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Prevalence of SEA and SEB producing *Staphylococcus aureus* isolated from foodborne- outbreaks in Iran

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Staphylococcus aureus **is an important pathogen causing a wide variety of diseases such as skin infections, food poisoning, pneumonia and septicemia in humans and animals. Among the bacterial agents of food poisoning, S. aureus is the most common agent worldwide. The aim of this study was to assess prevalence of SEA, SEB enterotoxin producing S. aureus isolated from foodborne outbreaks in Iran. In this study, 313 diarrheal samples from 120 outbreaks were collected from December 2017 to August 2018. Isolates were identified using classical methods. The antimicrobial resistance patterns of the S. aureus isolates were assessed using standard disk-diffusion and E-test methods. Presence of sea and seb genes was investigated using polymerase chain reaction (PCR). Data were analyzed using SPSS and Excel Software as well as statistical tests. In this study, 55 samples (17.6%) from an overall number of 313 samples from food outbreaks were identified as S. aureus, which were assessed for their antimicrobial susceptibility. The highest contamination belonged to Yazd (50.9%) and Semnan (29.1%) Provinces. However, no contaminations of S. aureus were seen in Zanjan Province. The S. aureus was more common in females (50.9%) than males. Furthermore, S. aureus isolates were mostly resistant to penicillin (81.8%) and completely susceptibile to vancomycin. Of 55 isolates of S. aureus, four isolates (7.3%) were positive for sea and one (1.8%) for seb genes. The current study has shown that S. aureus food infection is one of the most common foodborne diseases, caused by the ingestion of staphylococcal enterotoxins produced by enterotoxigenic strains of staphylococci. Therefore, further screening and monitoring programs are suggested for the prevention of staphylococcal infections.**

Key words: *Staphylococcus aureus*, foodborne outbreaks, antibiotic resistance, *sea*, *seb.*

INTRODUCTION

Foodborne infection is one of the most widespread public health problems worldwide, which can be caused by the consumption of foods contaminated with microorganisms or chemicals (World Health Organization (WHO), 2015). The WHO has described foodborne outbreak as “a foodborne outbreak happens when two or more people

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Table 1. List of primers used in PCR of *sea* and *seb* genes.

Primer	Sequence (5´–3´)	bp*	Ref.
seb-F	TTTTTCTTTGTCGTAAGATAA	477	15
seb-R	CCAACGTTTTAGCAGAGAAG		
sea-F	TAAGGAGGTGGTGCCTATGG	127	15
sea-R	CATCGAAACCAGCCAAAGTT		

*bp, amplicon size; ref., references.

show the same symptoms of an illness after consuming a common food or drink source" (World Health Organization (WHO), 2008; Kirk et al., 2015). *Staphylococcus aureus* is one of the most important microorganisms causing foodborne outbreaks (Masoumi et al., 2015). This bacteria is a Gram-positive aerobic bacteria discovered in the normal flora of various parts of the human and animal bodies (Lowy, 1998). In fact, *S. aureus* is an opportunistic pathogen that can induce food poisoning by producing enterotoxins (Hennekinne et al., 2012). Staphylococcal enterotoxins (SEs) are members of a family of more than 20 different staphylococcal and streptococcal exotoxins (Salgado-Pabon et al., 2014). These toxins share a common phylogenetic relationship, structure, function, and sequence homology (Soltan Dallal et al., 2016). Staphylococcal enterotoxins A and B (SEA and SEB) are the most significant enterotoxins produced by *S. aureus* (Pinchuk et al., 2010). SEA is often associated to foodborne outbreaks by *S. aureus* and SEB causes food poisoning (Rusnak et al., 2004). Many foods can be contaminated with SEs, particularly foods that contain carbohydrates and proteins (such as meats, eggs and dairy products). Most of the SE food poisoning cases are due to direct contact of hands with foods (Soltan Dallal et al., 2010). Production of SEs develops at optimum temperature (20-37°C) and pH (4–4.7). The most common symptoms of SE food poisoning are nausea, diarrhea, vomiting and abdominal cramps, which usually happen within 2-6 h after eating contaminated foods (Fletcher et al., 2015). Little is known about the implication of SEA/SEB-producing *S. aureus* in foodborne outbreaks in Iran. Therefore, the aim of this study was to determine the prevalence of SEA/SEB-producing *S. aureus* isolates from foodborne outbreaks in five Iranian provinces and to study the antimicrobial susceptibility patterns of these isolates.

MATERIALS AND METHODS

Sample collection

In this study, human diarrheal samples of foodborne outbreaks were collected from five Iranian Provinces of Tehran, Semnan, Kurdistan, Hamadan and Yazd from December 2017 to August 2018. The study was previously approved by the Ethical Committee of Tehran University of Medical Sciences (Code No. IR.TUMS.VCR.REC.1397.836). These samples were systematically sent to Accredited Food Microbiology Laboratory of the School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, by local health centers for microbiological identification. Results were reported to the centers confidentially. All activities within the laboratory were officially approved by the administrators.

Isolation and identification of *S. aureus*

Stool samples and rectal specimens were inoculated into Chapman culture media (Merck, Germany) and incubated at 37°C for 24 h. Routine biochemical tests of Gram staining, catalase, maltose fermentation, VP, DNase, coagulase and sensitivity to novobiocin and polymyxin B were carried out for the yellow colonies of *S. aureus* (Koneman et al., 2008).

Antimicrobial susceptibility test (AST)

The AST was carried out for the isolates that were phenotypically identified as *S. aureus* using disk diffusion and minimum inhibitory concentration (MIC). Disc diffusion test was carried out using Kirby-Bauer method. Bacterial suspensions of 0.5 McFarland turbidity provided were cultured on Muller-Hinton agar (MHA) (Merck, Germany). Routine clinically used antibiotic discs of penicillin (10 U), vancomycin (30 µg) and oxacillin (1 µg) (Merck, Germany) were used for the test. Results were reported based on the CLSI guidelines (Patra et al., 2011). The MIC test for vancomycin was carried out using E-tests. Bacterial suspensions of 0.5 McFarland turbidity were prepared from overnight cultures of *S. aureus* isolates on plate count agar (PCA) media. Suspensions were cultured on MHA plates. After a few minutes, vancomycin stripes (Liofilchem, MTS) were set on MHA plates and incubated at 37°C for 24 h. Results were reported based on the CLSI guidelines (Patra et al., 2011).

Detection of *sea* and *seb* genes using polymerase chain reaction (PCR)

The *sea*~~,~~ and *seb* genes were detected using PCR and specific primer pairs (Table 1). Briefly, DNA extraction was carried out using alkaline lysis method (Zouharova and Rysanek, 2008). The PCR reactions included 12.5 µl of Master Mix (Genfanavaran, Iran), 10.1 µl of distilled water, 0.5 µl (10 pM) of each primer (Genfanavaran, Iran), 50 ng of DNA template and 1 U of Taq DNA polymerase (Genfanavaran, Iran). The thermal cycling used for the PCR was as follows: initial denaturation at 95°C for 5 min; then, 35 cycles of denaturation at 95°C for 40 s, annealing at 58°C for 40 s and extension at 72°C for 90 s. The final extension was carried out at 72°C for 5 min. The *S. aureus* SINA strains were used as positive and DNA-free reactions as negative controls.

Statistical analysis

Data were statistically analyzed using SPSS Software v.18 (IBM

Analytics, USA), chi-square and Fisher exact tests with 95% confidence. A P-value of 0.05 was recognized as significant.

RESULTS

Prevalence of *S. aureus*

From December 2017 to August 2018, 313 diarrheas samples were collected from 120 foodborne outbreaks. In general, 55% of these isolates (17.6%) were identified as *S. aureus*. As shown in Table 2, *S. aureus* was mostly isolated from Yazd (28 isolates, 50.9%) and Semnan (16 isolates, 29.1%) Provinces. Prevalence of *S. aureus* was high in a patient age range of 20-29 years (17 patients, 30.9%); mostly from females (28 isolates, 50.9%). Most *S. aureus* were isolated in January and July (nine isolates each) with no strains isolated in March. Most isolates of *S. aureus* belonged to salad and rice-stew; however, food sources were unclear in most outbreaks. None of these results were statistically significant ($P > 0.05$). The most common infection symptom was watery diarrhea in 55 cases (100%); however, no dysentery symptom was reported. Diarrhea, nausea and fever symptoms showed a significant association with the isolation of *S. aureus* from foodborne outbreak samples ($P < 0.05$).

Antimicrobial susceptibility

The highest antibiotic resistance in *S. aureus* belonged to penicillin (45 isolates, 81.8%), while all isolates were susceptible to vancomycin (55 isolates, 100%) (Figure 1). Furthermore, nearly eight of the isolates (14.5%) were resistant to oxacillin.

Prevalence of *sea* and *seb* genes

The *sea* and *seb* genes were detected in four (7.3%) and one (1.8%) isolates, respectively (Figures 2 and 3). All detected *sea* genes belonged to isolates from Yazd Province.

DISCUSSION

S. aureus is a commensal and opportunistic pathogen that cause a wide range of infections, including superficial invasive infections (Lowy, 1998). This bacterium is the major pathogen of nosocomial and community-acquired infections (Chaibenjawong and Foster, 2011; Soltan Dallal et al., 2016). The current study was carried out on 313 foodborne diarrheal samples to isolate *S. aureus*. Fifty-five isolates (17.6%) were identified as *S. aureus*. In a study in Brazil, 2926 foodborne outbreaks were included (de Oliveira et al., 2018). The most common bacterial causes of the foodborne outbreaks were *Salmonella* (30%) and *S. aureus* (23.3%). Rates of *S. aureus* isolation mainly vary within various countries. Differences in hygiene, population and diet can result in differences in causative agent prevalence of the foodborne outbreaks. Unlike other bacteria, if a person is contaminated by *S. aureus* in the nose or skin, food contamination control protocols cannot be possible just by washing hands with soap and water while preparing and processing foods. Therefore, the infection control of *S. aureus* needs more precise monitoring protocols (de Oliveira et al., 2018; Gould et al., 2013; Kozak et al., 2013). In the current study, most of the *S. aureus* isolates were collected from 28 samples (50.9%) from Yazd and 16 samples (29.1%) from Semnan Provinces. In a study by Madahi et al. (2013) in Isfahan, the infection rate of chicken nuggets with *S. aureus* was 5.7% (Madahi et al., 2015). In another study by Feizi et al., the rate of *S. aureus* infection in chicken meats was 3.5% (23). As previously highlighted, the infection rate of *S. aureus* depends on hygiene level, weather condition, population, diet type and food source (Soltan Dallal et al., 2015). In this study, the highest age group with *S. aureus* infections was 20–29 year-old group (17 isolates, 30.9%). Furthermore, rates of infections in females and men included 50.9 and 49.1%, respectively. In a study in the United States, the predominant age group was reported as 40-49 year-old group (48%)(Bennett et al., 2013). Moreover, females were mostly infected by *S. aureus* (52%); similar to the present study. In a study in Japan, the most frequent age group infected with *S. aureus* foodborne outbreaks was less than ten years, mostly including girls (55.3%)(Asao et al., 2003). Based on the findings from the current study and similar studies, age groups of the patients infected with *S. aureus* differ in geographic regions.

In this study, the most common infection symptom was watery diarrhea in 55 cases (100%), followed by vomiting in 49 (89.1%), nausea in 49 (89.1%), abdominal cramp in 34 (61.8%), fever in three (5.5%) and headache in 22 (40%) cases. In other studies, similar symptoms were reported from the patients (Soltan Dallal et al., 2015; Guidi et al., 2018; Teague et al., 2013). In these studies, diarrhea has been shown as the most prevalent symptom of *S. aureus* infections. Normally, the *S. aureus* food toxicity is due to the production of bacterial enterotoxins. Staphylococcal enterotoxin contaminated foods have been shown to cause sudden vomiting, which may be followed by diarrhea after 4-6 h. Classic symptoms of the infection include nausea, vomiting, abdominal pain and diarrhea, while the headache is less common and fever occurs in only 25% of the patients (Landgraf and Destro, 2013). In the present study, the highest infection rate of *S. aureus* was seen in January and July (9 and 16.4%, respectively). In a study since 2006 to 2011 by Masoumi et al. (2015) foodborne outbreaks were studied; of which, the highest number of outbreaks occurred in August and September (17.8 and 13.6%, respectively) (Masoumi et al., 2015). In a study by Soltan Dallal et al. (2016) the

Table 2. Analysis of *S. aureus* isolated from foodborne outbreaks.

Prevalence	*S. aureus* (%)	*P*-value
Province		
Tehran	1 (1.8)	0.118
Semnan	16 (29.1)	
Kurdistan	5 (9.1)	
Hamadan	5 (9.1)	
Yazd	28 (50.9)	
Age group		
< 10	15 (27.3)	0.69
10–19	13 (23.6)	
20–29	17 (30.9)	
30–39	4 (7.3)	
40–49	4 (7.3)	
50 ≥	2 (3.6)	
Gender		
Female	28 (50.9)	0.42
Male	27 (49.1)	
Month		
January	9 (16.4)	0.42
February	5 (9.1)	
March	4 (7.3)	
April	6 (10.9)	
May	1 (1.8)	
June	1 (1.8)	
July	9 (16.4)	
August	8 (14.5)	
September	7 (12.7)	
October	3 (5.5)	
November	2 (3.6)	
December	0 (0)	
Clinical symptom		
Watery diarrhea	55 (100)	0.0001
Dysentery	0 (0)	0.05
Vomiting	49 (89.1)	0.045
Nausea	49 (89.1)	0.045
Abdominal cramp	34 (61.8)	0.097
Fever	3 (5.5)	0.0001
Headache	22 (40)	0.76
Food type		
Junk food	1 (1.8)	
Salad	5 (9.1)	
Spaghetti	4 (7.3)	
Rice-stew	5 (9.1)	
Chelo kebab	3 (5.5)	
Mushroom	2 (3.6)	
Falafel	3 (5.5)	
Omelet	4 (7.3)	

Table 2. Cont'd.

Chicken	5 (9.1)
Dairy	2 (3.6)
Water	1 (1.8)
Unknown	20 (36.4)

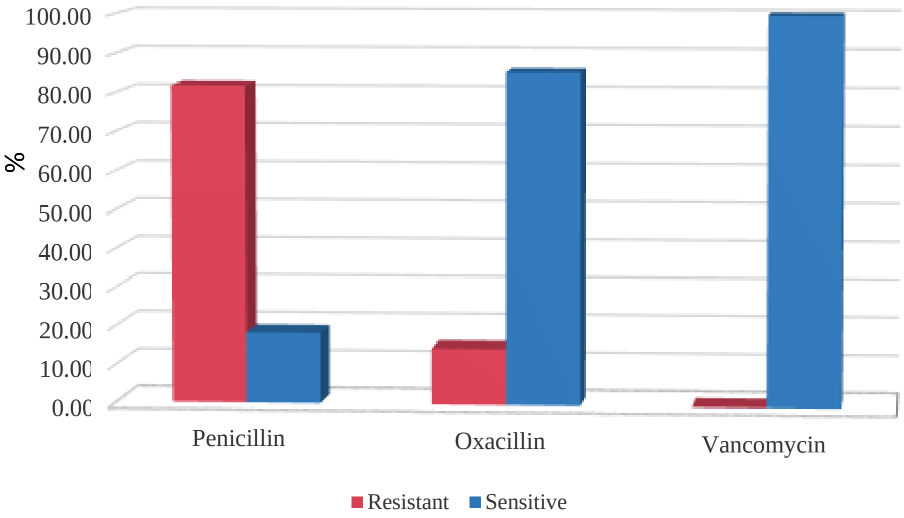

Figure 1. Antibiotic susceptibility of *S. aureus* isolated from foodborne outbreaks.

highest number of outbreaks was reported in September (30.13%) (Soltan Dallal et al., 2016). Studies have shown that the foodborne outbreaks mostly occur in warm months of the year; similar to that the current study has. In this study, the most common food sources for the outbreaks of *S. aureus* included salad, chicken and rice-stew for 15 samples (27.3%). In a study by Mossong et al., (2015) pasta salad (82%) was more likely to be contaminated with *S. aureus* (Mossong et al., 2015). Salad is one of the foods that need to be handled directly and can be easily contaminated by the carriers of *S. aureus*. A study in Germany showed that a food outbreak that caused infections in seven people was due to the consumption of ice cream contaminated with *S. aureus* (Fetsch et al., 2014). In the present study, two isolates (3.6%) of dairy foods were contaminated with *S. aureus*. Due to the uncertainty of contamination sources, it cannot be stated definitely that dairy products such as ice cream cause contaminations in a large number of cases in Iran. Ice cream is a high-risk food for *S. aureus* infections. Milk in ice creams is heated using a low temperature, allowing survival of the naturally occurring infectious bacteria. Other risk factors include additives in ice creams (Fetsch et al., 2014). However, the infection was mostly detected in salads, rice-stews and chickens in the current study.

As drug-resistant *Staphylococcus* spp. cause nosocomial and other communicable infections, these bacteria are considered one of the major threats to public health (Sarrafzadeh et al., 2014; Haran et al., 2012; Ateba et al., 2010). In the present study, the highest rate of resistance was observed toward penicillin (81.8%). Penicillin is widely used to treat infections in humans and animals. However, overuse of this antibiotic has led bacterial resistance. Unnecessary administration of antibiotics for therapeutic purposes and inappropriate and unscientific use of antibiotics are other factors that cause selection of antibiotic-resistant bacteria (Yavari, 2013). Antibiotic resistance restricts cure infection ability; therefore, strict rules must be used to prevent the spread of drug resistance. Antimicrobial drugs are used for the treatment, prevention and control of infectious diseases as well as dietary complements in foods. Relationships between the use of antimicrobial drugs in livestock and the selection of resistant bacteria in food chains have been debated widely (Yavari, 2013). In a study by Al-Bahry et al. (2014) in Oman, most of *S. aureus* isolates from food samples were multidrug resistant. The highest antibiotic resistance rate was observed for ampicillin and penicillin (86.3 and 85.93%, respectively) while vancomycin resistance was seen in a few isolates of *S. aureus* (5%) (Al-Bahry et al., 2014). Acco et al. (2003) showed that 70% of the *S. aureus* isolated from individuals involved in food baking were resistant to

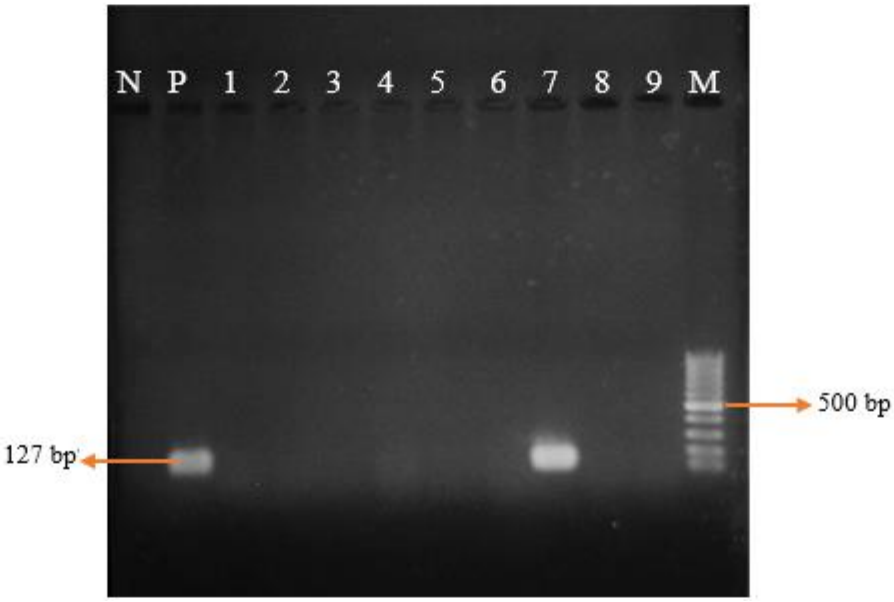

Figure 2. Agarose gel electrophoresis of *sea* gene amplicons of *S. aureus* isolated from foodborne outbreaks. Lane M, DNA ladder (100 bp); Lane N, negative control; Lane P, positive control (*S. aureus* strain SINA); and Lane 7, *sea*-positive *S. aureus* isolate from foodborne outbreaks.

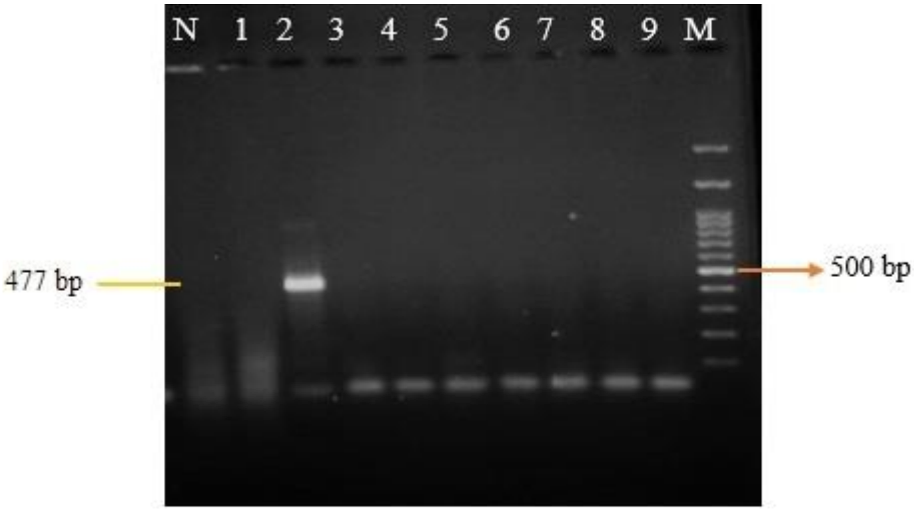

Figure 3. Agarose gel electrophoresis of *seb* genes of *S. aureus* isolated from foodborne outbreaks. Lane M, DNA ladder (100 bp); Lane N, negative control; and Lane 2, *seb*-postive *S. aureus* isolate from foodborne outbreaks

penicillin (Acco et al., 2003). In several countries, large-scale studies have detected penicillin resistance in approximately 60% of studied *S. aureus* isolates (Zarkesh et al., 2016). In this study, a high resistance rate to penicillin was observed; similar to other studies.

One of the most important virulence determinants of *S. aureus* are enterotoxins (SE). Over 15 types of SE with similar protein structures have been characterized (Pourmand et al., 2009). These toxins are not digested by the intestinal proteases such as pepsin and are highly resistant to high temperatures. For example, SEA preserves its biological properties after 28 min at 121°C (Nazari et al., 2014). Due to its thermo-resistance trait, SEA must be avoided in foods. As the taste and color of foods are not changed by SEA, usual cooking methods do not eliminate this toxin. In most parts of the world, nearly 50% of foodborne diseases are caused by the SEA. The enterotoxins in raw meats should be assessed before eating since these toxins are poisonous even at very low levels (0.5 ng/ml) (Alizadeh and Amini, 2015). Enterotoxins of *Staphylococcus* spp. can be identified using immunological methods (Pourmand et al., 2009); however, use of these methods is usually limited. Therefore, use of DNA-based methods has become further popular. In this study, *sea* and *seb* enterotoxin genes were detected in four (7.3%) and one (1.8%) *S. aureus* isolates, respectively. In a study by Johler et al. (2015) 14 patients with *S. aureus* intoxication were studied. The prevalence of *sea* enterotoxin gene was higher, compared to that of other enterotoxins (Johler et al., 2015). In Asgarpoor et al. (2018) study on 136 samples of *S. aureus* infected individuals, prevalence of *sea* and *seb* genes was 23.9 and 13%, respectively (Asgarpoor et al., 2018). Gholamzad et al. (2015) investigated 80 food samples suspected to *S. aureus* contamination and showed presence of the *seb* gene in 54 samples (Gholamzad et al., 2015). The *sea* gene is the most common gene within toxin-producing isolates. Moreover, all enterotoxins have similarities in gene structures and sequences; mostly encoded by mobile genetic elements (MGEs) such as bacteriophages, plasmids, transposons, insertion sequences (IS), integrons and pathogenic islands (PI)(Baba et al., 2002).

Conclusion

In this study, prevalence of *S. aureus* foodborne outbreaks in various regions of Iran was investigated for the first time. Overall, the current study showed that *S. aureus* accounts for a high rate of food contaminations. The *sea* and *seb* genes encoding SE were detected in eight *S. aureus* isolates; hence, elimination of the bacteria in foods is necessary. Furthermore, increased bacterial food poisoning and resistance to penicillin and oxacillin have raised public concerns. Despite reports on vancomycin resistance in *S. aureus* isolates, all our *S. aureus* strains were susceptible to this antibiotic.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Ethics committee

The study was previously approved by the Ethical Committee of Tehran University of Medical Sciences (Code No. IR.TUMS.VCR.REC.1397.836).

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