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Study on molecular characteristics of *Staphylococcus* from yak milk-Xizang

Fanxing Meng[1], Qingxia Wu[2†], Jiangyong Zeng[1†] and Hongcai Ma[1*]

Abstract

This study investigated the biological characteristics of *Staphylococcus* strains isolated from Xizangan yak milk by examining the antibiotic resistance phenotypes of 69 isolates against 18 antibiotics, detecting 31 associated resistance genes, identifying 16 virulence genes, and assessing biofilm formation capability. Furthermore, molecular typing techniques including spa typing, multilocus sequence typing (MLST), and *Staphylococcus* cassette chromosome mec (SCCmec) were used for detailed characterization of the isolates. The findings revealed a high penicillin resistance rate of 72.46%. Resistance genes such as *gyrA* (100.00%), *glrB* (92.75%), and *gyrB* (44.93%) were prevalent. The detection rates were 81.16% and 55.07% for the beta-lactamase gene *blaZ* and the *mecA* gene, respectively. Among the tested virulence genes, *lukS*, *lukF*, *hla*, *clfA*, and *icaD* were detected in 40.58% of isolates, while sec was detected in 24.64%. MLST typing identified four isolates belonging to the ST62623 type and 24 other isolates representing novel ST types not reported on PubMLST.org. Spa typing revealed spa types t1940 ($n=9$), t3022 ($n=8$), t4558 ($n=6$), t4236 ($n=3$), t4445 ($n=1$), and one unreported spa type. For SCCmec typing, 11 isolates were typed as SCCmec IVb and 9 as SCCmec V. These findings significantly enhance our understanding of the biological characteristics of *Staphylococcus* strains derived from Xizangan yak milk.

Keywords Yak milk, *Staphylococcus*, Antibiotic resistance, Resistance gene, Virulence gene, Molecular typing

Introduction

The yak is an ancient animal with a domestication history of millennia [1], and has progressively become an essential component of life for the residents of the Xizang Plateau and adjacent regions. Yaks have a crucial role in economic activities, religious rituals, folk traditions, and social frameworks. In the life of Xizang and other highland tribes, yaks are valued as diligent pack animals supply various resources, including meat, milk, wool, and leather [2]. However, the rudimentary breeding practices and inadequate management standards in yak farming in Xizang render these animals vulnerable to several bacterial infections that can lead to diseases. Therefore, this study aims to investigate the biological characteristics of *Staphylococcus* strains derived from yak milk, which is crucial for the prevention and treatment of related diseases.

[†]Qingxia Wu and Jiangyong Zeng contributed equally to this work.

*Correspondence:
Hongcai Ma
xzmahongcai@126.com
[1] Institute of Animal Husbandry and Veterinary, Xizang Academy of Agriculture and Animal Husbandry Science, Lhasa 850009, China
[2] College of Animal Science, Xizang Agriculture and Animal Husbandry University, Nyingchi 860000, China

Based on the presence of coagulase, *staphylococci* can be classified into coagulase-positive *staphylococci* (CPS) and coagulase-negative *staphylococci* (CNS). *Staphylococcus aureus* (*S. aureus*), a representative of coagulase-positive *staphylococci* (CPS), is the most clinically significant *staphylococcus* identified to date [3]. *S. aureus* exhibits strong virulence and adaptability, enabling it to evade immune responses and cause damage to the host via biofilm formation and generation of virulence factors [4]. *S. aureus* can induce a spectrum of diseases, including encephalitis, pericarditis, and pneumonia. It can also provoke systemic inflammatory responses such as sepsis and septicemia, posing serious threats to the health of humans and animals [5, 6]. In veterinary medicine, *S. aureus* is a source of great concern, frequently causing mastitis in cattle and sheep [7, 8], arthritis in chickens [9], and dermatitis in pigs [10], among other reported infections in animals like ducks [11]. Furthermore, *S. aureus* is one of the primary pathogens responsible for foodborne diseases [12], with the enterotoxins produced by this bacterium significantly contributing to gastroenteritis [13]. Antibiotics have historically served as an effective treatment for *S. aureus* infections; however, the misuse of antibiotics has resulted in the gradual emergence of resistant strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961 and rapidly disseminated [14]. By the 1980s, it had risen to become one of the three most challenging infectious diseases globally [15]. Research has demonstrated that the pathogenic mechanisms of MRSA are complex since the bacterium can colonize several anatomical sites in the host, including the throat [16], nasal cavity, armpits, groin [17], and intestines [18]. Notably, intestinal MRSA colonization can lead to infections in distant tissues [19]. Additionally, MRSA can cause chronic, persistent, and recurrent infections in the host, as well as invasive infections [20]. Therefore, MRSA poses a significant challenge to global public health systems [21].

There are several types of coagulase-negative *staphylococci* (CNS), including *Staphylococcus chromogenes* (*S. chromogenes*), *Staphylococcus pasteuri* (*S. pasteuri*), and *Staphylococcus epidermidis* (*S. epidermidis*). Over 30 subtypes of CNS have been identified, and this number continues expanding. Research has demonstrated that CNS contributes positively to the skin colonization of *S. aureus* [22], and serves as a gene reservoir that enhances the antibiotic resistance of *staphylococci* [23]. CNS are major components of the microbial flora on humans and animals' skin and mucous membranes. Although formerly considered non-pathogenic, recent studies have revealed that CNS could be potent and harmful to living organisms. Research indicated that most catheter-associated, device-associated, and prosthetic joint infections are caused by CNS, which generally exhibit high levels of antibiotic resistance [24]. Furthermore, methicillin-resistant coagulase-negative *staphylococci* (MRCNS) have been progressively identified in recent years [25, 26].

Staphylococcus agnetis (*S. agnetis*) is an emerging pathogen found in chickens but commonly isolated from cattle subclinical mastitis [27]. It is often derived from lesions associated with bacterial chondronecrosis with osteomyelitis (BCO) in broiler chickens [28]. This bacterium is classified as a coagulase-variable *staphylococcus* [29]. Therefore, monitoring *S. aureus* antimicrobial resistance in yak milk is essential to assess the risk patterns and develop efficient animal antibiotic treatments. To our knowledge, there is a scarcity of research on the contamination of *S. aureus* in yak milk. This study aims to isolate and identify *Staphylococcus* strains from yak milk to understand the biological characteristics of these strains.

Common molecular typing methods for *staphylococci* include multilocus sequence typing (MLST), staphylococcal protein A gene (spa) typing, staphylococcal cassette chromosome mec (SCCmec) typing, etc. [30]. This technique improves multilocus gel electrophoresis and combines phenotypic typing with protein electrophoresis. It can distinguish the genotypes of bacteria based on the nucleotide variations of seven housekeeping genes, namely *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*. In 1987, Pickenhahn et al. first used the spa typing method to type *Staphylococcus aureus* [31]. The spa protein is an important component of the cell wall of *Staphylococcus aureus*, including the X, C, and Fc regions. Spa typing is mainly evaluated based on the number and sequence variations of the repetitive sequences in the X region. The polymorphic X region encodes part of the staphylococcal protein A (Spa). It contains changes in the number of tandem repetitive sequences and base sequence changes within each repetitive sequence. That is, in any *Staphylococcus aureus* strain, each new 24-base pair sequence is assigned a unique repeat code, and the spa type is determined by the continuity and specific sequence of these repetitive sequences [32]. SCCmec typing was established in the early twenty-first century and has now become an important tool for studying the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA). The SCCmec is the core factor for MRSA to acquire drug resistance. It consists of three parts: the *mec* gene cluster, the *ccr* gene cluster, and three J regions of different sizes. Currently, SCCmec typing is achieved by detecting the different combinations between the *mec* gene cluster and the *ccr* gene cluster [33]. The convenience of the polymerase chain reaction (PCR)-based method has also led to the wide application of SCCmec typing.

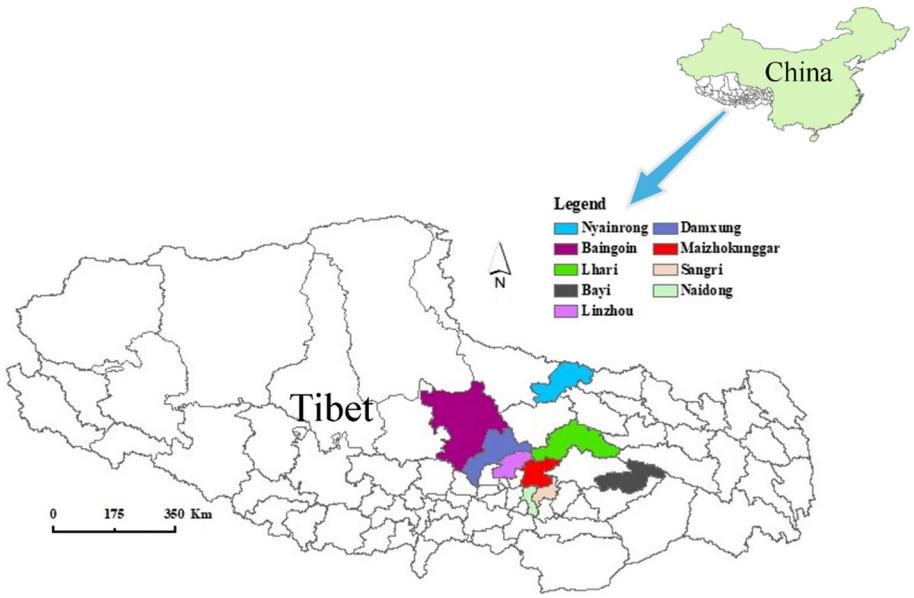

Fig. 1 Geographical locations from where samples were collected

Materials and methods

Sample

A total of 219 yak milk samples were collected from the primary grazing areas in Xizang from May 2023 to May 2024. The sampling was conducted across various locations, including Lhasa City ($n=81$), Nyingchi City ($n=7$), Nagqu City ($n=95$), and Shannan City ($n=36$), covering a total of nine counties or districts, as depicted in Fig. 1. Fresh yak milk samples were collected and stored in a vehicle refrigerator at −20 °C for transportation back to the laboratory of the Xizang Animal Husbandry and Veterinary Research Institute.

Isolation and culture of bacteria

Following the thawing and homogenization of the milk samples, 200 µL was uniformly distributed over Tryptic Soy Agar (TSA) (Hopebio, Qingdao, China) with 5% sterile defibrinated sheep blood (Chengrui, Zhengzhou, China) and incubated at 37 °C for 24 h. Typical colonies that were round, well-defined, smooth, and appeared milky white or golden yellow were selected for purification. After at least four subcultures for purification, Gram staining and microscopic examination were performed.

Biochemical identification tests

Biochemical identification test was carried out using *Staphylococcus* biochemical micro-identification tube. The specific bacterial colony was inoculated into biochemical identification tubes (Microbial Reagent, Hangzhou, China) according to the instructions and cultured at 37 °C for 18 to 24 h, and then results were observed and recorded.

Bacterial genome extraction

Purified single colonies were inoculated into Tryptic Soy Broth (TSB) (Hopebio, Qingdao, China) with 5% Fetal Bovine Serum (FBS) (Tianhang, Zhejiang, China) and cultured overnight at 37 °C at 130 rpm. Transferred 2 mL of the bacterial suspension, centrifuged at 10,000 rpm for 1 min, and discarded the supernatant. Added 500 µL of Tris–EDTA (TE) buffer (Yuanye, Shanghai, China) and vortexed until the bacterial pellet was completely resuspended. Added 20 µL of Proteinase K (Yuanye, Shanghai, China) (20 mg/mL) and 180 µL of lysozyme (Yuanye, Shanghai, China) (10 mg/mL), mixed thoroughly, and incubated at 37 °C for 10 min. After centrifugation at 10,000 rpm for 2 min, the supernatant was discarded. After adding 200µL of TE buffer and vertexing the bacterial pellet until it was fully reconstituted, it was placed in a metal bath set at 100℃ for 15 min before being swiftly moved to an ice box to cool for 5 min. Finally, centrifugation was performed at 10,000 rpm for 2 min, and the supernatant was collected and stored at −20 °C for future use.

PCR identification of *Staphylococcus*

The 16S rRNA primers were synthesized [34], which were used to amplify the genomic DNA of the isolated strains

through PCR. The primer sequences were 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-ACGGCTACCTTGTTACGACTT-3′, synthesized by Songon Biotech (Shanghai) Co., Ltd. The PCR amplification was conducted in a 25 μL reaction volume, consisting of 1 μL of the upstream primer, 1 μL of the downstream primer, 12.5 μL of PCR mix (Vazyme, Nanjing, Chian), 2 μL of DNA template, and 8.5 μL of ddH2O. The PCR products were then subjected to electrophoresis on a 1.0% agarose gel, and images were captured for analysis using a gel imaging system. Positive amplification products were sent to Tsingke Biotechnology (Chengdu) Co., Ltd. for sequencing, and further identification was performed using the NCBI website (https://www.ncbi.nlm.nih.gov/).

Antimicrobial susceptibility test

The Kirby-Bauer disk diffusion method was used to determine the sensitivity of the isolated bacteria to 18 antimicrobial agents [35]. The purified bacterial suspension was evenly spread on TSB (with 5% FBS), and then 18 antibiotic susceptibility disks (Microbial Reagent, Hangzhou, China) were placed on the agar. The plates were incubated at 37 °C for 24 h. The selected antimicrobial agents included commonly used drugs for livestock and humans: penicillin (10 U), cefotaxime (30 μg), trimethoprim-sulfamethoxazole (23.75/1.25 μg), tetracycline (30 μg), neomycin (30 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), florfenicol (30 μg), erythromycin (15 μg), rifampicin (5 μg), polymyxin B (300 IU), lincomycin (2 μg), oxacillin (1 μg), levofloxacin (5 μg), and vancomycin (30 μg). Finally, the diameters of the inhibition zones were measured, and the resistance of the isolated bacteria was determined according to the manufacturer's instructions.

Antibiotic resistance gene detection

Antibiotic resistance genes were detected in isolated strains using the PCR method [34]. Primers are listed in Table 1. These include resistance genes for macrolides, lincomycins, and streptogramins B (MLS_B) such as *ermA*, *ermB*, *ermC*, *ermF*, *erm33*, *mphC*, *msrA*, *lnuA*, and *lunB*; genes associated with pleuromutilins resistance (*vga*, *vgb*); β-lactam resistance genes (*blaZ*, *mecA*); tetracycline resistance genes (*tetM*, *tetO*, *tetL*, *tetK*); chloramphenicol resistance genes (*cfr*, *fexA*); aminoglycoside resistance genes (*aacA-aphD*, *aadD*); quinolone resistant genes (*gyrA*, *gyrB*, *glrA*, *glrB*); glycopeptide resistance genes (*VanA*, *VanB*, *VanC1*, *VanC2/3*); the oxazolidinone resistance gene (*optrA*); and the lifamycin resistance gene (*ropB*).

Biofilm detection

Qualitative detection of biofilm

Qualitative biofilm experiments were conducted following the methodology proposed by [34]. Inoculation of *S. aureus* onto Congo Red Agar (CRA) wad performed and incubated at 37 °C for 24 h. The color of the colonies was observed: black colonies indicate positive, while red colonies indicate negative for biofilm formation. The preparation method for CRA is as described previously. Briefly, 0.8 g of Congo Red (Beichen, Tianjin, China) was dissolved in 10 mL of deionized water and sterilized at 121 °C for 15 min for later use. Brain–Heart Infusion Broth (BHI) (Hopebio, Qingdao, China) 37 g, agar (Yuanye, Shanghai, China) 10 g, and sucrose (Sinopharm Chemical, Shanghai, China) 50 g were dissolved in 1L of deionized water, sterilized at 121 °C for 15 min, and then cooled to about 60 °C before adding 10 mL of Congo Red solution. Finally, mix thoroughly and pour equal amounts into sterilized Petri dishes.

Quantitative detection of biofilms

The biofilm quantification of *S. aureus* was performed using a microtiter plate assay (MPA) [36]. Firstly, single colony inoculation was performed in TSB (with 5% FBS) and incubated overnight at 37℃ with shaking at 130r/min. Afterward, the culture was mixed with TSB containing 0.25% glucose (Sinopharm Chemical, Shanghai, China) and 0.5% sodium chloride (Sinopharm Chemical, Shanghai, China) at a 1:100 ratio. 200μL of the mixture was transferred into each well of a 96-well plate and incubated at 37℃ for 24 h. Discarded the culture and washed the wells three times with sterile phosphate-buffered saline (PBS, PH 7.2) (Labgic, Beijing, China). After drying at room temperature, 150 μL of methanol (Jinshan, Chengdu, China) was added to each well and fixed for 20 min. Washed with PBS again and dried. Then, 150 μL of 0.1% crystal violet (Yuanye, Shanghai, China) solution is added to each well and stained for 15 min. Washed thrice with distilled water and dried at room temperature. Subsequently, 150 μL ethanol (Jinshan, Chengdu, China) was added to each well and waited for 30 min until the dye was completely dissolved. Finally, optical density was measured at 450 nm (OD_{450}). A culture medium was used without bacteria as a blank control and set three parallel samples. Average optical density (OD_{450}) and standard deviation (s) of the blank control were calculated. A strain is considered biofilm-positive if its OD_{450} is greater than the blank control OD_{450} plus 3 s.

Detection of virulence genes

Primers were synthesized (Table 2) for the detection of virulence genes in isolated *S. aureus* using the PCR method [34]. The targeted genes include enterotoxin

Table 1 Primer information of Antibiotic resistance gene

Drug type	Gene name	Primer sequence (5′−3′)	Fragment size (bp)
MLS_B	*erm(A)*	TATCTTATCGTTGAGAAGGGATT	139
		CTACACTTGGCTTAGGATGAAA	
	erm(B)	CTATCTGATTGTTGAAGAAGGATT	142
		GTTTACTCTTGGTTTAGGATGAAA	
	erm(C)	CTTGTTGATCACGATAATTTCC	190
		ATCTTTTAGCAAACCCGTATTC	
	erm(F)	AAGTTGTCGGTTGTGATT	477
		CAATTTGGGCATTAAGGTTT	
	erm(33)	TTGAAATTGGCTCAGGAA	404
		TACACTTGGCTTAGGATG	
	mph(C)	AAGTGCCGAATTGGAAAG	290
		GTCTGCCACGCCATAAGT	
	msr(A)	TTTTAGCCGAAGCGACAT	388
		TAATCCGAGTAACCACCC	
	lnu(A)	GGTGGCTGGGGTAGATGTATTAACTGG	323
		GCTTCTTTTGAAATACATGGTATTTTTCGA	
	lnu(B)	CCTACCTATTGTTTGTGGAA	365
		ATAACGTTACTCTCCTATTC	
Pleuromutilins	*vga*	AAACAAGAAAAGCGTCAT	430
		CAGAAGTGCCAATAATAAAA	
	vgb	AACATCATCAAAGCCACTG	299
		TTAATCGCACCCAACAAT	
β-lactams	*blaZ*	ACTTCAACACCTGCTGCTTTC	173
		TGACCACTTTTATCAGCAACC	
	mecA	TTTGTCTGCCAGTTTCTC	299
		AGCTGATTCAGGTTACGG	
Tetracyclines	*tet(M)*	GTGGACAAAGGTACAACGAG	406
		CGGTAAAGTTCGTCACACAC	
	tet(O)	AACTTAGGCATTCTGGCTCAC	515
		TCCCACTGTTCCATATCGTCA	
	tet(L)	TCGTTAGCGTGCTGTCATTC	267
		GTATCCCACCAATGTAGCCG	
	tet(K)	TCGATAGGAACAGCAGTA	169
		CAGCAGATCCTACTCCTT	
Chloramphenicols	*cfr*	CTACAGGCGACATTGGAT	310
		TTGCTGCGTTCCTCACTA	
	fexA	GTACTTGTAGGTGCAATTACGGCTGA	1272
		CGCATCTGAGTAGGACATAGCGTC	
Aminoglycosides	*aacA-aphD*	GAGCCTTGGGAAGATGAA	414
		CATTGCCTTAACATTTGTG	
	aadD	CTATTGGTGTTTATGGCTCT	386
		AAATGTTGTCGGTCCTTG	

Table 1 (continued)

Drug type	Gene name	Primer sequence (5′−3′)	Fragment size (bp)
Quinolones	*gyrA*	AATGAACAAGGTATGACACC	223
		TACGCGCTTCAGTATAACGC	
	gyrB	CAGCGTTAGATGTAGCAAGC	249
		CCAATTCCTGTACCAAATGC	
	glrA	AGGGAAGTGTTTCAGTCT	457
		CCATTCTCAATAATACCG	
	glrB	ATACACGTGAAGGTATGACAGC	779
		TCCAAGACCTTTGTATCGTGCA	
Glycopeptides	*VanA*	CATGAATAGAATAAAAGTTGCAATA	1030
		CCCCTTTAACGCTAATACGATCAA	
	Van B	CATCGCCGTCCCCGAATTTCAAA	297
		GATGCGGAAGATACCGTGGCT	
	Van C1	GGTATCAAGGAAACCTC	822
		CTTCCGCCATCATAGCT	
	Van C2/3	CTCCTACGATTCTCTTG	439
		CGAGCAAGACCTTTAAG	
Oxazolidinones	*optrA*	ATGGTGCCGTTTACTATGA	494
		AGTTCGCTGACCACCTGA	
Lifamycins	*ropB*	ACAGAGCGGACTTATTTG	156
		GGTATTGTATTTATCGAGGT	

genes (*sea*, *seb*, *sec*, *sed*, *see*), toxic shock syndrome toxin gene (*tsst*), leukocidin genes (*lukF*, *luk*S), hemolysin gene (*hla*), clumping factor genes (*clfA*, *clfB*), coagulase gene (*coa*), fibronectin-binding protein genes (*fnbA*, *fnbB*), and cell adhesion protein genes (*icaA*, *icaD*).

Molecular typing

SCCmec typing

SCCmec typing method is mainly suitable for *Staphylococcus* containing the *mecA* gene. Reference [34] synthesized primers (Table 3) and used the PCR method to perform SCCmec typing of MRSA and MRSCN strains.

Multilocus sequence typing

MLST typing on *S. aureus* was performed using primers obtained from the MLST database (http://www.mlst.net/) for the seven housekeeping genes of *S. aureus* (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, *yqiL*). After PCR amplification, the products of the seven housekeeping gene fragments were sequenced (Chengdu Tsingke Biotechnology Co., Ltd) and compared with the allele profiles in the *S. aureus* MLST database (https://pubmlst.org/) for the determination of their sequence type (ST). The primer sequences are shown in Table 4.

Spa typing

In order to amplify the polymorphic region X of the *S. aureus* spa gene was synthesized following the previously published protocol [37]. After purification, the sequencing was outsourced to Tsingke Biotechnology Co., Ltd. (Chengdu). The obtained sequences were submitted to the spa gene typing database (http://spatyper.fortinbras.us//) for comparison, leading to the identification of the spa type for each strain of *S.aureus*. The primer sequences are shown in Table 5.

Result

Isolation and identification of bacteria

A total of 219 samples of yak milk were analyzed, and 69 were found to be positive, resulting in a positive rate of 31.51%. Based on phenotypic screening, biochemical identification (Table 6), and 16S sequencing, five species of *Staphylococcus* were isolated: *S. aureus*, *S. chromogenes*, *S. pasteuri*, *S. agnetis*, and *S. epidermidis*. A total of 69 strains of *Staphylococcus* were chosen for further testing from each positive sample.

Table 2 Primer information of virulence resistance gene

Gene type	Gene name	Primer sequence (5′−3′)	Fragment size (bp)
Enterotoxin gene	*sea*	GTCTGAATTGCAGGGAACA	357
		TTACCGTCTAGCCATAAA	
	seb	AACTTGTATGTATGGTGGTG	217
		TTCATAAGGCGAGTTGTT	
	sec	ACCCAACGTATTAGCAGA	401
		GTAAGTTCCCATTATCAAAG	
	sed	ATTGTGATGGTGGTGAAA	393
		TATGAAGGTGCTCTGTGG	
	see	TATGAAGGTGCTCTGTGG	170
		TAACTTACCGTGGACCCTTC	
Toxic shock syndrome toxin gene	*tsst*	GTGGCGTTACAAATACTGA	290
		AGAAGAAGACGATGGGTT	
Leukocidin gene	*luks*	GCCGGATCCTCTATGGCATTATTATTAC	930
		GCCGTCGACCTATTTATTGTTTTCAGTTTC	
	lukf	GCGGGATCCATGCTTAAAAATAAAATA	948
		CCTGTCGACTCAATTCTGTCCTTTCAC	
Hemolysin gene	*hla*	CGCGGATCCAAAACACGTATAGTCAGC	960
		CGCGAGCTCATTTGTCATTTCTTCTTTTTC	
Clumping factor gene	*clfA*	TTTCAACAACGCAAGATAC	376
		ATTGGTGGCACTTTAGCA	
	clfB	AGTCCAGACCCAGAACCG	451
		CTGAATCTGAGTCGCTGT	
Coagulase gene	*coa*	CGGATTTACAAACTTATGAC	494
		TTGCTGGTTTCCAACTTTAGG	
Fibronectin-binding protein gene	*fnbA*	GTTGATGTAACGGCTGAA	309
		AACTTTTGGTTGATTTCC	
	fnbB	GAACTTTGATGAAGGGATT	365
		GTCCATTTACGGTTACGC	
Cell adhesion protein gene	*icaA*	AGCAGGAGCAATCAATAC	486
		CTGTCAATAAATAAGGCAAC	
	icaD	GGTCAAGCCCAGACAGAG	124
		ATAAACGAGTAGAACAAC	

Antibiotic sensitivity test results

The antibiotic susceptibility tests (Fig. 2, Table 7) indicated that penicillin exhibited the highest number of resistant strains, with 50 strains (72.46%). This was followed by tetracycline with 11 strains (15.94%), erythromycin with 10 strains (14.49%), lincomycin with 9 strains (13.04%), methicillin with 8 strains (11.59%), polymyxin B with 7 strains (10.14%), kanamycin with 6 strains (8.70%), and trimethoprim-sulfamethoxazole with 3 strains (4.35%). The resistance rates of *S.aureus* to penicillin and compound sulfamethoxazole were 67.86% and 3.57% respectively. The resistance rates of *S. chromogenes* to penicillin, tetracycline, and polymyxin B were 100.00%, 13.33%, and 13.33% respectively. The resistance rates of *S. pasteuri* to penicillin, tetracycline, erythromycin, lincomycin, oxacillin, and kanamycin were 100.00%, 69.23%, 69.23%, 53.33%, 53.33%, and 46.15% respectively. The resistance rates of *S. agnetis* to polymyxin B and lincomycin were 50.00% and 10.00% respectively. The resistance rates of *S. epidermidis* to penicillin, compound sulfamethoxazole, and erythromycin were 100.00%, 66.67%, and 33.33% respectively.

Antibiotic resistance gene detection

The results of the antibiotic resistance gene detection (Table 8) indicated that the resistance genes were detected in the following order, from highest to

Table 3 Primer sequences of SCCmec

Primer name	Primer sequence (5′−3′)	Fragment size (bp)
SCCmec I	GCTTTAAAGAGTGTCGTTACAGG	613
	GTTCTCTCATAGTATGACGTCC	
SCCmec II	CGTTGAAGATGATGAAGCG	398
	CGAAATCAATGGTTAATGGACC	
SCCmec III	GCCTTATTCGAAGAAACCG	280
	CCTTAGTTGTCGTAACAGATCG	
SCCmec IVa	CGTTGAAGATGATGAAGCG	776
	CTACTCTTCTGAAAAGCGTCG	
SCCmec IVb	TCTGGAATTACTTCAGCTGC	493
	AAACAATATTGCTCTCCCTC	
SCCmec IVc	ACAATATTTGTATTATCGGAGAGC	200
	TTGGTATGAGGTATTGCTGG	
SCCmec IVd	CTCAAAATACGGACCCCAATACA	881
	TGCTCCAGTAATTGCTAAAG	
SCCmec V	GAACATTGTTACTTAAATGAGCG	325
	TGAAAGTTGTACCCTTGACACC	

Table 4 Primer sequence of S.aureus housekeeping gene

Gene name	Primer sequence (5′−3′)	Fragment size (bp)
arcC	TTGATTCACCAGCGCGTATTGTC	456
	AGGTATCTGCTTCAATCAGCG	
aroE	ATCGGAAATCCTATTTCACATTC	456
	GGTGTTGTATTAATAACGATATC	
glpF	CTAGGAACTGCAATCTTAATCC	465
	TGGTAAAATCGCATGTCCAATTC	
gmk	ATCGTTTTATCGGGACCATC	429
	TCATTAACTACAACGTAATCGTA	
pta	GTTAAAATCGTATTACCTGAAGG	474
	GACCCTTTTGTTGAAAAGCTTAA	
tpi	TCGTTCATTCTGAACGTCGTGAA	402
	TTTGCACCTTCTAACAATTGTAC	
yqi L	CAGCATACAGGACACCTATTGGC	516
	CGTTGAGGAATCGATACTGGAAC	

Table 5 Primer sequence of S.aureus spa gene

Gene name	Primer sequence (5′−3′)	Fragment size (bp)
spa	AGACGATCCTTCGGTGAGC	350–850
	GCTTTTGCAATGTCATTTACTG	

lowest prevalence: Quinolone resistance gene *gyrA* (100.00%), *glrB* (92.75%), β-lactam resistance gene *blaZ* (81.16%), *mecA* (55.07%), Quinolone resistance gene *gyrB* (44.93%), MLS_B resistance gene *lnuA* (33.33%), Tetracycline resistance gene *tetK* (33.33%), Aminoglycoside resistance gene *aacA-aphD* (27.54%), Streptogramin resistance gene *vga* (13.04%), MLS_B resistance gene *ermC* (1.45%), Streptogramin resistance gene *vgb* (1.45%). These findings highlight the prevalence of specific resistance genes among the isolated strains, particularly those related to quinolone and β-lactam antibiotics.

In *S. aureus*, the distribution of *gyrA*, *gyrB*, and *glrB* is the most extensive, with a carriage rate of 100.00% for each. Followed by *blaZ* (71.43%), *mecA* (45.71%), and *tet(K)* (2.86%). In *S. chromogenes*, the carriage rates of *gyrA* and *blaZ* are both 100.00%. Next is *glrB* (80.00%). The carriage rates of *vga* and *vgb* are 60.00% and 6.67%, respectively, and the carriage rate of *tet(K)* is 13.33%. In *S. pasteuri*, the carriage rates of *blaZ*, *mecA*, *gyrA*, and *glrB* are all 100.00%. Next is *lnu(A),* with a detection rate of 92.31%. The detection rates of *tet(K)* and *aacA—aphD* are 69.23% and 61.54% respectively. In *S. agnetis*, *gyrA* has the most extensive distribution with a carriage rate of 100.00%. The carriage rates of *lnu(A), tet(K), aacA—aphD*, and *glrB* are all 80.00%, and the carriage rates of *blaZ* and *mecA* are both 70.00%. In *S. epidermidis*, the carriage rates of *lnu(A), blaZ, mecA, tet(K), aacA—aphD, gyrA, gyrB and glrB* are all 100.00%.

Biofilm detection results

The CRA method detected a total of 42 positive biofilm strains (60.87%) and 27 negative strains (39.13%). In contrast, the MPA method identified 54 positive biofilm strains (78.26%) and 15 negative strains (21.74%). The detailed results are shown in Table 9 and Table 10.

Virulence gene detection

The results of the virulence gene detection data (Table 11) indicated that the leukotoxin genes *lukS* and *lukF*, the hemolysin gene *hla*, the clumping factor gene *clfA*, and the cell adhesion protein gene *icaD* exhibited the highest detection rate, each at 40.58%.The remaining virulence genes, listed from highest to lowest detection rate, were as follows: enterotoxin gene *sec* (24.64%), *sed* (10.14%), cell adhesion protein gene *icaA* (5.80%), enterotoxin gene *sea* (4.35%), and fibronectin-binding protein gene *fnbA* (1.45%).

The detection rates of the *lukf*, *luks*, *hla,* and *clfA* in *S.aureus* were all 100.00%. Followed by *icaD* (96.43%), *sec* (50.00%), and *fnbA* (2.70%). Among *S. chromogenes*, the *sed* was the most widely distributed, with a detection rate of 46.67%. Followed by the *icaA* (26.67%) and *icaD* (6.67%). No virulence genes were detected in *S. pasteuri* and *S. agnetis*. In *S. epidermidis*, the detection rates of the *sea* and *sec* were both 100.00%.

Table 6 Biochemical identification results

Items	Results				
	S. aureus	***S. chromogenes***	***S. pasteuri***	***S. agnetis***	***S. epidermidis***
Maltose	+	-	+	-	+
Fructose	+	+	+	+	+
Sucrose	+	+	+	+	+
Mushroom sugar	+	+	+	+	-
Mannose	+	+	-	+	+
Xylose	-	-	-	-	-
Lactose	+	+	-	-	-
Mannitol	-	-	-	-	-
Urea	+	+	+	-	+
Nitrate reduction	+	+	+	+	+

+ Positive

-Negative

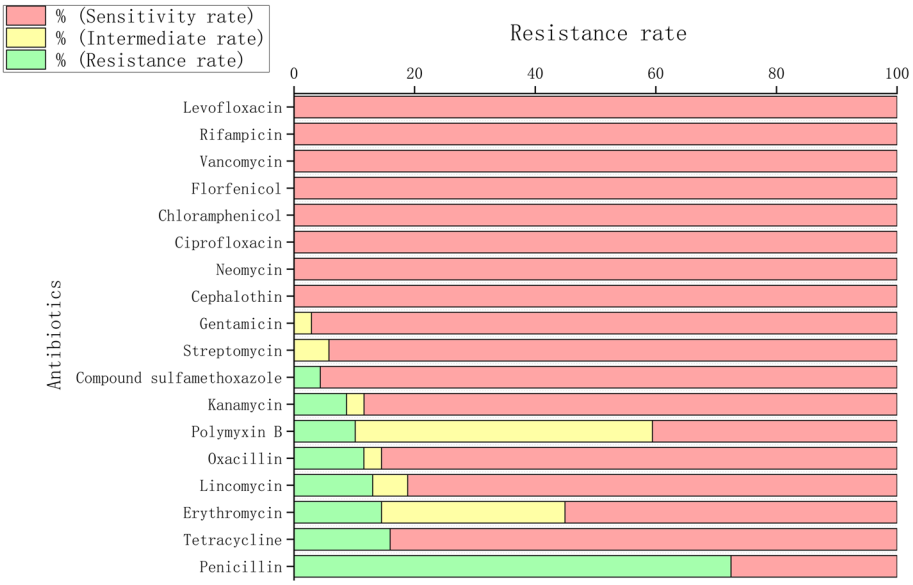

Fig. 2 Antibiotic sensitivity test results

Typing results

SCC typing

Among the 38 strains containing the *mecA* gene, the detection of SCCmec types revealed 11 stains of SCCmec IVb type and 2 strains of SCCmec V type (Table 12).

MLST typing

Among the 28 strains of *S. aureus*, ST6263 ($n=4$), ST9542 ($n=10$), ST9543 ($=n$),4 ST9544 ($n=1$), ST9545 ($n=1$), and ST9546 ($n=8$) were identified. Notably, except ST6263, all other STs represent novel sequence types (Table 13).

Spa typing

Among the 28 *S. aureus* strains, a total of 6 different spa types were identified. These included spa type t1940 ($n=9$), t3022 ($n=8$), t4558 ($n=6$), t4236 ($n=3$), t4445 ($n=1$), and one unreported spa type (Table 14).

Discussion

This study collected 219 samples of yak milk from the Xizang region and isolated 69 strains of *staphylococci*, with a detection rate of 31.51%. Among these, the highest proportion was *S. aureus* (40.58%), followed by *S. chromogenes* (21.74%), *S. pasteuri* (18.84%), *S. agnetis* (14.49%), and *S. epidermidis* (4.35%). These findings

Table 7 Antibiotic sensitivity test results

Antibiotic	Resistance rate(%)				
	S. aureus **(*n* = 28)**	***S. chromogenes*** **(*n* = 15)**	***S. pasteuri*** **(*n* = 13)**	***S. agnetis*** **(*n* = 10)**	***S. epidermidis*** **(*n* = 3)**
Penicillin	67.86% (19)	100% (15)	100% (13)	0% (0)	100.00% (3)
Cephalothin	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Compound sulfamethoxazole	3.57% (1)	0% (0)	0% (0)	0% (0)	66.67% (2)
Tetracycline	0% (0)	13.33% (2)	69.23% (9)	0% (0)	0% (0)
Neomycin	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Gentamicin	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Kanamycin	0% (0)	0% (0)	46.15% (6)	0% (0)	0% (0)
Streptomycin	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Ciprofloxacin	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Chloramphenicol	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Florfenicol	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Erythromycin	0% (0)	0% (0)	69.23% (9)	0% (0)	33.33% (1)
Rifampicin	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Polymyxin B	0% (0)	13.33% (2)	0% (0)	50.00% (5)	0% (0)
Lincomycin	0% (0)	0% (0)	53.33% (8)	10.00% (1)	0% (0)
Oxacillin	0% (0)	0% (0)	53.33% (8)	0% (0)	0% (0)

suggest a relatively serious contamination situation in yak milk from Xizang. Additionally, previous reports have indicated 47.2% isolation rate of *S. aureus* from bulk tank milk in dairy herds within the Lombardy region (Northern Italy) [38]. Another study found a 52% isolation rate of *S. chromogenes* in samples from commercial dairy herds in West Flanders, Belgium [39]. Moreover, previously it was reported an 18.1% isolation rate of *S. pasteuri* in nasal swabs from pig farms in Belgium [40], while 37.14% isolation rate of *S. epidermidis* in cheese samples made from unpasteurized milk purchased in Poland [41]. Overall, these data indicate that the isolation rate of *staphylococci* is closely related to the living environment of the host. This study shows that the isolation rate of *staphylococci* from yak milk in Xizang is lower compared to that from animals in other regions, which may be associated with differences in animal species. Furthermore, the unique environmental conditions of the Xizang region, including its high altitude, intense ultraviolet radiation, and low levels of airborne particulate pollutants, likely facilitate bacterial adhesion, thereby influencing bacterial survival and reproduction [42]. This phenomenon is further supported by a previous study conducted by Kumar S et al. which indicates that preferential growth of Gram-negative bacteria and a decrease in Gram-positive bacteria occur in high-altitude regions [43].

In recent years, the misuse of antibiotics has led to increased bacterial resistance and the emergence of multidrug-resistant strains. This study conducted phenotype resistance testing on the 69 isolated *staphylococci* against 18 antimicrobial agents. The results revealed that, except for *S. agnetis*, all *staphylococci* had a penicillin resistance rate of ≥ 50%. Specifically, the resistance rate for *S. aureus* was 64.29%, while *S. chromogenes*, *S. pasteuri*, and *S. epidermidis* exhibited a concerning 100% resistance rate to penicillin. A previous report indicated a 100% penicillin resistance rate in *S. aureus* isolated from ice cream [44], while a 90.62% resistance rate in *S. aureus* isolated from raw milk, pasteurized milk, and dairy-based beverages [45]. Although there are discrepancies between these results and our findings, they all indicate a generally high penicillin resistance rate in *S. aureus*. Our results are consistent with those of Regecová et al., who reported penicillin resistance in *S. chromogenes* isolated from sheep milk [46]. However, there was a difference in the penicillin resistance rate of *S. epidermidis* compared to the 74.40% rate in 78 strains isolated from mastitic milk [47], which may be attributed to the relatively small number of *S. epidermidis* strains isolated in our study. The resistance situation for *S. pasteuri* was particularly severe; besides penicillin, it showed nearly 50% resistance to six other antimicrobial agents, including erythromycin and tetracycline. The resistance rate for *S. epidermidis* to erythromycin reached 67%. Conversely, other *staphylococci* exhibited high sensitivity to antimicrobial agents, except for penicillin. Notably, *Staphylococcus* strains isolated in other studies exhibited significantly higher resistance rates to commonly used antibiotics such as erythromycin and demonstrated multidrug resistance compared

Table 8 Drug Resistance Gene Detection Results

Drug type	Gene name	Detection rate (%)					
		S. aureus **(*n* = 28)**	***S. chromogenes*** **(n = 15)**	***S. pasteuri*** **(*n* = 13)**	***S. agnetis*** **(*n* = 10)**	***S. epidermidis*** **(*n* = 3)**	**Total (*n* = 69)**
MLS_B	*erm(A)*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	erm(B)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	erm(C)	0% (0)	0% (0)	0% (0)	0% (0)	33.33% (1)	1.45% (1)
	erm(F)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	erm(33)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	mph(C)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	msr(A)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	lnu(A)	0% (0)	0% (0)	92.31% (12)	80.00% (8)	100.00 (3)	33.33% (23)
	lnu(B)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Pleuromutilins	*vga*	0% (0)	60.00% (9)	0% (0)	0% (0)	0% (0)	13.04% (9)
	vgb	0% (0)	6.67% (1)	0% (0)	0% (0)0	0% (0)	1.45% (1)
β-lactams	*blaZ*	64.29% (18)	100.00% (15)	100.00% (13)	70.00% (7)	100.00% (3)	81.16% (56)
	mecA	53.57% (15)	0% (0)	100.00% (13)	70.00% (7)	100.00% (3)	55.07% (38)
Tetracyclines	*tet(M)*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	tet(O)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	tet(L)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	tet(K)	3.57% (1)	13.33% (2)	69.23% (9)	80.00% (8)	100.00% (3)	33.33% (23)
Chloramphenicols	*cfr*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	fexA	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Aminoglycosides	*aacA-aphD*	0% (0)	0% (0)	61.54% (8)	80.00% (8)	100.00% (3)	27.54% (19)
	aadD	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Quinolones	*gyrA*	100.00% (28)	100.00% (15)	100.00% (13)	100.00% (10)	100.00% (3)	100.00% (69)
	gyrB	100.00% (28)	0% (0)	0% (0)	0% (0)	100.00% (3)	44.93% (31)
	glrA	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	glrB	100.00% (28)	80.00% (12)	100.00% (13)	80.00% (8)	100.00% (3)	92.75% (64)
Glycopeptides	*VanA*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	Van B	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	Van C1	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	Van C2/3	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Oxazolidinones	*optrA*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Lifamycins	*ropB*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)

Table 9 Qualitative detection results of biofilm

CRA	Result					
	S. aureus **(*n* = 28)**	***S. chromogenes*** **(*n* = 15)**	***S. pasteuri*** **(*n* = 13)**	***S. agnetis*** **(*n* = 10)**	***S. epidermidis*** **(*n* = 3)**	**Total (*n* = 69)**
Black	100.00 (28)	6.67% (1)	23.08% (3)	100% (10)	0% (0)	60.87 (42)
Red	0% (0)	93.33% (14)	76.92% (10)	0% (0)	100% (3)	39.13 (27)

to the findings of this study [48–50]. This suggests that the resistance of strains isolated from various regions is influenced by the types and quantities of antibiotics administered. In the Xizangan pastoral region, characterized by inadequate transportation and obsolete animal illness control protocols, the restricted and frequently misused veterinary pharmaceuticals have exacerbated bacterial resistance to specific drugs. Resistance genes detection results indicated that MRSA and MRCNS had detection rates of 53.57% and 51.61%, respectively, While

Table 10 Qualitative detection results of biofilm

MPA	Result					
	S. aureus **(*n* = 28)**	***S. chromogenes*** **(*n* = 15)**	***S. pasteuri*** **(*n* = 13)**	***S. agnetis*** **(*n* = 10)**	***S. epidermidis*** **(*n* = 3)**	**Total (*n* = 69)**
+	100.00 (28)	40.00% (6)	64.54% (8)	100% (10)	66.67% (2)	78.26% (54)
-	0% (0)	60.00% (9)	38.46% (5)	0% (0)	33.33% (1)	21.74% (15)

β-lactam resistance genes *blaZ* and *mecA* had detection rates of 81.16% and 55.07%. This is consistent with the high penicillin resistance observed in *staphylococci* and is significantly higher compared to the 8.3% MRSA isolation rate [51]. This discrepancy may be due to environmental factors, as well as the temporal development of *S. aureus* resistance. The elevated detection rates of MRSA and MRCNS in this study may pose potential threats to yaks and humans, indicating that Tibet should optimize antibiotic strategies by prioritizing *staphylococcus*-sensitives antimicrobials to mitigate public health risks. Among the isolated *S. pasteuri*, the detection rates of the MLS_B resistance gene *lnuA*, tetracycline resistance gene *tetK*, aminoglycoside resistance gene *aacA-aphD*, and quinolone resistance genes *gyrA* and *glrB* were all greater than 60%. However, some strains showed no resistance to the corresponding drugs. Multiple resistance genes were detected in *S. epidermidis*; however, it exhibited resistance only to penicillin, erythromycin, and trimethoprim-sulfamethoxazole. This suggests that resistance to these antimicrobial agents may be mediated by more than one gene, indicating that resistance phenotypes rarely consistently correlate with genotypes, which aligns with the previously published study [52]. Studies have shown that the expression of resistance genes is influenced by various environmental factors [53], and bacterial resistance cannot be completely explained from genetic and biochemical perspectives [54].

Bacterial biofilm infections have become a significant global healthcare issue, with clinical data indicating that approximately 80% of chronic and recurrent bacterial infections are associated with biofilms [55]. In this study, biofilm detection, results revealed that 42 strains tested positive for biofilm formation using the CRA method. In comparison, 54 strains were positive using the MPA method. The latter includes the former, which may be due to the limitations in nutritional conditions during the growth of strains in the CRA method, making it difficult to assess their biofilm-forming capability [56]. Biofilm-forming *staphylococci* demonstrated poor antibiotic resistance in susceptibility assessments. A previous study showed that among MSSA (Methicillin-Sensitive *Staphylococcus aureus*) and MRSA isolates, biofilm formation was not significantly associated with methicillin resistance. Furthermore, the sensitivity of biofilm-positive strains to antibiotics such as trimethoprim and gentamicin was non- significant from that of biofilm-negative strains [57]. In addition, analysis of MRSA biofilm-forming ability in China revealed that some biofilm-positive strains remained sensitive to antibiotics like dalbavancin, suggesting that biofilm presence does not universally confer resistance to all drugs [58]. These findings align with the results of our study. The formation of staphylococcal biofilms is primarily regulated by the expression of polysaccharide intercellular adhesin (PIA), whose synthesis is controlled by the *icaA/B/C/D* operon [59]. However, *staphylococci* lacking *ica* genes are capable of biofilm formation via the direct influence of surface proteins, including the bap protein [60]. This study revealed a detection rate of 46.38% for *icaA/D*; nevertheless, additional *staphylococci* lacking these genes still exhibited biofilm positive in the MPA test, suggesting that bacterial surface proteins may regulate their biofilm development. Previous research found that only 45% of biofilm-positive strains from rabbit-derived *staphylococci* contained *icaA/D* or *icaB/C* [61, 62]. A possible explanation for this phenomenon is that *icaA* and *icaD* may have undergone mutations during genetic evolution [63], or mutations at other loci on transposons could hinder the formation of PIA and biofilms [64]. The virulence gene detection results showed that out of 16 virulence genes tested, 11 were detected, primarily in *S. aureus*, with a small number of virulence genes found in *S. chromogenes* and *S. epidermidis*. No virulence genes were detected in *S. pasteuri* and *S. agnetis*. The pathogenicity of *staphylococci* is mostly determined by the virulence factors they produce; thus, the discovery of virulence genes indicates that the pathogenicity of the *S. aureus* isolated in this investigation is superior to that of other *staphylococci*. It is noteworthy that the coagulase gene (*coa*) was not detected in *S. aureus*, which may be due to the production of fibrinolysin by the isolated *S. aureus*, leading to the degradation of fibrin clots or insufficient amounts of free coagulase produced. The leukocidin genes *lukS* and

Table 11 Virulence Gene Detection Results

Gene type	Gene name	Detection rate (%)					
		S. aureus **(*n* = 28)**	***S. chromogenes*** **(*n* = 15)**	***S. pasteuri*** **(*n* = 13)**	***S. agnetis*** **(*n* = 10)**	***S. epidermidis*** **(*n* = 3)**	**Total (*n* = 69)**
Enterotoxin gene	*sea*	0% (0)	0% (0)	0% (0)	0% (0)	100.00% (3)	4.35% (3)
	seb	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	sec	50.00% (14)	0% (0)	0% (0)	0% (0)	100.00% (3)	24.64% (17)
	sed	0% (0)	46.67% (7)	0% (0)	0% (0)	0% (0)	10.14% (7)
	see	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Toxic shock syndrome toxin gene	*tsst*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Leukocidin gene	*luks*	100.00% (28)	0% (0)	0% (0)	0% (0)	0% (0)	40.58% (28)
	lukf	100.00% (28)	0% (0)	0% (0)	0% (0)	0% (0)	40.58% (28)
Hemolysin gene	*hla*	100.00% (28)	0% (0)	0% (0)	0% (0)	0% (0)	40.58% (28)
Clumping factor gene	*clfA*	100.00% (28)	0% (0)	0% (0)	0% (0)	0% (0)	40.58% (28)
	clfB	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Coagulase gene	*coa*	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Fibronectin-binding protein gene	*fnbA*	2.70% (1)	0% (0)	0% (0)	0% (0)	0% (0)	1.45% (1)
	fnbB	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Cell adhesion protein gene	*icaA*	0% (0)	26.67% (4)	0% (0)	0% (0)	0% (0)	5.80% (4)
	icaD	96.43% (27)	6.67% (1)	0% (0)	0% (0)	0% (0)	40.58% (28)

Table 12 SCC typing results

Type	Number					
	S. aureus	***S. chromogenes***	***S. pasteuri***	***S. agnetis***	***S. epidermidis***	**Total**
SCCmecI	0	0	0	0	0	0
SCCmecII	0	0	0	0	0	0
SCCmecIII	0	0	0	0	0	0
SCCmecIVa	0	0	0	0	0	0
SCCmecIVb	0	0	11	0	0	11
SCCmecIVc	0	0	0	0	0	0
SCCmecIVd	0	0	0	0	0	0
SCCmecV	0	0	0	0	2	2

Table 13 MLST typing results

ST type (number)	House-keeping gene type						
	arcC	***aroE***	***glpF***	***gmk***	***PTA***	***tpi***	***yqiL***
6263(*n* = 4)	4	1	1	15	12	1	40
9542*(*n* = 10)	1	1	1	1	12	27	1204
9543*(*n* = 4)	792	1	1	1	12	27	1204
9544*(*n* = 1)	3	115	46	1	7	5	1206
9545*(*n* = 1)	1	1	1	1	12	27	1205
9546*(*n* = 8)	4	1	625	15	12	1	40

*: Unreported ST type

Table 14 Spa typing results

Spa type	Number
t3022	8
t4236	3
t4558	6
t4445	1
t1940	9
*	1

*: Unreported Spa type

lukF, the hemolysin gene *hla*, and the clumping factor gene *clfA* were detected with a prevalence of 100% in *S. aureus*, while the enterotoxin gene *sec* was detected at a rate of 50%. Neelam et al. reported detection rates of 49% for the *hla* gene and 1.8% for the sec gene in *S. aureus* isolated from milk [65]. Likewise, Liu Yichen found detection rates of 81.25% and 78.13% for the *hla* and *clfA* genes, respectively, in *S. aureus* isolated from dead duck embryos [66]. The higher detection rates of the corresponding genes in this study suggest that the presence of virulence genes is geographically associated, with different strains prevalent in various areas and varying in their carriage of virulence genes.

Conclusion

In summary, this study explored the prevalence and biological characteristics of *staphylococci* isolated from yak milk in Xizang through antimicrobial susceptibility testing, biofilm assays, and the detection of virulence and resistance genes. Notably, the isolated *staphylococci* possessed several resistance genes. Despite the absence of significant multiple drug resistance, this circumstance warrants attention. It is imperative to abandon a monotherapy approach and rigorously regulate dosages. The information obtained from this study can provide a theoretical basis for the prevention and treatment of *staphylococci* in yaks.

Abbreviations

CPS	Coagulase-Positive *Staphylococci*
CNS	Coagulase-Negative *Staphylococci*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-**S**ensitive *Staphylococcus aureus*
MRCNS	Methicillin-Resistant Coagulase-Negative *Staphylococci*
BCO	Bacterial Chondronecrosis with Osteomyelitis
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
BHI	Brain–Heart Infusion
FBS	Fetal Bovine Serum
TE	Tris–EDTA
PBS	Phosphate Buffer Saline
MLS_B	Macrolides, Lincomycins, and Streptogramins B
CRA	Congo Red Agar
MPA	Microtiter Plate Assay
SCCmec	*Staphylococcus* Cassette Chromosome mec
MLST	Multilocus Sequence Typing

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Authors' contributions

FXM and HCM designed the research, carried out the experiments, obtained the bacterial isolates and wrote the manuscript. QXW and JYZ Edited and retouched the manuscript. All authors reviewed the manuscript.

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Data availability

The datasets generated during the current study are available in the GenBank, under accession number: PQ721055 ~ PQ721123. They are also available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The Laboratory Animal Welfare and Ethics Committee of Institute of Animal Husbandry and Veterinary, Xizang Academy of Agriculture and Animal Husbandry Science, ruled that no formal ethics approval was required to conduct this research. Before conducting the research, informed consent was obtained from all the owners of the yaks included in this study.
This article does not include any research conducted by any author on human participants or animals.

Consent for publication

Statement: Not Applicable.

Competing interests

The authors declare no competing interests.

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