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Staphylococcus aureus in bovine subclinical mastitis milk samples from different areas in and around Patna, Bihar

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Abstract
The present study was conducted for the isolation and identification of *Staphylococcus aureus* from bovine subclinical mastitis milk samples. A total of 236 milk samples from bovine subclinical mastitis milk were collected from a different region of Patna, Bihar during Jan. 2021 to March 2022. By conventional enrichment and plating, the characteristic colony of *S. aureus* was produced from 44.07% samples. By biochemical and molecular confirmation *S. aureus* was detected among 16.95% of subclinical mastitis milk. The isolates showed 100% resistant to penicillin and amoxicillin while higher resistant to ampicillin, oxacillin, amoxicillin/clavulanic acid, cefoxitin, erythromycin, and tetracycline.

Keywords: Bovine, sub clinical mastitis, *S. aureus*, 16SrRNA, ABST

Introduction
Staphylococcus aureus is a Gram-positive, non-motile, non-spore former, aerobic or facultative anaerobic bacterium. It is catalase and coagulase-positive coccal bacterium of 0.5-1.5 µm diameter having the appearance of grape-like clusters. It can colonize and infect a variety of host species, including farm, companion, and wild animals as well as humans. In cattle, it is known to cause subclinical and clinical mastitis and wound infections (Hamid *et al*. 2017) [1]. Mastitis is the inflammation of the parenchyma of the mammary gland characterized by physical, chemical, and frequent bacteriological changes in milk and unreasonable changes in glandular tissues (Radostits *et al*. 2000) [2]. Subclinical mastitis is characterized by no visible signs either in the udder or in the milk, however, it leads to a decrease in milk production with increased somatic cell count and has more effective in lactating animals. Because of the involvement of *S. aureus* in subclinical mastitis, infected animals may pose a risk of transmission of infection to other animals in the herd during each milking.
Watts (1988) [3] reported the involvement of 138 different pathogens as a cause of mastitis. More importantly, *S. aureus* is recognized as the most prevalent and economically significant contagious pathogen that is found in 30-40% of all mastitic cases (Asperger and Zangerl 2003) [4] and 80% of bovine subclinical mastitis. The annual losses in the dairy industry due to mastitis have been reported as approximately 2 billion dollars in the USA and about 35 billion US dollars, worldwide (Reshi *et al*. 2015) [5] while losses due to subclinical mastitis in India have been reported as about 526 million dollars in due to (Varshney and Naresh 2004) [6].
Antibiotics are widely used in the dairy industry to treat diseases and also to improve the performance of dairy animals. Antibiotics such as penicillin, cephalosporin, streptomycin, and tetracycline are used for the treatment and prevention of diseases affecting dairy cows caused by a variety of gram-positive and gram-negative bacteria. An increase in the incidence of disease in a herd generally results in increased use of antimicrobials, which in turn increases the potential for increased bacterial resistance to antimicrobials. Therefore, the present study was designed to assess the status of *S. aureus* in bovine sub-clinical mastitis with a generation of their antibiotic susceptibility profile.

Materials and Methods
Collection of samples
A total of 236 bovine subclinical mastitis milk samples were collected from cow and buffalo at different locations in Patna including Kautilya Nagar, Danapur, Digha, Maner, Phulwari Sharif, Ramana Road, ILFC-BASU, Khagaul, and Local Khatal-Raja Bazar, Patna. Subclinical

mastitis was diagnosed based on the finding of the onsite California Mastitis Test (CMT). The CMT-positive milk samples were collected into a screw-capped centrifuge tube and brought to the laboratory under cold conditions.

Enrichment and selective plating of samples

Approximately 1ml of milk samples were inoculated in sterile test tubes containing 10 ml of sterilized tryptone soya broth incorporated with 10% sodium chloride salt (TSB-S) and incubated overnight at 37 °C for 24 h. The samples which showed turbidity in TSB-S were streaked on mannitol salt agar (MSA) and incubated at 37 °C for 24 h. The plates were examined for the presence of mannitol fermenter, round-shaped, typical golden, yellow, or pale colour colonies of *S. aureus*. A part of the characteristic colony from the MSA plate was picked up and examined under oil immersion after Gram's staining as described by Agarwal *et al*. (2003) [7].

Biochemical confirmation of isolates

The presumptive *S. aureus* colonies that showed characteristic morphology as cocci in a bunch of grapes under microscopic examination were further confirmed by catalase and tube coagulase test using human plasma as per methods described by Agarwal *et al*. (2003) [7].

Molecular confirmation of isolates

The template DNA was prepared from the biochemically confirmed *S. aureus* isolates by the method of boiling and snaps chilling as described by Chai *et al*. (2007) [8]. A PCR assay was standardized for amplification of 16SrRNA gene fragment of *S. aureus* isolates as per the method described by Karmakar *et al*. (2016) [9] with some modification. The PCR reaction mixture was prepared in 25 µl reaction volume each containing 2.5 µl 10X PCR buffer, 0.5 µl of dNTP mixture (10 mM each), 2 µl (10 pmol/µl) of forward and reverse primers, 1 µl (1 U) Taq DNA polymerase, 2 µl of bacterial lysate and 15 µl nuclease-free water. The amplification of PCR products was performed in a PCR machine (Sure Cycler 8800, Agilent Technology) with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min with a final elongation phase at 72 °C for 5 min. The amplified products of 228 bp were analyzed by agarose gel (1.5%) electrophoresis stained with 0.5 µg/ml ethidium bromide. The gel image was visualized and documented in the gel documentation system (Vilber, France).

Antibiotic susceptibility profile of *Staphylococcus aureus*

The antibiotic susceptibility test was performed by the disc diffusion method (Wayne 2002) [10]. The test colony was inoculated overnight in nutrient broth at 37 °C. About 100 µl of the growth culture was spread on Mueller-Hilton agar plates with a sterile L-shaped spreader and the antibiotic discs of 5 mm were stuck to the plates with forceps, belonging to 13 antibiotics namely- ampicillin/AMP (10 µg), oxacillin/ OX (1 µg), vancomycin/VA (30 µg), cefoxitin (30 µg), penicillin/P (10 µg), amoxicillin/clavulanic acid (30 µg), tetracycline/TE (30 µg), linezolid/LZ (30 µg), erythromycin/ E (15 µg), chloramphenicol/C (30 µg), gentamicin (10 µg) enrofloxacin/EX (10 µg) and amoxicillin/AMX (10 µg). All antibiotic disc-containing plates were incubated for 18-24 h at 37 °C. The zones of inhibition were measured using a calibrated zone scale (Hi-media, India) and recorded for further interpretation according to the guidelines of CLSI (2013) [11].

Results and Discussion

Out of 236 bovine subclinical mastitis milk samples, the characteristic mannitol fermenter colony on MSA plates was isolated from a total of 104 (44.07%) samples. The area-wise sample analysis showed that *S. aureus* was isolated from the samples belonging to Kautilya Nagar (10), Danapur (7), Digha (19), Maner (13), Phulwari Sharif (6) samples from, Patna, Ramna Road (7), Institutional livestock farm complex, Bihar Animal Sciences University (26), Khagaul (7) and Local Khatal, Rajabajar (9), Patna.

By biochemical confirmation, a total of 38.46% (40/104) characteristic colony-producing isolates were confirmed as *S. aureus* that further produced 208 bp *S. aureus* species-specific amplicons in PCR followed by the agarose gel electrophoresis (Fig. 1). The sample-wise distribution of *S. aureus* among subclinical mastitis milk was recorded as 16.95% (40/236). The sampling area-wise distribution of *S. aureus* among subclinical mastitis milk samples showed that *S, aureus* was involved in bovine subclinical mastitis at 4.00% (1/25) in Kautilya Nagar, 13.33% (2/15) in Danapur, 29.63% (8/27) in Digha, 15.00% (3/20) in Maner, 13.04% (3/23) in Phulwari Sharif, 14.29% (2/14) in Ramna Road, 29.17% (14/48) in ILFC, 3.85% (1/26) in Khagaul, and 21.43% (6/28) in Local Khatal, Rajabajar, Patna (Fig. 2).

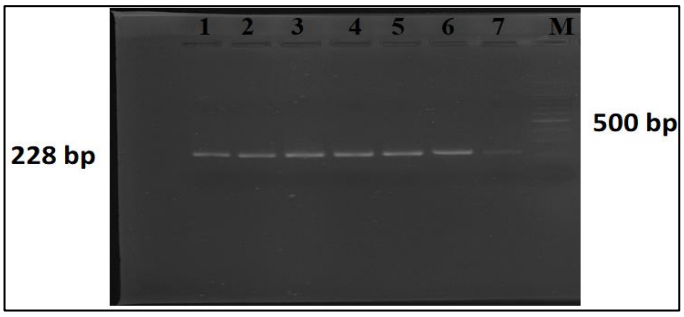
In the present work, involvement of *S. aureus* was detected as ~17% in bovine subclinical mastitis. In concordance with the findings of this study, a similar finding of 17.50% of *S. aureus* from Mathura (Sharma *et al*. 2015) [12] and 20.00% from Uttar Pradesh (Kutar *et al*. 2015) [13] in subclinical bovine milk samples were also reported. Accordingly, a similar prevalence of 16.5% from East Coast Malaysia (Saeed *et al*. 2022) [14], 18% from Flanders, Belgium (Piepers *et al*. 2007) [15], 19% from Sweden (Persson *et al*. 2011) [16], and 20.45% from Western Australia (Chung *et al*. 2021) [17] was reported by different workers from abroad. However, in contrast to the finding of the present study, a higher prevalence of 38.66% in Hisar, Haryana (Pankaj *et al*. 2012) [18], 40.74% in Bangalore (Mallikarjunaswamy and Murthy 1997) [19], 46.30% in Namakkal (Srinivasan *et al*. 2013) [20], 46.3% from Jaipur, Rajasthan (Jena *et al*. 2015) [21], 50% from Proddatur, Andhra Pradesh (Manasa *et al*. 2019) [22] and 58% from Ramanagara, Karnataka (Harini and Sumathi 2011) [23] were reported from India. Similarly, a higher prevalence of 30.76% from Aran, West Algeria (Benhamed *et al*. 2011) [24], 35.36% from the State of Rio de Janeiro (Vieira-da-Motta *et. al.* 2001) [25], 39.6% from Serbia (Zutic *et al*. 2012) [26], 58.04% from Thailand (Pumipuntu *et al*. 2017) [27], 72.73% - 80% from Sadat city, Egypt (Elsayed and Dawoud 2015) [28] and 77.38% from Southern Xinjiang, China (Ren *et al*. 2020) [29] were also reported from abroad. In contrast to the finding of the present study, a lower prevalence of 3.13% from Bangladesh (Rahman *et al*. 2010) [30], 4.69% from the Marmara Region of Turkey (Ikiz *et al*. 2013) [31], 13.8% from Holeta district, Ethiopia Ayano *et al*. (2013) [32] and 15.2% from Chitwan, Nepal (Shrestha *et al*. 2021) [33] of *S. aureus* were also reported from bovine subclinical mastitis.

The antibiotic susceptibility study of *S. aureus* isolates from bovine subclinical mastitic milk samples of the present study revealed that all isolates were resistant to penicillin and amoxicillin followed by 95.00% isolates resistant to ampicillin, 90.00% to oxacillin, 80.00% to amoxicillin/clavulanic acid, 77.50% to cefoxitin, 62.50% to

erythromycin, 55.00% to tetracycline, 20.00% to vancomycin, 15.00% to chloramphenicol and gentamicin and 5.00% to linezolid. The isolates showed a susceptibility of 95.00% to linezolid followed by 77.50% to enrofloxacin, 72.5% to chloramphenicol and gentamicin, 22.50% to vancomycin, cefoxitin, tetracycline and erythromycin, 20.00% to amoxicillin/clavulanic acid, 10.00% to oxacillin and 5.00% to ampicillin. The isolates also showed an intermediate susceptibility of 57.50% to vancomycin, 22.50% to tetracycline and enrofloxacin, 15% to erythromycin, and 12.50% to chloramphenicol and gentamycin.

The area-wise distribution of antibiotic resistance *S. aureus* showed that all isolates from Kautilya Nagar were resistant to ampicillin, oxacillin penicillin, amoxicillin/clavulanic acid, linezolid, and amoxicillin. All isolates from Danapur were resistant to ampicillin, penicillin, and amoxicillin while 50.00% were resistant to oxacillin, amoxicillin/clavulanic acid, and tetracycline. The isolates from Digha showed 100% resistance to penicillin, tetracycline, and amoxicillin followed by 87.5% to ampicillin, oxacillin, 75% to cefoxitin, 62.5% to amoxicillin/clavulanic acid, 37.5% to erythromycin and gentamicin, 12.5% to vancomycin, and chloramphenicol. All isolates from Maner were found resistant to ampicillin, cefoxitin, penicillin, amoxicillin/clavulanic acid, and amoxicillin followed by 66.67% resistant to oxacillin, tetracycline, erythromycin, and gentamicin and 33.33% resistance with vancomycin. The isolates from Phulwari Sharif were found 100% resistant to ampicillin, oxacillin, cefoxitin, penicillin, amoxicillin/clavulanic acid, and amoxicillin and 66.67% resistant to tetracycline and erythromycin. All isolates from Ramana Road were found resistant to ampicillin, oxacillin, penicillin, amoxicillin/clavulanic acid, tetracycline, erythromycin, and amoxicillin while 50.00% resistance to vancomycin and cefoxitin. All isolates from ILFC- BASU were found resistant to oxacillin, penicillin, amoxicillin/clavulanic acid, and amoxicillin while 92.86% to ampicillin and cefoxitin followed by 78.57% to erythromycin, 28.57% to vancomycin, 21.43% to tetracycline, 14.29% to chloramphenicol and 7.14% to linezolid. All isolates from Khagaul were found resistant to ampicillin, oxacillin, cefoxitin, penicillin, amoxicillin/clavulanic acid, and amoxicillin. All isolates from local khatal, Raja Bazar samples were resistant to ampicillin, penicillin, and amoxicillin. The isolates also showed 83.33% resistance to oxacillin and erythromycin followed by 66.67% to cefoxitin and tetracycline, 50.00% to chloramphenicol, 33.33% to amoxicillin/clavulanic acid, and 16.67% to gentamicin (Fig. 3).

In concordance with the findings of the present study 100% resistance of *S. aureus* isolates from subclinical bovine mastitis to various antibiotics including penicillin was also reported previously by Varela-Ortiz *et al*. (2018) [34], Gentilini *et al*. (2000) [35], Malinowski and Kłossowska (2002) [36]. Further multi-drug resistance of *S. aureus* isolates from bovine mastitis was also reported by Zayda *et al*. (2020) [37], Chandrasekaran *et al*. (2014) [38], Sharma and Brinty (2014) [39]. The widespread resistance to various antibiotics could be a consequence of the frequent use of antibiotics in intramammary infections without sensitivity testing. In cases of mastitis choosing the wrong antibiotic or applying an incomplete treatment to animals also contributes significantly to the development of bacterial resistance.



M: 100 bo DNA ladder
L1: Positive Control
L2-L7: Positive amplification of 228 bp *S. aureus* isolates from samples

Fig 1: PCR amplification of spices specific 16SrRNA gene to confirm *Staphylococcus aureus*

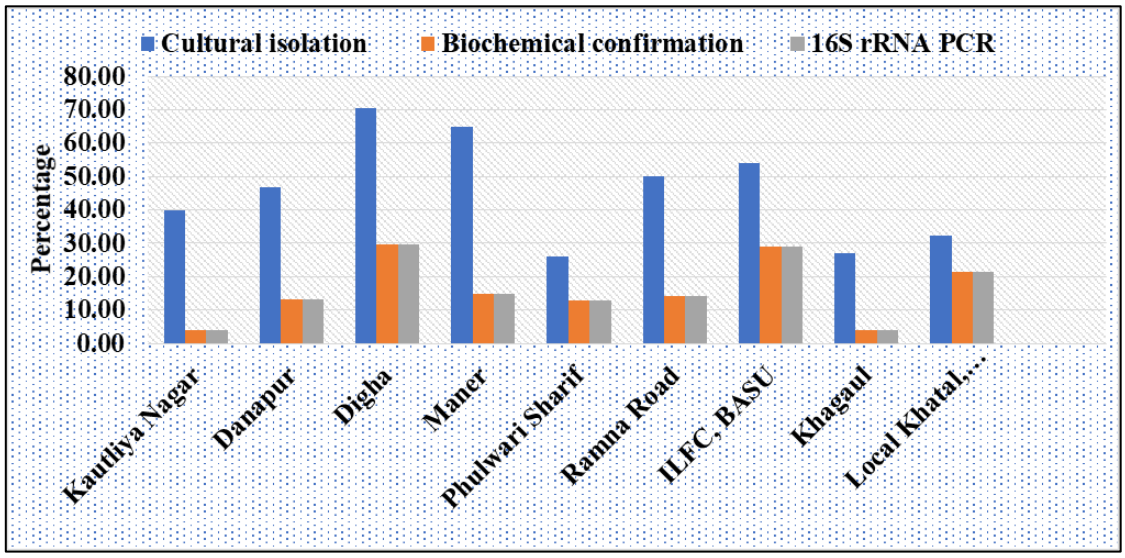


Fig 2: Area wise distribution of *Staphylococcus aureus* in milk samples of bovine subclinical mastitis

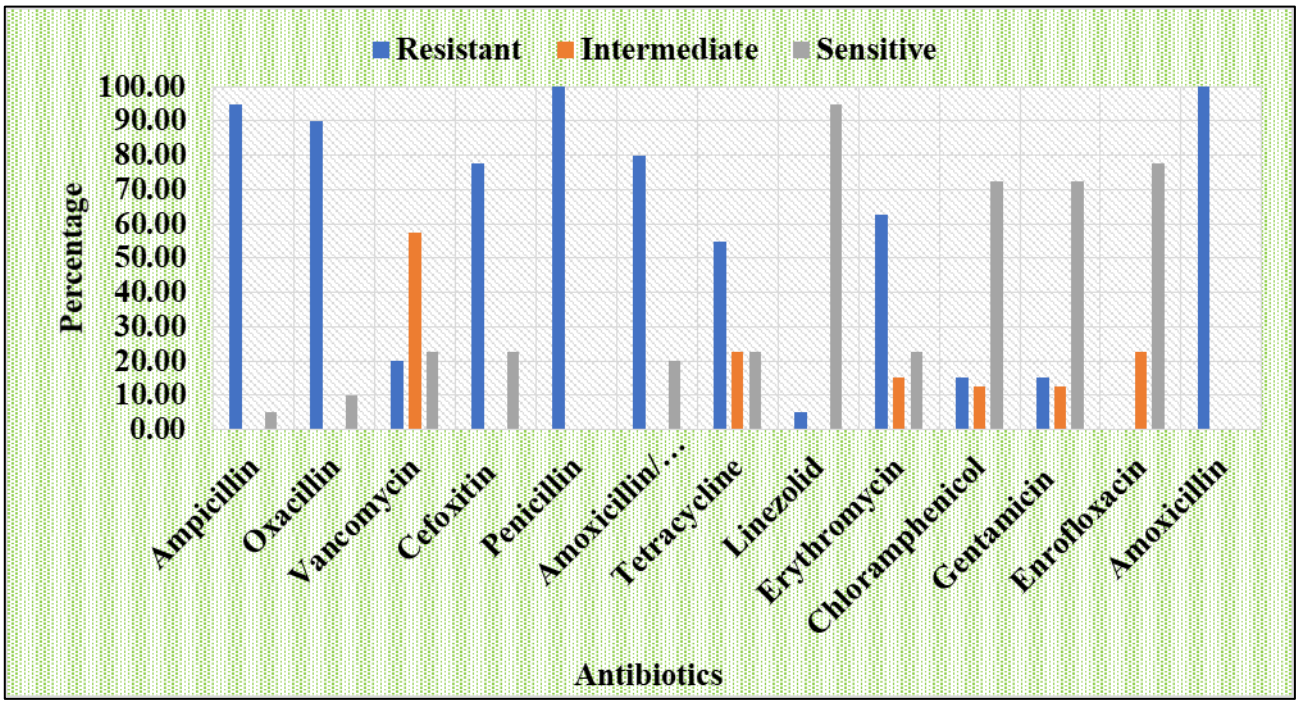


Fig 3: Antibiotic susceptibility profile of *Staphylococcus aureus* isolates of bovine subclinical mastitis milk

Conclusion

Based on the findings of present study it can be concluded that multi drug resistant *S. aureus* may be a major pathogen involved in bovine sub-clinical mastitis in and around Patna, Bihar. The study can provide the information about the selection of antibiotics for the treatment of bovine mastitis caused by *S. aureus.*

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References

1. Hamid S, Bhat MA, Mir IA, Taku A, Badroo GA, Nazki S *et al*. Phenotypic and genotypic characterization of methicillin-resistant *Staphylococcus aureus* from bovine mastitis. Veterinary world. 2017;10(3):363-367.
2. Radostits OM, Mayhew IG, Houston DM. Veterinary clinical examination and diagnosis. WB Saunders; 2000.
3. Watts JL, Owens WE. Evaluation of the rapid mastitis test for identification of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine mammary glands. Journal of clinical microbiology. 1988;26(4):672-674.
4. Asperger H, Zangerl P. *Staphylococcus aureus*. Roginski, H., Fuquay, J.W., Fox, P.F. (Eds), Encyclopaedia of Dairy Sciences, Academic Press and Elsevier Science, Amsterdam, Boston, London; c2003. p. 2563–2569.
5. 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;7(5):48.
6. Varshney JP, Naresh R. Evaluation of a homeopathic complex in the clinical management of udder diseases of riverine buffaloes. Homeopathy. 2004;93(01):17-20.
7. Agarwal RK, Bhilegaonkar KN, Singh DK, Kumar A, Rathore RS. Laboratory manual for the isolation and identification of food borne pathogens. 1st Edn., IVRI, Izatnagar; c2003. p. 38-39.
8. Chai LC, Robin T, Ragavan UM, Gunsalam JW, Bakar FA, Ghazali FM, *et al*. Thermophilic *Campylobacter* spp. in salad vegetables in Malaysia. International journal of food microbiology. 2007;117(1):106-111.
9. Karmakar A, Dua P, Ghosh C. Biochemical and molecular analysis of *Staphylococcus aureus* clinical isolates from hospitalized patients. Canadian journal of infectious diseases and medical microbiology. 2016, 2016.
10. Wayne PA. Performance Standards of Antimicrobial Susceptibility: National Committee for Clinical Laboratory Standards (NCCLS). NCCLS Approved Standards; c2002. p. 100-159
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement; c2013; p. M100–S23.
12. Sharma L, Verma AK, Kumar A, Rahat A, Neha, Nigam R. Incidence and pattern of antibiotic resistance of *Staphylococcus aureus* isolated from clinical and subclinical mastitis in cattle and buffaloes. Asian Journal of Animal Sciences. 2015;9(3):100-109.
13. Kutar K, Verma AK, Sharma B, Kumar A, Yadav SK. Analysis of mecA gene and antibiotic resistance in *Staphylococcus aureus* isolates from bovine mastitis. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 2015;36(1):22-27.
14. Saeed SI, Mat Yazid KA, Hashimy HA, Dzulkifli SK, Nordin F, Nik Him NA *et al*. Prevalence, Antimicrobial Resistance, and Characterization of *Staphylococcus aureus* Isolated from Subclinical Bovine Mastitis in East Coast Malaysia. Animals. 2022;12(13):1680.
15. Piepers S, De Meulemeester L, de Kruif A, Opsomer G, Barkema HW, De Vliegher S. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. Journal of Dairy Research. 2007;74(4):478-483.
16. Persson Y, Nyman AK, Grönlund-Andersson U. Etiology

and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. Acta Veterinaria Scandinavica. 2011;53(1):1-8.
17. Chung LK, Sahibzada S, Annandale HC, Robertson ID, Waichigo FW, Tufail MS *et al*. Bacterial pathogens associated with clinical and subclinical mastitis in a Mediterranean pasture-based dairy production system of Australia. Research in Veterinary Science. 2021;141:103-109.
18. Pankaj P, Sharma A, Chhabra R, Sindhu N. Prevalence of sub clinical mastitis in cows: Its etiology and antibiogram. Indian Journal of Animal Research. 2012;46(4):348-353.
19. Mallikarjunaswamy MC, Krishna Murthy GV. Antibiogram of bacterial pathogens isolated from bovine subclinical mastitis cases. Indian veterinary journal. 1997;74(10):885-886.
20. Srinivasan P, Jagadeswaran D, Manoharan R, Giri T, Balasubramaniam GA, Balachandran P. Prevalence and etiology of subclinical mastitis among buffaloes (*Bubalus bubalus*) in Namakkal, India. Pakistan Journal of Biological Sciences: PJBS. 2013;16(23):1776-1780.
21. Jena B, Pagrut NK, Sahoo A, Ahmed A. Subclinical bovine mastitis in Rural, Peri-urban and Suburban regions of Jaipur District of Rajasthan, India. Journal of Animal Research. 2015;5(1):175-182.
22. Manasa V, SaiKumar TV, Rao TP, Kumar KA. Incidence of clinical and sub-clinical bovine mastitis caused by *Staphylococcus aureus* in Proddatur region of Andhra Pradesh. International Journal of Chemical Studies. 2019;7(3):788-792.
23. Harini H, Sumathi BR. Screening of bovine milk samples for sub-clinical mastitis and antibiogram of bacterial isolates. Veterinary World. 2011;4(8):358-359.
24. Benhamed N, Moulay M, Aggad H, Henni JE, Kihal M. Prevalence of mastitis infection and identification of causing bacteria in cattle in the oran region West Algeria. Journal of animal and veterinary Advances. 2011;10(22):3002-3005.
25. Vieira-da-Motta O, Folly MM, Sakyiama CC. Detection of different *Staphylococcus aureus* strains in bovine milk from subclinical mastitis using PCR and routine techniques. Brazilian Journal of Microbiology. 2001;32:27-31.
26. Zutic M, Cirkovic I, Pavlovic L, Zutic J, Asanin J, Radanovic O. *et al*. Occurrence of methicillin-resistant *Staphylococcus aureus* in milk samples from Serbian cows with subclinical mastitis. African Journal of Microbiology Research. 2012;6(29):5887-5889.
27. Pumipuntu N, Kulpeanprasit S, Santajit S, Tunyong W, Kong-Ngoen T, Hinthong W, *et al*. Screening method for *Staphylococcus aureus* identification in subclinical bovine mastitis from dairy farms. Veterinary world. 2017;10(7):721.
28. Elsayed MS, Dawoud MA. Phenotypic and genotypic detection of virulence factors of *Staphylococcus aureus* isolated from clinical and subclinical mastitis in cattle and water buffaloes from different farms of Sadat City in Egypt. Veterinary world. 2015;8(9):1051.
29. Ren Q, Liao G, Wu Z, Lv J, Chen W. Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern Xinjiang, China. Journal of Dairy Science. 2020;103(4):3368-3380.
30. Rahman MM, Islam MR, Uddin MB, Aktaruzzaman M. Prevalence of subclinical mastitis in dairy cows reared in Sylhet district of Bangladesh. International Journal of Bio Research. 2010;1:23-28.
31. Ikiz S, Başaran B, Bingol EB, Cetin O, Kasikci G, Ozgur NY *et al*. Presence and antibiotic susceptibility patterns of contagious mastitis agents (*Staphylococcus aureus* and *Streptococcus agalactiae*) isolated from milks of dairy cows with subclinical mastitis. Turkish Journal of Veterinary & Animal Sciences. 2013;37(5):569-574.
32. Ayano AA, Hlriko F, Simyalew AM, Yohannes A. Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district. Journal of Veterinary Medicine and Animal Health. 2013;5(3):6772.
33. Shrestha A, Bhattarai RK, Luitel H, Karki S, Basnet HB. Prevalence of methicillin-resistant *Staphylococcus aureus* and pattern of antimicrobial resistance in mastitis milk of cattle in Chitwan, Nepal. BMC Veterinary Research. 2021;17(1):1-7.
34. Varela-Ortiz DF, Barboza-Corona JE, González-Marrero J, León-Galván M, Valencia-Posadas M, Lechuga-Arana AA, *et al*. Antibiotic susceptibility of *Staphylococcus aureus* isolated from subclinical bovine mastitis cases and in vitro efficacy of bacteriophage. Veterinary research communications. 2018;42(3):243-250.
35. Gentilini E, Denamiel G, Llorente P, Godaly S, Rebuelto M, DeGregorio O. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. Journal of dairy science. 2000;83(6):1224-1227.
36. Malinowski HL, Kłossowska A. Bulletin-Veterinary Institute in Pulawy. 2002;46:289-294.
37. Zayda MG, Masuda Y, Hammad AM, Honjoh KI, Elbagory AM, Miyamoto T. Molecular characterisation of methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* isolated from bovine subclinical mastitis and Egyptian raw milk cheese. International Dairy Journal. 2020;104:104646.
38. Chandrasekaran D, Venkatesan P, Tirumurugaan KG, Nambi AP, Thirunavukkarasu PS, Kumanan K *et al*. Pattern of antibiotic resistant mastitis in dairy cows. Veterinary World. 2014;7(6): 389-394.
39. Sharma I, Brinty A. Isolation and identification of *Staphylococcus aureus* from bovine mastitis milk and their drug resistance patterns in Silchar town dairy farms, NE India. Online International Interdisciplinary Research Journal. 2014;4:256-260.