



## Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolated from Shepherds and Apparently Healthy Goats and Sheep in Egypt



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### Abstract

THE OBJECTIVE of this study was to assess the prevalence, antimicrobial susceptibility, and distribution of virulent genes in *Staphylococcus aureus* (*S. aureus*) and MRSA isolates from healthy sheep and goats, as well as their shepherds. The study included 50 sheep, 50 goats, and 32 shepherds. Three types of samples were collected from each animal - milk samples, nasal swabs, and rectal skin swabs, while the shepherds provided thirty-two nasal swabs and 32 hand swabs. Biochemical tests were used to identify *S. aureus* colonies, and cefoxitin disk diffusion was employed to identify MRSA isolates. The isolates were then tested for *nuc*, *mecA*, *PVL*, and *VanA* genes. *S. aureus* was detected in 84.20% of the tested sheep samples, 51.33% in goats and 84% among shepherds. MRSA was present in 54% of milk samples, 58% of nasal swabs, and 48% of skin swabs in sheep. In goats, MRSA was found in 59%, 42%, and 58%, respectively. In shepherds, MRSA isolates were detected in 88% of nose swabs and 90% of hand swabs. All MRSA isolates were multi-drug resistant, with penicillin G showing the highest resistance (100%). In conclusion, sheep, goats, and their shepherds, may play a significant role in the transmission of *S. aureus*. Moreover, the presence of MRSA in raw milk, nose swabs, and skin swabs may contribute to the spread of the bacteria to other individuals.

**Keywords:** *S. aureus*, MRSA, small ruminants, *mecA*, panton valentine leukocidin, Antibiotics resistance.

### Introduction

Sheep are an important source of red meat in Egypt, accounting for 99,322 tons of fresh milk and 6% of the country's total red meat output [1]. Goat rearing has also become a significant aspect of animal production in Egypt, with goats being bred for both meat and milk, which accounts for 2.7% of the country's total meat production [2]. *Staphylococcus* bacteria are classified into various species and subspecies, with *S. aureus* being one of the most important opportunistic pathogens. This bacterium is the primary cause of nosocomial skin infections and community-associated infections, and its pathogenicity is primarily due to various components such as leucocidins, proteases, hemolysins, and toxins [3, 4]. *S. aureus* strains produce a variety of toxins, including staphylococcal enterotoxins (SEs), enterotoxin-like proteins (SEL), and staphylococcal toxic shock syndrome toxin (TSST) [4].

The close proximity of animals during lambing and kidding creates an ideal environment for infection to spread rapidly. As a result, small ruminants' milk, skin, feces, and nasal cavities are considered potential sources of staphylococcal infections and environmental contamination [5]. This bacterium has been linked to a variety of disorders in humans and animals [6]. *S. aureus* infections in humans can cause arthritis, sepsis, bacteremia, pneumonia, food poisoning, and nosocomial infections [7]. It has also been linked to mastitis in dairy animals, such as cattle, buffalo, sheep, and goats, resulting in significant economic losses in dairy farms due to reduced milk output and poor milk quality [8].

*S. aureus* has a high degree of variability, allowing it to spread at different periods and locations with varied clonal types and antibiotic resistance profiles across regions and countries [9]. Methicillin Resistance *Staphylococcus aureus*

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(MRSA) has emerged as a major health hazard for both humans and animals. Excessive and uncontrolled use of antimicrobial drugs causes these issues [10].

Some studies have investigated the prevalence of MRSA in milk from small ruminants, but the consumption of dairy products made from raw goat and sheep milk is still widespread, particularly in the Mediterranean region, where raw milk is used to make traditional cheeses [11]. Despite the limited research on *S. aureus* and MRSA in small ruminants, the current study aimed to provide up-to-date information on the prevalence of these organisms in healthy sheep and goats, as well as their shepherds, and to assess the susceptibility of the identified isolates to antimicrobials.

## **Material and Methods**

### *Samples Collection and Preparation*

The study involved 100 seemingly healthy sheep and goats (50 of each) and their contact shepherds (32 individuals). Each animal provided three types of samples: milk, nasal swabs, and skin swabs (from the rectum area), while the shepherds provided 32 nasal swabs and 32 hand swabs. The study was conducted in accordance with the ethical guidelines of Mansoura University's ethical committee and followed the 3rd edition of the Care and Use of Agricultural Animals in Research and Teaching (<<http://www.fass.org/>>). The animals were chosen based on convenience, and their owners gave their consent for the sampling procedure and the research plan. Detailed information about the sampling and processing for each type of sample is provided below.

### *Isolation and Identification of Staphylococcus aureus*

The obtained samples were deposited in an enriched broth (Tryptone soya broth, TSB) containing 70 mg of NaCl / ml and incubated at 37°C for 24 hours. After incubation, a loopful (10 µl) of each incubated broth was streaked onto selective media for *S. aureus*, Baird Parker agar base (Oxoid, CM 275) mixed with 5% egg yolk potassium tellurite and incubated at 37°C for 24-48 hours [12]. Colonies with black, shiny, convex surfaces were selected and streaked on Baird Parker agar for purification before incubating for 48 hours at 37°C. Suspected colonies were subjected to a variety of biochemical assays, including catalase, coagulase, and mannitol fermentation tests. The selected colonies were then

stored as glycerol stock at -20°C for further identification [13].

### *Detection of Methicillin-Resistant S. aureus (MRSA)*

Cefoxitin disk diffusion test was applied using 30µg discs. A bacterial inoculum was distributed on Muller Hinton agar medium, followed by loading a cefoxitin (30µg) disc and incubating the plates at 37°C for 24 hours. Following incubation, MRSA was detected by measuring the inhibitory zone diameters using the previously reported method [14].

### *Molecular Characterization of MRSA Strains*

Based on biochemical assays, the identified MRSA strains (n = 50) were subsequently screened using PCR targeting the *nuc*, *mecA*, *PVL*, and *VanA* genes.

### *DNA extraction*

Fifty overnight cultures of *S. aureus* were grown on mannitol salt agar. Two to three independent *S. aureus* colonies were harvested using a sterile loop in 200 µl of DNase/RNase-free water, vortexed, and boiled to isolate bacterial DNA [15]. The samples were heated at 95°C for 10 minutes before being centrifuged at 13000 rpm for 5 minutes using an iFuge M15K Microcentrifuge. Clear fluids were collected and kept at -20°C for future PCR amplification.

### *Method of DNA amplification*

Amplifications were done using DreamTaq™ Green PCR Master Mix (2X), Thermo Scientific, USA according to manufacturer instructions. The PCR cycle was carried out in the Mini PCR™ Mini16 Thermal Cycler (Ampliyus, Cambridge, MA, USA). The PCR reaction was done in a total volume of 50 µl, consisting of 25 µl of 2X Green master mix, 5 µl of DNA templates, and 2 µl of each primer, followed by adding DNA/RNA free water up to 50 µl. *S. aureus* strain was employed as the positive control in each reaction, while nuclease-free water served as the negative control. The positive control strain was isolated in our laboratory and was molecularly characterized using DNA microarray assays [12]. Primers and cycling conditions used for amplification of *nuc* according to Brakstad *et al.* [16], *mecA* according to Sallam *et al.* [17], *PVL* gene according to Algammal *et al.* [8] and *VanA* gene according to Thati *et al.* [18]. The amplified PCR products were run in 1.5% agarose gel in an electric field using 1X TBE buffer, then visualized and photographed using an ultraviolet transilluminator.

### Antibiotic Sensitivity Profiles of *S. aureus* Strains

The sensitivity of confirmed *S. aureus* isolates was tested using the disc diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines [19]. The antibiotics used were Penicillin G (P, 10 units), Clindamycin (DA, 2 µg), Gentamycin (CN, 10 µg), Kanamycin (K, 30 µg), Trimethoprim-Sulfamethazine (SXT, 1.25/23.75 µg), Ciprofloxacin (CIP, 5 µg), Chloramphenicol (C, 30 µg), Vancomycin (VA, 30 µg), Tetracycline (TE, 30 µg), Fusidic acid (FA, 5 – 10 µg), Erythromycin (E, 15 µg) and Linezolid (LNZ, 30 µg). Multi drug resistance has been defined as single isolate that expressed resistant to three or more antimicrobial agents. The following formula was used to calculate multiple antibiotic resistances (MAR) index for each strain [20]:

MAR index = Total no. of resistance / Total no. of tested antibiotics.

### Statistical Analysis

The data were analyzed with the Statistical Package for Social Science (SPSS) application for Windows (Standard version 26). Qualitative data were described in terms of numbers and percentages. The Chi-square test was used to assess the correlation between categorical variables. The Chi-square test has a predefined significance level of 5% (p-value). The results were considered significant at  $p < 0.05$ . The lower the p-value attained, the more significant the results.

### Results

Data in Table 1 illustrates the overall prevalence of *S. aureus* and MRSA among healthy sheep, goats, and shepherds. In the current study, *S. aureus* and MRSA were cultivated at different detection rates, regardless of animal species or sample source. For example, sheep had considerably more *S. aureus* (115/150; 77%) and MRSA isolates (62/115; 54%) than goats (77/150; 51%) and (40/77; 52%), respectively. Furthermore, nasal swabs of sheep were the greatest sources of *S. aureus* (45/50; 90%) and MRSA (26/45; 58%), followed by milk (39/50; 78%, and 21/39; 54%), respectively, then rectal skin swabs (31/50; 61%, and 15/31; 48%), respectively, while in goats, the majority of *S. aureus* (33/50; 66%) and MRSA (14/33; 42%) were recovered from nasal swabs, followed by milk (32/50; 64% and 19/32; 59%), respectively. Shepherds had higher detection rates for *S. aureus* (54/64; 84%) and MRSA (48/54; 89%) than the tested sheep and goats. Hand swabs

were the most common source of *S. aureus* (29/32; 91%) and MRSA (26/29; 90%), compared to nasal swabs (25/32; 78% and 22/25; 88%), respectively.

Table 2 representing that the majority (90%) of the *S. aureus* isolates (45/50) from various sources expressed *nuc* gene. The *nuc*-positive isolates were examined for *mecA*, *PVL*, and *VanA* genes. The results showed that 25 (56%) isolates carried the *mecA* gene and were identified as MRSA. Furthermore, the *PVL* gene was detected in nineteen (42%) *S. aureus* isolates (10 isolates from sheep, 4 isolates from goats and 5 isolates from human samples). Meanwhile, *VanA* gene wasn't detected in *S. aureus* isolates from different sources.

Table 3 reveals that molecularly identified *S. aureus* isolates (n = 45) are extremely resistant to penicillin G (100%), followed by kanamycin, tetracycline, and fusidic acid (96% each), erythromycin and gentamicin (93% each), sulfamethazine (82%), clindamycin (73%), and ciprofloxacin (60%). Linezolid (98%) was most effective against the tested *S. aureus* strains, followed by vancomycin (82%), and chloramphenicol (67%). In the present study, all *S. aureus* strains exhibited multi-drug resistance (MDR) to more than three antimicrobial drug classes as shown in table 4.

### Discussion

In this study, regardless of the animal type or sample source, *S. aureus* and MRSA were determined with various detection rates data presented in (Table 1). A recent study in Egypt reported a low detection rate of *S. aureus* in sheep [21]. The authors identified *S. aureus* in 58% of raw milk and 18% of nasal swabs, however the MRSA recovery rate was slightly higher (51.6% in milk samples and 66.7% in nasal swabs) than in our study. In this investigation, however, MRSA was discovered in 54% (62/115) of sheep samples and 52% (40/77) of goat samples.

Researchers in Tiwan Chu et al. [22], Egypt Fadel [23], Czech Republic Klimešová et al. [24], Italy Macori et al. [25], and Egypt Farag et al. [21] reported detection rates of 58% in goat nasal swabs, 50% in sheep milk, 50% in goat rectum surrounding, 57% in sheep milk, and 52% in sheep milk, respectively. In contrast, researchers from Middle Eastern countries such as Saudi Arabia Al zohairy [26], Egypt Fadel [23] and Farag et al. [21], and even Far-eastern countries such as India Fayaz et al. [27]

have reported a wide variety of MRSA detection rates. The authors found variable detection rates across sample sources, including 68% and 60% from sheep and goat nasal swabs, 71% from goat milk, 67% from sheep nasal swabs, and 100% from teat skin swabs and rectal swabs, respectively. Several European countries, notably Italy Cortimiglia *et al.* [28], the Czech Republic Klimešová *et al.* [24] have reported low detection rates of MRSA isolates in goat milk (2%, and 0%), respectively.

Several studies, including Gharsa *et al.* [29] from Tunisia, Klimešová *et al.* [24] from the Czech Republic, Jauro *et al.* [9] from Nigeria, and Venkatvasan *et al.* [30] from India, found low detection rates (varying from 3% to 26%) using samples originated from small ruminant nasal swabs. However, because each study used different samples and detection methodologies, direct comparisons of the wide prevalence range should be approached with care. Geographic location, the sensitivity of isolation procedures, antibiotic use, farm bio security, manufacturing processes, samples handling, and storage are all possible explanations for the disparity in occurrence between countries.

Interestingly, shepherds had higher detection rates for *S. aureus* (54/64; 84%) and MRSA (48/54; 89%) than the tested sheep and goats. Hand swabs were the most common source of *S. aureus* (29/32; 91%) and MRSA (26/29; 90%), compared to nasal swabs (25/32; 78% and 22/25; 88%), respectively. These findings were higher than those reported in Egypt Fadel [23], where *S. aureus* was detected in 19 of 30 (63%) of the human nose and hand swabs examined. In India, Fayaz *et al.* [27] discovered *S. aureus* in 11 out of 24 milkers' hand swabs (46%), with all recovered *S. aureus* being MRSA (100%, 11/11). In contrast, in an Italian study, the detection rate of *S. aureus* in nasal swabs from shepherds was 36% (97/275), while MRSA was 1.1% (3/275) [31]. Furthermore, in Nigeria, Jauro *et al.* [9] discovered *S. aureus* in 40% (40/100) of sheep handlers' nasal swabs, while MRSA was isolated in 17% (17/100). In India, Venkatvasan *et al.* [30] found *S. aureus* and MRSA in nasal swabs of goat handlers at a rate of 84% (26/31) and 19% (6/31), respectively. Taken together, the high percentages of *S. aureus* in human nose and hand swabs appear to be due to the prevalence of *S. aureus* on skin and nostrils, which are considered *S. aureus*' natural habitat [32]. However, the highest percentage of MRSA in contact humans could be related to the incorrect

administration of antimicrobial medications and uncontrollable antimicrobial feed additives [30].

In the current study, the vast majority (90%) of the *S. aureus* isolates (45/50) from various sources expressed *nuc* gene, while the remaining five isolates did not express *nuc* gene specific to *S. aureus*. According to Moustafa *et al.* [33], several *S. aureus* isolates that tested negative for the *nuc* gene could have been the result of gene mutation or deletion. It is probable that the amount of DNA that can be retrieved could be significantly reduced, or the DNA was destroyed during the boiling process.

The *nuc*-positive isolates were examined for *mecA*, *PVL*, and *VanA* genes. The results showed that 25 isolates (56%) carried the *mecA* gene and were identified as MRSA. Furthermore, the *PVL* gene was detected in nineteen *S. aureus* isolates (42%) (10 isolates from sheep, 4 isolates from goats and 5 isolates from human samples). The presence of *PVL* gene suggests a human origin and a recent, secondary transmission to sheep and goats. This finding may in part agreed with previous report [34].

Surprisingly, no *S. aureus* isolates contained the *VanA* gene, even though 8 isolates exhibited vancomycin resistance. A variety of variables, including inoculum size, pH, medium salt concentration, and incubation length, have been hypothesized to influence the phenotypic expression of antibiotic resistance [35]. In Egypt, Kamel [36] discovered that 64.4% (87/135) of the goats' mastitic milk samples tested positive for the *nuc* gene. Of these, 23 (26.4%) isolates were identified as MRSA using the *mecA* gene. Meanwhile, in Italy, Macori *et al.* [25] reported that all eight isolates (100%) from sheep bulk tank milk samples were validated phenotypically as MRSA carried *mecA* gene, while *PVL* gene was found in only one isolate (0.1%, 1/831) of sheep bulk tank milk. In Algeria, Agabou *et al.* [37] detected the *mecA* gene in 6/6 (100%) sheep nasal swabs that were phenotypically characterized as MRSA, as well as the *PVL* gene in 4 isolates out of 19 (21%) *S. aureus* isolates. Dastmalchi Saei and Panahi [38] were unable to detect the *PVL* gene in sheep and goat nasal swabs in Iran. In Tunisia, Ben Slama *et al.* [39] discovered that 2% (1/55) and 7% (4/55) of *S. aureus* isolates from analyzed nasal swabs of humans in contact with animals had the *mecA* and *PVL* genes, respectively.

Table 3 reveals that molecularly identified *S. aureus* isolates (n = 45) are extremely resistant to penicillin G (100%), followed by kanamycin,

tetracycline, and fusidic acid (96% each), erythromycin and gentamicin (93% each), sulfamethazine (82%), clindamycin (73%), and ciprofloxacin (60%). Linezolid (98%) was most effective against the tested *S. aureus* strains, followed by vancomycin (82%), and chloramphenicol (67%). Antibiotics such as vancomycin and linezolid are commonly used to treat MRSA infections. This is consistent with the findings of Algamal et al. [8], who found that linezolid was more effective than vancomycin in treating MRSA. In Egypt, Kamel [36] discovered that *S. aureus* isolated from raw goat milk samples was extremely resistant to amoxicillin (89.7%), followed by penicillin (88.5%) and ampicillin (86.2%); however, there was minimal resistance to ciprofloxacin (1.2%), levofloxacin (2.3%), and ofloxacin (4.5%).

In this study, all *S. aureus* strains exhibited multi-drug resistance (MDR) to more than three classes of antimicrobial drugs. Another study conducted in Jordan revealed that 87% of MRSA isolates from sheep milk samples and all MRSA isolates from goat milk samples exhibited MDR [11]. Additionally, 18.8-45.45% of human *S. aureus* isolates from Romania showed MDR [40]. The widespread use of antimicrobial agents in humans and animals contributes to the prevalence of MDR.

### **Conclusion**

This study suggests that small ruminants may act as a source of *S. aureus* transmission to humans, and vice versa. MRSA may spread from infected shepherds to small ruminants. Furthermore, the abuse

of antimicrobial drugs in small ruminants may increase the risk of MDR pathogen transmission between humans and animals. The presence of MDR *S. aureus* should raise public awareness about the threats these isolates pose to human health. As a result, health and veterinary authorities should enact stringent antibiotic prescription restrictions and laws, as well as prohibit raw dairy consumption.

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This study didn't received any funding support.

### *Conflict of interest*

The authors declare that there is no conflict of interest.

### *Ethical of approval*

This study follows the ethics guidelines of the faculty of veterinary medicine, Mansoura university, Egypt (ethics approval number; PhD/ 96).

### *Author contributions*

Th.S collected the samples, carried out the lab work and conducted data analysis. A. M, A. El-G, M.G and D.N conceptualized the study, planned for the research activity and revised the final version. All authors have read and approved the final version of the manuscript for publication.

TABLE 1. The overall prevalence of *S. aureus* and MRSA in healthy sheep and goats and their shepherds

Subjects	Source of samples	No. of examined samples	<i>S. aureus</i>			MRSA		
			No. of identified <i>S. aureus</i>	%	Chi-square test (p value)	No. of MRSA	%	Chi-square test (p value)
Goats	Milk	50	32	64	$\chi^2=22.46$ $P\leq 0.001$ (*)	19	59	$\chi^2=2.10$ $P=0.350$ (**)
	Nasal swabs	50	33	66		14	42	
	Skin swabs (rectum surrounding)	50	12	24		7	58	
	Total	150	77	51		40	52	
Sheep	Milk	50	39	78	$\chi^2=11.03$ $P=0.004$ (*)	21	54	$\chi^2=4.26$ $P=0.119$ (**)
	Nasal swabs	50	45	90		26	58	
	Skin swabs (rectum surrounding)	50	31	62		15	48	
	Total	150	115	77		62	54	
Shepherds	Nasal swabs	32	25	78	$\chi^2=1.89$ $P=0.168$ (**)	22	88	$\chi^2=0.037$ $P=0.847$ (**)
	Hand swabs	32	29	91		26	90	
	Total	64	54	84		48	89	
<b>Total</b>		364	246	68		150	61	

%: out of *S. aureus* isolate

(\*): significant

(\*\*): non-significant

TABLE 2. Molecular characterization of some *S. aureus* strains (n= 50) from different sources.

	Type of samples	No. of tested <i>S. aureus</i>	<i>Nuc gene</i>		<i>MecA gene</i>		<i>PVL gene</i>		
			No. of positive	Ratio	No. of positive	Ratio	No. of positive	Ratio	
<b>Animals</b>	Sheep	Milk	6	6/6	5	5/6	3	3/6	
		Nasal swabs	6	4	4/6	1	1/4	3	3/4
		Skin swabs	6	6	6/6	3	3/6	4	4/6
	Goats	Total	18	16	89	9	56	10	63
		Milk	6	6	6/6	4	4/6	2	2/6
		Nasal swabs	6	5	5/6	-	-	-	-
Total number of animal samples	Skin swabs	6	5	5/6	3	3/5	2	2/5	
	Total	18	16	89	7	44	4	25	
	Total	36	32	89	16	50	14	44	
<b>Human</b>	Nasal swabs	7	6	6/7	3	3/6	3	3/6	
	Hand swabs	7	7	7/7	6	6/7	2	2/7	
	Total	14	13	93	9	69	5	38	
<b>Total</b>		50	45	90	25	56	19	42	

Van A gene wasn't detected in *S. aureus* isolates from different sources.~ The ratio of each gene was calculated from positive *S. aureus* by *nuc gene*.

**TABLE 3. Antibiotic susceptibility test of PCR positive *S. aureus* strains (n=45).**

Antimicrobial agent	S		R	
	No.	%	No.	%
Penicillin G (p)	0	0	45	100
Erythromycin (E)	3	7	42	93
Clindamycin (DA)	12	27	33	73
Gentamycin (CN)	3	7	42	93
Kanamycin (K)	2	4	43	96
Vancomycin (VA)	37	82	8	18
Chloramphenicol (C)	30	67	15	33
Ciprofloxacin (CIP)	18	40	27	60
Trimethoprim-Sulfamethazine (SXT)	8	18	37	82
Tetracycline (TE)	2	4	43	96
Linezolid (LNZ)	44	98	1	2
Fusidic acid (FA)	2	4	43	96

S: Sensitive, R: Resistant

**TABLE 4. Antimicrobial resistance profile of *S. aureus* strains (n=45) with MAR index.**

Source	Antimicrobial resistance profile	MAR index
Human nasal swab	P, DA, CN, E, K, C, SXT	0.6
Human nasal swab	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Human nasal swab	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Human nasal swab	P, DA, CN, E, K, C, SXT, FA, TE	0.8
Human nasal swab	P, DA, E, K, SXT, CIP, FA, TE	0.7
Human nasal swab	P, CN, E, K, FA, TE	0.5
Human hand swab	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Human hand swab	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Human hand swab	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Human hand swab	P, DA, CN, E, K, SXT, CIP, VA, FA, TE	0.8
Human hand swab	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Human hand swab	P, DA, CN, K, SXT, FA, VA	0.6
Human hand swab	P, DA, CN, K, E, SXT, C, CIP, FA, TE	0.8
Sheep milk	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Sheep milk	P, DA, E, C, CIP, SXT, FA, TE	0.7
Sheep milk	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Sheep milk	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Sheep milk	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Sheep milk	P, DA, CN, E, K, FA, TE	0.6
Sheep nasal swab	P, E, K, SXT, FA, TE	0.6
Sheep nasal swab	P, CN, E, K, SXT, VA, FA, TE	0.7
Sheep nasal swab	P, CN, E, K, SXT, FA, TE	0.7
Sheep nasal swab	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Sheep skin swab	P, DA, CN, E, K, SXT, CIP, VA, FA, TE	0.8
Sheep skin swab	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Sheep skin swab	P, CN, E, K, SXT, FA, TE	0.6
Sheep skin swab	P, CN, E, K, SXT, CIP, VA, FA, TE	0.8
Sheep skin swab	P, DA, CN, E, K, C, CIP, SXT, FA, TE	0.8
Sheep skin swab	P, CN, E, K, SXT, FA, TE	0.6
Goat milk	P, DA, CN, E, K, C, SXT, CIP, VA, FA, TE	0.9
Goat milk	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Goat milk	P, CN, E, K, SXT, VA, FA, TE	0.7
Goat milk	P, CN, E, K, FA, TE	0.5
Goat milk	P, DA, CN, E, K, CIP, FA, TE	0.8
Goat milk	P, DA, CN, K, SXT, CIP, FA, TE	0.7
Goat nasal swab	P, DA, CN, E, K, CIP, FA, TE	0.7
Goat nasal swab	P, DA, CN, E, K, SXT, CIP, FA, TE	0.8
Goat nasal swab	P, CN, E, K, TE	0.4
Goat nasal swab	P, CN, E, K, FA, TE	0.5
Goat nasal swab	P, DA, CN, E, K, C, SXT, FA, TE	0.8
Goat skin swab	P, DA, CN, E, C, SXT, CIP, FA, TE	0.8
Goat skin swab	P, DA, CN, E, K, C, SXT, CIP, FA, TE	0.8
Goat skin swab	P, CN, K, FA, TE	0.4
Goat skin swab	P, DA, CN, K, E, C, SXT, VA, FA, TE	0.8
Goat skin swab	P, DA, CN, E, K, SXT, FA, TE	0.7

## References

- Ashour, G., Gad, A., Fayed, A.K., Ashmawy, N.A. and El-Sayed, A. Evaluation of growth performance, blood metabolites and gene expression analysis in Egyptian sheep breeds, in relation to age. *World Vet. J.*, **10**, 18-29 (2020).
- Aboul-Naga, A., Abo Amo, F., Abdel-Aal, E., Hassan, E. and Shafie, M. Review of Literature for Sheep and Goat RESEARCH and development in Egypt, Since the Forties: III. Local and Exotic Goat Breeds, Production Performance, and Breeding Programs. *J. Anim. Poult. Prod.*, **14**, 105-112 (2023).
- Katakweba, A.S., Muhairwa, A.P., Espinosa-Gongora, C., Guardabassi, L., Mtambo, M.M. and Olsen, J.E. *spa* typing and antimicrobial resistance of *Staphylococcus aureus* from healthy humans, pigs and dogs in Tanzania. *JIDC*, **10**, 143-148 (2016).
- Parco, A., Macaluso, G., Foti, M., Vitale, M., Fisichella, V., Tolone, M. and Loria, G.R. Phenotypic and genotypic study on antibiotic resistance and pathogenic factors of *Staphylococcus aureus* isolates from small ruminant mastitis milk in South of Italy (Sicily). *Ital. J. Food Saf.*, **10** (3), 9722 (2021).
- Haggag, Y. N., Nossair, M. A., Habib, H. M., El Naggat, A. L., Abdallah, M. and Farag, H. E. Prevalence of Subclinical Mastitis in Small Ruminants and Role of *Staphylococcus* Species in Such Infection. *Alex. J. Vet. Sci.*, **62**(2),96-104 (2019).
- Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A., Papa, A. and Sergelidis, D. Prevalence of *Staphylococcus aureus* and of methicillin-resistant *S. aureus* (MRSA) along the production chain of dairy products in north-western Greece. *Food Microbiol.*, **69**, 43-50 (2018).
- Zaher, H. A., El Baz, S., Alothaim, A. S., Alsalamah, S. A., Alghonaim, M. I., Alawam, A. S. and Eraqi, M. M. Molecular Basis of Methicillin and Vancomycin Resistance in *Staphylococcus aureus* from Cattle, Sheep Carcasses and Slaughterhouse Workers. *Antibiotics*, **12**(2), 205 (2023).
- Algammal, A.M., Enany, M.E., El-Tarabili, R.M., Ghobashy, M.O. and Helmy, Y.A. Prevalence, antimicrobial resistance profiles, virulence and enterotoxins-determinant genes of MRSA isolated from subclinical bovine mastitis in Egypt. *J. Pathog.*, **9** (5) 362 (2020).
- Jauro, S., Hamman, M. M., Malgwi, K. D., Musa, J. A., Ngoshe, Y. B., Gulani, I. A. and Fasina, F. O. Antimicrobial resistance pattern of methicillin-resistant *Staphylococcus aureus* isolated from sheep and humans in Veterinary Hospital Maiduguri, Nigeria. *Vet. World*, **15**(4), 1141 (2022).
- Lakhundi, S. and Zhang, K. 2018 Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *J. Clin. Microbiol.*, **31** (4), 10.1128/cmr. 00020-00018, (2018).
- Obaidat, M.M., Bani Salman, A.E. and Roess, A.A. High prevalence and antimicrobial resistance of *mecA Staphylococcus aureus* in dairy cattle, sheep, and goat bulk tank milk in Jordan. *Trop. Anim. Health Prod.*, **50**, 405-412 (2018).
- El-Ashker, M., Monecke, S., Gwida, M., Saad, T., El-Gohary, A., Mohamed, A. and Ehricht, R. Molecular characterisation of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* clones isolated from healthy dairy animals and their caretakers in Egypt. *Vet. Microbiol.*, **267**, 109374 (2022).
- Gwida, M. M., Saad, T. M., Elgohary, A. and Mohamed, A. Characterization of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* from healthy cattle and buffaloes in a linked community. *Mansoura Vet. Med. J.*, **22**(2), 76-81 (2021).
- Giacinti, G., Carfora, V., Caprioli, A., Sagrafoli, D., Marri, N., Giangolini, G. and Battisti, A. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* carrying *mecA* or *mecC* and methicillin-susceptible *Staphylococcus aureus* in dairy sheep farms in central Italy. *J. Dairy Sci.*, **100**(10), 7857-7863(2017).
- McClure-Warnier, J. A., Conly, J. M. and Zhang, K. Multiplex PCR assay for typing of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *JoVE*, **79**, e50779 (2013).
- Brakstad, O. G., Aasbakk, K. and Maeland, J. A. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J. Clin. Microbiol.*, **30** (7), 1654-1660 (1992).
- Sallam, K. I., Abd-Elghany, S. M., Elhadidy, M. and Tamura, T. Molecular characterization and antimicrobial resistance profile of methicillin-resistant *Staphylococcus aureus* in retail chicken. *J. Food Prot.*, **78** (10), 1879-1884 (2015).
- Thati, V., Shivannavar, C. T., & Gaddad, S. M. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. *Indian J. Med. Res.*, **134** (5), 704 (2011).
- CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing. In, Clinical and Laboratory Standards Institute, 30<sup>th</sup> ed., Wayne, PA, USA, (2017).



20. Chitanand, M. P., Kadam, T. A., Gyananath, G., Totewad, N. D. and Balhal, D. K. Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. *Indian J. Microbil.*, **50** (2), 216-220 (2010).
21. Farag, H.E., Ragab, M.T., Hafez, A.A., Osman, W.A. and Mansour, A.M. Molecular Study on Methicillin-Resistant *Staphylococcus aureus* Isolated from Sheep. *Alex. J. Vet. Sci.*, **77**, 83-83 (2023).
22. Chu, C., Wei, Y., Chuang, S. T., Yu, C., Changchien, C. H. and Su, Y. Differences in virulence genes and genome patterns of mastitis-associated *Staphylococcus aureus* among goat, cow, and human isolates in Taiwan. *Foodborne Pathog Dis.*, **10** (3), 256-262 (2013).
23. Fadel, H. Occurrence and zoonotic importance of methicillin-resistant *Staphylococcus aureus* in raw milk and some dairy products at Ismailia city, Egypt. *Zagazig Vet. J.*, **43**(3), 95-104 (2015).
24. Klimešová, M., Manga, I., Neješlebová, L., Horáček, J., Ponižil, A. and Vondrušková, E. Occurrence of *Staphylococcus aureus* in cattle, sheep, goat, and pig rearing in the Czech Republic. *Acta Vet. Brno*, **86**(1), 3-10 (2017).
25. Macori, G., Giacinti, G., Bellio, A., Gallina, S., Bianchi, D. M., Sagrafoli, D. and Decastelli, L. Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the ovine dairy chain and in farm-related humans. *J. Pathog.*, **9** (5), 161(2017).
26. Alzohairy, M. A. Colonization and antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia. *J. Bacteriol. Res.*, **3** (4), 63-68 (2011).
27. Fayaz, I.B., Hussain, S.A., Zehgeer, M.M., Showkat, P.R.P. and Shehriyar, Q. Investigations on Methicillin-resistant *Staphylococcus aureus* (MRSA) isolation and identification from milk and environmental sources. *J. Pharm Innov.*, **12** (8), 1072-1075 (2023).
28. Cortimiglia, C., et al., Bianchini, V., Franco, A., Caprioli, A., Battisti, A., Colombo, L., Stradiotto, K., Vezzoli, F., Luini, M. Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in bulk tank milk from dairy goat farms in Northern Italy. *J. Dairy Sci.*, **98**, 2307-2311 (2015).
29. Gharsa, H., Slama, K.B., Lozano, C., Gómez-Sanz, E., Klibi, N., Sallem, R.B., Gómez, P., Zarazaga, M., Boudabous, A. and Torres, C. Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. *Vet. Microbiol.*, **156**, 367-373 (2012).
30. Venkatvasan, R., Antony, P., Mukhopadhyay, H., Jayalakshmi, V., Srinivas, V.V., Thanissal, J. and Stephen, S. Characterization of methicillin-Resistant *Staphylococcus aureus* from goats and their relationship to goat handlers using multi-locus sequence typing (MLST). *Small Rum. Res.*, **186**, 106097 (2020).
31. Mascaro, V., Squillace, L., Nobile, C.G., Papadopoli, R., Bosch, T., Schouls, L.M., Casalnuovo, F., Musarella, R. and Pavia, M. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage and pattern of antibiotic resistance among sheep farmers from Southern Italy. *Infect. Drug Resist.*, **12**, 2561-2571(2019).
32. Suelam, I.I., Raslan, A.R. and Mohamed, M.E. Isolation of *Staphylococcus aureus* from Milk and Human with Reference to its Survival on Surfaces. *World J. Dairy Food Sci.*, **7**, 142-145 (2012).
33. Moustafa, A. E. D., Hammad, A. and Dawoud, M. Differences between phenotypic and genotypic characterization of *S. aureus* isolated from bovine mastitis in Egypt. *Assuit Vet. Med. J.*, **67** (169), 182-201 (2021).
34. El-Ashker, M., Gwida, M., Monecke, S., El-Gohary, F., Ehricht, R., Elsayed, M. and Maurischat, S. Antimicrobial resistance pattern and virulence profile of *S. aureus* isolated from household cattle and buffalo with mastitis in Egypt. *Vet. Microbiol.*, **240**, 108535(2020).
35. Agbo, M. C., Ezeonu, I. M., Onodagu, B. O., Ezech, C. C., Ozioko, C. A. and Emencheta, S. C. Antimicrobial resistance markers distribution in *Staphylococcus aureus* from Nsukka, Nigeria. *BMC Infect Dis.*, **24**(1), 320(2024).
36. Kamel, Y. Genotypic and phenotypic characterization, antibiotic resistance and virulence Patterns of *Staphylococcus aureus* isolated from goat mastitis. *Mansoura Vet. Med. J.*, **21**, 161-166 (2020).
37. Agabou, A., Ouchenane, Z., Ngba Essebe, C., Khemissi, S., Chehboub, M.T.E., Chehboub, I.B., Sotto, A., Dunyach-Remy, C. and Lavigne, J.P. Emergence of Nasal Carriage of ST80 and ST152 PVL+ *Staphylococcus aureus* Isolates from Livestock in Algeria. *Toxins*, **9** (10), 303 (2017).
38. Dastmalchi Saei, H. and Panahi, M. Genotyping and antimicrobial resistance of *Staphylococcus aureus* isolates from dairy ruminants: Differences in the distribution of clonal types between cattle and small ruminants. *Arch. Microbiol.*, **202** (1), 115-125 (2020).

39. Ben Slama, K., Gharsa, H., Klibi, N., Jouini, A., Lozano, C., Gómez-Sanz, E., Zarazaga, M., Boudabous, A. and Torres, C. Nasal carriage of *Staphylococcus aureus* in healthy humans with different levels of contact with animals in Tunisia: genetic lineages, methicillin resistance, and virulence factors. *Eur. J. Clin. Microbiol.*, **30**, 499-508 (2011).
40. Ungureanu, A., Zlatian, O., Mitroi, G., Drocaş, A., Țircă, T., Călina, D., Dehelean, C., Docea, A.O., Izotov, B.N. and Rakitskii, V.N. *Staphylococcus aureus* colonisation in patients from a primary regional hospital. *Mol. Med. Rep.*, **16**, 8771-8780(2017).

## الميكروب العنقودي الذهبي المقاوم للميثيسيلين المعزول من الرعاة والماعز و الأغنام السليمة صحيا ظاهريا في مصر

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### الملخص

الهدف من هذه الدراسة كان لتقييم مدى الانتشار، الحساسية لمضادات الميكروبات والمضادات الحيوية وجينات الضراوة في الميكروب العنقودي الذهبي والميكروب العنقودي الذهبي المقاوم للميثيسيلين المعزول من الأغنام والماعز السليمة صحيا ظاهريا ورعاتها. الدراسة شملت عدد 50 من الأغنام، 50 من الماعز و32 من رعاة الأغنام. تم تجميع ثلاث أنواع من العينات (مسحات من الأنف، مسحات من الجلد المحيط للمستقيم والألبان) من كل حيوان. بالنسبة لرعاة الأغنام تم تجميع عدد 32 مسحة من الأيدي و 32 مسحة من الأنف منهم. ثم تم عزل الميكروب علي الأوساط البكتيرية النوعية وتم التعرف علي الميكروب باستخدام الاختبارات الكيميائية الحيوية وتم التعرف والتميز علي عترات الميكروب العنقودي الذهبي المقاوم للميثيسيلين باستخدام اختبار نشر أقراص السيفوكسيتين. ثم تم اختبار العزلات بواسطة تفاعل البلمرة المتسلسل للكشف عن جينات الضراوة *nuc*, *mecA*, *PVL* and *VanA*

أوضحت النتائج أن الميكروب العنقودي الذهبي تم عزله بنسب 84.20% من عينات الأغنام، 51.33% من عينات الماعز و84% من عينات الرعاة. من ناحية أخرى وجد الميكروب العنقودي الذهبي المقاوم للميثيسيلين في 54% من الألبان، 58% من مسحات الأنف و 48% من مسحات الجلد المحيط للمستقيم للأغنام في حين أن وجد في 59%، 42% و58% من العزلات المصنفة من الماعز علي التوالي. بالنسبة لعينات والعزلات المصنفة من الرعاة وجد الميكروب العنقودي الذهبي المقاوم للميثيسيلين في 88% مسحات الأنف و90 من مسحات الأيدي. أثبتت النتائج أن كل عزلات الميكروب العنقودي الذهبي المقاوم للميثيسيلين كانت مقاومة للعديد من المضادات الحيوية المختبرة وكانت أعلاهم مقاومة للينسلين المائي بنسب 100%.

يستخلص من الدراسة أن الأغنام والماعز والرعاة تلعب دورا معنويا في انتقال وانتشار الميكروب العنقودي الذهبي. علاوة علي ذلك وجود الميكروب العنقودي الذهبي في عينات الألبان الخام، مسحات الأنف ومسحات الجلد يساهم في انتشار هذا الميكروب بين الحيوانات والأفراد.

**الكلمات المفتاحية:** مقاومة المضادات الحيوية، *mecA*, *PVL* الميكروب العنقودي الذهبي المقاوم للميثيسيلين، الميكروب العنقودي الذهبي، المجترات الصغيرة.