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Original article

# The prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in milk and dairy products in Riyadh, Saudi Arabia

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## ABSTRACT

In terms of life- menaced contagion, methicillin resistant *Staphylococcus aureus* (MRSA) is known to be one of which and it is truly notable in the contaminated food causing a community health anxiety. However, the occurrence of *S. aureus* and MRSA in diverse kinds of dairy products have been tested in this study. Samples from: raw milk (unpasteurized) from horse, goat, camel, and cow origins and unpacked cheese were checked for the recovered strains of such bacterium and MRSA. Wholly, MRSA isolates were verified for antimicrobial susceptibility and further characterized by *mecA* and staphylococcal cassette chromosome *mec* (SCCmec) typing. Also, Panton-Valentine Leukocidin (*PVL*), *Staphylococcus aureus* protein A (*spa*), and Staphylococcal enterotoxins (SEs) were also tested between all positive MRSA isolates in order to discover the virulence factors. Consequently, 70% of the 100 collected dairy products samples were contaminated by *S. aureus* bacteria and 72.9% of them were defined as MRSA. 9.8% of MRSA isolates contained *mecA* genes with SCCmec type II (80%) as the most common SCCmec type. Moreover, large number of MRSA isolates were identified as multidrug resistance and 28.6% of MRSA-*mecA* positive isolates were also carried vancomycin resistance genes (i.e., *vanB*). Too, *spa* gene was detected between 9.8% of MRSA isolates but *PVL* gene was not spotted at all. Additionally, the existing of SEs was variable between MRSA isolates and the most common type was SEH (51%). In general, our results confirmed that raw milk and unpacked cheese in the Kingdom of Saudi Arabia (Riyadh) is a potential vehicle for multidrug resistant MRSA transmission. It is a critical civic health menace and stresses, thus; the need of applying well cleanliness practices is essential.

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## 1. Introduction

*S. aureus* is considered to be an essential source for humanity contagion that causes food-poisoning globally. It (i.e., *S. aureus*) can be even a source for lethal diseases such as toxic shock syndrome, endocarditis, sepsis, and pneumonia (Otto, 2010). Nevertheless, great quantities of antimicrobial resistance to these bacteria constituted a critical civic health risk and approximately 13–74% of *S. aureus*'s contagions were defined as MRSA (Narayanan et al., 2019). The appearance of all  $\beta$ -lactam antibiotics resistance among MRSA isolates, in addition; was clarified via car-

rying *mecA* gene which locates on SCCmec (Yang et al., 2016). Because of the variances in the origins of the outbreaks, MRSA were organized into three groups which are healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA) (Dweba et al., 2018). HA-MRSA, CA-MRSA, and LA-MRSA strains showing different antimicrobial resistance profiles and SCCmec types, according to epidemiological studies. Thus, CA-MRSA strains carry SCCmec types IV and V but HA-MRSA strains carry SCCmec types I, II, and III and SCCmec V and SCCmec IV are the most common types between them (Shah et al., 2019). Then, SCCmec XI is detected in UK and Denmark among cattle (from mastitis cows) and humans which carries *mecC* gene and it is (i.e., *mecC*) identified later as *mecA* LGA251 (Butaye et al., 2016; Vindel et al., 2014). Furthermore, in 2003 and for the first time the virulence factor and genetic marker for MRSA existence (*PVL* gene) was explored and it is responsible for skin infection or serious diseases such as pneumonia. The later (i.e., *PVL*), found frequently in CA-MRSA strains rather than HA-MRSA and its rate of spread among strains is relying on bacteriophages (Kong et al., 2016; Loewen et al., 2017; Motamedi

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et al., 2015). As any infection, nonetheless, specific symptoms like red painful and filled with pus bump, fever, and swollen skin are commonly occurring. Regarding LA-MRSA, staphylococci species generally and *S. aureus* especially are reported vastly between livestock and it was found in the pig population in limitless countries of Europe after discovering it in France and the Netherlands. LA-MRSA can transmit to human via different ways like utilization of animal products, e.g., lamb, calve, and goat or direct interact with animals and the spread level of *S. aureus* was higher among lamb (29.7%) than it among goats and calves (12.5% and 1.4%, respectively). Furthermore, as HA-MRSA and CA-MRSA, LA-MRSA clones are various between countries, e.g., CC9 clone was uncovered in Asia but CC398 clone was uncovered in Europe and USA (Mama et al., 2019; Butaye et al., 2016; Yang et al., 2016).

MRSA infections spread vigorously throughout Saudi Arabia, a group of clinicians indicated that the percentage of such infections in King Fahad Medical City in Riyadh reached 50% during 2011, and there are no sufficient studies addressing such this problem (Monecke et al., 2012). Comparing to other countries around the world, MRSA infection in Saudi Arabia has been addressed inadequately; though, it was discovered in such country in the 1990s (Alrabiah et al., 2016). Despite the fact that large numbers of local research were about HA-MRSA, there is still a deficiency in the number of which among children and unexpectedly it is found in males more than females (Alrabiah et al., 2016). Apart from HA-MRSA, the number of CA-MRSA's patients was notably increased in Saudi Arabia from 9.9 per 10,000 in 2001 to 67 per 10,000 in 2008 and from 2000 to 2008 CA-MRSA spreaded six times more alongside Eastern region of Riyadh city (Alrabiah et al., 2016; Moussa and Hessian, 2010; Monecke et al., 2012). Farther, CA-MRSA ratios between 2006 and 2015 amplified steadily from 23% to 60% in Qatif city, Saudi Arabia. Lastly, the range of studies about LA-MRSA among MRSA's kinds is still low (Al-Hamad et al., 2018; Raji et al., 2016). Throughout Riyadh city, this study was planned to prove the incidents of CA-MRSA and LA-MRSA in dairy products and further molecular characteristics and antibiotic resistance tests.

## 2. Materials and methods

### 2.1. Sample collection

During the seven-month period (from August 2019 to March 2020), the whole one-hundred samples were gathered from different farms (raw milk samples) and local grocery shops (cheese samples), which are nine grocery shops: A, B, C, D, E, F, G, H, and I, throughout Riyadh city, Kingdom of Saudi Arabia. Fifty samples were from raw milk and the other fifty samples were from unpacked cheese which collected over a timeline and randomly. In addition, the raw milk samples were gained in labeled screwed top bottles from farms to prevent them from being contaminated and were kept in ice box under cold conditions to transfer them to the laboratory within 24 h and cheese samples were collected in labelled sterile bags (zip-seal bags), followed the same procedures of transporting to the laboratory. Nevertheless, raw milk samples' origins were from horses (11 samples), goats (20 samples), camels (15 samples), and cows (4 samples).

### 2.2. Bacterial growth conditions

500 µl of each raw milk sample were put in almost 5 ml of brain heart infusion (BHI) (Watin-Biolife factory) and then incubated at 37 °C for 24 h to grow *S. aureus*. Similarly, 0.5 g of unpacked cheese samples was grown overnight in 5 ml of BHI at 37 °C after cutting it into small pieces by sterilized scalpel and forceps.

### 2.3. Isolation and identification of *S. Aureus*

*S. aureus* colonies were tested phenotypically by traditional approaches. Therefore, mannitol salt agar (MSA) was chosen as a selective media to discriminate and isolate *S. aureus* bacteria through streaking such bacterium and incubating it at 37 °C for 24 h. Subsequently, all recovered *S. aureus* were gone under several tests and different medias, which are DNAs test, MRSA chromagar, and MSA supplemented with oxacillin (4.0 mg) (MSAO). Moreover, positive MRSA isolates in MRSA chromagar were confirmed in MSAO plates (all medias from Watin-Biolife factory).

### 2.4. Antimicrobial susceptibility testing

Relying on the Clinical Laboratory Standards Institute, Kirby Bauer disk diffusion method was applied to investigate the whole MRSA isolates for antimicrobial resistance. Therefore, eight different antibiotics from different classes were used, which are β-lactams (Oxacillin[1 µg], Ampicillin[25 µg], and penicillin [10 µg]), Glycopeptides (Vancomycin[30 µg]), Tetracyclines (Oxytetracycline[30 µg]), Aminoglycosides (Gentamicin [30 µg]), Sulfonamides (Trimethoprim-Sulphamethoxazole [25 µg]), and Macrolides (Erythromycin [15 µg]).

### 2.5. DNA extraction

5 ml of BHI was used as a nutrient for the two selected colonies from MRSA chromagar plates and incubated them for 24 h at 37 °C to grow. Next, by following the manufacturers' rules (Qiagen, Hilden, Germany), QIAamp DNA mini kit was used to isolate and purified DNA.

### 2.6. Discovery of *mecA*, *mecC*, and *mecB* and SCCmec typing of *mecA* positive MRSA isolates

*mecA* -responsible for methicillin resistance- *mecB* -plasmid-encoded transferable gene-mediated Methicillin resistance- and *mecC*, a different *mecA* homolog (named *mecALGA251*), were spotted by PCR using primers defined formerly (Genwiz, China) (Becker et al., 2018; Najjar-Peerayeh et al., 2014). Likewise, PCR technique was used with defined primers for SCCmec types among the *mecA*-positive MRSA isolates (McClure-Warnier et al., 2013).

### 2.7. Virulence factors of *Staphylococcus aureus* isolates

Panton-Valentine Leukocidin (*PVL*), *Staphylococcus aureus* protein A (*spa*), vancomycin resistance genes *vanA* and *vanB*, and the fourteen enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sen*, *seo*, *sem*, *sek*, and *sel*) were tested as virulence genes by PCR using primers defined previously (Chiang et al., 2006; Kariyama et al., 2000; Khairalla et al., 2017; Lee et al., 2007; McClure et al., 2006; Puah et al., 2016; Soares Casaes Nunes et al., 2015).

## 3. Results

### 3.1. Prevalence of *S. Aureus* and MRSA infection in food

A total of 100 samples were collected from dairy products being screened for sample contamination during the period of study (from August 2019 to March 2020). A standard food culture method and 16 s rRNA were used to identify *S. aureus*. From 100 food samples, 70% were contaminated by *S. aureus* bacteria. The contaminated samples of raw milk (i.e., 60%) were from: goat milk (16/20), horse milk (10/11), camel milk (12/15), and cow milk

(4/4). Furthermore, 40% of unpacked cheese samples were contaminated. 51/70 of *S. aureus* isolates from different types of food were confirmed as MRSA. Among the 51 MRSA isolates, 34/51 were from raw milk, while 17/51 were from unpacked cheese. As regard to the ratios of MRSA existence in different raw milk samples, precisely, they were from: goat milk (75%), horse milk (70%), camel milk (91.7%), and cow milk samples (100%) (Table 1). Furthermore, all subjected methicillin resistant *S. aureus* (MRSA) isolates were tested for *mecA*, *mecB*, and *mecC* genes. Neither *mecC* nor *mecB* genes were positive, while 5/51 of MRSA isolates were positive for *mecA* gene.

### 3.2. Antibiotic resistance profiles of MRSA isolates

Antimicrobial susceptibility test for all such isolates have been done by using eight antimicrobial agents from different antibiotic classes like beta-lactams (penicillin, ampicillin, and oxacillin), Aminoglycosides (gentamicin), Tetracyclines (oxytetracycline), Glycopeptides (vancomycin), Macrolides (erythromycin), and Sulfonylamides (trimethoprim-sulphamethoxazole) antibiotics. Consequently, the bacterial resistance to each antibiotic from the tested samples are: 23.5% to penicillin, 15.7% to ampicillin, 66.7% to oxacillin, 15.7% to erythromycin, 13.7% to gentamicin, 9.8% to oxytetracycline, 33.3% to trimethoprim-sulphamethoxazole, and 13.7% to vancomycin. According to vancomycin's resistance genes (i.e., *vanA* and *vanB*), they were checked and 28.6% (2/7) of MRSA isolates were carried *vanB* gene - from raw camel milk samples-, whereas *vanA* gene was not detected at all. Importantly, oxacillin was the predominant spotted resistance antibiotic among cheese samples, while gentamicin resistance was low among raw milk samples in MRSA isolates versus other types of food isolates (Fig. 1 and Table 2). However, large number of MRSA isolates were resistant to more than three antibiotics, involving 7.8% (4/51) of them were resistant to 5–7 antibiotics (one from raw goat milk,

one from raw camel milk, and two from cheese samples), 37.3% (19/51) of them were resistant to 2–4 antibiotics (14 from raw milk [eight from raw camel milk, two from each of raw horse, goat, and cow milk origins], and five from cheese) and 1.96% (1/51) of them were resistant to all eight antibiotics (from cheese).

On the other hand, 66.7% (34/51) of the isolates were sensitive to 5–7 antibiotics, 5.9% (3/51) of them were sensitive to 2–4 antibiotics, and 21.6% (11/51) of them were sensitive to all eight antibiotics.

### 3.3. Molecular typing of MRSA isolates

The MRSA positive *mecA* samples were exposed to identify SCCmec types. Five different types were identified among the positive *mecA* samples that are SCCmec types I, II, III, IV, and V.

Among 5 isolates, the type of one isolate from cheese samples could not be detected, while three isolates had two types, II + V, II + IVa and II + IVd. Moreover, one isolate had three types II + II I + V and the most presented type of MRSA positive *mecA* isolates was SCCmec type II, which was detected in 80% of isolates (4/5) (Table 3).

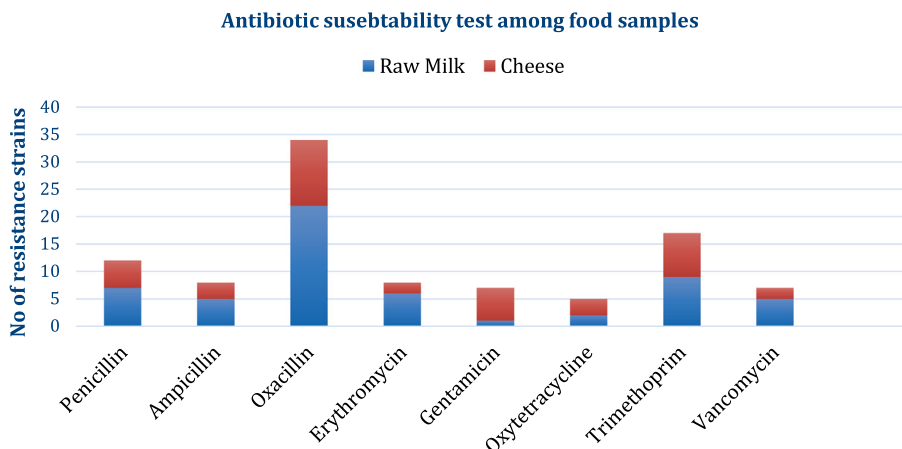
### 3.4. Distribution of virulence genes

*Staphylococcus aureus* protein A (*spa*) gene among the tested MRSA samples was recorded within 5 out of 51 (9.8%), while Pantone Valentine leukocidin (*PVL*) gene was not recorded at all. The fourteen examined enterotoxin genes (i.e., Staphylococcal enterotoxin A [SEA], B [SEB], C [SEC], D [SED], E [SEE], G (Becker *et al.*), H [SEH], I [SEI], J [SEJ], N [SEN], O [SEO], M [SEM], K [SEK], and L (Feßler *et al.*), also existed between such isolates. As a result, SEH showed the highest ratio between SEs (51% [26/51]) then SEE (27.5% [14/51]), SEM (21.6% [11/51]), SEO (19.6% [10/51]), SEA and SEN (17.6% [9/51]), SEB (13.7% [7/51]), SEG (11.8% [6/51]),

**Table 1**  
The occurrence of *S. aureus* and MRSA between the tested samples in Riyadh, Saudi Arabia.

| Kinds of samples | No of positive <i>S. aureus</i> | n/N (%)       | No of samples positive for MRSA | N/P (%)       |
|------------------|---------------------------------|---------------|---------------------------------|---------------|
| Raw Milk         | 42                              | 42/70 (60%)   | 34                              | 34/51 (66.7%) |
| Raw Goat Milk    | 16                              | 16/20 (80%)   | 12                              | 12/16 (75%)   |
| Raw Horse Milk   | 10                              | 10/11 (90.9%) | 7                               | 7/10 (70%)    |
| Raw Camel Milk   | 12                              | 12/15 (80%)   | 11                              | 11/12 (91.7%) |
| Raw Cow Milk     | 4                               | 4/4 (100%)    | 4                               | 4/4 (100%)    |
| Cheese           | 28                              | 28/70 (40%)   | 17                              | 17/51 (33.3%) |

n is the entire number of samples and N is the entire number of the contaminated samples.



**Fig. 1.** Antibiotics susceptibility examination within contaminated food samples collected from Riyadh, Saudi Arabia.

**Table 2**  
Antibiotics susceptibility examination within contaminated food samples collected from Riyadh, Saudi Arabia.

| The class of Antibiotic    | Antibiotics     | Raw Milk | Cheese | n/N (%)       |
|----------------------------|-----------------|----------|--------|---------------|
| β-lactams                  | penicillin      | 7        | 5      | 12/51 (23.5%) |
|                            | ampicillin      | 5        | 3      | 8/51 (15.7%)  |
|                            | oxacillin       | 22       | 12     | 34/51 (66.7%) |
| Macrolides<br>(15.7%)      | erythromycin    | 6        | 2      | 8/51          |
| Aminoglycosides<br>(13.7%) | gentamicin      | 1        | 6      | 7/51          |
| Tetracyclines<br>(9.8%)    | oxytetracycline | 2        | 3      | 5/51          |
| Sulfonamides<br>(33.3%)    | trimethoprim    | 9        | 8      | 17/51         |
| Glycopeptides<br>(13.7%)   | vancomycin      | 5        | 2      | 7/51          |

n is the entire number of samples and N is the entire number of the contaminated samples.

**Table 3**  
The detection of *mecA* gene (147 bp), SCCmec types II, III, IVa, IVb, IVd, and V between the tested samples in Riyadh, Saudi Arabia.

| Samples kinds  | Strains ID | <i>MecA</i> | SCCmec II | SCCmec III | SCCmec IV a | SCCmec IV b | SCCmec IV d | SCCmec V |
|----------------|------------|-------------|-----------|------------|-------------|-------------|-------------|----------|
| Raw Horse Milk | 17         | +           | +         | -          | +           | -           | -           | -        |
| Raw Horse Milk | 18         | +           | +         | +          | -           | -           | -           | +        |
| Raw Camel Milk | 24         | +           | +         | -          | -           | -           | +           | -        |
| Raw Camel Milk | 34         | +           | +         | -          | -           | -           | -           | +        |
| Cheese         | 40         | +           | -         | -          | -           | -           | -           | -        |

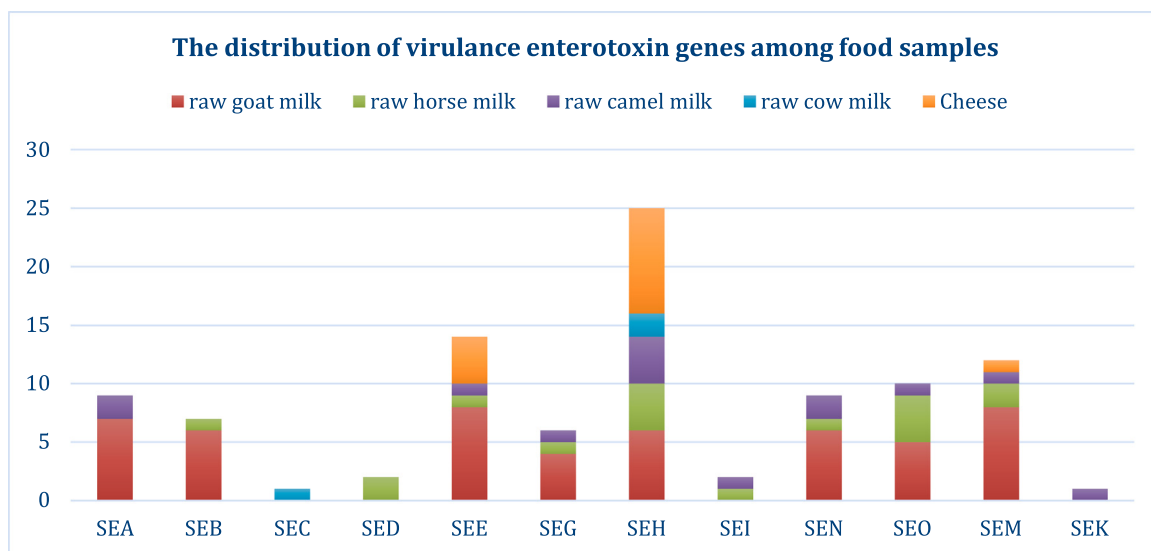
SCCmec = staphylococcal cassette chromosome mec.

SED and SEI (3.9% [2/51]), and lastly SEC and SEK (1.96% [1/51]). Whereas, neither SEJ nor SEL were existed in any of the MRSA isolates (Fig. 2).

Staphylococcal enterotoxins (SEA, SEB, SEC, SED, and SEE) and their origins were from: raw milk (29/33 [87.9%]) and cheese samples (4/33 [12.1%]). The majority of classical staphylococcal enterotoxins between raw milk samples; moreover, were from raw goat milk (21/29 [72.4%]) followed by raw horse milk (4/29 [13.8%]), raw camel milk (3/29 [10.3%]) and then raw cow milk (1/29 [3.4%]). Whereas 16 of 51 MRSA isolates (31.4%) harbored the genes of the enterotoxins gene cluster (*egc*) (SEG, SEI, SEN, SEO, and SEM) and their origins were from: raw milk (15/16 [93.8%]),

and cheese (1/16 [6.3%]). Raw goat milk also recorded the highest presence of *egc* (9/15 [60%]) among raw milk samples followed by raw horse milk (4/15 [26.7%]), and raw camel milk (2/15 [13.3%]). The SEC-SEL gene combination, typical of the SaPIbov pathogenicity island, was not noticed in any of the isolates, as Fig. 2 and Table 4 shown. In addition, four isolates from raw milk origins (goat [1], horse [2], and camel [1]) harbored variant virulence genes (i.e., *spa* and enterotoxins genes) and they were resistant to oxacillin as well.

Saudi researchers are focusing in their study on HA-MRSA more than CA-MRSA and LA-MRSA due to that CA-MRSA is described to be less serious outcome than HA-MRSA (Iyer et al., 2014; Raji et al.,



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

**Table 4**

The distribution of virulence enterotoxin genes within contaminated food samples collected from Riyadh, Saudi Arabia N is the entire number of positive samples P is the entire Number of MRSA samples.

| Samples types  | SEA  | SEB  | SEC  | SED | SEE  | SEG  | SEH | SEI | SEN  | SEO  | SEM  | SEK  |
|----------------|------|------|------|-----|------|------|-----|-----|------|------|------|------|
| Raw Goat Milk  | 7    | 6    | 0    | 0   | 8    | 4    | 6   | 0   | 6    | 5    | 8    | 0    |
| Raw Horse Milk | 0    | 1    | 0    | 2   | 1    | 1    | 4   | 1   | 1    | 4    | 2    | 0    |
| Raw Camel Milk | 2    | 0    | 0    | 0   | 1    | 1    | 4   | 1   | 2    | 1    | 1    | 1    |
| Raw Cow Milk   | 0    | 0    | 1    | 0   | 0    | 0    | 2   | 0   | 0    | 0    | 0    | 0    |
| Cheese         | 0    | 0    | 0    | 0   | 4    | 0    | 9   | 0   | 0    | 0    | 1    | 0    |
| N/P (%)        | 17.6 | 13.7 | 1.96 | 3.9 | 27.5 | 11.8 | 49  | 3.9 | 17.6 | 19.6 | 23.5 | 1.96 |

2016). LA-MRSA has been detected in this study among large number of four different kinds of raw milk samples (i.e., horse, goat, camel, and cow raw milk). Apart from sample origin, *S. aureus* has been identified to be an essential cause for food poisoning and animal diseases like LA-MRSA -which can be conveyed to people through livestock handling, animal-derived food utilization, or adjacent interaction (Bhedi et al. (2018); Cuny et al., 2019; Islam et al., 2019; Mama et al., 2019; Wang et al., 2018).

#### 4. Discussion

According to the conducted studies throughout Saudi Arabia on the whole and Riyadh city specifically and internationally, raw horse milk was examined for *S. aureus* and MRSA infection for the first time in this study and it illustrated a remarkable positive result. In spite of the social thought about asthmatic patients could be recovered by drinking raw horse milk (especially after birthing directly), *S. aureus* bacteria were detected in about 91% of the samples [10/11] and that may cause LA-MRSA infection were 70% [7/10]. The numbers were awkwardly spotted between the tested samples from origin, although samples were collected from four different locations. This means that such type of milk is not suitable for the consumption of human beings without being sterilized, and even if it has powerful remedial characteristics that can cure or at least lessen the severity of asthmatic patients' seizures, the bad effect of MRSA found in the samples examined is not questionable. The high percentages of *S. aureus*, LA-MRSA, and CA-MRSA found could be referred to the lack of hygiene of the animal per se or even that of the handlers as emphasized by Gopal, and Divya, (2017). Finally, such results were not according the researcher's knowledge and were not found in any other studies.

Moreover, goat, camel, and cow raw milk samples in this study presented also a great number of *S. aureus* (62.7% [32/51]) and LA-MRSA (26% [27/51]), which was less than that (57%) of the study that was done by Alzohairy (2011) (Alzohairy, 2011). Besides, LA-MRSA among raw goat milk samples (75%) was higher than the study was presented by El-Deeb et al. (73%) (2018) in an Eastern part of Saudi Arabia (El-Deeb et al., 2018). The outcome of this study also confirmed what Hirad et al. (2018) stated about the examined raw camel milk samples collected from Riyadh city, and the definite health risk of drinking that kind of raw milk (Hirad et al., 2018).

Comparing with the study that was performed by Abulreesh and Organji, 2011 in Makkah city, *S. aureus* multidrug resistant has also explored in this study among the total of such bacteria presence (i.e., 17%) from different kinds of unpacked cheese and it is slightly lower than it was discovered by Nusrat et al., 2015 (i.e., 20%). Remarkably, seventeen out of twenty-eight positive *S. aureus* among cheese samples was defined as CA-MRSA (61%) that is due to the lack of hygienic handling (Abulreesh and Organji, 2011; Nusrat et al., 2015).

For the first time, *MecA* gene -which is in charge of the resistance to methicillin and carried by SCCmec- was detected in Riyadh city between dairy products. It was found to be carried

by 5/51 of MRSA isolates (9.8%), although, the proportions of it were varied among samples (80% from raw milk, and 20% from cheese). Despite the fact that negative *mecA* strains are resistant to oxacillin rather than methicillin or could be a novel *mecA* homologue which is associated with cattle, 60% of the positive *mecA* isolates in our study were resistant to oxacillin as well (Osman et al., 2017). Furthermore, a group of scientists advised to consider alternative mechanisms for  $\beta$ -lactam resistance which may compete with *mecA* genes to discover the appearance of MRSA in the community (Elhassan et al., 2015).

##### 4.1. Antibiotic resistance profiles of MRSA isolates

This study was assessed the antibiotic susceptibility between 51 MRSA isolates for eight antibiotics from different classes and the high resistance was recorded against oxacillin (66.7%) compared with gentamicin (13.7%) within raw milk samples. On the other hand, 33.3% of MRSA isolates were sensitive to oxacillin which supported the study of Saeed et al. (2014) about Methicillin resistant *Staphylococcus aureus* (MRSA) is phenotypically presenting a minimum inhibitory concentration (MIC) of oxacillin greater than 2 mg/L. However, recently, cefoxitin/oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) has been explained internationally (Saeed et al., 2014).

Osman et al. (2017) attributed the reason of the huge resistance to the overusing of antibiotics in order to cure or promote animal growth (Osman et al. 2017). Moreover, other antibiotics from different groups (like  $\beta$ -lactams [penicillin, and ampicillin], Tetracyclines [Oxytetracycline], Glycopeptides [Vancomycin], Sulfonamides [Trimethoprim-sulphamethoxazole], and Macrolides [Erythromycin] antibiotics) were also examined and multidrug resistance phenotypes were determined (7.8% of MRSA isolates were resistant to 5–7 antibiotics [one from raw goat milk, one from raw camel milk, and two from cheese samples]), 37.3% from all types of tested samples were resistant to 2–4 antibiotics, and 1.96% were resistant to the all eight antibiotics (from cheese). Because of the significant number of pilgrims that may carry these drug resistant bacteria, the multidrug resistant phenotypes between MRSA isolates around Saudi Arabia was recorded, according to Adam and Abomughaid (2018) (Adam and Abomughaid, 2018). Surprisingly, this study uncovered 13.7% (7/51) were resistant to Vancomycin and 28.6% (2/7) were carried *vanB* gene (from raw camel milk); that is till now using as a drug to treat MRSA infections (Gade and Qazi, 2013).

To fulfill the goal of the epidemiology study for the MRSA isolates, SCCmec types (i.e., I, II, III, IV, and V) were also investigated in this study among the positive *mecA* samples. So, the majority of such carrier was from type II (80%) followed by type V (40%) and then types III, IVa, IVb, and IVd (20%) which completely agreed with studies conducted by Deurenberg, and Stobberingh (2008) and Moussa et al. (2012) about SCCmec types V and IV are associated with community transmission and CA-MRSA. But this study stood against Moussa et al. (2012) study about associating SCCmec



type III with HA-MRSA which is found as LA-MRSA strain currently (Deurenberg and Stobberingh, 2008; Moussa et al., 2012).

The main virulence agent (i.e., *PVL*), in addition; was not spotted in this study within the whole examined dairy products samples which opposed with Deurenberg and Stobberingh (2008) who described *PVL* as an indicator for the occurrence of CA-MRSA. In contrast, this study supported Herrera et al. (2016) view about most of LA-MRSA isolates do not carry *PVL* (Herrera et al., 2016). So that, *spa* gene as another virulence factors were examined and it formed 9.8% of the total number of LA-MRSA and CA-MRSA isolates. The fourteen variant Staphylococcal enterotoxins were also examined because of their ability to cause Staphylococcal food poisoning and health risks for consumers (Feßler et al., 2011; Jin & Yamada, 2016). 64.7% genes from classic staphylococcal enterotoxins (SEA, SEB, SEC, SED, and SEE) were detected to be harbored within the positive MRSA isolates and 31.4% genes were from the enterotoxins gene cluster (egc [SEG, SEI, SEN, SEO, and SEM]). SEA recorded the highest prevalence among raw milk samples in our study and it was similar to the outcome of Jin and Yamada (2016), who considered the spread of enterotoxin-producing MRSA between such samples is variant between countries and even districts within the same country (Jin & Yamada, 2016). SEH (51%) was the greatest spread enterotoxin followed by SEE (27.5%), SEM (21.6%), SEO (19.6%), SEA and SEN (17.6%), SEB (13.7%), SEG (11.8%), SED and SEI (3.9%), and lastly SEC and SEK (1.96% [1/51]), relying on the result of this study. Importantly, our results stood in the opposite site of Herrera's et al., (2016) declaration about most of LA-MRSA isolates do not carry Staphylococcal enterotoxin genes (Herrera et al., 2016). In other words, more than half of the total spread of staphylococcus enterotoxins (51% [26/51]) in our study was from LA-MRSA isolates.

## 5. Conclusion

To sum up, this inclusive investigation of the spread of *S.aureus* and MRSA in horse raw milk from Riyadh, Saudi Arabia have been done for the first time and it showed high percentages (90.9% and 70%, respectively). Also, our study announced a proportionately high percentage of *S. aureus* and MRSA contamination in Saudi dairy products and a high amount of antimicrobial resistance among the isolates, especially to oxacillin (i.e., 66.7%). Finally, our statements approve that the transition of multidrug resistant *S. aureus* strains and successful MRSA in Riyadh, Saudi Arabia are possible happening through consumption the tested products.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## 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.

## Ethics statement

This study was carried out in accordance with the recommendations of the PNU Institutional Review Board (IRB) number: 19-0169.

## Availability of data

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

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