Molecular detection and typing of methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative staphylococci isolated from cattle, animal handlers, and their environment from Karnataka, Southern Province of India

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Abstract

Background and Aim: Methicillin-resistant staphylococci are among the emerging pathogens which have become a threat to both human and animal health. The present investigation intended to examine the occurrence and the molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) recovered from cattle, its handlers, and their environment.

Materials and Methods: A total of 666 specimens were subjected to culture method and genus-specific polymerase chain reaction (PCR) for the identification of Staphylococcus. Methicillin resistance was substantiated by PCR identification of mecA and mecC resistance determinants. Species-specific identification of mecA positive isolates was conducted by multiplex PCR. The unidentified species were deciphered by 16S rRNA gene sequencing approach. The mecA positive isolates were further characterized by staphylococcal cassette chromosome mec (SCCmec) typing and multilocus sequence typing (MLST).

Results: Duplex PCR identified 728 Staphylococcus isolates, of which 66 (9%) were positive for mecA gene. MRSA constituted 24% of the total mecA positive isolates. Among MRCoNS, Staphylococcus epidermidis (42%), and Staphylococcus haemolyticus (11%) were the most common species identified. Overall, 47% of the mecA positive isolates belonged to SCCmec type V. MLST analysis showed eight different sequence types (STs) among MRSA isolates of which five were novel STs. Among methicillin-resistant S. epidermidis, 19 different STs were found, of which nine novel STs were detected.

Conclusion: The increase in the prevalence of mecA positive staphylococci, especially MRCoNS in cattle is a great concern in view of their transmission potential. Hence, continuous monitoring and molecular characterization of methicillin-resistant staphylococci should be elucidated in human and animal sectors so as to prevent the spread of these resistant pathogens.

Keywords: mecA/mecC gene, methicillin resistance, methicillin-resistant coagulase-negative staphylococci, methicillin-resistant Staphylococcus aureus.

Introduction

Over 50 species of the Staphylococcus genus have been described until now [1]. Staphylococcus aureus, a coagulase-positive species is a well-recognized nosocomial pathogen in both human and animal medicine. It is the most important pathogen recognized for intermittent infections and outbreaks [2], whereas coagulase-negative staphylococci (CoNS) signifying preponderance of species have been thought to be saprophytic or rarely pathogenic [3]. Over the past few decades, studies have revealed CoNS as the prime etiological agent of a series of infectious processes, ranging from hospital-acquired infection to livestock bacterial sepsis and mastitis [4].

S. aureus has a distinctive capacity to swiftly develop resistance to almost any antibiotics arriving into clinical practice. Methicillin resistance that demonstrates resistance to all available β-lactam antibiotics was initially described in 1961, which denoted the emergence of methicillin-resistant S. aureus (MRSA) [5]. It is presumed that determinants of methicillin resistance had emerged in CoNS and then disseminated horizontally among staphylococci [6]. Methicillin resistance is conferred
by an altered penicillin-binding protein 2a encoded by mecA gene sited on a mobile genetic element called staphylococcal cassette chromosome (SCC) [7]. In 2011, a different mecA homolog, designated as mecC (previously mecA<sub>1027</sub>) was identified in bovine milk and human clinical specimens in different parts of the world [8-10]. The mecC has about 70% comparability with mecA at the nucleotide level and 63% identity at the amino acid level. The mecA<sub>1027</sub> was discovered to carry a novel SCCmec element Type XI [11]. Since SCCmec is the element known to transfer the mecA and mecC gene among Staphylococcus species or between animals and humans and their environment; hence, it is imperative to detect the SCCmec type.

Over the years, the occurrence of MRSA has been progressively increasing across the globe [12]. In India, MRSA is recognized endemic with the prevalence rate of MRSA and methicillin-resistant CoNS (MRCoNS) varying from 17 to 70% and 22 to 73%, respectively [13,14]. Recent studies report the recovery of MRCoNS from diverse sources including medical devices, healthy humans, ambulatory patients, and bloodstream infections. The appearance of MRCoNS in animals was first explained in chicken [15]. However, MRSA and MRCoNS were also found in different animal species with clinical infections. Studies have detailed Staphylococcus epidermidis and Staphylococcus haemolyticus as the foremost pathogens engaged with a wide range of infections of humans and animals. Limited data are accessible on the prevalence and genomic characteristics of MRSA and MRCoNS from animal sector; thereby, it is likely to miss other spillover events of these pathogens between livestock and humans or vice versa [16].

Surveillance of MRSA and MRCoNS infections in both human and animal health-care settings is critical due to the changing epidemiological profile of organisms. Precise and quick detection of these pathogens allows application of efficient antimicrobial therapy and preventive infection control strategies [17]. The increasing significance of MRSA and MRCoNS serves the justification for more accurate species identification to allow the exact determination of host-pathogenic possibility of each of the different species [18]. The current study aimed to investigate the occurrence and characterize MRSA and MRCoNS recovered from cattle, animal handlers, and their environment.

Materials and Methods

Ethical approval

The study was approved by the Institutional Ethics Committee of ICAR-NIVEDI. All applicable international, national, and institutional guidelines for the animal’s care were followed during the sample collection.

Specimen collection

A total of 666 specimens were collected between 2015 and 2017 from five organized cattle farms (Kanakapura [12.9428°N, 77.5779°E], Kagalipura [12.7995°N,77.5101°E], Agara [12.9231°N, 77.6465°E], Bidadi [12.7968°N, 77.3839°E], and Arehalli [12.9117°N, 77.5412°E]), and eight unorganized cattle herds (Kodihalli [12.9654°N, 77.6493°E], Kadabagere [12.9969° N, 77.4331° E], Ramagondanahalli [12.9558°N, 77.7409°E], Hasanghatta [13.1500°N, 77.4900°E], Tapashalli [13.3907°N, 77.6859°E], Doddballapur [13.2895°N, 77.5342°E], Jakkanahalli [13.1783869 °N, 77.3467876°E], and Yediyur [12.9312 °N, 77.5723°E]) located in and around Bengaluru, India. The samples comprised cattle milk (n=371), cattle nasal swabs (n=109), extramammary site (n=90), wound samples from cattle (n=30), animal handlers hand swabs (n=32), and environmental swabs (n=34). The environmental samples included feed trough (n=13), floor of cattle shed (n=15), milking machine (n=4), and supplied water (n=2).

Isolation and tentative identification of Staphylococcus spp.

The specimens were inoculated into Brain Heart Infusion (BHI) Broth and transported to the Microbial Pathogenesis and Pathogen Diversity Laboratory, ICAR-NIVEDI, Bengaluru, India, within 2 h. Each sample was initially cultured on staphylococcus agar 110 (S110) (HiMedia, Mumbai) and incubated at 37°C for 24 h. Pure culture of isolates was obtained by subculture on BHI agar (HiMedia, Mumbai). Staphylococcus was identified based on colony characteristics, pigment production, Gram staining, catalase, and oxidase tests as per the standard protocol [19].

DNA extraction

The genomic DNA from the staphylococci was extracted using the QIAamp DNA Mini Kit (Qiagen, Duesseldorf, Germany) as per manufacturer’s recommendations. NanoDrop 2000c (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to determine the purity and concentration of the extracted DNA.

Duplex polymerase chain reaction (PCR) for detection of genus Staphylococcus and mecA gene

The extracted DNA from all the Gram-positive isolates was subjected to our in-house duplex PCR assay for the simultaneous detection of genus Staphylococcus and methicillin resistance determinant (mecA gene) (Table-1). The primers were synthesized based on the previously published sequences [20,21]. Duplex PCR assay was performed in a 25 µL reaction volume containing 1X PCR buffer, 1.5 U DNA Taq polymerase, 2mM MgCl<sub>2</sub>, and 200 µM deoxynucleotide triphosphates; (Fermentas, Glen Burnie, MD, USA), 0.6 µM and 0.5 µM of Staphylococcus genus and mecA specific primers, respectively, and 50 ng template DNA. The PCR cycling conditions comprised an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 45 s.
and final extension step at 72°C for 5 min. The ATCC 33591 MRSA reference strain was used for the optimization of the assay.

**Uniplex PCR for identification of mecC gene**

Uniplex PCR based testing of mecC gene (a homolog of mecA gene) was carried out for all the staphylococcal isolates (Table-1) [22]. PCR assay was done in a 15 μL reaction volume containing 1X PCR ready master mix (0.025U DNA Taq polymerase in reaction buffer, 2 mM MgCl₂, and 200 mM deoxynucleotide triphosphates [Fermentas, Glen Burnie, MD, USA]), 0.5 μM of mecC specific primers and 50 ng of extracted DNA. The PCR cycling conditions comprised an initial denaturation step at 95°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 30 s, and final extension step at 72°C for 4 min.

**Species-specific identification**

All mecA/mecC positive *Staphylococcus* isolates were deciphered to species level by species-specific multiplex PCR targeting five major *Staphylococcus* spp., namely, *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *Staphylococcus chromogenes*, and *Staphylococcus sciuri* (Table-1) [23].

**Partial 16S rRNA gene sequencing of *Staphylococcus* spp.**

The unidentified mecA/mecC positive strains were subjected to partial 16S rRNA gene sequencing for species-specific identification. The primer pair used was flanking five hypervariable regions (V2, V3, V4, V5, and V6) of 16S rRNA to amplify a 974 bp fragment (Table-1). A simplex PCR assay for amplification of DNA was performed in 50 μL reaction volume containing 0.5 μM of 16S rRNA specific primers. The reaction mixture and the cycling conditions were similar as previously published by Shome et al. [24]. The PCR amplicons were sequenced in bi-direction by Sanger sequencing approach at Eurofins, Bengaluru, India.

**Molecular characterization by SCCmec typing**

The mecA/mecC positive strains were subjected to PCR-directed SCCmec typing as earlier detailed by Kondo et al. [25]. The strategy comprised two multiplex PCR assays mPCR-1 for ccr typing and mPCR-2 for mec class typing (Table-1).
Results

Molecular detection of Staphylococcus and methicillin resistance

Out of 666 samples, a total of 762 Gram-positive bacteria presumptive to be Staphylococcus were recovered by the conventional culture method. Duplex PCR detected 728 isolates as Staphylococcus spp. and the majority were detected from milk samples (n=451) followed by nasal (n=111), and extramammary sites (n=75) (Table-2). Duplex PCR identified 66 Staphylococcus isolates harboring mecA gene (9%). The majority of the mecA positive isolates were recovered from milk (n=48) followed by animal handlers hand swabs (n=8) (Figure-1). The mecA gene was not detected in any of the environmental specimens. All the Staphylococcus isolates were negative for mecC gene.

Species-specific identification of Staphylococcus by multiplex PCR and partial 16S rRNA gene sequencing

The 66 mecA positive isolates were distributed into five different species by multiplex PCR, of which S. epidermidis (n=28) was the most predominant species subsequently proceeded by S. aureus (n=16), S. haemolyticus (n=7), S. chromogenes (n=3), and S. sciuri (n=1). The remaining 11 mecA positive isolates were unidentified by multiplex PCR (Figure-2).

Molecular characterization by SCCmec typing and MLST

SCCmec typing of 66 mecA positive Staphylococcus spp. identified 31 isolates as Type V, whereas the remaining 35 mecA positive isolates were non-typeable. The distribution of SCCmec types among various species of Staphylococcus is detailed in Table-3.

MLST analysis revealed ST 1687 (50%, 8/16) as the predominant ST. Further, six MRSA isolates were found to be novel STs, namely, ST 5217 (n=2), and one each as ST 5216, ST 5218, ST 5219, and ST 5220. Among MRSE, the most predominant ST was found to be ST 457 (14.3%, 4/28) followed by ST 575 (7.14%, 2/28). The other STs identified were ST 439, ST 110, ST 21, ST 226, ST 210, ST 114, ST 130, and ST 57. In addition, 14 MRSE isolates were distributed among nine novel STs, namely, ST 849 (n=3), ST 855 (n=3), ST 854 (n=2), and one each as ST 850, ST 851, ST 852, ST 853, ST 856, and ST 857 (Table-4).

Discussion

Methicillin-resistant Staphylococcus is an important pathogen which presently is receiving significant attention in the public and animal health sector. Recognition and discrimination of MRSA and MRCoNS is pre-requisite for implementing appropriate antimicrobial therapy and thereby controlling the advancement of the disease. The current study communicates on the occurrence and characteristics of MRSA and MRCoNS in cattle, cattle handlers, and their environment.

In the present study, the overall detection of methicillin-resistant staphylococci was found to be 9%. The mecA gene was observed in 16 isolates (2%) of S. aureus strains and 50 isolates of CoNS (7%). The rate of mecA positive isolates identified in the present study was low when compared to the other Indian studies, wherein methicillin resistance is recorded between 18 and 35% [26]. One of the reasons for the high rate of methicillin resistance in these studies could be the predominant inclusion of clinical samples. The incidence of MRSA and MRCoNS varied by sequencing fragments of seven housekeeping genes: arcC, aroE, gfpE, gmk, pta, tpi, and yqfL for S. aureus and arcC, aroE, gtr, mutS, pyr, tpi, and yqfL for S. epidermidis. Allele number and sequence types (STs) were assigned using the S. aureus and S. epidermidis MLST websites (https://pubmlst.org/saureus/ and https://pubmlst.org/epidermidis/).

Table-2: Details of samples collected from various sources.

| Source                  | Number of samples | Number of Staphylococcus | Number of mecA positive Staphylococcus |
|-------------------------|-------------------|--------------------------|----------------------------------------|
| Milk                    | 371               | 451                      | 48 (11%)                               |
| Nasal                   | 109               | 111                      | 2 (2%)                                 |
| Extramammary site       | 90                | 75                       | 5 (7%)                                 |
| Wound                   | 30                | 28                       | 3 (11%)                                |
| Animal handlers hand swab | 32              | 34                       | 8 (24%)                                |
| Environmental swabs     | 34                | 29                       | 0                                      |
| Total                   | 666               | 728                      | 66 (9%)                                |

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in various countries. In Turkey, Denmark, and China, the frequency of mecA positive Staphylococcus in humans was found to be 44%, 51%, and 60%, respectively [27-29]. The recovery of mecA gene (9%) among our study isolates should be considered a probable risk for public health, as these pathogens may gain access to the food chain. For this reason, it is mandatory to monitor the health status of animals and humans and expand to the hygienic environment. The presence of mecA in CoNS is obvious from the study, indicating the crucial role of CoNS in dissemination of methicillin resistance in the environment.

Recently, it has been reported that animals are often colonized with meticillin-resistant staphylococci; particularly, livestock has been a cause of worry, as it has exposed an expanded pool of meticillin resistance [30]. In the present study, 12% (n=8/66) and 88% (n=58/66) of mecA positive isolates were obtained from animal handlers and cattle population, correspondingly. Owing to close association of the animals with ecological microbiome and resistome, animal origin staphylococcal strains may serve as a dissemination source of resistance determinants. It has been reported that meticillin resistance can be transmitted to humans either by direct contact with animals, environmental contamination or by handling of products from infected animals [31]. Moreover, companion animals (cats and dogs) are also assumed

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**Figure-2**: Species diversity of methicillin-resistant Staphylococcus isolates recovered from various sample sources.

**Table-3**: Distribution of SCCmec types among MRSA and MRCoNS.

| Staphylococcus spp. | Source                      | Type V | Non-typeable |
|---------------------|-----------------------------|--------|--------------|
| S. epidermidis      | Milk                        | 8      | 11           |
|                     | Nasal                       | 0      | 1            |
|                     | Extramammary site           | 2      | 0            |
|                     | Animal handlers hand swab   | 3      | 3            |
| S. aureus           | Milk                        | 12     | 3            |
|                     | Wound                       | 1      | 0            |
| S. chromogenes      | Milk                        | 2      | 1            |
| S. sciuri           | Milk                        | 0      | 1            |
| S. haemolyticus     | Milk                        | 0      | 4            |
|                     | Nasal                       | 1      | 0            |
|                     | Extramammary site           | 2      | 0            |
| S. saprophyticus    | Milk                        | 0      | 1            |
|                     | Animal handlers hand swab   | 0      | 1            |
| S. warneri          | Extramammary site           | 0      | 1            |
| S. hominis          | Milk                        | 0      | 4            |
|                     | Wound                       | 0      | 1            |
| S. pasteuri         | Animal handlers hand swab   | 0      | 1            |
| S. equorum          | Milk                        | 0      | 1            |
| S. arlettae         | Milk                        | 0      | 1            |
| Total (66)          |                             | 31 (47%) | 35 (53%)     |

S. epidermidis=S. epidermidis, S. aureus=S. aureus, S. chromogenes=S. chromogenes, S. sciuri=S. sciuri, S. haemolyticus=S. haemolyticus, S. saprophyticus=S. saprophyticus, S. warneri=S. warneri, S. hominis=S. hominis, S. pasteuri=S. pasteuri, S. equorum=S. equorum, S. arlettae=S. arlettae, MRSA=M. aureus, MRCoNS=M. coagulase-negative staphylococci.
to acquire resistance from humans [32]. Thus, animals and humans are frequently colonized and both can act as reservoirs of methicillin resistance. Studies have shown that the spread of animal origin MRSA to veterinary personnel is more usual for large animal handlers than small animal handlers [33]. European institutions have played an important role in laying down and passing laws specific to the veterinary sanitary and food safety area for the eradication of certain infectious diseases of livestock [34-37]. The judicial enforcement of these laws will help to prevent the spread of bacterial population from animals to animal handlers or vice versa.

Precise estimations of the effect, sources, transmission dynamics, and control strategies for MRSA/MRCoNS necessitate the exact identification of species. The 66 mecA positive staphylococcal isolates identified in the present study were distributed into 11 different species with MRSA representing 24% of the total isolates. These findings are in concordance with the Prasanth et al. study [38]; wherein, the authors recorded 29% of the strains from bovine origin as MRSA. Methicillin resistance on an average is high in Indian dairy sectors probably due to lurking of methicillin-resistant genes in the dairy environment [38]. Independent studies from Saudi Arabia [39] and Iran [40] reported 56-57% of MRSA from farm animals and human clinical cases.

The authors suggested that the higher prevalence of MRSA in these studies may be due to the inappropriate use of methicillin, which aggravates the dissemination potential between humans and animals as well as to the community. Antoci et al. [41] from Southeastern Sicily reported 36% of humans, 61% of cattle, and 44% of milk samples positive for MRSA. In the current study, among MRCoNS, S. epidermidis (42%), and S. haemolyticus (11%) were the most predominant species identified. Huber et al. [42] reported 48% of samples from livestock and chicken carcasses positive for MRCoNS with S. sciuri (63%) and S. fleurettii (17%) representing the prime species. Sawanath et al. [43] and Jaglic et al. [44] found 30% and 50% of S. epidermidis strains harboring the mecA gene, respectively, from bovine milk samples. The high prevalence of CoNS may be attributed to the wide distribution of the organism inside the mammary gland and in the teats of the udder. In view of animal contact persons exclusively, we observed S. epidermidis (75%, n=6/8) as the most common species. A study from Mexico extended to human sector, identified S. epidermidis and S. haemolyticus as the most prevalent species among MRCoNS [45]. Similarly, results were obtained from human patients in Algeria, Mali, Moldova, and Cambodia in which S. epidermidis and S. haemolyticus comprised 98% of the MRCoNS [46]. Cattle and cattle handlers may share S. epidermidis

**Table-4:** MLST analysis of methicillin-resistant S. aureus and methicillin-resistant S. epidermidis.

| MRSA/MRSE strains with ST | Source | Number of isolates |
|---------------------------|--------|-------------------|
| MRSA-ST 5216 (novel ST)   | Cattle milk | 1                 |
| MRSA-ST 5217 (novel ST)   | Cattle milk | 2                 |
| MRSA-ST 5218 (novel ST)   | Cattle milk | 1                 |
| MRSA-ST 5219 (novel ST)   | Cattle milk | 1                 |
| MRSA-ST 5220 (novel ST)   | Cattle wound | 1                |
| MRSA-ST 1687              | Cattle milk | 8                 |
| MRSA-ST 3881              | Cattle milk | 1                 |
| MRSA-ST 2668              | Cattle milk | 1                 |
| MRSE-ST 849 (novel ST)    | Cattle milk | 3                 |
| MRSE-ST 850 (novel ST)    | Animal handler’s hand swab | 1            |
| MRSE- ST 851 (novel ST)   | Animal handler’s hand swab | 1            |
| MRSE-ST 852 (novel ST)    | Animal handler’s hand swab | 1            |
| MRSE-ST 853 (novel ST)    | Animal handler’s hand swab | 1            |
| MRSE-ST 854 (novel ST)    | Cattle milk | 1                 |
|                        | Cattle extramammary site | 1            |
| MRSE-ST 855 (novel ST)    | Cattle milk | 3                 |
| MRSE-ST 856 (novel ST)    | Cattle milk | 1                 |
| MRSE-ST 857 (novel ST)    | Cattle milk | 1                 |
| MRSE-ST 439              | Cattle milk | 1                 |
| MRSE-ST 110              | Cattle milk | 1                 |
| MRSE-ST 21               | Animal handler’s hand swab | 1            |
| MRSE-ST 226              | Cattle milk | 1                 |
| MRSE-ST 457              | Cattle milk | 3                 |
|                        | Cattle extramammary site | 1            |
| MRSE-ST 210              | Cattle milk | 1                 |
| MRSE-ST 114              | Animal handler’s hand swab | 1            |
| MRSE-ST 130              | Cattle nasal | 1                  |
| MRSE-ST 575              | Cattle milk | 2                 |
| MRSE-ST 57               | Cattle milk | 1                 |
| Total                    |         | 44                |

MRSA=Methicillin-resistant Staphylococcus aureus, MRCoNS=Methicillin-resistant coagulase-negative staphylococci, MLST=Multilocus sequence typing, S. epidermidis=Staphylococcus epidermidis, S. aureus=Staphylococcus aureus, MRSE-ST=Methicillin-resistant Staphylococcus epidermidis-sequence types

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strains, implying that bovine MRSE might be a zoonotic pathogen. It is hard to decipher the direction of interspecies transmission; however, it is suggested that *S. epidermidis* will probably spread from humans to dairy cows than the other way around [47].

SCCmec elements are genomic islands incorporated into the specific region of the *Staphylococcus* chromosome. In the present study, SCCmec type was assigned to 47% (n=31) of mecA positive isolates. SCCmec Type V was identified among 81% of MRSA, whereas among MRCoNS, 26% of *S. epidermidis*, 6% of *S. haemolyticus*, and 4% of *S. chromogenes* belonged to Type V. According to Ruppe et al. [46], SCCmec Type IV predominates in *S. epidermidis*, while Type V predominates in *S. haemolyticus* and Type III in *S. aureus* and variety of CoNS. Our observations are in align with the Fessler et al. study [48]; wherein, all the methicillin-resistant *S. haemolyticus* of bovine mastitis identified with SCCmec element Type V. Detection of SCC mec Type V (which is considered as a characteristic feature of CA-MRSA) among MRSA and MRCoNS belonging to the same farm supports the hypothesis for the possible transfer of SCCmec between CoNS and *S. aureus*. The increasing incidence of SCCmec type in certain CoNS species should be analyzed with caution. Studies have demonstrated that other than the prevailing SCCmec type, various other types may appear in members of the CoNS species. We observed 35 mecA positive isolates non-typeable for SCCmec element. The possible explanation could be either ccr genes might be anonymous types or may have undergone certain mutations in the primer-target sites or presumably lost [49]. SCCmec typing in MRCoNS is challenging due to its current identification of co-existed SCCmec and the existence of non-typeable components [7]. In spite of the fact that it may be hard to detect all SCCmec types carried by *Staphylococcus* spp, interpreting as many SCCmec types as possible will further help for epidemiological studies and in outlining the sources of MRSA/MRCoNS strains.

Among MRSA isolates, we identified ST 1687 as the most common ST which was detected earlier by Mistry et al. [50] as the novel ST type among milk samples obtained from mastitis affected cows of Telangana and Tamil Nadu regions from India. ST 2668 detected in our study among MRSA isolates were also previously reported in 3.5% of MSSA clones among environmental samples of Chinese Metro systems [51]. The study demonstrated that environmental surfaces may be a hazardous reservoir for transmission of methicillin-resistant *Staphylococci* to passengers. Cross transmissions of MRSA/MRCoNS isolates from various sources, including hospitals, communities, and livestock, are also possible. Thus, more stringent infection control and surveillance measures are needed. Moreover, we identified ST 457 as the most prevalent ST among MRSE. Previously, Armand-Lefevre et al. [52] reported ST 457 as the novel ST among pig farmers. The occurrence of the same STs among pig farmers and cattle probably suggests the host jump/adaptation and clonal evolution of the strains which further creates great havoc. The significance of this study is the detection of new STs, which will permit further analyses to keep pace with new evolutionary trends. Moreover, a high proportion of new STs among human population who were in close association with cattle may be explained by transmission of resistant determinants between humans and animals and vice versa. Further, whole-genome sequencing of these isolates will mark recognition of genetically closely related isolates within the STs, thereby tracing out the potential sources and identifying outbreaks.

**Conclusion**

The study highlights a high prevalence of methicillin resistance in the dairy environment with *S. epidermidis* as the most leading species. Prevalence of methicillin resistance among animal handlers was found to be 24% which was very high as compared to 8.7% in cattle. Thus, individuals with persistent animal contact should be educated on the risk of probable transmission of resistance from animals to humans and vice versa. As the transmission of resistant genes is dynamic and involves animals, humans, and their environment, it necessitates periodic surveillance of the resistance status of *S. aureus* and CoNS to control the spread of resistance and reduce disease burden associated with these resistant pathogens. Further, genomic characterization to find resistance level variations is essential to interpret human and animal transmission dynamics.

**Authors’ Contributions**

BRS supervised the group and executed the project, and contributed to the drafting and revision of the manuscript. RS and HR helped in the design of sample collection strategies and critical revision of the manuscript. NV collected samples, designed, and performed experiments, data analysis and contributed to the drafting and revision of the manuscript. SM and BRS supervised the group and executed the project. FG carried out data analysis and interpretation and contribution to drafting and critical revision of the manuscript. All authors read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.
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References
1. Pyorala, S. and Taponen, S. (2009) Coagulase-negative staphylococci emerging mastitis pathogens. Vet. Microbiol., 134(1-2): 3-8.
2. Mehdriratta, P.L., Bhalia, P., Ahmed, A. and Sharma, Y.D. (2009) Molecular typing of methicillin-resistant Staphylococcus aureus strains by PCR-RFLP of spa gene: A reference laboratory perspective. Indian. J. Med. Microbiol., 27(2): 116-122.
3. Mde, L.C., Sinzato, Y.K. and Silveira, L.V. (2004) Comparison of methods for the identification of coagulase-negative staphylococci. Mem. Inst. Oswaldo Cruz, 99(8): 855-860.
4. Becker, K., Pagnier, I., Schuben, B., Wenzelburger, F., Friedrich, A.W., Kipp, F., Peters, G. and von Eiff, C. (2006) Does nasal colonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible Staphylococcus aureus strains occur frequently enough to represent a risk of false-positive methicillin-resistant S. aureus determinations by molecular methods? J. Clin. Microbiol., 44(1): 229-231.
5. Molton, J.S., Tambyan, P.A., Ang, B.S., Ling, M.L. and Fisher, D.A. (2013) The global spread of healthcare-associated multidrug-resistant bacteria: A perspective from Asia. Clin. Infect. Dis., 56(9): 1310-1318.
6. Robinson, D.A. and Enright, M.C. (2004) Multilocus sequence typing and evolution of methicillin-resistant S. aureus. Clin. Microbiol. Infect., 10(2): 92-97.
7. Zong, Z., Peng, C. and Lu, X. (2009) Diversity of SCCmec elements in methicillin-resistant coagulase-negative staphylococcal clinical isolates. PLoS One, 4(5): e20191.
8. Kilic, A., Dogan, E., Kaya, S. and Baysallar, M. (2015) Investigation of the presence of mecC and pannon-valentine leucocidin genes in Staphylococcus aureus strains isolated from clinical specimens during seven years period. Mikrobiyol. Bul., 49(4): 594-599.
9. Kriegeskorte, A., Idelevich, E.A., Schlattmann, A., Layer, F., Strommenger, B., Denis, O., Paterson, G.K., Holmes, M.A., Werner, G. and Becker, K. (2017) Comparison of different phenotypic approaches to screen and detect mecC-harboring methicillin-resistant Staphylococcus aureus. J. Clin. Microbiol., 56(1): e00226-e00917.
10. Bietrix, J., Kolenda, C., Sapin, A., Haenni, M., Madc, J.Y., Bes, M., Dupieux, C., Tasse, J. and Laurent, F. (2019) Persistence and diffusion of mecC-positive CC130 MRSA isolates in dairy farms in meurthe-et-moselle county (France). Front. Microbiol., 10: 47.
11. Garcia-Alvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.J., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J. and Holmes, M.A. (2011) Methicillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect. Dis., 11(8): 595-603.
12. Shopsin, B. and Kreiswirth, B. (2001) Molecular epide-miology of methicillin-resistant Staphylococcus aureus. Emerg. Infect. Dis., 7(2): 323-326.
13. Peral, B.M.K., Korippella, R.R., Vani, S. and Lakshmi, N. (2016) Prevalence of methicillin-resistant Staphylococcus aureus in a tertiary care hospital. IOSR J. Dent. Med. Sci., 15(5): 29-31.
14. Aher, C.S. (2009) The isolation pattern, species distribution and antibiotic susceptibility profile of coagulase-negative staphylococci: Emerging opportunistic pathogens. Int. J. Biomed. Adv. Res., 5(1): 23-25.
15. Kawan, J., Shimizu, A., Saitoh, Y., Yagi, M., Saito, T. and Okamoto, R. (1996) Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. J. Clin. Microbiol., 34(9): 2072-2077.
16. Smith, T.C. (2015) Livestock-associated Staphylococcus aureus. The United States experience. PLoS Pathog., 11(2): e1004564.
17. Cooper, B.S., Stone, S.P., Kibbler, C.C., Cookson, B.D., Roberts, J.A., Medley, G.F. Duckworth, G., Lai, R. and Ebrahim, S. (2004) Isolation measures in the hospital management of methicillin-resistant Staphylococcus aureus MRSA: Systematic review of the literature. Br. Med. J., 329(7465): 533.
18. Heikens, E., Fleer, A., Pauw, A., Florijn, A. and Fluit, A.C. (2005) Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J. Clin. Microbiol., 43(5): 2286-2290.
19. Isenberg, H.D. (2004) Clinical Microbiology Procedures Handbook. 2nd ed., Vol. 2. ASM Press, Washington, D.C.
20. Shome, B.R., Natesan, K., Mitra, S.D., Venugopal, N., Mani, B., Ganaie, F., Shome, R. and Rahman, H. (2018) Development of simplex-PCR assays for accurate identification of nine staphylococcal species at genus and species levels. J. Microbiol. Infect. Dis., 8(3): 120-127.
21. Al-Talib, H., Yean, C.Y., Al-Khateeb, A., Hassan, H., Singh, K.K., Al-Jashamy, K. and Ravichandran, M. (2009) A pentaplex PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus and pannon-valentine leucocidin. BMC Microbiol., 9: 113.
22. Cuny, C., Layer, F., Strommenger, B. and Witte, W. (2011) Rare occurrence of methicillin-resistant Staphylococcus aureus CC130 with a novel mecA homologue in humans in Germany. PLoS One, 6(9): e24360.
23. Shome, B.R., Mitra, S.D., Bhuvana, M., Krithiga, N., Shome, R., Velu, D. and Prabhudas, K. (2012) Multiplex PCR for the detection of five important staphylococcus species in bovine subclinical mastitis milk. Indian J. Anim. Sci., 82(1): 9-14.
24. Shome, B.R., Mitra, S.D., Bhuvana, M., Krithiga, N., Velu, D., Shome, R., Isloor, S., Barbuddhe, S.B. and Rahman, H. (2011) Multiplex PCR assay for species identification of bovine mastitis pathogens. J. Appl. Microbiol., 111(6): 1349-1356.
25. Kondo, Y., Ito, T., Ma, X.X., Watanabe, S., Kreiswirth, B.N., Etienne, J. and Hiramatsu, K. (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: Rapid identification system for mec, ccr, and major differences in Junkyard regions. Antimicrob. Agents Chemother., 51(1): 264-274.
26. Manoharan, A., Lalitha, M.K. and Jesudason, M.V. (1997) In vitro activity of netilmicin against clinical isolates of methicillin-resistant and susceptible Staphylococcus aureus. Natl. Med. J. India, 10(2): 61-62.
27. Sareyuyugol, B., Ozurt, M., Haznedroglu, T. and Ardic, N. (2008) Detection of methicillin and mupirocin resistance in staphylococcal hospital isolates with a touchdown multiplex polymerase chain reaction. Folia Microbiol., 53(4): 363-367.
28. Poulsen, B., Skov, R. and Lars, P. (2003) Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA detection kit. J. Antimicrob. Chemother., 51(2): 419-421.
29. Yao, D., Yu, F.Y., Qin, Z.Q., Chen, C., He, S.S., Chen, Z.Q. and Wang, L.X. (2010) Molecular characterization of Staphylococcus aureus isolates causing skin and soft tissue infections (SSTIs). BMC Infect. Dis., 10: 133.
30. Pantosti, A. (2012) Methicillin-resistant Staphylococcus aureus associated with animals and its relevance to human health. Front. Microbiol., 3: 127.
31. Nunang, C. and Young, R. (2007) MRSA in Farm Animals

Available at www.veterinaryworld.org/Vol.12/November-2019/10.pdf
32. Morgan, M. (2008) Methicillin-resistant Staphylococcus aureus and animals: Zoonosis or humanosis? J. Antimicrob. Chemother., 62(6): 1181-1187.
33. Wulf, M.W., Sorum, M., Van, N.A., Skov, R., Melchers, W.J., Klaassen, C.H. and Voss, A. (2008) Prevalence of methicillin-resistant Staphylococcus aureus among veterinarians: an international study. Clin. Microbiol. Infect., 14(1): 29-34.
34. Bondoc, I. (2016) European regulation in the veterinary sanitary and food safety area, a component of the European policies on the safety of food products and the protection of consumer interests: A 2007 retrospective. Part one: The role of European institutions in laying down and passing laws specific to the veterinary sanitary and food safety area. Rev. Univ. Jurid. (Supplement -2016): 12-15.
35. Bondoc, I. (2016) European regulation in the veterinary sanitary and food safety area, a component of the European policies on the safety of food products and the protection of consumer interests: A 2007 retrospective. Part two: Regulations. Rev. Univ. Jurid. (Supplement 2016): 16-19.
36. Bondoc, I. (2016) European regulation in the veterinary sanitary and food safety area, a component of the European policies on the safety of food products and the protection of consumer interests: A 2007 retrospective. Part three: Directives. Rev. Univ. Jurid (Supplement 2016): 20-23.
37. Bondoc, I. (2016) European regulation in the veterinary sanitary and food safety area, a component of the European policies on the safety of food products and the protection of consumer interests: A 2007 retrospective. Part four: Decisions. Rev. Univ. Jurid. (Supplement 2016): 24-27.
38. Prashanth, K., Rao, R.K., Reddy, V.P.V., Saranathan, R. and Makki, A.R. (2011) Genotypic characterization of Staphylococcus aureus obtained from humans and bovine mastitis samples in India. J. Glob. Infect. Dis., 3(2): 115-122.
39. Alzohairy, M.A. (2011) Colonization and antibiotic susceptibility pattern of methicillin-resistance Staphylococcus aureus (MRSA) among farm animals in Saudi Arabia. J. Bacteriol. Res., 3(4): 63-68.
40. Davoodi, N.R., Yousef, J.V., Harzandi, N., Hajrafi, A., Rajaei, B., Nejad, S.G., Aghasadeghi, M.R., Moshiri, A., Bahremand, A.R. and Siadat, S.D. (2012) Molecular detection of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase-negative staphylococcus (CoNS) in Iran. Afr. J. Microbiol. Res., 6(16): 3716-3721.
41. Antoci, E., Pinzone, M.R., Nunnari, G., Stefani, S. and Cacopardo, B. (2013) Prevalence and molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) among subjects working on bovine dairy farms. Infez. Med., 21(2): 125-129.
42. Haber, H., Ziegler, D., Pfuger, V., Vogel, G., Zweifel, C. and Stephan, R. (2011) Prevalence and Characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat and contact persons. BMC Vet. Res., 7: 6.
43. Sawant, A.A., Gillespie, B.E. and Oliver, S.P. (2009) Antimicrobial susceptibility of coagulase-negative Staphylococcus species isolated from bovine milk. Vet. Microbiol., 134(1-2): 73-81.
44. Jaglic, Z., Michu, E., Holasova, M., Vikova, H., Babak, V., Kolar, M., Bardon, J and Schlegelova, J. (2010) Epidemiology and characterization of Staphylococcus epidermidis isolates from humans, raw bovine milk and a dairy plant. Epidemiol. Infect., 138(5): 772-782.
45. Moodley, A. and Guardabassi, L. (2009) Clonal spread of methicillin-resistant staphylococci among horses, personnel and environmental sites at equine facilities. Vet. Microbiol., 137(3-4): 397-401.
46. Ruppe, E., Barbier, F., Mesli, Y., Maiga, A., Cojocaru, R., Benkhalifa, M., Benchouk S, Hassaine, H., Maiga, I., Diallo, A., Kounmaré, A.K., Ouattara, K., Soumaré, S., Dufourcq, J.B., Nareth, C., Sarthou, J.L., Andremont, A. and Ruimy, R.(2009) Diversity of staphylococcal cassette chromosome mec structures in methicillin-resistant Staphylococcus epidermidis and Staphylococcus haemolyticus strains among outpatients from four countries. Antimicrob. Agents Chemother., 53(2): 442-449.
47. Thorberg, B.M., Kuhn, I., Aarestrup, F.M., Brandstrom, B., Jonsson, P. and Danielsson-Tham, M.L. (2006) Phenox and genotyping of Staphylococcus epidermidis isolated from bovine milk and human skin. Vet. Microbiol., 115(1-3): 163-172.
48. Fessler, A.T., Billerbeck, C., Kadlec, K. and Schwarz, S. (2010) Identification and characterization of methicillin-resistant coagulase-negative staphylococci from bovine mastitis. J. Antimicrob. Chemother., 65(8): 1576-1582.
49. Hanssen, A.M. and Sollid, J.U.E. (2006) SCCmec in staphylococci: Genes on the move. FEMS Immunol. Med. Microbiol., 46(1): 8-20.
50. Mistry, H., Sharma, P., Mahato, S., Saravananan, R., Kumar, P.A. 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.
51. Peng, Y., Ou, Q., Lin, D., Xu, P., Li, Y., Ye, X., Zhou, J. and Yao, Z. (2015) Metro system in Guangzhou as a hazardous reservoir of methicillin-resistant Staphylococci: Findings from a point-prevalence molecular epidemiologic study. Sci. Rep., 5: 16087.
52. Armand-Lefevre, L., Ruimy, R. and Andremont, A. (2005) Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. Emerg. Infect. Dis., 11(5): 711-714.