

# High prevalence and antimicrobial resistance of *mecA* *Staphylococcus aureus* in dairy cattle, sheep, and goat bulk tank milk in Jordan

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**Abstract** The aim of this study was to determine the prevalence and antimicrobial resistance of *mecA* and *mecC* methicillin-resistant *Staphylococcus aureus* (MRSA) in cattle, sheep, and goat dairy farms in Jordan. Milk samples were collected from bulk tanks at 117 dairy farms (44 cattle, 47 sheep, and 26 goat dairy farms) in each region of the country. MRSA were isolated on *mecA* and *mecC* chromogenic media and confirmed by PCR. The confirmed isolates were tested for resistance toward 15 antimicrobials by the disc diffusion method. None of the tested bulk milk samples were positive for *mecC* and 26% (95% CI 20–32%) were positive for *mecA* MRSA. Specifically, *mecA* MRSA was detected in 31.8% (95% CI 17.5–46.1) of cattle, 29.8% (95% CI 16.2–43.4) of sheep, and 11.5% (95% CI 1.6–24.7%) of goat dairy farms. All isolates ( $n = 86$ ) exhibited resistance to penicillin, oxacillin, cefoxitin; meanwhile, most isolates (70–85%) exhibited resistance toward gentamicin, clindamycin, rifampicin, neomycin, fusidic acid, erythromycin, tetracycline, and ciprofloxacin. All *mecA* MRSA isolates were resistant to at least one class of antimicrobials. Isolates from all goat milk, 88% of cattle milk, and 87% of sheep milk samples exhibited resistance to three classes of antimicrobials and were considered

multidrug resistant (MDR). These data demonstrate widespread MDR MRSA in dairy ruminants in Jordan, and these rates are higher than those reported in other countries. Such high prevalence of MDR MRSA and *mecA* MRSA could lead to economic losses in the dairy industry in Jordan and poses a possible public health risk.

**Keywords** Antibiotics · Ruminants · MRSA · Developing countries

## Introduction

*Staphylococcus aureus* is an important causative agent of foodborne illness in humans and mastitis in dairy ruminants ((CDC), 2014; Painter et al. 2013). *S. aureus* has a unique ability to adapt rapidly to antimicrobials and has developed resistance to methicillin and penicillin and most recently to daptomycin and linezolid which is a growing problem (Pantosti et al. 2007). Methicillin resistance by *S. aureus* is encoded by *mecA* and *mecC* genes. Those methicillin-resistant *Staphylococcus aureus* MRSA carry the staphylococcal cassette chromosome (SCC*mec*) and resistant beta-lactam antimicrobials. While both *mecA* and *mecC* exhibit resistance to cefoxitin, *mecC* is sensitive and *mecA* is resistant to oxacillin (Cartwright et al. 2013; Kim et al. 2012).

MRSA is a significant cause of mastitis in ruminants worldwide (Feßler et al. 2010; Vanderhaeghen et al. 2010a; Aras et al. 2012; Pilla et al. 2013; Guimaraes et al. 2017). In the case of subclinical mastitis, MRSA does not change the organoleptic characteristics of milk, thus it can be transmitted through milk and dairy products to humans. The prevalence of MRSA in bovine milk and its zoonotic transmission between farmers and ruminants have been reported by several studies (Feßler et al. 2010; Vanderhaeghen et al., 2010a, b; Spohr et al. 2011;

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Caruso et al. 2016). Studies have reported low prevalence of MRSA in cattle milk, ranging from 0.3% in the UK to 9% in Belgium (Vanderhaeghen et al. 2010a; Paterson et al. 2012; Parisi et al. 2016). Moreover, *mecC* MRSA has been isolated at low rates from bovine milk in European countries including Spain and UK (Ariza-Miguel et al. 2014; Paterson et al. 2014). In contrast, a limited number of studies have investigated the prevalence of MRSA in small ruminants' milk (Aras et al. 2012; Foti et al. 2012; Cortimiglia et al. 2015; Caruso et al. 2016). But, consumption of dairy products made from raw sheep and goats milk is widespread, especially in Mediterranean countries, where some traditional goat or sheep cheeses are made exclusively from raw milk (Hilali et al. 2011).

Anecdotal evidence reported by Jordanian field veterinarians suggests the high frequency of drug-resistant mastitis cases in dairy ruminants of Jordan. This study aimed to (1) determine the prevalence of *mecA*- and *mecC*-positive *S. aureus* (MRSA) in cattle, sheep, and goat bulk milk and (2) determine the antimicrobial resistance of the isolated *mecA* and *mecC*-positive *S. aureus*.

## Materials and methods

### Sample population

Milk production is an important part of the agricultural sector of Jordan and includes cattle, sheep, and goat milk. Dairy cattle have two production systems, large (intensive)- and small-scale systems. Both systems raise Holstein-Friesian dairy cows and contribute \$160 million annually to the national gross domestic product (DoS 2015).

The large production system uses modern management practices with zero-grazing and located in Al-Dulial area (East-Northern area of Jordan) which produces around 50–60% of the milk in Jordan (DoS 2015). The small-scale system houses cows in small traditional brick barns, with traditional management practices and is scattered throughout Jordan but mainly located in the Badia and the Northern highlands regions. Small ruminant dairy farms are more scattered in Jordan compared to the dairy cattle farms. The Badia region, which is an arid to semi-arid region, is located in eastern and southern Jordan and occupied by nomadic or pastoralist Bedouin people who raise small ruminants (Tarawneh and Kadioğlu 2003). It receives the highest amounts of rainfall in Jordan and occupied by small herders who rely on low production technologies (Tarawneh and Kadioğlu 2003). In contrast, the Northern highlands are characterized by a narrow strip which is rugged and intersected by deep valleys. The production system in the Highlands is extensive and uses rangelands under constant search for grass and water.

### Sample size

The prevalence of *mecA* and *mecC* MRSA in dairy ruminant milk is unknown in Jordan. To detect a prevalence of  $0.5 \pm 0.1$  at 95% confidence interval, using the formula  $n = z^2 p(1-p)/d^2$ , the required sample size would be 96 farms. A total of 117 farms were included in the study to account for possible refusal. Specifically, 44 cattle, 47 sheep, and 26 goat farms were randomly selected from different regions of Jordan. The studied sheep and goat farms included 26 in Northern Jordan, 22 in Southern Jordan, 15 in Badia, and 9 farms in the Jordan Valley. Cattle farms included 22 in Al-Dulial area and 22 in the Northern Highlands. Data collection occurred from December 2015 to March 2016 because during this period, parturitions in sheep and goats in Jordan usually occur (whereas, parturitions in cattle occur year around) (Talfha and Ababneh 2011). In each farm, we sampled one to two tanks based on availability for a total of 208 samples.

### Sampling approach

In each governorate, private veterinarians were recruited to assist with the data collection since they have strong ties with the farmers (their clients). Farms were randomly selected from a list of farms provided by the local veterinary associations for inclusion in this study. Once a farm was selected for inclusion in the study, we visited it and briefed the farm owner(s) about the aims and the voluntary nature of the study. Farm owners were assured that the study was for research purposes only and that any data pertaining to their specific farm would be available to them upon request free of charge. Consent to collect the milk samples was obtained from the owner of each farm prior to starting the study.

Milk samples (100 mL) from bulk tanks were aseptically collected by study personnel and samples were individually packed in sterile cups and transported daily in an ice box under cold conditions to the Food Safety and Zoonotic Diseases Laboratory, Faculty of Veterinary Medicine, Jordan University of Science and Technology. Upon arrival at the laboratory, the samples were registered in the sample log book and then kept in the refrigerator at 4 °C for 4 to 5 days then frozen at  $-20$  °C prior to testing to help release the intracellular *S. aureus* in milk somatic cells upon subsequent thawing as done by others (Paterson et al. 2014).

### Isolation of methicillin-resistant *S. aureus* from the milk samples

MRSA was isolated from the milk samples as described previously (Paterson et al. 2014). Briefly, frozen samples were thawed at 37 °C and 2 mL of milk was mixed with 8 mL of Mueller-Hinton broth (Oxoid CM0405, Hampshire, England) supplemented with 6.5% NaCl and incubated at 37 °C under

shaking at 200 rpm. Then, 100 µL of the culture was plated on the chromogenic MRSA Brilliance 2 plates (Oxoid, PO1210A) in triplicates and incubated at 37 °C for 24 h. Suspected MRSA colonies (blue color) were then subcultured on the chromogenic Staph Brilliance 24 plates (Oxoid, PO1186A). Isolated colonies were then stored in buffered glycerol at −20 °C.

### Molecular confirmation of methicillin-resistant *S. aureus*

Genomic DNA was extracted from the suspected isolates using the QIAamp DNA Mini Kit (product code: 51304, Qiagen, Germany) as specified by the manufacturer and using 20 mg/mL lysozyme (Sigma-Aldrich; product code: L7651) to lyse the bacterial cell wall. The DNA was then tested for *femB*, *mecA*, and *mecC* by uniplex PCR using the same primers and thermocycling conditions as previously described (Paterson et al. 2012). *mecA* and *mecC* produce 155- and 188-pb products, which are close to each other on gel electrophoresis (Paterson et al. 2012). Thus, uniplex PCR were performed. The *mecA*-positive NCTC 12493 and the *mecC*-positive isolate LGA251 were used as positive controls (courtesy of Paterson).

### Antimicrobial resistance testing

Antibiotic susceptibility profiles were determined for all of isolates by disc diffusion method on Mueller Hinton agar (Oxoid Ltd.) using 15 different antimicrobial discs (Oxoid Ltd.) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAT 2016). Briefly, pure frozen cultures were grown in on Mueller Hinton agar overnight at 37 °C and three to five colonies were adjusted to 0.5 McFarland turbidity in normal saline tubes. The isolates were tested for resistance to penicillins (penicillin; 10 units) and oxacillin (ox, 1 µg), cephalosporins (cefoxitin (FOX, 30 µg)), fluoroquinolones (ciprofloxacin (CIP, 5 µg)), tetracyclines (tetracycline (TE, 30 µg)), macrolides (clindamycin (DA, 2 µg) and erythromycin (E, 15 µg)), phenicols (chloramphenicol (C, 30 µg)), folate pathway inhibitors (sulfamethoxazole-trimethoprim (SXT, 25 µg)), aminoglycosides ((gentamicin (CN, 10 µg)), oxazolidinones (linezolid (LZD, 10 µg)), fusidic acid (FD, 10 µg), neomycin (N, 10 µg), mupirocin (MUP, 200 µg), and rifampicin (RD, 5 µg) (Oxoid) according to EUCAST Guidelines (2016). Only five discs per plate were tested to avoid overlapping zones of inhibition. *S. aureus* ATCC 29213 were used for quality control and were tested in each replicate. Inhibition zones were measured after 18 h of incubation at 35 °C and the diameter of inhibition around each disc was measured and interpreted according to EUCAST Guidelines (EUCAST 2016).

An isolate was considered resistant when it was resistant to one or more antimicrobials, and considered multidrug

resistant (MDR) when it was resistant to three or more classes of antimicrobials (Schwarz et al. 2010). Moreover, an isolate was classified as high or medium resistance if each tested resistant to > 50 or ≤ 50% of antimicrobials, respectively.

### Data analyses

The data were entered into MS Excel (Redmond, WA, USA) and prevalence, confidence intervals, and proportions were calculated using IBM SPSS 21.0 software (IBM SPSS Corp., Armonk, NY, USA).

## Results

### Prevalence of *mecA*- and *mecC*-positive MRSA in bulk tank milk

From the 208 bulk tank milk samples tested in this study, 54 samples tested positive for *mecA* *S. aureus* as confirmed by PCR and 86 unique *mecA* MRSA isolates were obtained as determined later by the antibiotic resistance profiles for isolates from the same milk sample. None of the samples carried *mecC* MRSA. The prevalence of *mecA* MRSA in bulk tank milk sampled from cattle, sheep, and goat milk was 20.0, 39.1, and 11.9%, respectively (Table 1). While, the farm level prevalence in cattle, sheep, and goat farms was 31.8, 30.4, and 15.4%, respectively (Table 1).

### Antimicrobial resistance of *mecA*-positive *S. aureus* isolated from cattle, sheep, and goat bulk tank milk

The percentage of antimicrobial-resistant isolates from bulk tank milk sampled is shown in Table 2. Nearly all isolates exhibited resistance toward penicillin, oxacillin, and cefoxitin, while most isolates exhibited resistance toward gentamicin (86.0%), clindamycin (86.0%), rifampicin (80.2%), neomycin (79.1%), fusidic acid (79.1%), erythromycin (75.6%), tetracycline (75.6%), and ciprofloxacin (70.9%). On the other hand, a lower percentage of isolates exhibited resistance toward mupirocin, sulfamethoxazole-trimethoprim, linezolid, and chloramphenicol, 41.9, 30.2, 20.9, and 15.1%, respectively (Table 2).

All (100%) of the *mecA* MRSA isolates were resistant to at least one class of antimicrobials (Fig. 1). However, all goats, 88% of cattle, and 87% of sheep isolates exhibited resistance to three classes of antimicrobials (i.e., exhibited multidrug resistance [MDR]). All goat isolates were also resistance to 8 and more classes of antimicrobials and 25 and 19% of cattle and sheep isolates exhibited resistance to 11 or more classes of antimicrobials. Four percent of sheep isolates were resistant to all 13 classes of antimicrobials tested (Fig. 1).

**Table 1** Bulk tank and farm-level prevalence of *mecA* *S. aureus* from cattle, sheep, and goats bulk milk in Jordan, 2015–2016. Data shown as percent; 95% CI

	Cattle	Sheep	Goats	Total
Bulk tank level	20.0% (11–29%) ( <i>n</i> = 80)	38.4% (27.9–48.9%) ( <i>n</i> = 87)	11.9% (1.7–22.1%) ( <i>n</i> = 42)	26% (20–32%) ( <i>n</i> = 208)
Farm-level	31.8% (17.5–46.1%) ( <i>n</i> = 44)	29.8% (16.2–43.4%) ( <i>n</i> = 47)	11.5% (–1.6–24.7%) ( <i>n</i> = 26)	26.5% (18.4–34.6%) ( <i>n</i> = 117)

### Antimicrobial resistance profiles

Forty-seven antimicrobial resistance profiles were exhibited by the MRSA isolates; 17 profiles by isolates from cattle, 32 profiles by isolates from sheep, and 5 profiles by isolates from goats (Supplementary Table). The profiles were diverse, especially for cattle isolates, and most profiles were exhibited by one isolate only. Three cattle and six sheep isolates exhibited the same profile, which corresponds to 14 antimicrobials (RdLzdNSxtEDaCipPOxTeCnFoxFdMup) (Supplementary Table).

### Discussion

The prevalence of MRSA and its resistance to a wide range of antimicrobials are growing worldwide concerns (Vanderhaeghen et al. 2010b). Over the last two decades, scientists have reported increasing prevalence of the *mecA* gene which carries resistance to multiple antibiotics (Pantosti et al. 2007; Spohr et al. 2011; Kreausukon et al. 2012; Al-Ashmawy et al. 2016). *mecA* MRSA strains in bovine milk samples have been reported in several countries (Kwon et al. 2005; Holmes and Zadoks 2011; Feßler et al. 2012). During this time period,

*mecA*-negative MRSA strains in bovine milk samples have also been reported (Kumar et al. 2010; Wang et al. 2014). This could be explained by a recent report that some MRSA *S. aureus* can possess *mecA* homologues like *mecC* or other mechanisms that lead to  $\beta$ -lactam resistance (Garcia-Alvarez et al. 2011; Paterson et al. 2012; Paterson et al. 2014). This study adds to the growing literature about the prevalence of *mecA* and *mecC* MRSA and presents the first set of data from Jordan on this issue.

This study did not recover *mecC*-positive *S. aureus* in cattle, sheep, and goat bulk milk. This corroborates a previous study that reported that no *mecC* was detected in bovine bulk milk from 625 farms in Scotland. However, the same study detected *mecC*-positive *S. aureus* in 10 of 465 dairy cattle farms sampled (prevalence 2.2%) in England and Wales (Paterson et al. 2014). Also, in Europe, one isolate of *mecC*-positive *S. aureus* was reported in sheep milk, where this isolate was detected from 601 *S. aureus* isolates obtained from milk samples of 229 dairy sheep farms in Spain (Ariza-Miguel et al. 2014). *mecC*-positive *S. aureus* was also isolated at a low rate in Finland (1 isolate from 135 *S. aureus* isolates) from bovine mastitic milk samples (Gindonis et al. 2013).

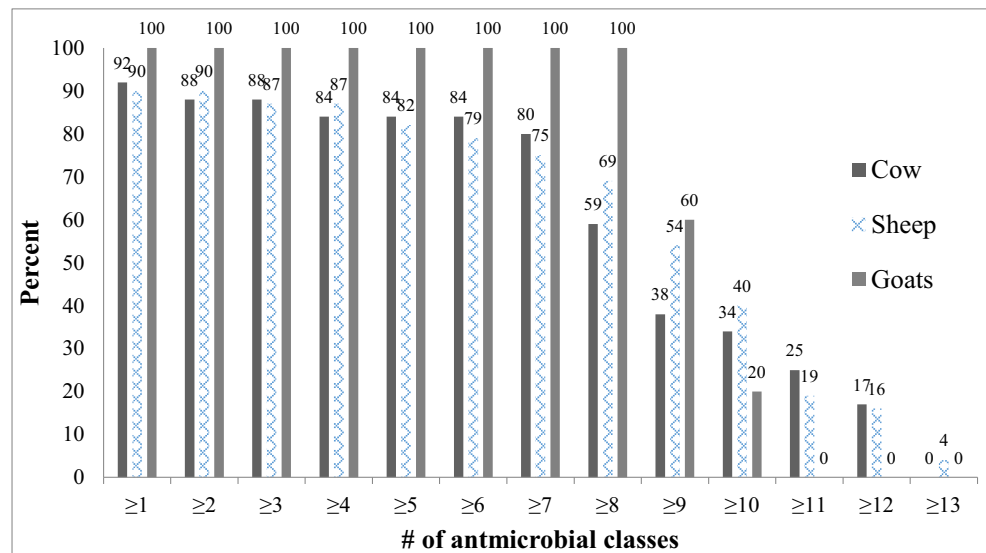
Our study detected *mecA* *S. aureus* in 31.8, 29.8, and 11.5% of the cattle, sheep, and goat dairy farms in Jordan.

**Table 2** Percentage of antimicrobial resistance *mecA* *S. aureus* isolates from cattle, sheep, and goats bulk tank milk sampled in Jordan, 2015–2016

Antimicrobial (breakpoint, mm)	Cattle <i>n</i> = 22	Sheep <i>n</i> = 59	Goat <i>n</i> = 5	Total <i>N</i> = 86
Penicillin ( $\leq 26$ )	100	100	100	100
Oxacillin ( $\leq 14$ )	100	100	100	100
Cefoxitin ( $\leq 22$ )	100	98.4	100	98.8
Gentamicin ( $\leq 18$ )	86.4	84.8	100	86.0
Clindamycin ( $\leq 19$ )	81.9	86.5	100	86.0
Erythromycin ( $\leq 23$ )	81.9	79.7	80	80.2
Neomycin ( $\leq 16$ )	77.3	78	100	79.1
Fusidic acid ( $\leq 24$ )	77.3	78.0	100	79.1
Erythromycin ( $\leq 18$ )	59.1	81.4	80	75.6
Tetracycline ( $\leq 19$ )	63.7	79.7	80	75.6
Ciprofloxacin ( $\leq 20$ )	41	84.8	40	70.9
Mupirocin ( $\leq 18$ )	54.6	37.3	40	41.9
Sulfamethoxazole-trimethoprim ( $\leq 14$ )	36.4	28.9	20	30.2
Linezolid ( $\leq 19$ )	22.8	22.1	0	20.9
Chloramphenicol ( $\leq 18$ )	13.7	17	0	15.1



**Fig. 1** Percentage of *mecA* *S. aureus* isolates from bulk tank milk samples exhibiting multidrug resistance by species in Jordan 2015–2016



Our sample size was based on the largest number of samples needed to detect  $50 \pm 10\%$ , and further research with larger sample sizes is needed to confirm these results. In contrast, most worldwide studies to date have reported low *mecA*-positive *S. aureus* prevalence. For example, an older US study did not detect MRSA in a nationally representative sample of bulk tank milk conducted by the US Department of Agriculture's (USDA) National Animal Health Monitoring System (NAHMS) using phenotypic and genotypic methods (USDA 2011). In Northern Italy, *mecA*-positive *S. aureus* was detected in 2% (4 out of 197) in bulk tank milk samples from dairy goat farms (Cortimiglia et al. 2015). In Southern Italy as well, the prevalence of MRSA in bovine bulk tank milk was 2.5% (12/486) (Parisi et al. 2016) and in 2009 and 2010, the prevalence was 4.4% (Kreausukon et al. 2012). No *mecA*-positive *S. aureus* was detected in 100 bulk milk tank and 200 raw cheese samples in Switzerland in 2009 (Huber et al. 2010). The wide range in prevalence reported by the different studies might be related to several factors including geographical location, sensitivity of the isolation methods, antimicrobial use practices, farm biosecurity, production techniques, and sample storage and handling (Sader et al. 2006; Chen et al. 2010). Our study targeted MRSA through primary enrichment media followed by isolation on MRSA selective media rather than isolating *S. aureus* then determining the *mecA* gene in the isolates. Thus, our approach, which was used by several recent studies, might lead to higher recovery of *mecA*-positive *S. aureus* from the milk samples (Paterson et al. 2014).

Despite their significant contribution as sources of milk in different countries worldwide, there is a scarcity of data on the prevalence of methicillin-resistant *S. aureus* in small ruminants' farms. Our study revealed high rates of *mecA*-positive *S. aureus* in sheep (29.8% farm level) and goat (11.5% farm

level) dairy farms. These rates are higher than those reported in other countries. For example, 0% of sheep farms and 0 to 2% of goat farms were positive in from Italy (Cortimiglia et al. 2015; Caruso et al. 2016). A low prevalence (0.3%) was also reported in bulk tank milk in Italian dairy sheep farms (Carfora et al. 2016). Our study showed that the prevalence in sheep is higher than that in cattle in Jordan. This is similar to findings from Iran (Rahimi et al. 2015). Although the latter study reports nasal carriage of *mecA*-positive *S. aureus* in ruminants, it showed that the prevalence in sheep and goat was 14.1 and 25%, but the prevalence in cattle was 5.1% (Rahimi et al. 2015).

In our study, nearly all *mecA*-positive *S. aureus* isolates exhibited resistance to penicillin, oxacillin, and cefoxitin; meanwhile most isolates exhibited resistance toward gentamicin (86.0%), clindamycin (86.0%), rifampicin (80.2%), neomycin (79.1%), fusidic acid (79.1%), erythromycin (75.6%), tetracycline (75.6%), and ciprofloxacin (70.9%). In Egypt, similar trends were reported. Specifically, about 87.9 and 65.2% of the *mecA*-positive *S. aureus* isolates from raw milk and dairy product were resistance to penicillin and tetracycline, respectively (Al-Ashmawy et al. 2016). Meanwhile, small percentages of the isolates exhibited resistance toward sulfamethoxazole/trimethoprim (25%), ciprofloxacin (30%), and gentamicin (37%) in Egypt (Al-Ashmawy et al. 2016). Similarly, *mecA*-positive *S. aureus* showed high resistance to penicillin-G, ampicillin, amoxicillin-clavulanate, tetracycline, erythromycin, and gentamicin from cattle milk in India and high resistance to tetracycline in studies from India and Germany (Kumar et al. 2010; Kreausukon et al. 2012). A German study also reported low rates of resistance to chloramphenicol and ciprofloxacin (Kreausukon et al. 2012). Resistance to gentamicin, clindamycin, and erythromycin is

of special concern, as these antimicrobials are among the few drugs used for treating bovine mastitis that are not based on  $\beta$ -lactams.

Our study showed that in total, 47 different resistance patterns were exhibited by the *mecA*-positive *S. aureus* isolates, of which 43 were exhibited by only one, two, or three isolates. These data from Jordan are noteworthy for the high resistant percentage and diverse resistance profiles of the *mecA*-positive *S. aureus* isolates to the tested antimicrobial agents. The findings of this study are similar to those of other studies. For example, ten resistance patterns were exhibited by 25 *mecA*-positive *S. aureus* isolates from bovine mastitis in Germany, in which resistance to only  $\beta$ -lactam antimicrobials and tetracyclines was the most common pattern and exhibited by 36% of isolates (Feßler et al. 2010). Other studies reported different antimicrobial resistance patterns by the *mecA*-positive *S. aureus* recovered from different raw milk and dairy products (Can and Çelik 2012; Al-Ashmawy et al. 2016; Xing et al. 2016). The heterogeneity of antimicrobial resistance patterns among the *mecA*-positive *S. aureus* isolates may reflect the microbial adaptive response to local antimicrobial usage practices, geographical location, and farm biosecurity and management (Cuny et al. 2015). Farm management and infrequent cleaning/sanitation could be associated with the presence of *mecA*-positive *S. aureus* in farms (Graveland et al. 2010; Dorado-García et al. 2013). This is supported by the persistence of the *mecA*-positive *S. aureus* in dairy goat farms, where the same the *mecA*-positive *S. aureus* strain was isolated from both bulk tank milk and the udder of three goats 1 year after the first isolation (Cortimiglia et al. 2015). Similarly, a study in South Korea found identical *mecA*-positive *S. aureus* strains in the same animals within the same farm after 1 year (Lim et al. 2013). This phenomenon was reported over two periods in Italian dairy sheep farms as well (Carfora et al. 2016).

The high prevalence of multidrug resistant *mecA*-positive *S. aureus* can have a serious impact on animal health and can result in an economic impact to the farmer. Moreover, there are serious public health implications for the high prevalence of multidrug-resistant *mecA*-positive *S. aureus*, namely, it can be disseminated into human populations and can lead to nosocomial MRSA infections. For example, a review of this topic showed that livestock-associated MRSA accounts for up to 10% of MRSA septicemia and 15% of MRSA wound infections in humans (Cuny et al. 2015). The rate of zoonotic transmission can pose greater risk for certain occupational groups particularly like farmers and veterinarians compared to the general population (Garcia-Graells et al. 2012; Klous et al. 2016) and isolates with identical MRSA genotype from farmers and goats' milk suggesting the zoonotic potential of MRSA have been reported (Caruso et al. 2016; Carfora et al. 2016). In addition, a study showed that MRSA carriage in people is strongly associated with both intensity of animal

contact and the percentage of MRSA-positive animals on a farm (Graveland et al. 2010). The latter study also reported that livestock-associated carriers lived significantly closer to a livestock farm compared to non-carriers (Zomer et al. 2016). Finally, the presence of *mecA*-positive *S. aureus* in raw milk in Jordan indicates that consuming raw milk and traditional dairy products like Bayda cheese could serve as a potential risk of foodborne infection especially in light of a recent study that found antimicrobial resistance was associated with harboring enterotoxin genes in *S. aureus* isolates (Obaidat et al. 2015). Thus, in Jordan, consumers may consider avoiding the consumption of unpasteurized milk products.

In conclusion, the high prevalence of multidrug-resistant *mecA*-positive *S. aureus* in this study shows that there is a need for strict hygienic measures in the production of raw milk and dairy products to prevent the spread of multidrug-resistant *mecA*-positive *S. aureus*.

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**Compliance with ethical standards** Farm owners were assured that the study was for research purposes only and that any data pertaining to their specific farm would be available to them upon request free of charge. Consent to collect the milk samples was obtained from the owner of each farm prior to starting the study.

**Competing interests** The authors declare that they have no competing interests.

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