

Isolation and identification of *Staphylococcus aureus* associated with skin infection in ruminant in Thi-Qar province

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Abstract

Acute skin infections are fairly common in ruminants and are important to the production and economy. The most important bacteria causing these infections are believed to be *Staphylococcus aureus* bacteria. The aim of this study was to isolate and identify the *Staphylococcus aureus* bacteria from the infected animals in ruminants, in which 60 samples were taken. A total of 18 samples were obtained from cows, 28 from sheep, 13 from goats and one from buffalo in Dhi Qar Governorate in Iraq. The bacteria were isolated from inflammatory infections (abscesses) by the traditional method (culture plate) and the modern method (the Vitek 2 system). *S. aureus* accounted for 14.3% of the bacteria. *S. aureus*, however, had no resistance (0%) against any antibiotic, and was 100% sensitive to all antibiotics (moxifloxacin, linezolid, teicoplanin, tigecycline, fusidic acid and rifampicin). This is due to the lack of resistance genes (or the genes that give resistance) and to the low previous exposure to antibiotics, resulting in less selective pressure and the emergence of resistant strains. This research will help in better control and treatment and in minimising economic losses due to this infection.

Keywords: Vitek 2, skin infections, staphylococci, sensitive , antibiotics.

الخلاصة:

تُعَدُّ التهابات الجلد لدى المجترات مشكلة صحية شائعة تؤثر على الإنتاج الحيواني والاقتصاد. وتلعب البكتيريا دوراً رئيسياً في هذه الالتهابات، ولا سيما المكورات العنقودية الذهبية. هدفت هذه الدراسة إلى عزل وتحديد المكورات العنقودية الذهبية من حالات التهابات الجلد لدى المجترات في محافظة ذي قار، العراق. وباستخدام الطرق التقليدية مع أطباق الزرع والطرق الحديثة باستخدام نظام Vitek 2، تم تحديد نسبة المكورات العنقودية الذهبية. في المقابل، لم تُظهر المكورات العنقودية الذهبية أي مقاومة (0%) لأي من المضادات الحيوية، وكانت حساسة بنسبة 100% لجميع المضادات الحيوية (موكسيفلوكساسين، لينزوليد، تيكونالين، تيجيسيكلين، حمض الفوسيديك، وريفامبين). تُساهم هذه الدراسة في تحسين استراتيجيات مكافحة العلاج والحد من الخسائر الاقتصادية المرتبطة بهذه العدوى. **الكلمات المفتاحية:** الحساسية، مضادات الحيوية، المكورات العنقودية، الالتهابات الجلدية.

Introduction

The skin is the largest organ in mammals and the outermost organ feature of the body, which divides the internal organisms and external surroundings, it is an essential part of homeostasis and protection against physical, chemical and biological attacks, such as pathogenic microorganisms (1). The skin is particularly susceptible to harm and infection due to constant exposure to the environmental factors, but livestock raised in field and intensive production settings is particularly vulnerable (2). The components of the skin vary from one animal to another, which affects the healing rate and appropriate treatment options for injuries (3). The environmental condition, management practices, hygiene, nutrition, and

infectious agent exposure contribute strongly to the skin health in animals that produce food (such as ruminants). Poor housing and mechanical damages, as well as the presence of ectoparasites on the skin, can weaken its surface and provide the best environment for the opportunistic and pathogenic bacteria to form new infections (4). Since the skin is considered a protective organ and protects the body from external conditions, it is exposed to many microorganisms, including bacteria, and there are two types of bacteria: Gram-positive and Gram-negative among (5). The gram-positive bacteria are another category of microorganisms that cause skin infections in ruminants, The feature of these bacteria is a thick peptidoglycan cell wall that makes them structurally stable and

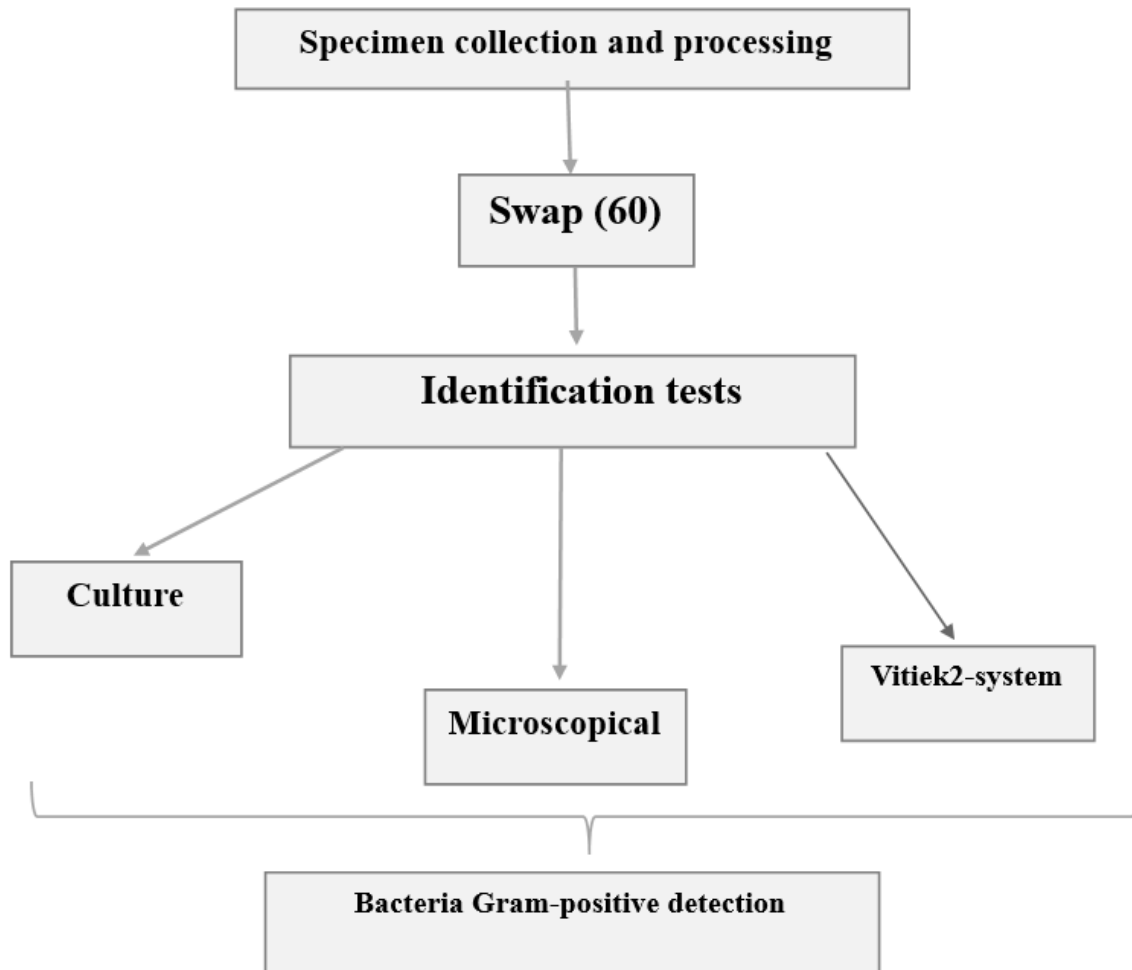
resistant to environment, most Gram-positive species belong to the normal skin flora, but in favorable conditions, they may serve as opportunistic pathogens and cause an extensive variety of skin infections (6). Staphylococci are considered a type of Gram-positive bacteria, classified as part of the flora, however, under certain conditions, they can become opportunistic and turn pathogenic, they are usually isolated from the skin and mucous membranes of animals and can cause skin infections (7). In ruminants, the species of the bacterial genus Staphylococcus are one of the most common cases of bacterial agents that lead to cutaneous infections, especially after skin traumas or infestation with ectoparasites, or due to poor hygienic and management status, Known breaks in the skin barrier promote entry of bacteria, which pose local inflammation and cause local lesions including dermatitis, folliculitis, wound infections, and abscesses (8) In Iraq, a study was carried out in the wounds of cattle affected by myiasis in the governorate of Basra, and Staphylococcus aureus was isolated in 32% (48 isolates from 150 samples), hence the importance of this bacterium in skin infections and wound in ruminant animals. Although these bacteria are important, the data on the spread of these bacteria and bacteriological characteristics in ruminants in Dhi Qar Governorate are limited and need local studies to identify these pathogens, characterize them in the laboratory and to determine the role of these in skin lesions in ruminants. (9) Staphylococci exhibit a high capacity for resistance to many classes of antibiotics, making the treatment of resulting infections more complex. High resistance has been recorded to beta-lactam antibiotics, particularly penicillin and methicillin, due to

their ability to produce enzymes that inhibit the effectiveness of these antibiotics (10). Some strains have also shown resistance to macrolides, tetracyclines, aminoglycosides, and fluoroquinolones, reflecting the development of multidrug resistance (11). The indiscriminate and repeated use of antibiotics is a major factor contributing to the spread of resistant strains, reducing available treatment options and increasing the importance of Staphylococci as a pathogen in human and animal infections (12). Many disease-fighting drugs lose their effectiveness microbes evolve resistance. Antibiotic-resistant diseases kill, over 700,000 people per year across the world. By 2050 up to 10,000 ,000 people a year could die of antimicrobial resistance (13). There are few studies deal with bacterial isolates associates with skin infection in ruminants in Thiqr province, therefore the current study covered this problem , The purpose of this study was to Isolation and identification of bacterial isolates from infected animals, through Culturing the swab samples on selective and enriched media, and staining the bacterial isolates, Confirmation of bacterial isolate diagnosis by using Vitek2 system, Conducting antibiotic sensitivity testing for the main bacterial isolates.

Materials and Methods

Sample collection and processing

In this study, skin swab samples were collected from Ruminants who visited various hospitals Thiqr Governorate, Iraq. (Thiqr Veterinary Hospital, Farm, Al-Graph Veterinary Clinic) The samples were collected in sterile swabs containing amies transport medium, isolated between October 2025 and February 2026, and their diagnoses were confirmed through routine biochemical tests.



Gram stain method

A few bacterial from a colony was distributed with a drop of normal saline on a clean slide, fixed by heat, and then dyed with crystal violet, Iodine, alcohol, and safranine before being magnified 100 times and studied (14).

Isolation of bacteria

To isolate the bacteria, the bacterial isolates were directly placed on Tryptone Soya agar plates , Inclusion criteria: Isolates of bacteria from Ruminants suffering from skin problems.

Confirmed test by Vitek2 compact system

The Vitek 2 System (ID) was used to identification the result of the manual biochemical test, but The Vitek 2 System (AST) this system used to antibiotic susceptibility tests. This system was performed according to the manufacturer's instructions (Biomerieux-France).

VITEK 2 System

The outcomes were verified using the VITEK 2 System. The isolates were cultivated on Tryptone soya agar, and after

being purified and transformed into pure colonies, they were incubated anaerobically for 24 hours at 37°C. The specimens were then added to the VITEK kit as suspension cells in 5 mL of normal saline. The results were checked between 5-7 hours later (15).

Statistical analysis:

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office , Excel 2010 statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using T test and Chi-square P. value ≤ 0.01 was considered to be all analyses. Independent sample t-test was used to study difference in mean between any two groups provided that the variable is normally distributed. Chi-square test was used to study association between any two categorical variables.

Ethical Approval

The necessary ethical approval was obtained with verbal consent, The publication ethics committee at the College

Result and Discussion

Skin infections in ruminants are a serious health problem that affects animal productivity and product quality (16). These infections result from a complex interplay of several factors, including pathogenic bacteria, parasites, environmental conditions, and weakened immunity (17). This study aims to isolate and identify the most important pathogenic bacteria associated with skin infections in ruminants, focusing on identifying the most common species and investigating their pathogenic role in causing skin lesions. A recent review showed that skin diseases in sheep can be caused by a wide range of factors, including bacteria (18). Bacteriological studies have shown that *Staphylococci*, such as *Staphylococcus aureus*,

of Veterinary Medicine, Al-Shatrah University (12), College of Veterinary Medicine, Department of Microbiology, Iraq, approved this study.

Limitation of the Study

The lack of molecular identification technique (polymerase chain reaction or PCR) to confirm *Staphylococcus* species is one of the major limitations of the present study. Considering the limited availability of molecular diagnostic facilities and financial constraints, conventional microbiological and biochemical methods were used for identification. These are all accepted methods and used routinely, but molecular methods are more accurate and specific for identification of bacteria. Thus, the absence of molecular evidence could be regarded as the limitation of this study.

Future studies are suggested to use molecular methods to further improve the accuracy and validity of results.

are among the most important causes of subcutaneous abscesses in sheep (19). Furthermore, recent reports indicate that the most common types of skin infections in sheep include bacterial abscesses and parasitic dermatitis, reflecting the diversity of causative agents and the complexity of diagnosis (20). The results of the current study showed that the isolation rate of *Staphylococci* was 14.3%, which is considered moderate compared to recent studies that addressed the isolation of *Staphylococcal* bacteria from the skin or injuries of ruminants. The appearance of this percentage may be attributed to the fact that *staphylococci* are part of the natural flora of the skin and mucous membranes in animals. However, they become opportunistic pathogens under suitable conditions such as wounds, humidity, weakened immunity, and poor

sanitary conditions. The results of the current study agreed with this, as the study showed the presence of multiple types *Staphylococci* on the skin of healthy sheep and goats. The isolation rate of some types from goat skin was about 13.6% for *Staphylococcus*, which is close to the results of the current study (21). The results of the current study were also lower than those of the study , which showed the spread of *Staphylococci* in milking goat herds at a rate of 27.7% for some dominant isolates. The study attributed this to the ability of staphylococci to colonize the skin, teats, and the environment

surrounding the animal, in addition to possessing virulence factors that help them adhere and form biofilms (22). On the other hand, the results of the current study were higher than some studies that recorded low rates of *Staphylococcus* isolation from animals, which recorded isolation rates of 10.3% in sheep and 8.3% in cattle. This difference may be attributed to the nature of the samples, the health condition of the animals, environmental differences, and the breeding and bacterial isolation methods used in each study (23).



Figure 2: *Staphylococcus* under the microscope after staining with Gram stain.

The results of the microscopic examination in the current study showed that the *Staphylococcus* isolates appeared spherical and clustered in irregular grape-like clusters after Gram staining, which are characteristic diagnostic features of *Staphylococcus* spp. This shape is attributed to the division of bacterial cells at multiple levels while remaining

interconnected after division, giving the characteristic cluster appearance. The results of the current study agreed with this study, which indicated that staphylococci appear in a Gram stain as Gram-positive cocci in clusters, and that this cluster arrangement is considered one of the important microscopic characteristics in the initial diagnosis of staphylococcal bacteria (24).

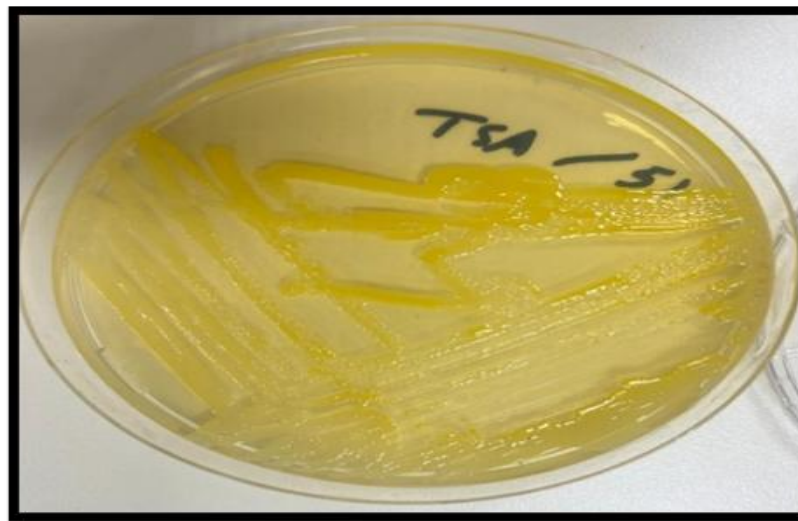


Figure 3: *Staphylococcus* after being cultured on (TSA) agar medium.

The current results showed the growth of staphylococcal isolates on TSA medium in the form of small, smooth, round colonies with a yellowish-golden color. These characteristics are among the common cultural properties used in the preliminary diagnosis of bacteria. The current study's results were consistent with

studies that demonstrated the ability of staphylococci to grow on selective and enriched media such as Tryptic Soy Agar, with the appearance of clear and smooth colonies that aid in the laboratory diagnosis of staphylococcal bacteria (25).

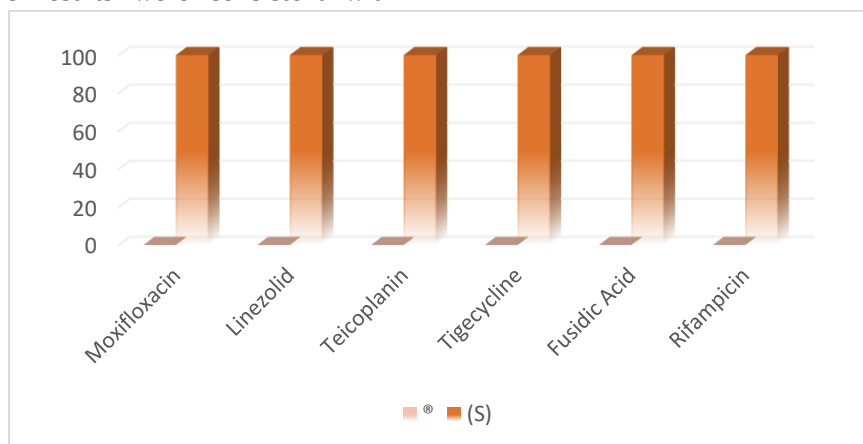


Figure 4: Antibiotic Susceptibility Test (vitek2)

The results of the current study showed that all *Staphylococcus aureus* isolates exhibited complete sensitivity (100%) to the antibiotics Moxifloxacin, Linezolid, Teicoplanin, Tigecycline, Fusidic acid, and Rifampicin, with no recorded resistance or intermediate

sensitivity to these antibiotics. These results indicate the high efficacy of these antibiotics in inhibiting the growth of the studied bacterial isolates, which may be due to the limited random use of some of these antibiotics in the veterinary field, contributing to their

effectiveness against staphylococci (26).The results of the current study were consistent with what was mentioned in previous studies, as the sensitivity rate of *Staphylococcus aureus* isolates to Tigecycline was approximately 98.6%, while the sensitivity rate to Linezolid was over 97%, with very low resistance rates recorded. This supports the current study's findings, which showed complete sensitivity to these antibiotics (27).In another recent study conducted on *Staphylococcus* isolates, it was found that the resistance rate to Linezolid was very low, not exceeding 2-3%, meaning that the sensitivity rate was over 97%, indicating the high efficacy of this antibiotic in controlling *Staphylococcus* isolates. This is consistent with the results of the current study, which showed a 100% sensitivity to this antibiotic (28).However, the marked differences in the patterns of antibiotic sensitivity of these bacteria can be attributed to the types of antimicrobials, geographical differences, differences in manufacturing sources, non-prescription purchases, prescriptions without laboratory confirmation, misuse such as inadequate concentrations, inappropriate treatment periods, and differences in study areas and types of bacteria (29-34)

Conclusion

The recorded isolation rate in this study (14.3%) indicates a moderate prevalence compared to previous recent studies, reflecting the impact of environmental factors, animal management, immune status, and health conditions on the occurrence of staphylococcal infections, Microscopic and culture examinations revealed distinctive morphological and developmental characteristics of the staphylococcal isolates, supporting the reliability of traditional bacteriological methods in the initial diagnosis of this bacterium. Additionally, the results of the antibiotic sensitivity test revealed complete sensitivity of the isolates to several important antimicrobial agents, including moxifloxacin,

linezolid, teicoplanin, tigecycline, fusidic acid, and rifampicin, indicating the continued effectiveness of these antibiotics against the studied isolates, The notable variation between this study and other studies regarding the spread of infection and antibiotic resistance patterns may be attributed to differences in geographical areas,Overall, this study highlights the importance of continuous monitoring of staphylococcal infections and their antibiotic resistance patterns in ruminants to support effective treatment strategies and improve animal health and productivity.

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