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RESEARCH ARTICLE OPEN ACCESS

The Prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* in Processed Food Samples in Riyadh, Saudi Arabia

Mashael J. Alghizzi[1], Maysoon Alansari[2] and Ashwag Shami[1]*

[1]Department of Biology Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.
[2]Department of Biology Science, King Saud University, Riyadh, Saudi Arabia.

Abstract

***Staphylococcus aureus* mainly Methicillin Resistant *Staphylococcus aureus*(MRSA) is a life-threatening infection that occurring in food and caused a public health concern. This study designed to examine the prevalence of *S. aureus* and MRSA in different types of processed food. Food samples were screened for the recovered strains of *S. aureus* and MRSA, and they were examined for antimicrobial susceptibility and by molecular characterization of *mecA* and staphylococcal cassette chromosome mec(*SCCmec*). Detection of virulence factors like Panton-Valentine Leukocidin (*PVL*), *Staphylococcus aureus* protein A(*spa*) and Staphylococcal enterotoxins(SEs) by PCR using specific primers. Among the 150 collected processed food samples, 62.7% were contaminated by *S. aureus* bacteria, 56.4% of which were proved as MRSA. 17% of MRSA isolates were positive for *mecA* genes with the *SCCmec* type IVb and V (11.1% each) as the solely existing types of *SCCmec*. None of the MRSA isolates carried *mecC* or *mecB* genes. Most of MRSA isolates were multidrug resistance and 33.3% of MRSA-*mecA* positive isolates also carried vancomycin resistance genes (i.e., *vanB*). In addition, *spa* gene was found among 7.5% of MRSA isolates; none of which were positive for *PVL* gene. Further, there were variant presence of SEs among MRSA isolates and the highest presence was from type SEH (49.1%). Generally, our results confirmed that processed foods in Saudi Arabia (Riyadh) are potential vehicles for multidrug resistant *S. aureus* and MRSA transmission; which are serious public health risks, and underlined the need for good hygiene practices.**

Keywords: MRSA, processed foods, multidrug resistance, *mecA*, CA-MRSA, LA-MRSA

***Correspondence:** ayshami@pnu.edu.sa

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INTRODUCTION

Staphylococcus aureus is a main source for food poisoning globally. In addition, it is a significant reason for human infection and it can be a reason for lethal diseases such as pneumonia, endocarditis, toxic shock syndrome, and sepsis[1]. Additionally, high levels of antimicrobial resistance to these bacteria pose a serious public health threat. Methicillin-Resistant *Staphylococcus aureus* (MRSA) forms around 13-74% of *S. aureus's* infections[2]. The acquisition of *mecA* gen via the mobile staphylococcal cassette chromosome *mec* (SCC*mec*) in MRSA strains is the reason for the emergence of resistance to all b-lactam antibiotics[3] . MRSA was classified to the three groups, due to the differences in the origin of the outbreaks, healthcare-associated MRSA (HA-MRSA) , community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA)[4]. Epidemiological reports have declared variance among HA-MRSA, CA-MRSA, and LA-MRSA strains counting antimicrobial resistance profiles and SCC*mec* types. For instance, *SCCmec* types IV and V are detected among CA-MRSA strains, whereas *SCCmec* types I, II, and III are detected among HA-MRSA strains and the most common types between them is *SCCmec* V and *SCCmec* IV, respectively[5]. In addition, *SCCmec* XI type is discovered newly in humans and cattle, especially from mastitis in cow in UK and Denmark and it carries *mecC* gene, which initially knew as *mecA* LGA251[6,7]. Furthermore, *PVL* gene is a genetic marker for the MRSA presence was recognized for the first time in 2003 and it produces strains that may cause skin infection or serious diseases such as pneumonia. However, *PVL* gene can be predominantly seen in CA-MRSA more than in HA-MRSA and it is found in around 60% to 100% of CA-MRSA strains. Consequently, *PVL* develops the pathogenicity of CA-MRSA strains and the prevalence of *PVL* between strains is depending on bacteriophages[8-10]. Nonetheless, specific types of symptoms such as red and swollen skin frequently occur with MRSA infection, which is painful and filled with pus bump fever.

With regard to MRSA's types, livestock is considered as a fertile source of staphylococci species generally and *S. aureus* especially, causing livestock-associated MRSA [LA-MRSA]. In addition, LA-MRSA can be occurred by direct contact with animals or utilization of animal products e.g., lamb, calve, and goat. Yet, the spread rate of *S. aureus* was lower in goat and calve (12.5% and 1.4%, respectively) than it in lamb (29.7%). LA-MRSA was discovered firstly in France and in the Netherlands then it was found in the pig population in countless countries of Europe. Further, as CA-MRSA and HA-MRSA, LA-MRSA clones are different between countries; e.g., clonal complex 398 (CC398) was detected in Europe and USA, whereas clonal complex 9 (CC9) was found in Asia[3,6,11].

However, MRSA infections spread vigorously throughout Saudi Arabia and reached 50% during 2011 in King Fahad Medical City in Riyadh city[12]. The number of studies about MRSA infection in Saudi Arabia is insufficient compared to the other countries around the world; although, MRSA in Saudi Arabia has been discovered in 1990s[13]. In spite of focusing on investigating and discussing HA-MRSA mainly, there is still a shortage in the number of studies on HA-MRSA in children and surprisingly it is found in males more than females[13]. Based on the prevalence of CA-MRSA disease, the quantity of patients significantly raised in Saudi Arabia from 9.9 per 10,000 people in 2001 to 67 per 10,000 people in 2008[13,14]. Furthermore, during the time between 2000 to 2008, the spread rate of CA-MRSA along Eastern region of Riyadh city increased by six times[12]. Also, CA-MRSA samples collected from Qatif city, Saudi Arabia showed that CA-MRSA cases increased gradually from 23% in 2006 to 60% in 2015. In terms of LA-MRSA infections, there is still a lack of studies that investigate this type of MRSA[15,16]. The purpose of this study was to investigate the prevalence of two types of MRSA: CA-MRSA and LA-MRS in processed foods collected from the capital city of Saudi Arabia and their antibiotic resistance and molecular characteristics of each isolate based on the identification of *MecA* gene, *SCCmec* types, *PVL, spa* gene, and SEs by PCR technique.

MATERIALS AND METHODS

Sample collection

A total of 150 samples of processed foods were collected during a period of August 2019 to March 2020 from different farms and local retail markets throughout Riyadh city, Kingdom of Saudi

Arabia, which are namely: A, B, C, D, E, F, G, H, and I. Samples were smoked Turkey (N=50), sausages (N=50), and salami (N=50).

Bacterial Growth Conditions

After cutting processed foods samples into small pieces by sterilized scalpel and forceps, 0.5g of each were added to approximately 5ml of specific liquid nutrient medium (i.e., brain heart infusion [BHI]) that were later incubated for 24 hours at 37°C in order to cultivate the bacteria.

Isolation and identification of *S. aureus*

In order to examine *S. aureus* colonies phenotypically, a loop of each cultivated sample was streaked on the Mannitol salt agar (MSA) -as a selective media to differentiate and isolate such bacteria- which were incubated for overnight at 37°C. As a confirmation stage, DNAs test was also performed for all *S. aureus* isolates. To detect the growth of MRSA isolates, we assessed the performance of MRSA chromagar as compared to that of mannitol salt agar supplemented with oxacillin (MSAO) for the recovery of MRSA from food specimens.

Antimicrobial susceptibility testing

All MRSA isolates were screened for antimicrobial resistance using disk diffusion method for the following antibiotics classes; β-lactams (penicillin, ampicillin, and oxacillin), macrolides (erythromycin), aminoglycosides (gentamicin), tetracyclines (oxytetracycline), sulfonamides (trimethoprim-sulphamethoxazole), and glycopeptides (vancomycin), were examined.

DNA extraction

Two colonies on MRSA chromagar plates were picked and used to be inoculated in 5 ml of BHI and grown overnight at 37°C. DNA was isolated and purified using a QIAamp DNA mini kit according to the manufacturers' instructions.

Detection of *mecA, mec*B , *mec*C and *SCCmec* typing of the *mecA* positive MRSA isolates

mecA, *mecB,* and *mecC* (also named *mec*ALGA251) were identified by PCR using primers defined previously[17,18] The *mecA*-positive MRSA isolates were also illustrated by *SCCmec* type using PCR using primers defined previously[19].

Virulence factors of *Staphylococcus aureus* isolates

The incidence of virulence genes 14 enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, sen, seo, sem, sek, sel*), Panton-Valentine Leukocidin (*PVL*), S*taphylococcus aureus* protein A (*spa*) and vancomycin resistance genes *vanA* and *vanB* were examined by PCR using primers defined previously[20-26].

RESULTS

Prevalence of *S. aureus* and MRSA infection in food

From 150 food samples 94 samples (62.7%) were contaminated by *S. aureus* bacteria. The contaminated samples were from turkey (27.7%), salami (42.6%), and sausages (29.8%). Despite the fact that *S. aureus* bacteria were detected in raw sausages samples, two samples were cooked for two different period of times (5 and 10 minutes) and they were still positive for *S. aureus* and MRSA infection as well. From the total of the 94 *S. aureus* isolates from different types of processed food, 53 (56.4%) isolates were confirmed as MRSA. Of the 53 MRSA isolates, 3/53 (5.7%) were from Turkey, 19/53(35.8%) were from Salami, and 31/53 (58.5%) were from Sausages (Table 1). In addition, *mecA, mecB* and *mecC* gene were examined in all subjected methicillin resistant *S. aureus* (MRSA) isolates. The result indicated that 17% of MRSA isolates (9/53) were positive for

Table 1. The prevalence of *S. aureus* and MRSA among the examined samples in Riyadh, Saudi Arabia

Types of samples	No of samples positive for *S. aureus*	N/P (%)	No of samples positive for MRSA	N/P (%)
Turkey	26	26/94 (27.7%)	3	3/53 (5.7%)
Salami	40	40/94 (42.6%)	19	19/53 (35.8%)
Sausages	28	28/94 (29.8%)	31	31/53 (58.5%)

N is the total Number of samples and P is the total number of positive samples

mecA genes, while none of them were positive for *mecC* or *mecB* genes.

Antibiotic resistance profiles of MRSA isolates

A total of 8 antimicrobial agents were examined for antimicrobial susceptibility in all MRSA isolates. Hence, the percentages of the bacterial resistance for each one of these antibiotics from all kinds of the examined samples are: 30.2% (16/53) to penicillin, 20.8% (11/53) to ampicillin, 86.8% (46/53) to oxacillin, 34% (18/53) to erythromycin, 9.4% (5/53) to gentamicin, 24.5% (13/53) to oxytetracycline, 47.2% (25/53) to trimethoprim-sulphamethoxazole, and 22.6% (12/53) to vancomycin. As regards to vancomycin resistance genes (i.e., *vanA* and *vanB*), vancomycin resistant isolates were tested and found that 33.3% (4/12) of them carried *vanB* gene but none of them were carried *vanA* gene and their origins were from salami (2), and sausages (2). Noticeably, oxacillin was the most commonly detected antibiotic among sausage samples, while trimethoprim-sulphamethoxazole resistance was detected at lower frequencies among turkey samples in MRSA isolates in comparison with other types of food isolates (i.e., salami origin) (Fig. 1 and Table 2). However, most of MRSA isolates were resistant to more than three antibiotics. For instances, 13.2% (7/53) of such isolates were resistant to 5-7 antibiotics (five from salami, and two from sausages), 43.4% (23/53) of them were resistant to 2-4 antibiotics (16 from sausages, six from salami, and one from turkey origin) and 5.7% (3/53) of them were resistant to all eight antibiotics (one from salami, and two from sausages). On the other hand, 64.2% (34/53) of the isolates were sensitive to 5-7 antibiotics, 11.3% (6/53) of them were sensitive to 2-4 antibiotics, and 7.5% (4/53) of them were sensitive to the all eight antibiotics.

Molecular typing of MRSA isolates

The positive *mecA* isolates were exposed

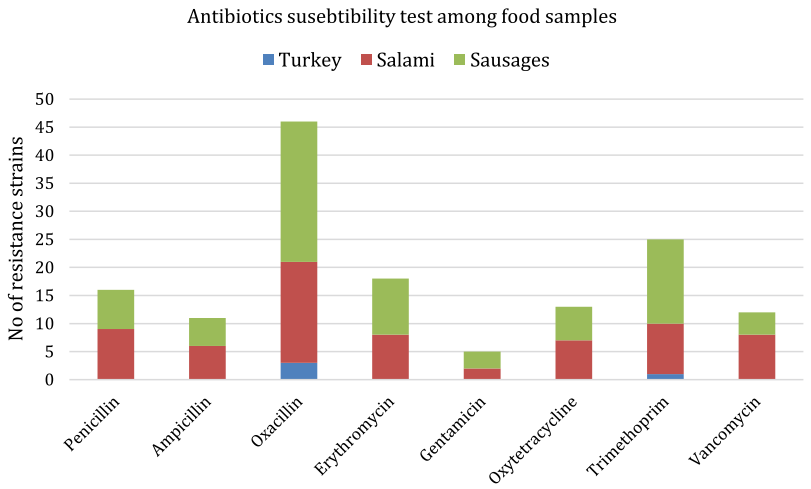

Fig. 1. Antibiotics susceptibility test among food samples collected from Riyadh, Saudi Arabia.

Table 2. Antibiotics susceptibility test among food samples collected from Riyadh, Saudi Arabia

Antibiotic Classes	Antibiotics	Turkey	Salami	Sausages	N/P (%)
β-lactams	penicillin	0	9	7	30.2
	ampicillin	0	6	5	20.8
	oxacillin	3	18	25	86.8
Macrolides	erythromycin	0	8	10	34
Aminoglycosides	gentamicin	0	2	3	9.4
Tetracyclines	oxytetracycline	0	7	6	24.5
Sulfonamides	trimethoprim	1	9	15	47.2
Glycopeptides	vancomycin	0	8	4	22.6

to identify five different types of *SCCmec*: type I, II, III, IV, and V. consequently, *SCCmec* types between seven out of nine isolates that carried *mecA* gene (from salami and sausages origins) could not be detected, whereas only two isolates had a single type which are *SCCmec* type V and IVb and they were from sausages origin, Table 3.

Distribution of virulence genes

In the tested MRSA samples (53 isolates), *Staphylococcus aureus* protein A (*spa*) gene was found between 4 out of 53 (7.5%) and none of them were positive to the Panton Valentine leukocidin (*PVL*) gene. Yet, a total of 53 methicillin resistant *Staphylococcus aureus* isolates were examined for the existence of virulence genes. Of the 14 investigated enterotoxin genes SE (i.e., Staphylococcal enterotoxin A [SEA], B [SEB], C [SEC], D [SED], E [SEE], G[18], H [SEH], I [SEI], J [SEJ], N [SEN], O [SEO], M [SEM], K [SEK], and L[SEL] [27]), there were variant presence of these types among such isolates. With regard to the presence of 14 investigated enterotoxin genes between examined samples, the highest proportion was from SEH (49.1%) followed by SEM (11.3%), SEA and SEN (7.5% each), SEO and SEK (5.7% each), SEE (3.8%), and then SEB, SEG, and SEI (1.9% each). Whilst, none of SEC, SED, SEJ, and SEL were existed in any of MRSA isolates (Fig. 2). Overall, MRSA isolates that were derived from turkey samples did not carry any of the 14 investigated enterotoxin genes. Staphylococcal enterotoxin (SEA, SEB, SEC, SED, and SEE) and their origins were from salami (4/6 [66.7%]) and sausages (2/6 [33.3%]) samples. The majority of classic staphylococcal enterotoxins

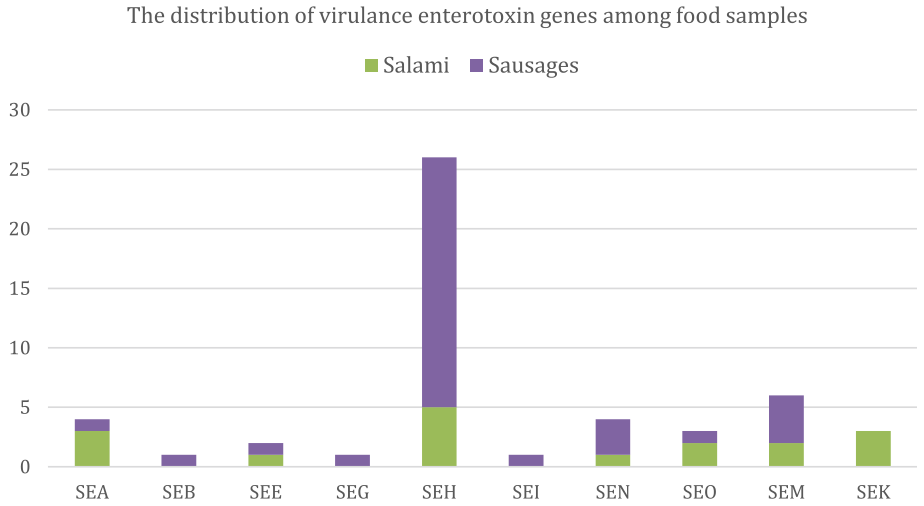

Fig. 2. The distribution of enterotoxin genes among food samples collected from Riyadh, Saudi Arabia.

Table 3. The discovery of *mecA* gene (147bp), *SCCmec* types II, III, IVa,IVb, IVd, and V among food smples in Riyadh, Saudi Arabia

Samples types	Strains ID	*MecA* gene	*SCCmec* II	*SCCmec* III	*SCCmec* IV a	*SCCmec* IV b	*SCCmec* IV d	*SCCmec* V
Salami	56	+	-	-	-	-	-	-
	57	+	-	-	-	-	-	-
	61	+	-	-	-	-	-	-
	63	+	-	-	-	-	-	-
	73	+	-	-	-	-	-	-
Sausages	86	+	-	-	-	+	-	-
	87	+	-	-	-	-	-	-
	88	+	-	-	-	-	-	-
	97	+	-	-	-	-	-	+

SCCmec= staphylococcal cassette chromosome mec

(i.e., SEA, SEB, SEC, SED, and SEE) were between salami samples. Moreover, 6 of 53 MRSA isolates (11.3%) harbored the genes of the enterotoxin gene cluster (egc) (SEG, SEI, SEN, SEO, and SEM) and their origins were from salami and sausages but the highest presence of egc was from sausages samples (i.e., 66.7%). Moreover, some described combinations of the virulence genes of *S. aureus* were spotted. The SEC-SEL gene combination, typical of the SaPIbov pathogenicity island, was not detected in any of the isolates, as shown in Fig. 2 and Table 4.

DISCUSSION

Because of CA-MRSA is reported to be less serious outcomes than HA-MRSA, HA-MRSA was investigated more than CA-MRSA and LA-MRSA locally[16,28]. Apart from samples origins, *S. aureus* has been known to be an important reason for causing animal illnesses like LA-MRSA (which can transmit to humans via livestock over handling, close contact, or animal-derived food consumption) and food poisoning; especially when such bacteria are multidrug resistant and have the ability to produce toxins like enterotoxins[11,29-32].

Hence, turkey, salami, and sausages have been checked for the first time for *S. aureus* and MRSA in this study; which were collected from different huge supermarkets around Riyadh city. This current study has found approximately 62.7% recorded presence of *S. aureus* between the tested samples and it was significantly higher than it (18%) in Islam *et al*. (2019) study in Dhaka, Bangladesh[29]. Further, more than half of such contaminated samples (56.4%) from processed food considered to be CA-MRSA infection and it occurred through the food preparing process or even hygienic handling circumstances, according to Kluytmans (2010)[33]. Moreover, turkey samples recorded as the lowest percentage of CA-MRSA (5.7%) followed by salami (35.8%) then sausages (58.5%) from all different collecting places. This may be due to the lower content of fat comparing with the rest of our processed food samples, considering to be rich nutrient environment for bacterial growth, as what confirmed by Raji *et al*. (2016) when poultry retail meat from Riyadh city were examined[16].

According to study done by El-Ghareeb *et al*. (2019), they examined 3 out of 20 isolates of camel meat collected from random supermarkets in Al-Hasa city and they were described as MRSA (15%), which proved our findings about CA-MRSA infection among examined raw camel meat sausages samples (6% [3/50])[34]. Furthermore, MRSA infection was still apparent among the four cooked samples of sausages from camel and lamb meat for two different periods (5 and 10 minutes) what supported Kluytmans (2010) study about appropriate cooking for longer time can reduce the risk of their consumption[33] .

MecA gene that is responsible for the resistance to methicillin and located on *SCCmec* was discovered for the first time in Riyadh city among processed food. In fact, *MecA* gene was found between 9 out of 53 of MRSA isolates (17%) and the ratios of which were diverse between samples (i.e., 55.6% from salami and 44.4% from sausages samples). In addition, our study found that about 86.8% of the positive *mecA* isolates were resistant to Oxacillin which was confirmed by Osman *et al*. (2017) about the negative *mecA* strains are resistant to Oxacillin rather than methicillin or could be a novel of *mecA* homologue that was linked with cattle[35]. Moreover, a study was done by Elhaaan *et al,* stated that it is extremely suggested to consider alternate mechanisms for β-lactams resistance that may compete with *mecA*

Table 4. The distribution of virulence enterotoxin genes among food samples collected from Riyadh, Saudi Arabia

Samples types	SEA	SEB	SEE	SEG	SEH	SEI	SEN	SEO	SEM	SEK
Turkey	0	0	0	0	0	0	0	0	0	0
Salami	3	0	1	0	5	0	1	2	2	3
Sausages	1	1	1	1	21	1	3	1	4	0
N/P (%)	7.5	1.9	3.8	1.9	49.1	1.9	7.5	5.7	11.3	5.7

SEA=staphylococcal enterotoxin A, SEB= staphylococcal enterotoxin B, SEE= staphylococcal enterotoxin E, SEG= staphylococcal enterotoxin G, SEH= staphylococcal enterotoxin H, SEI= staphylococcal enterotoxin I, SEN= staphylococcal enterotoxin N, SEO= staphylococcal enterotoxin O, SEM= staphylococcal enterotoxin M, SEK= staphylococcal enterotoxin K

gene to detect the emergence of MRSA in the community[36].

Antibiotic resistance profiles of MRSA isolates

The susceptibility test of a fifty-three MRSA isolates in this study was estimated for eight antibiotics from different classes and the resistance was detected against Oxacillin (86.8%), which was higher comparing with Gentamicin (9.4 %). It is worth noting that 13.2% of MRSA isolates were sensitive to Oxacillin. This result confirmed the study of Saeed *et al*. (2014) who stated that Methicillin resistant *Staphylococcus aureus* (MRSA) is phenotypically presenting minimum inhibitory concentration (MIC) of oxacillin greater than 2 mg/L. Nevertheless, lately, cefoxitin/oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) has been described globally[37].

Such giant resistance; in addition, was stated by Osman *et al*. (2017) to the over using of antibiotics in order to promote the animal growth or treatment[35]. The widespread of multidrug resistant phenotypes among MRSA isolates around Saudi Arabia generally was accounted by Adam and Abomughaid (2018) for the significant number of pilgrims whom may carry these such drug resistant bacteria[38]. Surprisingly, this study uncovered 22.6% (12/53) were resistant to Vancomycin and 33.3% (4/12) were carried *vanB* gene (from salami [2], and sausages [2]); which is still using as a drug of choice to cure MRSA infections, according to Gade and Qazi (2013)[39].

The *mec A* carrier (i.e., *SCCmec*) type I, II, III, IV, and V were also screened in this study among the positive *mec A* samples in order to achieve the goal of the epidemiology study for the MRSA isolates. It was found two types among two samples from salami and sausage (types IVb and V). In addition, this study completely support the studies done by Deurenberg, and Stobberingh (2008) and Moussa *et al*. (2012) which stated SCC*mec* types V and IV are coupled with community transmission and CA-MRSA and with Moussa *et al*. (2012) results about coupling *SCCmec* type III with HA-MRSA due to the absence of such type between LA-MRSA strains currently[40,41].

Panton-Valentine leukocidin (*PVL),* which is the major virulence factor*,* was not spotted in this study in all investigated processed food samples. Hence, our result was conflicted with Deurenberg and Stobberingh (2008) about using *PVL* as an indicator for the presence of CA-MRSA, but on the other hand, it was matching with Herrera *et al.,* (2016) who stated that most of LA-MRSA isolates do not carry *PVL*[42]. Following this reason, other virulence factors such as *spa* gene were examined and formed 7.5% of the total number of LA-MRSA and CA-MRSA isolates. Besides, fourteen variant Staphylococcal enterotoxins were investigated as factors causing Staphylococcal food poisoning and health hazards for consumers[27,43].

Positive MRSA isolates in our study found to be harbored one or more genes from classic staphylococcal enterotoxins (SEA, SEB, SEC, SED, and SEE) and the enterotoxin gene cluster (egc [SEG, SEI, SEN, SEO, and SEM]). In addition, our result confirms that SEH (49.1%) was the most spread enterotoxin followed by SEM (11.3%), 7.5% from each one of SEA and SEN, 5.7% from each one of SEO and SEK, SEE (3.8%), and 1.9% from each one of SEB, SEG, and SEI.

CONCLUSION

From the above, this is the first comprehensive investigation of the prevalence of *S. aureus* and MRSA in prossesed foods from Riyadh city, Saudi Arabia. This study declared a relatively high ratios of *S.aureus* and MRSA contamination in such foods (62.7% and 56.4%, respectively). Importantly, two cooked sausages samples were still contaminated with such bacteria and infection and none of MRSA isolates were positive for *PVL* gene. Whilst, nine of MRSA isolates were positive for *MecA* gene and *spa* gene was among four of which. High amount of antimicrobial resistance also found between the isolates ,therefore, our data approved the possible role of processed foods in the transmission of multidrug resistant *S.aureus* strains and successful MRSA in Riyadh , Saudi Arabia.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

MJA and AS contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. M.A conceived of the presented idea.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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