* also accounts for about 10% of all nosocomial infections [2, 7–10].

*S. aureus* elaborates an array of toxins and enzymes that assist it in overcoming the host defences. Some strains produce one or more of six serologically related enterotoxins designated A, B, C1, C2, D and E [11–13]. Ingestion of the preformed toxins in contaminated foods leads to the rapid development of vomiting and diarrhoea, the characteristic symptoms of staphylococcal food poisoning [11, 12, 14, 15]. Staphylococcal enterotoxins are heat stable and can withstand heating at 120 °C for 10 min or 100 °C for 2 h [13]. As a result of their heat stability most heating processes used in restaurants may not adequately inactivate preformed toxins although they may be sufficient to kill vegetative bacteria.

Enterotoxin-producing *S. aureus* is an important cause of food poisoning in many parts of the world [11–15]. It accounts for 14–20% of outbreaks involving contaminated food in the USA [8], and in the United Kingdom restaurants are the second most important place for acquiring staphylococcal food poisoning [10]. Although a mild disease with recovery usual in 1–3 days, fatalities associated with staphylococcal food poisoning have been reported in young children and adults [12]. Food poisoning outbreaks also result in huge financial losses to restaurants plus the loss of reputation and confidence by the public. It also impacts on society through loss of productive hours by the sick [14].

The human anterior nares and finger tips are important sources of enterotoxigenic and non-enterotoxigenic staphylococci [13, 16]. Nasal carriage

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of antibiotic-resistant *S. aureus* by hospital workers is an important source of outbreaks of staphylococcal infections in hospitals [3–6, 16–18]. Whereas nasal carriage of antibiotic-resistant *S. aureus* among hospital workers has been studied extensively, data on the nasal carriage of enterotoxigenic *S. aureus* is less extensive [19]. This study was conducted to detect nasal carriers of enterotoxigenic *S. aureus* among restaurant workers in Kuwait City which represents a section of the healthy population. The results revealed a carriage rate which was lower than that observed elsewhere for hospital workers [3–6, 17, 18] but which may be important for the restaurant industry.

## MATERIALS AND METHODS

### Sample collection

Five hundred food handlers consisting of cooks and waiters in 100 restaurants were screened. Sterile swabs (Atom Medical Ltd, Sussex, England) moistened in sterile normal saline were used to collect samples from the right anterior nares. The swabs were rotated firmly five to six times and used to inoculate mannitol salt agar (MSA) and blood agar plates (Oxoid, Basingstoke, Hampshire, UK) in duplicate and incubated at 37 °C. Plates were examined after 24 and 48 h incubation. A single swab was obtained from each worker. Isolates were identified as *S. aureus* by growth characteristics on blood agar and MSA, Gram stain reaction, catalase test, tube coagulase test and DNAse tests. Pure cultures of isolates were stored in skim milk broth at −70 °C. Working cultures were maintained on brain heart infusion agar slopes at 4 °C.

### Detection of enterotoxins

Staphylococcal enterotoxins were obtained by the optimal sensitivity plate method and detected by agar diffusion as published previously [7, 20]. Reference antitoxins A–E were kindly provided by Professor M. S. Bergdoll (Food Research Institute, Wisconsin, Madison, USA).

### Phage typing

Phage typing was performed using the International set of typing phages according to standard methods [21]. Phage typing was done at the staphylococcus phage typing centre at New Delhi (India) at routine test dilution (RTD) and 100 RTD.

Table 1. *Staphylococcal enterotoxins (SE) from food handlers*

| Types of enterotoxin | Number of *S. aureus* isolates (%) ($n$ = 116) |
|---|---|
| SEA | 33 (28·5) |
| SEB | 33 (28·5) |
| SEC | 19 (16·4) |
| SED | 17 (14·7) |
| SEE | 4 (13·5) |
| Combination SE (A–E) | 10 (8·6) |

## RESULTS

A total of 500 nasal swabs were obtained from 500 male workers in 100 restaurants in Kuwait City. The population studied comprised 294 (54·8 %) Arabs, 160 (38·6 %) people from different Asian countries and 46 (6·6 %) from other countries. The restaurants studied had less than ten workers each. Some restaurants had one cook and one or two attendants serving customers.

None of the swabs collected from 40 restaurants grew *S. aureus*. In the remaining 60 restaurants, a total of 134 isolates of *S. aureus* were obtained. This constituted 26·8 % of the total number of swabs taken.

One hundred and sixteen (86·6 %) of the 134 *S. aureus* isolates tested produced different staphylococcal enterotoxins (SE). These results are presented in Table 1.

Phage typing was used to study the relatedness of enterotoxin-producing *S. aureus* isolates [22–24]. All the 134 *S. aureus* obtained were studied. Ninety-nine (73·8 %) could not be lysed by any of the phages at 100 RTD. Twenty-three isolates were lysed by phage 29 or by the 29/52 complex (group I). Four isolates were lysed by group II phages and eight by group III phages. Of the 116 enterotoxin-producing strains, 85 were non-typable. The results of phage typing and enterotoxin production are shown in Table 2. No enterotoxins were detected in any of the phase group II isolates.

## DISCUSSION

The nasal carriage of enterotoxigenic strains of *S. aureus* among restaurant workers in Kuwait City was investigated. Only 26·8 % of the restaurant workers screened carried *S. aureus* in their noses. This incidence was low compared to the carriage rate in the general population and among hospital workers which

Table 2. *Relationship between phage types and enterotoxin production*

| Enterotoxins (N) | Phage types | | | | |
|---|---|---|---|---|---|
| | I | II | III | 94/95 | NT |
| SEA (33) | 12 | — | 4 | — | 17 |
| SEB (33) | 9 | — | 1 | — | 23 |
| SEC (19) | 1 | — | 2 | — | 16 |
| SED (17) | — | — | 1 | — | 16 |
| SEE (4) | 1 | — | — | — | 3 |
| Mixed toxins (10) | — | — | — | — | 10 |
| Total (116) | 23 | — | 8 | — | 85 |
| Total (%) | 19·8 | — | 6·9 | — | 73·2 |

is between 30 and 50% [3–6, 16–18]. However, 86·6% of the *S. aureus* strains produced enterotoxins. This figure was higher than that reported by Isigidi and colleagues [24] and Sokari [25]. The result is important for the restaurant industry because it may take only a single carrier to contaminate food with an enterotoxin-producing strain to start an outbreak [9, 19]. Richards and colleagues [9] found that the enterotoxin-producing *S. aureus* strains isolated from 18 students after an episode of staphylococcal food poisoning were similar to an isolate from a single food handler in the kitchen where the lunches were prepared.

Although *S. aureus* produces six different types of enterotoxins, A–E, enterotoxin A is commonly associated with food poisoning [9, 14, 22, 23]. In this study enterotoxin A was detected in 28·5% of the isolates (Table 1). This figure was higher than that reported by other workers [19, 22, 23]. Enterotoxin production in *S. aureus* has been associated with strains of phage groups I and III [22, 23, 25]. The majority (85 of 116) of the enterotoxin-producing strains in this study were untypable with the International Set of Typing phages. This is consistent with observations in recent isolates of multiply-resistant *S. aureus* from clinical samples. An increasing number of multiply-resistant *S. aureus* strains isolated from clinical samples are now non-typable with standard typing phages [26–29]. Virani and Noble [30] reported increases in the number of non-typable *S. aureus* isolated from normal individuals between 1964 and 1989. Nevertheless 17 of the non-typable strains in this study produced enterotoxin A (Table 2) in addition to the 19·8% and 6·9% of the strains belonging to phage groups I and III respectively, that also produced enterotoxin A. Thus the results show a high carriage rate of enterotoxin A producing *S. aureus* among the restaurant workers studied.

No case of food poisoning was reported in any of the restaurants investigated despite the presence of carriers among the food handlers. This would imply a satisfactory level of sanitation and care in the preparation and serving of food in these restaurants. Mere carriage of enterotoxin-producing strains by itself is not sufficient to initiate an outbreak if proper care is taken to prevent the contamination of foods including the exclusion of workers with open wounds from preparing and handling food. Staphylococcal food poisoning occurs when *S. aureus* is introduced into foods which are held under conditions that allow the organisms to multiply and produce a sufficient quantity of enterotoxins [19, 23].

Hospital workers who carry multiply-resistant *S. aureus* in their noses have been treated successfully with mupirocin [31, 32] to eradicate the organisms. However, treatment of nasal carriers of enterotoxin-producing *S. aureus* is unnecessary in the absence of disease.

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