Journal of Infection and Public Health (2016) 9, 153—160

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Food handlers as potential sources of dissemination of virulent strains of *Staphylococcus aureus* in the community

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Received 10 April 2015; received in revised form 26 June 2015; accepted 29 August 2015

KEYWORDS
Staphylococcus aureus;
Food handlers;
Hands and nose carriage;
Antimicrobial resistance;
Enterotoxin genes

Summary Food handlers may constitute a reservoir of virulent strains of *Staphylococcus aureus* and may be vehicles of their transmission to food.

One hundred and sixty-two volunteers were assessed for the presence of *S. aureus* on the hands and in the nose. *S. aureus* was isolated by routine procedures, and the isolates were tested for susceptibility against a panel of nine antimicrobial agents. The isolates were further characterized by SmaI-PFGE profiling and the presence of virulence factors.

Results: The prevalence of *S. aureus* was 19.8% in the nose and 11.1% on the hands; 6.2% of the individuals carried *S. aureus* both in their noses and hands, and three individuals had the same strain (PFGE type) in the nose and on the hands. Although 82% of the isolates were resistant to at least one antibiotic, none demonstrated the presence of either *mecA* gene or resistance to oxacillin (none identified as MRSA). Sixty-eight percent of the isolates from the nose and hands possessed enterotoxin genes.

This study revealed a high prevalence of antibiotic resistance and virulence determinants among the isolates, including not only classical and novel enterotoxin genes but also major virulence factors such as *tst*. Potential dissemination of these strains in the community is a matter of concern.

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Introduction

Staphylococcus aureus is one of the most important species in the field of food microbiology and has been considered a foodborne hazard for a

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http://dx.doi.org/10.1016/j.jiph.2015.08.001

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long time. In 2013, 386 staphylococcal outbreaks were reported by the EFSA, representing 7.4% of all outbreaks reported in the European Union [1]. Staphylococcal food poisoning, gastroenteritis with emesis and with or without diarrhea [2] characterized by a short incubation period, typically 2—4 h [3], is caused by the ingestion of food containing preformed enterotoxins. Not all strains are capable of producing staphylococcal enterotoxins [4], but up until now, 22 SEs have been described, 11 of them with known emetic action [5].

S. aureus can colonize the skin and the anterior nares of individuals and is carried by a significant proportion of the population [6]. As found by Kluytmans and Wertheim [6], *S. aureus* colonizes the nares of approximately 50% of healthy adults, either persistently or intermittently. In a study by Lues and Van Tonder [7], *S. aureus* was isolated from the hands of 88% of the population sampled. Human nasal or hand carriage of enterotoxigenic *S. aureus* during food processing is an important source of food contamination with *S. aureus* [5,8]. In fact, food poisoning outbreaks associated with post-process contamination of foods with *S. aureus* are in part the responsibility of food handlers who carry enterotoxigenic staphylococci in their nares or on their skin [7].

In recent decades, the increasing prevalence of antimicrobial-resistant *S. aureus* is receiving widespread attention. Strains of methicillin-resistant *S. aureus* (MRSA) are of particular concern given that they represent a significant cause of morbidity and mortality throughout the world. Methicillin-resistant *S. aureus* are resistant to all available penicillins and other β-lactam antimicrobial drugs [9]. Trends for the period 2009—2012 were calculated for 28 countries. Statistically significant increasing trends were observed for four countries, including Portugal, where in 2012, the percentage of MRSA isolates was greater than 50% [10].

Since Kluytmans et al. [11] described the first fatal foodborne outbreak of MRSA, food microbiologists now consider the possibility of foods as vectors of antimicrobial-resistant strains.

To identify MRSAs, the detection of the presence of the *mecA* gene and consequent resistance to methicillin is important not only in food isolates but also on food handlers who contribute to the cross-contamination of food products.

The combination of enterotoxin genes and the *mecA* gene could provide us with information about the presence of resistant strains in foodborne diseases and also the importance of food as a vehicle for antimicrobial resistance.

The purpose of this study was to evaluate the prevalence of *S. aureus* among healthy individuals working in a food company and to characterize isolates regarding their resistance to antibiotics and virulence factors. A potential clonal relationship between isolates from the nose and hands of the same individuals was also investigated.

Material and methods

Staphylococcus aureus sampling

One hundred and sixty-two volunteers from a food company were assessed for the presence of *S. aureus* on their hands and in their nose (a total of 324 samples were recovered). The definition of the sample was one of convenience and included 103 women and 59 men. This company sells food to numerous clients all over Portugal; raw meat is chopped and used within the company for further processed meat-containing foods or is sold to local shops.

The specimens were collected using a cotton-tipped swab previously moistened with sterile Ringers solution. The anterior nares were sampled by rotating the swab tip in both nostrils. Swabs were then spread onto Baird-Parker Egg Yolk Tellurite Medium (LabM, Bury, United Kingdom) and incubated aerobically at 37 °C for 48 h. Characteristic colonies were sub-cultured on Mannitol Salt Agar (MSA; Pronadisa, Madrid, Spain) incubated aerobically at 37 °C for 24 h. Presumptive *S. aureus* colonies on MSA (yellow colonies with yellow zones, Gram-positive, catalase positive, coagulase positive and DNase positive) were streaked on Tryptone Soy Agar (TSA; Pronadisa) before being stored at −80 °C in Brain Heart Infusion (BHI; LabM) broth containing 30% (v/v) glycerol.

DNA extraction

DNA was extracted from single colonies on TSA using the guanidine-isothiocyanate method [12]. DNA was quantified spectrophotometrically at 260 nm and 280 nm.

Identification of isolates by multiplex PCR

PCR multiplex to detect the simultaneous presence of 16S rRNA (*Staphylococcus* genus specific), *nuc* (*S. aureus* species specific) and *mecA* (determinant of methicillin resistance) genes was performed according to Zhang et al. [13]. *Staphylococcus aureus* DSM 11729 was used as a positive control for the gene *mecA*, *Staphylococcus epidermidis* DSM

20044 as a negative control for the gene *nuc* and *S. aureus* ATCC 29213 as a positive control for targeting 16S rRNA and the *nuc* gene and as a negative control for the gene *mecA*.

Detection of staphylococcal enterotoxin genes by multiplex PCR

The detection of enterotoxin genes, *sea* to *sej* and *tst*, was performed by multiplex PCR according to Løvseth, Loncarevic and Berdal [14]. The amplification of the target 16S rRNA gene was included as the internal control. As positive controls, different strains of *S. aureus* kindly supplied by Prof. Løvseth (National Veterinary Institute, Norway) were used: R2102/00 for the *sec*, *seg*, and *sei* genes; R4571/00 for the *sec* gene, FRI572 for the *seg* and *sei* genes; 3169 for the *sec*-bovine, *sed*, and *sej* genes; FRI472 for the *sed*, *seg*, *sei*, and *sej* genes; R5371/00 for the *sea*, *seg*, *seh*, and *sei* genes; R963/00 for the *sed*, *seg*, *sei*, and *sej* genes; R5460/00 for the *seb*, *seg*, *seh*, and *sei* genes; FRI913 for the *sea*, *sec*, and *see* genes; FRI445 for the *seg* and *sei* genes; R4071/00 for the *seb* gene; and R4774/00 as a negative control.

A 5-μl aliquot of DNA was added to a 20-μl PCR mixture containing 0.3 μM of each primer except 16S rRNA and *sei* (0.1 μM) and 12.5 μl Kapa 2G Fast master mix (Grisp, Porto, Portugal). Amplification was carried out as follows: an initial denaturation step at 95 °C for 3 min; 30 cycles at 95 °C for 15 s, 62 °C for 30 s and 72 °C for 30 s; and a final extension step at 72 °C for 1 min. The PCR products were resolved in 2% (w/v) agarose gels (1× Tris Acetic Acid, EDTA) at 60 V (constant voltage) for 3 h and visualized in a transilluminator.

Antibiotic susceptibility testing by agar dilution

The minimal inhibitory concentrations (MICs; μg/mL) for *S. aureus* isolates were determined by the agar dilution method described in the guidelines of the Clinical and Laboratory Standards Institute [15]. The inoculum was prepared from a 24 h culture on TSA by suspension in sterile Ringer's solution to obtain turbidity equivalent to the 0.5 McFarland standard. The antibiotics investigated were penicillin G, chloramphenicol (both obtained from Sigma, Steinheim, Germany), oxacillin (BioChemica, Billingham, UK), rifampin, gentamicin, tetracycline, erythromycin and ciprofloxacin (all kindly supplied by Labesfal, Tondela, Portugal) and Vancomycin (Fluka, Steinheim, Germany). The MICs were determined in Mueller-Hinton agar (MH; bioMérieux, Marcy l'Etoile, France) plus 2% (w/v) NaCl in the case of oxacillin, in cation-adjusted MH for penicillin and ampicillin and in MH for the other antibiotics investigated. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as controls. For each antibiotic susceptibility determination, at least two independent experiments were performed.

Detection of other of virulence factors

The production of hemolysin was evaluated on blood agar plates (COS, Columbia agar plus 5% (v/v) sheep's blood; bioMérieux). The isolates were streaked onto the plates and incubated at 37 °C for one to two days. The presence or absence of zones of clearing around the colonies was interpreted as β-hemolysis (positive) or gamma-hemolysis (negative) activity, respectively. Greenish zones around the colonies were interpreted as α-hemolysis [16].

Lipase activity was assessed as described by Tiago et al. [17]. A positive reaction was indicated by opacity around the colonies.

Gelatinase activity was assessed according to Tiago et al. [17].

For each virulence factor tested, at least two independent experiments were performed.

DNA-macrorestriction by pulsed-field gel electrophoresis (PFGE)

PFGE typing of the isolates was performed as previously described by Chung et al. [18] using the restriction enzyme SmaI (ThermoScientific, New York, USA) and *Salmonella enterica* ser. Braenderup H9812 as a standard and a CHEF Mapper XA (Bio-Rad, Laboratories, Hercules, CA, USA). PFGE image analysis and similarity clustering were performed with GelCompar software (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was done by the unweighted pair group method with average linkages (UPGMA), using the Dice coefficient to analyze the similarities of the banding pulsotypes.

Results

Fifty *S. aureus* isolates were recovered from the hand and nose samples of 162 individuals (Supplementary Table). Nearly one-quarter (24.7%, 40/162) of the individuals were *S. aureus* carriers; 60% of these (24/40) were female, and 40% (16/40) were male (Supplementary Table). Nasal carriage was found in 19.8% and hand carriage was found

Table 1 Hand and nasal carriage of *Staphylococcus aureus* among food handlers.

	Number of positive (%)
Hands	18 (11.1)
Nose	32 (19.8)
Hands and nose (simultaneously)	10 (6.2)
Hands but not nose	8 (4.9)

in 11.1% of the individuals; 6.2% had *S. aureus* in both their hands and nose. Eight individuals (4.9%) had *S. aureus* on their hands but not in their nose (Table 1).

Supplementary Table related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jiph.2015.08.001.

PFGE analysis was performed only for those isolates collected from the nose and hands of the same individual. Eighteen of these isolates were distributed among 15 PFGE types, and two were non-typeable (2095H and 2095N; Supplementary Table). In three individuals, the same strain was found in the nose and on the hands (Fig. 1).*seg* and *sei* were the most prevalent genes in the isolates recovered from both the nose (82.6%) and hands (70%), followed by the *tst* gene, which was recovered from 39.1% to 40% of noses and hands, respectively (Supplementary Table and Table 2). For all of the isolates, an association between the *seg* and *sei* genes was observed (Table 2).

Table 2 Arrangements of enterotoxin genes profiles of *Staphylococcus aureus* isolated from hands and nose of food handlers.

	N (%)
Nasal isolates	
secbov, *seg*, *sei*	6 (30.4)
seg, *sei*	3 (13.0)
sea, *seg*, *sei*, *tst*	2 (8.7)
seh, *seg*, *sei*, *tst*	2 (8.7)
tst	2 (8.7)
sej, *tst*	1 (4.3)
sea, *seh*, *seg*, *sei*	1 (4.3)
seg, *sei*, *tst*	1 (4.3)
sea, *tst*	1 (4.3)
sea, *sej*, *seg*, *sei*, *sed*	1 (4.3)
sec, *seg*, *sei*	1 (4.3)
sea, *sec*, *seg*, *sei*	1 (4.3)
sea, *seh*	1 (4.3)
Hands isolates	
secbov, *seg*, *sei*	3 (33.3)
sej, *tst*	2 (22.2)
seg, *sei*	2 (22.2)
seh, *seg*, *sei*, *tst*	1 (11.1)
seb	1 (11.1)
sec, *seg*, *sei*, *tst*	1 (11.1)

Table 3 Antibiotic sensitivity pattern of the *Staphylococcus aureus* isolates recovered from hands and nose of food handlers.

Antibiotic	Sensitive	Intermediate	Resistant
	N (%)		
Penicillin	26 (52.0)	[a]	24 (48.0)
Oxacillin	50 (100.0)	[a]	0 (0.0)
Tetracycline	48 (96.0)	0 (0.0)	2 (4.0)
Ciprofloxacin	37 (74.0)	3 (6.0)	10 (20.0)
Erythromycin	34 (68.0)	0 (0.0)	16 (32.0)
Gentamicin	49 (98.0)	0 (0.0)	1 (2.0)
Rifampin	46 (92.0)	0 (0.0)	4 (8.0)
Chloramphenicol	41 (82.0)	8 (16.0)	1 (2.0)
Vancomycin	(100)	0 (0.0)	(0.0)

[a] Antibiotic with no described value for the intermediate MIC.

The antibiotic resistance profile of the *S. aureus* isolates is presented in Table 3. Eighteen percent of the isolates were sensitive to all of the antibiotics investigated. Forty-eight percent and 26% of the isolates were resistant to two or three antibiotics of different classes, respectively. One isolate was resistant to five antibiotics, and another was resistant to six. Forty-eight percent of isolates were resistant to penicillin, 32.0% to erythromycin and 20.0% to ciprofloxacin. Four isolates were resistant to rifampin, two were resistant to tetracycline, one was resistant to gentamicin, and another was resistant to chloramphenicol. All of the isolates were sensitive to vancomycin and oxacillin (Table 3). The gene *mec*A was absent among all of the isolates (data not shown).

β-Hemolysin, γ-hemolysin, α-hemolysin, lipase and gelatinase were identified in 66%, 12%, 22%, 82% and 88% of isolates, respectively (Supplementary Table).

Discussion

The prevalence of *S. aureus* in the nose of the workers at the food company investigated (19.8%) is in accordance with the mean nasal colonization of healthy adults (20—30%) who are persistent carriers [6]. Similar values have been reported by other authors [19,20]. Nevertheless, other authors had reported higher [21—24] and lower prevalences [25]. A lower prevalence of *S. aureus* was found on the hands (11.1%) than in the nose of the food handlers. This is consistent with the findings of previous studies [21,22]. The reported prevalence of *S. aureus* on the hands of food handlers is highly variable. While values similar to or lower

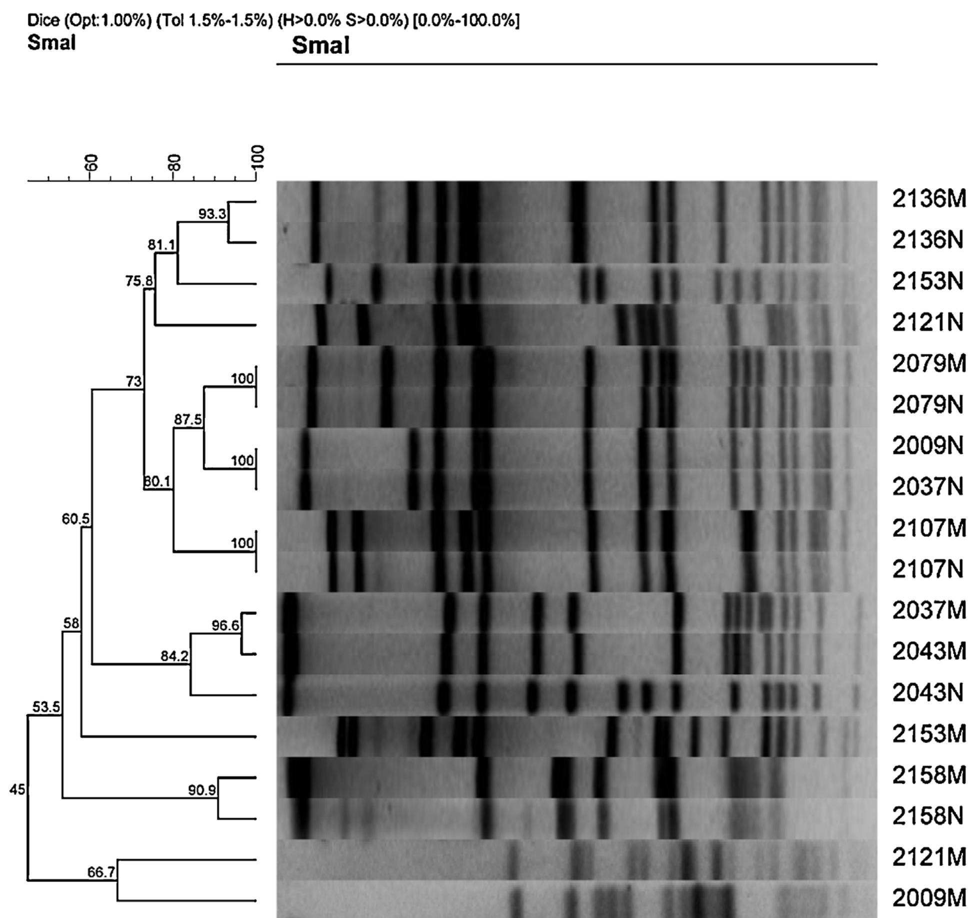

Figure 1 Dendrogram of *Staphylococcus aureus* isolates recovered from the hands and nose of the same individual.

than those found in this study have been reported [21,26], prevalences higher than 50% have also been reported [7,24,27,28]. A high carriage level had been found for food handlers working in hospitals [24,27] where *S. aureus* is highly disseminated.

Ten individuals carried *S. aureus* both nasally and on their hands, and three of these individuals had the same strain in their nose and hands. The presence of *S. aureus* on the hands but not in nose and the detection of isolates in the nose and hands of the same individual with different PFGE types indicate that hand contamination may result from sources other than the individual [21]. Food is naturally contaminated, at least temporarily, when raw or even through exposure during processing or refrigeration to temperatures that allow the growth of *S. aureus*. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *S. aureus* [29].

Eighty-two percent of the isolates were resistant to at least one antibiotic, and 48%, 26% and 8% were resistant to one, two and three or more antibiotics of different classes, respectively. None demonstrated the presence of either the *mecA* gene or resistance to oxacillin. MRSAs have frequently been isolated from health professionals [30], although the isolation of MRSAs from food handlers is rare [22,23,25]. The rate of penicillin resistance was lower in our study relative to that found for isolates recovered from foods commercialized in Portugal [31] and also from food handlers in other studies [22,24,25]. In contrast, higher percentages of isolates resistant to ciprofloxacin and to erythromycin were observed. Working in hospitals seems to be a risk factor for the carriage of resistant strains by food handlers, as demonstrated in the study by Ferreira et al. [24]. The high prevalence of antibiotic resistance among food handlers and the carriage of multidrug-resistant strains highlights the growing problem of antibiotic resistance in the ''healthy'' community.

S. aureus produces a wide array of cell surface and extracellular proteins (proteases, lipases and cytotoxins such as hemolysins — alpha, beta,

gamma and delta) involved in virulence that enable it to invade and destroy host tissues and metastasize to other sites [2]. *Staphylococcus aureus* Hlb (gene of beta-hemolysin) also plays an important role in skin colonization by damaging keratinocytes, in addition to its well-known hemolytic activity for erythrocytes [32]. The results from this study are consistent with those observed in previous studies. Among the *S. aureus* isolated from the oral cavity, 100% produced gelatinase, 77% lipase, 59% beta-hemolysins and 41% alpha-hemolysins [33]. Saising et al. [34] reported that 65.6% of the strains isolated from acne lesions were lipase positive, and Wu et al. [35] showed that 80% of *S. aureus* isolated from corneal ulcers produced gelatinase. Beta-hemolysin is secreted by certain strains of *S. aureus*, especially strains isolated from corneal infections [33].

S. aureus produces a wide variety of toxins including staphylococcal enterotoxins (SEs; SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity and staphylococcal-like (SEl) proteins, which are not emetic in a primate model (SElL and SElQ) or have yet to be tested (SElJ, SElK, SElM to SElP, SElU, SElU2 and SElV). SEs and SEls have been traditionally subdivided into classical (SEA to SEE) and new (SEG to SElU2) types. Each of these toxins is known to have potent effects on the cells of the immune system, but many of them have other biological effects as well. Their primary function *in vivo* may be to inhibit host immune responses to *S. aureus* [2]. SEs are the causative agents of staphylococcal food poisoning resulting from the ingestion of contaminated food. Due to their extraordinary stability in denaturing conditions, such as heat and low pH levels, SEs are not completely destroyed by mild cooking or the digestion of food in the stomach. Nausea, emesis, abdominal pain or cramping and diarrhea ensue after a short incubation period. The disease is usually self-limiting [36].

The majority (71.9% and 55.6%, respectively) of isolates recovered from the nose and hand samples possessed enterotoxin genes. A high prevalence of enterotoxigenic isolates recovered from food handlers has been reported by several studies [19,22,37]. Although *sea* is the most commonly reported enterotoxin gene [19,37], this was not observed in the present study in which *seg* and *sei* were the most prevalent enterotoxin genes and were associated with all of the isolates. This association has been previously reported [19] and is justified by the fact that they belong to an operon of the *egc* enterotoxin gene cluster, which contains five enterotoxin genes (*seg*, *sei*, *sem*, *sen*, and *seo*) [38]. The *tst* genes were also highly prevalent. *tst* genes have been previously detected in isolates recovered from food handlers [22,37], though normally in lower percentages. TSST-1-producing *S. aureus* was detected for the first time on a food service worker's hand by Sospedra et al. [39]. TSST-1 was the first marker identified for Staphylococcal Toxic Shock Syndrome (TSS), which is an acute and potentially fatal illness that is characterized by a high fever, diffuse erythematous rash, desquamation of the skin one to two weeks after onset (if not fatal before this time), hypotension, and the involvement of three or more organ systems [36].

Food has to meet high food safety and food quality standards. Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP) systems are applied to improve the microbial safety and quality of food. However, even with the best control measures in place, a food product may still pose a risk to the consumer. The presented data have certain limitations, as the population sampled in this study is not representative of the food handler population as a whole in Portugal. Nevertheless, the food company analyzed followed all of the measures referred to above, and yet, the food handlers' hands were contaminated with enterotoxigenic and antibiotic-resistant *S. aureus* strains.

Conclusion

This study revealed a high prevalence of antibiotic resistance and virulence determinants, including not only classical and novel enterotoxin genes but also major virulence factors such as *tst*, in the studied population, which is one of the major sources of contamination/recontamination of food with *S. aureus* during processing. Potential dissemination of these strains in the community is a matter of concern.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

This work was supported by National Funds from the Fundação para a Ciência e a Tecnologia (FCT) through project Pest-OE/EQB/LA0016/2013. Financial support for Ana Castro was provided by FCT through a doctoral fellowship BD/39315/2007. Editing of this paper by Dr. P.A. Gibbs is gratefully acknowledged.

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