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RESEARCH ARTICLE

Molecular Insights into Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Goat Mastitis

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ABSTRACT

Mastitis is a significant factor that influences the integrity and production of milk, with *Staphylococcus aureus* (*S. aureus*) being the most important mastitic pathogen of goat herds. The research aimed to demonstrate the prevalence of antibiotic resistance profiling, MRSA, and *S. aureus* in goat milk samples from the study district. For this, 384 *Capra hircus* milk samples were taken from goat herds and subjected to analysis to isolate *S. aureus*. The bacteria isolates were recognized as MRSA employing the cefoxitin disc in a disc diffusion experiment, and the *mecA* gene was detected using PCR. The MRSA-positive isolates were characterized by bioinformatics tools like Clustal Omega for alignments, MEME for motif identification, and Swiss software for 3D protein structure analysis. In contrast, the protein interactions were predicted via STRING, and SOPMA and PDBsum tools were used for secondary structure and amino acid conformations. The in vitro antimicrobial susceptibility pattern was evaluated by the Kirby-Bauer disc diffusion technique. The results outlined that 50.26 % and 19.27 % of milk samples were positive for SCM and *S. aureus*, respectively. The phenotypic prevalence of MRSA was found to be 52.70%, while PCR protocol confirmed 31.08 % of isolates as MRSA. The findings of the antimicrobial susceptibility testing of MRSA isolates were found highly resistant against cefoxitin, followed by oxytetracycline and gentamicin, while linezolid and fusidic acid were found highly sensitive drugs against the tested isolates. The current study deduced that MRSA is a common and growing disease in dairy goats that has a greater risk of spreading to people. MRSA's impending threat of zoonosis is highlighted by the presence of several virulence factors and antibiotic resistance, underscoring the need for additional investigation.

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INTRODUCTION

The consumption of goat milk and its byproducts is boosted by its better digestibility and lesser allergenicity in comparison to cow's milk (Al Kaisy *et al.*, 2023). The expansion of the industry can be attributed to the pros of goat milk in third-world countries, even if it only makes up around 3% of the world's milk supply (Coimbra-E-Souza *et al.*, 2019). Mastitis is one of the most severe barriers because of the increased expense of treatment and low milk production in small ruminants (Gelasakis *et al.*, 2015). Mastitis in small ruminants is provoked by various pathogens. *Staphylococcus aureus* (*S. aureus*) is believed to be the commonest pathogenic

organism isolated from their milk (Abdalhamed *et al.,* 2018).

Staphylococcus aureus carries the nuc gene, the gold standard for *S. aureus* detection (Torres *et al.,* 2019). It encodes the thermostable nuclease that lyses host cell DNA and RNA, causing damage to tissue and its dissemination throughout the body (Hu *et al.,* 2013). It also encourages the outflow of *S. aureus*, which hence enables the bacteria to escape the host defense when captured by neutrophil extracellular traps (Kenny *et al.,* 2017). Additionally, the bacteria possess pathogenic chemicals that aid in their establishment, survival, and enhancement of host harm. *S. aureus* also contains a coagulase enzyme, which transforms plasma fibrinogen into fibrin clots, preventing bacteria from phagocytosing and inhibiting other host defense systems (Andrade *et al.,* 2021). Over time, *S. aureus* became resistant to penicillin in over 90% of strains. Methicillin drugs are semi-synthetic beta-lactam antibiotics, which are resistant to the bacterium producing the β-lactamase enzyme developed as a result of their increased resistance to penicillin (Michalik *et al.,* 2025). It was believed to be extensively effective on *S. aureus* resistant to penicillin. Despite its successful application against penicillin-resistant *S. aureus*, in 1961, the first incident of the MRSA strain was reported (Javed *et al.,* 2024). Since then, MRSA has become an endemic organism and a great hazard for human health in the whole world. *S. aureus* acquires methicillin resistance when it enters the *mecA* gene; this gene codes for a penicillin-binding protein (PBP2a or PBP2) found on bacterial cell walls and is responsible for the bacteria's poor affinity for β-lactam antibiotics (Katayama *et al.,* 2000). Antibiotic resistance genes in *S. aureus* that cause mastitis in dairy animals have become a serious problem due to limited treatment options. Consuming raw goat milk can expose consumers to zoonotic infections that spread from animals to people, endangering their health. With the rising cases of antimicrobial resistance and contamination of raw milk with a pathogenic bacterium, notably MRSA, it has attracted global attention (Eid *et al.,* 2022). The present study intended to determine the *S. aureus* prevalence and MRSA and the patterns of antimicrobial sensitivity of isolated *S. aureus* from dairy goats in the district of Muzaffargarh, Punjab, Pakistan.

MATERIALS AND METHODS

Study area: The study district, Muzaffargarh, is 71.19º East longitude, 30.08º North Latitude, 114m above sea level, and located in the southwest of the province of Punjab at the Chenab River of Pakistan. Due to the high population of goats in that area and to make the research study more diverse and demographic, the sampling was conducted at various farms and veterinary teaching hospitals situated in and around the territories of the district of Faisalabad, Punjab, Pakistan.

Sampling Strategy and Sample Size Determination: Overall, 384 milk samples from lactating goats were assembled aseptically using the convenient sampling technique as per the guidance of Thrushfield (2013). The qualitative analysis was done using the California mastitis test to confirm subclinical mastitis in the observed samples (Javed *et al.,* 2023). Sub-clinically positive milk samples were sent to the University of Veterinary and Animal Sciences, Lahore, for analysis at the Medicine Research Laboratory while being stored at 4◦C which maintains the cold chain.

Bacterial Isolation and Confirmation: Processing was done on the subclinical positive milk samples for harvesting the *Staphylococcus aureus* strain (Ahmed *et al.,* 2022). *S. aureus* was confirmed phenotypically using colony morphology, Gram staining, and different biochemical assays, including tube coagulase, catalase, and D-mannitol. The genotypic confirmation was carried out using PCR, which targeted the *nuc* gene, and verified *S. aureus* strains were kept at -20°C in Trypticase Soy broth with 15% (v/v) glycerol for subsequent phenotypic and genotypic confirmation (Aras *et al.,* 2012).

Phenotypic identification of methicillin-resistant *S. aureus:* Methicillin-resistant and sensitive *S. aureus* were phenotypically recognized using the disc-diffusion antibiotic susceptibility test. An actively growing culture of *S. aureus* was swabbed on Mueller-Hinton Agar for phenotypic detection. A cefoxitin disc (30µg) was then placed on the agar and incubated at 37 ˚C for 24 hours. Then, isolates were classified as either methicillin-sensitive or methicillin-resistant *S. aureus*, focused on the examination of the inhibition zones and comparison with the CLSI, 2019 standards (Javed *et al.,* 2023). As per CLSI guidelines, isolates exhibiting a zone of ≥21 mm surrounding the cefoxitin disc were classified as MRSA, whereas isolates displaying a zone of clearing ≥22 mm fall in the category of MSSA.

Molecular confirmation of MRSA: Aerobic BHI broth culture of *S. aureus* strains during the night was done at 37°C, until reaching a closest of 0.5 McFarland, after thawing. Genomic DNA was extracted using the Thermo Scientific GeneJET kit genomics DNA purification kit following the manufacturer's instructions. and the yield was estimated through the use of a Nanodrop machine (Thermo ScientificTM–Nanodrop2000). Genotypic MRSA identification was based on the PCR

amplification of the *mecA* gene using previously validated and tested primers and conditions on the extracted DNA (Javed *et al.,* 2023). A reference strain of MRSA, as described by Javed *et al.,* (2023), was employed as a positive control. The isolates that showed a band on 310 bp were considered *mecA*-positive and hence MRSA, while the isolates that didn't carry the *mecA* gene were named methicillin-sensitive *S. aureus.* The PCR reaction mix for each reaction was 20 µl containing 10 µl of 2x master mix, 2 µl of 20 pmol forward and reverse primer, 3 µl of nuclease-free water, and 3 µl of template DNA.

Evolutionary analysis of *the mecA* gene in *S. aureus*: Clustal Omega software was employed for multiple alignments of nucleic and amino acids. Nucleic acid and amino acid motifs were constructed by using the MEME (Multiple EM for Motif Elicitation) suite. Furthermore, Swiss software was used to investigate the three-dimensional structure of the protein. Using the STRING tool, protein-protein interactions were anticipated, and PBP2a physicochemical properties were assessed by utilizing ProtParam. With the assistance of a bioinformatic tool named SOPMA, the local fold comparison was performed. In addition, Ramachandran plots were outlined to imagine the possible conformation of amino acid residues within the protein, utilizing the PDBsum tool.

***In-vitro* antimicrobial susceptibility testing of MRSA isolates:** The *in-vitro* sensitivity trials against MRSA isolates were done using the Kirby–Bauer test. For this purpose, MRSA growth of 0.5 McFarland standards was taken, and swabbing was performed on Mueller-Hinton agar. The antibiotic discs of various groups were analyzed against these isolates and were aseptically placed under a biological safety cabinet, providing incubation for 24 hours at 37°C. The diameters of the inhibition zones resulting from the use of each isolate were quantified and compared to the employer by CLSI breakpoint values (CLSI, 2019).

Statistical Analysis: The formula outlined by Thrusfield (2013) was used for prevalence demonstration. The estimation of *in vitro* antibiotic susceptibility was carried out by descriptive statistics using SPSS version 22.

RESULTS

Prevalence of SCM and *S. aureus*: The present investigation showed a prevalence of 50.26% (193/384) for subclinical mastitis (SCM) samples using the surf field mastitis test (SFMT) from goats of district Faisalabad. The prevalence of SCM was found to be larger in tehsil Jaranwala (55.47%) than in tehsil Sumundri (42.19%) and Tandlianwala (53.13%), as shown in Table 1.

Out of 384 milk samples, 19.27% (74/384) samples were found positive for *S. aureus* after morphological characteristics and biochemical identification were authenticated as the thermostable nuclease producers by harboring the *nuc* gene and showed 100% prevalence towards that gene. The prevalence of *S. aureus* was also found to be higher in tehsil Jaranwala (26.56%) than in tehsil Sumundri (17.97%) and Tandlianwala (13.28%), as shown in Table 1.

Table 1. Prevalence of subclinical mastitis, *S aureus*, and MRSA from goats of the study district

Study Area	No of samples	SCM (%)	S. aureus (%)	MRSA	
				Phenotypic	Genotypic
Jaranwala	128	71(55.47)	34(26.56)	17(50.00)	11(32.35)
Samundri	128	54(42.19)	23(17.97)	13(56.52)	07(30.43)
Tandlian-wala	128	68(53.13)	17(13.28)	09(52.94)	05(29.41)
Total	384	93(50.26)	74(19.27)	39(52.70)	23(31.08)

Phenotypic and genotypic prevalence of MRSA: The findings of MRSA revealed that 52.70% (39/74) of *S. aureus* isolates were resistant to cefoxitin discs, declaring them MRSA on a phenotypic basis. The confirmation of MRSA by the *mecA* gene revealed that 31.08% (23/74) of isolates were declared as MRSA, while 51 isolates were found negative based on PCR and were declared as methicillin-sensitive *S. aureus* (MSSA).

***In silico* analysis of Penicillin-binding protein 2a:** Multiple sequence alignment of Nucleic acid was done by Clustal Omega, exhibiting significant variation between local isolates and reference sequences (Figure 1). The nucleotide motif analysis revealed that all three conserved motifs are identical in study isolates and reference sequences with a *p*-value ranging between 1.02e-20 to 5.77e-22 as shown in (Figure 1) and the colored bars represent different motif types in each sequence highlighting their conserved binding sites but different E- value, and motif width as shown in Table 5. Moreover, Amino acid sequence alignment of the study sequence and reference sequence was found to be varied as given in (Figure 2), and motif analysis of PBP2a of the study and reference sequences was shown in different colors with a p-value ranging between 1.32e-5 and 5.32e-93 (Figure 2). All three motifs are conserved in all sequences except MZ359762 and KY467026 isolates, and variation was found in binding sites, E-value, and motif width as shown in Table 6.

Table 2. Antibiogram of MRSA isolates (n=10)

Antibiotics discs	MRSA isolates (%) n=10		
	Sensitive	Intermediate	Resistant
Oxytetracycline (30 µg)	40	0	60
Ciprofloxacin (5 µg)	40	20	40
Gentamicin (10 µg)	30	0	70
Amikacin (30 µg)	60	0	40
Tylosin (30 µg)	80	0	20
Fusidic acid (10 µg)	90	0	10
Moxifloxacin (5 µg)	80	10	10
Cefoxitin (30 µg)	0	0	100
Linezolid (30 µg)	90	10	0
Trimethoprim+ Sulfamethoxazole (1.25 µg, 23.75 µg)	40	10	50

Table 3: Secondary structure comparison of PBP2a by SOPMA

Sample ID	Alpha helix	Extended strand	Beta turn	Random coil
Seq1	34.31	24.51	13.73	27.45
Seq2	34.31	24.51	13.73	27.45
Seq3	33.65	26.92	9.62	29.81

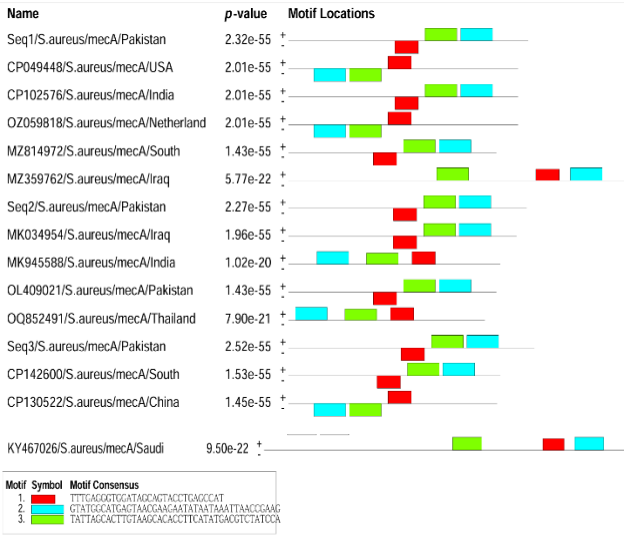


Fig. 1: Conserved motifs in the *mecA* gene of study isolates and reference sequences

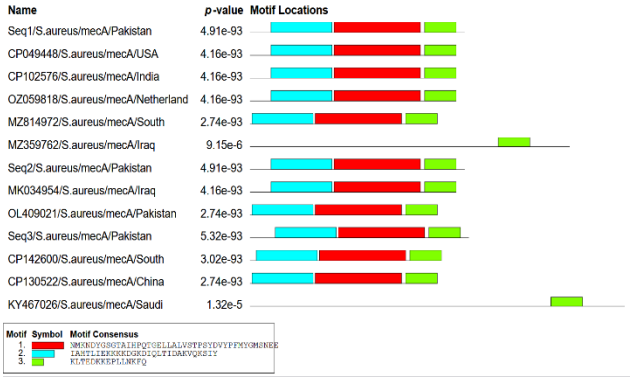


Fig. 2: Conserved motifs in PBP2a of study isolates and reference sequences

The 3D model for the PBP2a protein of all study isolates is shown in Figure 3. Applying STRING (Search Tool for the Retrieval of Interacting Genes/ Proteins), the network's interaction of the studied protein (Penicillin-binding protein 2a) demonstrated salient association with other proteins like *mec1* (methicillin resistance regulatory protein), *mecR* (methicillin resistance protein) and *bla1* (penicillinase repressor) with a score of 0.959, 0.892 and 0.892 respectively as shown in (Figure 4). Furthermore, a secondary structure comparison of PBP2a exhibited that seq3 showed variation from Seq1 and Seq2 in terms of beta-turn, extended strand, alpha helix, and random coil, as shown in Table 3, and the physicochemical properties of PBP2a were shown in Table 4. The Ramachandran plot favored the 3D model of *PBP2a* because 93.1% of amino acids fall in the most favored regions, indicating a structural stability of the protein, as shown in Figure 5. Apart from this, 1.1% of residues fall in the disallowed region, and Glycine and Proline residues are 5 and 4 in number, respectively.

Table 4: Physical and chemical properties of PBP2a protein by Protparam

Sample ID	MW	AA	Theoretically	TNC	TPC	Half-life (hours)	(II)	(AI)	GRAVY
Seq1	11542.06	102	5.61	12	14	100	30.79	83.14	-0.647
Seq2	11542.06	102	5.61	12	14	100	30.79	83.14	-0.647
Seq3	11758.26	104	5.91	15	12	1	32.24	81.54	-0.676

MW=Molecular weight, AA=Number of Amino Acids, TNC=Total Negatively charged residues, TPC=Total Positively Charged residues, II=Instability index, AI=Aliphatic index

Table 5: Discovered common motifs for the *mecA* gene

Discovered candidate motifs	E- value	Binding sites	Width
	1.4e-106	15	30
	7.0e-142	15	41

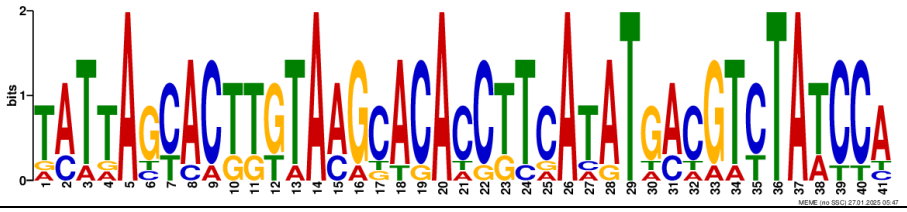
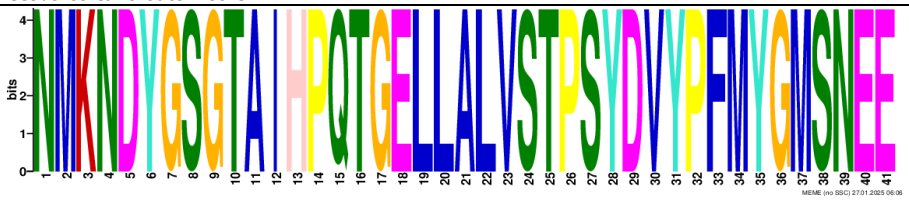
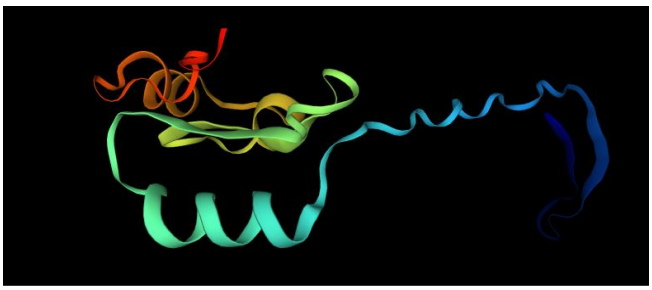
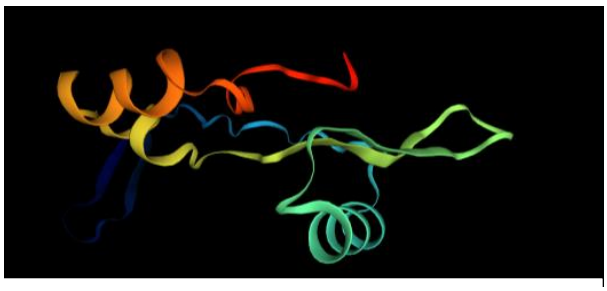
	1.0e-121	15	41

Table 6: Discovered common motifs for PBP2a

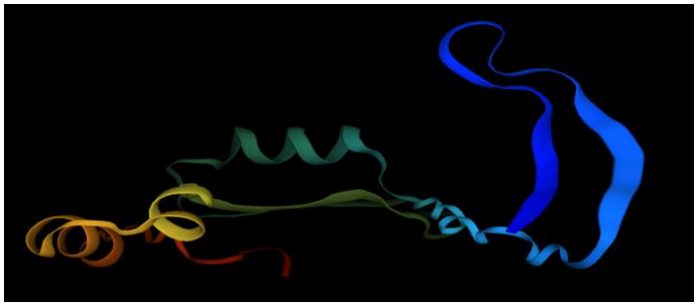
Discovered candidate motifs	E- value	Binding sites	Width
	8.9e-330	11	41
	8.8e-185	11	29
	7.8e-086	13	15



(A) 3D model of PBP2a formed by sample ID Seq1



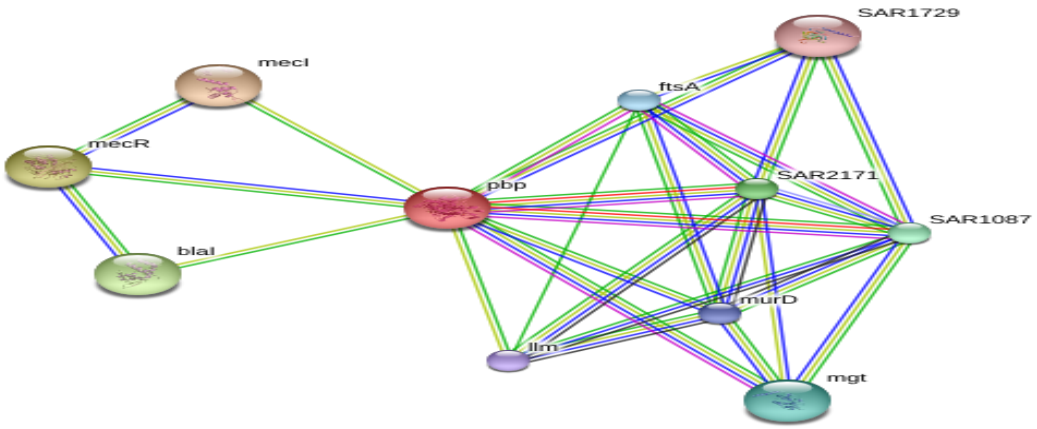
(B) 3D model of PBP2a formed by sample ID Seq2



(C) 3D model of PBP2a formed by sample ID Seq3

Figure 3: Three-dimensional models of Penicillin-binding protein 2a. (A) Seq1 (B) Seq2 (C) Seq3

(A)



(B)

Your Input:

pbp penicillin-binding protein 2 prime (668 aa)
(Staphylococcus aureus MRSA252)

Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
mecI	methicillin resistance regulatory protein MecI; Transcriptional repressor that constitutively b [...] (114 aa)									0.959
mecR	methicillin resistance protein MecR1; Penicillin-interactive protein and potential antirepresso [...] (585 aa)									0.892
blaI	penicillinase repressor; Transcriptional repressor that constitutively blocks expression of bet [...] (126 aa)									0.892
SAR2171	hypothetical protein (400 aa)									0.892
SAR1087	putative cell division protein (408 aa)									0.850
mgt	glycosyltransferase; Involved in the biosynthesis of cell wall peptidoglycan. Responsible for t [...] (269 aa)									0.837
ftsA	putative cell division protein; This protein may be involved in anomalous filament growth. May [...] (468 aa)									0.759
murD	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase; Cell wall formation. Catalyzes the additi [...] (449 aa)									0.758
llm	putative glycosyl transferase (351 aa)									0.752
SAR1729	rod shape-determining protein MreC (280 aa)									0.745

Figure 4: (A) Protein-Protein interaction by STRING (Network view) and (B) Interaction of reference protein with other proteins (predicted functional partners)

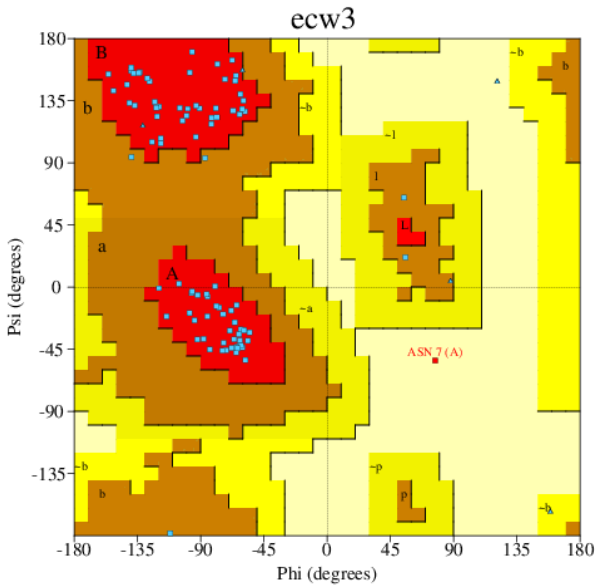


Figure 5: Ramachandran plot of PBP2a

Antimicrobial susceptibility pattern of MRSA isolates: The MRSA's susceptibility profiling against a variety of antibiotics was done by placement of antibiotics discs including cefoxitin (30 µg), oxytetracycline (30 µg), linezolid (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), tylosin (30 µg), amikacin (30 µg), moxifloxacin (5 µg), fusidic acid (10 µg), and TMP+sulpha on pre-set Mueller Hinton agar plates swabbed with activated growth of MRSA. The findings of the susceptibility profile of MRSA showed that 100% of MRSA isolates were found resistant towards cefoxitin, while the next highly resistant antibiotic was gentamicin, oxytetracycline, and trimethoprim + sulphamethoxazole, while the most sensitive antibiotics were found to be linezolid, fusidic acid, and moxifloxacin (Table 2).

DISCUSSION

In Pakistan, goat breeding represents an important substitute for livestock production, and its population was estimated to be 82.5 million, contributing to the industry's growth. Different pathogens can be spread to humans via milk and its products due to the dairy industry's decline to compete in international markets (Obaidat *et al.*, 2018).

The results obtained from the present investigation revealed that 50.26% of samples of milk from *Capra hircus*, (193/384), were positive for subclinical mastitis, that is in agreement with the outcomes of (Begum *et al.*, 2016; Spigelman *et al.*, 2020), but unlike the findings of other authors (Altaf *et al.*, 2020; Pirzada *et al.*, 2019) who explored 39.22% and 38% prevalence. The differences may be

attributed to different sampling procedures; in the present investigation, samples were collected from healthy goats without any mastitis history, or it could be a result of management or therapeutic measures.

The research revealed that the prevalence of *S. aureus* in goats with mastitis was determined to be 26.82%. This result corresponds to the results of (Hussein *et al.,* 2020), who showed a prevalence of 24.37%. However, the prevalence accounted in this study is significantly lower from that of (Cortimiglia *et al.,* 2016) 43.1% of *S. aureus* in goat milk (Altaf *et al.,* 2020) on the contrary, reported a much high prevalence of 80.79 % in their samples attributing this increased incidence to the exopolysaccharides (slime) produced by *S. aureus*.

MRSA identification in affected goats with subclinical mastitis presents a double risk since it reduces both the quantity and quality produced and introduces significant zoonotic risk. This bacterium is transmitted to humans at a rapid rate, which is of concern about the spread of antibiotic resistance (Altaf *et al.,* 2020). The main mode of transmission is raw milk drinking-when proper hygiene requirements are not met carefully. If people consume raw milk without caring for their safety measures, *S. aureus* and MRSA can infect them, making the already active AMR problem worse (Ganai *et al.,* 2016). In addition, goat milk and its derivatives are very susceptible to microbial contamination during processing, particularly in poor hygienic conditions, which leads to an escalation of the chance of human exposure to resistant strains (Lopes Júnior *et al.*, 2021).

In the current study, the *mecA* gene was identified in a large percentage of goats (35.92%) and humans (10.71%) *S. aureus* isolates, where MRSA was confirmed. The genetic marker, *the mecA gene in the study bacteria S. aureus*, is responsible for methicillin resistance due to encoding a penicillin-binding protein (PBP2a) that has a weak affinity for the β-lactams and thus makes β-lactam antibiotics ineffective in the treatment of MRSA infections. This genetic characteristic allows *S. aureus* to evade routinely used antibiotics and make treatment regimens more cumbersome for animals and humans. Some *S. aureus* strains isolated from goat milk may carry *mecA-associated* β-lactam resistance even though the previous research proved that the gene is not present (Titouche *et al.*, 2019). For instance, 11.9% prevalence of *mecA* was reported by (Obaidat *et al.,* 2018), in the bulk tank goat milk, and prevalence of *mecA* in mastitic goat milk samples was 8.96% (Rana *et al.,* 2020) and 9.2% (El-Deeb *et al.,* 2018). These results emphasize the widespread presence of MRSA in goat milk with serious implications for public health. Multiple factors lead to the high incidence of *S. aureus* and MRSA in the targeted species, goats, studied in this paper. These will be unsanitary farming practices such as poor utensil sanitation, filthy udders, mixed farming systems, and a high percentage of goats with sub-clinical mastitis. Subclinical mastitis is particularly dangerous since it may be overlooked, but it will also help spread germs in milk. Raw milk contamination with *S. aureus* and MRSA bacteria is also promoted substantially by poor milk handling practices. These factors have been observed as major contributors to increased bacterial load in unpasteurized milk as well as dairy products (Ganai *et al.,* 2016). In addition, the increased MRSA frequency in this research has been contributed to by environmental pollution and extensive careless use of antibiotics in the livestock agricultural practices in Pakistan. In agriculture, the misuse and abuse of antibiotics, therefore, not only speed the resistance process, but they also encourage the spread of resistant strains such as MRSA. If there are no stricter restrictions and no better control over the use of antibiotics, the situation may reach to worst extremity, rising drug resistance both among animals and humans. Improved biosecurity measures, improved hygiene practices, and smarter use of antibiotics are essential steps in minimizing the risks posed by MRSA in cattle, especially in areas where transmission from animal to human is an issue.

The findings of antibiogram profiling of MRSA revealed that the maximum isolates were explored resistant to cefoxitin (100%), followed by gentamicin (70%) and oxytetracycline (60%). The antibiotics linezolid and fusidic acid (90%) had the highest sensitivity against the MRSA isolates. These findings are consistent with previous research undertaken in Iran. (Rahimi and Alian, 2013), Egypt (Ali *et al.,* 2017), Jordan (Obaidat *et al.,* 2018), and China (Wu *et al.,* 2019). Similar findings have been observed in Pakistan across a variety of species. (Javed *et al.,* 2023; Sabir *et al.,* 2024). The prevalence of antibiotic-resistant MRSA strains is connected to the overuse and misuse in both veterinary and human medicine (Javed *et al.,* 2023). Enhanced concentration of antibiotic resistance has been reported in staphylococci involved in caprine mastitis, while this study isolates showed a high sensitivity towards Ciprofloxacin, Linezolid, and levofloxacin, which aligns with the outcomes of (Altaf *et al.,* 2019).

The infected goats are reservoirs for transferring the resistant strains to humans and other animals. Antimicrobial resistance may occur as a result of the medical treatments applied to Pakistani farm animals. These antibiotic-resistant bacteria are another

outcome of the practice of farmers using more antibiotics without consultation with a veterinarian, and incorrect or inadequate treatment of mastitis. A significant possibility for the induction of caprine mastitis is implied by the combination of exogenous and antibiotic-resistant genes identified in our research.

Conclusion

The increased prevalence of MRSA is responsible for the subclinical mastitis causation in goats, and the consumption of raw milk highlights the potential for food poisoning caused by this pathogen, which provides a vehicle for transmitting potential pathogens. The association of multidrug-resistant *S. aureus* has gained significant community health attention as this pathogenic strain can be disseminated to the human population. Additionally, during the treatment of the infected animal, frequent surveillance systems and policies in study areas are needed to strengthen and promote the responsible use of antimicrobial drugs as well as effective control measures for minimizing the bacterial infection load among animal handlers to prevent the AMR issue in native animals.

Conflict of Interest: The authors declared no conflict of interest.

Authors' contribution: NZG and SK provided the research idea, performed the experiments, and wrote the manuscript. FM handled the revision.

REFERENCES

Abdalhamed AM, Zeedan GSG and Zeina HAAA, 2018. Isolation and identification of bacteria causing mastitis in small ruminants and their susceptibility to antibiotics, honey, essential oils, and plant extracts. Vet World 11:355-362.

Ahmed A, Ijaz M, Khan JA, *et al.*,2022. Molecular characterization and therapeutic insights into biofilm-positive Staphylococcus aureus isolated from bovine subclinical mastitis. Pak Vet J 42:584-590.

ALKaisy QH, Al-Saadi JS, Al-Rikabi AK, *et al.,* 2023. Exploring the health benefits and functional properties of goat milk proteins. Food Sci Nutr 11:5641-5656.

Altaf M, Ijaz M, Iqbal MK, *et al.,* 2020. Molecular characterization of Methicillin-Resistant Staphylococcus Aureus (MRSA) and associated risk factors with the occurrence of goat mastitis. Pak Vet J 40:1-6.

Andrade NC, Laranjo M, Costa MM, *et al.,* 2021. Virulence factors in Staphylococcus associated with small ruminant mastitis: Biofilm production and antimicrobial resistance genes. Antibiotics 10:1-10.

Aras Z, Aydin I and Kav K, 2012. Isolation of methicillin-resistant Staphylococcus aureus from caprine mastitis cases. Small Ruminant Res 102:68-73.

Begum M, Hossain M, Ershaduzzaman M, *et al.,* 2016. Epidemiological studies on subclinical mastitis in dairy goats in northern regions of Bangladesh. Bangladesh J Livest Res 19:112-122.

CLSI, 2019. CLSI M100-ED29: 2019 Performance standards for antimicrobial susceptibility testing, 29th Edition.

Coimbra-E-Souza V, Rossi CC, Jesus-de Freitas LJ, *et al.,* 2019. Short communication: Diversity of species and transmission of antimicrobial resistance among Staphylococcus spp. Isolated from goat milk. J Dairy Sci 102:5518-5524.

Cortimiglia C, Luini M, Bianchini V, *et al.,* 2016. Prevalence of Staphylococcus aureus and of methicillin-resistant *S. aureus* clonal complexes in bulk tank milk from dairy cattle herds in Lombardy Region (Northern Italy). Epidemiol Infect 144:3046-3051.

Eid HM, El-Mahallawy HS, Mohammed NEY, *et al.,* 2022. Multidrug-resistant and enterotoxigenic methicillin-resistant Staphylococcus aureus isolated from raw milk of cows at small-scale production units. J Adv Vet Anim Res 9:113-121.

El-Deeb W, Fayez M, Elmoslemany A, *et al.,* 2018. Methicillin-resistant Staphylococcus aureus among goat farms in Eastern province, Saudi Arabia: Prevalence and risk factors. Prev Vet Med 156:84-90.

Ganai AW, Kotwal SK, Wani N, *et al.,,* 2016. Detection of the *mecA* gene of methicillin-resistant Staphylococcus aureus by PCR assay from raw milk. Indian J Anim Sci 86:508-511.

Gelasakis AI, Mavrogianni VS, Petridis IG, *et al.,* 2015. Mastitis in sheep–The last 10 years and the future of research. Vet Microbiol 181:136-146.

Hu Y, Meng J, Shi C, *et al.,* 2013. Characterization and comparative analysis of a second thermos nuclease from Staphylococcus aureus. Microbiol Res 168:174-182.

Hussein HA, Fouad MT, Abd El-Razik KA, *et al.,* 2020. Study on prevalence and bacterial etiology of mastitis, and effects of subclinical mastitis and stage of lactation on SCC in dairy goats in Egypt. Trop Anim Health Prod 52:3091-3097.

Javed MU, Ijaz M, Durrani AZ, *et al.,* 2023. On-farm epidemiology, virulence profiling, and molecular characterization of methicillin-resistant Staphylococcus aureus at goat farms. Microb Pathog 185:106456.

Javed MU, Ijaz M, Ahmed A, *et al.,* 2024. Molecular dynamics and antimicrobial resistance pattern of β-lactam-resistant coagulase-positive Staphylococcus aureus isolated from goat mastitis. Pak Vet J 44:423-429.

Kenny EF, Herzig A, Krüger R, *et al.,* 2017. Diverse stimuli engage different neutrophil extracellular trap pathways. Elife 6: e24437.

Lopes Júnior WD, Monte DFM do, de Leon CMGC, *et al.,* 2021. Logistic regression model reveals major factors associated with total bacteria and somatic cell counts in goat bulk milk. Small Ruminant Res 198:0-7.

Michalik M, Podbielska-Kubera A and Dmowska-Koroblewska A, 2025. Antibiotic resistance of Staphylococcus aureus strains—searching for new antimicrobial agents. Pharmaceutics 18:81.

Obaidat MM, Bani Salman AE and Roess AA, 2018. High prevalence and antimicrobial resistance of *mecA* Staphylococcus aureus in dairy cattle, sheep, and goat bulk tank milk in Jordan. Trop Anim Health Prod 50:405-412.

Rahimi E and Alian F, 2013. Presence of enterotoxigenic staphylococcus aureus in cow, camel, sheep, goat, and buffalo bulk tank milk. Vet Arhiv 83:23-30.

Rana EA, Das T, Dutta, *et al.,* 2020. Coagulase-positive methicillin-resistant Staphylococcus aureus circulating in clinical mastitic goats in Bangladesh. Vet World 13:1303-1307.

Sabir MJ, Ijaz M, Ahmed A, *et al.,* 2024. First report on genotypic estimation of MRSA load in udder of nomadic sheep flocks affected with subclinical mastitis in Pakistan. Res Vet Sci 166:105107.

Thrusfield, M. 2013. Veterinary epidemiology. 3rd Edition. Blackwell Science Ltd; Oxford. 150 p.

Titouche Y, Hakem A, Houali K, *et al.,* 2019. Emergence of methicillin-resistant Staphylococcus aureus (MRSA) ST8 in raw milk and traditional dairy products in the Tizi Ouzou area of Algeria. J Dairy Sci 102:6876-6884.

Torres G, Vargas K, Sánchez-Jiménez M, *et al.,* 2019. Genotypic and phenotypic characterization of biofilm production by Staphylococcus aureus strains isolated from bovine intramammary infections in Colombian dairy farms. Heliyon 5: e02117.

Wu Y, Li J, Qiao M, *et al.,* 2019. Characteristic profiles of biofilm, enterotoxins and virulence of Staphylococcus aureus isolates from dairy cows in Xinjiang Province, China. J Vet Sci 20:1-7.