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RESEARCH ARTICLE

PREVALENCE OF CLASSICAL ENTEROTOXIN GENES IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FOOD HANDLERS IN MAKKAH CITY KITCHENS

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ABSTRACT

Staphylococcus aureus (S. aureus) is Gram-positive cocci that can produce staphylococcal enterotoxins (SEs). The SEs are emetic toxins and are the causes of Staphylococcal food poisoning (SFP). The aim of this study was to investigate the presence of classical SEs genes in methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from 200 adult male workers in Makkah, by polymerase chain reaction (PCR). Our results showed that a total of 165 (40.3 %) of the swabs from nasal cavities and hands were positive for *S. aureus* and 20.0% of the *S. aureus* isolates were MRSA. The incidence of toxin genes in MRSA and MSSA isolates was found to be 90.9 % and 43.9 % respectively. SEA was the predominant enterotoxins in both MRSA (36.4%) and MSSA (30.3%). Multiple combinations of exotoxin genes were also seen in both MRSA and MSSA isolates. It was concluded that SEs genes were widespread during Hajj season and more predominant in MRSA, the SEA gene was most encountered followed by SEB.

INTRODUCTION

S. aureus is a Gram-positive cocci that belongs to the *Staphylococcus* genus which is subdivided into 32 species and subspecies. MRSA is considered to have emerged from *S. aureus* through the acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*), which carries the *mec*A gene for methicillin resistance. The intrinsic resistance to these antibiotics is attributed to the presence of *mecA*, whose product is a 78-kDa protein called penicillin binding protein 2a (Mehrotra *et al*., 2000). *S. aureus* does not form spores that is why contamination can be readily avoided by heat treatment of food. *S. aureus* is able to contaminate food products during preparation and processing, so that it is considered a major cause of food borne disease. SFP has been reported as the third most prevalent cause of food borne illness worldwide (Le Loir *et al*., 2003, Zhang *et al* 1998). Common SFP symptoms include nausea, vomiting, abdominal cramps and diarrhoea (Stewart 2003). In humans, *S. aureus* is present on external sites, such as the nostrils or the skin and also transiently in the oropharynx and faeces (Bhatia *et al*., 2007) and more than 70% of isolates recovered from healthy population were enterotoxigenic (Omoe *et al* 2002).

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Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are considered as the major source of food contamination, via manual contact or through respiratory secretions. *S. aureus* can produce exotoxins including exfoliative toxins, toxic shock syndrome toxin-1 and staphylococcal enterotoxins (SEs). The SEs are emetic toxins and are the causes of SFP. They have been divided into five serological types (SEA through SEE) on the basis of their antigenicities (Bergdoll, 1983). In recent years, the existence of new types of SEs (SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, and SEO) has been reported (Omoe *et al* 2002). The methods most frequently used for the detection of staphylococcal toxins are immunodiffusion, agglutination, radioimmunoassay, and enzyme-linked immunosorbent assay (Iandolo *et al*., 1989, Johnson *et al*., 1991). PCR have been reported to be very successful and reliable for detection of the genes that responsible for production of enterotoxins in *S. aureus* bacteria. (Johnson *et al*., 1991). The aim of this study was to investigate the presence of classical SEs genes by PCR in MSSA and MRSA strains isolated from food handlers during hajj season 1435H (Septemper 2014) in Makkah, Saudi Arabia.

MATERIALS AND METHODS

S. aureus isolates

S. aureus isolates were recovered from food handlers working in Makkah city kitchens during Hajj 1435 H (September-

October 2014). They consisted of 165 isolates from 400 nasal cavities and hand swabs from 200 adult male workers in 50 kitchens. Samples were collected from the hands (interdigital region, index fingers, thumbs and palms of both right and left hands) and anterior nares of 200 food handlers, distributed among 50 kitchens in Makkah, during meal preparation. One swab was used in each region. After sampling, swabs were immediately transferred into 5 mL nutrient broth and incubated for 18-24 h at 37ºC. Ten 10 µl of the enriched cultures were streaked on Baird Parker Agar; a Staphylococcus selective medium. The plates were incubated at 37°C for 18-24 h after which single colonies were streaked onto blood agar plates and further incubated at 37 °C for 12-18 h. Identification of *S. aureus* was confirmed on the basis of Gram stain, catalase, culture properties on mannitol salt agar, detection of hemolysis on blood agar and coagulase reaction. The isolates were stored at –70 C in Tryptic Soy broth with 20% glycerol till further investigations. All isolates were screened for the resistance *mec*A and SE genes by PCR, with specific primers shown in table1. All the primers were synthesized by IDT (Integrated DNA technologies, Interleucvenlaan, 12A, B3001, Belgium).

centrifugation for 20 min and precipitated with ethanol and then resuspended with 50 ul TE (Bollet *et al*., 1991).

PCR Conditions

For detection of *mec*A and SE genes, a 50µl PCR mixture containing 8 µl of DNA template, 1µl (100 pmol) of each primer and a 25µl of Taq PCR Master Mix polymerase containing 100mM Tris-HCl, 500mM KCl at pH 8.3 at 20°C, 1.5 mM MgCl2, 200M of each of deoxyribonucleoside triphosphate and 0.025U Taq polymerase (Qiagen, USA) was prepared. Amplification was performed using Mastercycler PCR machine (Eppendorf, Germany)) which consisted of initial denaturation for 5 minutes at 94ºC and 30 cycles at 94ºC for 2 minutes for denaturation and 72ºC for 1 minute for extension. Annealing temperatures used each step are shown in Table 1. Final extension was performed at 72ºC for 5 minutes. About 25µl of the PCR products were mixed 10µl of loading dye and analyzed by electrophoresis in 1% agarose gels (for 35 minutes at 90 V using 5 X TBE running buffer. Also, 100 bp DNA ladder was included in each run and DNA bands were viewed under UVP BioDoct It Imaging System after staining with ethidium bromide (2 g/ml).

Table 1. Primers and temperature used for the detection of *S. aureus* genes

Gene"	Primer	Oligonucleotide (5'-3')	Size of amplified product	Temp	Reference
*mec*A	MECAP4 MECAP7	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	162	53°C	Milheiriç *et al.,* 2007
sea	SEA-1 SEA-2	TTGGAAACGGTTAAAACGAA GAACCTTCCCATCAAAAACA	120 bp	50°C	Johnson *et al*., 1991
seb	SEB-1 SEB-2	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCC	478 bp	50°C	Johnson *et al*., 1991
sec	SEC-1 SEC-2	GACATAAAAGCTAGGAATTT AAATCGGATTAACATTATCC	257 bp	50°C	Johnson *et al*., 1991
sed	SED-1 SED-2	CTAGTTTGGTAATATCTCCT TAATGCTATATCTTATAGGG	317 bp	50°C	Johnson *et al*., 1991
see	SEE-1 SEE-2	TAGATAAAGTTAAAACAAGC TAACTTACCGTGGACCCTTC	170 bp	50°C	Taj et al., 2014

Table 2. Staphylococcal enterotoxins genes distribution among MRSA and MSSA isolates.

Genes	MRSA (33)	MSSA (132)
Sea	12 (36.4%)	40 (30.3%)
Seb	2 (6 %)	2 (1.5 %)
Sea, Seb	3 (9.1%)	1 (0.8%)
Sea, Sec	5 (15.2%)	11 (8.3%)
Sec, Sed	2 (6 %)	2 (1.5%)
Sea, See	2 (6 %)	2 (1.5%)
Sea, Seb, Sec	4 (12.1%)	0 (0%)
Total	30 (90.9%)	58 (43.9%)

DNA extraction

DNA was extracted by taking a single colony from a nutrient agar plate (Oxoid) that had been incubated overnight. Cell suspensions were centrifuged at 4,500 rpm for 5 min at 4°C. Cell pellets were washed with 1 ml of TE (10 mM Tris, pH 8, 10 mM EDTA) and were re-suspended in 100 ul of TE. After addition of 50 u1 of 10% SDS, the mixture was incubated for 30 min at 65 C. The lysates were centrifuged and supernatants were removed. The micro tubes were then placed in a microwave oven and heated three times for 1 min at 750 W. The pellets were dissolved in 200 ul of TE and were extracted with an equal volume of phenol / chloroform / isoamyl alcohol (25:24:1) for 15 min. The aqueous phase was recovered by

RESULTS

Our results showed that a total of 165 (40.3 %) of food handlers were positive for *S. aureus*. The number of MRSA isolates detected by amplifications of the *mec*A gene was 33 (20.0 %) out of 165 *S. aureus* isolates (Figure 1). Detection of enterotoxin genes by PCR showed that 88 (53.3%) of all *S. aureus* isolates were positive for one or more of these genes. The incidence of toxin genes in MRSA and MSSA isolates was found to be 90.9 % and 43.9 % respectively. The prevalence of genes for SE in MRSA and MSSA isolates is shown in (Table 2). Multiple combinations of SE genes were also seen in both MRSA and MSSA isolates (Figures 2 & 3).

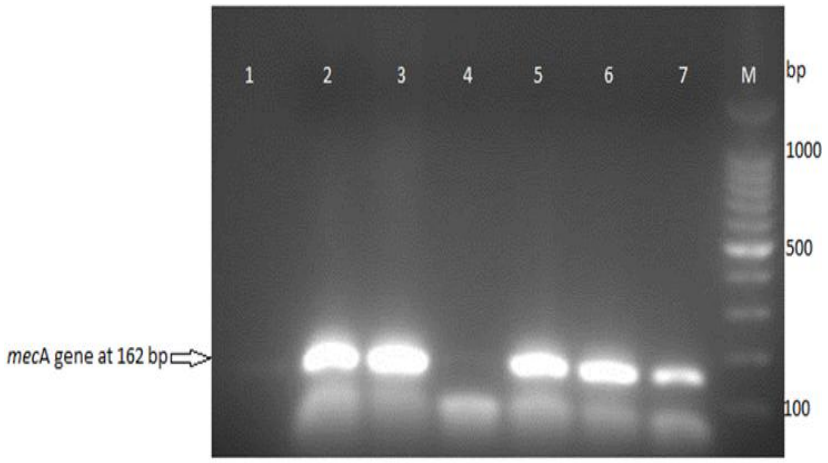

Figure 1. *mec*A genes after PCR on 1 % agarose gel electrophoresis. Lanes 2, 3, 5, 6 and 7; Positive *mec*A genes at 162 bp. lanes 1 and 4; negative *mec*A genes. Lane M; 100-bp DNA ladder

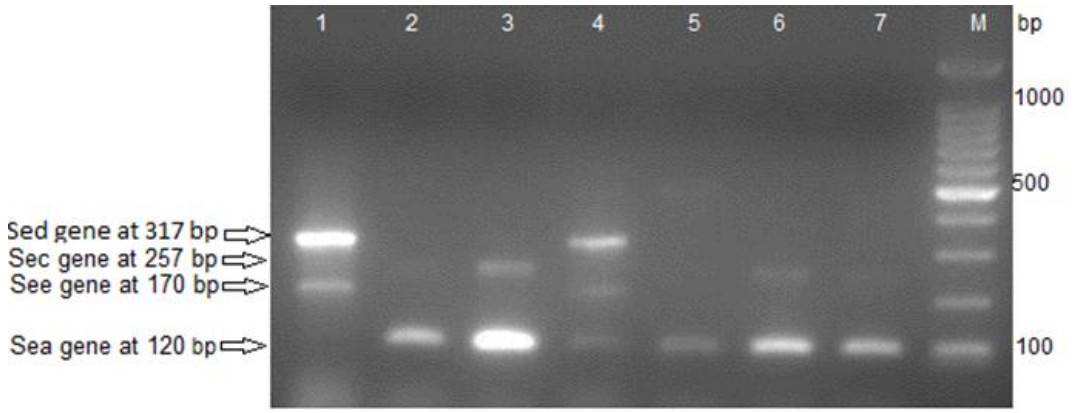

Figure 2. SE genes after PCR on 1 % agarose gel electrophoresis. Lanes 1-7; Positive SE genes at 120 bp (SEA), 170 bp (SEE), 257 bp (SEC), and 317 bp (SED). Lane M; 100-bp DNA ladder.

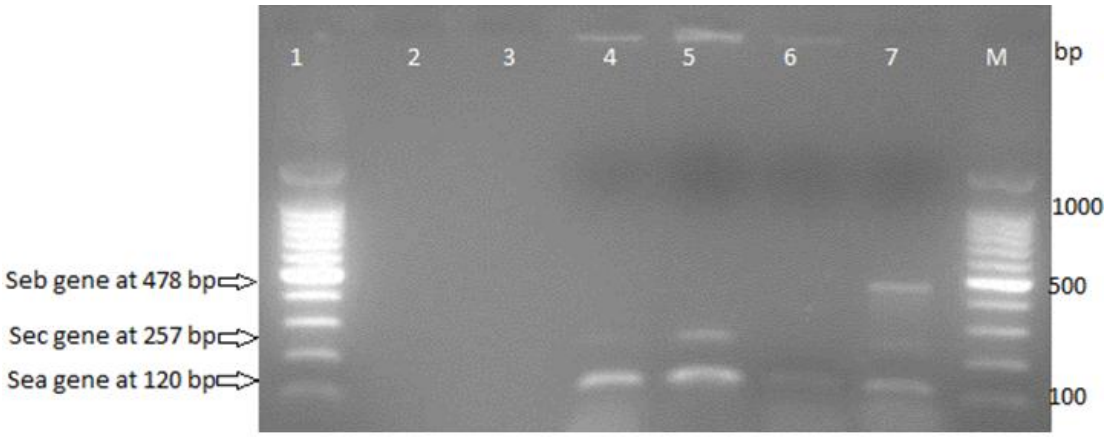

Figure 3. SE genes after PCR on 1 % agarose gel electrophoresis. Lanes 2 and 3; negative SE genes. Lanes 4-7; Positive SE genes at 120 bp (SEA), 257 bp (SEC), and 478 bp (SEB). Lanes 1 and M; 100-bp DNA ladder.

DISCUSSION

Hajj is the annual pilgrimage to Makkah, Saudi Arabia, and the largest mass gathering in the world, on which huge amounts of food is prepared and consumed. Food handlers may be vectors of food borne disease spreading, due to inadequate personal hygiene or cross contamination (pdf (Bass *et al*., 2006). In this study, it was found that 40.3 % food handlers were positive for *S. aureus* which in agreement with Vanderbergh *et al*. (1999) who reported that the isolation of *S. aureus* could vary from 20 to 55% in a healthy adult population and higher than that reported by Asghar *et al*. (2006) (22.4%) in Makkah. Prevalence rates of MRSA in the carriers (food handlers) in this study were 20.0 %. Higher *S. aureus* prevalence among food handlers, of 44.6%, 53.2% and 23.1% was noted in Botswana, Kuwait, and South-eastern

Anatolia, respectively (Uzunov *et al*., 2013). These high results may be due to the transmission mode of *S. aureus* and MRSA through hands, which may become contaminated by contact with colonized or infected individuals or through contact with colonized or infected body sites of other persons. Other factors contributing to transmission include close skin-to-skin contact, crowded conditions, and poor hygiene. Thus during hajj, ordinarily, food handlers are subjected to medical examination before assignment to work. However, they are mostly lacking proper training in food handling operations, mass feeding, and sanitary practices (Dablool and Al-Ghamdi, 2011). SFP is an intoxication that is caused by the ingestion of food containing pre-formed SE (Argudin *et al*. 2010). PCR is a rapid and sensitive tool, which can show the presence of enterotoxigenic *S.aureus* in food on the basis of specific gene sequences and detect the potential source of contamination before enterotoxins are produced (Bystroń *et al*., 2005). In this study, detection of enterotoxin genes by PCR showed that 88 (53.3 %) out of 165 *S. aureus* isolates were positive for one or more of these genes. Prevalence of enterotoxigenic *S. aureus* in food handlers is variable between industries and countries.

Prevalence estimates from several small studies range from 2% of food handlers in Italy (Talarico *et al*., 1997), 12% of flight-catering staff in Finland (Hatakka *et al*. 2000), 19% of restaurant workers in Chile (Figueroa *et al*. 2002) to 62% of fish processing factory workers in India (Simon and Sanjeev, 2007). The predominant enterotoxins genes in this study were SEA followed by SEB in all isolates. No other of single detection for SE encoded genes was observed. No available data about genetic detection of SEs in Makkah during Hajj season. Worldwide, SEA has been described as the most common by many other authors (Taj Y *et al*., 2014, Adwan *et al*., 2008; Al Bustan *et al*., 1996, Normanno *et al*., 2005, Peacock *et al*., 2002). In Kuwait, *S. aureus* strains isolated food handlers were shown to produce toxin SEB followed by SEA, SEC and SED (Al Bustan *et al*., 1996). Nashev *et al*. (2004) observed low positivity for SED gene among the food handlers. In conclusion, this is the first study detected genes encoding the classic (SEA to SEE) in *S. aureus* strains isolated from food handlers in Makkah city. SEA was the predominant gene followed by SEB. The occurrence of multiple genes carried by the same isolate indicating the pathogenic potential of *S. aureus* specifically MRSA strains.

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