DOI: https://doi.org/10.54393/fbt.v5i3.184

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 03, (July-Sep, 2025)

Original Article

OPEN ACCESS

Molecular-Based Investigation of Methicillin-Resistant *Staphylococcus Aureus* from Bovine Mastitis in Kasur

Abdul Qadeer Haider[1], Husnain Ali[1], Farooq Ahmad[1], Noor Fatima Tareen[1], Mahnoor Basit[1], Muhammad Naveed Anjum[2] and Numan Javed[1]

[1]Institute of Microbiology and Molecular Genetics, University of Punjab, Lahore, Pakistan
[2]Center of Excellence in Molecular Biology, University of Punjab, Lahore, Pakistan

ARTICLE INFO

Keywords:
Staphylococcus Aureus, Molecular-Based Investigation, Bovine Mastitis, Biochemical Tests

How to Cite:
Haider, A. Q., Ali, H., Ahmad, F., Tareen, N. F., Basit, M., Anjum, M. N., & Javed, N. (2025). Molecular-Based Investigation of Methicillin-Resistant Staphylococcus Aureus from Bovine Mastitis in Kasur: Molecular-Based Investigation of Methicillin-Resistant Staphylococcus Aureus. Futuristic Biotechnology, 5(3), 42-47. https://doi.org/10.54393/fbt.v5i3.184

***Corresponding Author:**
Numan Javed
Institute of Microbiology and Molecular Genetics, University of Punjab, Lahore, Pakistan
numan.mmg@pu.edu.pk

Received Date: 31st July, 2025
Revised Date: 23rd September, 2025
Acceptance Date: 26th September, 2025
Published Date: 30th September, 2025

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant threat to the dairy industry through mastitis, causing substantial economic losses. MRSA is a zoonotic pathogen that transmits between livestock and humans through direct contact, contaminated environments, or animal products. Its prevalence is further exacerbated by inadequate research and the misuse of antibiotics. **Objectives:** To find molecular-based investigation of methicillin-resistant *Staphylococcus aureus* from bovine mastitis in Kasur. **Methods:** This study characterized MRSA isolates from bovine mastitis using biochemical tests and genotypic analysis of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*), which carries the *mec*A gene that makes the bacteria resistant to β-lactam antibiotics. SCC*mec* typing distinguishes hospital-associated (HA-), community-associated (CA-), and livestock-associated (LA-) MRSA strains, the latter (notably CC398) exhibiting rising zoonotic concern. **Results:** Among 100 mastitic milk samples, 85% (85/100) carried *mec*A, confirming MRSA. Alarmingly, 78% (78/100) co-harbored the Panton-Valentine Leukocidin (PVL) gene, a key virulence determinant linked to severe infections in humans. **Conclusions:** The high co-occurrence of *mec*A (mediating multidrug resistance) and PVL (enhancing pathogenicity) in LA-MRSA isolates underscores a critical one health challenge, necessitating urgent interventions in antibiotic resistance and farm hygiene to mitigate transmission risks.

INTRODUCTION

Bovine mastitis, or a mammary gland inflammation, is a major problem affecting the dairy industry in the world because it comes with a decrease in milk production, quality, and the cost of treatment [1]. In countries like Pakistan, where the dairy sector is economically vital, mastitis causes annual losses in the billions due to milk wastage, veterinary expenses, and culling [2]. Staphylococcus aureus, especially methicillin-resistant strains (MRSA), is a leading mastitis pathogen, known for its antibiotic resistance and zoonotic potential. Subclinical infections, poor diagnostics, and unregulated antibiotic use have accelerated the emergence of multidrug-resistant strains on dairy farms [3]. MRSA resistance is primarily driven by the mecA gene, which encodes PBP2a, a penicillin-binding protein with low β-lactam affinity [4]. This gene resides within the SCCmec element, classified by MRSA types: HA-MRSA, CA-MRSA, and livestock-associated MRSA (LA-MRSA) [5]. Another key virulence factor is the *PVL* gene, encoding a cytotoxin that destroys immune cells and promotes tissue damage. While well-documented in human infections, *PVL*-positive MRSA is increasingly found in livestock, including bovine mastitis cases. The co-detection of *mecA* and *PVL* genes signals the emergence of highly virulent, multidrug-resistant strains,

DOI: https://doi.org/10.54393/fbt.v5i3.184

posing significant zoonotic and public health risks [6]. Transmission from infected cattle to humans can occur through direct contact, contaminated environments, or unpasteurized milk. In Pakistan, where veterinary infrastructure and hygiene standards are often lacking, farm workers and consumers are particularly vulnerable. Studies have shown genetic overlap between human and animal MRSA strains, emphasizing the need for One Health–based surveillance and control strategies [7]. Despite growing concerns, MRSA detection in Pakistan's dairy industry remains limited to conventional tools like culturing and CMT, which lack sensitivity and fail to identify key resistance genes. Phenotypic assays may miss genotypic resistance, leading to treatment failures and prolonged infections. In contrast, PCR-based molecular diagnostics offer rapid and precise detection of *mecA* and PVL, guiding more targeted treatments and reducing empirical antibiotic use [8]. This study aims to fill a critical gap in MRSA epidemiology by using PCR to characterize resistance and virulence in bovine mastitis isolates from Pakistan. Beyond laboratory insights, the findings can inform veterinary policies, antibiotic stewardship, and molecular diagnostic adoption. Addressing MRSA's dual threat of antibiotic resistance and virulence requires urgent, integrated responses to protect public health and sustain dairy productivity.

This study aims to find molecular-based investigation of methicillin-resistant *Staphylococcus aureus* from bovine mastitis in Kasur.

METHODS

This study utilized a descriptive cross-sectional design with a laboratory-based component. This study was conducted in the Department of Microbiology at the University of Punjab (May 2022 to April 2023), Lahore, with a focus on understanding the phenotypic and genotypic traits of MRSA in cases of bovine mastitis. The sampling technique was purposive (non-random). Samples were collected specifically from animals showing clinical signs of mastitis or positive California Mastitis Test (CMT) results to ensure the selection of infected individuals for MRSA analysis. This design was appropriate as it characterizes the prevalence, resistance patterns, and genetic profiles of MRSA in a defined population at a single point in time. The analytical methods, including descriptive statistics and correlation, align with this study design. A total of 100 raw milk samples were collected aseptically from mastitic animals in the Kasur district, comprising 65 from cows and 35 from buffaloes. The sample size of 100 was justified based on an expected high MRSA prevalence of approximately 80-90% reported in prior regional studies. This provides a precision (margin of error) of roughly ±8% at a 95% confidence level for prevalence estimates, which was deemed sufficient for this initial investigation. The collection was performed using sterile vials, and the samples were immediately. The samples were immediately transported in an insulated box maintained at 4°C, and processing was completed within 4 hours of collection to preserve bacterial viability. To isolate *Staphylococcus aureus*, each milk sample was cultured on Mannitol Salt Agar (MSA). MSA is both selective and differential: its high salt concentration inhibits the growth of most other bacteria, allowing only halotolerant organisms such as staphylococci to grow. The differential aspect lies in its ability to distinguish *S. aureus* from coagulase-negative staphylococci based on mannitol fermentation. Colonies of *S. aureus* that fermented mannitol turned the medium yellow due to acid production. Presumptive isolates were further confirmed using Gram staining, biochemical tests (catalase, coagulase, DNase), and PCR-based molecular identification targeting the mecA and PVL genes. Following initial screening, isolates were sub-cultured on Blood Agar Plates (BAPs) to assess their hemolytic activity. Hemolysis was categorized as α (partial), β (complete), or γ (no hemolysis). *S. aureus* commonly exhibits β-hemolysis, a useful identifying trait [9]. Presumptive Staphylococcus colonies were subjected to Gram staining. Under microscopic examination, Gram-positive cocci arranged in clusters were suggestive of *Staphylococcus aureus* [10]. Further biochemical confirmation was carried out through: Catalase Test: A drop of hydrogen peroxide (3%) was placed on a glass slide with the bacterial colony. Immediate bubbling indicated the presence of the catalase enzyme, confirming the staphylococcal species. Coagulase Test: A definitive test for *S. aureus*, this was done by mixing the bacterial culture with rabbit plasma. The presence of clot formation within 4 hours indicated coagulase-positive S. All tests were performed in duplicates. aureus. DNase Test: To assess the ability of isolates to produce Deoxyribonuclease, cultures were streaked on DNase agar plates and incubated. Plates were flooded with 1N HCl to precipitate DNA [11]. Clear zones around colonies confirmed DNase activity, supporting *S. aureus* identification. Laboratory Standards Institute) guidelines. The antibiotics used included: Oxacillin (1 µg), Cefoxitin (30 µg), Vancomycin (30 µg) [12]. The bacterial lawn was prepared on Mueller-Hinton Agar (MHA), and disks were placed aseptically. After incubation at 35℃ for 24 hours, zones of inhibition were measured. Resistance to cefoxitin and oxacillin indicated MRSA. The zone diameter interpretive criteria followed CLSI standards: for oxacillin (1 µg), a zone ≤10 mm indicates resistance; for cefoxitin (30 µg), a zone ≤21 mm indicates resistance. Vancomycin susceptibility was interpreted according to CLSI guidelines. Vancomycin-resistant isolates, if any, were noted for further molecular confirmation. Control strains

DOI: https://doi.org/10.54393/fbt.v5i3.184

included S. aureus ATCC 25923 (MSSA) and ATCC 43300 (MRSA) for validating antibiotic testing and PCR. Cefoxitin and oxacillin were used as surrogate markers for the detection of MRSA phenotypically. Isolates showing reduced susceptibility to these antibiotics were selected for genotypic confirmation of the *mecA* gene, a critical determinant of methicillin resistance. Genomic DNA was extracted using the CTAB method with a lysozyme pretreatment step to break the thick peptidoglycan layer of Gram-positive bacteria, ensuring high-quality DNA for downstream PCR. Briefly, bacterial pellets were lysed using lysozyme, followed by treatment with CTAB buffer. Proteins were precipitated using chloroform-isoamyl alcohol, and DNA was precipitated with isopropanol. The DNA was then washed with ethanol and resuspended in TE buffer. DNA quality and quantity were assessed using a NanoDrop spectrophotometer and 1% agarose gel electrophoresis. Only samples with an A260/280 ratio of 1.8–2.0 and an A260/230 ratio >1.8 were used for PCR amplification [12]. The presence of the *mecA* and Panton-Valentine Leukocidin (PVL) genes was confirmed via polymerase chain reaction (PCR) using gene-specific primers. mecA primers: Forward: 5'-AAA ATC GAT GGT AAA GGT TGG C-3', Reverse: 5'-AGT TCT GCA GTA CCG GAT TTG C-3'. PVL primers: Forward: 5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCCA-3', Reverse: 5'-GCA TCA AST GTA TTG GAT AGC AAA AGC-3'. PCR conditions: Initial denaturation at 94°C for 5 minutes. 30 cycles of: Denaturation at 94°C for 30 seconds, Annealing at 55°C (mecA) / 57°C (PVL) for 30 seconds, Extension at 72°C for 30 seconds, Final extension at 72°C for 5 minutes. PCR products were analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. PCR bands (mecA ~533 bp, PVL ~433 bp) were confirmed using a MRSA-positive control, and representative products were sequenced for validation. In this study, statistical analyses were carried out using IBM SPSS Statistics version 25. The prevalence of MRSA was determined by expressing the number of *mecA*-positive isolates as a percentage of the total samples. Inhibition zone diameters from the disk diffusion assays for oxacillin, cefoxitin, and vancomycin were summarized using descriptive statistics, calculating means and standard deviations to capture central tendency and dispersion. The data analysis plan was explicitly tailored to meet the study's objectives and handle the specific types of data collected. Descriptive statistics characterized the basic features of the data, the Chi-square test assessed crucial categorical associations, the independent t-test compared means between two animal species, and Pearson's correlation quantified relationships between key continuous variables, providing a comprehensive analytical approach. To evaluate the association between phenotypic resistance (disk diffusion results) and genotypic confirmation (mecA presence), a Chi-square test of independence was employed, with $p<0.05$ indicating statistical significance. Differences in inhibition zone diameters between cow- and buffalo-derived isolates were compared using a one-way ANOVA. Finally, Pearson's correlation coefficient (r) was calculated, with r = 0–0.3 considered weak, 0.3–0.6 moderate, and 0.6–1.0 strong correlation. "Finally, Pearson's correlation coefficient (r) was calculated to assess the strength and direction of the relationship between *mecA* and PVL gene carriage and between antibiotic inhibition zones. The strength of correlation was interpreted using the following thresholds: |r| = 0.00–0.30 was considered weak, |r| = 0.31–0.60 moderate, and |r| = 0.61–1.00 strong." to assess the strength and direction of the relationship between *mecA* and PVL gene carriage. All microbiological work was performed in a Biosafety Level 2 laboratory under sterile conditions. Positive and negative controls were run with each PCR batch to ensure validity. Autoclaved materials and proper aseptic techniques were employed throughout the study.

RESULTS

Although 100 milk samples were collected for MRSA analysis, these were obtained from 104 animals because some animals contributed samples from multiple udders. Mastitis was detected in 103/104 (99%) of animals. Herds were generally small-to-medium (2–5 animals; 85%), with infrequent milking (0–2 times/day) and limited udder sampling (1–3 udders/animal). Hygiene practices were poor: only 19/104 (18.3%) of samples reflected proper milker hand hygiene, and 28/104 (26.9%) were collected under hygienic milking conditions. Weak correlations between hygiene and mastitis positivity ($r \approx 0.05$–0.06) suggest that current practices are insufficient to control infection. Phenotypic resistance profiling confirmed universal oxacillin resistance (0mm, median; range 0–2mm) in all 100 isolates. Cefoxitin resistance was observed in 60% of isolates (mean inhibition zone: 15.3 ± 3.2mm; range 10–22 mm), while 51% showed vancomycin resistance (mean: 19.77 ± 5.89mm; range 12–28mm). Vancomycin showed variable susceptibility (median zone: 21 mm), contrasting with minimal inhibition for oxacillin/cefoxitin (median: 0 mm). A moderate correlation existed between cefoxitin and vancomycin responses ($r > 0.4$), while oxacillin resistance was independent. Correlation of the surf field mastitis test with sampling parameters like herd distribution, Udder number, Frequency of lactation, Herd size, and hygiene graphs is made using pandas and Matplotlib libraries for statistical analysis (Figure 1).

DOI: https://doi.org/10.54393/fbt.v5i3.184

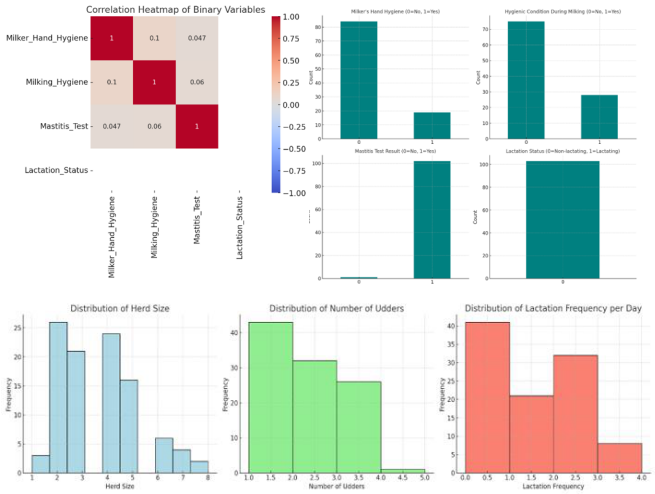

Figure 1: Correlation of Surf Field Mastitis Test, with Sampling Parameters Like Herd Distribution, Udder Number, Frequency of Lactation

Genotypic analysis detected the *mecA* gene (encoding PBP2a) in 85% (85/100) of MRSA isolates, confirming β-lactam resistance. The remaining 15% (15/100) *mecA*-negative MRSA implies alternative resistance mechanisms (e.g., modified PBPs). The virulence-associated PVL gene was prevalent in 78% (78/100) of isolates. Strain profiling revealed significant divergence: HL-SK exhibited hyper-virulence with robust mecA/PVL co-occurrence and high enzymatic activity (catalase: 27/30, DNase: 22/30, coagulase: 18/30). In contrast, HL-DS and HL-PK showed minimal enzymatic/genotypic virulence. Strains like HL-KK (high *mecA*, low PVL) suggested Biochemical heterogeneity was evident, with catalase/DNase activity ranging from high (HL-DH, HL-KK, HL-KP) to low (HL-PK, HL-DS)(Figure 2).

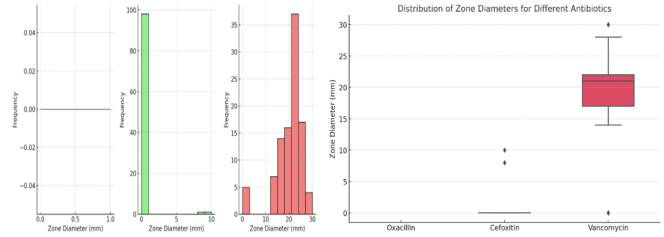

Figure 2: Correlation of Oxacillin, Cefoxitin, and Vancomycin Distribution among MRSA Strains

The convergence of widespread *mecA*-mediated resistance (85%), high PVL virulence (78%), and deficient on-farm hygiene underscores significant zoonotic risks, particularly from hyper-virulent strains like HL-SK in dairy settings. Vancomycin resistance (51%) further threatens last-line therapy efficacy (Figure 3).

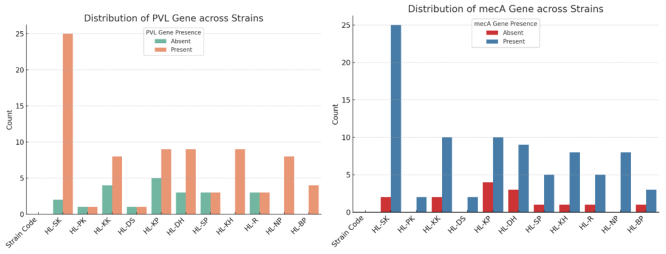

Figure 3: Distribution of mecA and PVL Gene among MRSA Samples

DISCUSSION

This study offers an in-depth analysis of antimicrobial resistance (AMR) patterns in *Staphylococcus aureus*, specifically targeting MRSA strains isolated from bovine mastitis milk samples [13, 14]. The resistance prevalence data will be revised to include 95% confidence intervals (95% CI) for all percentage estimates. For example, the prevalence of the **mecA** gene will be reported as 85% (95% CI: 76.2% - 91.1%). This addition will provide a measure of the precision and reliability of the prevalence estimates reported in the study [15, 16]. Complete resistance to oxacillin and notable resistance to cefoxitin and vancomycin reflect the growing clinical and zoonotic risks posed by these strains in livestock environments. Universal phenotypic resistance to oxacillin validates the MRSA classification of all isolates. However, only 85% harbored the *mecA* gene, indicating a possible divergence between phenotypic and genotypic resistance. This suggests the presence of alternative resistance mechanisms such as PBP mutations, efflux pumps, or epigenetic modifications that enable methicillin resistance without *mecA* involvement [17]. Alterations in native PBPs, such as PBP2, can reduce affinity for β-lactam antibiotics, leading to resistance without the presence of *mecA* [18]. The Discrepancy underlines the necessity of using combined diagnostic approaches rather than relying solely on genetic markers. Cefoxitin resistance in 60% of isolates, typically a reliable marker for *mecA* presence, supports its diagnostic value, though 40% of isolates remained susceptible despite being phenotypically resistant to oxacillin. This may reflect suppressed *mecA* expression or phenotypic heterogeneity. Vancomycin resistance in 51% isolates is especially alarming, as it compromises one of the last-resort antibiotics for MRSA [12]. Such resistance indicates the emergence of VISA/VRSA strains, presenting a direct threat to veterinary and public health. The molecular detection of virulent genes further clarifies the pathogenic profile of these isolates. The *mecA* gene, encoding the penicillin-binding protein PBP2a, was prevalent in 85% of strains, while 78% carried the PVL gene, a bicomponent leukotoxin associated with increased necrosis and immune system evasion (Itodo, 2023). The co-presence of mecA and PVL genes, particularly in strains like HL-SK, suggests

DOI: https://doi.org/10.54393/fbt.v5i3.184

simultaneous selection of resistance and virulence traits, potentially increasing pathogenicity and complicating treatment, although direct clinical outcome data were not assessed in this study. Biochemical testing supported the genotypic data, with HL-SK exhibiting strong enzymatic activity and maximum *mecA/PVL* expression, classifying it as a hypervirulent MRSA variant. In contrast, HL-DS and HL-PK showed low enzymatic and genotypic virulence. These observations suggest a potential role for biochemical assays in screening strain virulence and resistance levels. Epidemiological findings contextualized these results, revealing a nearly universal mastitis prevalence in herds despite partial hygiene measures. Weak correlations between hygiene practices and mastitis incidence suggest that current efforts are inadequate or inconsistently applied [19, 20]. Poor milk-hand hygiene and unsanitary environments likely contribute to persistent transmission of resistant strains. Biofilm formation was not assessed in this study, but it is recognized as a key factor in bacterial persistence and antimicrobial resistance in dairy settings. Resistance profiling through statistical and graphical analysis revealed distinct susceptibility patterns. Cefoxitin and oxacillin showed narrow, compressed inhibition zones reflecting widespread resistance. In contrast, vancomycin displayed a broader range, indicating mixed susceptibility. Heatmap correlations showed moderate linkage between cefoxitin and vancomycin resistance, implying possible shared regulatory pathways [10]. Oxacillin resistance, with minimal correlation to other antibiotics, suggests independent or unique resistance mechanisms. These insights emphasize the need for comprehensive AMR surveillance, multi-target diagnostics, and enhanced farm biosecurity to control hypervirulent MRSA in dairy settings.

CONCLUSION

This study on MRSA from bovine mastitis cases in Kasur highlights a complex antimicrobial resistance profile that necessitates integrated control strategies. All 100 isolates showed phenotypic resistance to oxacillin, confirming widespread MRSA prevalence. However, only 85% carried the *mecA* gene, suggesting alternative resistance mechanisms such as beta-lactamase overproduction or mutations in penicillin-binding proteins. Cefoxitin resistance in 60% of isolates reinforces its diagnostic relevance, though the remaining 40% may involve heteroresistance or suppressed *mecA* expression. Vancomycin resistance in 51% of isolates raises serious concerns, indicating the emergence of VISA/VRSA strains in livestock. The PVL gene, detected in 78% of isolates, points to significant virulence potential, particularly in co-*mecA/PVL* positive strains like HL-SK, which also exhibited peak enzymatic activity, marking it as hypervirulent. Epidemiological data showed generally poor farm hygiene, including limited handwashing and unsanitary milking practices, which were only weakly associated with mastitis prevalence.

Authors Contribution

Conceptualization: AQH
Methodology: HA, NJ
Formal analysis: NFT, MB
Writing review and editing: AQH, FA, MNA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Reshi AA, Husain I, Bhat SA, Rehman MU, Razak R, Bilal S *et al.* Bovine Mastitis as an Evolving Disease and Its Impact on the Dairy Industry. International Journal of Current Research and Review. 2015 Mar; 7(5): 48.

[2] Malik MH. Economic Losses Due to Selected Diseases of Dairy Animals in Punjab (Doctoral Dissertation, PhD, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India). 2018.

[3] Otenga FO. Risk Factors of Sub-Clinical Mastitis, Antibiogram and Genotypic Analysis of Staphylococcus Spp. And Enterobacteria Resistant Bacteria Isolated from Humans and Lactating Dairy Cows from Small-Holder Farms in Gatundu Sub-County, Kenya (Doctoral dissertation, JKUAT-COPAS). 2023. doi: 10.18203/2394-6040.ijcmph20222897.

[4] Lade H and Kim JS. Molecular Determinants of β-Lactam Resistance in Methicillin-Resistant Staphylococcus Aureus (MRSA): An Updated Review. Antibiotics. 2023 Aug; 12(9): 1362. doi: 10.3390/antibiotics12091362.

[5] Ghodasara SN. Phenotypic and Molecular Characterization of Staphylococcal Cassette Chromosome Mec (Sccmec) Types of Methicillin-Resistant Staphylococci from Animal and Human Origin. 2018. doi: 10.20546/ijcmas.2019.810.107.

[6] Adeyemi FM, Oyedara OO, Yusuf-Omoloye NA, Ajigbewu OH, Ndaji OL, Adegbite-Badmus MK *et al.* Guardians of Resistance and Virulence: Detection of mec, femA, Van, pvl, hlg and spa genes in Methicillin and Vancomycin-Resistant Staphylococcus Aureus

DOI: https://doi.org/10.54393/fbt.v5i3.184

from Clinical and Food Samples in Southwestern Nigeria. BioMed Central Microbiology. 2024 Nov; 24(1): 498. doi: 10.1186/s12866-024-03660-3.

[7] Ali S, Tahir MW, Sultan A, Naseem MA, Habib MS, Qayyum HM *et al*. Methicillin-Resistant Staphylococcus Aureus (MRSA) and Its Intersection with Animals. Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan. 2023; 4: 163-71. doi: 10.47278/book.zoon/2023.145.

[8] Banerjee R and Patel R. Molecular Diagnostics for Genotypic Detection of Antibiotic Resistance: Current Landscape and Future Directions. Journal of Antimicrobial Chemotherapy-Antimicrobial Resistance. 2023 Feb; 5(1): dlad018. doi: 10.1093/jacamr/dlad018.

[9] Chakrabortty T. Isolation, Identification and Antibiogram Profiling of Staphylococcus Aureus from Fresh Cow Milk Sample (Doctoral Dissertation, Department of Microbiology and Parasitology, Sher-E-Bangla Agricultural University, DHAKA-1207). 2019.

[10] COCCI CF. Staphylococcus: Cluster-Forming Gram-Positive Cocci Jagriti Bansal. Bacterial Enemies of Human Health. 2025 Mar: 234.

[11] Farheen S. Isolation of Methicillin-resistant Staphylococcus aureus from the environmental samples in Dhaka City (Doctoral dissertation, BRAC University). 2017.

[12] Hanumantharaju MV. Comparison of Cefoxitin, Oxacillin Disc Diffusion Test with mecA Gene Detection Method Along with Demonstration of Vancomycin Resistance Among the MRSA Isolates at a Tertiary Care Hospital (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)). 2014.

[13] Nelli A, Voidarou C, Venardou B, Fotou K, Tsinas A, Bonos E *et al*. Antimicrobial and Methicillin Resistance Pattern of Potential Mastitis-Inducing Staphylococcus Aureus and Coagulase-Negative Staphylococci Isolates from the Mammary Secretion of Dairy Goats. Biology. 2022 Oct; 11(11): 1591. doi: 10.3390/biology11111591.

[14] Touaitia R, Ibrahim NA, Touati A, Idres T. Staphylococcus Aureus in Bovine Mastitis: A Narrative Review of Prevalence, Antimicrobial Resistance, and Advances in Detection Strategies. Antibiotics. 2025 Aug; 14(8): 810. doi: 10.3390/antibiotics14080810.

[15] Neri TA, Park H, Kang S, Baek SH, Nam IS. Comparative Antimicrobial Resistance and Prevalence of Methicillin Resistance in Coagulase-Positive Staphylococci from Conventional and Organic Dairy Farms in South Korea. Antibiotics. 2024 Jul; 13(7): 617. doi: 10.3390/antibiotics13070617.

[16] Neri TA, Park H, Kang S, Baek SH, Nam I. Antimicrobial Resistance and Prevalence of Methicillin-resistant Staphylococcus Aureus in Bovine Mastitis Milk from Conventional and Organic Dairy Farms in South Korea. 2023 Jun. doi: 10.20944/preprints202306.1843.v1.

[17] Miragaia M. Factors Contributing to the Evolution of Meca-Mediated B-Lactam Resistance in Staphylococci: Update and New Insights from Whole Genome Sequencing (WGS). Frontiers in Microbiology. 2018 Nov; 9: 2723. doi: 10.3389/fmicb.2018.02723.

[18] Gitman MR, Alburquerque B, Chung M, Van de Guchte A, Sullivan MJ, Obla A *et al*. Modified Methicillin-Resistant Staphylococcus Aureus Detected in Neonatal Intensive Care Patients. Journal of Antimicrobial Chemotherapy. 2021 Nov; 76(11): 2774-7. doi: 10.1093/jac/dkab266.

[19] Khanal S, Boonyayatra S, Awaiwanont N. Prevalence of Methicillin-Resistant Staphylococcus Aureus in Dairy Farms: A Systematic Review and Meta-Analysis. Frontiers in Veterinary Science. 2022 Dec; 9: 947154. doi: 10.3389/fvets.2022.947154.

[20] ALI D. Phenotypic and Genotypic Characterization of Methicillin Sensitive and Resistant Staphylococcus Aureus (MSSA and MRSA) Isolated from Bovine Mastitis. 2020.