Prevalence of *SEA* and *SEB* enterotoxin producing methicillin-resistant *staphylococcus aureus* strains among primary school children in Sari, Iran

Sahar Khalili[1], Nikou Bahrami[2], Shaghaygh Rezai[3], *Iman Pouladi[4]

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Abstract

Background: Staphylococcus aureus is a frequent hospital and community-acquired infection. It has different types of virulence factors and provides host invasion requirements through the release of various toxins, the super-antigenic enterotoxins, *SEA* and *SEB* being the most likely to cause pathogenicity.

Objectives: To assess the prevalence of *SEA* and *SEB* enterotoxin producing *MRSA* strains among primary school children in Sari, northern Iran.

Method: In this descriptive cross-sectional study, 140 nasal isolates of primary school children were collected for 4 months in 2017 in Sari city. First, isolates were identified utilising biochemical and laboratory methods. Minimum inhibitory concentration of isolates to oxacillin was next determined using phenotypic and molecular methods. The presence of *SEA* and *SEB* genes were then detected using polymerase chain reaction sequence specific primers (PCR-SSP).

Results: We identified 70 *Staphylococcus aureus* isolates through standard microbiological procedures. In the phenotypic antibiotic susceptibility assay of 70 isolates of *Staphylococcus aureus*, 42 isolates were identified as oxacillin-resistant isolates according to the CLSI guidelines, and were reported as methicillin-resistant *staphylococcus aureus* (*MRSA*) nasal carriers. Also, 23 cases of oxacillin-resistant isolates lacked the mecA gene (false positive). Nineteen isolates were definite *MRSA* using PCR, of which 8 isolates (21.42%) had *SEA* gene and 3 isolates (7.14%) had *SEB* gene. Also, three isolates (7.14%) carried both the *SEA* and *SEB* genes.

Conclusions: In primary school children in Sari, northern Iran, of the 19 definite *MRSA* isolates using PCR, 21.4% had the *SEA* gene, 7.1% had the *SEB* gene and 7.1% carried both the *SEA* and *SEB* genes.

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Introduction

Staphylococcus species are a common cause of nosocomial infection worldwide[1]. Among the many species of staphylococcus genus, *Staphylococcus aureus* (*S. aureus*) is the most invasive species that can contribute to the development of skin infections, sinusitis infections and food poisoning[2]. The anterior nares are the primary source of *S. aureus* and 20-40% of healthy individuals carry nasal *S. aureus*. Nasal carriage is the principal cause of staphylococcal infection[3]. Antibiotic resistance is now a major global concern because of potential pathogenicity and increased prevalence of methicillin-resistant *Staphylococcus aureus* (*MRSA*). *MRSA* has been shown to pose a serious threat to nosocomial infections, and treatment failure[4,5]. Resistant to methicillin in MRSA strains is due to a *mecA* gene embedded in a staphylococcal cassette chromosome mec (SCCmec)[6]. This gene encodes an alternative penicillin-binding protein, PBP2a that has a low affinity for binding β-lactam antibiotics[3,7]. Community-acquired *MRSA* (CA-MRSA) normally expresses lower levels of antibiotic resistance compared to hospital-acquired *MRSA* (HA-MRSA)[8,9]. Over the past few years, the prevalence of nosocomial infections caused by this strain has significantly increased. As such, an average of 40% of *S. aureus* strains are *MRSA*[10]. Many virulence factors are produced by different strains of *S. aureus* (*SEA* and *SEB)*[11].

[1]*MSc Student in Microbiology, Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran,* [2]*MSc Graduate, Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Iran,* [3]*Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran,* [4]*MSc in Medical Microbiology, Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran*

**Correspondence:* imanpouladi96a@gmail.com

https://orcid.org/0000-0002-0640-2727

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Most diseases are caused by *SEA strains*, whilst *SEB* is a main cause of pseudomembranous colitis (PMC)[12]. These enterotoxin-producing strains of *S. aureus* are more resistant to host immunity and antibiotics compared to other strains[11,13].

Objectives

To assess the prevalence of *SEA* and *SEB* enterotoxin producing *MRSA* strains in primary school children in Sari, northern Iran.

Method

A cross-sectional study was carried out on 140 primary school children aged 6-12 years for four months in 2017 in Sari city. Sampling was performed using stratified random sampling method.

Clinical specimen collection and bacterial identification: A sterile wet swab was inserted into the nostril, and specimens were collected after rotating swabs several times against the nasal wall. The swabs of samples were placed into the Stuart's transport media, and were immediately transferred to the microbiology laboratory of Mazandaran University of Medical Sciences. Bacterial identification was performed using colony morphology, gram stain, catalase testing, coagulase assay and mannitol salt agar, and were confirmed by molecular methods.

Antibiotic susceptibility assay, isolation of MRSA strains: MRSA-resistant isolates of *S. aureus* were phenotypically detected by Kirby-Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A suspension of the organism equivalent to a 0.5 McFarland standard was prepared and inoculated at final concentration of 10^5 CFU/ml in Müller-Hinton agar. Agar plates with oxacillin were used to detect *MRSA* strains. *S. aureus* strains were cultured on Müller Hinton agar containing 4% sodium chloride and 6 mg/l oxacillin, and were then incubated for 24 hours.

DNA extraction: Boiling was used to extract the DNA of the *S. aureus*. Bacterial colonies were placed in sterile microtubes, containing 1 ml of distilled water. They were then boiled at 100° C for 5 minutes, and the microtubes were then frozen for 5 minutes and boiled again for 5 minutes. Afterwards, the microtubes were centrifuged at 3000 rpm for 10 minutes. The supernatant was then stored as purified DNA at -20 °C.

Application of molecular PCR for detection of mecA, SEA, and SEB genes: After genomes extraction of the samples and primer BLAST on the selected DNA primers, the *mecA*, *SEB* and *SEB* genes were detected by polymerase chain reaction using sequence-specific primers (PCR-SSP) (Table 1) at final concentration of 25 µL, including an initial denaturation at 95 °C for 1 min, 37 cycles comprised of denaturation at 94° C for 30 seconds, primer binding to the target DNA at 59 °C for 30 seconds, elongation at 72 °C for 1 minute, as well as a final elongation at 72° C for 7 minutes, according to the protocol for detection of *mecA* genes. In addition, for detection of *SEA* and *SEB* genes, PCR-SSP was performed at final concentration of 25 µL, including an initial denaturation at 94 °C for 5 min, 35 cycles comprised of denaturation at 94 °C for 45 seconds, primer binding to target DNA at 56 °C for *SEA* gene and 60 °C for gene *SEB* for 45 sec, incubation at 72° C for 45 seconds, and the final elongation at 72 °C for 10 min, according to the protocol. The PCR products were finally electrophoresed.

Ethical issues: This study was approved by the Ethics Committee of Qaemshahr branch, Azad University of Qaemshahr (378. ID Code: 10730548952006). Written informed consent was obtained from the parents of the participants.

Table 1: ***The nucleotide sequences and DNA primers***

Gene	Sequence	Product size	Reference
mecA	FP: 5'- TCCAGATTACAACTTCACAGG RP: 5'- CCACTTCATATCTTGTAACG	162bp	14
SEA	FP: 5'- AAA GTC CCG ATC AAT TTA TGG RP: 5'-GTA ATT AAC CGA AGG TTC TGT	210bp	15
SEB	FP: 5'-TCG CAT CAA ACT GAC AAA CG RP: 5'-GCA GGT ACT CTA TAA GTG CC	478bp	15

Results

This study was conducted on 140 primary school children aged 6-12 years in Sari city. There were 70 isolates of *S. aureus* identified. Then, DNA extraction was performed, and PCR products of *mecA*, *Sea*, and *Seb* genes were obtained using PCR-SSP. In the phenotypic antibiotic susceptibility assay of 70 isolates of *S. aureus*, 42 isolates were identified as oxacillin-resistant isolates and were reported as *MRSA* nasal carriers. Also, 23 cases of oxacillin-resistant isolates were shown to have no *mecA* gene (false positive). Nineteen isolates were definite *MRSA* using PCR, of which eight isolates (21.42%) had *SEA* gene (figure 1) and three isolates (7.14%) had *SEB* gene (figure 2). Also, three isolates (7.14%) carried both the *SEA* and *SEB* genes (table 2).

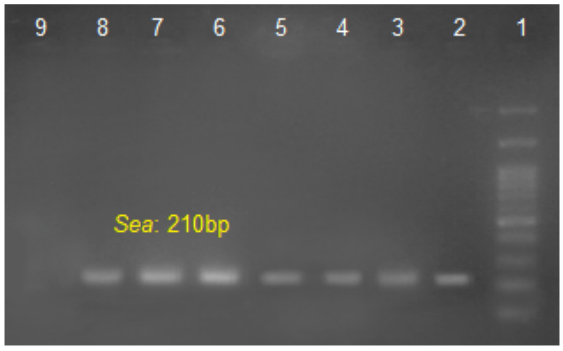

Figure 1: ***The schematic of agarose gel electrophoresis bands of SEA gene for S. aureus species: raw 1: 100bp DNA ladder mark; raw 2: amplified PCR products of SEA gene for standard S. aureus strains; raw 3 to 8: amplified DNA products of clinical SEA -positive isolates of S. aureus; raw 9: amplified PCR products of negative control***

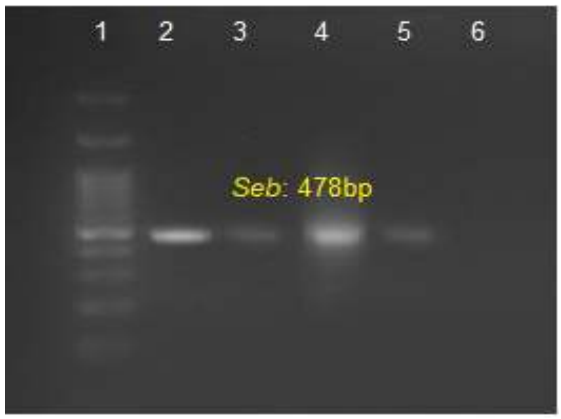

Figure 2: ***The schematic of agarose gel electrophoresis bands of SEB gene for S. aureus species: raw 1: 100bp DNA ladder mark; raw 2: amplified PCR products of SEB gene for standard S. aureus strains; raw 3 to 5: amplified DNA products of clinical SEB -positive isolates of S. aureus; raw 6: amplified PCR products of negative control***

Table 2: ***Patterns of enterotoxins of MRSA isolates obtained from the participants***

Type of enterotoxin	Percentage of enterotoxin genes among isolates
SEA	21.4
SEB	7.1
SEA and SEB	7.1

Discussion

MRSA strains are more resistant to other antibiotics as well as to methicillin and beta-lactam antibiotics[7]. *S. aureus* acquired from the CA-MRSA clones, mostly affect children and young adults who are in close contact with community members. These isolates can cause many infections, such as necrotizing pneumonia and skin infections. A major factor associated with community-acquired infections is presence in crowded areas[3]. This study was conducted to assess the enterotoxin-producing CA-MRSA in primary school children in Sari city. Results showed that 42 isolates were identified as oxacillin-resistant and 23 were reported as nasal *MRSA* carriers, according to CLSI guidelines. Also, 23 cases of oxacillin-resistant isolates were shown to have no *mecA*. Nineteen isolates were identified as definite *MRSA* by PCR. Gardella *et al.* (2010) conducted a study on 316 healthy children in Argentina, and found that 31% were nasal carriers of *S. aureus* and 4.4% were nasal *MRSA* carriers, which is in accordance with results of our study[16]. Stanley *et al.* (2014) investigated 400 isolates of *S. aureus*, and reported that 31.3% and 38% were identified as *MRSA* nasal carrier using phenotypic technique and PCR, respectively[17]. Asgary *et al.* (2017) examined 102 clinical isolates of *S. aureus*, and indicated that 58.8% of the isolates were methicillin-resistant by PCR, which is consistent with our study[7].

S. aureus functions both as potent toxins as well as super-antigens (SAgs). Staphylococcal enterotoxins are a superfamily of proteins produced by *S. aureus*, and appear in different serological types, including staphylococcal enterotoxins and toxic shock syndrome toxin[18,19]. The potential role of *S. aureus* SAgs has been shown in allergic respiratory diseases. Deregulation of the immune response, proliferation of autoreactive T cells and development or exacerbation of many chronic autoimmune diseases may result from the effects of these SAgs[20]. Sensitivity to specific *S. aureus*-derived *SEA*/*SEB* SAgs has been linked with development and increased odds of exacerbation of

the disease. Enterotoxins *A* and *B* are the commonest produced by *S. aureus*. Therefore, it is important to assess the frequency and timely control of enterotoxin-producing *S. aureus* strains, especially *SEA* and *SEB* genes which are virulence factors associated with diseases caused by *S. aureus*[19,20].

In the present study, of 19 *MRSA* clinical isolates, 8 (21.4%) had *SEA* gene, 3 (7.1%) had *SEB* gene, and 3 (7.1%) carried both SEA and SEB genes. Abolghasemi *et al* (2017) examined 65 isolates of *S. aureus* from skin samples, and reported the *SEA* gene frequency as 86.2% and *SEB* gene frequency as 15.4% using phenotypic technique and PCR, which is consistent with our study, as we showed higher rate of *SEA* compared with *SEB*[18]. Valizadeh *et al* (2016) evaluated production of enterotoxins in 60 *S. aureus* specimens collected from human cases with purulent infection, poisoning symptoms and their skin. When multiplex PCR was performed on the samples, 50% of the isolated *S. aureus* were positive for one or more enterotoxin genes, with *SEA* gene as the most frequent isolated gene (30%), which was consistent with the results of our study[21]. Sadeghi *et al.* (2019), collected and examined 350 nasal swab samples from nasal carriers (210 patients) and multiple sclerosis patients (140 patients). They identified *S. aureus* SAgs by multiplex PCR, and reported *Seb* (7.7%), *SEA* (5.31%), and Eta (16.6%) as the most common SAgs, which were higher compared to our findings[20].

Conclusions

In primary school children in Sari, northern Iran, of the 19 definite *MRSA* isolates using PCR, 21.4% had the *SEA* gene, 7.1% had the *SEB* gene and 7.1% carried both the *SEA* and *SEB* genes.

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