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Detection and Molecular Characterization of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) Nasal Carriage Isolates from Healthy Domestic Animal in Duhok Province

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THE study was aimed to determine and characterize the nasal carriage rate of *S. aureus* among apparently healthy farm animals including cattle, sheep, and goats using traditional and molecular characterization. The study was conducted in Duhok province during the period from November 2021 to March 2022. Furthermore, the isolates were screened for the presence of MRSA using the standard Kirby-Bauer disk diffusion method (oxacillin discs) and genotypically by PCR targeting the *mecA* gene. Among the 300 tested nasal swabs, 209 (69.7%) samples were positive for *S. aureus* isolation using traditional methods. The isolation rate was 62.7% (47/75), 66.7% (100/150), and 82.7% (62/75) in cattle, sheep, and goats, respectively. Amplification of species-specific *nuc* gene confirmed that 119 of 209 (56.9%) animals carried *S. aureus* in their nasal cavity. The isolates showed variation toward different antibiotics used in this study and the highest resistance rate was recorded toward penicillin at a ratio of 72.3% (86/119). This study confirms the occurrence of MRSA for the first time in nasal swabs from a healthy animal in Duhok Province. The MRSA isolates were found only in cattle (7/119) and none of the nasal carriages isolates from sheep and goats were carried MRSA isolates. The presence of MRSA and multidrug-resistant MRSA among healthy cattle could be considered as a potential reservoir for transmission of multidrug-resistant MRSA to humans especially farm workers and they could act as a reservoir to spread MRSA in livestock. Further studies are required for a better understanding of pathogenic transmission and for confirming the origin of the strains, whether are of human origin or vice versa.

Keywords: MRSA, Nasal carriage, Healthy animals, Multidrug-resistant MRSA, Duhok

Introduction

Staphylococcus aureus is a commensal and a major opportunistic pathogen causing a wide range of diseases in both humans and animals. It causes a high impact on public health and the livestock industry [1]S. aureus is a Gram-positive, non-motile, non-spore-forming, facultative anaerobic cocci commensal pathogen that colonizes different parts of the body, including the skin, nares, and mucosal surfaces of humans and animals [2]. Worldwide, S. aureus is considered a major pathogen causing clinical or subclinical mastitis in lactating sheep, goats, and cows [3]. It has also been suggested that nasal carriage may represent a major reservoir for S. aureus to contaminate the udder and milk in dairy farms [4]. This pathogen has been frequently reported from the nares of farm animals including cattle, sheep, and goats [5-8]. The major concern about S. aureus is the

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adaptation to the diverse environment and the development of antimicrobial resistance toward multiple antibiotics. The level of resistance is growing very quickly, especially to commonly used antibiotics against staphylococcal infections such as β-lactams, macrolides, and tetracyclines [9]. Methicillin-resistant S. aureus (MRSA) strains are resistant to all β-lactam antibiotics and the resistance is mediated by the acquisition of mecA, which encodes a penicillin-binding protein (PBP) [10]. mecA is localized within the mobile genetic element known as the staphylococcal cassette chromosome (SCCmec) [10]. One of the public health concerns is MRSA in animals and food and there could be a subsequent transmission of MRSA to humans from animals because they could act as reservoirs of MRSA. Colonization of people in contact with infected or colonized animals has been widely reported for small animals [11]. It has also been shown that MRSA especially multidrug- resistant was distributed among the examined healthy farm animals and could represent a potential reservoir for multidrug-resistant MRSA with public health implications [9]. Yet, to our knowledge, no studies have been conducted in Duhok Province to investigate the prevalence rate and characterization of S. aureus from the nasal cavity of healthy ruminates. Therefore, this study aimed to determine the prevalence and nasal carriage rate of S. aureus among apparently healthy farms animals; to determine the susceptibility profile to different antibiotics; to detect the occurrence rate of multidrug-resistant S. aureus among the isolates, and investigate the presence of methicillin-resistant Staphylococcus aureus (MRSA) among the healthy animals.

Material and Methods

Sample collection

Three hundred (300) samples of nasal swabs were collected randomly from apparently healthy animals from different areas of Dohuk governorate for the period from November 2021 to March 2022. The sample were distributed as follow: cattle (75), sheep (150), and goats (75). The samples were collected from the nostrils of healthy animals by using sterile cotton swabs. The swab was first moistened with normal saline and inserted into nares and then rotated gently so it can make contact with the nasal septum as mentioned by Hakim et al.[12]. The swabs were directly steaked on mannitol salt agar and then the plates were transported directly to Microbiology Laboratory at Duhok Research Centre/ College of Veterinary Medicine and incubated for 24 h at 37°C.

Phenotypic characterization of S. aureus using standard conventional methods

Isolation and identification of *S. aureus* were performed using traditional methods . The suspected colonies (mannitol fermenter) were subcultured on MSA to obtain pure culture and incubated for 24 h at 37°C. Colonies with typical morphology including mannitol fermenter colonies were selected and subjected to Gram-staining, catalase, and coagulase test [13]. Phenotypically suspected *S. aureus* isolates including Gram-positive grape-like cocci, catalase, and coagulase-positive colonies were maintained and stored in 50% glycerol and brain heart infusion broth (BHIB) stocks at -20°C for further processing including molecular characterization and antibiotic susceptibility test.

Molecular detection of S. aureus

DNA extraction

Suspected colonies of *S. aureus* by traditional methods were further confirmed by the detection of the *S. aureus*-specific thermostable nuclease gene (nuc) by using PCR [14]. DNA was extracted according to the literature [15,16]. Briefly, a few bacterial colonies were resuspended in 500 µl of deionized doubled distilled water and mixed very well using vortex. The mixture was then boiled in a heating block for 15-20 min. The tubes were immediately put on ice and then centrifuged at 10,000 X for 3 min. The supernatant was collected and used as a DNA template PCR. The purity and concentration of DNA were checked using a NanoDrop 2000C spectrophotometer (Thermo Scientific) and the DNA samples were stored at -20°C for further analysis.

Detection of S. aureus specific nuc gene

The *nuc* gene (226bp) was amplified using primer pair nuc-F1 (AGCGATTGATGGTGATACGG) and nuc-R1 (ATACGCTAAGC-

CACGTCCAT)[14]. The PCR reactions were performed in a total volume of 20 µl. Each reaction has consisted of 10 µl of Add Taq Master (ADDBIO Inc, Korea), 2 µl of each primer, 3 µl of DNA, and 3 µl of deionized sterile dH_2O. The following PCR parameters of 35 cycle reactions were used as follows: initial denaturation at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 55°C for 30 s, extension at 72°C for 2 min and a final extension at 72°C for 5 min [14]. Confirmation of amplified *nuc* gene was performed using a 2% agarose gel electrophoresis prepared with TAE (1X) buffer containing Prime Safe Dye (GeNet Bio, Korea). Finally, UV light was used to visualize the fragment size of the *S. aureus* specific *nuc* gene.

Antimicrobial susceptibility of S. aureus isolates

The confirmed *S. aureus* isolates by PCR were selected to investigate the susceptibly patterns of the following 11 antimicrobial agents from different antimicrobial classes using the standard Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) [17]: penicillin (10 µg), oxacillin (1 µg), vancomycin (30 µg), gentamicin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (30 µg), rifampicin (5µg). Briefly, confirmed *S. aureus* isolates were plated out onto MSA from -20°C stock cultures and incubated overnight at 37°C. Pure colonies from overnight MSA cultures were selected to prepare a suspension of 0.5 McFarland turbidity standards in 5 ml of nutrient broth. Uniform bacterial growth was prepared on Mueller-Hinton agar plates using a sterile cotton swab. Antibiotic discs were applied on plates seeded with bacterial lawn and incubated at 37 °C for 24 h. The results were interpreted based on the size of the inhibition zone according to the guidelines that are provided by CLSI [17]. Detection of multidrug-resistant *S. aureus* strains was confirmed by resistance to three or more three antibiotics. The confirmation was done phenotypically by Kirby-Bauer disk diffusion results based on the size of the inhibition zone according to the guidelines provided by CLSI [17].

Molecular detection and confirmation of Methicillin-resistant S. aureus (MRSA) isolates among healthy animals

The molecular confirmation of MRSA was carried out through the amplification of a 533-bp *mecA* gene according to Murakami *et al.* [18]. The PCR reaction was carried out using *mecA*1 (AAAATCGATGGTAAAGGTTGGC) and mecA2 (AGTTCTGCAGTACCGGATTTGC) primers. Amplification was performed in a total reaction of 25 µl including 12.5 µl of Add Taq Master (ADDBIO Inc, Korea), 2 µl of each primer, 3 µl of DNA, and 5.5 µl of deionized sterile dH_2O. The following PCR parameters: initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min [18]. The detection of the *mecA* gene was confirmed using a 2% agarose gel.

Results

Nasal carriage of S. aureus among healthy animals

The results showed that out of the 300 samples of nasal swabs, 209 at the rate of 69.7% were positive for *S. aureus* by using phenotypic characterization. The recovery rate was different; *S. aureus* was isolated from 47 of 75 cattle, 100 of 150 sheep, and 62 of 75 goats at the rate of 62.7%, 66.7%, and 82.7%, respectively (Table 1). Based on phenotypic characteristics all the suspected 209 isolates provided typical characteristics of *S. aureus* where the colonies appeared yellow with yellow background on MSA as a result of mannitol fermentation. Mannitol fermenter isolates were further confirmed by Gram staining and they appeared Gram-positive grape-like cocci. The 209 isolates were catalase positive and finally the results showed that 209 were found to be coagulase positive using slide coagulase test as shown in Table 1

Molecular characterization of S. aureus isolates

Amplification of the *nuc* gene (226 bp) showed that among 209 suspected isolates, 119 isolates were confirmed as *S. aureus* (Table 1). The PCR results confirmed that the prevalence of *S. aureus* was 28/47(59.6%) in cattle, 50/100 (50%) in

sheep, and 41/62 (66.1%) in goats. Therefore, 56.9% (119/209) of the total animals carried *S. aureus* in their nasal cavity (Table 1).

Antibiotic sensitivity test of S. aureus isolates

Antibiotic susceptibility profiles of the 119 *S. aureus* isolates from healthy cattle, sheep, and goats were determined against 11 antibiotics, including beta-lactams and non-beta lactams antibiotics. The sensitivity test revealed that none of the isolates were resistant toward rifampin, trimethoprim/sulphamethoxazole, and gentamicin as shown in Figure 1. Moreover, higher resistance ratios can be seen toward ciprofloxacin 2.5% (3/119), chloramphenicol 4.20% (5/119), clindamycin 5.1% (6/119), oxacillin 5.9% (7/119), vancomycin 6.7% (8/119), erythromycin 22.68% (27/119) and tetracycline 45.37% (54/119). The highest resistance rate was recorded toward penicillin with a ratio of 72.3% (86/119).

The overall susceptibility ratio was the highest for gentamicin (100%), followed by trimethoprim/sulphamethoxazole (99.15%), then two antibiotics that had equal ratios, rifampin, and oxacillin (94.1%). Other isolates showed sensitivity to other antibiotics including ciprofloxacin, chloramphenicol, clindamycin, vancomycin, erythromycin, and tetracycline. However, the sensitivity was with lower ratios as follows ciprofloxacin (88.23%), chloramphenicol (87.39%), clindamycin (78.99%), vancomycin (72.6%), erythromycin (52.1%) and tetracycline (34.45%). Penicillin showed the lowest susceptibility ratio (27.73%) (Figure 1 & Table 2)

Occurrence of multidrug-resistance S. aureus among healthy animals

According to the antibiotic susceptibility test, among 119 isolates, 22 (18.48%) isolates were classified as multidrug-resistant *S. aureus* (MDR-SA) strains because they were found resistant to at least three or more antibiotics used in this study. The resistant profiles were different among the isolates. Among 22 MDR-SA, 16 isolates were resistant to 3 antibiotics, while 5 isolates were resistant to 4 antibiotics. Only one isolate from sheep was found to be resistant to 5 antibiotics. The results identified 12 different resistant patterns as shown in Table 3.

Screening for methicillin (Oxacillin) resistance S. aureus among healthy animals:

The results showed that among the 119 isolates only 7 isolates from cattle were positive and classified as MRSA isolates as they were positive for the *mecA* gene. However, none of the isolates from sheep and goats were found to be *mecA* gene positive and they were classified as MSSA (Table 4). These isolates (7 isolates) from cattle were also found to be resistant to oxacillin phenotypically by antibiotic susceptibility test. Furthermore, the 7 isolates exhibited resistance not only to oxacillin, but they were resistant to several antibiotics, four of the MRSA isolates were resistant to oxacillin, erythromycin, and penicillin, and one of them was resistant to oxacillin, penicillin, and tetracycline. Only one MRSA isolate was resistant to four antibiotics including oxacillin, erythromycin, penicillin, and tetracycline. Therefore, these isolates were classified as multidrug- resistant MRSA isolates.

TABLE 1. Phenotypic and molecular characterization of *S. aureus* nasal carriage isolates from healthy cattle, sheep, and goats.

Animals Species	No. of Samples	Phenotypic characterization					Molecular characterization (PCR)	
		Growth on MSA	Gram staining	Biochemical tests		Rate (%)	No. of confirmed isolates	Rate (%)
				Catalase test	Coagulase test			
Cattle	75	47	47	47	47	62.7	28	59.6
Sheep	150	100	100	100	100	66.7	50	50
Goat	75	62	62	62	62	82.7	41	66.1
Total	300	209	209	209	209	69.7	119	56.9

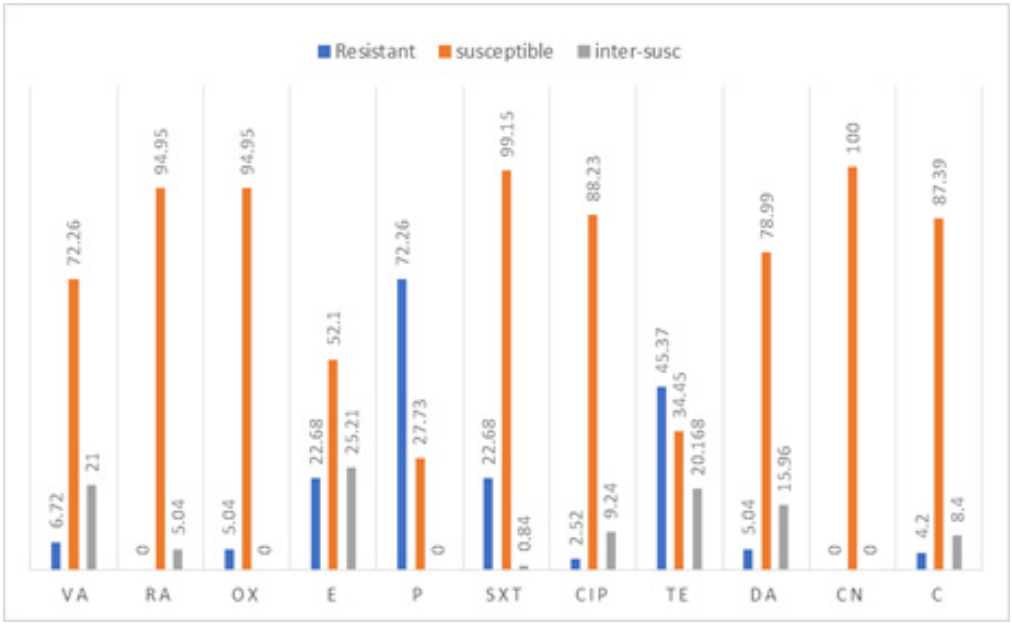

Fig. 1. Antimicrobial sensitivity profile for *S. aureus* nasal carriage isolates

TABLE 2. Antimicrobial sensitivity profile for *aureus* nasal carriage isolates (no: 119)

Antibiotics	Concentrations (µg/disc)	Antimicrobial susceptibility profile					
		Sensitive		Intermediate		Resistance	
		No. of isolates	Rate (%)	No. of isolate	Rate (%)	No. of isolate	Rate (%)
Vancomycin (VA)	30	86	72.6	25	21	8	6.7
Rifampin (RA)	5	113	94.9	6	5.1	0	0
Oxacillin (OX)	1	112	94.1	0	0	7	5.9
Erythromycin (E)	15	61	51.3	31	26.1	27	22.7
Penicillin (P)	10	33	27.7	0	0	86	72.3
Trimethoprim/ sulphamethoxazole (SXT)	25	118	99.2	1	0.8	0	0
Ciprofloxacin (CIP)	5	105	88.2	11	9.24	3	2.52
Tetracycline (TE)	30	41	34.5	24	20.2	54	45.4
Clindamycin (DA)	2	94	78.9	19	15.9	6	5.1
Gentamicin (CN)	10	119	100	0	0	0	0
Chloramphenicol (C)	30	104	87.4	10	8.1	5	4.2

TABLE 3. Multidrug-resistance patterns among 22 *S. aureus* nasal carriage isolates from healthy animals

Multidrug-resistance profiles	Pattern	Number of isolates (%)
OX, E, P	1	4 (18.2)
E, P, TE	2	6 (27.3)
P, TE, DA	3	3 (13.6)
OX, P, TE	4	1(4.5)
P, TE, C	5	1(4.5)
VA, P, TE	6	1(4.5)
OX, E, P, TE	7	1(4.5)
E, P, TE, DA	8	1(4.5)
E, P, TE, C	9	1(4.5)
P, TE, DA, C	10	1(4.5)
VA, P, TE, C	11	1(4.5)
VA, E, P, TE, C	12	1(4.5)
Total		22

TABLE 4. MRSA and MSSA nasal carriage rate among healthy cattle, sheep, and goats

Animal species	No. of nasal swabs	No. of confirmed *S. aureus* isolates	No. of MRSA isolates (rate %)	No. of MSSA isolates (rate %)
Cattle	75	28	7 (25%)	21 (75%)
Sheep	150	50	0 (0%)	50 (100%)
Goats	75	41	0 (0%)	41 (100%)
Total	300	119	7 (5.9%)	112 (94.1%)

MRSA: methicillin resistance *S. aureus*; MSSA: methicillin-sensitive *S. aureus*

Discussion

S. aureus has been recognized as a major pathogen affecting humans and animals. The prevalence and manifestation rate of this pathogen is increasing worldwide [19, 20]. However, there are significant differences between the rate of S. aureus appearance and the rate of MRSA. Moreover, the susceptibility to antibiotics has different outcomes and ratios, and this can be noticed from the difference between the regions utilizing large quantities of antibiotics and regions with lower antibiotic usage [21].

This study is conducted to determine the prevalence of nasal carriage rate of *S. aureus* in healthy farm animals, to investigate the sensitivity profile of *S. aureus* and the occurrence of (MDR-SA) and to detect the occurrence of MRSA among *S. aureus* nasal carriage isolates from cattle, sheep, and goats. Both conventional and molecular methods are important for identifying and diagnosing bacteria. Molecular characterization has the advantage of being a more reliable and sensitive methods for diagnosis [22]. In the present study, all the animals were healthy and show no sign of infection. However, they were carriers of *S. aureus* as it was detected in their nasal cavity. Among 300 nasal swabs using phenotypic characterization, 69.6% (209/300) of the samples tested positive. However, utilizing PCR showed that among 209 isolates, 119 isolates were confirmed as *S. aureus*.

In the current study, S. aureus isolates were found in 59.6% of cattle, 50% of sheep, and 66.1% of goats. Therefore, 56.9% (119/209) of the total animals carry S. aureus in their nasal cavity. Comparing the results to other studies conducted in Iraq and other countries, it can be

noticed that rates of S. aureus prevalence have various rates. For instance, a study conducted in Basra, Iraq by Khudaier et al. [23] showed that the prevalence of S. aureus was lower and the rate was 4%, 12.5%, and 5% in cattle, sheep, and goats, respectively. Furthermore, a previous study conducted in Iran showed lower ratios because the study reported that the ratio of S. aureus in nasal swabs taken from cattle, sheep, and goats 5.06%, 14.1%, and 25%, respectively (1). A study in Pakistan by Anueyiagu et al. [24] included similar farm animals and the results were based on a nasal carriage, revealing that the prevalence rates were 40%, 80%, and 50% in cows, sheep, and goats, respectively. The reason behind the variation in the occurrence of S. aureus in different hosts, might due to various virulence factors that aid the pathogen in invading and colonizing different hosts [25].

The 119 *S. aureus* isolates were tested against several antibiotics. The antibiotics included were from the beta-lactam group and the non-beta-lactam group. The resistance rate toward the well-known beta-lactam antibiotic. Penicillin was the highest among all the animals. In the present study, *S. aureus* from cattle resistance rate was 64.28%. The rate from different regions varies. However, penicillin resistance was the highest among all the antimicrobial drugs. A study from Northern Ethiopia by Kalayu et al. [26] including dairy cows. It is the most common cause of intramammary infections in dairy cows. Its public health importance increases inline to the continuous emergence of drug-resistant strains; such as Methicillin-resistant S. aureus (MRSA noticed that penicillin showed the highest resistance rate, which was 91.7%. Moreover, another study of cattle isolates in Northern Greece by Kotzamanidis et al. [27] revealed that the resistance toward penicillin was the highest (25%). The resistance toward other drugs in *S. aureus* isolates from cattle was also recorded. The penicillin was followed by erythromycin (42.85%) then tetracycline (17.85%). On the other hand, it has been shown that penicillin was followed by tetracycline (35.4%) and then erythromycin (2.1%) [26]including dairy cows. It is the most common cause of intramammary infections in dairy cows. Its public health importance increases inline to the continuous emergence of drug-resistant strains; such as Methicillin-resistant S. aureus (MRSA. However, Kotzamanidis et al. [27]. recorded that tetracycline showed a similar rate as penicillin at a rate of 25%. Another study by Kashoma et al. [28] reported higher resistance toward tetracycline (73%) than erythromycin (16.2%).

The nasal carriage *S. aureus* isolates from sheep and goats showed the highest resistance toward penicillin, being 82% for sheep and 65.85% for goats, followed by tetracycline (74% for sheep, 29.6% for goats) then erythromycin (14% for sheep and 19.51% for goats). These results were in agreement with Ünal et al. [29], where the resistance in sheep and goats isolates showed the same descending resistance pattern: the highest for penicillin (19%) followed by tetracycline (4.8%) and erythromycin (4%).

All the isolates from cattle, sheep, and goats were 100% susceptible to gentamicin. These results were in line with Kashoma et al. [28] and *S. aureus* isolates from cattle showed no resistance toward gentamycin. 100% sensitivity to gentamicin was also recorded by Bendahou et al. [30]. A low rate of resistance to gentamycin was recorded and it was only 4.8% (in sheep and goats) [29]. Moreover, all the isolates were not resistant to rifampin and the same results were recorded by Anueyiagu et al. [24] and the total resistance rate to rifampin was 0%. Furthermore, 100% susceptibility to SXT was found in this study, similar results were presented by Abdel-Moein & Zaher [9]195 nasal swabs from apparently healthy farm animals (52 sheep, 51 goats, 47 cattle and 45 buffalo and they reported that isolates from cattle, sheep, and goats were susceptible to SXT.

Generally, *S. aureus* is susceptible to ciprofloxacin and chloramphenicol. In this study, the resistance rate to ciprofloxacin and chloramphenicol was 2.52% and 4.2%, respectively. Almost similar results were reported by Anueyiagu et al. [24], as being 0% susceptibility in three species) for both antibiotics. Similar results were reported in three species for these both antibiotics and they were found to be 4% resistance to ciprofloxacin and 100% susceptibility to chloramphenicol [23].

Total resistance to clindamycin was also low, as it was 5.04%. Khudaier et al. [23] reported that the total resistance was 8.33%, and Abdel-Moein & Zaher [9]195 nasal swabs from apparently healthy farm animals (52 sheep, 51 goats, 47 cattle and 45 buffalo showed that all the isolates from cattle, sheep, and goats were highly susceptible to clindamycin. Moreover, it has been reported that *S. aureus* from cattle has a 97.9% susceptibility

to clindamycin [26]including dairy cows. It is the most common cause of intramammary infections in dairy cows. Its public health importance increases inline to the continuous emergence of drug-resistant strains; such as Methicillin-resistant S. aureus (MRSA.

Susceptibility to vancomycin was noticed to be high as it was 72.26%. However, variation in results was indicated in different studies. For instance, a study showed that the *S. aureus* isolates were 40.28% susceptible to vancomycin [23]. In contrast, it was reported that sensitivity to vancomycin was 100% in all three species [31]. Vancomycin has been selected as an alternative antibiotic of choice for treating *S. aureus* infection mostly MRSA infections and increases in the resistance to vancomycin might cause risk for treatment of infection caused by MRSA, especially multidrug-resistance MRSA infection.

The differences among the antimicrobial indicated diversity in resistance rates among different regions in the world. The interpretation of diversity is increased hygienic procedures and risen surveillance measures in some countries more than others [25]. Improved hygienic procedures help with reducing the opportunity for *S. aureus* to spread. Surveillance helps in the early identification of *S. aureus* emergence, hence earlier treatment and isolation of infected species [32]. Moreover, high antibiotic usage plays an important role in the resistance rate. For instance, it was believed that vancomycin was highly effective toward *S .aureus*. However, in developed countries, where there is better access to drugs, the increased usage of vancomycin led to the appearance of vancomycin-resistant strain of *S. aureus*. In contrast, in developing countries, where vancomycin is barely available, vancomycin resistance is not problematic [33].

Among 119 isolates, 22 isolates demonstrated multidrug-resistance characteristics, and within the 22 isolates only 7 isolates from cattle harbored the *mecA* gene and none of the sheep and goats isolates contained the *mecA* gene. All 7 isolates were resistant to oxacillin phenotypically by disc diffusion test and screening for the *mecA* gene. This is because of the *mecA* gene function which is a production of PBP 2a, which in turn increase the resistance toward beta-lactams such as methicillin and oxacillin. The results of this study indicated that only (7/119) 5.9% of the animals carried MRSA strains. These findings alert the personnel and owners of the farm because these animals can be possible reservoirs of this pathogen in the farm and the community. Comparing *mecA* gene results to the other studies, 0% of MRSA strains were detected [23], and also it has been reported that no MRSA was found in the nasal swabs of goats and cattle [12]. It has also been reported only 5/73 (6.84%) nasal swabs from sheep were MRSA [5]. It has been recorded that the *mecA* gene detection and the occurrence of MRSA were very low among healthy cattle, sheep, and goats [34]. The rate was only 0.49% (1/201) and one MRSA was found only in a nasal swab from sheep [34]. On the other hand, it has been reported the presence of MRSA in 3 species at the rate of 4.3% in cattle, 3.8% in sheep, and 3.9% in goats [9].

The 7 MRSA isolates showed resistance not only to oxacillin but also to other antimicrobials, including penicillin, erythromycin, and tetracycline, making them multidrug-resistant MRSA. Gharsa et al. [5] also reported that MRSA isolates from sheep, presented resistance to more than three antibiotics, in addition to beta-lactams, to streptomycin, kanamycin, erythromycin, and clindamycin, tetracycline thus being multidrug-resistant MRSA as well. It was presented that all the MRSA isolates from sheep, cattle, and goats exhibited resistance to 3-5 antibiotics [9]. The occurrence multidrug-resistant MRSA among apparently healthy cattle, sheep, and goats points out the role of such animals in the epidemiology of multidrug-resistant MRSA strains which may act as a threat to public health [9].

Conclusion

This study showed high nasal carriage of *S. aureus* among apparently healthy animals. Cattle isolates harbored multidrug-resistant MRSA. The presence of MRSA and multidrug-resistant MRSA among healthy cattle could be considered as a potential reservoir for the transmission of multidrug-resistant MRSA to humans, especially farm workers. None of sheep and goats isolates were found to be positive *mecA* gene.

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Conflict of Interest

The authors declare that no conflict of interest.

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الكشف والتوصيف الجزيئي لعزلات المكورات العنقودية وMRSA المتواجدة في أنف الحيوانات المستأنسة السليمة في محافظة دهوك

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هدفت هذه الدراسة الى تحديد وتوصيف نسبة تواجد المكورات العنقودية في الانف لحيوانات المزرعة السليمة ظاهرياً والتي تشمل الأبقار الضأن والماعز باستخدام التوصيف المظهري والجزيئي. أجريت هذه الدراسة في محافظة دهوك/ إقليم كردستان العراق فى الفترة مابين تشرين الثاني٢٠٢١ و آذار٢ ٢٠٢ .فضلا عن ذلك فقد تم الكشف عن تواجد MRSA باستخدام طريقة Kirby-Bauer لفحص الحساسية للمضادات الحيوية بطريقة الانتشار في الاقراص (oxacillin discs) وتوصيفها جينياً باستخدام تقنية تفاعل البلمرة المتسلسل PCR للكسف عن الجين mecA. فمن بين ٣٠٠ عزلة أنفية تم فحصها كانت ٢٠٩ (٦٩,٧٪) موجبة للمكورات العنقودية باستخدام الطرق المظهرية. وكانت نسبة العزل ٦٢,٧٪ (٤٧/٧٥) و ٦٦,٧٪ (١٠٠/١٥٠) و ٨٢,٧٪ (٦٢/٧٥) في الابقار والضأن والماعز على التوالي. واظهر تضخيم الجين *nuc* والخاص بتحديد النوع ان ١١٩ من٢٠٩ (٥٦,٩٪) من الحيوانات تحمل المكورات العنقودية في التجويف الانفي. كما واظهرت العزلات تنوع في مقاومتها لمختلف المضادات الحيوية المستخدمة في هذه الدراسة. وسجلت اعلى نسبة مقاومة للبنسلين وبنسبة ٧٢,٣٪ (١١٩/٨٦). اثبتت هذه الدراسة وجود MRSA في مسحات التجويف الانفي للحيوانات السليمة في محافظة دهوك ولأول مرة. وأن عزلات MRSA وجدت فقط في الابقار (١١٩/٧) ولم تكن العزلات الأنفية من الضأن والماعز حاملة للنوع MRSA. إن وجود MRSA و MRSA ذات المقاومة المتعددة في الابقار السليمة يمكن ان تيعتبر خازن مهم لانتقال MRSA ذات المقاومة المتعددة للإنسان وخصوصاً العاملين في المزارع والذين بدورهم يمكن ان يكونوا خازناً لانتشار MRSA في الماشية. واخيرا، هناك حاجة إلى مزيد من الدراسات من أجل فهم أفضل لانتقال العوامل الممرضة ولإثبات مصدر العترات الممرضة سواء كانت من الانسان أو العكس من ذلك.

الكلمات الدالة, MRSA**:** حيوانات السليمة ظاهرياً MRSA , ذات المقاومة المتعددة ,دهوك