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ORIGINAL ARTICLE

Investigation of the virulence, antibiotic resistance, and enterotoxin genes of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from nugget and salad samples

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ABSTRACT

Background and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant cause of illness from consuming contaminated food. MRSA is mainly known for its ability to develop resistance to antibiotics including methicillin. This research examined the antimicrobial resistance pattern, enterotoxigenic dispensation, virulence factors, and biotyping for MRSA isolates.

Materials and Methods: Susceptibility of *S. aureus* isolates to 13 types of antibiotics were assessed, and the genes associated with the resistance were investigated. Disk diffusion was used to identify the phenotypic tenet of antibiotic resistance. PCR is instrumental in detecting genes that confer resistance to antibiotics, virulence and enterotoxin genes.

Results: *S. aureus* were found in 167 out of 363 nugget and salad samples, representing 46% of the total sample count. Seventy-eight isolates (46.71%) were identified as MRSA bacteria. Its prevalence in different sources was as follows: 10% in bovine, 0% in ovine, 30% in poultry, and 56% in humans. MRSA displays high prevalence of resistance to cefotaxime and tetracycline (100%). *coa* was the most prevalent virulence factor (100%) in MRSA.

Conclusion: Distribution of antibiotic resistance genes in MRSA, highlights a serious health issue, as the presence of different antibiotic resistance genes exacerbates multidrug resistance in MRSA isolates.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Antibiotic resistance; Enterotoxin genes; Nugget; Salad; Polymerase chain reaction

INTRODUCTION

Food-related health problems resulting from contamination have a devastating impact on health and the economy worldwide. Foodborne diseases (FBDs) can result in significant public health problems. Numerous epidemics and infectious diseases are associated with different types of foods (1). The economic impact of FBDs on industry and healthcare systems is significant, leading to lost productivity, increased medical costs, and inadequate food waste management. FBDs cause 600 million people worldwide to become seriously ill each year and cause approximately 420,000 deaths (2). *Staphylococcus aureus*, espe-

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cially methicillin-resistant *Staphylococcus aureus* (MRSA), is recognized as one of the most important pathogens responsible for human food poisoning in the world (3). Since its discovery, MRSA has become one of the most significant antibiotic-resistant pathogens affecting humans. Previously known as a nosocomial severe infection, it has recently become a foodborne epidemic. Besides its ability to form typical biofilms, MRSA can produce various toxins and is responsible for a variety of human diseases (4). MRSA has been reported to cause several foodborne outbreaks in recent years (5). The prevalence of antibiotic resistance typically increases with foodborne *S. aureus* bacteria. Today, MRSA poses a significant challenge in healthcare facilities as well as the community. According to reliable data and reports, about 70% of *S. aureus* strains are resistant to penicillins. In addition, diseases caused by MRSA exhibit a high level of resistance to various antibiotics, including tetracyclines, aminoglycosides, macrolides, penicillins, and fluoroquinolones. The clinical importance of MRSA in popular foods is increasing due to the rise in popular contaminated food sources (6). Foods containing MRSA are viewed as essential sources of genes encoding resistance to antibiotics. A significant number of resistance genes contribute to antibiotic resistance, including those for tetracycline (*tetK* and *tetM*), lincosamides (*linA*), folic acid inhibitors (*dfrA1*), aminoglycosides (*aacA-D*), phenicol (*cfr*), macrolides (*ermA* and B, *msrA*, and B, and *mefA*), fluoroquinolones (*girA* and *grlA*), penicillin (*blaZ*), and ansamycins (*rpoB*) (7). *S. aureus* has the potential to be a significant pathogen in humans. Staphylococcal food poisoning (SFP) can result in a range of illnesses, including toxic shock syndrome, pneumonia, and other mild infections that may exhibit minimal symptoms (8). The infection and pathogenicity of this bacterium are facilitated by several pathogenic factors, including staphylococcal enterotoxin (SE), hemolysin, and the fibronectin-binding protein (9). MRSA is the most pathogenic strain of Staphylococcus and is among the top 10 contaminants and causes of foodborne bacterial diseases (FBDs) (10), making it a significant contributor to food hygiene issues (11). *S. aureus* strains that produce structurally and functionally similar enterotoxins produce a superfamily of exotoxins known as staphylococcal enterotoxins (SEs), which lead to vomiting and diarrhea. 28 SEs have been discovered so far. (12). Foods commonly monitored in SFP include meat, milk, poultry, eggs, salads, and bakery products (13). The relationship between SFP and food varies by country due to different consumption habits (14). *S. aureus* contamination occurs through contaminated raw materials, improper food handling, and failures in maintaining the cold chain (15). Furthermore, it is essential to understand all the characteristics of *S. aureus*, given its ability to contaminate food and develop antibiotic resistance (16). This is due to its capability to get diverse resistance mechanisms versus antimicrobial agents such as methicillin (17). The specific *mecA* gene, encoding penicillin-binding protein 2a (PBP2a), is situated within the staphylococcal chromosomal cassette mec (SCCmec). PBP2a has a high affinity for beta-lactam antibiotics (18). MRSA can be detected in animal-derived foods, including pork, poultry, and meat, suggesting that food could act as a potential source of MRSA (19). Transmission of MRSA can occur not only through direct contact between humans and animals—where animals act as the natural reservoir for this bacterium—but also through the exposure to or consumption of contaminated food (20, 21). Recent reports of methicillin-resistant strains have become increasingly alarming due to the increasing number of new resistant isolates, highlighting the need to track and trace the resulting contamination to ensure food safety (22, 23). Identifying source of *S. aureus* in contaminated foods and designing preventive measures is one of the most important aspects of investigating food contamination with this bacterium. In foods of animal origin, it is crucial to determine the origin of *S. aureus*. *S. aureus* is capable of spreading in a variety of hosts. The existence of different biotypes of this organism, including bovine, ovine, poultry, and human biotypes, each with distinct and unique biochemical and microbiological characteristics, underscores the importance of identifying the source of contamination. Identifying the phenotypic and genotypic characteristics of different biotypes isolated from food samples provides a practical and reproducible method for tracing the source of contamination in the environment and food, as well as understanding the extent of similarities and differences. Biotyping *S. aureus* is essential for determining their origin, assessing public health significance, exploring the relationships among different strains, and analyzing their diversity within and between samples (6). Despite the importance of screening for these factors in public health, more data on MRSA in food samples is needed. This study investigated

the prevalence, antibiotic resistance, and enterotoxin genes of MRSA biotypes isolated from different types of nuggets and salads in Shahrekord City, Iran.

MATERIALS AND METHODS

Samples. Between August and November 2023, we carried out a comprehensive sampling project that involved collecting 363 samples of nuggets and salads from different retail locations and reputable restaurants in Shahrekord Province, Iran. The samples included chicken nuggets (n = 127), meat nuggets (n = 36), olivier salad (n = 65), season's salad (n = 70), and caesar salad (n = 65). After being collected, the samples were promptly transported in ice packs to Food Hygiene laboratory, Islamic Azad University branch in Shahrekord. Each food sample displayed standard physical traits, such as smell, color, and texture.

***S. aureus* isolation and identification.** In the first step, the collected samples were weighed using a sensitive laboratory balance in a completely sterile manner. In the second step, 20 g of each of the weighed samples was transferred to a previously prepared sterile plastic bag. In the third step, 230 ml of BPW, buffered peptone water (Sigma, USA), was added to the bag to bring the volume to 250 ml. In the fourth step, the resulting suspension was thoroughly mixed and homogenized for 120 seconds in a 400 W Stomacher high-power bag blender (Interscience, France). In the fifth step, 5 ml of enriched homogenate solution was transferred to 50 ml of TSB: Trypticase Soy Broth (Sigma, USA) (containing 1% sodium pyruvate and 10% NaCl). In the sixth step, following an 18-hour incubation period at 35°C, a circle of the culture medium was plated onto Baird-Parker agar that was supplemented with egg yolk tellurite emulsion (Merck, Germany) and incubated for approximately 18 hours at 37°C. In the final step, the presence of shiny black colonies with a clear halo, measuring 2 to 5 mm in diameter, was confirmed through laboratory techniques, including Gram staining, catalase and oxidase tests, and blood agar hemolytic activity (24).

MRSA identification. Susceptibility tests for cefoxitin (30 µg) and oxacillin (1 µg) were performed adhered to CLSI to distinguish MRSA strains (25). The identification of MRSA isolates was achieved through PCR of the *mecA*. Subsequently, these MRSA isolates transferred to TSB (Sigma, USA) then kept at 37°C for a period of 48 hours. Genomic DNA from the MRSA colonies extracted using DNA extraction kit (Yektatajhiz, Iran), according to the manufacturer's instruction. The PCR reaction was set up in a 20 µL volume, comprised of 10 µL of master mix (Yektatajhiz, Iran), 2 µL of extracted DNA, 0.5 µL of each primer (*mecA*-F: 5'-ACGAGTAGATGCTCAATATAA-3' and *mecA*-R: 5'-CTTAGTTCTTTAGCGATTGC-3') (NC_003923M), and 7 µL of dH2O. The PCR cycling protocol included 1 step initial denaturation at 94°C/6 min, 30 steps of 94°C/45 sec, 57°C/60 sec, and 72°C/45 sec, and 1 step final extension at 72°C/4 min.

MRSA biotyping. MRSA strains were biotyped by means of the improved version of the method described by Bruhn et al. (26). MRSA isolates were evaluated for β-hemolysin and staphylokinase production, their ability to grow on crystal violet agar, and coagulation of bovine plasma. To evaluate staphylokinase production, MRSA isolates were cultured on bovine fibrin plates enriched with plasminogen, sourced from dog serum. The formation of a transparent zone on these plates, which contain dog serum, serves as an indicator of staphylokinase production. Meanwhile, beta-hemolysin production was assessed by culturing the MRSA isolates on agar media with 5% sheep blood. β-hemolysin production was confirmed by the identification of broad, sharply demarcated areas of discoloration at 4°C. The coagulation process of bovine plasma was evaluated by introducing 0/1 ml of Heart Infusion broth (Sigma, USA) containing MRSA into tubes with 3-fold dilutions (1:3) of bovine plasma. After 6 hours, the medium was inspected for the presence of clots. The detection of large clots was regarded as a positive result. MRSA was cultured on crystal violet agar, and the development of three distinct types of spots was assessed: type A, yellow spots with blue edges; type C, blue or purple spots that may have an orange hue; and type E, white spots with blue edges.

MRSA phenotypic evaluation of antibiotic resistance. Disk diffusion method used to assess the antibiotic resistance pattern of MRSA in nuggets and salads. Mueller-Hinton agar culture medium was used to culture bacteria. CLSI 2021 guidelines were followed

to determine the antimicrobial resistance pattern. MRSA was cultured on Mueller Hinton Agar (Sigma, USA). To evaluate antibiotic resistance, several antibiotics were utilized, including penicillin; Pen (10 µg), tetracycline; Tet (10 µg), enrofloxacin; Enr (5 µg), ciprofloxacin; Cip (5 µg), trimethoprim; Tri (5 µg), cotrimoxazole; Cot (25 µg), cephalexin; Cep (30 µg), chloramphenicol; Chl (30 µg), nitrofurantoin; Nit (300 µg), gentamicin; Gen (10 µg), oxacillin; Oxa (5 µg), cephalothin; Cef (30 µg), and erythromycin; Ery (15 µg). Test plates were inoculated with *S. aureus* at a concentration corresponding to 0.5 McFarland standard and incubated with antibiotic discs at 37°C /24 h. Following incubation, the diameters of the zones of inhibition surrounding bacterial growth were measured and compared to CLSI standards, by using *S. aureus* ATCC 29213 as a control.

Genotypic evaluation of antibiotic resistance and distribution of virulence genes in MRSA. The PCR method was used to investigate the existence of antibiotic resistance and virulence genes in MRSA. PCR can accurately identify even a single antibiotic resistance gene from different antibiotic classes and investigate key pathogenic factors of staphylococcal infections. DNA was extracted by subculturing the MRSA onto TSB media (Sigma, USA) and incubating at 37°C/48 h. Then DNA was isolated from the MRSA using DNA extraction kit (Yektatajhiz, Iran). The purity of DNA was assessed using NanoDrop device (Thermo Scientific, Germany), and the quality was verified through electrophoresis on a 1% agarose gel. PCR was carried out by programmable thermo-cycler (Eppendorf, Germany). The products were run on a 1.5% horizontal agarose gel and stained using green viewer. The outcomes of PCR were assessed with the UVI Doc gel documentation system (Jencons PLC, UK). Table 1 provides details on the PCR conditions and primers used to amplify virulence, antibiotic resistance, and enterotoxin genes in MRSA strains obtained from different samples.

Statistical analysis. Statistical evaluations were performed using GraphPad Prism version 9. To assess the significance of relationships between the occurrence of MRSA, biotypes, and antibiotic resistance genes, the chi-square test and Fisher's exact two-tailed test were utilized. P-value <0.05 was considered as statistically significant.

RESULTS

***S. aureus* and MRSA prevalence.** This research investigated the occurrence of *S. aureus* across various food categories, including nuggets and salads, as shown in Table 2. One hundred sixty-seven of 363 samples (46%) were positive for *S. aureus.* Chicken nuggets had high prevalence of *S. aureus* (55.90%,71/127) among all studied samples. Caesar salad had the highest prevalence of *S. aureus* (41.54%, 27/65) among all analyzed salad samples. Seventy-eight isolates from 167 *S. aureus* (46.71%) were recognized as MRSA. MRSA prevalence in season's, olivier and caesar salad was 44.83, 47.82 and 37.03%, respectively. MRSA prevalence in chicken and meat nugget, was 47.89 and 58.82%, respectively. Caesar salad had the lowest prevalence of MRSA (37.03%). All of them harbored the *mecA*. A significant difference in the prevalence of *S. aureus* was observed between chicken nuggets and salads.

MRSA biotypes in different samples. The proportion of different biotypes found in MRSA samples from assorted nuggets and salads is shown in Table 3. Among the MRSA strains identified in nugget and salad samples, 10% were bovine-based, 0% ovine-based, 30% poultry-based, and 56% human-based, with 4% classified as unknown biotypes. These results indicate that infected humans can transmit MRSA to food.

MRSA phenotypical antibiotic resistance. Antibiotic resistance pattern findings indicate that all 336 isolates were resistant to cefotaxime and tetracycline, showing a 100% resistance rate (Table 4). Oxacillin resistance was found in 98.72% of the isolates, penicillin resistance in 75.64%, and gentamicin resistance in 55.12%. A study conducted in Kenya found that 71.4% of *S. aureus* were resistant to ampicillin. When the load of the infectious food reaches to approximately 5 Log CFU/g, it can be transmitted to humans, and its enterotoxins may result in serious illnesses. In the current study, frequency and pathogenic potential of the enterotoxigenic genes of *S. aureus* were investigated. Our results confirmed that MRSA isolates from nuggets and salads exhibit significant resistance to antibiotics such as penicillin, cefoxitin, tetracycline, and gentamicin, which is associated with a high prevalence of resistance genes, such as *mecA, aacA-D, tetK,* and *msrA*.

Table 1. Target genes, primers and PCR program

	***S. aureus* virulence gene primers**		
Target gene	**Sequence (5′-3′)**	**Product (bp)**	**Program**
coa	F: CGAGACCAAGATTCAACAAG	730	30 steps (94°C/2 min; 58°C/1
	R: AAAGAAAACCACTCACATCA		min; 72°C/1 min)
clfA	F: GGCTTCAGTGCTTGTAGG	980	35 steps (94°C/2 min; 57°C/1
	R: TTTTCAGGGTCAATATAAGC		min; 72°C/1 min)
X-region	F: CAAGCACCAAAAGAGGAA	320	30 steps (94°C/1 min; 60°C/1
	R: CACCAGGTTTAACGACAT		min; 72°C/1 min)
IgG binding region	F: CACCTGCTGCAAATGCTGCG	920	30 steps (94°C/2 min; 58°C/1
	R: GGCTTGTTGTTGTCTTCCTC		min; 72°C/1 min)
tsst-1	F: ATGGCAGCATCAGCTTGATA	350	30 steps (94°C/1 min; 55°C/1
	R: TTTCCAATAACCACCCGTTT		min; 72°C/1 min)
etA	F: CTAGTGCATTTGTTATTCAA	119	30 steps (94°C/30 s; 55°C/1
	R: TGCATTGACACCATAGTACT		min; 72°C/1 min)
etB	F: ACGGCTATATACATTCAATT	200	30 steps (94°C/30 s; 55°C/30
	R: TCCATCGATAATATACCTAA		s; 72°C/1 min)
agrI	F: ATGCACATGGTGCACATGC	441	26 steps (94°C/45 s; 55°C/45
	R: GTCACAAGTACTATAAGCTGCGAT		s; 72°C/1 min)
agrII	F: ATGCACATGGTGCACATGC	575	26 steps (94°C/1 min; 55°C/1
	R: TATTACTAATTGAAAAGTGGCCATAGC		min; 72°C/1 min)
agrIII	F: ATGCACATGGTGCACATGC	323	26 steps (94°C/45 s; 55°C/45
	R: GTAATGTAATAGCTTGTATAATAATACCCAG		s; 72°C/1 min)
PVL	F: ATCATTAGGTAAAATGTCTGGACATGATCCA	433	25 steps (94°C/45 s; 50°C/1
	R: GCATCAAGTGTATTGGATAGCAAAAGC		min; 72°C/1 min)
	***S. aureus* antibiotic resistance gene primers**		
mecA	F: AAAATCGATGGTAAAGGTTGGC	532	30 steps (94°C/1 min; 57°C/1
	R: AGTTCTGCAGTACCGGATTTGC		min; 72°C/1 min)
ermA	F: AAGCGGTAAACCCCTCTGA	190	30 steps (94°C/30 s; 54°C/30
	R: TTCGCAAATCCCTTCTCAAC		s; 72°C/30 s)
ermC	F: AATCGTCAATTCCTGCATGT	299	30 steps (94°C/45 s; 55°C/45
	R: TAATCGTGGAATACGGGTTTG		s; 72°C/45 s)
msrB	F: TATGATATCCATAATAATTATCCAATC	595	30 steps (94°C/1 min; 56°C/1
	R: AAGTTATATCATGAATAGATTGTCCTGTT		min; 72°C/1 min)
linA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG	323	28 steps (94°C/45 s; 58°C/45
	R: GCTTCTTTTGAAATACATGGTATTTTTCGA		s; 72°C/45 s)
aacA-D	F: TAATCCAAGAGCAATAAGGGC	227	30 steps (94°C/30 s; 60°C/30
	R: GCCACACTATCATAACCACTA		s; 72°C/30 s)
tetK	F: GTAGCGACAATAGGTAATAGT	360	30 steps (94°C/45 s; 59°C/45
	R: GTAGTGACAATAAACCTCCTA		s; 72°C/45 s)
tetM	F: AGTGGAGCGATTACAGAA	158	30 steps (94°C/30 s; 55°C/30
	R: CATATGTCCTGGCGTGTCTA		s; 72°C/30 s)
msrA	F: GGCACAATAAGAGTGTTTAAAGG	940	25 steps (94°C/2 min; 54°C/1
	R: AAGTTATATCATGAATAGATTGTCCTGTT		min; 72°C/1 min)
vatA	F: TGGTCCCGGAACAACATTTAT	268	30 times (94°C/30 s; 55°C/30
	R: TCCACCGACAATAGAATAGGG		s; 72°C/30 s)
vatB	F: GCTGCGAATTCAGTTGTTACA	136	30 steps (94°C/30 s; 56°C/30
	R: CTGACCAATCCCACCATTTTA		s; 72°C/45 s)
vatC	F: AAGGCCCCAATCCAGAAGAA	323	30 steps (94°C/1 min; 58°C/1
	R: TCAACGTTCTTTGTCACAACC		min; 72°C/1 min)

Table 1. Continuing...

	***S. aureus* enterotoxin genes primers**		
sea	F: ACGATCAATTTTTACAGC R: TGCATGTTTTCAGAGTTAATC	544	30 steps (94°C/1 min; 58°C/1 min; 72°C/1 min)
seb	F: GAATGATATTAATTCGCATC R: TCTTTGTCGTAAGATAAACTTC	416	30 steps (94°C/1 min; 56°C/1 min; 72°C/1 min)
sec	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCCA	257	30 steps (94°C/30 s; 55°C/30 s; 72°C/30 s)
sed	F: TTACTAGTTTGGTAATATCTCCTT R: CCACCATAACAATTAATGC	334	30 steps (94°C/45 s; 58°C/45 s; 72°C/1 min)

Table 2. *S. aureus* and MRSA prevalence

Food Category		**Number of samples**	**% of *S. aureus***	**% of MRSA**
Nuggets	Chicken	127	55.90% (71/127)	47.89% (34/71)
	Meat	36	47.22% (17/36)	58.82% (10/17)
Salads	Olivier	65	35.38% (23/65)	47.82% (11/23)
	Season's	70	41.43% (29/70)	44.83% (13/29)
	Caesar	65	41.54% (27/65)	37.03% (10/27)
Total		363	46% (167/363)	46.71% (78/167)

Table 3. Prevalence of different biotypes MRSA

N of MRSA		**N and % of positive samples**				
		Bovine	**Ovine**	**Poultry**	**Human**	**Unknown**
Nugget	Chicken (10)	-	-	8 (80%)	2 (20%)	-
	Meat (10)	5 (50%)	-	-	4 (40%)	1 (10%)
Salad	Olivier (10)	-	-	4 (40%)	6 (60%)	-
	Season's (10)	-	-	-	9 (90%)	1 (10)
	Caesar (10)	-	-	3 (30%)	7 (70%)	-
Total	50	5 (10%)	0 (0%)	15 (30%)	28 (56%)	2 (4%)

Table 4. Antibiotic resistance pattern of MRSA

Samples		**Resistance to antibiotics (n)**												
Nugget		**Nit**	**Cot**	**Gen**	**Ery**	**Tet**	**Cip**	**Enr**	**Oxa**	**Pen**	**Cep**	**Chl**	**Tri**	**Cef**
	Chicken (n=34)	2	19	22	18	34	14	15	33	27	16	3	14	34
Salad	Meat (n=10)	1	6	4	3	10	5	5	10	7	5	1	4	10
	Olivier (n=11)	2	5	5	4	11	4	4	11	8	4	1	4	11
	Season's (n=13)	1	5	7	6	13	6	7	13	10	6	2	5	13
Total	Caesar (n=10)	1	5	5	3	10	4	4	10	7	4	1	4	10
	78	7	40	43	34	78	33	35	77	59	35	8	31	78
		(8.97%)	(51.28%)	(55.12%)	(43.60%)	(100%)	(42.30%)	(44.87%)	(98.72%)	(75.64%)	(44.87%)	(10.26%)	(39.74%)	(100%)

Table 5. Antibiotic resistance, virulence and enterotoxins genotypic pattern of MRSA

Origins (N of MRSA strains)		Antibiotic resistance genes (n)											
		aacA-D	***tetK***	***tetM***	***msrA***	***msrB***	***ermA***	***ermC***	***vatA***	***vatB***	***vatC***	***LinA***	***mecA***
Nugget	Chicken (n=10)	8 (80%)	10 (100%)	4 (40%)	10 (100%)	8 (80%)	2 (20%)	5 (50%)	2 (20%)	–	2 (20%)	7 (70%)	9 (90%)
Salad	Meat (n=10)	9 (90%)	10 (100%)	4 (40%)	10 (100%)	5 (50%)	1 (10%)	3 (30%)	1 (10%)	–	1 (10%)	6 (60%)	10 (100%)
	Olivier (n=10)	8 (80%)	10 (100%)	5 (50%)	10 (100%)	5 (50%)	1 (10%)	4 (40%)	1 (10%)	–	1 (10%)	7 (70%)	10 (100%)
	Season's (n=10)	10 (100%)	10 (100%)	6 (60%)	10 (100%)	7 (70%)	1 (10%)	2 (20%)	–	–	1 (10%)	8 (80%)	8 (80%)
	Caesar (n=10)	9 (90%)	10 (100%)	4 (40%)	10 (100%)	5 (50%)	–	3 (30%)	–	–	1 (10%)	5 (50%)	10 (100%)
Total	50	44 (88%)	50 (100%)	23 (46%)	50 (100%)	30 (60%)	5 (10%)	17 (34%)	4 (8%)	0 (0%)	6 (12%)	33 (66%)	47 (94%)

Origins (N of MRSA strains)		Virulence genes (n)										
		PVL	***coa***	***clfA***	**X-region**	**IgG binding region**	***tsst-1***	***etA***	***etB***	***agrI***	***agrII***	***agrIII***
Nugget	Chicken (n=10)	10 (100%)	10 (100%)	4 (40%)	6 (60%)	6 (60%)	1 (10%)	2 (20%)	1 (10%)	3 (30%)	2 (20%)	6 (60%)
Salad	Meat (n=10)	10 (100%)	10 (100%)	4 (40%)	7 (70%)	5 (50%)	1 (10%)	3 (30%)	1 (10%)	2 (20%)	1 (10%)	6 (60%)
	Olivier (n=10)	10 (100%)	10 (100%)	5 (50%)	4 (40%)	5 (50%)	1 (10%)	4 (40%)	1 (10%)	3 (30%)	1 (10%)	7 (70%)
	Season's (n=10)	10 (100%)	10 (100%)	5 (50%)	6 (60%)	6 (60%)	1 (10%)	2 (20%)	1 (10%)	1 (10%)	1 (10%)	7 (70%)
	Caesar (n=10)	9 (90%)	10 (100%)	5 (50%)	6 (60%)	5 (50%)	1 (10%)	3 (30%)	–	1 (10%)	1 (10%)	4 (40%)
Total	50	49 (98%)	50 (100%)	23 (46%)	29 (58%)	27 (54%)	5 (10%)	14 (28%)	4 (8%)	10 (20%)	6 (12%)	30 (60%)

Origins (N of MRSA strains)		Enterotoxin genes (n)			
		Sea	***seb***	***sec***	***sed***
Nugget	Chicken (n=10)	7 (70%)	6 (60%)	3 (30%)	4 (40%)
Salad	Meat (n=10)	8 (80%)	7 (70%)	3 (30%)	2 (20%)
	Olivier (n=10)	8 (80%)	6 (60%)	4 (40%)	4 (40%)
	Season's (n=10)	7 (70%)	6 (60%)	3 (30%)	3 (30%)
	Caesar (n=10)	7 (70%)	6 (60%)	5 (50%)	3 (30%)
Total	50	37 (74%)	31 (62%)	18 (36%)	16 (32%)

MRSA genotypical antibiotic resistance, virulence, and enterotoxin genes. Table 5 presents the genotypic antibiotic resistance patterns found in MRSA strains isolated from nuggets and salads, as illustrated in Fig. 1a. The analysis shows that multiple antimicrobial resistance genes, including *linA*, *tetK* and *M*, *msrA*, *vatA* and *B*, *vatC*, *ermA* and *C*, *mecA*, *msrB*, and *aacA-D*, are present among these MRSA strains. Notably, there is a high distribution of specific resistance genes: *tetK* (100%), *msrA* (100%), *mecA* (94%), *aacA-D* (88%), *linA* (66%), and *msrB* (60%), as shown in Fig. 1a. In reverse, the prevalence of *vatB* (0%), *vatA* (8%), *ermA* (10%), and *vatC* (12%) were lower. Table 5 presents the phenotypic antibiotic resistance patterns in MRSA. Table 5 presents the distribution of virulent genes in MRSA. The most detected virulence genes in MRSA were *COA* (100%), *PVL* (98%), and *agrIII* (60%), as illustrated in Fig. 1b. The total prevalence of *etB*, *tsst-1*, and *agrII* among MRSA were 8%, 10%, and 12%, respectively. Table 5 describes the distribution of enterotoxins among MRSA isolated from nuggets, and salads. *sea* (74%) and *seb* (62%) were the most detected enterotoxins among the MRSA isolated from nuggets and salads, as shown in Fig. 1c.

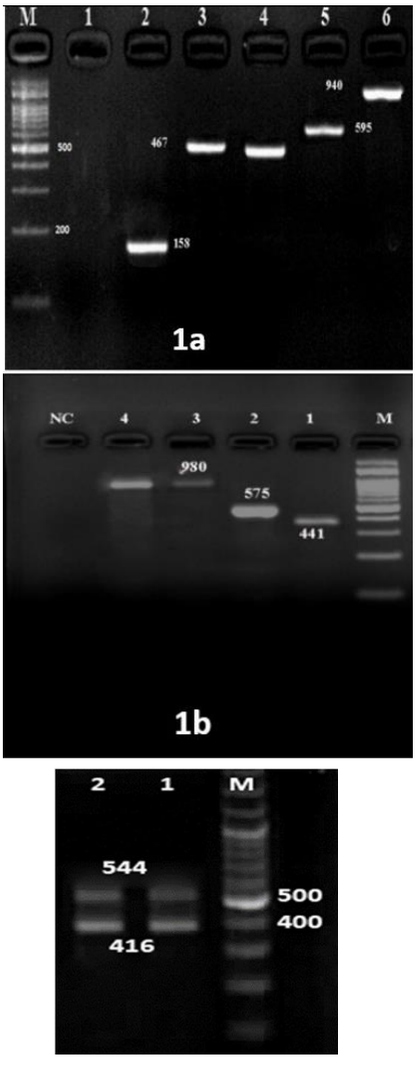

Fig. 1. a. The gel obtained from electrophoresis of the PCR product related to the detection of *msrA* (940 bp), *msrB* (595 bp), *vatC* (467 bp) and *tetM* (158 bp) genes in *Staphylococcus* isolates (M: 100 bp DNA marker, columns 1: negative control (NC), 2-6: studied isolates) b. The gel obtained from electrophoresis of the PCR product related to the detection of *clfA* (980 bp), *agrI* (441 bp) and *agrII* (575 bp) genes in *Staphylococcus* isolates (M:100 bp DNA marker, 1-4: studied isolates, NC: negative control). c. The gel obtained from the electrophoresis of the PCR product related to the detection of the *sea* (544 bp) and *seb* (416 bp) genes in *Staphylococcus* isolates (M: 100 bp DNA marker, 1 and 2: studied isolates)

DISCUSSION

This research investigated the occurrence of *S. aureus* across various food categories, including nuggets and salads. One hundred sixty-seven of 363 samples (46%) were positive for *S. aureus.* To better understand the patterns of antibiotic resistance and its transmission, it is essential to distinguish between the various methods of detection (27). Foods can transmit antibiotic-resistant bacteria to humans. Nuggets and salads are highly consumed and important staple foods worldwide. Antibiotic-resistant strains of *S. aureus* are frequently found in these items (28). The objective was to evaluate the biotyping, phenotypic, and genotypic features related to antibiotic resistance and virulence factors found in MRSA. MRSA prevalence was found to be 50% (44/88) in nuggets and 43.04% (34/79) in salads. Notably, this research marks the first time MRSA has been isolated from these food items. Contamination of various foods with *S. aureus* can be caused by animals producing milk and meat, and poor hygiene in production, sale, and storage cycle. Moreover, humans can also be the carriers of these microorganisms and transmit contamination to foods during processing. This research provides the initial account of the biotype and antibiotic resistance in MRSA obtained from different categories, such as nuggets and salads. The findings revealed that the prevalence of *S. aureus* in the differ-

ent food samples examined was 46%. This rate is significantly higher than those reported in Spain (6.1%) (29) and Portugal (11.1%) (30, 31). The origins of *S. aureus* taken from food can effectively be traced using biotyping. By identifying the various biotypes of *S. aureus*, we are able to pinpoint specific pathways of food contamination and analyze the similarities and differences in the microbiological and epidemiological aspects of these strains. Understanding these origins and their implications for public health is essential, and biotyping plays a key role in this analysis. Furthermore, it enables the exploration of the relationships between different strains and helps assess their diversity across samples. In our research, we observed that a majority of MRSA was isolated from samples originated from humans. Among the MRSA strains identified in nugget and salad samples, 10% were bovine-based, 0% ovine-based, 30% poultry-based, and 56% human-based, with 4% classified as unknown biotypes. Previous research has shown that food handlers can transmit MRSA strains into food items (32, 33). A study by Kitai (34) supports the high prevalence of poultry biotypes, showing that poultry-based strains accounted for 80% of *S. aureus* found in food products. In comparison, human-based biotypes accounted for 20%. In another report on *S. aureus* isolates from Italian food, the breakdown of biotypes was as follows: human (50.40%), ovine (23.20%), not-host-specific (17.60%), bovine (7.20%), and poultry-based (1.60%) (35). This is the first study examining the presence of 27 genes associated with the pathogenicity of *S. aureus* in food samples, Shahrekord, Iran. Some MRSA isolates demonstrated resistance to multiple drugs. Antibiotics excessive and unplanned use can lead to rapid spread of antibiotic-resistant genes. Our findings suggest that certain strains of MRSA isolated from salads show a higher prevalence of resistance compared to the antibiotics commonly used to treat clinical infections. This situation may be associated with transmission from infected restaurant staff or workers. Furthermore, the significant resistance levels seen in MRSA found in chicken nuggets could be attributed to contamination from animal manure or the water used during the production of the nuggets (36, 37). Previous studies have concurrently evaluated both phenotypic and genotypic antibiotic resistance in MRSA. For example, Wu (38) reported that the resistance against antibiotic in *S. aureus* was significantly high across a broad range of antibiotics. Similarly, González (39) documented elevated resistance rates of *S. aureus* in salads from Greece against ampicillin, clindamycin, and penicillin, with resistance rates of 94%, 89.40%, and 82.60%, respectively. Our results are consistent with earlier studies from Iran (40) and Korea (41), which revealed high levels of resistance in *S. aureus* from various sources against antibiotics such as cefoxitin, penicillin, tetracycline, gentamicin, and erythromycin.

The MRSA recovered from nugget and salads had not been previously tested for antibiotic-resistance genes. Our results revealed higher phenotypic resistance profile prevalence than the genotypic pattern. There are various mechanisms by which bacteria can spread antibiotic resistance. These include: 1. Excretion of antibiotics through active efflux pumps, 2. Reduction of the bacteria's selective permeability to antibiotics, 3. Inhibition of antibiotics via hydrolysis or significant structural changes, 4. Alteration of the antibiotic's target site, 5. Activation of secondary metabolic pathways, 6. Genetic mutations. MRSA showed the highest rates of resistance to cefotaxime (100%), tetracycline (100%), oxacillin (98.72%), penicillin (75.64%), and gentamicin (55.12%). MRSA isolated from salads showed a higher antibiotic resistance rate than MRSA isolated from nuggets. MRSA prevalence was found to be 36%, with 32% of the isolates expressing the *sec* and *sed* genes. The study by Safarpoor highlighted a significant prevalence of the genes *tetK*, *msrA*, *vatA*, *aacA-D*, and *linA* among MRSA isolates from hospital food, with rates of 62.16%, 72.97%, 27.02%, 64.86%, and 43.24%, respectively. In a similar context, Rahi confirmed a striking prevalence of the genes *ermB*, *msrA*, *gyrA*, *cfr*, *aacA-D*, and *rpoB* among MRSA isolates from raw milk specimens, with rates of 100%, 67.85%, 50%, 25%, 35.71%, 10.71%, and 28.75%, respectively (42).

CONCLUSION

Among the samples examined, particularly those of salads, a notably high prevalence of MRSA was observed, as indicated by the findings. In Tanzania, herbal product samples were found to contain antibiotic-resistant bacteria. The growing prevalence of MRSA, along with the significant presence of antibiotic resistance genes and multidrug resistance results in critical public health issues related to the

consumption of salads and nuggets. The rising adoption of antibiotics in both human and veterinary medicine, along with the movement of antibiotic resistance genes via mobile genetic elements, clarifies the reasons behind the increasing occurrence of resistant bacteria today. It is crucial to address the alarming levels of antimicrobial resistance in MRSA highlighted in this research. Implementing a controlled approach to the use of antimicrobials could be instrumental in preventing further development of drug resistance, particularly given the high incidence of MRSA in samples associated with resistance to various antibiotic classes. This study tracked antibiotic resistance and virulence genes, marking it as first of its kind to evaluate these characteristics and biotypes among MRSA isolated from nuggets and salads. The MRSA detected in salads displayed elevated levels of antibiotic resistance, virulence, and the presence of enterotoxin genes. The coexistence of virulence and antibiotic resistance in MRSA represents a significant threat that must not be overlooked. The identification of MRSA in chicken nuggets and salad samples highlights the risks associated with consuming these foods, as they may serve as vectors for transmitting both antibiotic-resistant and pathogenic MRSA. Resistance prevalence to commonly used human antibiotic highlights the potential origins of MRSA isolates. In Iran, foodborne illnesses caused by *S. aureus* are inadequately treated with cefoxitin, penicillin, tetracycline, or gentamicin, underscoring the urgent need for improved treatment strategies. Our study identifies chicken nuggets and salads as significant sources of virulent and resistant MRSA. Analysis of Panton-Valentine leukocidin (PVL) distribution reveals that the majority of MRSA isolated from chicken nuggets are classified as healthcare-associated MRSA (HA-MRSA). At the same time, most MRSA found in salads fall under the community-associated MRSA (CA-MRSA) category.

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