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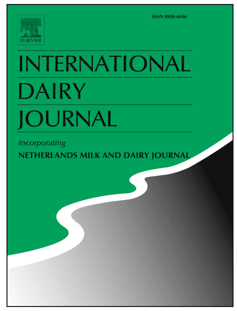

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Antibiotic-resistance, enterotoxin gene profiles and farm-level prevalence of S*taphylococcus aureus* in cow, sheep and goat bulk tank milk in Jordan

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ABSTRACT

Bulk tank milk was collected from 44 cow, 47 sheep and 26 goat farms to determine antibiotic resistance, enterotoxin gene profiles, and *Staphyococcus aureus* prevalence in Jordan. *S. aureus* (n = 169) was detected in 69.2% of farms; 65.9% cow, 68.1% sheep, and 76.9% goat farms. Thirty-three (19.5%) isolates harboured enterotoxin genes, *sec* was the most prevalent (75.8%) and six profiles were observed. Resistance to $\geq$1 antibiotic class and multidrug resistance were exhibited by 93.5% and 59.2% of isolates, respectively. One goat and two sheep isolates resisted seven classes. More than 50% of the isolates resisted penicillin, ampicillin, and clindamycin and 30–40% resisted tetracycline, gentamicin, rifampin and erythromycin. About 20% resisted cefotaxime, doxycycline and amoxicillin–clavulanic acid. Meanwhile, 10% resisted chloramphenicol, sulfamethoxazole-trimethoprim and ciprofloxacin. The resistance found is alarmingly higher than elsewhere and necessitates programs to promote judicious use of antibiotics in Jordan.

1. Introduction

Staphylococcus aureus can cause several diseases in human and animals, including foodborne intoxication in humans and mastitis in animals (Hennekinne, De Buyser, & Dragacci, 2012; Ruegg, 2012). Staphylococcal foodborne intoxication, which results from ingesting food contaminated with staphylococcal enterotoxin, is an important public health issue worldwide (Hennekinne et al., 2012; Painter et al., 2013). Several food types provide a suitable environment for *S. aureus* growth and enterotoxin production, including milk and milk products (Cretenet, Even, & Le Loir, 2011; Hennekinne, et al., 2012; Kadariya, Smith, & Thapaliya, 2014; Sabike, Fujikawa, Sakha, & Edris, 2014). *S. aureus* contaminates milk through contaminated containers, unhygienic farm environments, unhygienic handling, mastitic udders and other routes (Jørgensen, Mørk, Høgasen, & Rørvik, 2005; Kümmel, et al., 2016). Even though *S. aureus* can be killed during pasteurisation, the heat stable enterotoxins may survive the pasteurisation process and cause intoxication (Hennekinne et al., 2012; Oliver, Boor, Murphy, & Murinda, 2009).

In dairy ruminants, *S. aureus* can cause contagious mastitis, which decreases milk production and leads ultimately to economic and financial losses for the dairy industry (Botaro et al., 2015; Tesfaye, Regassa, & Kelay, 2010). The dairy industry in Jordan, which includes cow, sheep and goat milk, is an important economic sector and contributes $160 million yearly to the national gross domestic product (DoS, 2015). A recent report from Jordan found that mastitis is very common in dairy ruminants and between 30–51% of mastitis cases require veterinary interventions (Obaidat, Al-Zyoud, Bani Salman, & Davis, 2017; Obaidat, Bani Salman, Davis, & Roess, 2018). Several antibiotics are used to treat mastitis in Jordan including amoxicillin, tetracyclines and gentamicin (Obaidat et al.,

2017) and this may result in emerging resistance in Jordan as it has elsewhere (Pantosti, Sanchini, & Monaco, 2007).

Globally, studies usually determine the prevalence of *S. aureus* in individual herds or in a few preselected herds (or characterise isolates from mastitis cases or milk samples with no reference to prevalence (Cortimiglia et al., 2015; Fessler et al., 2010; Vanderhaeghen et al., 2010). In Jordan, anecdotal evidence from field veterinarians and from our diagnostic laboratory observed a significant proportion of drug resistant mastitis cases in dairy ruminants that require expensive and lengthy treatment and in many cases culling (Jebreen and Zuraikat, personal communication). In addition to its animal health impact, in subclinical mastitis, *S. aureus* infection does not change the organoleptic characteristics of milk (Le Maréchal, Thiéry, Vautor, & Le Loir, 2011). Thus, the probability of foodborne intoxication through the consumption of milk and dairy products exists. Moreover, the production of unpasteurised cheese (Bayada cheese) from bulk milk is widely practiced in Jordan and other Middle Eastern countries (Hilali, El-Mayda, & Rischkowsky, 2011).

Bulk tank milk (BTM) testing can be a valuable and inexpensive approach to determine the herd prevalence of *S. aureus* compared with testing individual milk samples. This study aimed to shed light on the magnitude of the animal and public health impact of *S. aureus* in dairy ruminants in Jordan. Specifically, the objectives were to (1) determine the prevalence of *S. aureus* in bulk tank milk from cow, sheep and goat farms in Jordan, (2) investigate the proportion of *S. aureus* isolates that carry enterotoxin genes, (3) determine the antibiotic resistance percentages, multidrug resistance of *S. aureus* among isolates and (4) determine the antibiotic resistance pattern the *S. aureus* isolates.

2. Materials and methods

2.1. Population

In Jordan, the dairy cow production system is divided into two systems; large and small scale, and both systems raise Holstein-Friesian dairy cows. The large system depends on zero-grazing, utilises modern management practices and is located in the East-Northern area of Jordan (Al-Dulial area) that produces approximately 60% of the country's milk (DoS, 2015). The small system houses cows in small brick barns, with traditional management practices, and is scattered in various regions of Jordan, mostly in the Northern Highland area.

Small ruminant farms in Jordan can be divided into two major production systems; extensive and semi-extensive and these are scattered throughout the country. The extensive system is located primarily in the Northern highlands that receive high amounts of rainfall and are occupied by small herders who use rangelands under constant search for grass and water (Tarawneh & Kadıoğlu, 2003). But, the semi-extensive system is located in the Badia in eastern and southern Jordan which is arid to semi-arid and occupied by nomadic or pastoralist Bedouin (Tarawneh & Kadıoğlu, 2003). Both systems rely on low production technologies (Tarawneh & Kadıoğlu, 2003).

2.2. Sample size

The prevalence of *S. aureus* in dairy ruminant farms in Jordan is unknown. Using the sample size formula $n= z^2 p(1-p)/d^2$; where n is the sample size, z is the statistic for a level of confidence (equal 1.96 at 95% level of confidence), p is the expected prevalence (p = 0.5) and d is precision (d is 0.1), the needed sample size is 96 farms. We randomly selected 117 farms (44 cow; 47 sheep; 26 goat farms) from a list of farms provided by local veterinary associations to account for an expected 10–15% refusal rate based on our previous experience conducting similar surveys in Jordan. An equal number of farms from each region were selected to ensure representativeness. We selected 22 dairy cow farms in Al-Dulail area and 22 from the Northern highlands and 35 sheep and goat farms from Northern Jordan and 37 farms from Southern Jordan and the Badia. Samples were collected between December 2015 and March 2016. This period was chosen because parturitions in sheep and goats occur between December to March in Jordan, while parturitions in cow usually occur year around.

2.3. Sampling approach

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

2.4. Isolation of S. aureus *from the milk samples*

Frozen samples were thawed at 37 °C and 2 mL of milk were mixed with 8 mL of Mueller–Hinton broth (Oxoid, Hampshire, England) supplemented with 6.5% NaCl and incubated at 37 °C under shaking at 200 rpm. Samples were then streaked onto Baird-

Parker agar (Oxoid) supplemented with egg yolk tellurite emulsion (Oxoid). Agar plates were incubated at 35 °C for 48 h. Suspected *S. aureus* colonies (black surrounded with halo zone) were stored in TSB with 20% buffered glycerol at –20 °C for subsequent DNA isolation, molecular confirmation, enterotoxin gene profiling and antibiotic resistance testing.

2.5. Molecular confirmation of S. aureus *and detection of enterotoxin genes*

Each *S. aureus* isolate was revived in 1 mL of Tryptone Soya Broth (TSB) **(**Oxoid) with 6.5% NaCl and incubated at 37 °C for 24 h (Obaidat, Bani Salman, & Lafi, 2015). The genomic DNA of each isolate was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions after using 20 mg mL^{-1} lysozyme (Sigma-Aldrich, St. Louis, MO, USA) to lyse the bacterial cell wall. *S. aureus* isolates were confirmed using the *nuc* gene as previously described (Obaidat et al., 2015). To detect enterotoxin genes, two multiplex PCR reactions were performed (Obaidat et al., 2015). The first reaction was carried out to detect the "classical" enterotoxin genes (*sea, seb, sec, sed, see)*, and the second reaction to detect three of the new enterotoxin genes (*seg, seh,* and *sei)*. Multiplex PCR reactions were carried out using the same primers, concentration and cycling conditions as described elsewhere (Vázquez-Sánchez, López-Cabo, Saá-Ibusquiza, & Rodríguez-Herrera, 2012). The positive controls for the first reaction were *S. aureus* ATCC 13565 for *sea* and *sed*, ATCC 19095 for *sec*, ATCC 14458 for *seb*, and ATCC 27664 for *see* and the positive control for the second reaction was ATCC 19095 (Obaidat et al., 2015).

2.6. Antibiotic susceptibility testing

S. aureus isolates were tested for resistance towards thirteen antibiotics that belong to nine antibiotic classes. Resistance testing was performed by the disk diffusion method on Mueller-Hinton agar (Oxoid, Hampshire, UK) according to the Clinical and Laboratory Standards Institute Standards (CLSI, 2014). Bacterial suspensions were adjusted to a 0.5 McFarland turbidity in normal saline tubes. Isolates were tested for resistance against (Oxoid Ltd.) β-lactams [penicillin, (P, 10 units), ampicillin (Amp, 10 μg), amoxicillin–clavulanic acid (Amc, 30 μg), and cefotaxime (Ctx, 30 μg)], quinolones [ciprofloxacin (Cip, 5 μg)], aminoglycosides [Cn, gentamicin (10 μg)], phenicols [chloramphenicol (C, 30 μg)], folate pathway inhibitors [sulfamethoxazole–trimethoprim (Sxt, 25 μg)], tetracyclines [tetracycline (Te, 30 μg), doxycycline (DA, 30 μg)], lincosamides [clindamycin (Da, 2 μg)], ansamycins [rifampin (Rd, 5 μg)] and macrolides [erythromycin, (E, 15 μg)].

Zones of inhibition were measured after 18 h of incubation at 35 °C. *S. aureus* ATCC 25923 was tested in every replicate for quality control. Any isolate with intermediate susceptibility to a tested antibiotic was considered susceptible for this study's purpose. Any isolates that demonstrated resistance to at least one antibiotic were considered resistant and isolates that were resistant to at least three classes of antibiotics were considered multi-drug resistant (Magiorakos et al., 2012). An isolate was classified as highly resistant if it was resistant to ≥ 50% of antibiotics, moderate if it was resistant to < 50% to ≥ 10%, and low if it was resistant to < 10%.

2.7. Statistical analysis

Data were entered in Microsoft Excel and analysed using IBM SPSS 20.0 software for windows (IBM SPSS Corp., Armonk, NY, USA). If no suspected *S. aureus* colonies grew on the Baird Parker plates from a farm milk sample, this farm was considered negative for *S. aureus*. Meanwhile, if suspected *S. aureus* colonies grew, up to three colonies from that farm sample were further confirmed and tested for enterotoxins and antibiotics resistance. Frequencies were calculated to determine the percentage of isolates resistant to the tested antibiotics. Chi-square and Fisher's exact test were used as appropriate to determine statistically significant differences.

3. Results

3.1. Prevalence of S. aureus *in dairy ruminant farms*

S. aureus was isolated from bulk tank milk in 69.2% (81/117) of farms (95% CI, 60.4–76.9 %), specifically the prevalence in cow, sheep and goat farms were 65.9% (95% CI, 51.1–78.1%), 68.1% (95% CI, 53.8–79.6%), and 76.9% (95% CI, 57.9–88.9%), respectively. A total of 169 unique *S. aureus* isolates were obtained and further analysed. Isolates were obtained from each type of animal milk (cow, n = 53), (sheep, n = 78), and (goat, n = 38).

3.2. Enterotoxigenicity of S. aureus

In total, 19.5% (33/169) of the isolates harboured enterotoxin genes; specifically, *sec* was the most frequently carried gene (25/ 169; 14.8%), followed by *sei* (9/169, 5.3%) and *seg* (5/169, 3%). Only two and one isolates carried the *sea* and *sed*; respectively. None of the isolates carried the *seb*, *see* and *seh* genes (Table 1). Isolates obtained from cows had the most diversity in enterotoxin profiles (Fig. 1).

3.3. Antibiotic resistance

Most of the *S. aureus* isolates (94%; 158/169) demonstrated antibiotic resistance to one and more of the tested antibiotics (Table 2). A high percentage of cow, sheep and goat isolates were resistant to penicillin G (92%, 80%, and 90%, respectively), ampicillin (70%, 77%, and 76%, respectively) and clindamycin (70%, 59%, and 66%, respectively). A higher percentage of the cow isolates demonstrated resistance to tetracycline (60%) and gentamicin (53%), compared with sheep (41% and 22%, respectively) and goat (26% and 32%, respectively) isolates. About one-third of isolates from the three animal species exhibited resistance to rifampin (32%, 33%, and 29%, respectively), erythromycin (30%, 33%, and 21%, respectively) and cefotaxime (23%, 22%, and 21%, respectively). The cow, sheep and goat isolates exhibited resistance to doxycycline (24%, 24%, and 13%, respectively) and amoxicillin-clavulanic acid (11%, 24%, and 21%, respectively). In general, a low percentage (< 10%) of isolates exhibited resistance to chloramphenicol, sulfamethoxazole-trimethoprim and ciprofloxacin (Table 2).

The multi-antibiotics resistance by *S. aureus* isolates is shown in Fig. 2. In general, 94% of the isolates were resistant to one or more of the antibiotic classes tested, 74% toward two or more classes and 59.2% toward three or more classes (multidrug resistance) (Fig. 2). One goat and two sheep (from two separate farms) isolates were resistant to seven classes of antibiotics and 21% of isolates were resistant to five or more classes of antibiotics. A greater percentage of cow isolates demonstrated resistance to at least one antibiotic class; 98% of cow compared with 91% of sheep and 92% of goat isolates. More cow isolates also demonstrated resistance to three or more antibiotic classes (multidrug resistance); 71%, 66% and 48% of cow, sheep and goat isolates; respectively (Fig. 2).

Forty antibiotic resistance profiles were exhibited by the *S. aureus* isolates from the three animal species. *S. aureus* from the sheep exhibit the highest number of patterns (29 patterns), followed by cow (27 patterns) and goat (19 patterns). Eleven resistance patterns were shared by isolates from the three animal species (Supplementary table). The most prevalent resistance pattern was penicillin G- ampicillin- clindamycin- rifampin – erythromycin-amoxicillin–clavulanic acid (P-Amp-Da-Rd-E-Amc) (18 isolates, 11%) followed by penicillin- ampicillin (10 isolates, 6%) (Supplementary material, Table S1). Four cow isolates had resistance to seven antibiotics; namely, penicillin- ampicillin- clindamycin- rifampin- erythromycin -amoxicillin–clavulanic acid (P-Amp-Da-Te-Cn-Rd-Ctx) (Supplementary material, Table S1).

4. Discussion

Worldwide raw cows' milk is a common source of *S. aureus* (Oliver et al., 2009). In this study, a high prevalence (66%) of *S. aureus* was found in the bulk tank milk of dairy cow farms. High rates were reported on dairy cow farms in both resources-rich and resources-poor countries. For example, high rates were reported in Minnesota (84%; Haran et al., 2012), Wisconsin, USA (73%; Sato, Bennedsgaard, Bartlett, Erskine, & Kaneene, 2004) and in other parts of the USA (55%; Cicconi-Hogan et al., 2013), Denmark (85%; Sato et al., 2004), Norway (75%; Jørgensen et al., 2005), China (50%; Bi et al., 2016), Northern Italy (47.2%; Cortimiglia et al., 2015), Prince Edward Island, Canada (73; Olde Riekerink et al., 2006) throughout Canada (74%; Olde Riekerink, Barkema, Scholl, Poole, & Kelton, 2010), Tunisia (50%; Ben Said et al., 2016), São Miguel Island, (Azores) (59%;

Azevedo et al., 2016), north-western Greece (40%; Papadopoulos et al., 2018) and Northern Ethiopia (34%; Tarekgne et al., 2016). This contamination level might be attributed to improper farms management and milking practices (Ruegg, 2003).

Despite the widespread use of small ruminant dairy products, often a main source of dairy in resource-poor settings, there are a limited number of studies on the prevalence and antibiotic resistance patterns of *S. aureus* in small ruminants. In this study, a high prevalence of *S. aureus* was detected in sheep (68%) and goat (77%) farms. Similar high prevalence rates were reported elsewhere; 96% of goat milk samples in Norway (Jørgensen et al., 2005), 63.2% sheep and 80% goat (80%) bulk tank milk in north-western Greece (Papadopoulos et al., 2018) and 46% of sheep and goat bulk tank milk samples in Switzerland (Merz, Stephan, & Johler, 2016). The high prevalence of *S. aureus* in sheep and goat bulk tank milk highlight the risk from unpasteurised milk and milk products, which are commonly consumed in Jordan and in other Middle Eastern countries (Hilali et al., 2011).

Alarmingly high numbers of antibiotic resistant *S. aureus* isolates were detected from bulk tank milk from all three animal species studied in Jordan. Specifically, 94% of *S. aureus* isolates from dairy ruminants' bulk milk exhibited antibiotic resistance to one or more of the tested antibiotics. Lower percentages of antibiotic resistant isolates were reported from other resources-poor countries. For example, 41 and 28% of cow isolates showed resistance to at least one antibiotic in Iran and Malaysia, respectively (Jamali, Paydar, Radmehr, Ismail, & Dadrasnia, 2015; Shamila-Syuhada, Rusul, Wan-Nadiah, & Chuah, 2016). Meanwhile, 46% of sheep milk isolates exhibited resistance to at least one antibiotic in Iran (Jamali et al., 2015). However, the only antibiotic resistant isolate identified from cow, sheep and goat bulk milk in Australia was a single isolate which was resistant to penicillin (McMillan, Moore, McAuley, Fegan, & Fox, 2016).

Resistance to penicillin G and ampicillin was exhibited by a large number of *S. aureus* isolates from the three ruminant species in this study. This corroborates the findings of several other studies that reported a high rate of resistance (≥ 70%) to these antibiotics; from cow, sheep and goat milk in north-western Greece (Papadopoulos et al., 2018), from cow milk in Ethiopia, Iran and China (Daka, G/silassie & Yihdego, 2012; Jamali, Radmehr, & Ismail, 2014; Shi, Hao, Zhang, Wulan, & Fan, 2010) and from cow and sheep milk in Iran (44% and 51% for penicillin G; Jamali et al., 2015), and Taiwan (70% for penicillin; Chu et al., 2013). In contrast, a low prevalence (≤17%) of resistance to ampicillin and penicillin were exhibited by *S. aureus* isolates from raw cow milk in Vermont, USA (D'Amico & Donnelly, 2011). Similarly, a low prevalence of penicillin resistance (7%) was exhibited by sheep milk isolates in from transhumant farms in Greece (Zdragas et al., 2015). The differences by country might be related to the regulation and use of these antibiotics for food animal production, with resources-rich countries (USA) generally enforcing more stringent regulations while resources-poor countries having fewer regulations and enforcement of antibiotic use in food animal production. Moreover, the low prevalence of resistance in Greece might be attributed to the sampled sheep farms that use ‘transhumant breeding system (Zdragas et al., 2015). The general agreement in the literature about high resistance toward β-lactam antibiotics may be because these antibiotics were the first against which *S. aureus* developed resistance.

In our study, 20 to 30% of *S. aureus* isolates exhibited resistance to tetracycline, gentamicin, rifampin, erythromycin, cefotaxime, and doxycycline. Other studies reported similar percentages of *S. aureus* resistance to tetracycline and gentamicin (Chu et al., 2013; Kumar, Yadav, & Singh, 2010). Studies in China and Mexico reported somewhat similar percentages of resistance to rifampin (Ochoa-Zarzosa et al., 2008; Shi et al., 2010). In contrast, lower percentage (≤12%) of the cow milk isolates from studies in Greece and

Malaysia were resistant to tetracycline and erythromycin (Pexara, Solomakos, Sergelidis, Angelidis, & Govaris, 2016; Shamila-Syuhada, et al., 2016). In contrast again, about 50% of cow and sheep milk isolates were resistant to tetracycline and a low percentage (≤8%) of isolates resistant to gentamicin, erythromycin and kanamycin in Iran (Jamali et al., 2015) which are similiar to reported perecentages in north-western Greece for isolates from cow, sheep and goat bulk tank milk (Papadopoulos et al., 2018). Another study from Iran reported that a low percentage of cow, sheep and goat milk isolates were resistant to tetracycline and erythromycin (Alian et al., 2012). In addition, a low percentage (<3) of sheep milk isolates were resistant to tetracycline and erythromycin in transhumant farms Greece (Zdragas et al., 2015).

Similar to our study several others report no to low resistance to sulfamethoxazole-trimethoprim (SXT), chloramphenicol, ciprofloxacin and amoxicillin–clavulanic acid in *S. aureus* isolates from milk sample. In Greece, for example, two studies reported no resistance to SXT in cow, sheep and goat milk isolates (Papadopoulos et al., 2018; Pexara et al., 2016), while another study there reported that about 1% of sheep milk isolates from transhumant farms were SXT resistant (Zdragas et al., 2015). No resistance to chloramphenicol and ciprofloxacin was exhibited by cow, sheep and goat milk isolates in Iran but a low percentage were resistant (8%) to SXT (Alian et al., 2012). Similar findings were reported from Ethiopia (no resistance to ciprofloxacin, 8% to SXT resistant cow milk *S. aureus* isolates (Daka et al., 2012) and from Iran (no resistance to ciprofloxacin and ≤5% chloramphenicol resistant cow and sheep milk isolates) (Jamali et al., 2015).

Due to the variation in the panel of tested antibiotics among studies, comparing our resistance patterns with previous studies is somewhat difficult. In addition, a limitation to our study is that the isolates were not tested for oxacillin or methicillin resistance. Nonetheless, forty different antibiotic resistance patterns were exhibited by the 169 *S.*

aureus isolates in our study and the two most prevalent patterns were penicillin G- ampicillin- amoxicillin–clavulanic acid - rifampin - erythromycin – clindamycin and penicillin- ampicillin. In other studies, the number and structure of the patterns differ, but some similarities are noted. For example, Ochoa-Zarzosa et al. (2008) detected 32 different patterns; where penicillin- ampicillin was the most prevalent pattern exhibited by *S. aureus* isolates from dairy cows with subclinical mastitis in Mexico.

In this study 20% of *S. aureus* isolates from each of the three animal species carried enterotoxin genes; specifically, 13% of dairy, 22% of sheep and 18% of goat isolates. Similar percentages (10–28%) were reported in isolates from US dairy cow milk (Oliveira, Rodrigues, Hulland, & Ruegg, 2011), Brazilian goat milk (Ferreira, Carvalho, Nardelli, Sousa, & Oliveira, 2014), Serbian cow milk (Rajic-Savic, Katic, Velebit, Colovic, 2015) and cow milk in Brazil (de Freitas Guimarães et al., 2013). In contrast, a number of studies detected a high percentage of enterotoxigenic *S. aureus* isolates in milk. For example, a high percentage (45–75%) was reported in milk isolates in Italy, France, Norway, Spain, Sweden and Ethiopia (Bianchi et al., 2014; Haenni et al., 2011; Jørgensen et al., 2005; Linage, Rodriguez-Calleja, Otero, Garcia-Lopez, & Santos, 2012; Rosengren, Fabricius, Guss, Sylven, & Lindqvist, 2010; Tarekgne et al., 2016). These findings of high percentages of enterotoxin carriage among *S. aureus* isolates necessitate the maintenance of the cold chain to prevent the production of the *S. aureus* pasteurisation, protease and processing- stable enterotoxins (Hennekinne et al., 2012).

In our study, the most frequent gene carried by the *S. aureus* isolates from the three animal species was *sec* (15% of the isolates). This result is consistent with other studies, which were carried out in Italy, Brazil and Switzerland (Basanisi et al., 2016; Cremonesi et al., 2006; Ferreira et al., 2014; Riva et al., 2015; Scherrer, Corti, Muehlherr, Zweifel, & Stephan, 2004; Vimercati et al., 2006) and all demonstrated a high prevalence of the *sec* gene among *S. aureus* isolated from cow, sheep and goat milk samples. Several studies showed that around 20% *S. aureus* dairy cow isolates carried *sea*; specifically, in Turkey, Ethiopia and Italy (Boynukara, Gulhan, Alisarli, Gurturk, & Solmaz, 2008; Rall et al., 2014; Tarekgne et al., 2016; Vimercati et al., 2006) and 53% in Brazil (Rall et al., 2014). We found the presence of *sea* in one isolate from sheep milk and one from goat milk but none from cow milk. The presence of enterotoxin genes on genetic mobile elements (phages, plasmids and pathogenicity islands) allows them to transmit among different isolates by horizontal gene transfer (Malachowa & DeLeo, 2010; Moore & Lindsay, 2001).

Here, six different enterotoxin genes patterns were detected in the 33 enterotoxin – positive *S. aureus* isolates and the most diversity was observed in isolates obtained from cow milk. Similarly, in Italy 15 different enterotoxin profiles were identified in 35 cow, sheep and goat milk isolates and there was also more diversity in the cow milk samples (Carfora et al., 2015). In addition, 35 different enterotoxin gene profiles were distinguished among 255 *S. aureus* isolates from milk and dairy products in Italy (Bianchi et al., 2014). While 18 profiles were found in 57 *se*-positive isolates in Ethiopia (Tarekgne et al., 2016). This variation likely results from differences in the country or geographical location, and the number of tested enterotoxin genes. For example, the most recent studies test for all of the new enterotoxin and the enterotoxin-like genes; so the comparison with different studies could be somehow difficult.

5. Conclusions

The findings of this study suggest that urgent interventions are needed to control the emergence and spread of *S. aureus* in dairy ruminants to ultimately protect the milk supply from contamination with this pathogen. Further, in Jordan, as in much of the world, there is a growing demand for raw cows' milk and coupled with the fact that raw milk is a common source of *S. aureus* and other pathogens a growing number of raw milk consumers may be at an increased risk for food intoxication. Monitoring resistance patterns is important given that resistant *S. aureus* causes subclinical and clinical mastitis that are difficult to treat, result in poor animal health and economic losses for the farmer. Surveillance systems should also consider monitoring enterotoxins in the milk supply. Finally, interventions should be adopted to reduce the sources of milk contamination with *S. aureus* such as more frequent cleaning of animal housing areas, and educating famers on udder hygienic scoring charts. Moreover, adopting uniform milking practices such as pre-dipping with iodine and forestripping should be considered.

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Figure legends

Fig. 1. Number (n) of *S. aureus* isolates bearing SE gene profiles from (a) cow, (b) sheep, and (c) goat bulk tank milk samples in Jordan, Dec 2015–March 2016.

Fig. 2. Percentage of *S. aureus* isolates from cow (■), sheep (■) and goat (■) bulk tank milk (and, on the left of each set, total: ■) that exhibited resistance to one or more antibiotic classes in Jordan, Dec 2015–March 2016.

Table 1

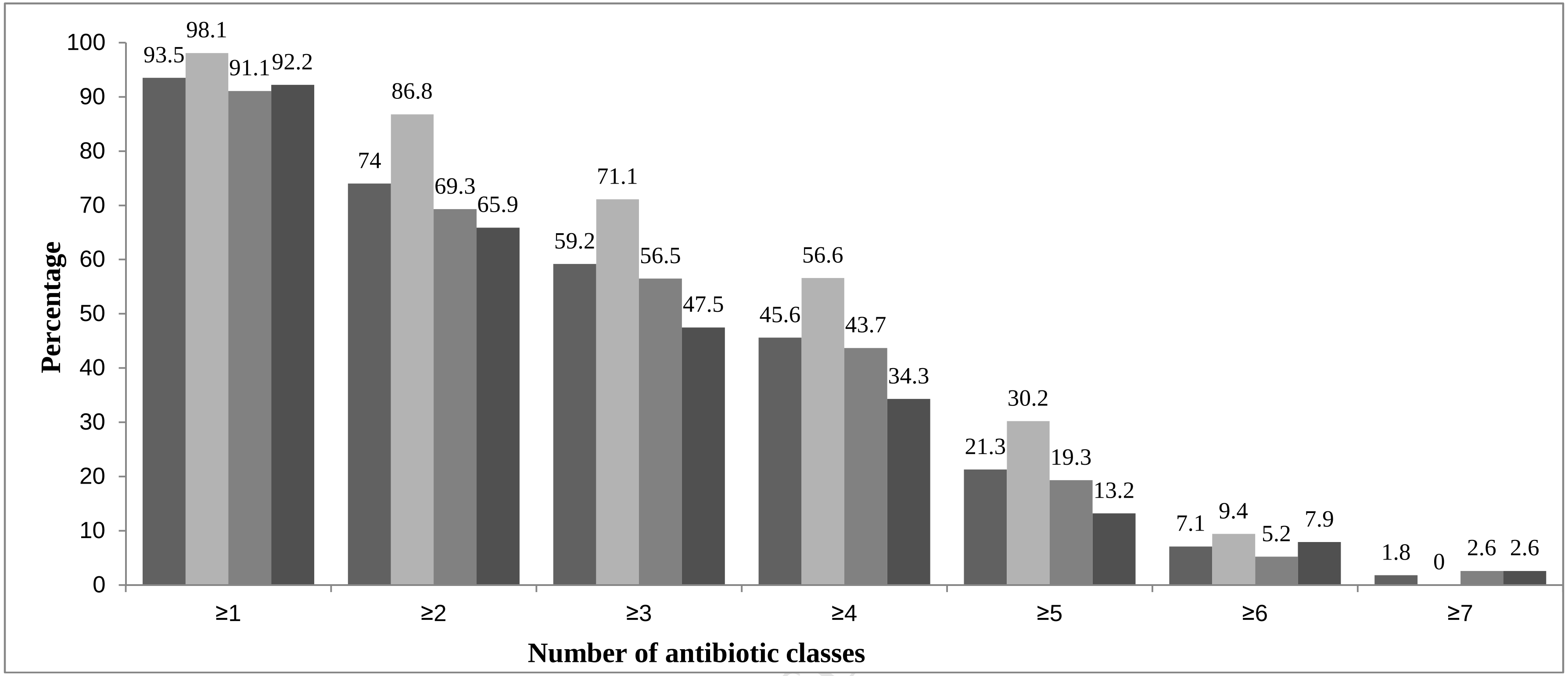
Number and percentage of enterotoxin gene profiles of *S. aureus* isolates from cow, sheep and goat bulk tank milk samples in Jordan, Dec 2015–March 2016.

Enterotoxin genes	Cow (n = 53)	Sheep (n = 78)	Goat (n = 38)	Total (n = 169)
sea		1 (1.3%)	1 (2.6%)	2 (1.2%)
seb				
sec	5 (9.4%)	12 (15.4%)	8 (21.1%)	25 (14.8%)
sed		1 (1.3%)		1 (0.6%)
see				
seh				
sei	2 (3.8%)	5 (6.4%)	2 (5.3%)	9 (5.3%)
seg	1 (1.9%)	3 (3.8%)	1 (2.6%)	5 (3.0%)
Enterotoxin profiles				
sec	5 (9.4%)	11 (14.1%)	6 (15.8%)	22 (13.0 %)
sei	1 (1.9 %)	2 (2.6%)	1 (2.6 %)	4 (2.4%)
sec-sei-seg		1 (1.3%)	1 (2.6%)	2 (1.2%)
sei-seg	1 (1.9%)	2 (2.6%)		3 (1.8%)
sea-sec			1 (2.6%)	1 (0.6%)
sea-sed		1 (0.6%)		1 (0.6%)

Table 2

Percentage of antibiotic resistant *S. aureus* isolates from cow, sheep and goat bulk tank milk samples in Jordan, Dec 2015–March 2016.

Antibiotics (breakpoints, mm)	Cow (n = 53)	Sheep (n =78)	Goat (n = 38)	Total (n =169)
Penicillin (≤ 28)	92.5	79.5	89.5	85.8
Ampicillin (≤ 28)	69.8	76.9	76.3	74.6
Clindamycin (≤ 14)	69.8	59.0	65.8	63.9
Tetracycline (≤ 14)	60.4	41.0	26.3	43.8
Gentamicin (≤ 12)	52.8	21.8	31.6	33.7
Rifampin (≤ 16)	32.1	33.3	28.9	32.0
Erythromycin (≤ 13)	30.2	33.3	21.1	29.6
Cefotaxime (≤ 14)	22.6	21.8	21.1	21.9
Doxycycline (≤ 12)	24.5	24.4	13.2	21.9
Amoxicillin–clavulanic acid (≤ 19)	11.3	24.4	21.1	19.5
Chloramphenicol (≤ 12)	3.8	3.8	2.6	4.1
Sulfamethoxazole-trimethoprim (≤ 10)	7.5	3.8	0.0	4.1
Ciprofloxacin (≤ 15)	1.9	5.1	2.6	3.6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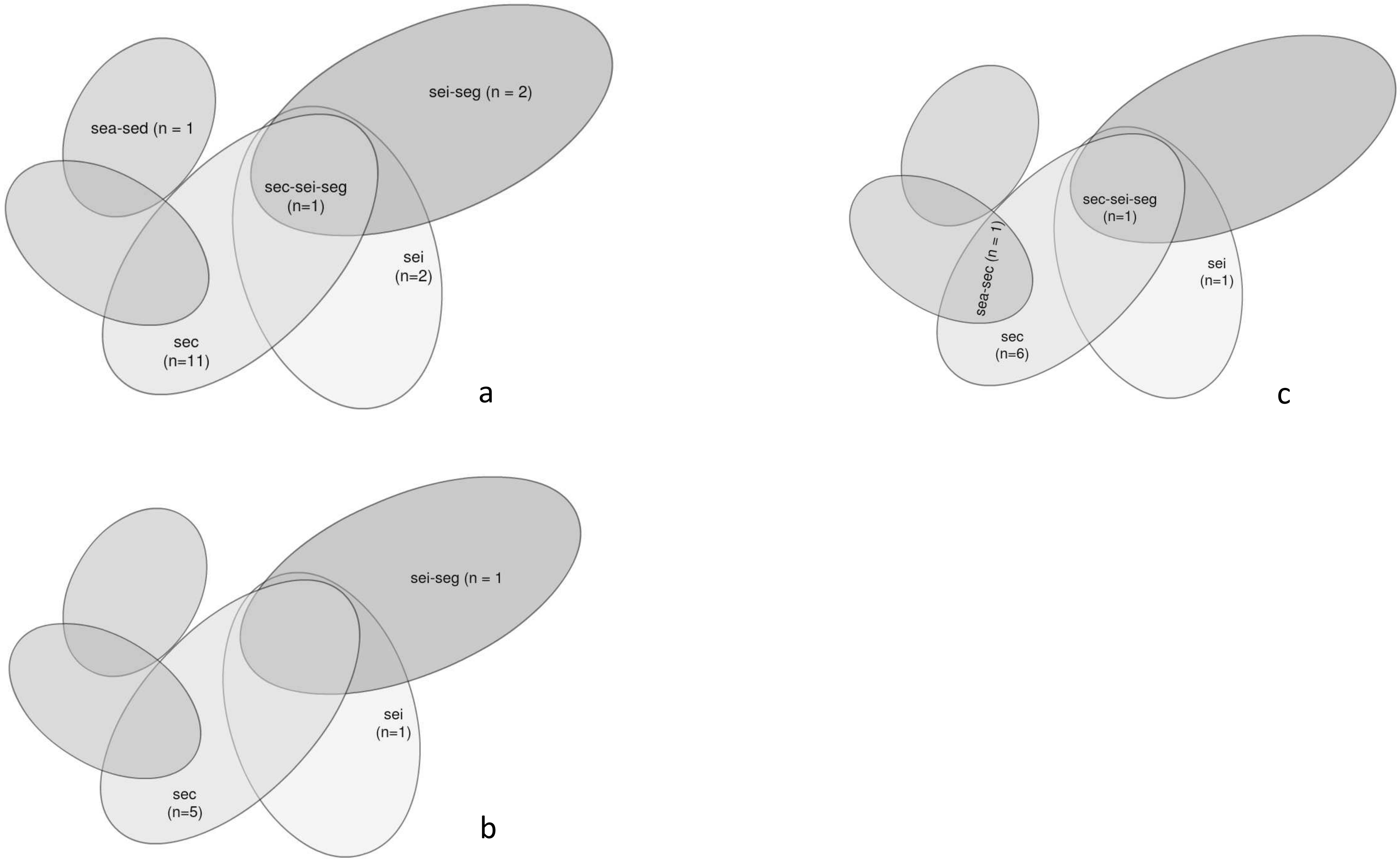
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