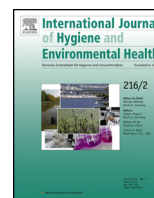




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Occupational exposure to raw meat: A newly-recognized risk factor for *Staphylococcus aureus* nasal colonization amongst food handlers

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ABSTRACT

Staphylococcus aureus contaminating raw meat may increase nasal colonization risk for occupationally-exposed food handlers. Food handlers from six catering establishments were nasally sampled for *S. aureus* and completed a questionnaire on carriage risk factors. Isolates were characterized for antibiotic susceptibility, *spa* type and, for methicillin-resistant strains, SCCmec type. Of 434 food handlers, 99 (22.8%) were colonized with *S. aureus*. Five isolates were methicillin-resistant belonging to SCCmec IV (2) and V (3). Resistance to tetracycline (20%), and erythromycin (16%) was high, but <10% to other antibiotics. *Spa* typing revealed 17% of isolates as t189, with 8% each t127 and t1081. Food handlers ever handling raw meat had a significantly higher colonization risk (OR=2.7; 95% CI: 1.7–4.5), increasing to 3.7 (95% CI: 2.0–6.8) for those always exposed. This is the first report of increased colonization risk in food handlers exposed to raw meat. This occupational hazard may increase infection risk, so improved compliance with workplace hygiene may be required.

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Introduction

Nasal carriage of *Staphylococcus aureus* in food handlers is the major risk for staphylococcal food poisoning and thus a concern for food safety. Although at any one time approximately 20% of healthy individuals carry the organism, only a proportion of the population is persistently colonized and these subjects harbour a heavier bacterial load which acts as a reservoir for spread of *S. aureus*. The remaining population may be transiently colonized (Van Belkum et al., 2009). If there is poor compliance with hygiene measures, hands can be contaminated and the organism transferred to food where products of strains positive for enterotoxin genes can lead to food poisoning (Argudin et al., 2012). Consumption of these foods results in acute gastroenteritis. In various food poisoning outbreak investigations, both epidemiologic and molecular data have indicated that *S. aureus* carried by food handlers was the source of contamination (Do Carmo et al., 2004; Greig et al., 2007).

In recent years, livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) has emerged and become widely disseminated, initially among pigs and poultry (Graveland et al., 2011; Larsen et al., 2012) and, subsequently has been isolated from retail meats (O'Brien et al., 2012; Boost et al., 2013; de Boer et al., 2009). Whilst various genotypes have been identified, most

isolates from Europe and Asia belong to two dominant clonal lineages, designated ST398 and ST9 respectively (Graveland et al., 2011; Guardabassi et al., 2009). A significantly higher risk of acquiring MRSA of porcine origin has been observed in pig farmers who have regular contact with pigs and this may persist even after a period during which there is no pig contact (Voss et al., 2005; Köck et al., 2012). MRSA contamination of meats and meat products has been reported from several countries (Kitai et al., 2005; de Boer et al., 2009; Bhargava et al., 2011; Fessler et al., 2011; Boost et al., 2013), although there have been limited reports of enterotoxin gene carriage in these strains (Argudin et al., 2011; Ho et al., 2012).

Occupational exposure to raw meat may predispose workers to contamination and increase their risk for colonization, as was observed in a recent survey of butchers in wet markets (Boost et al., 2012). In a large scale survey investigating *S. aureus* isolates from food, fresh meat was found to have the highest contamination rate (Di Giannatale et al., 2011). This study aimed to determine if food handlers employed in large scale catering establishments and regularly in contact with raw meats exhibit an increased risk for carriage of *S. aureus* and whether livestock-associated strains of *S. aureus* are more common in these food handlers.

Materials and methods

Sample population

In this cross-sectional study, food handlers are defined as workers in commercial kitchens who handle raw and/or cooked foods on

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a daily basis. Twelve of the largest catering establishments in Hong Kong, each employing at least 60 food handlers, were invited to participate, of whom six responded positively. These consisted of a centralized kitchen supplying meals for 40% of Hong Kong hospitals, caterers servicing canteens for staff and students at Universities, a centralized kitchen for attendees and staff of major sporting facilities, and two large hotels each with >400 rooms and three restaurants. Based on an *S. aureus* carriage rate of 24% in the general population (Zhang et al., 2011), a sample size of 416 subjects was calculated to yield 100 isolates for characterization. To determine if there was a difference in carriage rates between food handlers exposed to raw meat and those non-occupationally exposed, 175 subjects of each type would be required to determine prevalence with a 2% error. At each establishment, the manager invited all staff involved in direct handling of food to participate in the study. Overall, there was a 94% response rate, with a total of 434 food handlers successfully recruited between June 2011 and June 2012. All participants were provided with a short description of the study and signed informed consent. Ethical approval was obtained from the University Human Subjects Ethics Committee.

Risk estimation

All food handlers were asked to complete a one-page questionnaire to determine risk factors for *S. aureus* colonization. The questionnaire sought information on demographics, smoking, hand washing habits, years of work experience in the catering industry, daily working hours, frequency and type of raw meat handled at work, use of gloves at work, hand washing practice, pet ownership, employment of family members as healthcare workers, presence of chronic illnesses, use of antibiotics within the last three months, and hospitalization in the last 12 months. Reliability of the questionnaire was assessed by pilot testing at the first establishment where the questionnaire was repeated two weeks after sampling.

Sample collection and isolation of *S. aureus*

All nasal specimens were collected by one investigator using Transwabs (Oxoid, UK) which were moistened with sterile normal saline, inserted into the anterior nares and rotated against the nasal septum. Specimens were enriched in brain heart infusion broth (Oxoid, Basingstoke, UK) supplemented with 5% sodium chloride at 37 °C for 24 h and then plated onto SA *Select* chromogenic agar (Biorad UK Ltd., Hemel Hempstead, UK).

S. aureus was identified by latex agglutination test (Remel, Lennexa, KS, USA) and confirmed by amplification of the 16S-rRNA gene as previously described (Monday and Bohach, 1999).

Antibiotic susceptibility testing and typing of *S. aureus* strains

Susceptibility to cefoxitin and oxacillin, to indicate methicillin resistance, and to a range of non-beta lactam antibiotics, including tetracycline, erythromycin, gentamicin, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, linezolid, and quinupristin-dalfopristin, was determined using disc diffusion (CLSI, 2012). Fusidic acid susceptibility was determined using EUCAST guidelines (www.euca.org).

Presence of the *mecA* gene was confirmed in isolates phenotypically resistant to cefoxitin and/or oxacillin and SCCmec typing performed by identification of *ccr* type and *mec* complex class as previously described (Kondo et al., 2007). Staphylococcal protein A gene (*spa*) typing was performed and the oligonucleotide sequences matched with the online Ridom database (www.spaserver.ridom.de).

Table 1

Nasal carriage rates of *S. aureus* at individual catering establishments.

Site ^a	Number of subjects (% of total)	<i>S. aureus</i> isolates (% carriage rate)
1	40 (9.2)	10 (25.0)
2	53 (12.2)	9 (17.0)
3	40 (9.2)	11 (27.5)
4	16 (3.7)	3 (18.8)
5	187 (43.1)	42 (22.5)
6	98 (22.6)	24 (24.5)
Overall	434 (100)	$p = 0.86^b$

^a Sampling Site 1. University Canteen A; 2. University Canteen B; 3. Centralized Hospital Kitchen; 4. Hotel A; 5. Hotel B; 6. Centralized kitchen for local sporting facilities.

^b Chi-square test.

Detection of genes for classical staphylococcal enterotoxins

All *S. aureus* isolates were investigated for the presence of *sea*-*see* by PCR using primers as previously described (Monday and Bohach, 1999). Amplified products were visualized by agarose gel electrophoresis and compared with control strains included in each batch.

Statistical analysis

Carriage rates in various establishments were compared using Chi-square test. Risk factors for *S. aureus* carriage were analyzed by Pearson Chi-square test or Fisher's exact test using SPSS version 19.0 for Windows. Variables significant or approaching significance ($p \leq 0.15$) in the univariate model were further analyzed by multiple backward logistic regression to identify independent risk factors. A p -value <0.05 was considered as statistically significant.

Results

There were a total of 434 participants, 288 (66%) male and 146 (34%) female. Overall, 99 (22.8%) were nasally colonized with *S. aureus*, of whom 74 were meat handlers. Types of raw meat handled were predominantly pork, beef and chicken. The carriage rate observed at different workplaces did not differ significantly (Table 1).

Colonization rates were considerably higher in workers handling raw meats (30%) than in non-exposed workers (13.4%) (OR = 2.7, 95% CI: 1.7–4.5) (Table 2). Other factors including gender, hand washing after handling raw meat, recent antibiotic use, and acne approaching though not reaching statistical significance were included in the logistic regression model, but results revealed handling raw meat regularly at work as the only independent risk factor for *S. aureus* colonization in food handlers (Table 2).

There was a positive trend of increased colonization between workers who never, sometimes and always handled raw meat ($P_{\text{trend}} < 0.001$). "Sometimes" was defined as one to two days per week, while "always" meant on at least three days each week. When compared to food handlers who were never exposed, those sometimes handling raw meat had a two-fold higher colonization risk, which increased to almost four-fold for regular handlers (Table 3).

Five isolates were MRSA belonging to SCCmec type IV ($n = 2$) and V ($n = 3$). Resistance to non-beta-lactam agents was also observed including tetracycline 20%, erythromycin 16%, gentamicin 10%, chloramphenicol 7%, ciprofloxacin 7%, fusidic acid 6%, clindamycin 4%, and co-trimoxazole 2%. All isolates were sensitive to linezolid and quinupristin-dalfopristin. Resistance to clindamycin was all constitutive.

Although a total of 42 *spa* types were detected, the three types most frequently observed were t189 (17%), t127 (8%) and t1081 (8%). Colonization with these *spa* types and t034 appeared higher

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