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Livestock, pets and humans as carriers of methicillin-resistant *Staphylococcus aureus* and comparative evaluation of two PCR protocols for detection

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Abstract

Staphylococcus aureus are Gram positive bacteria known to acquire antibiotic resistance rapidly and pose a major challenge to clinicians worldwide. Infections by methicillin resistant *Staphylococcus aureus* (MRSA) are usually associated with increased mortality and prolonging of treatment. Samples (n = 706) from diverse sources (livestock, pets, animal handlers, human hospital) were collected and screened for the presence of MRSA by phenotypic and genotypic methods. The incidence of *Staphylococcus aureus* was greater in goats (42.00%; 28.20 - 56.80%, confidence interval [CI] 95.00%) followed by cattle (13.50%; 9.20 - 18.80%, CI 95.00%), humans (12.90%; 9.30 - 17.40%, CI 95.00%) and dogs (12.90%; 8.10 - 19.20%, CI 95.00%). Significantly higher incidence of MRSA was observed in dogs (65.00%; 40.80 - 84.60%, CI 95.00%), compared to other hosts namely cattle (48.00%; 26.50 - 64.30%, CI 95.00%), humans (35.00%; 20.20 - 52.50%, CI 95.00%) and goats (10.00%; 1.20 - 30.40%, CI 95.00%). All the *S. aureus* isolates were further screened for thermostable nuclease (*nuc* gene) by polymerase chain reaction (PCR). The incidence of *nuc* gene in cattle, dog, goat and human were found to be 3.30% (1.30 - 6.60%, CI 95.00%), 5.20% (2.30 - 9.90%, CI 95.00%), 28.00% (16.20 - 42.50%, CI 95.00%) and 9.10% (6.00 - 13.00%, CI 95.00%), respectively. Comparative evaluation of two PCR primers (*mecA*-162 and *mecA*-310) indicated the former one as more rational choice for detection of MRSA. Overall, the results of our study indicated possible risk of zoonotic transmission of MRSA from canines.

Introduction

Staphylococcus aureus is a significant pathogen of humans and animals and is also a commensal found on body surfaces of hosts.[1] The organism is responsible for a variety of infections both animals and humans. In humans *S. aureus* causes skin and soft tissue infections, food poisoning by enterotoxic strains, toxic shock syndrome and scalded skin syndrome among others. In animals the organism causes mastitis in livestock, skin and soft tissue infections in companion animals.[1-3]

One notable feature of *S. aureus* is the emergence of methicillin resistance strains which have the ability to withstand a wide range of antibiotics causing palpable clinical concern.[1] Owing to the zoonotic nature of *S. aureus*, global dissemination of methicillin resistant *Staphylococcus aureus* (MRSA) raised the risks of transfer of not only the pathogen, but its antimicrobial resistance traits also. Methicillin resistance in *S. aureus* is mediated by altered form of penicillin binding protein (PBP), encoded by *mecA* gene.[1-3] However, another determinant, *mecC* has also been identified in *S. aureus*.[4] In addition to antimicrobial resistance traits, MRSA are also known to carry virulence determinants including thermostable nuclease encoded by *nuc* gene which reportedly help the organism to escape host defence. The *nuc* gene had previously been reported in MRSA from Iran and India.

During last decades, several reports of MRSA from various sources have been reported and well-reviewed by a number of researchers.[1-5] Compared to global landscape,

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reports on MRSA in domestic animals and pets from India are rarer considering the vast livestock numbers and under-regulated use of antimicrobials in human and veterinary medicine.[5] However, researchers have documented prevalence of MRSA in various hosts, though reports from bovines tend to predominate.[5-8] In a systematic meta-analysis, Krishnamoorthy *et al*.[9] estimated that prevalence of MRSA was around 10.00% in livestock in India. Despite these available reports, there is even a smaller number of reports available from north-eastern parts of the country, which is witnessing a surge in dairy husbandry due to government impetus.[10] Available reports from north-eastern part of India are mainly from pigs.[11]

Reports of MRSA in humans in India have been reviewed by several authors and a large multi-year, multicentre assessment pegged the prevalence of MRSA in humans at 4.50%.[12-15] As in livestock associated MRSA, reports of MRSA in humans are infrequent from north-eastern part of India, though some researchers documented the occurrences in healthcare settings.[16-18]

In addition to available phenotypic methods for detection of MRSA, a number of molecular tools are also available including polymerase chain reaction (PCR), real-time PCR, and other assays.[19-20] Among, many available methods, PCR based methods have their appeal in terms of simplicity, rapidity and falling costs per sample. Of the PCR based methods, two protocols, one reported by Geha *et al*.[21] and another by Oliveira and de Lencastre.[22] have been widely used and the latter have even been recommended by Danish Technical University of European Union for detection of *mecA*. In order to enable researchers and laboratorians to make informed decision on choice of PCR protocols it is necessary to have comparative analysis of popular PCR protocols which is lacking.

Considering these gaps in data on prevalence of MRSA in north-eastern India and the paucity of data on comparative evaluation of PCR protocols, in the present study we reported the occurrence of MRSA in various hosts including humans, pet dogs and livestock. Further, we reported the comparative evaluation of two popular PCR protocols for evidence-based decision making for choice of assay.

Materials and Methods

Sample collection. During the study, 706 samples were collected from cattle (n = 215), goat (n = 50), dog (n = 155), people associated with animal and clinical samples (n = 286) from human hospital (Table 1). Bovine samples were collected from various cases of mastitis from cattle farms in and around Guwahati city, India (26.1445° N, 91.7362° E). Goat samples were collected from clinical mastitis cases and wound infections from organized goat farm (26.0515° N, 91.8696° E). Canine samples were collected from Teaching Veterinary Clinical Complex, College of Veterinary Science, Guwahati, India. On the other hand, human samples (nasal swabs and hand swabs) were collected from people associated with animals and anonymized culture isolates were obtained from the Gauhati Medical College and Hospital, Guwahati. Prior to collection of samples informed consents were obtained in all cases. The study was approved by the Institutional Ethics Committee of Gauhati Medical College and Hospital, Guwahati (MC/190/2007/Pt.II/36) and Institute Animal Ethics Committee of Veterinary College, AAU, Khanapara, Guwahati (770/ac/CPCSEA/FVSc/AAU/IAEC/16-17/446). All samples were collected aseptically in sterile vials, brought to laboratory under chilled conditions and were processed within 6 hr for microbiological analysis.

Isolation and identification. Samples were processed for isolation of *S. aureus* employing standard bacteriological procedures. Briefly, samples were enriched overnight in brain heart infusion (BHI) broth (HiMedia, Mumbai, India) at 37.00˚C. A loopful of enriched broth was streaked onto mannitol salt agar (MSA; HiMedia) and incubated at 37.00˚C for 24hr. Suspected colonies were further streaked to Baird-Parker agar (BPA) plates (Himedia). Typical shiny jet-black colonies with a halo around them were further characterized for biochemical and morphological features including Gram's staining, catalase and coagulase production, fermentation of sugars as per methods described previously.[23] Culture isolates obtained from hospital were also checked for their purity and identification by subjecting them to all tests described above. In all tests, *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used as controls as appropriate.

DNA extraction. DNA from isolates was extracted with GeneJET Genomic DNA purification kit (Thermo Fisher Scientific, Mumbai, India), as per manufacturer's guidelines. Extracted DNA was checked for purity using NanoDrop (Thermo Fisher Scientific).

Molecular detection of *Sa442* gene in *S. aureus* isolates. Molecular confirmation of isolates was undertaken as described by Martineau *et al*. previously by targeting *Sa442* genes of *S. aureus* using specific primer which gives amplification of 108 bp DNA (Table 2).[24] The PCR blend comprised of 2.00 µL of extracted DNA, 10.00 µL 2X master mix (Thermo Fisher Scientific, Waltham, USA) 10.00 pmol each of forward (5′-AATCTTTGTCG-GTACACGATATTCTTCACG-3′) and reverse (5′-CGTAATG-AGATTTCAGTAGATAATACAACA-3′) primers in 20.00 µL volume made up with nuclease free water. Cycling condition of the PCR consisted of preheating at 95.00 ˚C for 3 min, followed by 40 cycles of denaturation (94.00 ˚C for 30 sec), annealing (58.00 ˚C for 30 sec) and extension (72.00 ˚C for 30 sec) followed by final extension of 72.00 ˚C at 3 min. Appropriate positive and negative controls

(*Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922) were included in all PCR runs. On completion of PCR, amplicons were electrophoresed in 1.50% agarose and visualized under ultraviolet (UV) illumination (DNR Sigma, Neve Yamin, Israel).

***In vitro* susceptibility testing for cefoxitin.** *In vitro* susceptibility testing of all the isolates of *S. aureus* was performed by disc diffusion technique according to European Committee on Antimicrobial Susceptibility Testing (EUCAST).[25] Antimicrobial disc (HiMedia) of cefoxitin (30.00 µg) were used. Isolates grown overnight in Muller Hinton broth (HiMedia) were layered on Muller-Hinton (MH) agar plate (HiMedia) and antimicrobial discs were placed onto MH agar plates at a distance of approximately 2.50 cm and incubated overnight at 37.00 ˚C. The zone of inhibition around disc was measured to the nearest millimetre and interpreted as per EUCAST reading guidelines.[25] An inhibition zone of < 22.00 mm for cefoxitin (30.00 µg) was interpreted as methicillin resistant.

Molecular detection of *mecA* gene in *S. aureus* isolates. For detection of *mecA* gene two separate primer pairs (Table 2) were employed as described previously by Oliveira and de Lencastre,[22] and Geha *et al.*[21] producing amplicons of 162 bp and 310 bp, respectively. Amplification mix of the reaction was prepared with 2.00 µL extracted DNA, 10.00 µL 2X master mix (Thermo Fisher Scientific) 10.00 pmol each of forward and reverse primers in a final volume of 20.00 µL. The PCR mix were thermocycled (Applied Biosystems, San Francisco, USA) for 40 cycles 94.00 ˚C for 45 sec, 59.00 ˚C for 1 min, 72.00 ˚C for 40s followed by 72.00 ˚C at 7 min. For all PCR runs, known positive control DNA from laboratory and appropriate negative control were employed. Amplified products were gel electrophoresed and visualized in a Gel documentation system (DNR Sigma).

Detection of *nuc* gene. The PCR assay for *nuc* gene was performed with primer pairs (Table 2) described by Zhang *et al.*[26] The reaction blend (20.00 µL) was optimized with 1.00 µL of extracted DNA with 10.00 µL of 2X Master-mix (Thermo Fisher Scientific) 10.00 pmol each of forward (5'-GCGATTGATGGTGATACGGTT-3') and reverse (5′-AGC-CAAGCCTTGACGAACTAAAGC-3′) primers. The PCR was performed in a thermocycler (Applied Biosystems, Foster City, USA) with annealing at 52.00 ˚C. The PCR amplified products were electrophoresed on 1.50% agarose gel and visualized under UV illumination (DNR Sigma). Previously known positive control DNA from laboratory and negative control (nucleic acid blank) were employed in each run.

Table 1. Details of samples analyzed, MRSA isolated (cefoxitin resistant) and genes detected among the isolates in the study.

Species	Sample type	Number collected	*S. aureus* isolated	MRSA isolated	*mecA* (310 bp)	*mecA* (162 bp)	*nuc*
Dog (n = 155)	Nasal swab	107	2	0	0	0	1
	Dermatitis	21	5	3	3	3	2
	Abscess	27	13	10	10	10	5
Cattle (n = 215)	Mastitic milk	17	15	3	3	5	2
	Milk	140	5	2	1	2	1
	Abscess	12	7	7	6	6	3
	Nasal swab	46	2	1	1	1	1
Goat (n = 50)	Mastitic milk	4	1	1	0	1	0
	Milk	6	1	1	0	1	0
	Abscess	2	0	0	0	0	0
	Nasal swab	38	19	0	0	0	14
Human (n = 286)	Hand swab	23	1	0	0	0	0
	Nasal swab	23	0	0	0	0	0
	Urine culture isolates*	85	14	3	3	3	9
	Pus isolates*	63	16	7	7	8	12
	Blood isolates*	91	5	2	1	3	4
	Sputum isolates*	1	1	1	1	1	1
Total (n = 706)		**706**	**107**	**41**	**36**	**44**	**55**

* Isolates from hospital.

Table 2. Oligonucleotide primers used in the study.

Target gene	Primer sequences (5′-3′)	Amplicon size (bp)	References
Sa442	F: 5′-AATCTTTGTCGGTACACGATATTCTTCACG-3′ R: 5′-CGTAATGAGATTTCAGTAGATAATACAACA-3′	108	24
***mecA*-162**	F: 5'-TCCAGATTACAACTTCACCAGG-3' R: 5'-CCACTTCATATCTTGTAACG-3'	162	22
***mecA*-310**	F: 5'-GTAGAAATGACTGAACGTCCGATAA-3' R: 5'-CCAATTCCACATTGTTTCGGTCTAA-3'	310	21
nuc	F: 5'-GCGATTGATGGTGATACGGTT-3' R: 5′-AGCCAAGCCTTGACGAACTAAAGC-3′	279	26

Statistical analysis. Univariate analyses (Fisher's exact test, Pearson's chi-square test, Cohen's Kappa) were performed with SPSS Software (version 27; IBM Corp., Armonk, USA). A *p* value of ≤ 0.05 was considered statistically significant.

Results

Out of 706 samples, 107 presumptive isolates were obtained which showed biochemical reactions and cellular morphology conforming to *S. aureus* (Fig. 1). These presumptive were subjected to PCR amplification of the conserved gene using *Sa442* primers which yielded a single amplicon of 108 bp (Fig. 2) confirming the identity of the all isolates as *S. aureus*. Among the 107 *S. aureus* isolates, 29 (13.50%; 9.20 - 18.80%, confidence interval [CI] 95.00%) were isolated from cattle and 20 (12.90%; 8.10 - 19.20%, CI 95.00%), 21 (42.00%; 28.20 - 56.80%, CI 95.00%) and 37 (12.90%; 9.30 - 17.40%, CI 95.00%) were isolated from dog, goat and human, respectively (Table 1).

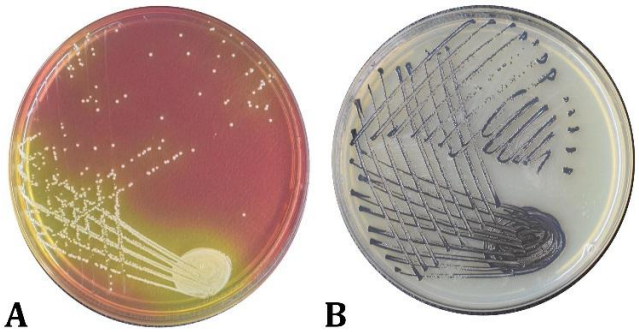

Fig. 1. Colony morphology of *S. aureus* on **A)** mannitol salt agar and **B)** Baird-Parker agar.

In order to detect MRSA, all 107 isolates of *S. aureus* were subjected for *in vitro* susceptibility test as per EUCAST guidelines. Standardization of PCRs for *mecA* gene yielded clear unambiguous specific amplification of *mecA* gene with product sizes of 162 bp and 310 bp (Fig. 2). Upon optimization of PCR protocols of *mecA* gene all isolates of *S. aureus* were screened and results indicated that a total of 44 isolates were identified as MRSA by *mecA*-162 PCR while 36 isolates were identified as MRSA by *mecA*-310 PCR (Table 1). The analyses indicated that a substantial fraction of *S. aureus* isolated from canine (65.00%; 40.80 - 84.60%, CI 95.00%) and cattle samples (48.00%; 26.50 - 64.30%, CI 95.00%) were MRSA (Fig. 3). While proportion of MRSA in human origin *S. aureus* isolates was 35.00% (20.20 - 52.50%, CI 95.00%), the same was the lowest in case of caprine isolates (10.00%; 1.20 - 30.40%, CI 95.00%).

The PCR amplification of the *nuc* gene produced a single amplicon of 279 bp (Fig. 2). Our results indicated that overall, 51.40% (41.50 - 61.20%, CI 95.00%) of the *S. aureus* isolates possessed *nuc* gene (Table 1). Species-wise, the highest carriage rate was observed among samples of caprine origin (28.00%; 16.20 - 42.50%, CI 95.00%) followed by human (9.10%; 6.00 - 13.00%, CI 95.0.0%), canine (5.20%; 2.30 - 9.90%, CI 95.00%) and bovine isolates (3.30%; 1.30 - 6.60%, CI 95.00%).

Considering the discrepancy, we went ahead with comparative analysis of *mecA* primers to adjudge the applicability of the primers for routine detection of MRSA employing PCR. We assumed the results of cefoxitin disc test as standard as recommended by EUCAST.[25] Results revealed that *mecA*-162 PCR was more sensitive compared to *mecA*-310 PCR, though, the latter appeared more specific (Table 3).

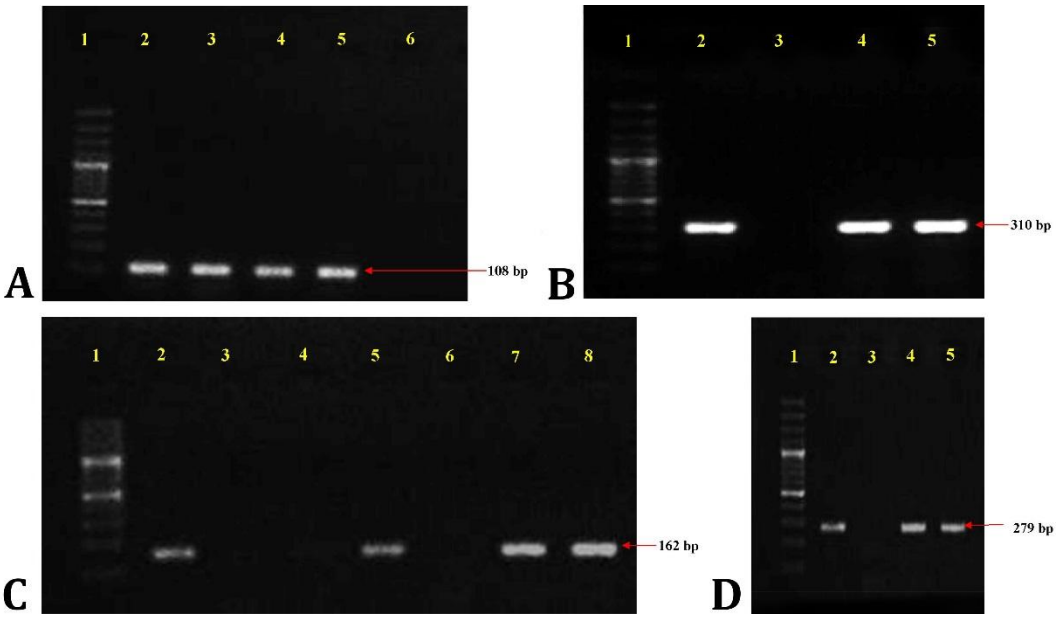

Fig. 2. Molecular detections of **A)** *S. aureus Sa442* gene (108 bp), **B)** *mecA* gene 310 bp, **C)** *mecA* gene (162 bp), and **D)** *nuc* gene (279 bp).

Table 3. Sensitivity, specificity and measures of agreement of PCR protocols evaluated in the study

PCR protocol	Sensitivity	Specificity	Kappa (measure of agreement)	References
***mecA*-162**	1.00 (0.89 - 1.00)*	0.95 (0.86 - 0.98)*	0.941	22
***mecA*-310**	0.88 (0.73 - 0.95)*	1.00 (0.93 - 1.00)*	0.899	21

* indicates lower limit and upper limit at 95.00% confidence interval.

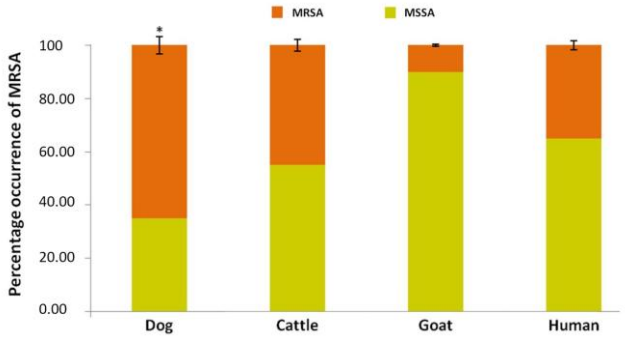

Fig. 3. Relative incidence of methicillin resistant *S. aureus* among various hosts. (MRSA – methicillin resistant *Staphylococcus aureus*; MSSA – methicillin susceptible *Staphylococcus aureus*). * indicates significant difference compared to the other species ($p < 0.05$).

Discussion

Indiscriminate uses of antibiotics in human and veterinary medicines have been implicated for emergence of antimicrobial resistant bacteria worldwide. Of the many pathogens, which acquired resistance to antibiotics, *S. aureus* was notable due to its amphixenotic transmission potential. The MRSA is now a global concern owing to its ability to withstand antibiotics and consequent therapeutic failures in both human and veterinary medicine.[1-3]

In our study, incidence of *S. aureus* in cattle were slightly higher (13.50%, 9.20 - 18.80%, CI 95.00%) compared to the previous finding of Fagundes *et al*.[27] Healthy cows being carriers can harbour *S. aureus* in the nasal cavity, whereas infected cows tend to shed the bacteria in milk.[3] In our study, the incidence of *S. aureus* in bovine milk samples was found to be 12.74% (20/157, 7.96 - 18.99%, CI 95.00%) out of which occurrence in mastitic milk samples were found to be 88.24% (15/17, 63.56 - 98.54%, CI 95.00%). Previous reports on occurrence of *S. aureus* in mastitis and sub-clinical mastitis varied. Anderson *et al.* while studying the molecular epidemiology of *S. aureus* in dairy heifers, reported similar occurrence of 13.60% among milk samples in North Carolina, United States.[28] Likewise, Pu *et al*. observed similar occurrence (77.00%) of *S. aureus* in sub-clinical mastitis milk samples in China.[29] In India, Mitra *et al*. reported isolation of 173 *S. aureus* from 294 sub-clinical mastitis milk samples indicating prevalence of 59.00%.[30] Besides, in a review analysis Karzis *et al*. reported that 7.00 – 40.00% mastitis cases between 1975 - 2018 were due to *S. aureus* in dairy herds in South African.[31] The wide range of occurrence of *S. aureus* reported by various authors and the composite array of samples makes it difficult for an effective comparison with previous findings, however, our results were within documented range.

In case of goat, majority of isolates (90.00%) were obtained from nasal canal. Occurrence of *S. aureus* in nasal swabs was 50.00% (33.38 – 66.62%, CI 95.00%). This appears to be considerably higher than previous reports by Shittu *et al*. and Zhou *et al*. who observed nasal carriage of *S. aureus* in goats in the range of 12.00 – 43.00%.[32,33] In addition to the fact that small ruminants suffer from mastitis and septicaemia caused by *S. aureus,*[3] goats are also known to be source of *S. aureus* for zoonotic transmission following professional exposure.[34] Therefore high level of occurrence on *S. aureus* in the nasal cavities of goats pose a risk to public health especially for occupationally exposed persons.

In our study half of the canine origin, *S. aureus* were isolated from abscess exudates. *S. aureus* is a prominent pathogen involved in abscessation in animals including pets.[35] Previously, Hoekstra and Paulton[36] in a 6-year study reported isolation *S. aureus* of abscess origin in 14.40% of all cases. Generally, dogs are not colonized by *S. aureus* and their infection with the organism is reported due to transient infections which are usually of human origin.[3] However, dogs along with goats and cattle are known to be moderate to highly likely sources of zoonotic transfer of *S. aureus*.[3]

The incidence of *S. aureus* in human samples was 12.90% (9.30 - 17.40, CI 95.00%) in our study. Of these, majority were derived from culturing of pus (n = 16, 25.40%; 15.30 - 37.90%, CI 95.00%) and urine (n=14, 16.50%; 9.30 - 26.10%, CI 95.00%). In humans, *S. aureus* is the most common cause of purulent infections of skin and soft tissue.[37-40] Additionally, *S. aureus* was isolated from 5.49% (1.80 - 12.36%, CI 95.00%) of blood samples in the present study. Compared to the magnitude of *S. aureus* occurrence observed in our study, higher incidences (55.60% in pus samples and 11.28% in blood samples) of the pathogen were reported previously from a tertiary care hospital.[38] On the contrary, *S. aureus* is considered to be a rather minor pathogen in bacteriuria.[39] In our study, 16.90% of urine samples harboured *S. aureus*. This was on the higher side considering previous reports that indicated occurrences in the range of 1.00 - 6.90% of cases of urinary tract infection (UTI)/bacteriuria.[39] However, higher incidence (34.00%) of *S. aureus* in UTI cases was also documented previously. S. *aureus*, being a multi-host organism, is capable of infection in multiple organs/ systems and is expected to vary in its occurrences across sampled cohorts under study. Our results were, therefore, largely within reported ranges.

Since the first description of MRSA in mastitis of cattle in Belgium, reports of the pathogen in various animals including companion animals such as cats and dogs surfaced from all over the world.[2] Subsequent reports proved the zoonotic nature of the organism and potential transmission of the MRSA from animals to humans.[1] As in our study, Yadav *et al*. isolated *S. aureus* in 50.00% of clinical pyogenic cases of which 57.50% were MRSA.[7] In companion animals, MRSA is known to be principally associated with skin and soft tissue infections and often transmitted between pet parents and pet animals.[3] On the other hand, Davis *et al.* reported lower prevalence (5.00%) of *S. aureus* in healthy canines and felines though 78.00% of the isolates were MRSA.[41] The difference in the prevalence of MRSA in these studies, including ours, might be due to that in our study samples were from clinical cases. Additionally, the usage pattern of antibiotics such as oxacillin and methicillin may also play a significant role.[42] In case of cattle, most of the MRSA isolates were from abscess which was not unexpected. Previous report from India documented varying prevalence (approximately 10.00%) of MRSA in cattle, though higher prevalence was also reported.[8,42] On the contrary, reports from other part of the world (United States) documented lower incidence rate around 2.00% of MRSA in cattle.[43] Nonetheless, compared to previous reports from India, we observed a lower rate of prevalence which might be due to lower use of antibiotics as well as non-intensive system of dairying practices in the region and both these factors are well known risk factors for MRSA infection in cattle.[46] The incidence of *mecA* gene in goat was found to be 9.52% (1.20 - 30.40%, CI 95.00%). Since the incidence of MRSA in goats is known to range between 1.20% and 17.70%,[45,46] the result of present study was within the limits. Among goat samples, the detection of MRSA from milk reasserted the role played by goat milk as one of the sources of MRSA to humans. Occurrence of MRSA is less in goat perhaps due to limited use of antimicrobial drugs in goat husbandry.[45] Moreover, lower isolation rate of MRSA might be due to variation in host and immune status of the host.

In our study, approximately 4.50% (2.40 - 7.70%, CI 95.00%) of the human samples harboured MRSA which appears to be lower than a large networked study reported in 2013 (Indian Network for Surveillance of Antimicrobial Resistance group, 2013).[15] However, in our study a substantial share of the isolates were of urine culture origin and occurrence of MRSA was known to be low in urinary origin isolates.[39] The complexity of distribution of MRSA, including community-associated and healthcare-associated clones, in Indian healthcare settings is not yet fully understood and requires further investigations.[6]

The Comparison of PCR primers for detection of MRSA was done. In relation to EUCAST approved cefoxitin disc test, Kappa value (measure of agreement) was higher for *mecA*-162 PCR (0.941) in detecting MRSA compared to the *mecA*-310 PCR (Kappa value 0.899). For detection of MRSA, a number of molecular methods are available.[19,20] While each individual assays have their own pros and cons, PCR is by and large choice of method for many laboratories. Therefore, having a quantitative analysis for the primers is useful and our results indicated that for routine screening of samples *mecA*-162 PCR might be a reasonable and balanced option. However, a study involving larger number of isolates will be highly recommended to arrive at a definitive conclusion.

The PCR for *nuc* gene among *S. aureus* isolates with previously reported primer pairs[26] indicated that overall, 51.40% (41.50 - 61.20%, CI 95.00%) of the *S. aureus* isolates possessed *nuc* gene (Table 1). Species-wise, the highest carriage rate was observed among samples of caprine origin (28.00%; 16.20 - 42.50%, CI 95.00%) followed by human (9.10%; 6.00 - 13.00%, CI 95.00%), canine (5.20%; 2.30 - 9.90%, CI 95.00%) and bovine isolates (3.30%; 1.30 - 6.60%, CI 95.00%). The gene (*nuc*) encoding a heat stable nuclease is one of the virulence genes of *S. aureus* which helps the organism to escape neutrophil mediated extracellular immune response of the host.[47] Considering this, we observed a clear association between host (goat) and possession of *nuc* gene (2-sided Pearson Chi-Square value 16.90; $p < 0.01$). Though, *nuc* gene was proposed earlier to be *S. aureus* specific and subsequent reports were contradictory.[26] Sahebnasagh *et al*. reported occurrence of *nuc* gene in 101 of 126 isolates,[48] while Karmakar *et al.* observed a prevalence of only 27.00% among community acquired *S. aureus* isolates.[49] Therefore, our finding with overall occurrence *nuc* gene in 51.00% (41.50 - 61.20%, CI 95.00%) of *S. aureus* isolates was within reported limits.

In conclusion, results of our study showed that *S. aureus* incidence rate was the highest among goat samples (42.00%; 28.20 - 56.80%, CI 95.00%), whereas, majority of the isolates of *S. aureus* of canine origin were MRSA implying that while goats might be putative source of *S. aureus* for humans, the pet dog might be potential source for zoonotic transfer of MRSA to their owners and handlers. Moreover, *S. aureus* of caprine origin were more likely to harbour *nuc* gene, a virulence determinant affording immune-evasion by the organism. Further, our results clearly revealed that *mecA*-162 PCR was a more rational choice for routine molecular screening of *S. aureus* isolates for methicillin resistance especially in terms of Kappa value vis-a-vis EUCAST approved test for MRSA.

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Conflict of interest

Authors declare no competing interest.

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