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Prevalence, Antibiotic Susceptibility, and Molecular Typing of *Staphylococcus aureus* and Methicillin-resistant *S. aureus* (MRSA) among Domestic Animals in Dharan, Nepal

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Abstract

Staphylococcus aureus, a commensal bacterium found on the skin, skin glands, and mucous membranes of humans and animals. It affects the skin, soft tissues, bloodstream, and lower respiratory tract, causing diseases such as endocarditis, osteomyelitis, and generalized scalded skin disease in animals. This study investigates the prevalence and antibiotic susceptibility patterns of *S. aureus* and Methicillin-resistant *S. aureus* (MRSA) among domestic animals in Dharan, Nepal. A total of 320 skin swab samples were collected from various domestic animals, revealing a 12.5% prevalence rate of *S. aureus.* Strikingly, MRSA prevalence was notably high at 60% among the isolated *S. aureus* strains. Antimicrobial susceptibility testing unveiled widespread resistance to commonly used antibiotics, with notable exceptions such as vancomycin. Molecular analysis using the *spa* gene as a marker for strain typing detected its presence in a small subset of MRSA isolates, suggesting the possibility of human colonization and further underscoring the interconnectedness of animal and human health. Only 5% of the MRSA isolates carried the *spa* gene, confirmed by PCR and gel documentation in samples from buffalo skin swabs. The study highlights the importance of interdisciplinary collaboration between veterinary and human medicine sectors to address the growing threat of antimicrobial resistance. Overall, this research contributes valuable insights into the epidemiology of *S. aureus* infections in livestock and advocates for stringent hygiene practices, prudent antibiotic usage, and routine veterinary monitoring to mitigate the spread of MRSA and protect public health interests.

Keywords: *Staphylococcus aureus*, domestic animals, antibiotic susceptibility, *Spa* gene

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Introduction

Staphylococci, which are prevalent pathogens, are Gram-positive bacteria typically present on the skin or mucous membranes of humans and animals. They are part of the Micrococcaceae family and are characterized by their round shape and grape-like clusters, ranging from 0.5 to 1.5 µm in diameter [1]. *S. aureus* has been closely linked with livestock, which can serve as carriers of the bacterium or become infected themselves. Livestock, including pigs, cattle, and poultry, are susceptible to *S. aureus* infection, with dairy cows and chickens posing significant challenges in infection control [2].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that has acquired specific genetic elements, namely the *mecA* or *mecC* gene. These genes enable the bacteria to produce an altered penicillin-binding protein, PBP2a, which reduces the efficacy of most beta-lactam antibiotics, including methicillin, penicillin, and cephalosporins. As a result, MRSA infections are particularly challenging to treat and often require alternative antibiotics, like vancomycin or linezolid, which can have more severe side effects or limited efficacy in certain cases. MRSA is primarily transmitted through direct contact, often via contaminated hands, making it a common concern in healthcare settings where patients and healthcare workers frequently come into close contact. It can also spread through shared personal items, such as towels, razors, or athletic equipment, and public facilities like gym benches and locker rooms, where bacteria can survive on surfaces for extended periods. Because of this, athletes, military personnel, and individuals in communal living situations are at heightened risk of MRSA colonization or infection. Though rare, droplet-borne transmission of MRSA is also possible, particularly among individuals with compromised respiratory control, such as those with tracheostomies or chronic respiratory conditions. Once colonized, MRSA can inhabit various body sites, including the skin, nasal passages, and even the throat. While colonization does not always lead to active infection, the bacteria can become opportunistic, entering the bloodstream, urine, or other sterile sites and causing conditions such as cellulitis, abscesses, septicemia, or pneumonia. This

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versatility and persistence make MRSA a significant pathogen of concern in both community and healthcare environments, as it poses substantial health risks and necessitates rigorous infection control measures [3]. Additionally, domestic animals like horses and pigs can serve as reservoirs for MRSA transmission. Livestock-associated methicillin-resistant *S. aureus* become the most emerging problem in many parts of the world while it may be considered a possible change in the zoonotic transmission of LA-MRSA [4]. MRSA is categorized into hospital-acquired (HA-MRSA), community-acquired (CA-MRSA), and livestock-associated (LA-MRSA) strains, with the latter being identified in humans in 2005 in the Netherlands [5]. MRSA is frequently detected in nasal swabs and skin infections of livestock, specifically in case of poultry and dairy animals [6,7]. As there are number of reported cases of its cross-species transmission, there is potential risks of transmission between humans and animals as well [8]. For example, the CC-398 strain of *S. aureus* is frequently reported in livestock as well as in humans [9]. In this way, economic losses in dairy and poultry is significantly higher. In case of milk industry, clinical mastitis is a severe livestock disease that particularly affects cows, goats, and sheep thus hampers on milk production quality and yield [10].

MRSA is not only a significant cause of human infections but also a notable pathogen in veterinary medicine, responsible for various animal diseases. In dairy cows, MRSA is a common cause of mastitis, an infection marked by inflammation of the mammary glands. Mastitis is a costly condition for the dairy industry due to its impact on milk production, quality, and animal welfare. The inflammation in the udder is often due to bacterial infections that cows may contract during the milking process or from contaminated environmental sources, such as bedding, manure, and feed. Mastitis caused by MRSA can present in two primary forms: contagious and environmental. Contagious mastitis is often the result of persistent infections within the udder, leading to subclinical infections, where cows may appear healthy but have lower milk yields and increased somatic cell counts. These infections can be difficult to detect and treat, allowing MRSA to spread from cow to cow during milking. In contrast, environmental mastitis typically occurs when environmental pathogens come into contact with the udder. Unlike the persistent infections caused by MRSA, environmental pathogens are usually quickly targeted by the host's immune response and eradicated, causing less long-term damage to the udder tissue. Managing MRSA-related mastitis requires strict biosecurity measures, including regular milking equipment sanitation, isolation of infected animals, and monitoring of milk quality to minimize the risk of transmission within herds. Additionally, because MRSA is resistant to common antibiotics, treatment options are limited, complicating efforts to control the spread of the bacteria in dairy herds. This situation underscores the importance of preventive strategies, such as maintaining clean environments and practicing effective milking hygiene, to protect animal health and ensure the safety and productivity of milk supplies. The presence of MRSA in livestock also has public health implications, as it raises concerns about potential transmission to humans through direct contact or consumption of contaminated dairy products [11]. Suppurative ailments, predominantly impacting cattle, entail inflammation within the central nervous system, resulting in significant mortality rates. Conditions induced by toxins, such as staphylococcal food poisoning, toxic shock syndrome (linked to tampon usage), and staphylococcal scalded skin syndrome (mainly affecting humans), stem from toxins produced by *S. aureus* [12].

Protein A is a surface protein bound to the peptidoglycan of the *S. aureus* cell wall [13,14]. This protein has a role in the mechanism of bacteria infecting the host body. Among them play a role in adhesion (adhesion), colonization and destruction of cells in various body tissues. Besides the biological effects caused by slow hypersensitivity reactions and inhibit anti-phagocytosis opsonization. Furthermore, protein A can bind to the Fc receptor part of immunoglobulin (Ig) in most mammalian species [15]. The gene that encodes for protein A *(spa)* is the most widely used marker for molecular typing because it contains polymorphic units. *Spa* genes are also a good choice to be able to identify and distinguish *Staphylococcus aureus* strain variability [16,17]. Protein A (*spa*) has been the most widely used markers for molecular typing as they contain highly polymorphic repeat units [18]. *Spa* typing relies on the variability within the gene encoding Protein A (*spa*), a crucial virulence factor of *S. aureus*, encompassing five IgG binding sites and a C-terminal cell wall attachment portion. The gene comprises two regions: one encoding the Fc-binding domain and the other containing the X region, which includes variable repeats and the cell wall attachment sequence [19]. Variability in the X region, attributed to repeat deletion, duplication, or point mutations, results in the *spa* gene's length variation among strains, forming the basis of *spa* typing for strain differentiation [20].

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Epidemiological studies of *Staphylococcus aureus*, including MRSA strains, frequently utilize *Spa* typing, a molecular method that focuses on sequencing the polymorphic X region of the *spa* gene. The *spa* gene encodes protein A, a cell wall-associated protein that has critical roles in bacterial virulence and immune evasion. This protein includes two significant regions: an Fc-binding region, which enables protein A to bind to the Fc region of immunoglobulins, allowing the bacteria to evade immune detection, and the polymorphic X region, which consists of highly variable 24-base pair (bp) repeat sequences. The variability in these repeats enables researchers to distinguish between different strains of *S. aureus*, making *Spa* typing a valuable tool in tracing infection sources and transmission patterns. During *Spa* typing, the X region is amplified and sequenced, with the pattern of repeat units used to assign a specific *Spa* type to the bacterial strain. This repeat-based approach provides a high-resolution tool for studying genetic diversity within *S. aureus* populations and is particularly useful in investigating outbreaks in both healthcare and community settings. Different *Spa* types are often associated with specific epidemiological characteristics or geographic locations, which can help public health authorities identify the spread of MRSA strains and implement targeted control measures. *Spa* typing is not only cost-effective but also offers a high degree of reproducibility and discriminatory power, making it an ideal choice for surveillance and outbreak investigations. In combination with other molecular typing techniques, such as multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), *Spa* typing can provide a comprehensive view of the genetic makeup and evolutionary relationships of *S. aureus* strains. This approach is invaluable in monitoring the emergence of new MRSA strains and understanding their adaptation to different environments, including hospitals, farms, and communities. Such insights contribute significantly to infection prevention strategies and the development of more effective public health responses to the ongoing challenge posed by MRSA [21]. They had also reported the detection of the *spa* gene in 248 out of 250 isolates (99.2%), with the remaining 2 isolates classified as non-typable; these 248 *S. aureus* isolates were further categorized into 39 *spa* types, forming six distinct *spa* clonal clusters and eight singletons [21].

The research outcomes underscore the significance of mitigating superfluous contact with cattle and adhering to stringent hygiene practices. Moreover, it underscores the imperative for judicious antibiotic employment to mitigate the emergence of staphylococcal bacterial resistance. Furthermore, the study contributes to veterinarians' and medical practitioners' comprehension of the prevalence of *S. aureus* infections among animals. It serves as a reminder against the unauthorized use of antibiotics and advocates for routine veterinary examinations and vaccination protocols for cattle. Fundamentally, the study's objective was to ascertain the prevalence of *S. aureus* infections in livestock and elucidate their impact on animal health.

Materials and Methods

Sample collection and processing

This research employed a laboratory-based cross-sectional approach, conducted within various locations in Dharan, with all activities undertaken at the Microbiology Laboratory of Central Campus of Technology, Dharan. Simple random sampling was utilized, with cattle selected through a lottery method following random site selection. Specifically, cattle from Dharan Sub-Metropolitan City, free from disease and lacking oral, skin, or nasal lesions, and not administered antibiotics for four weeks, were included. Any cattle failing to meet these criteria were excluded from the study. Skin swab samples were gathered randomly from diverse locations in Dharan, using sterile cotton swabs under aseptic conditions. Swabs were then rolled over the back skin, collected in sterile 1 mL nutrient broth tubes (HiMedia, India), and preserved for one hour in sterile vials containing nutrient broth under refrigeration before transportation to the microbiology laboratory at the Central Campus of Technology, Dharan.

Identification and Biochemical tests

The specimens, preserved in vials with nutrient broth, were transferred onto Mannitol Salt Agar plates and subsequently placed in an incubator at 37°C for 24 hours. Identification of *S. aureus* was based on the distinctive growth characteristics, observed as colonies with a golden yellow hue. Additional confirmation was conducted through Gram staining, catalase, citrate, indole, MRVP, and coagulase tests.

MRSA and MSSA Identification

Initially, *S. aureus* was suspended in peptone water and kept at 37°C for four hours. The bacterial suspension's turbidity was kept at 0.5 McFarland by adding regular saline if it was higher and continuing to incubate if it was lower. Next, using the Kirby Bauer disk diffusion technique, an antibiotic sensitivity test was conducted on MHA. MRSA and MSSA strains are identified based on the size of the zone of inhibition on cefotaxime. If the zone

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of inhibition is less than 21 mm, the strain is MRSA and if the zone is greater than 21mm, the strain is MSSA.

Antibiotic Susceptibility Testing (AST)

Positive *S. aureus* samples were processed for antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method on Muller-Hilton agar, following CLSI guidelines (2012). These samples were first cultured in nutrient broth at 37°C for 18–24 hours to achieve 0.5 McFarland turbidity. Muller-Hilton agar plates were then prepared and inoculated with fresh cultures using sterile cotton swabs. Antibiotic discs, including cefotaxime, chloramphenicol, ceftriaxone, erythromycin, clindamycin, gentamycin, penicillin, and vancomycin, were placed on the plates and allowed to diffuse for 15 minutes before incubating at 37°C for 24 hours. Following incubation, zones of inhibition were measured, and results were interpreted as susceptible, intermediate, or resistant according to CLSI guidelines (2012).

DNA extraction

DNA extraction was carried out using the phenol-chloroform method. A total of 2.5mL from a 5ml overnight culture in Luria-Bertani (LB) broth was centrifuged at 33,000 rpm for 30 sec. The supernatant was discarded and the pellet was re-suspended in 700µL extraction buffer (1.4M NaCl; 100mM Tris-HCl [pH8.0]; 200mM EDTA [pH 8.0]; 40%PVP (polyvinylpyrrolidone); 2% CTAB (cetyltrimethylammonium bromide), 20mg/mL Proteinase K; 0.2% β- Mercaptoethanol). The tube was incubated at 65°C for 30min with occasional mixing at every 10min. Then, 650µL chloroform-isoamyl alcohol (24:1) was added and the solution was centrifuged at 33,000 rpm for 7min. The upper aqueous phase was transferred to a 1.5-mL tube and 200µL extraction buffer without proteinase K was added. The solution was gently mixed and 650µL chloroform-isoamyl alcohol (24:1) was added. The tube was centrifuged at 33,000 rpm for 7 min after which the upper aqueous phase was transferred for a fresh tube. Chloroform-isoamyl alcohol (24:1) extractions was performed twice using 650µL of the chemicals. The DNA was precipitated by adding an equal volume of isopropanol at room temperature. The solution was mixed and centrifuged at 33,000 rpm for 7min. The isopropanol was removed and the pellet was washed twice with 70µL 70% ethanol. The DNA pellet was air-dried and re-suspended in 40µl TE buffer (10mM Tris-HCl [pH 8.0]; 1mM EDTA [pH 8.0] + 10µgmL^{-1}RNAse) and incubated at 37°C for 30 min [22].

PCR/ Electrophoresis/ Gel documentation

The primers for the PCR were *spa*-1113F "5–TAAAGACGATCCTTCGGTGAGC-3" and *spa*-1514R "5-C AGCAGTAGTGCCGTTTGCTT –3" [23]. The PCR master mixture consists of 1 mmol/L magnesium chloride, 0.2 mmol/ L dNTPs, PCR buffer, 1µmol/ L of primers, and 1 unit of Taq-DNA polymerase in a final volume of 50 µL. Samples were denaturated at 94°C for 4min followed by 35 cycles using the following parameters: denaturation at 94°C for 1min, annealing at 56°C for 1min, and extension at 72°C for 3min, with a final extension at 72°C for 5min. Following the PCR thermal cycling, a gel electrophoresis was conducted, and the resulting bands were visualized under UV light. DNA fragment was observed in the gel, which had been stained with ethidium bromide.

Statistical analysis

Descriptive analysis was performed in MS Excel 2013. Shapiro test was used to observe the normality of data. R programming was used to conduct one-way ANOVA of prevalence of *S. aureus* among the domestic animals.

Results and Discussion

Identification of *S. aureus*

S. aureus is a significant cause of infection and disease in animals leading to a potential risk to public health and agriculture [24]. Infections in animals can harm the animal health and may transfer staphylococcal infections from the livestock to community and hospitals [25]. In the present study, a total of 320 skin swab samples were collected from different domestic animals (85 pigs, 83 buffaloes, 77 cows, and 75 goats) were processed for this study (**Figure**. 1). *S. aureus* was found to have colonies with golden yellow pigmentation, Gram-positive cocci, catalase-positive, citrate-negative, indole-negative, MR-positive, VP-negative, and coagulase-positive. Of the 320 samples, 40 were positive for *S. aureus* among the different animals (**Table**1).

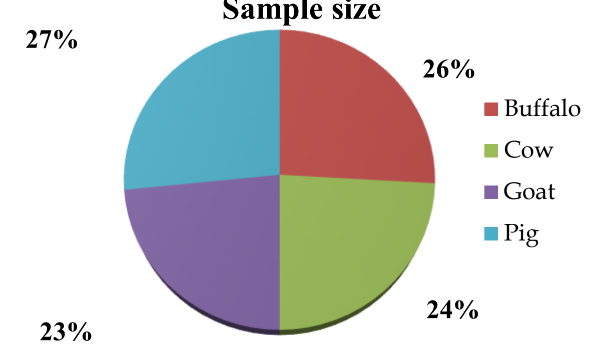

Figure 1. Sample size of studied population

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Table 1. Prevalence of *S. aureus* according to animal types

Animals	*S. aureus*		Total	P value
	Isolated	Not isolated		
Buffalo	9	74	83	0.385
Cow	11	66	77	
Goat	6	69	75	
Pig	14	71	85	
Total	40	280	320	

Prevalence of *S. aureus* and MRSA

Among the tested samples, pig samples were mostly found positive (n = 14) for *S. aureus*, followed by cow (n = 11), buffalo (n = 9) and goat (n = 6) (**Table 1**). However, there was not any significant difference (p value = 0.385, level of significance = 0.05) observed in the samples which were positive for *S. aureus*. The highest percentage of samples were obtained from pigs, followed by buffaloes. The occurrence of *S. aureus* was identified as 12.5%, aligning with earlier investigations, which reported rates of 12.5% [26] and 15.2%, respectively [27]. These results demonstrated a high probability of recovering *Staphylococcus aureus* from animal samples. However, the prevalence rate is higher in other countries: 32.7% in Bangladesh [28], 35.6% in India [29], and 43.24% in China [30]. These differences might be due to the study population, the sample size, antibiotic prescription patterns, and animal husbandry practices in different regions.

Out of the 40 isolated bacterial samples, 60% were MRSA and 40% were found to be MSSA. Among the 24 MRSA isolates, 9 (64.28%) were isolated from pigs, 6 (54.54%) from cow, 5 (83.33%) from goat, and 4 (44.4%) from buffalo. However, a significant difference (p value = 0.001) was observed in the number of MRSA found in the different samples of the animal (**Table 2**). MRSA has gained considerable attention as a zoonotic organism due to research indicating that animals may serve as reservoirs for MRSA infections in humans [24]. The high prevalence of MRSA, accounting for 60% of the isolated *S. aureus* strains, is a concerning finding.

Table 2. Prevalence of MRSA according to animals

Animals	MRSA	MSSA	Total	P-Value
Buffalo	4	5	9	0.001
Cow	6	5	11	
Goat	5	1	6	
Pig	9	5	14	
Total	24	16	40	

MRSA possess a significant public health threat due to its resistance to multiple antibiotics, making treatment challenging. Contrary to our findings, the lower prevalence was reported by several studies conducted in Nepal: 11.25% by Joshi et al. [31], 6.9% by Shrestha et al. [27], and 10% by Chaudary et al. [32]. The high prevalence of MRSA might be due to the improper use of beta lactam antibiotics against MRSA infection [31]. The high prevalence rate 83.33% for MRSA isolates was obtained among the isolated bacterial samples from goats. Moreover, the observed variation in MRSA prevalence among different animal species suggests potential differences in transmission dynamics or management practices. Individuals who frequently encounter MRSA-colonized animals can cause infections in humans [25]. However, those living in regions with high livestock density are also more likely to be colonized with Livestock-associated MRSA, even without direct animal contact [33,34]. Consequently, domestic animals as a source of MRSA infection have become an increasing public health concern.

Antibiotic susceptibility test of *S. aureus*

Out of given antibiotics, every bacterial sample showed some resistant properties against all antibiotics except against Vancomycin (100% sensitive to all the samples). Most of the isolated *S. aureus* were (n = 38, 95%) resistant to Penicillin, followed by Tetracycline (80%.), Gentamycin (72.5%), and Chloramphenicol (70%) (**Figure. 2**). However, half of the samples were sensitive and half of them were resistant to cefotaxime.

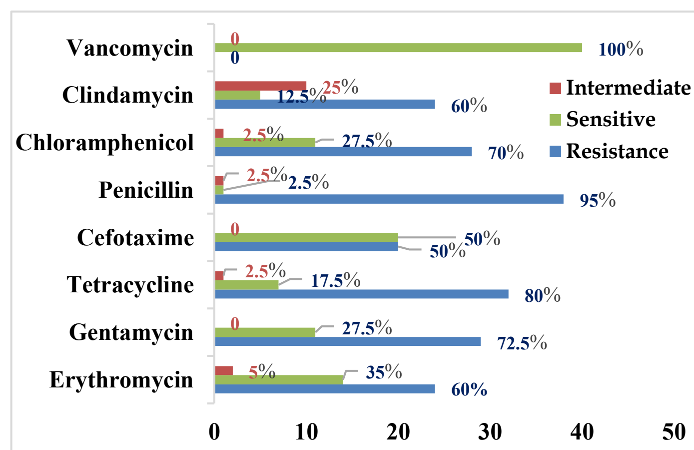

Figure 2. Bar graph representing the overall antibacterial susceptibility test of *S. aureus*

The interpretation of antimicrobial susceptibility results for specific types of *Staphylococcus aureus*, including MRSA and MSSA, indicates substantial differences in resistance patterns between these strains. For MRSA, there is complete resistance to Penicillin due to the presence of the *mecA* or *mecC* gene, which enables the bacteria to produce an altered penicillin-binding protein (PBP2a) that beta-lactam antibiotics cannot effectively target. This high resistance is one of the defining characteristics of MRSA and limits treatment options significantly, often necessitating the use of more potent antibiotics. Additionally, MRSA exhibited some level of resistance against other antibiotics, including

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Tetracycline, Cefotaxime, Chloramphenicol, and Clindamycin, suggesting that MRSA strains possess various mechanisms of resistance that allow them to withstand multiple drug classes. Interestingly, however, the numbers of MRSA samples that were resistant and those that were sensitive to these antibiotics were approximately equal, as shown in **Table** 3, indicating variability in the resistance profiles of MRSA strains within the study population.

Table 3. Antibacterial susceptibility test of MRSA and MSSA against seven standard antibiotics

Antibiotics	MRSA			MSSA		
	R	*I*	*S*	*R*	*I*	*S*
Erythromycin	12	1	11	12	1	3
Gentamycin	18	0	6	11	0	5
Tetracycline	19	1	4	13	0	3
Cefotaxime	15	0	9	5	0	11
Penicillin	24	0	0	14	1	1
Chloramphenicol	16	1	7	12	0	4
Clindamycin	17	6	1	7	4	4
Vancomycin	0	0	24	0	0	16

R, I, and S refers to resistance, intermediate and sensitive respectively

On the other hand, MSSA, which lacks the *mecA* gene and is typically more susceptible to beta-lactam antibiotics, also demonstrated notable resistance to Penicillin. This is a common finding, as *S. aureus* frequently produces beta-lactamase enzymes that hydrolyze Penicillin, leading to reduced efficacy of this drug even in MSSA strains. Beyond Penicillin, many MSSA samples showed resistance to other antibiotics as well, with high resistance rates observed for Tetracycline, Chloramphenicol, and Erythromycin. This pattern of resistance suggests that while MSSA is generally more treatable than MRSA, certain MSSA strains have also acquired resistance mechanisms that complicate treatment options. Nevertheless, MSSA samples were highly sensitive to Vancomycin, with 100% susceptibility observed, making it a reliable option for treating MSSA infections. Additionally, 69% of MSSA samples were sensitive to Cefotaxime, further supporting its use as an effective alternative in treating these strains. These findings underscore the importance of antimicrobial susceptibility testing in tailoring treatment approaches for *S. aureus* infections. As resistance patterns vary even among MRSA and MSSA strains, clinicians must carefully select antibiotics based on local susceptibility data to improve therapeutic outcomes. The variability in resistance profiles also highlights the ongoing challenge of managing *S. aureus* infections and the need for prudent antibiotic use to minimize further resistance development.

The antibiotic susceptibility testing revealed widespread resistance among *S. aureus* isolates, particularly against commonly used antibiotics such as Penicillin, Tetracycline, and Gentamycin. This highlighted the urgent need for prudent antibiotic use in veterinary medicine to curb the spread of antimicrobial resistance. *S. aureus* isolates were susceptible to Vancomycin therefore, Vancomycin may be the recommended medication for treating infections caused by *S. aureus* isolates. The findings of our study are consistent with other studies reported from Nepal [32] and Ethiopia [26]. Antibiotic susceptibility studies based on domestic animals conducted in different regions of Nepal show that the resistance pattern is serious. The study conducted on dairy farms in Pokhara found increasing resistance of *S. aureus* to beta-lactam antibiotics, suggesting the emergence of MRSA [31]. A similar study conducted on dogs at Central Veterinary Hospital, Kathmandu also indicated MRSA as an emerging pathogen [35]. These findings point out that the increasing prevalence of MRSA is gradually becoming a serious threat to human and animal health.

The acquisition of mobile genetic elements containing resistance genes, poor hygiene, and the inappropriate and overuse of antibiotics for growth promotion or treatment in livestock without the veterinarians' advice or prescription are the common factors leading to drug resistance in *S. aureus* [36]. Such resistance can potentially be transmitted to humans through food and other means of transmission [37,38]. The antibiogram results that MRSA isolates were resistance to most of the tested antibiotics such as Penicillin, and Tetracycline. MRSA strains were more resistant towards single or multiple antibiotic combinations; and were cross-resistant to many other antibiotic families other than β-lactam antibiotics. Therefore, the carriage of MRSA in humans and animals poses a significant threat to the effectiveness of antimicrobial treatment [39]. Furthermore, the higher sensitivity of *S. aureus* isolates, particularly MRSA to Vancomycin underscores its importance as a critical antibiotic for treating MRSA infections in animals. Further research to examine the genetic diversity of MRSA strains in this area considering environmental factors, antibiotic-use practices, and animal hygiene will help to identify particular risk factors or control measures for MRSA transmission. Additionally, significant relation might be established by correlating the status of animal infection or animal health with antibiotic susceptibility patterns.

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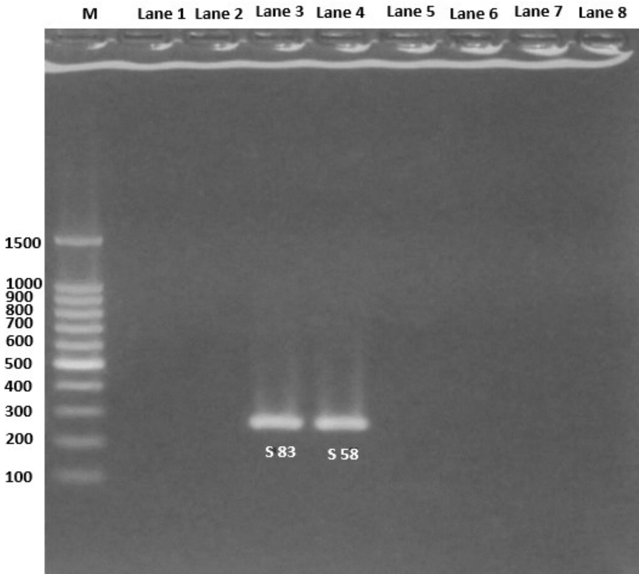

Figure 3. *Spa* gene Lane M: Marker DNA (100 bp) ; Lane 3 and 4: *spa* positive isolates (S83 and S58)

Spa gene detection

The *spa* gene is a widely used molecular method for genotyping mastitis causing *Staphylococcus* isolates [40]. This method is quick and simple. The selected region of the *spa* gene is typically a short sequence repeat with enough variability to enable isolate typing [41]. The *spa* gene was detected in 5% (2/40) of the total isolates (**Figure** 3). Among 24 MRSA isolates, 2 isolates exhibited the *spa* gene, while none of the MSSA isolates showed the *spa* gene. The S83 and S58 isolates showed the *spa* gene with the size of 300 bp PCR product. The *spa* gene detected in the MRSA was found to be isolated from the samples obtained from buffalo, where none of the samples from other animals were found to present the *spa* gene. The previous study showed that 63.3% of *S. aureus* isolates with *spa* gene which is higher compared to our study [42]. This *spa* gene type can lead to infection through human colonization, particularly in regions with extensive livestock farming. This creates a vulnerable environment where infections can easily spread between humans and the surrounding area [43]. Detection of the *spa* gene, a marker for molecular typing of *S. aureus* strains, in a small subset of MRSA isolates suggests potential human colonization and underscores the interconnectedness of human and animal health. Further research is needed to elucidate the transmission dynamics of MRSA between humans and animals and to implement effective control measures.

Conclusion

The findings of this study shed light on the prevalence and antibiotic susceptibility patterns of *S. aureus* and MRSA among domestic animals in Dharan, Nepal. With a notable prevalence rate of *S. aureus* and an alarmingly high occurrence of MRSA, this research underscores the urgent need for effective measures to address antimicrobial resistance veterinary medicine. The widespread resistance to commonly used antibiotics emphasizes the importance of prudent antibiotic usage and the development of alternative treatment strategies. Additionally, the detection of the *spa* gene in a subset of MRSA isolates highlights the potential for human colonization and underscores the interconnectedness of animal and human health. By implementing stringent hygiene practices, routine veterinary monitoring, and judicious antibiotic usage, we can mitigate the spread of MRSA and protect veterinary health interests. Moreover, this study emphasizes the importance of surveillance programs to monitor antimicrobial resistance trends and guide appropriate treatment strategies.

In conclusion, this research contributes valuable insights into the epidemiology of *S. aureus* infections in livestock and advocates for a multifaceted approach to combat antimicrobial resistance. By adopting a One Health approach, encompassing human, animal, and environmental health, the work towards preserving the efficacy of antibiotics and safeguarding animal health can be achieved.

Author's contribution

RK and HK designed and conceived the study. RK and BS executed the experiment and analyzed the samples. RK, SK, and MS analyzed the data and drafted the manuscript. All authors interpreted the data, critically revised the manuscript for important intellectual contents, and approved the final version.

Competing interests

The authors declare to have no conflict of interest.

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Ethical Considerations

Ethical Considerations have been taken into account during the overall process of research to ensure the safety

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of animals as well as the environment. Informed consent has been taken with the owner before sample collection. All the samples are handled and discarded following the biosafety guidelines.

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