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

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**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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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

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