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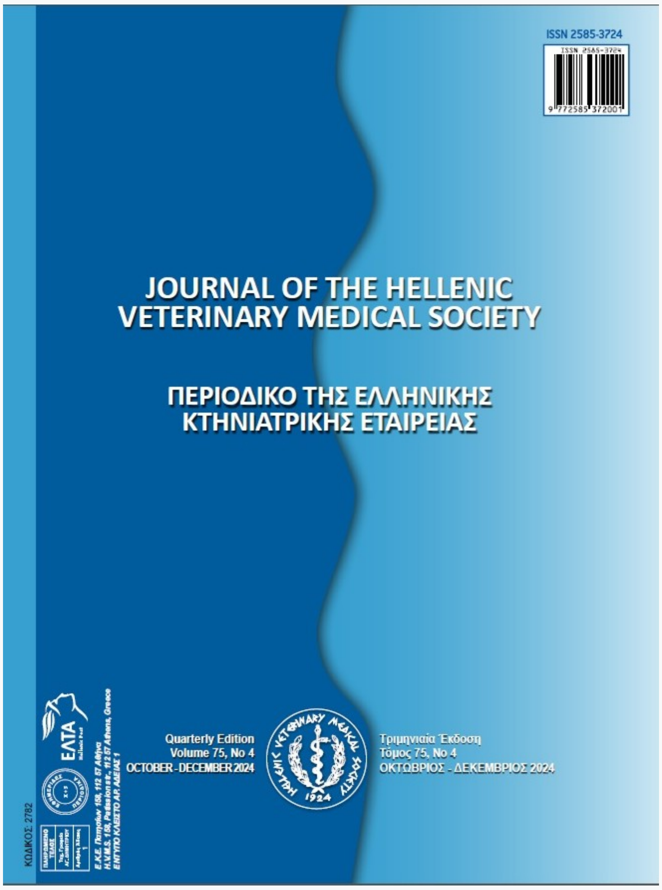

Detection of Staphylococcal Enterotoxin, Methicillin-Resistant, Panton- Valentine Leukocidin Genes and Antibiotic Susceptibility in Staphylococci and Current Bacteria Associated with Subclinical Mastitis in Cattle

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Detection of Staphylococcal Enterotoxin, Methicillin-Resistant, Panton-Valentine Leukocidin Genes and Antibiotic Susceptibility in Staphylococci and Current Bacteria Associated with Subclinical Mastitis in Cattle

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ABSTRACT: Bovine mastitis is a significant infectious disease affecting dairy animals, resulting in substantial economic losses. Staphylococci are the predominant causative agents of mastitis. This study aimed to identify the bacteria isolated from cow's milk with subclinical mastitis using MALDI-TOF mass spectrometry, assess their antibiotic susceptibility, and examine the presence of staphylococcal enterotoxin, *pvl* (Panton-Valentine Leukocidin), and *mec*A genes in *Staphylococcus* spp. Out of 301 milk samples, 73 isolates were identified as bacteria, with 40 (54.8%) being staphylococci. The antibiotic resistance rates of staphylococci were as follow: benzylpenicillin 57.5% (n:23), tetracycline 22.5% (n:9), erythromycin 17.5% (n:7), gentamicin 12.5% (n:5), cefoxitin 10.0% (n:4), ciprofloxacin 7.5% (n:3), tigecycline 7.5 % (n:3), trimethoprim/sulfamethoxazole 5.0% (n:2), and rifampicin 2.5% (n:1). Among the *Staphylococcus haemolyticus* isolates, 5 (11.62%) carried the *mec*A gene, and interestingly, 3 of these were phenotypically sensitive to cefoxitin despite harboring the *mec*A gene. However, none of the isolates carried the enterotoxin and *pvl* genes. This study was emphasized that *mec*A, staphylococcal enterotoxin and *pvl* genes were not found in staphylococci isolated from subclinical cows with mastitis.

Keywords: Subclinical mastitis; staphylococci; Panton-Valentine Leukocidin; methicillin resistance; animal health

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INTRODUCTION

Mastitis, an inflammation of the udder gland, is a pervasive issue in dairy herds worldwide. It manifests in two primary forms, clinical and subclinical, each with distinct characteristics. Clinical mastitis is characterized by noticeable systemic changes in milk, the mammary gland, or even the cow's overall health. In contrast, subclinical mastitis presents with no discernible symptoms in milk or the mammary gland, yet it leads to reduced milk production and altered milk composition (Erskine, 2020).

Subclinical mastitis (SCM) is notably more prevalent than clinical mastitis and is believed to inflict more substantial economic losses on the dairy industry (Paramasivam et al., 2023). Among the bacterial pathogens responsible for mastitis, *Staphylococcus aureus,* Coagulase-Negative Staphylococci (CNS), *Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus agalactiae,* and *Escherichia coli* are the most frequently identified agents (Ali et al., 2021). Of these, *Staphylococcus* species, particularly *Staphylococcus aureus* (*S. aureus*), stand out as major contributors to bovine mastitis (BM), inflicting considerable economic losses on dairy farms and posing public health concerns (Ünal et al., 2012; Ünal and Cinar, 2012; Yang et al., 2023).

Although CNS are often considered smaller pathogens in comparison to *S. aureus*, recent research highlights their substantial role in subclinical mastitis in ruminants (Deng et al., 2023; Raspanti et al., 2016; Ünal et al., 2012). Understanding the prevalence and significance of these bacterial pathogens is crucial for effective mastitis control strategies, emphasizing the need for ongoing research and surveillance in both the dairy industry and public health sectors.

The widespread use of antibiotic for the prevention and treatment of mastitis in dairy cows, contributing to the emergence and dissemination of antibiotic-resistant bacteria, notably methicillin-resistant *Staphylococcus aureus* (MRSA). Methicillin-resistance arises due to the acquisition of the *mec*A gene, which encodes an alternative penicillin-binding protein, PBP2a (or PBP2'), which has a low affinity for most β-lactam antibiotics (Morgan, 2008). Epidemiological evidence suggests horizontal transfer of resistance, such as methicillin, to initially susceptible *S. aureus* strains (Harkins et al., 2017; Lee et al., 2018). MRSA is a significant concern for nosocomial infections, foodborne illnesses, and post-surgical infections worldwide, with dairy cows considered potential reservoirs for these resistant bacteria (Kulangara et al., 2017).

Staphylococcal enterotoxins (SEs), including classical (SEA, SEB, SEC, SED, and SEE) and novel SEs (SEG, SEH, and SEI), along with staphylococcal enterotoxin-like (SElJ, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SElR, SElU, and SElV), play a pivotal role in staphylococcal food poisoning, one of the most common foodborne illnesses in humans (Wang, 2009).

Panton-Valentine leukocidin (PVL), a β-pore-forming toxin associated with leukocyte destruction and tissue damage, enhances the virulence of *Staphylococcus* spp. While the *pvl* gene rapidly spreads among human staphylococci, its sporadic presence has been noted in staphylococci isolated from cows with subclinical mastitis (Unal et al., 1992; Ünal et al., 2012; Ünal and Cinar, 2012; Kulangara et al., 2017; Şeker et al., 2019).

In conclusion, the detection of virulence and *mec*A genes in bovine milk poses potential risks to public, animal, and environmental health, aligning with the 'One Health' approach. This study aimed to use MALDI-TOF for the identification of bacteria isolated from cow's milk with subclinical mastitis, assess their antibiotic susceptibility, and investigate the presence of staphylococcal enterotoxin and *pvl* genes in *Staphylococcus* spp., providing insights into the multifaced aspects of this critical issue.

MATERIALS AND METHODS

Bacterial strains

Between May and August 2022, a comprehensive study was conducted across three distinct dairy farms in Ankara. A total of 301 milk samples were meticulously collected from 78 cows and transported to our laboratory for analysis. Each milk sample was processed by inoculating ten microliters onto blood agar plates (Merck, Germany), with subsequent aerobic incubation at 37°C for 24 hours. The infection status of the samples was determined using protocols recommended by the National Mastitis Council.

Bacterial colonies were identified based on Gram staining, morphological characteristics, hemolysis patterns, and additional tests including tube coagulase, catalase, and oxidase. Furthermore, the identification of bacterial strains was refined using MALDI-TOF mass spectrometry. All identified isolates were preserved in brain heart infusion broth supplemented with 15% glycerol at -20°C, awaiting further analysis and investigation (Merck, Germany).

Antimicrobial susceptibility test of *Staphylococcus* spp.

In this study, 40 *Staphylococcus* spp. strains, comprising 4 *Staphylococcus aureus* and 36 coagulase-negative staphylococci (CNS), were cultivated from 24-hour fresh cultures. Suspensions with McFarland 0.5 turbidity in sterile saline (0.85% NaCl) were prepared from these strains and evenly spread on Mueller Hinton agar medium using sterile swabs. Susceptibility testing for *Staphylococcus* spp. species was performed against a panel of antibiotics, including benzylpenicillin (1U), gentamicin (10 µg), erythromycin (15 µg), rifampicin (5 µg), cefoxitin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.7 µg), tetracycline (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), and tigecycline (15 µg) using a disc diffusion method. The resulting inhibition zones were meticulously measured and evaluated in accordance with EUCAST standards following the incubation period. To ensure the quality control of the testing procedure, *Staphylococcus aureus* ATCC 29213.

Genomic DNA Extraction

In preparation for PCR analysis, the DNA of *Staphylococcus* spp. was extracted following a protocol. Initially, fresh bacterial suspension of the isolates were subjected to centrifugation at 5,000 rpm for 10 minutes. The resulting cell pellets were subsequently washed twice in 1 ml of TE buffer (comprising 10 mM Tris-HCl and 1 mM EDTA, pH 8.0). For DNA extraction, a stepwise process was employed. Initially, 50 microliters of lysostaphin enzyme (100 µg/ml) were added to the cell pellet, followed by the addition of 50 µl of proteinase K enzyme (100 µg/ml). This mixture was then incubated for 10 minutes at a time at 37°C each step. Finally, to inactivate proteinase K, the sample was heated at 100°C for 10 minutes. The extracted DNA samples were then stored at -20°C for subsequent use (Unal et al., 1992).

Detection of Staphylococcal enterotoxins, *mec*A and *pvl* genes

In this study, we use employed a protocol for PCR amplification to detect the presence of genes, including staphylococcal *sea*, *seb, sec, sed, see, seg, seh, sei, sej* enterotoxin genes, the 16S rRNA gene, the *mec*A gene, and the *pvl* gene. The primer sequences used for these genes were based on previous literature references (Monday and Bohach, 1999; Lovseth et al., 2004; Malik et al., 2006; Lina et al., 1999). For the amplification of the *mec*A and *pvl* genes, a PCR mix was prepared, consisting of 1x Taq polymerase buffer, 4 mM MgCl2, 1U Taq polymerase, 400 µM deoxynucleoside triphosphate each, and 300 nM of each primer. To this mix, 5 µl of the DNA sample was added to initiate the PCR amplification. The amplification program included an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 55°C for 30 seconds, 72°C for 1 minute for extension, and a final extension step at 72°C for 5 minutes.

To differentiate PCR products of certain enterotoxin genes with similar sizes, ten primers were divided into two groups: reaction mixture 1 included primers for *sea*, *seb*-*sec*, *sec*, *seh*, and *sej*, while reaction mixture 2 contained primers for *sed*, *see*, *seg*, *sei*, and 16S rRNA. Multiplex-PCR (m-PCR) amplification was performed by adding 5 µl of DNA to 45 µl of PCR mix, which included 1x Taq polymerase buffer, 4 mM MgCl2, 400 µM deoxynucleoside triphosphate each, 300 nM of each primer, and 2 U of Taq polymerase. The m-PCR program consisted of an initial DNA denaturation at 95°C for 10 minutes, followed by 30 cycles of 95°C for 1 minute, 64°C for 45 seconds, 72°C for 1 minute, and a final extension at 72°C for 1 minute using a thermocycler (amplified using TC-PRO/Boeco).

To visualize the amplified PCR products, agarose gel electrophoresis (1.5%) was conducted at 100 V for 100 minutes, followed by UV transillumination (Syngene G:Box). The sizes of the PCR products were determined relative to a 100-bp marker (Vivantis, NL0401).

In this study, reference strains of *S. aureus*, including D4508 (*sea*, *seh*), FRI913 (*see*), RIMD 31092 (*seb*, *seg*, *sei*), NTCC9393 (*sej*, *sed*, *seg*, *sei*), FRI137 (*sec*, *seh*), and ATCC 49775 (*pvl*), as well as *S. aureus* 43300 (*mecA*), were utilized for quality control and validation purposes.

RESULTS

A total of 301 milk samples were collected from 78 animals, resulting in the isolation of 73 bacterial strains from 36 animals across 62 udder lobes. The identification of these bacterial strains was carried out by MALDI-TOF mass spectrometry, revealing a diverse array of species. Among the isolates, 4 were identified as *Staphylococcus aureus*, 14 as *Staphylococcus haemolyticus*, 9 as *Staphylococcus chromogenes*, 9 as *Staphylococcus epidermidis*, 2 as *Staphylococcus borealis*, 2 as *Staphylococcus hominis*, 11 as *Aerococcus viridans*, 3 as *Streptococcus agalactiae*, 2 as *Streptococcus dysgalactiae*, 5 as *Streptococcus uberis*, 6 as *Acinetobacter* spp., and 4 as other bacterial species (Table 1).

Antimicrobial susceptibility test of *Staphylococcus* spp.

In this study, 40 *Staphylococcus* spp. to susceptibility for tested antibiotics was determined by disc diffusion method. Thirty-four of a totally 40 isolates were determined to be resistant against one or more antibiotics. Cefoxitin discs were used for phenotypic detection of MRSA strains. Three *S. haemolyticus* and one *S. chromogones* isolates have phenotypic cefoxitin resistant in this study were determined. The resistance rates to *Staphylococcus* spp. isolates to benzylpenicillin, tetracycline, erythromycin, gentamicin, cefoxitin, ciprofloxacin, tigecycline, trimethoprim/sulfamethoxazole and rifampicin were found as 57.5% (n:23), 22.5% (n:9), 17.5% (n:7), 12.5% (n:5), 10.0% (n:4), 7.5% (n:3), 7.5 % (n:3), 5.0% (n:2), and 2.5% (n:1) respectively (Table 2). Enrofloxacin was effective against all staphylococci. Besides thirteen isolates were be susceptible all antibioticts in this study. Multi-drug resistance profile was observed in fourteen isolates (2 and/or more), with one *S. chromogenes* isolate resistant to all eight antibiotics.

Exotoxins profiles of *Staphylococcus* spp.

In this study, none of the isolates harbored enterotoxin and *pvl* genes. All isotes were harbored 16S RNA gene. A total of 5 (11.62%) staphylococci isolates were harboring the *mec*A gene. All staphylococci carrying the *mec*A gene were *S. haemolyticus*. 2 *S. haemolyticus* isolates were phenotypically resistant to cefoxitin and carried the *mec*A gene.

DISCUSSION

Subclinical mastitis stands as a significant concern within the dairy industry, incurring substantial economic losses worldwide, as highlighted by recent studies (Abed et al., 2021). In this current investigation, we observed a prevalence rate of 43.59% (34/78)

Table 1. Bacteria isolated in the study

Identified bacteria by MALDITOFF	Number of isolates	Prevalance %
S.aureus	4	5,47
Coagulase negative *Staphylococcus* spp.		
S.borealis	2	2,73
S.chromogenes	9	12,32
S.epidermidis	9	12,32
S.haemolyticus	14	19,17
S.hominis	2	2,73
Catalase negative coccus		
Aeroccoccus viridans	11	15,06
Enterococcus casselifilavus	1	1,36
Enterococcus faecium	1	1,36
Streptococcus agalactiae	3	4,1
Streptococcus dysgalactiae	2	2,73
Streptococcus uberis	5	6,84
Acinetobacter spp.		
Acinetobacter johnsonii	1	1,36
Acinetobacter indicus	1	1,36
Acinetobacter pseudowoffii	2	2,73
Acinetobacter towneri	2	2,73
Others		
Clostridium cochlearium	1	1,36
Bacillus licheniformis	1	1,36
Corynebacterium amycolatum	1	1,36
Corynebacterium xerosis	1	1,36
Total	73	100

Table 2. Levels of resistance to tested antibiotics in *Staphylococcus* spp.

Antibiotics	Resistant (%)	Susceptible (%)
Benzylpenicillin	23 (57,5)	17 (42,5)
Tetracycline	9 (22,5)	31 (77,5)
Erythromycin	7 (17,5)	33 (82,5)
Gentamicin	5 (12,5)	35 (87,5)
Cefoxitin	4 (10)	36 (90)
Ciprofloxacin	3 (7,5)	37 (92,5)
Tigecycline	3 (7,5)	37 (92,5)
Trimethoptim/ sulfametoxazole	2 (5)	35 (95)
Rifampicin	1 (2,5)	39 (97,5)
Enrofloxacin	0 (0)	40 (100)

for subclinical mastitis, consistent with the range reported in the literature (Abed et al., 2021; Ibrahim et al., 2023; Patel et al., 2023), which underscores the persistent challenge posed by this condition.

More than 135 distinct microbial pathogens have been identified as potential contributors to mastitis, including notable culprits such as *Staphylococcus aureus*, CNS, *Streptococcus* spp. (particularly *S. agalactiae* and *S. dysgalactiae*), and *Escherichia coli* (Constable et al., 2017). In our study, we identified a similar pattern of bacterial pathogens, with CNS being the most frequently isolated agents, followed by *Aerococcus viridans*, *Streptococcus* spp., *Acinetobacter* spp., and *S. aureus*. Prevalence of *S. aureus* was 6.06 % in this study. In addition, *A. viridans* isolates reported in the current literatures were found at a rate of 16.67% in this study (Song et al., 2020; Sun et al., 2017). In conclusion, CNS are still the most frequently isolated pathogens, while the prevalences of *A.viridans* and *Acinetobacter* spp. are increasing. *A. viridans* and *Acinetobacter* spp. are becoming an important cause of subclinical bovine mastitis similar to Song et al. (2020) reported. In this study, 49.32 % (36 of them) of 73 bacteria isolated from the milk of cows with subclinical mastitis were identified as non-coagulase *Staphylococcus* spp. Interestingly, we observed a low prevalence of *S. aureus* and the absence of *E. coli*, potentially attributed to improved milking hygiene practices and the utilization of milking machines. This bacterial analysis provides insights into the diverse microbiological landscape of cow's milk affected by subclinical mastitis. Understanding the distribution of these bacterial agents is crucial for the development of effective management and control strategies to mitigate the economic losses associated with this condition in dairy cattle.

Furthermore, we examined revealed varying degrees of resistance, with penicillin resistance being the most prominent, consistent with previous findings (Yilmaz and Şeker, 2022). Importantly, a substantial portion of staphylococci strains displayed resistance to at least one antibiotic (85%), with 42.5% exhibiting resistance to two or more antibiotics. These findings underscore the global challenge of antibiotic resistance and the importance of prudent antibiotic use in veterinary medicine.

Antibiotic resistance crisis has become a rising problem as a result of the global spread of resistant bacteria. Antibiotics are predominantly administration for control of udder diseases by local and/or systemic. In this study, the resistance of *Staphylococcus* species to the antibiotics tested ranged from 57.5% to 2.5%. The penicillin resistance to *Staphylococcus* spp. isolates of our current study closely proximate with the findings of Yilmaz and Şeker (2022), but slightly lower than the findings of Demil et al. (2022) and Bentayeb et al. (2023). Penicillin generally showed the highest rate of resistance in most studies, but rates of resistance to other antibiotics varied. Additionally, antimicrobial susceptibility testing showed that 85% staphylococci strains were resistant to at least one antibiotic, 42.5% to two or more antibiotics. Other studies detecting multiple resistance to antibiotics in staphylococci isolates declerated that Algeria, South Africa, Bangladesh, and Canada showed MDR rates of 77.0%, 51.0%, 49.0%, and 15.0%, respectively (Hoque et al., 2018; Phophi et al., 2019; Saini et al., 2012). Differences in antibiotic resistance in mastitis-derived staphylococci may differ depending on regions, herds within the same region, and the origin of the samples taken (Hoque et al., 2018).

Methicillin-resistant staphylococci remain an important health problem for the environment, human and animal health. *mec*A-positive staphylococci are resistant to penicillins, penems, carbapenems, and cephalosporins, complicating therapy if linked with infections (Chambers, 1997). Five CNS were found to have the *mec*A gene in our study. All these isolates were *S. haemolyticus* (11.62%). In addition, cefoxitin resistance was determined phenotypically in two of the isolates. The same findings were reported by Ünal and Cinar (2012) and 2 of the isolates carrying *mec*A were found to be *S. haemolyticus*. *mec*A carriage rates in isolates other than MRSA are typically low, which is corroborated by certain investigations in the literature (Bal et al., 2010; Xu et al., 2015). Although few *S. aureus* (4) isolates were studied in our current analysis, none of them had the *mec*A gene. Similar results were obtained by Şeker et al. (2019), Ren et al. (2020) and Munive Nuñez et al. (2023) who did not identify the *mec*A gene in *S. aureus*. Contrary to these studies, Singh et al. (2023) reported a higher rate of *S. aureus* carrying *mec*A from bovine suffering with mastitis.

Staphylococcus species carrying virulence, enterotoxins (*sea, seb, sec, sed, see, seg, seh, sei*) and cytotoxin (*pvl*) genes are very important in the pathogenicity of infections in humans and animals. No isolates in this study carried the enterotoxin and *pvl* genes. Contrary to this study, it has been stated in the literature that the *pvl* gene is carried at a rate of up to 3-47% in *Staphylococcus* spp. (Ünal, 2013; Roshan et al., 2022; Rossi et al., 2019; Ünal et al., 2012; Ünal and Cinar, 2012).

CONCLUSION

Staphylococci are still among the important pathogens in subclinical bovine mastiti*s*. CNS were isolated more frequently than *S. aureus,* and the prevalences of *A. viridans* and *Acinetobacter* spp. were increased in milks with subclinical mastitis. Beside, staphylococcal enterotoxin genes and *pvl* gene were not detected among isolated staphylococci strains. This was an extremely positive outcome for both animal and human health. This study showed that bacteria species causing subclinical mastitis in cows, antibiotic resistance profiles and *se* and *pvl* genes carriers of staphylococci vary according to years and regions. So, it should be managed by ongoing surveillance research.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data related to this study are available from the corresponding author upon request.

REFERENCES

Abed AH, Menshawy AMS, Zeinhom MMA, et al (2021) Subclinical Mastitis in Selected Bovine Dairy Herds in North Upper Egypt: Assessment of Prevalence, Causative Bacterial Pathogens, Antimicrobial Resistance and Virulence-Associated Genes. Microorganisms 9(6). https://doi.org/10.3390/microorganisms9061175

Ali T, Kamran RA, Wazir I, et al (2021) Prevalence of Mastitis Pathogens and Antimicrobial Susceptibility of Isolates From Cattle and Buffaloes in Northwest of Pakistan. Front Vet Sci 8:746755. https://doi.org/10.3389/fvets.2021.746755

Bal EB, Bayar S, Bal MA, (2010) Antimicrobial susceptibilities of coagulase-negative staphylococci (CNS) and streptococci from bovine subclinical mastitis cases. J Microbiol 48(3): 267-274. https://doi.org/10.1007/s12275-010-9373-9

Bentayeb L, Akkou M, Zennia SS, et al (2023) Impacts of subclinical mastitis on milk quality, clotting ability and microbial resistance of the causative Staphylococci, LAR, 29: 105-111. https://www.largeanimalreview.com/index.php/lar/article/view/633

Chambers HF, (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 10(4): 781-791. https://doi.org/10.1128/CMR.10.4.781

Constable PD, Hinchcliff KW, Done SH, et al (2017): Veterinary Medicine-e-Book: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats 11th ed.,Elsevier, Amsterdam, The Netherlands, pp. 220-465.

Demil E, Teshome L, Kerie Y, et al (2022) Prevalence of subclinical mastitis, associated risk factors and antimicrobial susceptibility of the pathogens isolated from milk samples of dairy cows in Northwest Ethiopia. Prev Vet Med 205, 105680. https://doi.org/10.1016/j.prevetmed.2022.105680

Deng J, Liu K, Wang K, et al (2023) The prevalence of coagulase-negative staphylococcus associated with bovine mastitis in China and its antimicrobial resistance rate: a meta-analysis. J Dairy Res 90(2): 158-163. https://doi.org/10.1017/S0022029923000365

Erskine RJ (2020) Mastitis in cattle. In: MSD Manual. Veterinary Manual. Available from: https://www.msdvetmanual.com/reproductive-system/mastitis-in-large-animals/mastitis-in-cattle?query=mastitis%20in%20cattle [accessed 14 August 2023].

Harkins CP, Pichon B, Doumith M, et al (2017) Methicillin-resistant Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. Genome Biol 18(1): 130. https://doi.org/10.1186/s13059-017-1252-9

Hoque MN, Das ZC, Rahman A, et al (2018) Molecular characterization of Staphylococcus aureus strains in bovine mastitis milk in Bangladesh. Int J Vet Sci Med 6(1):53-60. https://doi.org/10.1016/j.ijvsm.2018.03.008

Ibrahim N, Regassa F, Yilmaz T, et al (2023) Impact of subclinical mastitis on uterine health, reproductive performances and hormonal profile of Zebu x Friesian crossbred dairy cows in and around Jimma town dairy farms, Ethiopia. Heliyon 9(6):e16793. https://doi.org/10.1016/j.heliyon.2023.e16793

Kulangara V, Nair N, Sivasailam A, et al (2017) Genotypic and phenotypic beta-lactam resistance and presence of PVL gene in Staphylococci from dry bovine udder. PLoS One, 12(11): e0187277. https://doi.org/10.1371/journal.pone.0187277

Lee AS, de Lencastre H, Garau J, et al (2018) Methicillin-resistant Staphylococcus aureus. Nat Rev Dis Primers 4: 18033. https://doi.org/10.1038/nrdp.2018.33

Lina G, Piémont Y, Godail-Gamot F, et al (1999) Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 29(5): 1128-1132. https://doi.org/10.1086/313461

Lovseth A, Loncarevic S, Berdal KG (2004) Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. J Clin Microbiol 42(8): 3869-3872. https://doi.org/10.1128/JCM.42.8.3869-3872.2004

Malik S, Peng H, Barton MD (2006) Partial nucleotide sequencing of the mecA genes of Staphylococcus aureus isolates from cats and dogs. J Clin Microbiol 44(2): 413-416. https://doi.org/10.1128/JCM.44.2.413-416.2006

Monday SR, Bohach GA (1999) Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. J Clin Microbiol 37(10): 3411-3414. https://doi.org/10.1128/JCM.37.10.3411-3414.1999

Morgan M (2008) Methicillin-resistant Staphylococcus aureus and animals: zoonosis or humanosis?. J Antimicrob Chemother 62(6): 1181-1187. https://doi.org/1093/jac/dkn405

Munive Nuñez, KV, d Silva Abreu AC, Gonçalves JL, et al (2023) Virulence and antimicrobial resistance genes profiles of spa type t605 methicillin-susceptible Staphylococcus aureus isolated from subclinical bovine mastitis. J Appl Microbiol 134(4). https://doi.org/10.1093/jambio/lxad057

Paramasivam R, Gopal DR, Dhandapani R, et al (2023) Is AMR in Dairy Products a Threat to Human Health? An Updated Review on the Origin, Prevention, Treatment, and Economic Impacts of Subclinical Mastitis. Infect Drug Resist 16: 155-178. https://doi.org/10.2147/IDR.S384776

Patel BR, Vagh AA, Vala KB, et al (2023): Prevalence of Subclinical Mastitis in Gir Cows in and around Junagadh District of Gujarat. Indian Vet J 19(4): 50-54. https://doi.org/10.48165/ijvsbt.19.4.10

Phophi L, Petzer IM, Qekwana DN, et al (2019) Antimicrobial resistance patterns and biofilm formation of coagulase-negative Staphylococcus species isolated from subclinical mastitis cow milk samples submitted to the Onderstepoort Milk Laboratory. BMC Vet Res 15(1): 420. https://doi.org/10.1186/s12917-019-2175-3

Raspanti CG, Bonetto CC, Vissio C, et al (2016) Prevalence and antibiotic susceptibility of coagulase-negative Staphylococcus species from bovine subclinical mastitis in dairy herds in the central region of Argentina. Rev Argent Microbiol 48(1):50-56. https://doi.org/10.1016/j.ram.2015.12.001

Ren Q, Liao G, Wu Z, et al (2020) Prevalence and characterization of Staphylococcus aureus isolates from subclinical bovine mastitis in southern Xinjiang, China. J Dairy Sci 103(4): 3368-3380. https://doi.org/10.3168/jds.2019-17420

Roshan M, Parmanand Arora D, Behera M, et al (2022) Virulence and enterotoxin gene profile of methicillin-resistant Staphylococcus aureus isolates from bovine mastitis. Comp Immunol Microbiol Infect Dis 80, 101724. https://doi.org/10.1016/j.cimid.2021.101724

Rossi BF, Bonsaglia ECR, Castilho IG, et al (2019) Genotyping of long term persistent Staphylococcus aureus in bovine subclinical mastitis. Microb Pathog 132, 45-50. https://doi.org/10.1016/j.micpath.2019.04.031

Saini V, McClure JT, Leger D, et al (2012) Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. J Dairy Sci 95(8): 4319-4332. https://doi.org/10.3168/jds.2012-5373

Singh I, Roshan M, Vats A, et al (2023) Evaluation of Virulence, Antimicrobial Resistance and Biofilm Forming Potential of Methicillin-Resistant Staphylococcus aureus (MRSA) Isolates from Bovine Suspected with Mastitis. Curr Microbiol 80(6): 198. https://doi.org/10.1007/s00284-023-03303-2

Song X, Huang X, Xu H, et al (2020): The prevalence of pathogens causing bovine mastitis and their associated risk factors in 15 large dairy farms in China: An observational study. Vet Microbiol 247, 108757. https://doi.org/10.1016/j.vetmic.2020.108757

Sun M, Gao J, Ali T, et al (2017) Characteristics of Aerococcus viridans isolated from bovine subclinical mastitis and its effect on milk SCC, yield, and composition. Trop Anim Health Prod 49(4): 843-849. https://doi.org/10.1007/s11250-017-1271-2

Şeker E, Özenç E, Baki A, et al (2019) Prevalence of Methicillin Resistance and Panton-Valentine Leukocidin Genes in Staphylococci Isolated from Pirlak Sheep with Subclinical Mastitis in Turkey. Kocatepe Vet J 1-1. https://doi.org/10.30607/kvj.617025

Ünal N, (2013) Subklinik mastitisli inek sütlerinden izole edilen Staphylococcus aureus izolatlarında bazı toksin genleri ve metisilin direnç geninin araştırılması. Vet J Ankara Univ, 60, 21-26.

Ünal N, Askar S, Macun HC, et al (2012) Panton-Valentine leukocidin and some exotoxins of Staphylococcus aureus and antimicrobial susceptibility profiles of staphylococci isolated from milks of small ruminants, Trop. Anim. Health. Prod., 44(3), 573-579. https://doi.org/10.1007/s11250-011-9937-7

Ünal N, Cinar OD, (2012) Detection of stapylococcal enterotoxin, methicillin-resistant and Panton-Valentine leukocidin genes in coagulase-negative staphylococci isolated from cows and ewes with subclinical mastitis. Trop Anim Health Prod 44(2):369-375. https://doi.org/10.1007/s11250-011-0032-x

Unal S, Hoskins J, Flokowitsch JE, et al (1992) Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. J Clin Microbiol 30(7): 1685-1691. https://doi.org/10.1128/jcm.30.7.1685-1691.1992

Wang SC, Wu CM, Xia SC, et al (2009) Distribution of superantigenic toxin genes in Staphylococcus aureus isolates from milk samples of bovine subclinical mastitis cases in two major diary production regions of China. Vet Microbiol 137(3-4): 276-281. https://doi.org/10.1016/j.vetmic.2009.01.007

Xu J, Tan X, Zhang X, (2015) The diversities of staphylococcal species, virulence and antibiotic resistance genes in the subclinical mastitis milk from a single Chinese cow herd. Microb Pathog 88, 29-38. https://doi.org/10.1016/j.micpath.2015.08.004

Yang F, Shi W, Meng N, et al (2023): Antimicrobial resistance and virulence profiles of staphylococci isolated from clinical bovine mastitis. Front microbiol 14:1190790. https://doi.org/10.3389/fmicb.2023.1190790

Yilmaz M, Şeker E, (2022) Investigation of mecA, vanA and pvl genes in Staphylococcus aureus strains isolated from bovine mastitis in smallholder dairy farms. Etlik Vet Mikrobiyol Derg 33(1): 50-55. https://doi.org/10.35864/evmd.1008728