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Enterotoxigenicity, distribution of enterotoxigenic genes and antibiotic resistance pattern of *Staphylococcus aureus* isolated from traditional sweet

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Abstract. Resistant and enterotoxigenic *Staphylococcus aureus* strains are considered to be one of the major causes of foodborne diseases due to the consumption of sweet. The present research was done to study the distribution of enterotoxin types, enterotoxigenic genes and antibiotic resistance pattern of *S. au*reus strains isolated from traditional sweet samples. Eight-hundred and fifteen sweet samples were cultured and *S. aureus* strains were identified. Antibiotic resistance, enterotoxigenicity and enterotoxigenic gene profile were studied using disk diffusion, Enzyme Link Immunosorbent Assay and PCR, respectively. One-hundred and seven out of 815 (13.12%) sweet samples were positive for *S. aureus*. Prevalence of *S. aureus* in dried and semi-dried sweet samples were 15.08% and 11.13%, respectively (P <0.05). Forty-six out of 107 *S. aureus* strains (42.99%) were determined as enterotoxigenic. A (41.30%) and C (17.39%) were the most commonly detected enterotoxin types. *Sea* (20.56%), *sec* (14.95%) and *seb* (11.21%) were the most commonly detected enterotoxigenic genes. There were no positive sample for the *sej* enterotoxin gene. *S. aureus* strains harbored the highest prevalence of resistance against penicillin (88.78%), tetracycline (83.17%), ceftaroline (75.70%) and doxycycline (71.02%). Simultaneous presence of enterotoxins and enterotoxigenic genes in multi-drug resistant *S. aureus* strains indicates important public health issue regarding the consumption of contaminated traditional sweet samples.

INTRODUCTION

Sweets contain both hard and soft tissues and also both dried and semi-dried consolidations, which cover with tasty sugar or same materials with additional compatible components (Kawo and Abdulmumin, 2009; Nateghi *et al.*, 2013). Iranian traditional sweets are mostly shaped from diverse kinds of flour with some supplementary ingredients such as milk, honey, herbal extracts, chocolate, cardamom, and nuts (Kawo and Abdulmumin, 2009). Human involvement in the preparation of sweet increased the risk of microbial contamination and occurrence of food-borne diseases (Kawo and Abdulmumin, 2009).

There were several important types of food-borne bacteria responsible for severe kinds of food poisonings (Atapoor *et al.*, 2014; Ghorbani *et al.*, 2016; Hemmatinezhad *et al.*, 2015; Momtaz *et al.*, 2013a; Momtaz *et al.*, 2012; Nejat *et al.*, 2015; Rahimi *et al.*, 2014a; Rahimi *et al.*, 2014b; Ranjbar *et al.*, 2018; Safarpoor Dehkordi *et al.*, 2013a; Safarpoor Dehkordi *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2014a; Safarpoor Dehkordi *et al.*, 2012; Safarpoor Dehkordi *et al.*,

2014b). *Staphylococcus aureus* (*S. aureus*) is a Gram-positive, catalase positive, and cocci-shaped bacterium usually originate from nose and respiratory system and on the skin (Dehkordi *et al.*, 2017; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017). *S. aureus* is responsible for nosocomial and community-acquired infections, food-borne diseases and food poisoning (Dehkordi *et al.*, 2017; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017). Occurrence of different types of gastrointestinal diseases which are known by vomiting, nausea, abdominal cramps, weakness and diarrhea and also toxic shock syndrome are attributed to *S. aureus* strains (Dehkordi *et al.*, 2017; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017).

Staphylococcal food poisoning is an intoxication that results from the consumption of foods containing sufficient amounts of one (or more) enterotoxin (Hennekinne *et al.*, 2012; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017). The staphylococcal enterotoxins (SEs) are a group of low-molecular-mass and single-chain super antigenic proteins that are similar in composition and biological activity, which differ in antigenicity (Hennekinne *et al.*, 2012; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017). Up to know, 23 known main kinds of SEs (SEA to SEV) have been recognized. Different enterotoxins are classified into 5 diverse groups regarding the sequences of amino acids, in which group 1 includes the SEA, SED, SEE, SEJ, SEN, SEO, SEP and SES, group 2 includes the SEB, SEC, SEG, SER and SEU, group 3 includes the SEI, SEK, SEL, SEM, and SEQ and groups 4 and 5 include only the SEV and SEH, respectively. These enterotoxins are mainly coded by certain eneterotoxigenic genes (Hennekinne *et al.*, 2012; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017).

Food-borne *S. aureus* strains are classically resist against several types of antibiotics and particularly penicillins, aminoglycosides, tetracyclines, cephalosporins, macrolides, lincosamides, quinolones and streptogramins (Dehkordi *et al.*, 2017; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017). Higher pathogenicity of resistant *S. aureus* strains and also their food-borne aspects have also been described previously (Dehkordi *et al.*, 2017; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017).

High pathogenicity of *S. aureus* strains and general conditions of sweets for growth and survival of bacteria make it necessary to assess their microbiological quality. Furthermore, there were no formerly available data on the *S. aureus* in sweet samples. Consequently, the current research was done to study the prevalence rate, distribution of enterotoxins and enterotoxigenic genes and antimicrobial resistance properties of the *S. aureus* strains isolated from various types of Iranian traditional sweet samples.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Ethical Council of Research of the Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Verification of this research project and the licenses related to sampling process were approved by the Prof. Amir Shakerian and Prof. Ebrahim Rahimi.

Samples

From June to August 2017, a total of 815 Iranian traditional sweet samples including dried (n= 411) and semi-dried (n= 404) were randomly collected from shopping centers of the Isfahan province, Iran. Sweet samples were categorized in terms of shelf life and moisture content. Produced sweet samples were prepared using wheat, rice or chickpea flours and also mixture of oil, sugar, egg and some aromatic seasonings and fruits (ISIRI 2395, 2012). Semi-dried sweet samples with spongy and soft texture were prepared using jam, marmalade, dried fruits and oil beans. Dried sweet samples with harder texture and lower moisture were prepared using

flour, dried fruits and oil beans. Samples were immediately transferred to the Food Hygiene Research Center of the Islamic Azad University of Shahrekord Branch in cooler with ice-packs. Collected traditional sweet samples indicated normal physical characters such as odor, color and consolidation.

Isolation and identification of S. aureus

Each sample was aseptically weighed in an analytical balance and twenty-five grams were transferred into a sterile plastic bag. Then, 225 mL of buffered peptone water (Merck, Germany) was added and homogenized in a Stomacher Bagmixer 400W (Interscience, Saint-Nom, France) for two min. Five milliliter aliquot of the enriched homogenate was transferred into 50 mL Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate. After incubation at 35°C for 18 h, a loopful of the culture was plated onto Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and incubated overnight at 37°C. Black shiny colonies surrounded by 2 to 5-mm clear zones were further identified on the basis of Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, voges-proskaver (Merck, Germany) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests (Safarpoor Dehkordi *et al.*, 2017).

Antibiotic resistance pattern

Pattern of antimicrobial resistance of *S. aureus* strains were studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Susceptibility of *S. aureus* isolates were examined against several types of antibiotics including penicillin (10 µg/disk), ceftaroline (30 µg/disk), gentamicin (10 µg/disk), azithromycin (15 µg/disk), erythromycin (15 µg/disk), tetracycline (30 µg/disk), doxycycline (30 µg/disk), ciprofloxacin (5 µg/disk), clindamycin (2 µg/disk), trimethoprim-sulfamethoxazole (25 µg/disk), chloramphenicol (30 µg/disk) and rifampin (5 µg/disk) antibiotic agents (Oxoid, UK). Instructions of the Clinical and Laboratory Standards Institute (CLSI, 2015) were used for this purpose. The plates containing the discs were allowed to stand for at least 30 min before incubated at 37°C for 24 h. The diameter of the growth inhibition zone produced by each antibiotic disc was measured and interpreted using the CLSI standards (CLSI, 2015). *S. aureus* (ATCC 43300) was used as quality control organism in antimicrobial susceptibility determination.

DNA extraction

S. aureus isolates were sub-cultured on Tryptic Soy Broth media (TSB, Merck, Germany) and further incubated for 48 h at 37°C. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, Germany) according to manufacturer's instruction. After extraction, the DNA samples were quantified (NanoDrop, Thermo Scientific, Waltham, MA, USA), their purity checked (A260/A280), and their concentrations adjusted to 50 ng/µL. The integrity of the DNA was evaluated on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, Germany). The DNA concentration was also estimated by spectrophotometric absorbance at 257 nm (Hach, DR5000, USA). The DNA was stored at -20°C pending subsequent PCR analysis.

Detection of classical enterotoxins

To detect staphylococcal enterotoxins, *S. aureus* isolates were cultured aerobically in 10 ml nutrient broth (Merck, Germany) at 37°C. Overnight culture supernatants of the isolated bacteria were used for detection of *S. aureus* enterotoxins. Enzyme Linked Immunosorbent Assay (ELISA) kit (RIDASCREEN1 SET A, B, C, D, E; R-Biopharm AG, Darmstadt, Germany)

was used for detection of Staphylococcal entrotoxins SEA, SEB, SEC, SED and SEE. Detection was performed based on manufacturer's instructions of ELISA kit. The detection limit was 0.1 mg/ml. *S. aureus* and *S. epidermidis* strains were used as positive and negative control organisms, respectively (Rahimi and Safai, 2010).

Detection of enterotoxigenic genes

Table 1 represents the oligonucleotide primers and PCR conditions used for amplification of enterotoxigenic genes amongst the *S. aureus* strains isolated from various types of traditional sweet samples (19). A programmable DNA thermocycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. All runs included a negative DNA control consisting of sterile PCR grade water (Thermo Fisher Scientific, Germany) and positive DNA control consisting of positive DNA of each target gene. Fifteen microliters of amplified PCR products were subjected to electrophoresis in a 2% agarose gel in 1× TBE buffer at 90 V for 30-40 min, stained with SYBR Green (Thermo Fisher Scientific, Germany).

Table 1. Oligonucleotide primers and PCR conditions used for amplification of enterotoxigenic genes amongst the *S. aureus* strains isolated from various types of traditional sweet samples

Target gene	Primer sequence (5'-3')	PCR programs	PCR volume (50µL)
sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	1 cycle: 94°C —— 2 min 30 cycles: 94°C —— 120 s 55°C —— 120 s 72°C —— 60 s 1 cycle: 72°C —— 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCC		
sec	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC		
sed	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG		
see	F: AGGTTTTTTCACAGGTCATCC R: CTTTTTTTTCTTCGGTCAATC	1 cycle: 94°C —— 2 min 35 cycles: 94°C —— 120 s 57°C —— 120 s 72°C —— 60 s 1 cycle: 72°C —— 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
seg	F: AAGTAGACATTTTTGGCGTTCC R: AGAACCATCAAACTCGTATAGC	1 cycle: 94°C —— 2 min 30 cycles: 94°C —— 30 s 55°C —— 30 s 72°C —— 60 s 1 cycle: 72°C —— 10 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
seh	F: GTCTATATGGAGGTACAACACT R: GACCTTTACTTATTTCGCTGTC		
sei	F: GGTGATATTGGTGTAGGTAAC R: ATCCATATTCTTTGCCTTTACCAG		
sej	F: CATCAGAACTGTTGTTCCGCTAG R: CTGAATTTTACCATCAAAGGTAC	1 cycle: 94°C —— 1 min 30 cycles: 94°C —— 60 s 62°C —— 60 s 72°C —— 60 s 1 cycle: 72°C —— 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template

Statistical analysis

Statistical analysis was done using the SPSS 25.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's exact two-tailed tests were used to assess any significant relationship for prevalence of *S. aureus* and distribution of enterotoxins, enterotoxin genes and antibiotic resistance pattern between different types of sweet samples. *P* value <0.05 was considered as statistical significant level.

RESULTS

Distribution of S. aureus *strains and staphylococcal enterotoxins*

Table 2 represents the distribution of *S. aureus* strains and staphylococcal enterotoxins in different types of traditional sweet samples. One-hundred and seven out of 815 (13.12%) sweet samples were positive for *S. aureus*. Prevalence of *S. aureus* in dried and semi-dried sweet samples were 15.08% and 11.13%, respectively. Figure 1 indicates the black shiny colonies of the *S. aureus* strains in Baird-Parker agar medium. Statistically significant difference was seen for the prevalence of *S. aureus* between dried and semi-dried sweet samples (p<0.05). Forty-six out of 107 *S. aureus* strains (42.99%) were determined as enterotoxigenic *S. aureus*. Prevalence of enterotoxigenic *S. aureus* strains in dried and semi-dried sweet samples were 41.95% and 44.44%, respectively. A (41.30%) and C (17.39%) were the most commonly detected enterotoxin types amongst the enterotoxigenic S. aureus strains. Prevalence of A+C, A+D and C+D enterotoxins amongst the enterotoxigenic *S. aureus* strains were 10.86%, 6.52% and 6.52%, respectively.

Distribution of enterotoxigenic genes

Table 3 represents the distribution of enterotoxigenic genes amongst the *S. aureus* strains isolated from different types of traditional sweet samples. *Sea* (20.56%), *sec* (14.95%) and *seb* (11.21%) were the most commonly detected enterotoxigenic genes amongst the *S. aureus* strains isolated from different types of traditional sweet samples. There were no positive sample for the *sej* enterotoxin gene. *Seh* (0.93%) and *sei* (0.93%) had the lowest prevalence. *S. aureus* strains isolated from dried sweet samples harbored the higher and more diverse distribution of enterotoxigenic genes (p<0.05).

Antibiotic resistance pattern

Table 4 represents the antibiotic resistance pattern of *S. aureus* strains isolated from different types of traditional sweet samples. *S. aureus* strains harbored the highest prevalence of resistance against penicillin (88.78%), tetracycline (83.17%), ceftaroline (75.70%) and doxycycline (71.02%). *S. aureus* strains exhibited the lowest prevalence of resistance against chloramphenicol (9.34%) and rifampin (27.10%). Statistically significant difference was seen between the type of samples and prevalence of antibiotic resistance (p<0.05).

DISCUSSION

S. aureus is considered as one of the most common causes of nosocomial infections, as well as the food poisonings with emergence of antibiotic resistance (Dehkordi *et al.*, 2017; Madahi *et al.*, 2014; Momtaz *et al.*, 2013b; Safarpoor Dehkordi *et al.*, 2017). Foodstuff contamination with

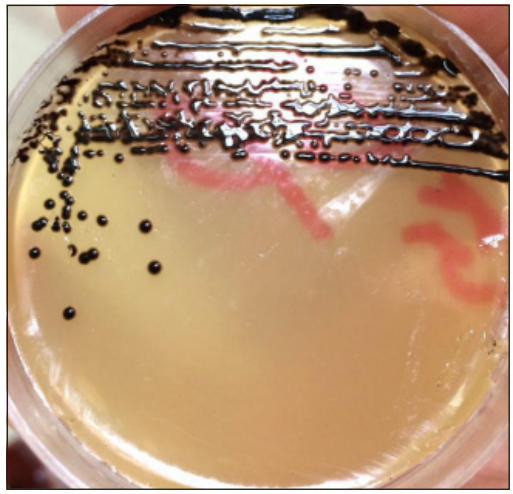

Figure 1. Black shiny colonies of the *S. aureus* in Baird-Parker agar medium.

Table 2. Distribution of *S. aureus* strains and staphylococcal enterotoxins in different types of traditional sweet samples

Samples	N samples collected	N of *S. aureus* positive samples (%)	N samples negative for enterotoxigenic *S. aureus* (%)	N samples positive for enterotoxigenic *S. aureus* (%)	N samples positive for each enterotoxin (%)							
					A	B	C	D	E	A+C	A+D	C+D
Semi-dried	404	45 (11.13)	25 (55.55)	20 (44.44)	8 (40)	1 (5)	3 (15)	2 (10)	–	2 (10)	2 (10)	2 (10)
Dried	411	62 (15.08)	36 (58.05)	26 (41.93)	10 (38.46)	3 (11.53)	5 (19.23)	2 (7.69)	1 (3.84)	3 (11.53)	1 (3.84)	1 (3.84)
Total	815	107 (13.12)	61 (57.00)	46 (42.99)	19 (41.30)	4 (8.69)	8 (17.39)	4 (8.69)	1 (2.17)	5 (10.86)	3 (6.52)	3 (6.52)

Table 3. Distribution of enterotoxigenic genes amongst the *S. aureus* strains isolated from different types of traditional sweet samples

Samples (N of *S. aureus* strains)	N (%) isolates positive for each enterotoxin gene								
	sea	*seb*	*sec*	*sed*	*see*	*seg*	*seh*	*sei*	*sej*
Semi-dried (45)	8 (17.77)	4 (8.88)	5 (11.11)	3 (6.66)	2 (4.44)	1 (2.22)	–	–	–
Dried (62)	14 (22.58)	8 (12.90)	11 (17.74)	6 (9.67)	3 (4.83)	2 (3.22)	1 (1.61)	1 (1.61)	–
Total (107)	22 (20.56)	12 (11.21)	16 (14.95)	9 (8.41)	5 (4.67)	3 (2.80)	1 (0.93)	1 (0.93)	–

Table 4. Antibiotic resistance pattern of *S. aureus* strains isolated from different types of traditional sweet samples

Samples (N of *S. aureus* strains)	N (%) isolates resistant to each antibiotic											
	P10*	Cef	Gen	Az	Ert	Tet	Dox	Cip	Cln	Trsu	C30	Rif
Semi-dried (45)	40 (88.88)	38 (84.44)	35 (77.77)	20 (44.44)	27 (60)	39 (86.66)	31 (68.88)	25 (55.55)	16 (35.55)	24 (53.33)	4 (8.88)	12 (26.66)
Dried (62)	55 (88.70)	43 (69.35)	39 (62.90)	26 (41.93)	34 (54.83)	50 (80.64)	45 (72.58)	32 (51.61)	22 (35.48)	28 (45.16)	6 (14.28)	17 (27.41)
Total (107)	95 (88.78)	81 (75.70)	74 (69.15)	46 (42.99)	61 (57)	89 (83.17)	76 (71.02)	57 (53.27)	38 (35.51)	52 (48.59)	10 (9.34)	29 (27.10)

*P10: penicillin (10 µg/disk), Cef: ceftaroline (30 µg/disk), Gen: gentamicin (10 µg/disk), Az: azithromycin (15 µg/disk), Ert: erythromycin (15 µg/disk), Tet: tetracycline (30 µg/disk), Dox: doxycycline (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Cln: clindamycin (2 µg/disk), Trsu: trimethoprim-sulfamethoxazole (25 µg/disk), C30: chloramphenicol (30 µg/disk), Rif: rifampin (5 µg/disk).

S. aureus may occur directly from infected food-producing animals (or their products such as milk and meat) or may result from poor hygiene conditions during production processes, or the retail and storage of food, since humans may also harbor micro-organisms. The present investigation is the first report of the study the prevalence, pattern of antibiotic resistance and distribution of enterotoxins and enterotoxigenic genes of the *S. aureus* strains isolated from diverse kinds of Iranian traditional sweet samples.

Our results showed that the prevalence of *S. aureus* in all studied samples was 13.12%. Additionally, prevalence of *S. aureus* in dried and semi-dried sweet samples were 15.08% and 11.13%, respectively. Traditional sweets are extensively handled manually and are not submitted to any subsequent thermal or anti-microbial processing. Thus, this kind of food is frequently contaminated by *S. aureus*. Higher prevalence of *S. aureus* in dried sweet samples is maybe due to the fact that *S. aureus* can resist against low levels of activated water (AW). Therefore, *S. aureus* can easily growth in dried sweet samples which have a hard condition for survival of other bacteria. As far as we know, this is the highest prevalence rate of *S. aureus* in traditional sweet samples al-around the world (13.12%). The prevalence rate of the *S. aureus* in Iranian traditional sweet samples of our research was higher than that of Pakistan (6.70%) (Sahir *et al.*), Portugal (11.10%) (Castro *et al.*, 2016), Brazil (12.00%) (Kroning *et al.*, 2016) and Spain (6.10%) (Gutiérrez *et al.*, 2012), while lower than that of Japan which was conducted on industrial sweet samples (19.40%) (Shimamura *et al.*, 2006). High prevalence of *S. aureus* strains in studied samples is maybe due to using unpasteurized milk in their preparation. The possibility of transmission of *S. aureus* strains from infected staffs of sweet producing factories is additional reason for the high prevalence of *S. aureus*. Sahir *et al.* (2017) (Sahir *et al.*) described that the prevalence of *S. aureus* in industrial candy, candy mix, date, formula milk, lentils, mayonnaise, paratha and samosa samples were 80.95%, 80.00%, 56.25%, 66.67%, 47.50%, 40.00%, 64.00% and 55.00%, respectively. Kroning *et al.* (2016) (Kroning *et al.*, 2016) reported that 12% of diverse kinds of handmade sweets were positive for *S. aureus* strains. All stated authors point out that proper hygiene of the food handlers, as well as correct cleaning of the food preparation surfaces, will decrease contamination by *S. aureus*.

Iranian traditional sweet samples are mainly produced from different types of flour with some supplementary ingredients such as milk, honey, herbal extracts, chocolate, cardamom, and nuts. Semi-dried sweet samples with spongy and soft texture were prepared using jam, marmalade, dried fruits and oil beans, while dried sweet samples with harder texture and lower moisture were prepared using flour, dried fruits and oil beans. Contamination in one of these ingredients caused survival of *S. aureus* even in produced sweet samples. Therefore, hygienic condition of raw ingredients should observe.

S. aureus strains of our research had a higher prevalence of resistance especially against human-based antibiotics including ceftaroline, doxycycline, azithromycin, doxycycline, erythromycin, ciprofloxacin, clindamycin and rifampin. This outcome presented the human-based origin for the *S. aureus* strains isolated from sweet samples. The finding is indirectly approved that the *S. aureus* strains are probable transferred from infected food handlers. Some strains harbored the significant prevalence of resistance against chloramphenicol. It may be due to the high prescription of chloramphenicol in Iran. Accordingly, the resistance levels found in this study can be explained by the indiscriminate use of antimicrobials in humans treating diseases, which is a worrying trend. High prevalence of resistance against human-based antibiotics have also been reported from Nigeria (Fowoyo and Ogunbanwo, 2017), Italy (Paludi *et al.*, 2011), Iran (Safarpoor Dehkordi *et al.*, 2017), Brazil (Kroning *et al.*, 2016), Egypt (Sallam *et al.*, 2015) and

USA (Jackson *et al.*, 2013). Rong *et al.* (2017) (Rong *et al.*, 2017) described that the prevalence of antibiotic resistance in the *S. aureus* strains isolated from different types of food samples against ampicillin, penicillin, amoxicillin-clavulanic acid, cefoxitin, ceftazidime, cefepime, kanamycin, streptomycin, amikacin, gentamicin, norfloxacin, ciprofloxacin, erythromycin, tetracycline, clindamycin, chloramphenicol, trimethoprim-sulfamethoxazole, vancomycin and rifampicin were 88.20%, 88.20%, 73.90%, 8.40%, 10.90%, 8.40%, 22.70%, 14.30%, 1.70%, 4.20%, 6.70%, 5.00%, 53.80%, 26.90%, 12.60%, 7.50%, 7.50%, 0% and 2.50%, respectively. Ma *et al.* (2018) (Ma *et al.*, 2018) reported that the prevalence of resistance of *S. aureus* strains isolated from food samples against penicillin, erythromycin, clindamycin, tetracycline, chloramphenicol, ciprofloxacin and gentamicin were 92.47%, 58.06%, 78.49%, 25.81%, 20.43%, 10.75% and 2.15%, respectively. Chinese investigation (Zhuge *et al.*, 2015) showed the resistance to penicillin was 95.92%, tetracycline 85.71%, chloramphenicol 81.63% and erythromycin 77.55%. Another study in the United States (Abdalrahman *et al.*, 2015) reported the resistance to penicillin was 52.5%, tetracycline 53.5%, kanamycin 25.7% and erythromycin 32.7%, which were somewhat similar to our findings. An extensive use of antibiotics in humans as well as their use for disease prevention and growth promotion in agriculture has led to the emergence of antibiotic-resistant strains. *S. aureus* has an outstanding ability to acquire resistance to antibiotics, which is considered a major public health concern. Selection of antibiotic agents in the present investigation was done according to the principles of the CLSI (CLSI, 2015) and also their application in veterinary and medical sciences.

S. aureus strains have the ability to produce enterotoxins by activity of enterotoxigenic genes which pose an important public health issue. We found that majority of *S. aureus* strains harbored both enterotoxins and also enterotoxigenic genes. Simultaneous presence of two or more types of enterotoxins and also enterotoxigenic genes is another important finding of the present study. A, C and D and also *sea*, *seb*, *sec* and *sed* were the most commonly detected enterotoxin types and enterotoxigenic genes, respectively. High prevalence of enterotoxin A and its gene (*sea*) represented the high ability of *S. aureus* strains to cause staphylococcal food poisoning. A comparatively boost percentage of enterotoxin-forming *S. aureus* strains from food samples found in our study was approved by the previous findings (Kroning *et al.*, 2016; Schelin *et al.*, 2011; Song *et al.*, 2016; Wang *et al.*, 2017). About 95% of staphylococcal food poisoning (SFP) are associated with the classical SEs (SEA to SEE) which had the high prevalence in the *S. aureus* strains of our study. Low prevalence of enterotoxin E and also *seh*, *sei* and *sej* enterotoxigenic genes is another important finding of our study which was similar to those of Iran (Rahimi, 2013), France (Kérouanton *et al.*, 2007) and Brazil (Pelisser *et al.*, 2009). Song *et al.* (2015) (Song *et al.*, 2016) reported that 5.60% of *S. aureus* isolates from ready to eat food samples carried the *sea* and 3.50% harbored the *seb* genes. Similarly, Zhang *et al.* (2013) (Zhang *et al.*, 2013) represents that the *sea* gene found in 24.10% and the *seb* gene in 4.20% of *S. aureus* strains isolated from different types of raw and cooked ready to eat food samples. These researchers also reported that 20.50% of *S. aureus* isolates carried the *sed* gene, 6.80% carried the *sec* gene and 0.60% carried the *see* gene. Kroning *et al.* (2016) (Kroning *et al.*, 2016) reported the high prevalence of *sea* (33.4%) and *seb* (16.6%) enterotoxigenic genes amongst the *S. aureus* strains isolated from handmade sweet samples. Our studied isolates have enterotoxigenic potential because they may possess the *sec*, *sea*, *sed* and *seb* genes. The *sea* and *sed* genes are the most usually reported in contaminated food samples and also measured the major causes of staphylococcal food poisoning worldwide, while the *seb* gene is known for endorsing more severe poisoning than other entero-

toxins (Argudín *et al.*, 2010; Carfora *et al.*, 2015). We also found that 107 *S. aureus* isolates harbored at least one of the enterotoxin types and also entertotoxigenic genes, which is similar to those reported in the USA (Ge *et al.*, 2017) and Malaysia (Puah *et al.*, 2016). High prevalence of virulent, toxigenic and antibiotic resistant foodborne bacteria has also been reported in different types of food samples (Dehkordi *et al.*, 2012; 2013; 2014a; 2014b; Ranjbar *et al.*, 2017; Safarpoor Dehkordi *et al.*, 2011a; 2011b).

CONCLUSION

To put it in a nutshell, we identified boost prevalence of *S. aureus* and enterotoxigenic *S. aureus* amongst the Iranian traditional sweet samples. This study was also provided detail epidemiological estimations of prevalence of antibiotic resistance and entyerotoxigenic genes amongst the *S. aureus* strains isolated from sweet samples. A, B and C types of enterotoxins, *sea*, *seb*, *sed* and *sed* enterotoxigenic genes and finally boost resistance against penicillin, tetracycline, ceftaroline and doxycycline were the most important finding of the current research. Simultaneous presence of enterotoxigenic genes, enterotoxins and multi-drug resistant *S. aureus* strains had also high clinical and epidemiological importance. Furthermore, our research demonstrated that most *S. aureus* were found to be resistant to commonly used antimicrobial agents which raised concerns regarding transmission risk following the consumption of traditional sweet contaminated with these bacteria. Our research highlights the importance of monitoring the antimicrobial susceptibility and enterotoxigenicity of *S. aureus* in the food chains including traditional sweet and especially dried sweet samples and these data could be used proactively to assist government and industries in Iran to develop improved food safety measures, designed to reduce the contamination and transmission of this bacterium. Moreover, a future large-scale, multi-population-based study must be conducted to obtain more comprehensive data on the prevalence and distribution of *S. aureus* in different Iranian ethnic populations.

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