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Molecular Comparison of Different Types and Antibiotic Resistance Profile of *Staphylococcus aureus* Isolated from Infected Wounds

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ABSTRACT

Staphylococcus aureus is one of the most prevalent microorganisms in both medical and veterinary field. This bacterium often could infect in areas of damaged skin, such as abrasions and open wound. This research aims to detect the common type and antibiotic resistant pattern of isolated *S. aureus* in wound specimens. One hundred and fifty wound swabs were collected from human, sheep and goat (50 equal samples) in West and East Azarbaijan provinces (Iran). All swabs' samples were transferred to the laboratory in peptone water broth near ice box. The samples were cultured aerobically on sheep blood agar medium at 37 °C for 24 h. Then polymerase chain reaction was used for amplification 16S rRNA and mecA genes to identify the *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Then antimicrobial susceptibility test was done for antibiotic resistant patterns configuration using some antibiotic discs. *S. aureus* was isolated from 19 wound samples and 6 isolates were identified as methicillin-resistant *S. aureus*. The higher and lower isolate was belonged to human (52 %) and goat (8%), also 40 % of sheep wound samples were infected by *S. aureus*. No animal wound samples had MRSA while six human specimens were infected by MRSA. All isolates were sensitive against cefepime, imipenem and carbapenem and the higher resistances were seen against penicillin, vancomycin and cefazolin. It's concluded that MRSA is common in infected human wound and today new generation of cephalosporins are useful for treatment of many multidrug resistant bacteria.

Introduction

Staphylococcus aureus is one of a critical causative agent in both livestock and human pathogenesis, leading to considerable economic losses within the livestock industry. Despite the provision of antimicrobial treatments and advances in healthcare, which notably contributed to decreasing the prevalence and fatalities associated with staphylococcal diseases during the 20th century, *Staphylococcus aureus* remain noteworthy pathogens within hospital settings. Staphylococci's primary natural niche resides on the body surfaces of mammals, where these organisms proliferate in significant numbers. In line with their parasitic nature, staphylococci emerge as remarkably adept and accomplished pathogenic microorganisms. It's when the host's defense barriers are compromised due to injuries or surgical procedures that these organisms can unveil their latent invasive potential. Their true strength becomes apparent when they infiltrate the underlying tissues.[1]

The key attribute that has sustained the survival of these microorganisms, despite the availability of numerous anti-staphylococcal antibiotics over the past four decades, is their capacity to develop resistance,

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especially to penicillin and its derivatives. Moreover, their connection with widespread outbreaks of severe hospital-acquired infections has garnered significant attention.[2]

Over time, a decline in the clinical efficacy of penicillin ensued, and in parallel with the advent of novel antibiotics, the rapid emergence of resistance to these agents became evident. Resistance to recently developed antibiotics is primarily linked to the production of penicillinase and the development of multidrug resistance in certain strains that have become entrenched within hospital settings. The rise in the population of vulnerable individuals within hospitals plays a role in the onset of epidemics stemming from staphylococcal infections. In 1960, Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially identified as a hospital-acquired pathogen, and its prevalence within society continues to escalate.[3] MRSA strains possess a compact chromosomal cassette responsible for penicillin resistance, denoted as *SCCmec*, encompassing five distinct types. Within the fourth type of this gene, there are four individual segments: *SCCmec Iva*, *SCCmec IVb*, *SCCmec IVc*, and *SCCmec IVd*. This gene plays a role in inhibiting xenophagy and indirect cellular immunity, while also generating an enzyme primarily responsible for undermining the efficacy of penicillin and methicillin-based therapies.[4]

Various molecular techniques are employed for the typing and identification of both *Staphylococcus aureus* and MRSA strains. Noteworthy among these approaches are DNA fingerprinting through the PFGE method, *SCCmec* typing, and sequencing-based methodologies like spa-typing and MLST. The spa-typing technique involves the amplification and sequencing of the x region within the spa gene, responsible for encoding surface protein A. The x region's pronounced polymorphism renders it suitable for distinguishing investigations and typification. Although this method exhibits lesser discriminatory capacity in comparison to PFGE, it surpasses MLST in terms of its discriminatory prowess. Furthermore, the spa-typing approach proves to be more cost-effective when contrasted with methods like MLST, which demands sequencing of no less than seven genes, as well as the PFGE technique.[1]

Staphylococcus aureus readily adheres to highly proteinaceous surface of chronic wounds and forms matrix-encased communities resulting in present chronic wound infection that is recalcitrant to antibacterial therapies.[5] Wounds are defined as a breach in the skin or tissues structural integrity that affect the skin ability to defend itself. As one of the most common causes of death and morbidity in surgical patients, wound infection accounts for 70% to 80% of deaths after burn injuries. Bacteria that cause pus production or wound infection include *S. aureus*, *Clostridium spp*., *Actinomyces spp*., *Escherichia coli*, *Proteus* spp. *Neisseria* spp., *Vibrio vulnificus* and *Candida* spp.[6]

This study sheds light on the current prevalence of *S. aureus* and MRSA in wound, the antimicrobial susceptibility pattern of the isolated *S. aureus* in human and animal wound samples.

Materials and Methods

Sample Preparation

In this research, we examined 150 wound swab samples of human (50), sheep (50) and goat (50) from West and East Azarbaijan provinces (west north, Iran) from January 2023 to January 2024. Human samples were taken from hospitalized patients in burning section of Emam Khomaini (Urmia) and Sina (Tabriz) hospitals and animal samples were collected from north, west, east and south parts of both provinces. Then swabs transferred to the laboratory on ice box immediately in peptone water (Merck, Germany; transfer medium). All swabs cultured on 5% sheep blood agar (Merck, Germany) and incubated aerobically at 37 °C for 24-48 h. All suspected colonies to *Staphylococcus aureus* subjected for pure culture and primary identification was done using gram staining, morphology and some biochemical tests such as catalase, coagulase, nitrate reduction, motility, mannitol fermentation and gelatin.[7]

Molecular Assay

Polymerase chain reaction was used for *16s rRNA* and *mecA* genes amplification also spa typing of isolated *S. aureus* from wound swabs. *16s rRNA* gene was used for *Staphylococcus aureus* detection and *mecA* gene used to confirmation methicillin-resistant *S. aureus*. At the first step pure colony of each isolated *S. aureus* was cultured in tryptic soy broth (TSA; Merck, Germany) and incubated aerobically at 37 °C for 18-24 h. Then the medium centrifuged at 3000 round per minute for 15 min and the precipitant washed twice with phosphate buffer solution (PBS). Then bacterial chromosomal DNA extracted using a commercial DNA extraction kit (Favorgen, Taiwan). Extraction process was done according to the instruction of kit and the quality and quantity of extracted DNA was assayed by nanodrop (Thermo Fisher Scientific, USA) at 260 and 280 nm wavelength. Finally, all extracted DNA from isolated *S. aureus* stored at -20 °C until examination by PCR.

All reaction mixtures for *16s rRNA*, *mecA* and spa typing were adjusted in 25 µl volume containing 12.5 µl 2X master mix PCR (Yekttajhiz, Iran), 0.5 µl for each forward and reverse primers, 5 µl template DNA and 6.5 µl DEPC water. Thermal program for PCR was set according to the Table 1 in a thermal cycler (Corbett,

Table 1. Nucleotide sequences of the primers used for amplification *16s rRNA* and *mecA* genes.

Gene	primer	Sequences (5' to 3')	Product size (bp)
16s rRNA	forward	ACGGTCTTGCTGTCACTTATA	257
	reverse	TACACATATGTTCTTCCCTAATAA	
mecA	forward	ACTGCTATCCACCCTCAAAC	163
	reverse	CTGGTGAAGTTGTAATCTGG	

Australia). Also, primers sequences for *16s rRNA*, *mecA* and spa typing were designed using Amplifix software (version 7) and primers properties were showed in Table 2.[8] Finally, all PCR products run on agarose gel electrophoresis (1.5-2.5% based on expected PCR products size) and visualized using gel doc apparatus (Thermo Fisher Scientific, USA).

Antimicrobial Susceptibility Test

To evaluate the antibiotic susceptibility of isolates, a range of antibiotic disks employed, including penicillin (10 µg), ampicillin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), ceftriaxone (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), methicillin (10 µg), trimethoprim-sulfamethoxazole (25 µg), gentamicin (10 µg), amikacin (30 µg), imipenem (5 µg), cefoxitin (15 µg), vancomycin (30 µg) and oxacillin (30 µg). The susceptibility testing was conducted following the standard disc diffusion method in accordance with CLSI (2024) guidelines.[9]

Statistical Analysis

The results were analyzed through descriptive statistical methods, including frequency and percentage calculations.

Results

Staphylococcus aureus Isolation from Wound Swabs

In this investigation from 150 wound swab samples 16.66% were infected with *S. aureus*. The results showed in 25 swab samples *S. aureus* isolated and identified based on common bacteriological tests and PCR. Out of 25 wound swab samples 19 isolates were sensitive against methicillin, but 6 isolates were resistant against it. Human wound samples had higher contamination with *S. aureus* (13 isolates, 52%) and all methicillin-resistant types (6 isolated) belonged to human wounds. Goat and sheep wound swab samples only were infected with methicillin-sensitive type of *S. aureus* 8% and 40% respectively.

Amplification of *16s rRNA* gene by polymerase chain reaction for *S. aureus* revealed a band size of 598 base pair in comparison with 100 bp DNA marker (Figure 1). All 25 isolates of *S. aureus* had this band size on agarose gel. On the other hand *mecA* gene amplification produced a 563 bp product in PCR and only 6 isolates out of 25 isolated *S. aureus* showed this band in agarose gel (Figure 2).

Antibiotic Sensitivity Pattern

The resistance patterns of *S. aureus* strains from human, got ad sheep wound swab samples were showed in Table 3. All isolated *S. aureus* strains showed resistance to penicillin (100%), ampicillin (95%), tetracycline (57%) and gentamicin (38 %). The isolates exhibited susceptibility to vancomycin (81%), ciprofloxacin (75%), cefoxitin (65%), and oxacillin (58%).

Out of 25 isolates of *S. aureus*, 21 exhibited multidrug resistance (MDR). The highest number of MDR *S. aureus* isolates (11/13) was found in the human samples. In addition, 3 isolates of sheep wound samples showed resistance to 6 antibiotics (penicillin, ampicillin, nalidixic acid, tetracycline, gentamycin and amikacin). Only one isolates of *S. aureus* from goat was resistant against four antibiotics (penicillin, ampicillin, tetracycline and gentamycin).

Table 2. PCR reaction programming for both *16s rRNA* and *mecA* amplification.

PCR steps	Temperature (degrees Celsius)	Time (Seconds)
Primary Denaturation	94	180
Denaturation	94	30
Primer Annealing	58	30
Extension	72	60
Final Extension	72	300

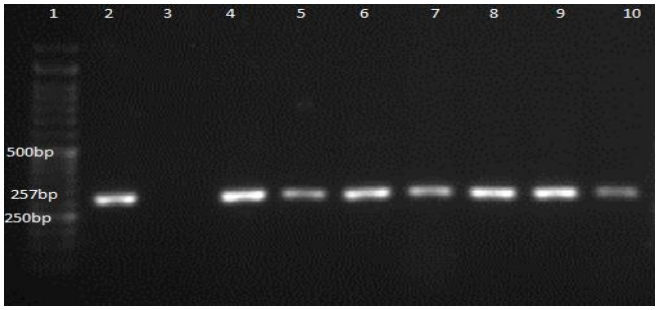

Figure 1. The results of *16s rRNA* gene amplification on 2% agarose gel. Lane 1: 50 bp DNA marker; lane 2: positive control (PTCC* 1431; lane 3: negative control; lane 4-10: positive samples for *S. aureus*.
* Persian Type Culture Collection.

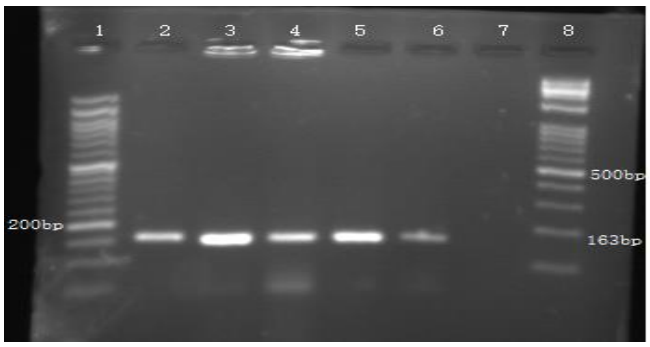

Figure 2. The results of *mecA* gene amplification on 2% agarose gel. Lane 1 and 8: 50 bp DNA marker; lane 2-6: positive isolates for methicillin-resistant *S. aureus* and lane 7: negative *S. aureus*.

Table 3. Antibiotic sensitivity pattern of isolated S. aureus from wound.

Antibiotic (µg per disc)	Number of isolates (%)		
	resistant	intermediate	sensitive
Penicillin (10)	22 (88)	3 (12)	0
Ampicillin (10)	21 (84)	2 (8)	2 (8)
Nalidixic acid (30)	17 (68)	7 (28)	1 (4)
Tetracycline (30)	13 (52)	8 (32)	4 (16)
Ceftriaxone (30)	13 (52)	6 (24)	6 (24)
Cefepime (30)	11 (44)	9 (36)	5 (20)
Ciprofloxacin (5)	9 (36)	10 (40)	6 (24)
Clindamycin (2)	9 (36)	9 (36)	7 (28)
Erythromycin (15)	8 (32)	9 (36)	8 (32)
Trimethoprim-Sulfamethoxazole (25)	8 (32)	5 (20)	12 (48)
Methicillin (10)	6 (24)	0	0
Amikacin (30)	6 (24)	15 60)	4 (16)
Imipenem (5)	5 (20)	17 (68)	3 (12)
Cefoxitin (15)	4 (16)	15 (60)	6 (24)
Vancomycin (30)	4 (16)	10 (40)	11 (44)
Oxacillin (30)	4 (16)	12 (48)	9 (36)

Discussion

Many bacteria are implicated in wound infection, with *S. aureus* identified as one of the common and widespread causal agents in worldwide.[10] A thorough understanding of wound's infection pathogen pattern is necessary for the effective management in human and animals.[11]

Several studies have reported a high prevalence of *S. aureus* in wound infection. The rise of MDR *S. aureus* strains is a growing concern worldwide. This situation has been documented in various studies from Iran and other parts of the world. The presence of these resistant strains complicates treatment efforts and leads to prolonged infections, higher treatment costs, and increased culling rates, all of which have significant economic implications for the public health. The present study reveals a prevalence of 16.66% for *S. aureus* in wound among human, goat and sheep in the northwest region of Iran.

The use of antibiotics has long been a common practice in effectively treating wound infection induced by bacteria. Cephalosporines, macrolides, and tetracyclines are common antimicrobial agents in managing staphylococcal infection in human and animals. Nonetheless, their efficacy is compromised by the rise of multidrug-resistant strains.[12] The outcome of treatment for infections caused by *S. aureus*, particularly when involving MDR strains is often less favorable and more complex compared to non-resistant.[13] The presence of MDR strains significantly reduces the effectiveness of standard antibiotic therapies, leading to prolonged treatment durations, higher doses of antibiotics, and sometimes the necessity to use more potent or last-resort antibiotics, such as vancomycin. However, even these aggressive treatments may not guarantee success due to the high adaptability and resilience of MDR *S. aureus*.

The combination of MDR and chronic infection often leads to poor treatment outcomes, with higher rates of relapse, prolonged infections, and in some cases, the necessity to cull affected animals due to the inability to control the infection effectively.[14]

All isolated *S. aureus* strains underwent antimicrobial susceptibility testing. In this study, penicillin showed 88 % resistance to *S. aureus*, followed by ampicillin and nalidixic acid. On the other hand, vancomycin, oxacillin and cefoxitin found most sensitive to the isolated *S. aureus*. Our results align with previous studies, which consistently document resistance to these antibiotics.[15] In our investigation, 6 out of 25 isolates were identified as resistant against methicillin (MRSA). A significant contributing factor is the extensive and sometimes indiscriminate administration of antibiotics for bacterial disease treatment in human and animals. This selective pressure fosters the emergence of resistance genes in *S. aureus*, enabling their survival despite subsequent antibiotic interventions.[16] Additionally, *S. aureus* possesses a natural ability to acquire resistance determinants through horizontal gene transfer from other bacteria within the udder environment. These factors combined contribute to the concerning rise of MDR-SA in clinical samples from human and animals. MDR *S. aureus* has been associated with increased morbidity and mortality, as well as longer hospital stays in human patients and severe illness in animals.[16] Despite the challenges posed by MDR pathogens, recent studies have shown promising results in both in vitro and in vivo investigations of *S. aureus* infections, demonstrating effective antimicrobial strategies and the potential for successful treatment, providing hope for better control of diseases caused by these resistant strains.[18]

The emergence of MRSA from the human, goat and sheep source poses a threat to human health due to the possibilities of transferring from animals to human.[18]

Methicillin-resistant *S. aureus* isolates are also MDR which bear resistance genes on their chromosome cassette mec carries the *mecA* gene. The presence of resistance genes such as *mecA* exacerbates the issue by reducing the efficacy of commonly used antibiotics like β-lactams, necessitating the use of more potent drugs, which may not always be available or may lead to adverse effects.[20] In this study, 4 out of 25 *S. aureus* strains (16 %) exhibited phenotypic resistance to oxacillin. However, only 6 isolates (24 %) were genotypically confirmed to possess the methicillin-resistance gene (*mecA*). This discrepancy underscores the complex nature of antibiotic resistance in *S. aureus*, where phenotypic resistance does not always correlate with the presence of known resistance genes like *mecA*. The variation observed may be attributed to several factors, including differential expression of the *mecA* gene, which can be influenced by regulatory elements and environmental conditions. Some *S. aureus* strains may carry alternative resistance mechanisms, such as mutations in other genes like *mecC* or modifications in penicillin-binding proteins (PBPs) that confer resistance to β-lactam antibiotics independently of *mecA*.[21] Additionally, phenotypic resistance could arise from non-genetic factors, such as the presence of biofilms or changes in cell wall structure, which can inhibit antibiotic efficacy even in the absence of *mecA*. This finding highlights the challenges in diagnosing and treating methicillin-resistant *S. aureus* (MRSA) infections, as reliance solely on genotypic methods may overlook strains that display resistance through other pathways. Therefore, a comprehensive diagnostic approach that includes both phenotypic and genotypic assessments is essential for accurately identifying and managing MRSA infections, particularly in agricultural settings where varied environmental factors may influence resistance expression. Our results are consistent with the findings of other researchers, [22-25] who reported a 47.72 % presence of the *mecA* gene in 220 *S. aureus* isolates. Similarly, Havaei *et al*.[26] observed a positive *mecA* test in 18.52 % of the samples, with 10 out of 54 *S. aureus* isolates carrying the gene. These studies further emphasize the need for a deeper understanding of the molecular mechanisms governing antibiotic resistance in *S. aureus* to improve treatment outcomes and resistance mitigation strategies.

In conclusion, this study highlights the significant role of *S. aureus* as a leading cause of wound infection in northwest Iran, reflecting global trends and its impact on veterinary and public health. The identification of *S. aureus* strains with high antibiotic resistance level including MRSA, raises concerns about the effectiveness of current treatment protocols for human medicine and the potential threat to animal health and productivity. These isolates showed diverse resistance patterns, particularly to antibiotics commonly used in human and veterinary settings. The presence of MRSA in human wound also indicates a potential risk to human health. These findings emphasize the urgent need for antimicrobial stewardship and surveillance in public health to reduce the spread of antibiotic resistance. Continuous monitoring of antibiotic resistance patterns and virulence factors of *S. aureus* from human and animal is crucial for developing evidence-based strategies to manage and prevent wound infection, ensuring food safety and the health of animals and humans.

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Conflict of Interest

The authors declare no conflict of interest.

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