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Detection and Identification of Staphylococcus Species Isolated From Skin Wounds of Cow in Al-Shatrah City-Southern Iraq

Haider S. Abdulhusein[1] and Baidaa M. Kadim[2]

[1]Department of Microbiology, College of Veterinary Medicine, Shatrah University, Iraq.

[2]Department of Pathological Analysis, College of Applied Science, Shatrah University, Iraq.

Email haidersa@shu.edu.iq[1], baidaamezher@shu.edu.iq[2]

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Abstract

A wide variety of commensal microorganisms reside on the skin, many of which secrete proteolytic enzymes that might have detrimental effects following an injury. These bacteria are considered opportunistic pathogens. The aim of this study is a detection and identification of *Staphylococcus* spp. isolated from animal skin (cows) and conduct molecular characterization of the isolated strains in order to examine their enzymatic production capabilities, and to determine the ideal conditions for bacterial development. A total of 25 swab samples were obtained from animal skin wounds in Al-Shatrah city (from veterinary clinic after the permission had been taken from the animal breeder). Each sample was inoculated into each of nutrient agar, blood agar, and Brain Heart Infusion Agar (enrichment media); and Mannitol salt agar (differential and selective medium). All isolates were subjected to molecular detection using the PCR (The conventional polymerase chain reaction) technique. Moreover, 10 isolates were sequenced using the Sangerdideoxy sequencing method targeting the 16S rRNA gene. Enzyme production properties were analyzed, and the sensitivity of bacteria to six antibiotics was studied. Analysis of 25 swab samples taken from animal skin wounds yielded a total of 18 isolates. According to the results, 18 bacterial strains were isolated from the samples. Biochemical tests identified the majority of these strains as *S. aureus, S. haemolyticus, S. epidermidis,* and *S. borealis*. Among these isolates, *S. warneri* and *S. epidermidis* showed sensitivity to Novobiocin, with inhibition zones of 22mm and 21mm, respectively. However, all other isolates tested exhibited resistance to novobiocin. *Staphylococcus* is categorized as an opportunistic bacterium, meaning it can cause many clinical symptoms in animals and can be transmitted to humans

through animal products. Additionally, the use of antibiotics in food contributes to the development of antibiotic resistance among *Streptococcus* species. The current study is the first to isolate and identify *Staphylococcus* sp. from superficial wounds on cows' skin in Thi-Qar province/Shatrah city

Keywords

Staphylococcus sp., Antibiotics, Enzymes, Amino acids, and carbon sources.

1. Introduction

Staphylococcus is one of the genres that can survive with or without oxygen. It is round-shaped, stain purple when exposed to a certain dye, and produces an enzyme called catalase. *Staphylococcus* belongs to the *Micrococcaceae* family. *S. aureus*, which is responsible for mastitis, is prevalent among cattle, goats, and sheep. In cattle, the infection is frequently asymptomatic, resulting in decreased milk output and quality. However, it can sometimes manifest as severe catarrhal or even gangrenous inflammation. Enzootic acute gangrenous mastitis is frequently observed in goats and sheep. *S. hyicus* is responsible for exudative epidermitis, sometimes known as "greasy pig disease," which is a frequently acute and widespread skin illness in piglets. Additionally, there are systemic manifestations of the disease that lead to the mortality of the animals (Werckenthin*et al*., 2001).

Staphylococci commonly belong to the typical bacteria found on the skin and mucous membranes of mammals and birds in the respiratory, upper digestive, and urogenital systems. Staphylococci can be rapidly transmitted from animals to people under specific circumstances through contact with bodily secretions like saliva or airborne particles emitted during sneezing and coughing. In addition, Staphylococci can be transmitted through animal products, specifically non-pasteurized milk (Jordan and Pattison, 2005).

S. aureus is responsible for conditions such as septicemia and bone infections in commercially raised broiler chickens (Kumbhar*et al*., 2018). The precise process by which *S. aureus* infection spreads through poultry flocks remains incompletely known (Thompson *et al.*, 2001). A wound is a break in the skin that exposes the underlying tissue. This creates a moist, warm, and nourishing environment that promotes the growth of microorganisms (Bowler *et al*., 2001). An infection in a wound can impede the healing process and could lead to wound disintegration, herniation of the wound, and complete wound dehiscence. The prevalence of *S. aureus* infection was highest in wound samples collected from buffalos (70.00%), followed by donkeys (40%), chickens (46.66%), goats (33%), and dogs (3%) (Kumbhar *et al*., 2018). The most significant species of coagulase-negative staphylococci that have been linked to bovine mastitis in Turkey include *S. chromogenes, S. simulans, S. epidermidis, S. xylosus, and S. caprae* (Hadimli *et al*., 2014). The skin in live mammals has multiple purposes, such as providing insulation against extreme temperatures, protection against physical impacts, and defense against harmful microbes such as bacteria, viruses, parasites, and fungi. It is regarded as the largest and most intricate organ of the animal body. It plays a

crucial role in thermoregulation and aids in preventing dehydration in animals (Singh*et al.*, 2013), also acts as a vital shield, safeguarding the body from several detrimental elements like ultraviolet (UV) radiation, chemical substances (Menon, 2002; Krinsky *et al*., 2004; Lademann *et al*., 2011), and physical stress (Endris and Feki, 2021). Skin possesses three self-repair processes that activate in response to the extent and kind of damage incurred. The skin microbiome encompasses a community of bacteria that reside on the outer layer of skin in healthy mammals (Abdulhusein and Kadim, 2024).

The aim of this study is to identify the specific *Staphylococcus* species that make up a portion of the microbiome found on animal skin. The study determined the antibiotic susceptibility, and to identify the ideal conditions for bacterial proliferation. Additionally, the study aims to examine the isolates' capacity to metabolize 11 different sugars and investigate the enzymatic characteristics of the *Staphylococcus* sp.

2. Methods

Twenty-five swab samples were obtained from the skin of cows and cultivated in Brain Heart Infusion Agar. The samples were then divided into two groups based on the results of the Gram stain. The Gram positive isolates were further cultured in mannitol salt agar and blood agar. We conducted an investigation on the enzyme characteristics of all our isolates and assessed their ability to utilize 11 different carbon sources. Ultimately, all acquired isolates were diagnosed at the molecular level using the PCR technique. Following the extraction and PCR experiment, DNA sequencing was performed.

2.1. Inoculation of skin wound swabs

Following the protocol mentioned by MacFaddin (2000), pus and liquids were meticulously taken from the infected area from cows (depending on the primary diagnosis by the veterinarian). The swab samples were subjected to culturing on Brian heart infusion agar supplemented with 7.5% NaCl (as non-selective an enrichment medium). The cultures were then incubated aerobically at a temperature of 37℃ for duration of 48 hours. Following that, the samples were categorized into two groups using the gram stain technique, namely gram positive and gram negative (Moyes *et al*., 2009; Sabah and Kadim, 2024).

2.2. Identification of Bacteria

The Gram-positive specimens were cultured on blood agar and Mannitol saline agar (MSA) to assess their hemolysis characteristics and isolate *Staphylococcus*, respectively. The study utilized the *Staphylococcus* sp. API20 specifically for precise diagnostic purposes.

2.3. Morphological and Microscopic examination

The analysis of colony morphology was conducted using blood agar. Following incubation at a temperature of 37℃ for a period of 24 hours, many features of the colonies were examined and documented. The factors examined were the edge morphology, color, colonies' size, and the category of hemolysis on blood agar.

In addition, a solitary colony of each isolate was affixed onto a pristine slide to perform a gram stain examination using a light microscope (Seddiq *et al*., 2023). An examination was conducted on the structure and coloring of bacterial colonies. This was accomplished by examining the morphology and pigmentation of colonies on Petri plates following their cultivation and overnight incubation at a temperature of 37°C (Sánchez-Porro *et al*. 2011).

2.4. The Enzymatic Properties of *Staphylococcus* sp.

In this experiment, five types of agar media were used, including casein, Tween80, cellulose, gelatine, and urea agar media. The isolates were examined for the presence of Caseinase, lipase, cellulase, protease, and urease using the medium mentioned above. Every individual sample was obtained using a sterile loop and then applied onto these media. The Petri plates and test tubes were incubated overnight at a temperature of 35 ℃, as described by Harley and Prescott in (2002).

2.4.1. Urea Agar test:

The urea agar was inoculated in a test tube and then incubated for a period of 24 hours. The presence of the urease enzyme was deduced from the alteration in color to pink in the urea agar test tubes, as documented by Bilgehan (2014) and Abdulhusein (2023).

2.4.2. Oxidase Test:

In the experimental protocol for oxidase, the filter paper was first treated with tetra-dimethyl-para-phenylenediaminedihydrochloride. Subsequently, bacterial colonies were applied onto the same filter paper by smearing. Following that, the results were read by blue-violet color formation within 20 seconds (Abdulhusein 2023; Harley and Prescott 2002).

2.4.3. Catalase Test:

To test for catalase activity, two drops of hydrogen peroxide (H_2O_2) with a concentration of 3% were added to the colonies that had developed on the growth medium. The colonies that displayed a favorable response generated gas bubbles, but the negative isolates did not exhibit any bubble formation. The objective of this experiment was to distinguish between *Staphylococcus* sp. and *Streptococcus* sp. by employing a sophisticated test that exclusively focuses on aerobic and microaerophilic bacteria (Abdulhusein 2023; Harley and Prescott 2002).

2.4.4. Coagulase Test:

The coagulase test involves inoculating bacterial growth into a test tube containing rabbit plasma, which is then incubated overnight at 37℃. The presence of a clot is regarded a positive result, whereas the absence of a clot is interpreted as a negative result (Rakotovao-Ravahatra*et al*., 2019).

2. 5. Mannitol Fermentation

Mannitol agar, with its sodium chloride (NaCl) concentration of 7.5%, has demonstrated its efficacy as a selective medium for specific bacteria, including *Staphylococcus* sp. The isolates grown on Mannitol Salt Agar (differential and selective medium) supplemented with mannose and phenol red exhibited positive catalase activity and hemolysis after 24 hours of incubation at 37℃. Yellow colonies seen on Mannitol agar following the incubation period were regarded as evidence of *S. aureus* exhibiting positive Mannitol activity. The transition from red to pink was seen as a positive indicator of Mannitol activity by other *Staphylococcus* sp. (Santos *et al*., 2015; Saab *et al*., 2018).

2.6. Utilization of carbon sources by *Staphylococcus* sp.

The metabolic activity of pathogenic bacterial isolates was assessed by employing 11 carbohydrates. The bacterial isolates were separately inoculated into test tubes and cultured at a temperature of 35℃ for duration of 24 hours (Abdulhusein 2023; Harley and Prescott 2002). The utilization of 11 sugar sources by the tested isolates was evaluated independently using a 1% (w/v) concentration of Ribose, Galactose, Lactose, Mannose, Cellobiose, Maltose, Fructose, Trehalose, Arabinose, and Sucrose. The transition from the color red to yellow was viewed as a favorable outcome for the utilization of carbohydrates (Abdulhusein 2023; Harley and Prescott 2002).

2.7. Molecular diagnosis

The chromosomal DNA was isolated and purified using the QIAampDNA and QIAquickPCR Kits from Qiagen, Germany. The isolation and purification process followed the provided instructions. Subsequently, the DNA was amplified using the PCR technique and specific forward and reverse primers designed for *Staphylococcus* sp. The forward primer sequence was GTAGGTGGCAAGCGTTACC and the reverse primer sequence was CGCACATCAGCGTCAG (Al-Musawi*et al*., 2014).

2.8. DNA sequencing

A total of ten isolates were submitted for sequencing using the DideoxySanger sequencing method.

3. Results

The analysis revealed that out of the 25 swab samples, a total of 25 distinct bacterial species were identified. Among these, 18 isolates were classified as Gram positive and were utilized in this study, while the other 7 isolates were Gram negative and were not considered for further analysis. The identification of 11% *Staphylococcus haemolyticus* (2 isolates), 11% *Staphylococcus warneri* (2 isolates), 16.6% *Staphylococcus epidermidis* (3 isolates), 22% *Staphylococcus aureus* (4 isolates), 11% *Staphylococcus hominis* (2 isolates), 11% *Staphylococcus xylosus* (2 isolates), 11% *Staphylococcus sciuri* (2 isolates), and 5.5% *Staphylococcus borealis* (1 isolate) was determined based on biochemical tests, including selective media (Mannitol Salt Agar), enzyme production (Catalase, Coagulase, Oxidase, Protease, Lipase, Caseinase, Urease, Esterase, and Cellulase), utilization of sugar sources, and the AP20 test. Table 1.

Table 1. Isolates code, Species, and Numbers of isolates.

Isolates code	Species	Numbers	%
*SS1	*Staphylococcus haemolyticus*	2 isolates	11
SS2	*Staphylococcus warneri*	2 isolates	11
SS3	*Staphylococcus epidermidis*	3 isolates	16.6
SS4	*Staphylococcus aureus*	4 isolates	22
SS5	*Staphylococcus hominis*	2 isolates	11
SS6	*Staphylococcus xylosus*	2 isolates	11
SS7	*Staphylococcus sciuri*	2 isolates	11
SS8	*Staphylococcus borealis*	1 isolates	5.5
Total:	18 isolates are belonging to *Staphylococcus* sp. of 25 swab samples, S* skin Swab		

The enzymatic properties of *Staphylococcus* sp. were examined, and the findings indicated that 2 isolates, 3 isolates, 6 isolates, and one isolate, respectively, exhibited the production of Coagulase, Esterase, Protease, Urease, Oxidase, Caseinase, and Lipase. All isolates exhibited catalase production, but none of them had the ability to produce cellulase (Table,2).

Table 2. The enzymatic properties of *Staphylococcus* sp.

No	**Similar species**	Oxidase	Protease	Caseinase	Lipase	Cellulase	Urease	Esterase	Coagulase	Catalase
1	*Staphylococcus haemolyticus*	-	+	-	-	-	-	+	-	+
2	*Staphylococcus warneri*	-	-	-	-	-	+	-	-	+
3	*Staphylococcus epidermidis*	-	+	-	-	-	+	-	-	+
4	*Staphylococcus aureus*	-	+	-	-	-	+	+	+	+
5	*Staphylococcus hominis*	-	+	-	-	-	+	-	-	+
6	*Staphylococcus xylosus*	-	-	-	-	-	+	+	-	+

7	*Staphylococcus sciuri*	+	+	+	-	-	-	+	+	+
8	*Staphylococcus borealis*	-	+	-	+	-	+	-	-	+
Positive		1	6	1	1	0	6	3	2	8

A total of eleven sugars were employed in this experiment, and the findings revealed that only *S. sciuri* shown the ability to metabolize Cellobiose as a carbon source. All of our isolates possess the capacity to use Sucrose and Maltose. Nevertheless, all of our samples were unable to metabolize Arabinose. Mannitol, Mannose, Trehalose, Fructose, Galactose, Lactose, Ribose, and Cellobiose were identified individually utilized by (7, 4, 6, 3, 3, 2, and 1)(Table,3).

Table 3. Utilization of carbohydrates by *Staphylococcus* sp.

No	Species	Cellobiose	Fructose	Galactose	Arabinose	Maltose	Sucrose	Lactose	Ribose	Mannitol	Mannose	Trehalose
1	*Staphylococcus haemolyticus*	-	-	+	-	+	+	-	-	+	-	+
2	*Staphylococcus warneri*	-	+	-	-	+	+	-	-	+	-	+
3	*Staphylococcus epidermidis*	-	+	-	-	+	+	-	+	-	+	-
4	*Staphylococcus aureus*	-	+	-	-	+	+	-	-	+	+	+
5	*Staphylococcus hominis*	-	-	-	-	+	+	-	-	+	-	-
6	*Staphylococcus xylosus*	-	+	-	-	+	+	+	-	+	-	+
7	*Staphylococcus sciuri*	+	-	+	-	+	+	+	-	+	+	+
8	*Staphylococcus borealis*	-	+	+	-	+	+	+	+	+	+	+
Positive		1	5	3	0	8	8	3	2	7	4	6

Out of the ten isolates that were submitted for sequencing based on their 16S rRNA sequence, only two isolates were successfully sequenced according to the DNA sequencing results. The sequencing results indicated a similarity of 98.12% for *S.aureus* and 99.56% for *S.haemolyticus*. The results indicated that the base pair of both sequenced isolates were 1128 and 1059, respectively. Furthermore, the accession numbers for both of these isolates were duly recorded in GeneBank(Table,4).

Table 4. The similar species depending on the results of 16S r RNA sequences.

Isolates	*Staphylococcus haemolyticus*	*Staphylococcus aureus*
Similar species	99.56%	98.12%
Base pair	1128	1059

Strain	*EB17 16S* rRNA	*S33 16S r RNA*
Blast name	Firmicutes	Firmicutes
Starting sequences	"CTCCACCGGC"	"ACTCCACCGG"
Molecular weight	346768.06 Da	325684.52 Da
GC content	51%	51%
Tm (nn model)	91.5°C	91.3°C

Six kinds of antibiotics discs which are including Cephalothin, Cefotaksime, Rifampicin, Spectinomycin, Tetracycline, and Novobiocin, were tested against *S. haemolyticus*, *S. warneri*, *S. epidermidis*, *S. aureus*, *S. hominis*, *S. xylosus*, *S. sciuri*, and *S. borealis*. The findings indicated that *S. warneri* and *S. epidermidis* exhibited susceptibility to Novobiocin, with inhibition zones measuring 22 mm and 21 mm, respectively. *S. hominis* exhibited resistance to tetracycline, while the other isolates shown sensitivity to tetracycline with varying sizes of inhibitory zones. All of our isolates were susceptible to Rifampicin and Spectinomycin. In addition, only *S. haemolyticus* showed resistance to Cephalothin and Cefotaksime (Table 5).

Table 5.Diameters of Inhibition Zones (mm) of the test isolates against antibiotics

No	Species	Types of Antibiotics					
		Cephalothin	Cefotaksime	Rifampicin	Spectinomycin	Tetracycline	Novobiocin
		Inhibition zone (mm)					
1	*Staphylococcus haemolyticus*	R	R	≥28	≥8	≥20	R
2	*Staphylococcus warneri*	≥23	≥10	≥28	≥11	≥22	≥22
3	*Staphylococcus epidermidis*	≥21	≥36	≥33	≥12	≥12	≥21
4	*Staphylococcus aureus*	≥29	≥30	≥36	≥15	≥33	R
5	*Staphylococcus hominis*	≥16	≥18	≥21	≥22	R	R
6	*Staphylococcus xylosus*	≥17	≥14	≥19	≥18	≥21	R
7	*Staphylococcus sciuri*	≥20	≥21	≥26	≥20	≥19	R
8	*Staphylococcus borealis*	≥18	≥19	≥21	≥14	≥24	R

The optimal growth conditions for *Staphylococcus* sp. were examined, and the results revealed that the pH range for growth was between 5 and 10. However, the optimal pH for all investigated isolates was determined to be 7. The optimal concentration of NaCl was 2%, with a range of NaCl concentrations varying from 0 to 12. All *Staphylococcus* species that were identified during this experiment were categorized as mesophilic, with an optimal temperature range of 35℃ to 40℃. The optimum temperature was ranging from 35℃ to 40℃(Table,6).

Table 6. The Optimum conditions for *Staphylococcus* sp. growth.

No	Species	pH range	Optimum pH	NaCl range %	Optimum NaCl %	Temperature range	Optimum Temperature
1	*Staphylococcus haemolyticus*	5-10	7	0-12	2%	18-60°C	35°C-40°C
2	*Staphylococcus warneri*	5-10	7	0-12	2%	18-60°C	35°C-40°C
3	*Staphylococcus epidermidis*	5-10	7	0-12	2%	18-60°C	35°C-40°C
4	*Staphylococcus aureus*	5-10	7	0-12	2%	18-60°C	35°C-40°C
5	*Staphylococcus hominis*	5-10	7	0-12	2%	18-60°C	35°C-40°C
6	*Staphylococcus xylosus*	5-10	7	0-12	2%	18-60°C	35°C-40°C
7	*Staphylococcus sciuri*	5-10	7	0-12	2%	18-60°C	35°C-40°C
8	*Staphylococcus borealis*	5-10	7	0-12	2%	18-60°C	35°C-40°C

4. Discussion

Although not all members of the genus *Staphylococcus* are pathogens, it is widely recognized as one of the most significant genus among pathogenic species. *S. aureus* is a gram-positive bacterium that induces a wide variety of infections in both humans and animals. A primary etiological agent of mastitis in dairy herds, exudative dermatitis in pigs, and arthritis and osteomyelitis in poultry is *S. aureus*, which is extensively recognized (Ho *et al*., 2012). Additionally, *S. aureus* is regarded as a dangerous bacterium for humans due to its potential to cause a variety of illnesses, including respiratory infections, food poisoning, nosocomial bacteremia, and surgical incision infections. This is due to the fact that it possesses a variety of virulence factors.The study revealed that out of the 25 isolates obtained from cows' wounded skin, *S. aureus* accounted for 22% (4 isolates) representing the most isolated species, while *S. borealis* represented the least isolated species with 5.5% (1 isolate). Prior study found that 48 isolates were confirmed as *S. aureus*isolated from skin wounds associated with myiasis infestation in cattle in Basrah province/ Iraq (Sayhood*et al*., 2022). The enzymatic properties of *Staphylococcus* sp. were investigated and the results

discovered that all our isolates do not produce cellulase; whereas Coagulase, Esterase, Protease, Urease, Oxidase, Caseinase, and Lipase were produced by 2 isolates, 3 isolates, 6 isolates, 6 isolates, 1 isolate, 1 isolate, and one isolate, respectively.

The previous investigation indicated that *S. aureus* was the most prevalent species, accounting for 48% of the bacterial isolates obtained from milk and cheese samples; whereas *S. chromogenes* and *S. epidermidis* showed 41% and 11%, respectively. Also, the enzymatic activities of *S. aureus*were investigated and the results explained that all tested isolates exhibited positive results for protease, lipase, gelatinase, and urease; whereas, 80% of these isolates showed positive results for haemolysin. On the other flip side, *S. epidermidis* tested positive for lipase and urease in 100% of cases, and for protease in 78% of cases. However, it tested negative for both haemolysin and gelatinase (Al-Khafaji*et al*., 2013).

Also, the ability of isolated species for using carbohydrates was investigated and the results found that while only *S.sciuri* shown the ability to use Cellobiose, all of our isolates possess the capacity to use Sucrose and Maltose. Nevertheless, all of our samples were unable to metabolize Arabinose. Mannitol, Mannose, Trehalose, Fructose, Galactose, Lactose, Ribose, and Cellobiose were identified individually utilized by (7, 4, 6, 3, 3, 2, and 1).

The results of biochemical tests, the morphology of colonies and pulsed-field gel electrophoresis exhibited that 12, 17, 30, 73, 81, 10, 23, 20, and 80 isolates were isolated respectively from pigs', horse's, cows', chickens', dogs', mice's, pigeons' and humans' skin and identified as *Staphylococcus* sp., these isolates were distributed within five species which are including *S. warneri, S. hominis, S. xylosus, S. epidermidis,* and *S. sciuri*; categorized into two groups novobiocin-resistant species and novobiocin-sensitive species (Nagase *et.al*, 2002).

Other prior investigation 11, 23, and 2 isolates were isolated from animal skins which were belonging to *S. felis, S. epidermidis,* and *S. pseudointermedius*. Also *S. aureus, S. sciuri, S. succinus,* and *S. equorum*were isolated from animal skin (Thomson*et. al*., 2022).

Cephalothin, Cefotaksime, Rifampicin, Spectinomycin, Tetracycline, and Novobiocin, were applied against our isolates and the results found that *S. warneri* and *S. epidermidis*affected byNovobiocin, with inhibition zones measuring 22 mm and 21 mm, respectively. *S. hominis* exhibited resistance to tetracycline, while the other isolates shown sensitivity to tetracycline with varying sizes of inhibitory zones. All of our isolates were susceptible to Rifampicin and Spectinomycin. Previous investigation showed that *S. aureus* which isolated from soft tissue and skin exhibited resistance against ten used antibiotics which were including penicillin, oxacillin, ciprofloxacin, gentamicin, ofloxacin, ampicillin, chloramphenicol,

doxycycline, azithromycin, and tetracycline (Mohanty *et al*., 2018). Previous study found that the optimum conditions for *Staphylococcus* sp. proliferation were 3M NaCl, pH 7, and 35°C (Abdulhusein *et al*., 2023).

5. Conclusion

Staphylococcus is a bacterium that causes a wide range of infections in both animals and humans. This is due to the presence of many virulence factors that enable these bacteria to thrive in various types of environments. This work is the first to isolate and identify *Staphylococcus* sp. from superficial wounds on animal skin in Thi-Qar province/Shatrah city. The study also involves conventional and molecular characterization of *Staphylococcus* utilizing 16S rRNAgene sequence analysis. The present results clearly showed that *Staphylococcus aureus* is the most species that is isolated compared to other species followed by *Staphylococcus epidermidis*. To reduce the development of antibiotic resistance in *Staphylococcus* sp., it is crucial to continuously monitor the patterns of antibiotic susceptibility and the controlling use of antibiotics as well as other bacterial infections. The sensitivity of *Staphylococcus* to antibiotics might differ due to factors such as geographic location, patterns of antibiotic usage, and the existence of antibiotic-resistance genes or mechanisms.

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Ethical approval:

The experimental procedures were approved by the scientific committee of the College of veterinary medicine/ Shatrah University (According to No. 744 dated 8/9/2024).

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