Indian J Dairy Sci 77(5): 448-454
https://doi.org/10.33785/IJDS.2024.v77i05.008

RESEARCH ARTICLE

Isolation and Characterization of Methicillin Resistant *Staphylococcus aureus* (MRSA) from milk samples

Jubeda Begum[a] (✉) and Nasir Akbar Mir[b]

Received: 04 November 2023/ Accepted: 03 March 2024 / Published online: 23 October 2024

Abstract: This study reports the isolation and molecular characterization of methicillin-resistant *S. aureus* (MRSA) from sub-clinical mastitic milk samples of cows and does followed by the detection of multi-drug resistance profile of the isolates. The occurrence of MRSA was confirmed phenotypically as well as by molecular detection of the *mecA* gene. A total of 165 suspected mastitic milk samples were collected from cows (116) and does (49) in 2022 in Uttarakhand India. Out of 165 samples, 107 (64.8%) were positive for *S. aureus* (74 from cow and 32 from does samples) and 21 (19.6%) were positive for MRSA out of 107 (15 cow and 6 does samples). All the MRSA isolates were multidrug resistant. However, both MRSA and non-MRSA isolates were most sensitive to Chloramphenicol and complete resistance was observed against eight antibiotics including methicillin. The present study also revealed the resistance of MRSA isolates to antibiotics not used in veterinary medicine earlier. Our findings also highlights the detection of a significant percentage of MRSA in sub-clinical mastitic milk samples for the first time in the state of Uttarakhand, India; growing trends of multidrug resistance due to indiscriminate use of antibiotics and possible diffusion into the human food chain.

Keywords: Antibiotic resistance, sub-clinical mastitis, *mecA* gene, MRSA

Introduction

Staphylococcus aureus causes several infections in domestic animals but mastitis is the most significant one from dairy economy and public health point of view (Peacock and Paterson 2015). The *S. aureus* infections become even more complicated with the emergence of methicillin resistant *S. aureus* (MRSA). Though, methicillin is no longer used clinically, it represents the resistance against most of the β-lactam antibiotics in addition to some other alternative antimicrobials used commonly in clinical settings (Peacock and Paterson 2015; Begum and Mir 2023). The emergence of MRSA is associated with the acquisition of large mobile genetic element – staphylococcal cassette chromosome *mec* encoding a low-affinity penicillin binding protein having lower affinity for all β-lactam antibiotics which are mostly used in dairy animal mastitis treatment (Mahanti et al. 2020; Girmay et al. 2020; Derib et al. 2017; Srednik et al. 2019). Because of the strain exchange between humans and animals, the epidemiology of MRSA infections is quite complex (Parisi et al. 2016). Initially MRSA was confined to hospital and community settings, but now the human originated strains have adapted to livestock resulting in livestock-associated MRSA (LA-MRSA) (Mahanti et al. 2020; Price et al. 2012). The shedding of organisms in milk without organoleptic changes, predominance of backyard system of cattle rearing involving direct contact with animals, and continuous detection of MRSA in meat and milk (Mahanti et al. 2020; Giovanni et al. 2020; Carfora et al. 2016) have raised serious public and veterinary health concerns. Though, not reported in India, yet there are the reports showing MRSA transmission between humans and animals (Ferreira et al. 2011; Christaine et al. 2015; Smith, 2015).Therefore, there is a requirement of strict monitoring of bacterial populations at the human-animal interface. Owing to the potential zoonotic transmission of MRSA, there is a need of surveillance of antibiotic sensitivity profile, genotypic detection of pathogenic strains, and detection of cross resistance to other antibiotics to devise and adopt new therapeutic approaches (Mistry et al. 2016). The present study was carried out by using the samples collected from the suspected cases of mastitis presented at the veterinary clinical complex of College of Veterinary and Animal Sciences GBPUAT, Pantnagar and around Pantnagar campus. Therefore, this study was conducted to determine the prevalence of *S. aureus* induced mastitis by cultural

---

[a]Department of Veterinary Microbiology, Govind Ballabh Pant University of Agriculture & Technology, College of Veterinary & Animal Sciences, Pantnagar-263145, India
[b] Department of Animal Husbandry, Govt. of Jammu and Kashmir
Jubeda Begum (✉)
Email: jubedavet@gmail.com

and biochemical tests followed by detection of MRSA among the Staphylococcal isolates by antibiotic sensitivity testing and confirmation by detection of *mecA* gene by PCR technique.

Materials and Methods

Sampling

During 2021-22, 165 milk samples of each 10-15 mL volume were collected from 116 cows and 49 goats from Veterinary clinical complex and around Pantnagar campus. All the samples were collected from the suspected cases of mastitis and confirmed by California mastitis test. The milk samples from the affected animals were collected directly in sterile vials from all the teats equally and mixed. Before collection, first few strips of milk were discarded from each teat to prevent the contamination. The milk samples were transported to laboratory by maintaining cold chain and stored at 4 °C till further processing.

Isolation and identification of S. aureus

For isolation and maintenance of *S. aureus*, the method of Missiakas et al. (2013) was used with suitable modifications. The milk samples were inoculated into Brain heart infusion (BHI) broth (HiMedia, India) and incubated at 37 °C for overnight enrichment followed by subsequent inoculation on BHI agar (HiMedia, India). The colonies showing different morphologies were picked up and subjected to Gram staining; and the Gram positive colonies with bunch of grape appearances were further inoculated into selective media – Manitol salt agar (MSA) and Baird parker agar (BPA) for further identification. The pure culture of selected colonies of golden yellow colour on MSA and jet black colour with clear surrounding halo on BPA were further streaked on nutrient agar slant (HiMedia, India), incubated at 37 °C for 18-24 hrs, and stored at 4 °C for further biochemical confirmation. Also, the motility of the isolated bacterial isolates was checked by hanging drop method.

Biochemical confirmation of S. aureus

Pure culture isolates of *S. aureus* were further confirmed by various biochemical tests – catalase test, oxidase test, urease test, nitrate reduction test, indole test, methyl red test, Voges-prausker test, citrate utilization test, and fermentation of different sugars (glucose, sucrose, lactose, maltose, mannitol, mannose, trehalose and xylose) (Holt et al. 1994). The biochemical tests were conducted by using reference strain of *S. aureus* ATCC 25923 (Hi-Media) for confirmation of the species.

Antimicrobial susceptibility testing of S. aureus

Culturally and biochemically confirmed pure isolates of *S. aureus* were subjected to antimicrobial susceptibility testing by the agar disc diffusion method (Bauer et al. 1966) on Mueller-Hinton agar after adjusting the turbidity of the bacterial cultures to 0.5 Mc Farland turbidity standard. *Staphylococcus aureus* (ATCC 6538), standard methicillin-susceptible *Staphylococcus aureus* (MSSA, ATCC 25923) and methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33591) were used as reference strain for conducting the test. The isolates were tested against 17 different antibiotics (Hi-Media, India) of standardized concentrations (Table 1).The diameter of zone of inhibition around each antibiotic disc were measured to determine the sensitivity of the *S. aureus* isolates against each antibiotic following CLSI guidelines.

Molecular identification and confirmation of methicillin resistance in S. aureus

A loopful of *S. aureus* colonies from the nutrient agar slant were inoculated into BHI broth and incubated at 37°C overnight. The DNA extraction of *S. aureus* was done as per Hassanzadeh et al. (2016) with suitable modifications. The bacterial broth cultures were subjected to centrifugation at 10,000 rpm for 5 minutes and the pellets obtained were re-suspended in 500 µl of sterile phosphate buffer saline (PBS)solution (pH 7.4). The bacterial pellets were washed thrice with sterile PBS (pH 7.4) solution by centrifugation and finally re-suspended in 400 µl of nuclease free water (NFW). The suspension was boiled in a water bath for 10 minutes followed by snap chilling on ice for 10 minutes and centrifugation at 8,000 rpm for 10 minutes. The supernatant was collected in a sterile eppendorf tube and used as template for PCR reaction. Purity of the extracted DNA of each sample was measured by Nano Drop spectrophotometer (Thermo scientific) at OD 260/280.

The determinant of methicillin resistance in *S. aureus* is *mecA* gene which codes for Penicillin binding protein 2 (PBP-2). For the detection of *mecA* gene in the *S. aureus* isolates, the forward and reverse oligonucleotide primer sequences used in this study were 5’ACTGCTATCCACCCTCAAAC3' and 5'CTGGTGAAGTTGTAATCTGG3', respectively, with the product size of 169 bp (Oliveira and de Lencastre, 2002). The reaction mixture used in PCR for the detection of *mecA* gene was prepared by using PCR master mix (Thermo Scientific) following the manufacturer’s instructions. Final reaction mixture of 25µl was prepared by taking 12.5 µl of 2x PCR master mix (Thermo Scientific), 1 µl each of forward and reverse primers (10 µM), 5 µl of template DNA, and 5.5 µl of NFW.

Amplification parameters as described for *mecA* PCR were used with suitable modifications (Arakere et al. 2005). The amplification cycle consisted of denaturation at 95 °C for 5 minutes, followed by 40 cycles of initial denaturation at 94°C for 30 seconds, annealing at 57 °C for 1 min and extension at 72ºC for 1 minute, followed by final extension at 72ºC for 7 min and holding at 4°C. The confirmation of PCR product was done by electrophoresis of amplified products in 1.2 % agarose gel using 1x Tris acetate-EDTA (TAE) buffer in horizontal electrophoresis unit. For each sample 10 µl PCR product was mixed with 2 µl gel loading dye blue (6x) (Biolabs, #B7021S) and 5 µl of each mixed product were loaded in different wells. A positive (ATCC 25923) and negative control PCR product were also loaded in between the wells of samples. 5 µl of GeneRuler 100 bp plus DNA ladder (Thermo Scientific, SM0323) was also loaded in ladder lane.

Results and Discussion

Cultural and staining characteristics of bacterial isolates

Gram-staining of the bacterial colonies from culture media revealed the presence of Gram positive round shaped cocci, arranged in clusters or bunch of grape appearance. Motility test by hanging drop method revealed that the organisms are non-motile. On MSA, yellow colonies with yellow zones were observed after overnight incubation at 37°C due to the fermentation of mannitol and production of an acidic byproduct that causes the phenol red in the agar to turn yellow (Fig 1a). On BPA, black colonies surrounded by opaque halos due to tellurite reduction and lecithinase break down of the egg yolk in the media (Fig 1b) were observed. Similarly, on triple sugar iron agar both slant and butt of media were completely yellow coloured because of the drop in pH due to the fermentation byproducts of glucose, lactose, and sucrose in the medium. There was no blackening of the media as well as no gas produc-tion. All these characteristics features were indicative of *S. aureus*. However, among 165 confirmed cases of mastitis; 75 cases of cows and 32 cases of goats (107 = 64.8%) were positive for *S. aureus*. The remaining mastitis cases were of non-staphylococcal origin.

Biochemical profile of bacterial isolates

All the 107 pure culture isolates of *S. aureus* identified by Gram staining were positive for catalase, urease, nitrate, methyl red, voges-prausker, and citrate utilization tests while indole test and oxidase test were negative for all the isolates. The total 107 isolates fermented glucose, sucrose, maltose, lactose, mannitol, mannose, sucrose, and trehalose; but not xylose which confirm the presence of *S. aureus* microorganism.

Antibiogram of S. aureus

Among the 17 antibiotics tested, *S. aureus* was most sensitive to chloramphenicol followed by nitrofurantoin, vancomycin, and gentamicin (Fig 2; Table 1). Intermediate sensitivity was shown against gentamicin, vancomycin, tetracycline, enrofloxacin, bacitracin, penicillin G, and ciprofloxacin in the decreasing order. However, complete resistance was shown by *S. aureus* isolates against methicillin, trimethoprim, oxacillin, erythromycin, ceftriaxone, amoxycillin, azithromycin, and ampicillin.

Confirmation of MRSA by detection of *mecA* gene

Out of 107 confirmed pure isolates of *S. aureus*, 21 (19.6%) were positive for *mecA* gene expression with a product size of 169 kb (Fig 3, Table 2) (15 cow sample and 6 goat sample isolates) which confirms the presence of MRSA in mastitis cases of the study area. The overall prevalence of MRSA observed in this study was 12.7%. All the 21 confirmed MRSA isolates were resistant to at least four antibiotics tested and all the isolates showed resistance against the methicillin and oxacillin. This confirms the prevalence of multi-drug resistant MRSA in the mastitic animals of the study area and strong concurrence was observed between phenotypic and genotypic resistance.

Wide range of *Staphylococcus* species have been found to be associated with mastitis in dairy animals in addition to *S. aureus*

Table 1: Antibiotic sensitivity testing

S. No.	Antibiotics	Concentration/disc	Zone of inhibition (mm)*	Susceptibility
1.	Chloramphenicol	30 mcg	25 mm	S
2.	Vancomycin	10 mcg	17 mm	I
3.	Tetracycline	10 mcg	16 mm	I
4.	Penicillin G	10 units	13 mm	I
5.	Methicillin	30 mcg	-	R
6.	Bacitracin	10 units	15 mm	I
7.	Trimethoprim	30 mcg	-	R
8.	Gentamicin	10 mcg	17 mm	S
9.	Oxacillin	5mcg	-	R
10.	Erythromycin	15 mcg	-	R
11.	Ceftriaxone	10mcg	-	R
12.	Amoxycillin	30 mcg	-	R
13.	Azithromycin	15 mcg	-	R
14.	Nitrofurantoin	100 mcg	20 mm	S
15.	Ciprofloxacin	10 mcg	10 mm	I
16.	Ampicillin	25 mcg	-	R
17.	Enrofloxacin	10 mcg	16 mm	I

S: Susceptible, I: Intermediate, R: Resistant

* Represents mean values of samples against each antibiotics

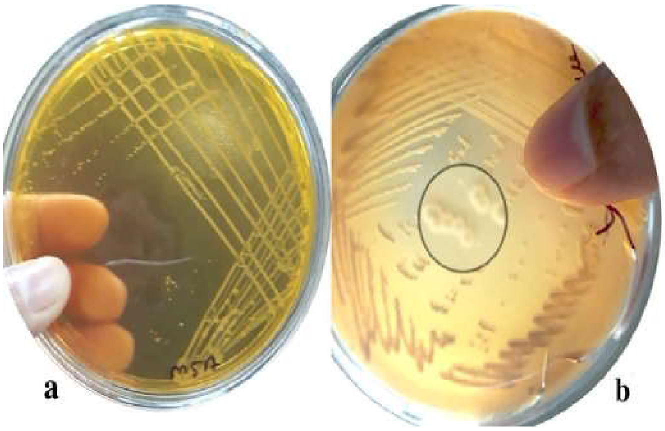

Fig. 1 a) Yellow colonies of *S. aureus* on MSA
b) Black colonies of *S. aureus* surrounded by opaque halos

Fig 2: Antibiotic sensitivity of *S. aureus* against different antibiotics

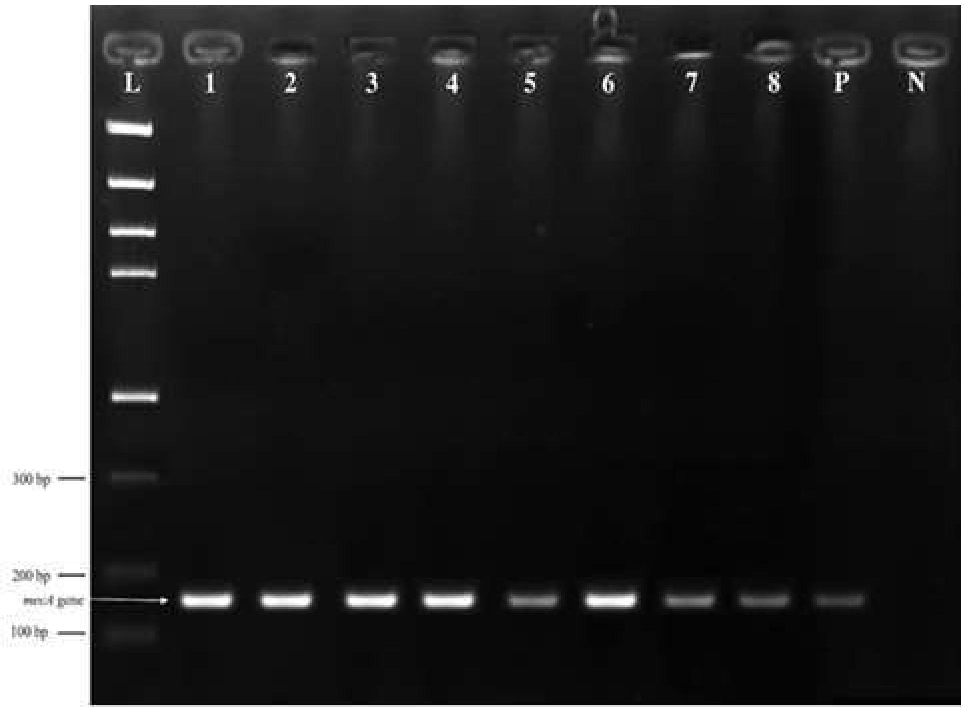

Fig. 3 Gel electrophoresis analysis of PCR amplification product showing presence of *mecA*gene (169 bp) in all the phenotypically identified MRSA isolates
L: Ladder(GeneRuler 100 bp plus DNA ladder)
1-8: MRSA isolates(All cow isolates)
P: Positive control(ATCC 25923)
N: Negative control

and have been reported to carry the genetic determinants of methicillin resistance (Shrivastava et al. 2017; Febler et al. 2010). On the other hand, various researcher have reported the occurrence of MRSA strains sensitive to oxacillin (Mistry et al. 2016; Pu et al. 2014; Pournaras et al. 2013; Kumar et al. 2013) which has put a question mark on the reliability of phenotypic tests in the detection of MRSA in food animals. Therefore, accurate identification of *Staphylococcus* species carrying methicillin resistance determinant by employing phenotypic tests as well as by molecular assays simultaneously cannot be overlooked.

In the present study, isolates *S. aureus* were observed as Gram-positive, round shaped cocci, arranged in clusters or bunch of grape appearance with yellowish colonies on MSA, black colonies surrounded by opaque halos on BPA, and there was production of acidic butt as well as slant in triple sugar iron agar. All the pure culture isolates obtained in this study revealed typical biochemical characteristics of *S. aureus*. Similar staining, cultural, and biochemical characteristics of *S. aureus* were also reported earlier (Habib et al. 2015; Konuku et al. 2012). This study revealed very high prevalence (64.8%) of *S. aureus* in mastitic milk of cattle and goat. However, owing to different animal

Table 2: MRSA prevalence in milk

Species	No. of samples collected	No. of samples positive for *S. aureus*	No. of samples positive for mecA gene	Over all prevalence of MRSA
Goat milk	49	32 (65.30%)	15 (20%)	21/165x100
Cow milk	116	75(64.65%)	6 (18.75%)	= 12.70%
Total	165	107 (64.8%)	21 (19.6%)	

husbandry practices, animal health delivery systems, sample collection procedures, study areas, and bacterial isolation techniques, the prevalence of *S. aureus* in milk and milk products vary widely from 7.0% to 84.0% (Mahanti et al. 2020; Girmay et al. 2020; Giovanni et al. 2020; Ananya and Pranab, 2015). In India, a detailed survey of the existing literature on mastitis infection in dairy animals from northern and north-eastern regions revealed very high prevalence of *S. aureus* (47.86% – 83.72%) which is comparatively higher than other Asian countries.

The worrisome aspect of mastitis infection in India is the much higher prevalence of *S. aureus* and thus, the consequent possibility of widespread MRSA in animals as well as humans. Though, there is no evidence of MRSA anthropozoonosis in India, the probability cannot be ruled out because the zoonotic transmission of MRSA between farm animals and humans have been reported outside India (Ferreira et al, 2011; Christaine et al. 2015; Smith, 2015; Pillai and Reji 2023). In this study, 19.6% of *S. aureus* were positive of *mecA*gene and the prevalence of MRSA in sub-clinical mastitic milk was 12.7%. These findings are alarming in highlighting the dangers of collecting milk from diseased animals as these *mecA* possessing *S. aureus* may enter the human food chain. In India, the prevalence of MRSA in dairy animals based on the molecular detection of genetic determinants of methicillin resistance fall in a wide range of 5.4% to 29.41% (Mahanti et al. 2020; Shrivastava et al. 2017; Kumar et al. 2010; Chandrasekaran et al. 2014; Kutar et al. 2015). The variation in the prevalence of *mecA* gene may be as a result of differential selection pressure imposed by the differences in the preference of antibiotic use in different places. In Turkey, MRSA prevalence of 9% was reported from buffalo milk and milk products by detection of *mecA* gene determinant (Saka and Terzi Gulel, 2018) and in Italy 4% dairy farms and 3% bulk tank milk samples tested positive for MRSA (Giovanni et al. 2020).

In this study, all the isolates of *S. aureus* were most sensitive to chloramphenicol followed by nitrofurantoin, vancomycin, and gentamicin. Complete resistance was observed against methicillin, trimethoprim, oxacillin, erythromycin, ceftriaxone, amoxycillin, azithromycin, and ampicillin. All the phenotypically identified MRSA possessed *mecA* gene determinant and this concurrence between phenotypic and genotypic detection was reported by other researchers too (Mahanti et al. 2020; Karmakar et al. 2016; Bhattacharyya et al. 2016). Even resistance was shown against the antibiotics which are not used in treatment of dairy animals, such as azithromycin, which indicates the environmental transfer of resistance genes from human to animal settings. In fact, resistance against the antimicrobials can be found which are never used in animal settings (Gebreyes et al. 2006; Mollenkopf et al. 2014) and similarly a tobramycin resistant MRSA was detected in buffalo milk, an antibiotic which is rarely used in veterinary medicine (Giovanni et al. 2020). However, all the *S. aureus* isolates were completely susceptible to chloramphenicol and nitrofurantoin because they are used rarely in animals and similar sensitivity pattern was observed earlier (Mahanti et al. 2020). Furthermore, the most worrisome finding of this study was that, all the MRSA isolates were multidrug resistant with resistance to at least four antibiotics tested. This multidrug resistance have been reported earlier (Mahanti et al. 2020; Girmay et al. 2020; Carfora et al. 2016; Bhattacharyya et al. 2016) and is emerging at a much rapid rate now.

Conclusion

This study revealed high prevalence of *S. aureus* as a major cause of sub-clinical mastitis in dairy animals and detection of MRSA isolates for the first time in the state, Uttarakhand, India. Though, chloramphenicol and nitrofurantoin were most effective antibiotics, all the MRSA isolates were multidrug resistant. Detection of multidrug resistant MRSA in milk samples represents a serious food safety and public health concern because of the possibility of diffusion of methicillin resistance genes into human health settings via adulterations in food chain. This study also points out the potential transfer of resistance genes from humans to animals because antibiotic resistance of *S. aureus* isolates against the antibiotics not used in veterinary medicine were observed and thus, warrants the judicious use of antimicrobials in animal and human health systems, simultaneously.

Conflict of interest

The authors declare no conflict of interest of any kind arising out of this manuscript

Acknowledgement:

The authors would like to acknowledge Dean, College of Veterinary and Animal Sciences and Head of Department of Veterinary Microbiology, GBPUAT, Pantnagar for the moral support and other lab facilities.

References

Ananya M, Pranab BM (2015) Detection of staphylococci in raw milk and milk products and evaluation of their antibiotic sensitivity: a report from Southern Assam, India. IOSR J Environ Sci Toxicol Food Technol 9(1): 17-22

Arakere G, Nadig S, Swedberg G, Macaden R, Amarnath SK and Raghunath D (2005) Genotyping of methicillin-resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, South India. J Clin Microbiol 43(7): 3198-3202

Bauer AW, Kirby WM, Sherris JC and Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45(4): 493-496.

Begum J, Mir NA (2023) Molecular mechanisms of biofilm resistance against antibiotics. Lett Anim Biol 03(1): 17-27

Bhattacharyya D, Banerjee J, Bandyopadhyay S, Mondal B, Nanda PK, Samanta I. Mahanti A, Das AK, Das G, Dandapat P and Bandyopadhyay S (2016) First report on vancomycin-resistant Staphylococcus aureus in bovine and caprine milk. Microb Drug Resist 22(8): 675-681

Carfora V, Giacinti G, Sagrafoli D, Marri N, Giangolini G, Alba P, Feltrin F, Sorbara L, Amoruso R, Caprioli A, Amatiste S and Battisti A (2016) Methicillin- resistant and methicillin-susceptible *Staphylococcus aureus* in dairy sheep and in- contact humans: an intra-farm study. J Dairy Sci 99: 4251-4258

Chandrasekaran D, Venkatesan P, Tirumurugaan KG, Nambi AP, Thirunavukkarasu PS, Kumanan K, Vairamuthu S and Ramesh S (2014) Pattern of antibiotic resistant mastitis in dairy cows. Vet World 7(6): 389-394

Christaine C, Lothar HW and Wolfgang W (2015) Livestock-Associated MRSA: The Impact on Humans. Antibiotics (Basel) 4(4): 521-543

Derib BT, Birhanu BT, Tessema TS and Wubshet AK (2017) Isolation and identification of methicillin resistant *Staphylococcus aureus* from bovine mastitic milk in and around Wolaita Sodo, Southern Ethiopia. J Vet Sci Res 2(3): 000136. https://medwinpublishers.com/OAJVSR/OAJVSR16000136.pdf

Febler AT, Billerbeck C, Kadlec K and Schwarz S (2010) Identification and characterization of methicillin-resistant coagulase-negative staphylococci from bovine mastitis. J Antimicrob Chemother 65: 1576-1582

Ferreira JP, Kevin LA, Maria TC, Roberta L, Felicia R and Reller B (2011) Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. PloS One 6(11): e26978

Gebreyes WA, Thakur S, Morrow WE (2006) Comparison of prevalence, anti- microbial resistance, and occurrence of multidrug-resistant salmonella in anti- microbial-free and conventional pig production. J Food Protect 69: 743-748

Giovanni, N, Marta C, Elisa S, Rosa F, Loredana C, Alessandra B and Antonio P (2020) Occurrence and characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in buffalo bulk tank milk and the farm workers in Italy. Food Microbiol 91: 103509. https://doi.org/10.1016/j.fm.2020.103509

Girmay W, Gugsa G, Taddele H, Tsegaye Y, Awol N, Ahmed M and Feleke A (2020) Isolation and Identification of Methicillin-Resistant Staphylococcus aureus (MRSA) from Milk in Shire Dairy Farms, Tigray, Ethiopia. Vet Med Int 8833973. https://doi.org/10.1155/2020/8833973

Habib F, Rind R, DuraniN, Bhutto AL, BuriroRS, TunioA, Aijaz N, Lakho SA, Bugti AG and Shoaib M (2015) Morphological and cultural characterization of *Staphylococcus aureus* isolated from different animal species. J Appl Environ Biol Sci 5(2): 15-26

Hassanzadeh S, Pourmand MR, Afshar D, Dehbashi S, Mashhadi R (2016) TENT: A rapid DNA extraction method of *Staphylococcus aureus*. Iran J Pub Health 45(8): 1093-1095

Holt JG, Krieg NR, Sneath PHA, Stanley JT and William ST (1994) Bergey's Manual of Determinative Bacteriology. Williams and Wilikins, Baltimore, pp 786-788.

https://doi.org/10.1089/fpd.2014.180

Karmakar A, Dua P, Ghosh C (2016) Biochemical and molecular analysis of *Staphylococcus aureus*clinical isolates from hospitalized patients. Can J Infect Dis Med Microbiol 9041636: 1-7. http://doi.org/10.1155/2016/9041636

Konuku S, Rajan MM and Muruhan S (2012) Morphological and biochemical characteristics and antibiotic resistance pattern of *Staphylococcus aureus* isolated from grapes. Int J Nut Pharmacol Neurol Dis 2: 70-73

Kumar R, Yadav BR and Singh RS (2010) Genetic determinants of antibiotic resistance in Staphylococcus aureus isolates from milk of mastitic crossbred cattle. Curr Microbiol 60(5): 379-386. https://doi.org/10.1007/s00284-009-9553-1

Kumar VA, Steffy K, Chatterjee M, Sugumar M, Dinesh KR, Manoharan A, Karim S and Biswas R (2013) Detection of oxacillin- susceptible *mecA*-positive *Staphylococcus aureus* isolates by use of chromogenic medium MRSA ID. J ClinMicrobiol 51(1): 318-319. https://doi.org/10.1128/JCM.01040-12

Kutar K, Verma AK, Sharma B, Kumar A and Yadav SK (2015) Analysis of *mecA*gene and antibiotic resistance in *Staphylococcus aureus* isolates from bovine mastitis. Indian J Comp Microbiol Immunol Infect Dis 36(1): 22-27. https://doi.org/10.5958/0974-0147.2015.00005.7

Mahanti A, Joardar SN, Bandyopadhyay S, Banerjee J, Ghosh S, Batabyal K, Sar TK, Dutta TK and Samanta I (2020) Characterization of methicillin-resistant and enterotoxins producing *Staphylococcus aureus* in bovine milk in India. J Agri Food Res 2: 100017. https://doi.org/10.1016/j.jafr.2019.100017

Missiakas, Dominique M, Olaf Schneewind (2013) Growth and laboratory maintenance of Staphylococcus aureus. In: *Current protocols in microbiology*. Chapter 9: Unit 9C.1. doi:10.1002/9780471729259.mc09c01s28

Mistry H, Sharma P, Mahato S, SaravananR, Kumar PA and Bhandari V (2016) Prevalence and characterization of oxacillin susceptible *mecA*- positive clinical isolates of *Staphylococcus aureus*causing bovine mastitis in India. PLoS One 11(9): e0162256. https://doi.org/10.1371/journal.pone.0162256

Mollenkopf DF, Cenera JK, Bryant EM, King CA, Kashoma I, Kumar A, Funk JA, Rajashekara G and Wittum TE (2014) Organic or antibiotic-free labelling does not impact the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken. Foodborne Pathog Dis 11(12): 920-929

Oliveira DC and de Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the *mec*element in methicillin resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 46(7): 2155-2161

Parisi A, Caruso M, Normanno G, Latorre L, Sottili R, Miccolupo A, Fraccalvieri R, Santagada G (2016) Prevalence, antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in bulk tank milk from southern Italy. Food Microbiol 58: 36-42. https://doi.org/10.1016/j.fm.2016.03. 004

Peacock SJ, Paterson GK (2015) Mechanisms of methicillin resistance in *Staphylococcus aureus*. Ann Rev Biochem 84: 577-601.

Pournaras S, Stathopoulos C, Tsakris A (2013) Oxacillin-susceptible MRSA: could it become a successful MRSA type? Future Microbiol 8(11): 1365-1367. https://doi.org/10.2217/fmb.13.118

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al (2012) *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 3(1): e00305 e00311.https://doi.org/10.1128/mBio.00305-11

Pu W, Su Y, Li J, Li C, Yang Z, Deng H, Ni C (2014) High incidence of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) associated with bovine mastitis in China. PLoS One 9(2): e88134. https://doi.org/10.1371/journal.pone.0088134

Saka E, Terzi Gulel G (2018) Detection of enterotoxin genes and methicillin-resistance in Staphylococcus aureus isolated from water buffalo milk and dairy products. J Food Sci 83: 1716-1722

Pillai VPS, Reji SA (2023) The role of veterinary research in human health promotion. Lett Anim Biol 03(2): 16-22

Shrivastava N, Sharma V, Nayak A, Shrivastava AB, Sarkhel BC, Shukla PC, Arpita S (2017) Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) mastitis in dairy cattle in Jabalpur, Madhya Pradesh. J Anim Res 7(1): 77-84

Smith TC (2015) Livestock- Associated *Staphylococcus aureus*: The United States Experience. PLoS Pathog 11(2): e1004564. https://doi.org/10.1371/journal.ppat.1004564

Srednik ME, Crespi E, Testorelli MF, Puigdevall T, Pereyra AMD, Rumi MV, Caggiano N, Gulone L, Mollerach M, Gentillini ER (2019) First isolation of a methicillin-resistant *Staphylococcus aureus* from bovine mastitis in Argentina. Vet Anim Sci 7: 100043. https://doi.org/10.1016/j.vas.2018.11.004