BACTERIAL PATHOGENICITY

Enterotoxin production by coagulase-negative staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning

E. E. UDO, M. A. AL-BUSTAN*, L. E. JACOB and T. D. CHUGH

Departments of Microbiology and *Community Medicine, Faculty of Medicine, Kuwait University, Kuwait

Staphylococcus aureus and coagulase-negative staphylococci (CNS) were isolated from the hands of food handlers in 50 restaurants in Kuwait City and studied for the production of staphylococcal enterotoxins, toxic shock syndrome toxin-1, slime and resistance to antimicrobial agents. One or a combination of staphylococcal enterotoxins A, B or C were produced by 6% of the isolates, with the majority producing enterotoxin B. Toxic shock syndrome toxin-1 was detected in c. 7% of the isolates; 47% produced slime. In all, 21% of the isolates were resistant to tetracycline and 11.2% were resistant to propamidine isethionate and mercuric chloride. There was no correlation between slime and toxin production or between slime production and antibiotic resistance. The detection of enterotoxigenic CNS on food handlers suggests that such strains may contribute to food poisoning if food is contaminated by them and held in conditions that allow their growth and elaboration of the enterotoxins. It is recommended that enterotoxigenic CNS should not be ignored when investigating suspected cases of staphylococcal food poisoning.

Introduction

Staphylococcus aureus and coagulase-negative staphylococci (CNS) inhabit the human skin and mucous membranes where they exist mostly as commensal flora [1–3]. However, some of them also cause different types of infections in man and animals. S. aureus is an important cause of food poisoning through the elaboration of one or more of six serologically related, heat-stable enterotoxins designated staphylococcal enterotoxins (SE) A-E [4–8]. Ingestion of these preformed toxins in contaminated food leads to symptoms of food poisoning [6–8].

Some CNS species are recognised human pathogens and can cause serious opportunistic infections, especially in immunocompromised patients and premature babies [9–13], with the patients' skin flora serving as a major source of the infecting micro-organisms. They also elaborate a number of important toxins and haemolysins implicated in serious disease conditions. For example, some CNS produce staphylococcal toxic shock syndrome toxin-1 (TSST-1) which causes toxic shock syndrome [14–16], δ-toxins associated with necrotising enterocolitis in premature infants [17] and δ-haemolysin associated with chronic orofacial muscle pain [18]. Furthermore, staphylococcal enterotoxins (SE) have been detected in CNS isolates from food [16–21], goat's milk [22] and other animal products [23,24]. Despite these findings, studies that have investigated the sources of staphylococcal food poisoning have focused almost exclusively on the carriage of enterotoxigenic S. aureus [7,8,25–28], and rarely on enterotoxin-producing CNS [16,25], in food handlers. As the human nares and fingers are the important sources of S. aureus contaminating food in restaurants [25,26] and because CNS inhabit the human skin and mucous membranes, they can also contaminate food from the hands of food handlers if proper care is not taken when preparing and serving food. Therefore, enterotoxigenic CNS strains may contribute to staphylococcal food poisoning, as any enterotoxigenic staphylococcal strain can be involved in food poisoning [29]. In a study of nasal carriage of S. aureus by restaurant workers in Kuwait City, it was found that 26% of them carried S. aureus in their nares and 86% of these S. aureus isolates produced either TSST-1 or SE. The nasal swabs were not screened for CNS carriage or their toxins.

In the present study, nasal and hand swabs from workers in different restaurants in Kuwait City were
cultured for the presence of *S. aureus* and CNS and the isolates were examined for the production of staphylococcal enterotoxins, TSST-1 and slime and for resistance to antimicrobial agents.

**Materials and methods**

**Sample collection**

A total of 500 nasal and hand swabs was obtained from 250 food handlers in 50 restaurants. The personnel included those who handle food in some way such as cutting, washing, cooking and serving. Sterile swabs (Atom Medical, Sussex) were moistened in sterile nutrient broth. One swab was used to swab areas in between fingers and the wrist area of the hand and another swab was used to swab the nose. The swabs were used to inoculate mannitol salt agar (MSA) and blood agar plates in duplicate and the plates were incubated at 35°C for 24 h. Propamidine isethionate was a gift from Foratom, Marcy l’Etoile, France. Pure cultures of the isolates were preserved in skimmed milk at -20°C. They were subcultured in brain heart infusion broth and incubated at 35°C. Cultures on blood agar were examined after 24 h and those on MSA were examined after incubation for 48 h. Representatives of each colony type were subcultured on blood agar and incubated at 35°C for 24 h for identification.

**Identification of staphylococcal strains**

The isolates were identified on the basis of cultural characteristics, Gram's stain reaction and the results of catalase and tube coagulase tests. Tube coagulase negative, catalase positive, gram-positive coccal isolates were identified further with biochemical tests (API 20 Staph identification system; bioMérieux SA, Marcy l'Etoile, France). Pure cultures of the isolates were preserved in skimmed milk at -20°C. They were subcultured in brain heart infusion broth and incubated at 35°C. Working cultures were maintained on agar slopes at 4°C. *S. aureus* strain ATCC 25923 and *S. epidermidis* strain ATCC 12228 were used as control strains.

**Susceptibility to antimicrobial agents**

Susceptibility to antimicrobial agents was tested by the disk diffusion method as described previously [30], with Mueller Hinton Agar (Unipath, Basingstoke). The disks were impregnated with the following antibacterial agents (μg): methicillin (5), penicillin (10), gentamicin (10), kanamycin (30), neomycin (30), streptomycin (30), erythromycin (15), clindamycin (2), chloramphenicol (30), tetracycline (10), minocycline (30), trimethoprim (2.5), fusidic acid (10), rifampicin (5), ciprofloxacin (5), mupirocin (200), teicoplanin (30), vancomycin (30), cadmium acetate (50), mercuric chloride (100), propamidine isethionate (50) or ethidium bromide (60). The plates were incubated at 35°C for 24 h. Propamidine isethionate was a gift from Rhône-Poulenc Rorer, UK.

**Detection of staphylococcal toxins**

SE and TSST-1 production were detected by the reverse passive latex agglutination kits SET-RPLA and TSTRPLA (Unipath), respectively. Both tests were performed by following the manufacturer's instructions. Known positive and negative controls accompanied each test sample.

**Slime production**

Slime production was detected by the qualitative method as described by Jones et al. [31]. Pure cultures of the organisms were grown in 5 ml of trypticase soy broth supplemented with glucose 10% w/v and incubated at 35°C for 18 h in glass test tubes in a slanting position. The broth culture was then decanted and the slime layer adherent to the glass surface was stained with 5 ml of safranin (0.25 g in 100 ml of ethanol 95%). The tubes were allowed to dry before the results were read. When no stained slime or only a trace of film was observed, the isolate was reported as slime-negative. Organisms forming an extensive film of slime were designated slime-positive.

**Results**

**Distribution of staphylococcal isolates**

A total of 174 isolates of staphylococcal species consisting of 32 *S. aureus* and 142 CNS was isolated from the hands of healthy food handlers in different restaurants in Kuwait City. In contrast 157 *S. aureus* (92.3%) and only 13 CNS (7.6%) isolates were cultured from the nares of the workers. The 13 nasal CNS isolates consisted of six *S. hominis* (3.8%), four *S. epidermidis* (2.5%) and three *S. warneri* (1.9%). The nasal *S. aureus* isolates were not studied further as part of this report.

CNS obtained from the workers' hands consisted of 41 *S. hominis* (28.8%), 36 *S. warneri* (25.3%), 17 *S. saprophyticus* (11.9%), 17 *S. xylosus* (11.9%), 12 *S. schleiferi* (8.4%), six *S. epidermidis* (4.2%), six *S. haemolyticus* (4.2%), two *S. cohnii* (1.4%) and one each of *S. capitis*, *S. intermedius* and *S. lentus*. Two isolates could not be identified with the API-Staph identification system.

**Toxin production**

Twenty-four isolates (13.8%), consisting of four *S. aureus* (12.5%) and 20 CNS (14.1%), produced either SE or TSST-1. Thirteen isolates — two *S. aureus*, 10 CNS (5.6%) from the hands and one nasal *S. hominis*—produced TSST-1. Fifteen isolates — four *S. aureus* and 11 CNS—produced different SEs. Eleven of the 15 SE-isolates produced SEB; only three isolates produced SEA. Three isolates produced both SEA and SEB. None of them produced SED or SEE. Four isolates, two *S. aureus* and one each of...
**ENTEROTOXIN PRODUCTION BY CNS FROM RESTAURANT WORKERS**

S. saprophyticus and S. warneri, produced both SE and TSST (Table 1).

**Antimicrobial resistance**

Resistance to different antimicrobial agents was detected in all CNS species. However, only a small number were resistant to antibiotics. All were susceptible to vancomycin, teicoplanin, rifampicin, gentamicin, streptomycin and ciprofloxacin and 47 (33.1%) were susceptible to all antimicrobial agents tested. Thirty (21.1%) were resistant to tetracycline, 10 to methicillin, five to erythromycin, six to trimethoprim and nine to chloramphenicol; and three isolates (one S. saprophyticus and two S. xylosus) expressed high-level resistance to mupirocin.

Sixteen isolates (11.2%) were resistant to the nucleic acid binding compound, propamidine isethionate, and to mercuric chloride. Sixty-two isolates were resistant to cadmium. Twenty-six isolates (18.3%), were resistant to three or more of the antimicrobial agents and were classified as multi-resistant. Five of the nasal CNS isolates were resistant to tetracycline and six were resistant to cadmium. Of the 32 S. aureus strains isolated from the workers' hands, 14 and 10 of them were resistant to penicillin G and tetracycline, respectively, five were resistant to chloramphenicol and four were resistant to mercuric chloride and propamidine isethionate.

**Slime production**

Slime production was demonstrated in 67 (46.1%) of the CNS isolates. These consisted of 16 (39.0%) of 41 S. hominis, 15 (41.6%) of 36 S. warneri, 14 (82.3%) of 17 S. xylosus, six of 17 S. saprophyticus, five of 12 S. schleiferi, four of six S. haemolyticus, three of six S. epidermidis, both S. cohnii and one of the two unspeciated isolates. None of the S. capitis, S. intermedius and S. lents isolates produced slime. There was no correlation between slime and toxin production, as the majority of the isolates that produced slime did not produce toxin and, as summarised in Table 1, many of the isolates that produced toxins did not produce slime, although some slime-producing isolates also produced different toxins. Also, there was no correlation between slime production and antibiotic resistance or between toxin production and antibiotic resistance.

**Discussion**

This study investigated S. aureus and CNS isolates from the hands of food handlers working in restaurants in Kuwait City, for SE and slime production and for resistance to antimicrobial agents. The study revealed that CNS were more prevalent than S. aureus as hand colonisers among these restaurant workers. In contrast, CNS constituted only 7% of staphylococci from the noses of the same workers. In Spain, CNS constituted

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Species</th>
<th>TSST</th>
<th>SE</th>
<th>Slime</th>
<th>Resistance</th>
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<tbody>
<tr>
<td>52</td>
<td>S. aureus</td>
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<td>ND</td>
<td>Meth, Pen G, Tet, Tmp, Fcd, Cd, Pi</td>
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<td>ND</td>
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<td>S. aureus</td>
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<td>ND</td>
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</tr>
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<td>-</td>
<td>B</td>
<td>-</td>
<td>Cd</td>
</tr>
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<td>48</td>
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<td>-</td>
<td>+</td>
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<td>S. hominis</td>
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<td>-</td>
<td>+</td>
<td>Susceptible</td>
</tr>
<tr>
<td>29*</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>Ery, Cd</td>
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<td>Cd</td>
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<td>B</td>
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<td>-</td>
<td>Tet</td>
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<td>S. schleiferi</td>
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<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>Susceptible</td>
</tr>
<tr>
<td>136</td>
<td>S. warneri</td>
<td>-</td>
<td>B</td>
<td>-</td>
<td>Susceptible</td>
</tr>
<tr>
<td>138</td>
<td>S. warneri</td>
<td>+</td>
<td>B</td>
<td>-</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

Total 24 13 15 8 14

+, produced; -, not produced; Cd, cadmium; Ery, erythromycin; Fcd, fusidic acid; Hg, mercuric chloride; Km, kanamycin; PenG, penicillin G; Pi, propamidine isethionate; Meth, methicillin; Tet, tetracycline; Tmp, trimethoprim; ND, not determined.

*Nasal isolates.*
39.3% and *S. aureus* 27.6% of the nasal flora of food handlers [25]. This may reflect differences in the distribution of CNS flora on different body sites [2] and in different populations living in different geographical regions.

The species distribution of CNS on the hands of the food handlers in this study showed a higher incidence of *S. hominis* (23.6%) and *S. warneri* (20.6%) than *S. epidermidis* (3.4%). These results are similar to those reported by Larson and Burke [13], who isolated *S. hominis* more frequently from the skin flora of healthy adults and *S. haemolyticus* more frequently among hospitalised patients, but differ from the distribution of CNS on the skin of hospitalised patients where *S. epidermidis* is the most common species [30–35]. As the restaurant workers performed different duties relating to cooking and serving of different types of food, it is possible for some of them to have acquired staphylococcal flora from some of the food in addition to their own flora. However, it is not known if any of the foodstuffs contributed to the CNS flora detected in this study.

The present study found that 8% of the CNS (including a nasal isolate of *S. hominis*) and 12.5% of *S. aureus* from the hands of the food handlers produced one or a combination of staphylococcal enterotoxins (Table 1). Despite the fact that only a small number of the CNS produced SE, their detection is significant because it confirms that CNS from different sources can produce SE [20]. It is also important because SE has not been studied extensively in CNS from food handlers. Whereas 10 of the 11 SE-producing CNS produced SEB, three of the four *S. aureus* isolates produced SEC. In a previous study, the majority of nasal *S. aureus* isolates from food handlers in Kuwait City produced either SEA or SEB [27]. The finding that *S. aureus* isolates from the hands produced mostly SEC would indicate that they did not originate from the workers’ noses.

With the exception of tetracycline resistance, the incidence of antibiotic resistance was lower than that obtained from the skin flora of hospitalised patients [13, 30–35]. However, detection of resistance to propamidine isethionate in 11.2% of the isolates was significant. Propamidine isethionate and quaternary ammonium compounds are components of household disinfectants and antiseptics [36, 37], and resistance to propamidine isethionate is usually linked to resistance to quaternary ammonium compounds [36, 37]. Therefore their resistance may diminish the usefulness of antiseptics containing them as hand-washing agents for removing enterotoxigenic staphylococci from the hands and preventing their cross-contamination.

No correlation was observed between slime production and antibiotic multi-resistance or between slime production and toxin production. Similarly, Alexander and Rimland [38] could not establish a correlation between slime production and antibiotic resistance or between slime production and pathogenicity in peritonitis caused by CNS, although slime production has been shown to correlate with antibiotic multi-resistance [30–33] and with pathogenicity [34, 39] in CNS from hospital patients.

The detection of SE in CNS colonising the hands of these restaurant workers raises the important question as to whether these SEs can cause food poisoning. Although SEs have been detected previously from food incriminated in food poisoning [16, 20, 21], from milk [22] and from the noses of food handlers [25], they have not been directly linked with food poisoning. However, as indicated previously, any staphylococcal strain producing SE can be involved in food poisoning [29]. In addition, the present study has demonstrated that enterotoxigenic CNS can co-exist with enterotoxigenic *S. aureus*, colonising both nasal and hand surfaces. Therefore, it is possible for enterotoxigenic CNS to contribute to food poisoning given the right conditions. It is possible that they have been involved in food poisoning in the past in association with *S. aureus*, but have been overlooked once enterotoxigenic *S. aureus* was isolated. As cautioned by Crass and Bergdoll [16], CNS should not be ignored but should be considered with *S. aureus* in suspected cases of staphylococcal food poisoning. Also, increased awareness of enterotoxigenic CNS carriage among food handlers is necessary to avoid contamination of food with these strains.

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