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Molecular detection of methicillin resistant *Staphylococcus aureus* associated with mastitis in goats

J. N. Bhagya[1], P. V. Tresamol [2], K. Vijayakumar [3], V. H. Shyma [4] and M. Mini [5]
Department of Veterinary Epidemiology and Preventive Medicine
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651
Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Antimicrobial resistance is an emerging concern in the world while combating with a wide range of pathogen associated with various disease conditions. It is considered as a silent pandemic nowadays. A constant monitoring and measures for controlling emergence of antimicrobial resistance are crucial in the current situation. The present study was conducted with the objective of detection of methicillin resistant Staphylococcus aureus (MRSA) associated with mastitis in goats. Milk samples were collected from 66 goats suffering from mastitis and screened for the presence of S. aureus. Conventional microbiological tests and species specific PCR were employed for the species confirmation. A total of 10 samples (15.15%) were found to be positive for S. aurues. Among the ten isolates of S. aureus obtained two isolates (3.03%) were found to harbour mecA gene suggestive of the presence of MRSA phenotype. All the isolates were negative for the presence of pvl gene which is present in highly virulent strains. In short, even though the study demonstrated the presence of MRSA at a lower rate, the results are of a marked public health significance due to the zoonotic significance of the pathogen.

Keywords: *Mastitis, caprine, MRSA, PCR*

Staphylococcus aureus is an opportunistic pathogen of human and other mammals which has increasing clinical and veterinary importance as it has capability to develop antimicrobial resistance rapidly (Foster, 1996). The injudicious use of antibiotics has led to the emergence of antibiotic resistant *S. aureus* strains, most significant being methicillin resistant *S. aureus*. Methicillin resistant *S. aureus* (MRSA) is an emerging zoonotic pathogen of clinical significance worldwide. It can lead to several infections that are difficult to treat due to multidrug resistance. Methicillin is a beta lactam antibiotic that inhibits penicillin binding proteins that are involved in the synthesis of cell wall component of bacterium, peptidoglycan (Stapleton and Taylor, 2002). The methicillin

1. *MVSc Scholar*
2. *Professor , Department of Veterinary Epidemiology and Preventive Medicine*
3. *Professor and Head, Department of Veterinary Epidemiology and Preventive Medicine*
4. *Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine,CVAS,Mannuthy*
5. *Professor and head, Department of Veterinary Microbiology , CVAS,Mannuthy*

**Corresponding author: jbhagya93@gmail.com , Ph: 9497716756*

resistance emerges due to the expression resistance emerges due to the expression of penicillin-binding protein (PBP2a), a foreign PBP that can resist the action of methicillin at the same time perform function of host PBPs (Kitti *et al*., 2011). The emergence of MRSA is a public health threat of utmost importance. The emergence of antimicrobial resistance is considered as a silent pandemic. (Mahoney *et al.,* 2021). Panton- valentine leukocidin is a beta pore forming cytotoxin is related with tissue necrosis and also causes disruption of leukocyte membranes. *Staphylococcus aureus* strains carrying PVL are highly virulent and rapidly transmissible strains than PVL negative *S. aureus* (Karmakar *et al*., 2018). The presence of this virulent factor in *S. aureus* is important in pathogenicity of bacteria.

Mastitis is an inflammation of the mammary gland and is the most debilitating disease in dairy goats which demands huge economic investment in treatment (Marogna *et al.,* 2010).

In developing countries goats are remarkable for the livelihood of the large population especially to disadvantaged sections of society who are prone to penury and undernourishment. According to the National Livestock census (2019), the total goat population in India was 148.88 million which forms about 27.8 per cent of the total livestock population. Milch animals constitute 69.65 per cent of the total goat population and this is the population susceptible to mastitis. The occurrence of MRSA in goats has to be constantly monitored as it can lead to serious public health issues. Hence the present study has undertaken to assess the prevalence of MRSA in caprine mastitis.

Materials and methods

Sixty-six goats suffering from clinical mastitis that had been presented at University Veterinary Hospitals (UVH), Mannuthy and Kokkalai during the period from April 2019 to March 2021 formed the subjects of the study**.** Milk samples were collected from the udder halves with mastitis after taking all aseptic precautions. Primary isolation of bacteria was performed in Brain Heart infusion agar (BHIA; Himedia, M211) by directly streaking on to agar and incubating at 37℃ for 24 hr. The isolates obtained were identified using the colony morphology gram staining and biochemical tests as per Barrow and Feltham (1993). Deoxyribonucleic acid was isolated from the cultures of bacteria using snap chill method. Polymerase chain reaction was performed to confirm the *S. aureus* isolates using species specific primers that target the *23S rRNA* gene of *S. aureus* (El-Razik *et al*., 2010). The presence of *mec*A and *PVL* in *S. aureus* isolates were detected by PCR using specific primers as per Kobayashi *et al.* (1994) and Pajic *et al.* (2014), respectively (Table 1.).

The PCR reaction mix was made of 5 µL of template DNA, 12.5 µL of PCR master mix (2X PCR Smart mix, Takara, Japan) forward and reverse primer of 100nM/ µL concentration 1µL (Sigma Aldrich), sterile nuclease free water 5.5µL and the total volume was made to 25µL. The amplification procedure followed for 23SrRNA was an initial denaturation at 94℃ for 5 min followed by 30 cycles of denaturation (94℃ for 45 s), annealing (55.8℃ for 45 s), extension (72℃ for 90 s), followed by final extension at 72℃ for 10 min. The amplification procedure followed for *mecA* gene was an initial denaturation (95℃ for 3 min) followed by 30 cycles of denaturation (95℃ for 45 s), annealing (60℃ for 60s), extension (72℃ for 60 seconds) and a final extension at 72℃ for 7 min. The amplification procedure followed for *pvl* gene was an initial denaturation (95℃ for 5 min) followed by 30 cycles of denaturation (95℃ for 30 s), annealing (59℃ for 30s), extension (72℃ for 60s) and a final extension at 72℃ for 5 min). The DNA isolated from known *S. aureus* constituted the positive control (Tresamol *et al*., 2018) and DNA from known *Escherichia coli* constituted the negative control (confirmed at Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy). The PCR products were subjected to 1.5 per cent agarose gel electrophoresis for visualisation and documentation of the amplified products.

Results and discussion

Milk samples from all the 66 lactating goats with clinical mastitis yielded growth on

primary isolation on brain heart infusion agar. Gram's staining revealed that 80.31 per cent of the cases had (53/66) gram positive cocci and 19.69% (13/66) isolates were gram negative bacilli. The colonies of gram positive cocci were subjected to catalase and coagulase test to identify *S. aureus* and this was confirmed by sub culturing on mannitol salt agar. Based on colony morphology, Gram's staining and biochemical tests, 10 isolates were tentatively identified as *S. aureus*. The isolates obtained were further confirmed by species-specific PCR targeting 23S *rRNA* (Hunt *et al*., 2006), in which all the isolates yielded amplicons of 1318 bp confirming species identification (Fig.1.). Similar to our work, Yamagishi *et al*. (2007), El-Razik, *et al*. (2010) and Tresamol *et al*. (2018) used the same PCR technique for species level identification of *S. aureus* from bubaline and bovine milk respectively. In short, the prevalence of caprine mastitis was observed to be 15.15 per cent during a period of 8 months in the present study.

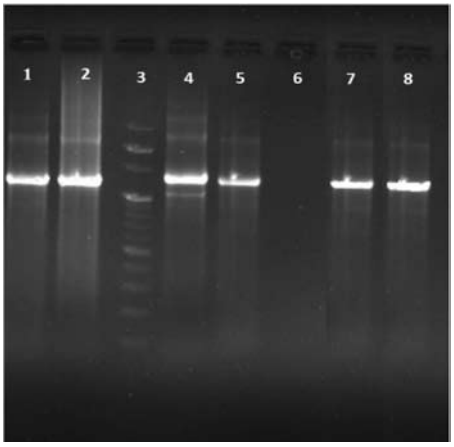

Fig. 1. Agarose gel electrophoresis of *23S rRNA* specific PCR of *S. aureus* (Lane 1: Positive control, Lane 6:Negative control Lane 2,4,5,7,8: Positive samples (1318 bp),Lane 3:DNA Marker (100 bp)

Fig. 2. Agarose gel electrophoresis of *mecA* specific PCR of *S. aureus* (Lane 6: DNA Marker (100 bp), Lane 4 and 5: Positive samples (303 bp), Lane 7: Negative control Lane 8: Positive control

A variable prevalence rate of *S. aureus* has been reported in lactating animals by different authors. Kini *et al.* (2019) reported a 51 per cent occurrence of *S. aureus* in goat mastitis from Wayanad, Kerala. Danmallam and Pimenov (2019) reported a 20 per cent prevalence of *S. aureus* in caprine mastitis from Moscow, Russia. Mugabe *et al.* (2017) reported an 83 per cent prevalence in goat mastitis from Botswana, Pakistan. Islam *et al.* (2011) reported *S. aureus* (36.36%) as the major pathogen associated with caprine mastitis from Mymensingh, Bangladesh. Najeeb *et al*. (2013) reported a 61.64 per cent *S. aureus* prevalence in caprine mastitis. Jose *et al.* (2021) reported 32.84 per cent occurrence of *S. aureus* in bovine mastitis from Thrissur, Kerala. Low prevalence of 23 per cent and 18.18 per cent were reported by Kumar *et al.* (2016) and Nabih *et al.* (2018) from Pakistan and Egypt, respectively.

Ten isolates yielded amplicons of size 1318bp confirming *S. aureus*. These ten isolates were subjected to PCR targeting *mecA* gene and *pvl* gene. Only 2 isolates yielded amplicons of size 303bp whereas none yielded amplicons for *pvl* gene. Detection of methicillin resistance in staphylococci is a tedious process in the clinical microbiology laboratory due to the heterogeneity of the bacterium under test. The detection of resistance in these isolates has been disturbed due to variability in the standard techniques used in determining resistance to methicillin. Resistance to methicillin is due to *mec*A gene which is part of a mobile genetic element called the staphylococcal cassette chromosomes (SCC) *mec* (Wu *et al*., 1996), and it represents a marker of methicillin resistance when molecular techniques such as PCR are used for screening the methicillin resistant gene in *S. aureus.* In the present study methicillin resistance was detected by using

PCR targeting *mec*A gene. All the 10 species confirmed isolates were when subjected to PCR for detection of presence of *mec*A gene, only two yielded *mec*A gene amplicons (Fig. 2.). Accordingly, the prevalence of MRSA in caprine mastitis cases was calculated as 3.03 per cent. Similarly, Aras *et al*. (2012) reported a low prevalence of 4.76 per cent in caprine mastitis cases reported in Turkey. The prevalence of *mec*A gene reported by Obaidat *et al*. (2018) and Altaf *et al.* (2020) in *S. aureus* isolates from goat milk were 11.5 and 6.55 per cents, respectively. In contrast, a higher prevalence of 50 per cent was reported by Suchithra *et al*. (2015) in *S. aureus* isolates from caprine clinical mastitis cases of Thrissur, Kerala whereas Persson *et al.* (2021) could not detect *mec*A gene in milk samples and nasal swab of goats.

High prevalence of MRSA had been detected in bovine mastitis (Kumar *et al*., 2011., Jisha *et al*., 2020.). The reason for the variation in occurrence between the species can be due to multiple causes such as the use of antibiotics, hygiene practices, and disease control programmes which influences the occurrence. The group under study constituted only a small proportion of a large population which might be the reason for the low percentage of detection of MRSA in the present study. None of the isolates yielded amplicons for *pvl* when subjected to PCR specific for *pvl* gene. This result was in line with the findings of Tegegne *et al.* (2019) where no *S. aureus* from goat mastitis samples were reported to harbour the *pvl* gene. The presence of MRSA in goats even at a low rate is of great public health significance. Hygienic precautions to control and constantly monitor the scene for the presence of methicillin resistance are very important as this pathogen is zoonotic and can lead to a pandemic if left unnoticed.

Conclusion

Antimicrobial resistance is an emerging issue in treating infectious diseases. It can be considered as a silent threat that could reverse the entire development that has been achieved in the field of treating infectious diseases. Constant monitoring and control measures are equally essential in controlling the emergence of antimicrobial resistance**.**

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Conflicts of interest

The authors have no conflicts of interest to declare.

References

Altaf, M., Ijaz, M., Iqbal, M.K., Rehman, A., Avais, M., Ghaffar, A. and Ayyub, R.M. 2020.

Molecular characterization of methicillin resistant *Staphylococcus aureus* (MRSA) and associated risk factors with the occurrence of goat mastitis. *Pak. Vet. J*. **40** (1):1-6.

Aras, Z., Aydin, I. and Kav, K. 2012. Isolation of methicillin-resistant *Staphylococcus aureus* from caprine mastitis cases. *Small Rumin. Res*. **102** (1):.68-73.

Barrow, C.I. and Feltham, R.K.A. 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria.* (3rd Ed.). Cambridge University Press, Great Britain, 331p.

Danmallam, F.A. and Pimenov, N.V. 2019. Study on prevalence, clinical presentation, and associated bacterial pathogens of goat mastitis in Bauchi, Plateau, and Edo states, Nigeria, *Vet. World*. **12** (5): 638-64.

El- Razik, A.K.A., Abdelrahman, K.A., Ahmed, Y.F., Gomaa, A.M. and Eldebaky, H.A. 2010. Direct identification of major pathogens of the bubaline subclinical mastitis in Egypt using PCR. *J. Anim. Sci*. **10**: 652 - 660.

Foster, T. 1996. Staphylococcus. In: Baron S. (ed.), *Medical Microbiology*. (4th Ed).

Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 12.

Available from: https://www.ncbi.nlm.nih.gov/books/NBK8448/

Hunt, D.E., Klepac-Ceraj, V., Acinas, S.G., Gautier, C., Bertilsson, S. and Polz, M.F. 2006. Evaluation of 23S *rRNA* PCR primers for use in phylogenetic studies of bacterial diversity. *Appl. Environ. Microbiol*. **72**(3): 2221-2225.

Islam, M.A., Samad, M.A. and Rahman, A.K.M.A. (2011). Bacterial pathogens and risk factors associated with mastitis in Black Bengal goats in Bangladesh. *Bangl. J. Vet. Med.* **9**: 155-159.

Jisha, S., Suja, R.S., Bibu, J. K., Surya, S., Ambily, R., Reni, J., Thresia, Vidya , V.K., Gayathri, P. and Usha, P. T.A. 2020. *In vitro* antimicrobial activity of lemongrass (Cymbopogon flexuosus) oil and citral against Methicillin Resistant *Staphylococcus aureus (*MRSA) from bovine mastitis. *J. Vet. Anim. Sci.* **51**(2): 184-188.

Jose, K. R., Vijayakumar, K., Justin, D. K., Shyma, V. H. and Ambily, R. 2021. Comparative efficacy of different treatment regimens against bovine mastitis caused by *Staphylococcus aureus. J. Vet. Anim. Sci.* **52**(1): 55-59.

Karmakar, A., Jana, D., Dutta, K., Dua, P. and Ghosh, C. 2018. Prevalence of Panton-Valentine leukocidin gene among community acquired *Staphylococcus aureus*: a real-time PCR study. *J. Pathog*. Article ID 4518541https://doi.org/10.1155/2018/4518541

Kini, M.M.A., Smitha, J.P., Deepa, P.M., Vijaykumar, K and Prejit. 2019. Aetiology of clinical goat mastitis and their occurrence in Wayanad District. *Pharm. Innov*. **8**(1): 405-406.

Kitti, T., Boonyonying, K. and Sitthisak, S. 2011. Prevalence of methicillin-resistant *Staphylococcus aureus* among university students in Thailand. *Southeast Asian J. Trop. Med.* **42**(6): 1498.

Kobayashi, N., Wu, H., Kojima, K., Taniguchi, K., Urasawa, S., Uehara, N., Omizu, Y., Kishi, Y., Yagihashi, A. and Kurokawa, I. 1994. Detection of *mec*A, *fem*A, and *fem*B genes in clinical strains of staphylococci using polymerase chain reaction. *Epidem. Infect*. **113**(2): 259-266.

Kumar, R., Yadav, B.R. and Singh, R.S. 2011. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. *J. Biosci.* **36**(1): 175-188.

Kumar, R., Gupta, D.K., Bansal, B.K., Singh, S., Sharma, S., Kumar, A. and Uppal, S.K. 2016. Prevalence, current antibiogram and risk factors associated with mastitis in dairy goats in Punjab. *Int. J. Sci .Environ. Tech.* **5**(6**)**: 4580-4593.

Mahoney, A.R., Safaee, M.M., Wuest, W.M. and Furst, A.L. 2021. The silent pandemic: Emergent antibiotic resistances following the global response to SARS-CoV-2. *Iscience.* p.102304.

Marogna, G., Rolesu, S., Lollai, S., Tola, S. and Leori, G. 2010. Clinical findings in sheep farms affected by recurrent bacterial mastitis. *Small Rumin. Res.* **88**(2-3**)**: 119- 125.

Mugabe, W., Nsoso, S.J., Mpapho, G.S., Kamau, J.M., Mahabile, W., Shah, A.A., Nazar, M., Khan, I.U., Kaka, N.A. and Shah, I.A. 2017. Occurrence of caprine mastitis and its etiological agents and associated selected risk in mid lactating goats in the oodi extension area of Kgatleng district, Botswana. *Acad. Web J. Agric. Res*. **2**(1): 14-20.

Nabih, A.M., Hussein, H.A., El-Wakeel, S.A., Abd El-Razik, K.A. and Gomaa, A.M. 2018. *Corynebacterium pseudotuberculosis* mastitis in Egyptian dairy goats. *Vet. World*. **11**(11): 1574.

Najeeb, M.F., Anjum, A.A., Ahmad, M.U.D., Khan, H.M., Ali, M.A. and Sattar, M.M.K. 2013. Bacterial etiology of subclinical mastitis in dairy goats and multiple drug resistance of the isolates. *J. Anim. Plant Sci.* **23**(6**)**: 1541-1544.

National Livestock Census. 2019. Published by Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare. *20th Livestock Census-2019. All India Report*. Government of India, New Delhi.

Obaidat, M.M., Salman, A.E.B. and Roess, A.A. 2018. High prevalence and antimicrobial resistance of *mec*A *Staphylococcus aureus* in dairy cattle, sheep, and goat bulk tank milk in Jordan. *Trop. Anim. Health Prod.* **50**(2): 405-412.

Pajić, M.J., Rašić, Z.B., Velebit, B.M., Boboš, S.F., Mihajlović-Ukropina, M.M., Radinović, M.Ž., Galfi, A.L., Petković, J.M. and Trojačanec, S.I. 2014. The prevalence of methicillin resistance and Panton-Valentine leukocidin synthesis genes in *Staphylococcus aureus* isolates of bovine and human origin. *Vet. Arhiv*. **8456**: 205-214.

Persson, Y., Börjesson, S., Myrenås, M. and Pedersen, K. 2021. No Detection of Methicillin-Resistant *Staphylococcus aureus* in Dairy Goats. *Dairy*. ***2*(1)**:.65-70.

Stapleton, P. D. and Taylor, P. W. 2002. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. *Sci. Prog.* **85**(1): 57-72.

Suchithra, S., Rathish R.L., Tresamol, P.V., Vinodkumar, K. and Seemanthini. R. 2015. Occurrence of MRSA in clinical mastitis of goats [abstract]. In: Compendium, XXXIII-ISVM Annual Convention and National Symposium On New Dimensions in Veterinary Medicine: Technological Advances, One Health Concept and Animal Welfare Concerns; 22 to 24 January 2015, Pookode. Kerala Veterinary and Animal Sciences University. p.227. Abstract No. PFI 5.4.

Tegegne, H.A., Florianová, M., Gelbíčová, T., Karpíšková, R. and Koláčková, I. 2019. Detection and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from bulk tank milk of cows, sheep, and goats. *FPD*. **16**(1): 68-73.

Tresamol, P.V., Suchithra, S., Rathish, R. and Saseendranath, M.R. 2018. Molecular identification of Staphylococcus aureus isolates from Subclinical Mastitis in Cattle. *Int. J. Livest. Res*. **8**(6): 67-71. doi.10.5455/ijlr20170804045412.

Yamagishi, N., Y. Jinkawa, K. Omoe, S. Makino, and K. Oboshi. 2007. Sensitive test for screening for *Staphylococcus aureus* in bovine mastitis by broth cultivation and PCR. *Vet. Rec.* **161**: 381–383. ■