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ORIGINAL ARTICLE

High prevalence of methicillin resistant and enterotoxin gene-positive *Staphylococcus aureus* among nasally colonized food handlers in central Iran

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Abstract

This study defined the prevalence of enterotoxin gene-positive *Staphylococcus aureus* strains among food handlers and non-food processing healthy nasal *S. aureus* carriers in central Iran. Meticillin-resistant *S. aureus* (MRSA) strains were diagnosed by cefoxitin disk diffusion. PCR was used to detect the *mecA*, *Sa442*, and enterotoxin genes. Out of the 1113 food handlers, 224 (20.1%) were nasal carriers of *S. aureus* and 157 (70.1%) of these isolates were positive for one or more enterotoxin genes. The most prevalent enterotoxin gene was *sei* (40.2%), followed by *seg* (35.3%), *sea* (23.5%), *seb* (15.2%), *sec* (5.5%), and *seh* (2.7%). *See* and *sed* genes were not found. Sixty seven (42.7%) of enterotoxin gene-positive isolates possessed a single enterotoxin gene, and 64 (40.8%), 23 (14.7%), and 3 (1.9%) contained two, three, or four enterotoxin genes, respectively. The most frequently detected gene combination was *sei*/*seg* ($n = 35$, 22.3%). Thirty seven (16.5%) isolates were diagnosed as MRSA, and 27 (73%) of these strains were positive for at least one enterotoxin gene. Out of 546 healthy controls, 100 individuals were identified as *S. aureus* nasal carriers; among the strains, 39 (39%) were positive for at least one enterotoxin gene. Only one (1%) CA-MRSA was identified among the strains from the volunteers. A high prevalence of meticillin resistant and enterotoxin-positive *S. aureus* were documented in food handlers. We suggest that this may be due to the frequent handling of contaminated foodstuffs and that this is possibly related to the elevated frequencies of acquired staphylococcal food poisoning in this population.

Keywords Nasal carriage · *Staphylococcus aureus* · Enterotoxin genes · Food handlers

Introduction

Globally, a large number of consumers suffers from foodborne illnesses [1]. Toxigenic strains of *Staphylococcus aureus* produce enterotoxins that may cause such food poisoning upon ingestion [2], and staphylococcal food poisoning (SFP) is one of the most common gastrointestinal diseases [3]. Symptoms develop within 1 to 6 hours, and patients typically experience vomiting, diarrhea, and abdominal pain [4]. Direct food contamination by toxin-producing *S. aureus* is one of the frequent threats to food safety [5], and improper food processing is the main cause for SFP outbreaks [2].

About 20–30% of humans is persistently and 40–60% is intermittently colonized with *S. aureus* [6]. The nasal cavity is the primary ecological niche, but skin folds in the groin, the perineum, the axillary area, the nasopharynges, and the hands are often colonized or contaminated as well [7]. Hence, colonized food handlers are frequent and can shed enterotoxigenic bacteria during food preparation [6]. Staphylococcal enterotoxins (SEs) are low molecular weight superantigens, insensitive to heat, irradiation, denaturing agents, and a wide pH range [8]. These toxins are divided into the so-called classical enterotoxins (five serotypes, SE-A to E) and the staphylococcal enterotoxin-like proteins (SE-G to -I, SEI-I to -R, SEI-U,

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SEI-V, and SEI-X). The distinction is based on the ability to invoke emesis [9, 10]. More than 95% of *S. aureus* producing enterotoxins causing SFP generate the classical emetic ones [11]. SEs and SEIs are usually encoded by mobile genetic elements (MGE) and enterotoxin genes are widespread among *S. aureus* due to horizontal gene transfer [9]. Certain strains of *S. aureus* express resistance to beta-lactam antibiotics such as methicillin, penicillin, and other penicillin-like antibiotics, which are referred to as methicillin-resistant *S. aureus* (MRSA). Resistance is due to the *mecA* gene that encodes the protein penicillin-binding protein (PBP) 2A [12].

Data obtained from Iranian centers of public health in the central province of Iran indicate that the prevalence of SFP in this region is considerable. Figure 1 shows the number of clinical specimens obtained from patients with diarrhea in central Iran over the past few years, which were considered to be negative for *Salmonella* spp., *Shigella* spp., amoeba, and *Campylobacter* spp. Therefore, the aim of the present study was to determine the frequency of *S. aureus* nasal carriage among food handlers and to quantify enterotoxin gene presence in isolated strains.

Materials and methods

Sample collection and *S. aureus* identification

The city of Arak is the capital of the central province in Iran. It lies at 49° 41′ east longitude and 34° 5′ north latitude, 284 km southwest of Tehran. Food handlers from Arak ($n = 1113$) were assessed for nasal carriage of *S. aureus* in a cross-sectional study (February to August 2017). During the same episode, 546 healthy people residing in the same region were screened as controls. The presence of the *mec*A gene and staphylococcal enterotoxin genes was investigated for all isolates. Respecting ethical principles (IRB application and validation: IR.ARAKMU.REC.1395.270), nasal swab samples were obtained from both anterior nares using the Transwab system and Amies transport medium (Medical Wire and Equipment Company, Corsham, UK). Swabs were transferred to the Laboratory of Microbiology, Faculty of Medicine, Arak University of Medical Sciences within 6 hours. Swabs were incubated at 37 °C for 6–12 h and then streaked on sheep blood agar and mannitol salt agar. Plates were aerobically incubated at 37 °C for 24–48 h. *S. aureus*-suspect colonies were Gram stained, and biochemical tests for catalase, clumping factor, coagulase, DNase, and thermos table nuclease were performed. All isolates were subjected to *ssa442* PCR for final species assessment. According to the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2016), all isolates were tested for cefoxitin resistance (30 μg discs, MASTDISCS) based on disk diffusion. Cefoxitin-resistant strains were tested by *mecA* PCR [13]. All *S. aureus* isolates were stored at − 20 °C in Luria-Bertani (LB) broth with 20% glycerol.

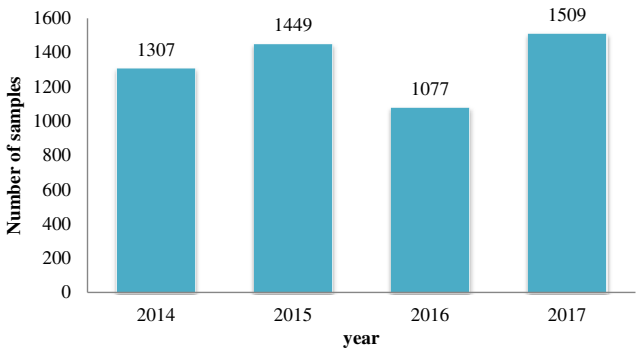

Fig. 1 The number of diarrhea specimens in central Iran during 214–217, specimens was negative for Salmonella spp., Shigella spp., Amoeba and Campylobacter spp. The data has been provided by centers of public health of Arak University of Medical Sciences

PCR of enterotoxin genes

DNA purification was done using the Tissue Genomic DNA Extraction mini kit (FavorPrep, FavorGen, Taiwan) applying the manufacturer's instructions. The PCRs were performed in a total volume of 25 μL containing 12.5 μl Super PCR mastermix 2X)Taq DNA polymerase, dNTP, $MgCl_2$), 1 μl forward and reverse primer (10 μM each), 1 μl template DNA (100 ng), and 9.5 μl nuclease-free water. All PCRs were run on a peQlab thermocycler (peQlab, UK). The conditions were: pre-denaturation at 94 °C for 5 min and then 30 cycles at 94 °C for 1 min with 1 min of annealing at 72 °C in between. This was followed by an extension at 72 °C for 5 min [14–17]. The PCR-amplified material was analyzed by electrophoresis in 1% agarose gel in 1X Tris-EDTA buffer. All *S. aureus* isolates were PCR-tested for the eight most prevalent enterotoxin genes: *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, and *sei*).

Statistical analysis

Differences in enterotoxin gene content between groups of isolates were statistically analyzed using SPSS version 24 (Microsoft office, Chicago, IL; USA ver 24) by applying the Pearson chi-square test. The significance level was set at a $P \leq 0.05$.

Results

Prevalence of enterotoxin gene among food handlers

From a total of 1113 food handlers, 224 *S. aureus* strains (20.1%) were isolated. Enterotoxin gene PCR demonstrated that 157 (70.1%) of these isolates were positive for one or

more enterotoxin genes. The most frequently detected enterotoxin gene was *sei* (40.2%) followed by *seg* (35.3%), *sea* (23.5%), *seb* (15.2%), *sec* (5.5%), and *seh* (2.7%). *See* and *sed* were not found (Table 1). Altogether, 67 (42.7%) of enterotoxin gene-positive isolates were positive for a single enterotoxin gene, 64 (40.8%) contained two enterotoxin genes, 23 (14.7%) contained three enterotoxin genes, and 3 (1.9%) contained four enterotoxin genes (Table 2). The most frequently encountered gene combination was *sei* plus *seg*, where all *seg*-positive isolates ($n = 35$, 22.3%) were also positive for *sei*.

Prevalence of enterotoxin genes among healthy carriers

Hundred *S. aureus* strains (18.3%) were found among the 546 healthy controls. Enterotoxin gene PCRs showed that 39 (39%) of the isolates from healthy carriers were positive for one or more enterotoxin genes. The most commonly detected enterotoxin gene marker was *sea* (17.9%), followed by *sei* (11.8%), *seg* (7%), *seb* (2.3%), *sec* (1.6%), *sed* (1.6%), and *see* (0.8%) (Table 1). None of the isolates possessed *seh*. Altogether, 26 (66.7%) of enterotoxin gene-positive isolates were positive for a single enterotoxin gene, 12 (30.8%) harbored two enterotoxin genes, 1(2.5%) harbored three enterotoxin genes (Table 3). The most commonly encountered gene combination was *sei* plus *seg*; all *seg*-positive isolates ($n = 6$, 15.4%) were also contained of *sei*.

Prevalence of meticillin-resistant *S. aureus*

Thirty seven (16.5%) of the 224 isolates from food handlers were recognized as MRSA. Overall, 27 (73%) of these MRSA harbored at least one enterotoxin gene. A single (1%) CA-MRSA was diagnosed among isolated from healthy controls, this strain was positive for enterotoxin gene.

Table 1 The pattern of enterotoxin genes among S. aureus isolates from food handlers and healthy carriers

Genes	Food handlers ($n = 224$)	Healthy carriers ($n = 100$)	*P* value
sea	52 (23.5%)	23 (17.9%)	0.292
seb	34 (15.2%)	3 (2.3%)	**0.001**
sec	12 (5.4%)	2 (1.6%)	0.240
see	0	1 (0.8)	ND
sed	0	2 (1.6%)	ND
seg	79 (35.3%)	9 (7%)	**0.0001**
sei	90 (40.2%)	15 (11.8%)	**0.0001**
seh	6 (2.7%)	0	ND
≥ 1	157 (70.1%)	39 (39%)	**0.0001**
A	37 (16.5%)	1 (1%)	**0.0001**

ND not determined. ≥ 1, positive for one or more enterotoxin genes. Boldface values indicate statistically significant values

Statistical comparison

The prevalence of nasal *S. aureus* carriers between food handlers and volunteers did not differ significantly ($P > 0.05$). The prevalence of enterotoxin gene-positive *S. aureus* strains among food handlers (70.1%) significantly higher from volunteers (39%) ($P = 0.0001$). Also, there was a significantly higher count of *sei*, *seg*, and *seb* genes in strains from food handlers as compared to controls ($P \leq 0.05$). The presence of *sea*, *sec*, *sed*, *see*, and *seh* genes was not significantly different between both groups ($P > 0.05$). Significantly, higher MRSA-colonization frequency was found among strains isolated from food handlers ($P = 0.0001$) (Table 1).

Discussion

Nasal carriage

Nasal carriage of *S. aureus* among food handlers in our study was 20.1%, as was also reported from Ethiopia, Brazil, and Turkey (20.5, 20.7, and 23.1%, respectively) [18–21]. In contrast, our carriage rate is lower than reported from Chili, Nigeria, and Botswana (65, 60, and 57.5%, respectively) [22–24]. We documented 16.5% MRSA which is lower than the 22% recorded by Loeto et al. [24]. A previous study from central Iran showed values of 4.5% in 2013 (15) and 7.9% in 2014 [12, 25]. This implies that MRSA colonization rates may be on the rise locally. We observed a considerably higher rate of MRSA colonization in food handlers (16.5%) than in the volunteers group (1%) ($P = 0.0001$). This shows that food handlers are at an elevated risk for MRSA colonization due to the presence of MRSA in several veterinary products. Ho et al. already put forward that regular exposure to raw meat increased risk for *S. aureus* colonization in food handlers [26].

Overall enterotoxin gene frequencies

It has been shown that there is no absolute correlation between enterotoxin gene presence and the amount of toxin actually produced. Expression strongly depends on the environment [27], but there seems to be a good correlation between gene presence and the possibility to express the toxins. For this reason, we have used diagnostic PCRs to establish the opportunity for a strain of being able to synthesize enterotoxins. Seventy percent of the *S. aureus* isolates from food handlers were positive for one or more enterotoxin genes, which is significantly higher than the 39% for healthy carriers isolates ($P = 0.0001$). In support of our findings, Nashev et al. [28] reported 67.9% enterotoxin gene-positive strains among

Table 2 Enterotoxin gene combination profiles of gene-positive *S. aureus* strains from food handlers

Number of enterotoxins genes come together	Enterotoxin profiles	Frequencies
S. aureus isolates with single enterotoxin gene	*sea* (20), *seb* (12), *sec* (1), *seg* (15), *seh* (2), and *sei* (17)	67 (42.7%)
S. aureus isolates with two enterotoxin genes	*sea*, *seb* (8); *sea*, *seg* (1); *sea*, *sei* (6); *seb*, *sei* (4); *seb*, *seg* (4); *seb*, *seh* (2); *sec*, *sei* (3); *seg*, *sei* (35), and *seh*, *sei* (1)	64 (40.8%)
S. aureus isolates with three enterotoxin genes	*Sea*, *sec*, *seg* (1); *sea*, *seh*, *sei* (1); (12); *seb*, *seh*, *seg* (2); *seb*, *seg*, *sei* (4), and *sec*, *seg*, *sei* (3)	23 (14.7%)
S. aureus isolates with four enterotoxin genes	*Sea*, *sec*, *seg*, *sei* (3)	3 (1.9%)

carriage and infectious isolates. Becker et al. [29] recorded 73% from blood derived and nasal strains. Similarly, a prevalence of 71.0% was reported for *S. aureus* from food handlers in Kuwait [30] and 74.1% from food poisoning cases in Taiwan [31]. In contrast, Alhashimi et al. found a lower 38% of enterotoxin-positive *S. aureus* isolates among food handlers in Kerbala city (Iraq) [32].

Presence of individual enterotoxin genes

In a study conducted in Kuwait, the frequencies of *sei*, *seg*, and *seh* genes were 38.5, 24.0, and 21.5%, respectively [30]. Silva et al. reported similar figures for *sei* and *seg* genes (both 29.3%) and 7.3% for the *seh* gene among *Staphylococcus spp.* isolated from Brazilian food handlers (18). On a global scale, *seh* and *sei* genes seem to be the top reported classical enterotoxin genes. In the present study, the frequency of *sei* and *seg* genes was higher in strains isolated from food handlers than healthy carriers ($P = 0.0001$). In the study of Nashev et al., the prevalence of *sei* and *seg* genes ($n = 22$, 52.4%) were lower in infection-associated isolates of *S. aureus* than in isolates from healthy carriers ($n = 33$, 73.4%) ($P = 0.048$), and the prevalence of the *seh* gene (21.4%) was higher in carrier isolates but this was not significant ($P = 0.063$) [28].

The frequencies of occurrence of classical enterotoxin genes vary in previous studies. *Sea* gene frequency recorded 16%, *seb* 18%, and *sec* 8% among *S. aureus* isolates from food handlers in Iraq [32]; in another study in Kuwait, isolates contained 11% *sea*, 12.5% *seb*, and 23% *sec* [30]. Asgarpoor et al. reported the frequency of *sea*, *seb*, and *sec* genes in 23.9, 13 and 10.8% *S. aureus* isolates from food handlers in Iran [33]. In the present investigation, the frequency of the *seb* gene was higher among isolates from food handlers than from healthy carriers (P value = 0.001). In the study by Becker et al., the *sea*, *seb*, and *sec* genes frequencies were 17.4, 5.9, and 8.7% among *S. aureus* isolates from blood; the authors also reported 15.9, 6.8, and 11.2% of *sea*, *seb*, and *sec* genes in nasal isolates. The overall prevalence of these genes was not significantly different among blood and nasal isolates [29].

Sed and *see* genes are rare and absent in the present set of strains. The frequency of *sed* was 2% in strains from nasal swabs in Switzerland [25]. *Sed* gene absence was reported in Brazil [34]. A study from The Netherlands found 1.9% *sed* gene positivity, whereas *see* was absent [35]. *Sed* and *see* genes were not found in nasal swab strains from healthy individuals in Iran [36]. In the study by Nashev et al., *sed* gene was detected in 2.4 and 6.7% of nasal *S. aureus* isolates from clinical samples and carriers, and the *see* gene was not found in the two groups [28]. Peacock et al. showed that 5% of disease *S. aureus* isolates and 5% of carriage isolates were positive for *sed* gene, all isolates did not score positive for *see* [37].

Ratios between enterotoxin genes

Udo et al. published interesting data with respect to multiple toxin genes occurring in single strains. The authors demonstrated that 35% of enterotoxin gene-positive *S. aureus* isolates contained a single enterotoxin gene, 38% contained two enterotoxin genes, 20% contained three enterotoxin genes,

Table 3 Enterotoxin gene combination profiles of gene-positive *S. aureus* strains from healthy carriers

Number of enterotoxins genes come together	Enterotoxin profiles	Frequencies (%)
S. aureus isolates with single enterotoxin gene	*sea* (16), *seb* (1), *seg* (4), *sei* (4), and *sed* (1)	26 (66.7%)
S. aureus isolates with two enterotoxin genes	*sea*, *seb* (2); *sea*, *see* (2); *sea*, *sec* (1); *sea*, *sed* (1), and *seg*, *sei* (6)	12 (30.8%)
S. aureus isolates with three enterotoxin genes	*sea*, *seg*, *sei* (1)	1 (2.5%)

and 7% contained four enterotoxin genes [30]. A higher prevalence was recorded among the isolates positive for enterotoxin genes in Brazil, so that 69% of these isolates were positive for only one enterotoxin gene, 27.6% positive for two enterotoxin genes, and 6.9% positive for three enterotoxin genes [19]. The coexistence of *sei* and *seg* genes on an enterotoxin gene cluster (egc) was identified by Jarraud et al. [38]. Aydin et al. presented similar findings for the *seg-sei* combination among *S. aureus* isolates, they observed that all *seg*-positive isolates also were containing *sei*, however, 3 isolates containing *sei* did not positive for *seg* [39]. MacLauchlin et al. detected 19 *S. aureus* isolates positive for both *sei* and *seg* genes, interestingly, 1 isolate containing *sei* was negative for *seg* [40]. These genes are located on a common genetic element so are usually found together.

Conclusion

MRSA colonization is 16 times more frequent in food handlers than it is in healthy volunteers. Also, the frequency of enterotoxin gene-positive *S. aureus* was found to be significantly higher among food handlers than in healthy carriers. Elevated enterotoxin gene numbers were more common in strains from food handlers. Significant differences between the groups were observed for *sei*, *seg*, and *seb* genes only. Consequently, possible food contamination with MRSA or enterotoxigenic *S. aureus* through the handlers suggests that strong enforcement of hygiene measures is mandatory in the battle against SFP. One of the main approaches to reducing SFP may be nasal decolonization.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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