Int.J.Curr.Microbiol.App.Sci (2019) *8*(10): 916-927

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 10 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.810.107

Molecular Characterization of Major Types of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) in Methicillin-Resistant Staphylococci (MRS) from Animal and Human Origin

S.N. Ghodasara[1]*, J.H. Purohit[2], A.R. Baria[3], A.S. Patel[3], B.B. Javia[1], D.B. Barad[1], J.A. Kathiria[4] and A.R. Bhadania[5]

[1]Department of Veterinary Microbiology, College of Veterinary Science and A. H., Junagadh Agricultural University, Junagadh, Gujarat, India
[2]Department of Veterinary Microbiology, College of Veterinary Science and A. H., Anand Agricultural University, Anand, Gujarat, India
[3]Department of Livestock Products Technology, College of Veterinary Science and A. H., Junagadh Agricultural University, Junagadh, Gujarat, India
[4]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and A. H., Junagadh Agricultural University, Junagadh, Gujarat, India
[5]Department of Veterinary Pathology, College of Veterinary Science and A. H., Junagadh Agricultural University, Junagadh, Gujarat, India

**Corresponding author*

Keywords

SCC*mec*, Methicillin, Community associated, Hospital associated, Nasal swab

Article Info

Accepted:
10 September 2019
Available Online:
10 October 2019

A B S T R A C T

A total of 202 animal samples (167 milk and 35 pus/exudate samples from cattle and buffalo) were collected and examined from dairy cattle and buffaloes. Simultaneously, 100 nasal swabs were collected from the closely associated personnel and farm workers. Out of these, the *Staphylococcus* spp. were isolated from86 (42.57%) animal and 62 (62%) human samples. Of these, total 9 isolates from animals and 20 isolates from human were identified as MRS positive. Out of 9 and 20 MRS isolates from animal and human, 1 (11.11%) and 2 (10%) isolate were Methicillin-Resistant Coagulase Positive Staphylococcus (MRCoPS), whereas 8 (88.89%) and 18 (90%) isolates were Methicillin-Resistant Coagulase Negative Staphylococcus (MRCoNS), respectively. The SCC*mec* typing of 16 and 40 MRS (Including departmental isolates) isolates from animal and human were carried out. Out of these, the study revealed 18.75% (3/16) and 25% (10/40) isolates were classified as hospital associated methicillin-resistant staphylococci (HA-MRS), whereas 68.75% (11/16) and 45% (18/40) isolates were classified as community associated methicillin resistant staphylococci (CA-MRS) and 12.5% (2/16) and 30% (12/40) MRS isolates remained untypable, respectively. The overall percentage of CA-MRS (63.04%) was higher as compare to HA-MRS (28.26%) among both the species.

Introduction

Staphylococcus aureus is one of the most extensively studied bacteria of genus *Staphylococcus.* During recent years, Coagulase Negative Staphylococci (CNS) referred as "*Environmental Staphs"* have become the most common bovine mastitis isolates in many countries and are regarded as emerging mastitis pathogens (Jakee *et al.,* 2013).

Amongst the Staphylococci group, *Staphylococcus aureus* strains are more dangerous to dairy animals and are of greatest attention for scientific community worldwide as they have ability to resist antibiotic therapy due to production of beta-lactamases a group of enzymes that inactivate penicillin and closely related antibiotics. To overcome infections caused by beta-lactamase producing Staphylococci, narrow spectrum semi-synthetic penicillin (methicillin) was introduced. However, soon after introduction, Methicillin-resistant *Staphylococcus aureus* (MRSA) strain was identified. Initially, MRSA strains were encountered only in the hospitals, but in the late 1990s first virulent community-acquired MRSA (CA-MRSA) clones, characterized by the presence of the toxin Panton-Valentine Leukocidin (*PVL*), appeared rapidly and unexpectedly. They quickly spread worldwide, initially only in the community, but later on also in the healthcare facilities, displacing in some countries typical HA-MRSA. For this reason, nowadays, distinction between CA-MRSA and mostly multi resistant HA-MRSA become challenging (Chambers and Deleo, 2009).

MRSA needs to be identified below the species level by rapid and reliable typing methods. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing accompanied with overall genotyping has already provided strong evidence for the independent origins of healthcare associated MRSA and community acquired MRSA (Naimi *et al.,* 2003). To date, eleven different types of SCC*mec* (I–XI) have been defined on the basis of the combination of *ccr* and *mec* complexes, but only type I–V are globally distributed, while others appear to exist as local strains in the country of origin (Zhang *et al.,* 2009). Among this five types, SCC*mec* types I, II and III are mainly associated with healthcare associated MRSA (HA-MRSA) strains, whereas SCC*mec* type IV and Vare associated with community associated MRSA (CA-MRSA) strains, as well as with the Pediatric clone MRSA strains (Shore *et al.,* 2005).

The literature reviewed that there are lots of reports on staphylococcal cassette chromosome *mec* (SCC*mec*) types in Methicillin-resistance Staphylococci of human origin across the globe including India, but in compare to human, few reports on SCC*mec* typing have been published from animals across the globe and still very few from India. The identification and molecular SCC*mec* typing and epidemiological studies have been conducted from pig and human, but scanty of references available regarding animal and human SCC*mec* types, epidemiological studies and their relationship in the world and in India.

Materials and Methods

The present investigation was carried out to optimize and standardize uniplex PCR for various types of Staphylococci SCC*mec* types from MRS prevalent in this region in animals and closely associated human beings that will facilitate the study of epidemiological correlation between human and animal isolates on the basis of hospital acquired methicillin resistance staphylococci (HA-MRS) or community acquire methicillin resistance staphylococci (CA-MRS).

Collection of samples from milk, pus/exudate (abscess) from animal and human nasal swabs

A total of 202 animal samples (167 milk and 35 pus/exudate samples from cattle and buffalo) were collected and examined from dairy cattle and buffaloes. Simultaneously, 100 nasal swabs were collected from the closely associated personnel and farm workers aseptically as per the guidelines given by Peacock *et al*., (2001). The milk samples were collected from clinical and sub clinical mastitis as per the guidelines laid by European Food Safety Authority (EFSA, 2009). Simultaneously, the isolates of Staphylococci previously recovered from subclinical and clinical mastitic milk from bovines and human nasal swabs and maintained at the Department of Veterinary Microbiology, Junagadh Agricultural University, were included in the study for SCC*mec* typing.

The isolates presumably identified as *Staphylococcus* spp. based on cultural, colony and biochemical characteristics (Result not shown) and amplified with different pairs of 4 primers by m-PCR for the identification of Methicillin-Resistance coagulase positive staphylococci (MRCoPS) or Methicillin-Resistance coagulase negative staphylococci (MRCoNS).

The isolates were confirmed as methicillin resistant staphylococci (MRS) using *mecA*-positive gene amplification. The isolates confirmed as MRS were further subjected to SCC*mec* typing using four primer-pairs designed to identify the five main known SCC*mec* types.

Isolation of bacterial genomic DNA from bacterial culture by Proteinase K-SDS method

The genomic DNA of staphylococci from bacterial broth culture was extracted according to Parayre *et al.*, (2007) with minor modifications.

Molecular detection of MRSA isolates from animal and humans

Four predominant genes (*16S rRNA*, *Nuc*, *Coa* & *MecA* gene targets) (Table 1) identified based on the outcome of phenotype-based speciation were considered to be included for detection by m-PCR. From all genes, *16S rRNA* was used to identify genus i.e. Staphylococci, *Nuc* gene encodes for thermonuclease was used to identify species i.e. *Staphylococcus aureus*, *Coa* gene encodes for coagulase was used as pathogenicity indicator and *mecA* gene target encodes for modified penicillin binding protein 2B was used for genotypically identification of methicillin resistance in *Staphylococcus* spp. Five microliters of the extracted DNA were used as a template in a25 µL PCR mixture containing 14.25µLof 2X PCR master mix (Thermoscietific) (0.05 U/µL Taq DNA polymerase, reaction buffer,4 mM MgCl2, 0.4 mM of each dNTP (dATP, dCTP, dGTPand dTTP), 0.5 µL of each forward and reverse primer except *mecA*(1.25 µL of each forward and reverse primers) and 0.1 µLTaq Polymerase (5U/ µl) (Thermo scientific).

The reference strain of *Staphylococcus aureus* ATCC 43300 and ATCC 25923 (Himedia Pvt. Ltd., Mumbai) was used as negative and positive control, respectively for *mecA* gene. The Steps and cycling conditions for m-PCR were Initial denaturation at 94 °C for 5 min followed by 40 cycle of Denaturation at 94 °C for 30 sec, Annealing at 52 °C for 1 min., Extension at 72 °C for 1 min and Final extension at 72 °C for 10 min in m-PCR. Using m-PCR Methicillin Resistant Coagulase Positive Staphylococci (MRCoPS) as well as Methicillin Resistant Coagulase Negative Staphylococci (MRCoNS) were identified in one single reaction.

Molecular detection of SCC*mec* types from methicillin-resistant staphylococci from animal and humans

Four primer sets were used to ensure amplification of two DNA targets from SCC*mec* type IV and two targets from SCC*mec* type V. The targets were chosen so that one target would be amplified from each of SCC*mec* types I–III (Table 2). The PCR of MRS colonies was carried out in final reaction volume of 25 µl in thermal cycler (verity, Applied Biosystems by life technology, Singapore).

Three microliters of the extracted DNA were used as a template in a25 µL PCR mixture containing 12.5 µL of 2X PCR master mix (Thermoscientific) (0.05 U/µL Taq DNA polymerase, reaction buffer,4 mM MgCl2, 0.4 mM of each dNTP (dATP, dCTP, dGTPand dTTP), 1 µL of each forward and reverse primer and 7.5 µl nuclease free water.

The Steps and cycling conditions for PCR were Initial denaturation at 94 °C for 4 min followed by 30 cycle of Denaturation at 94 °C for 30 sec, Annealing at 55 °C for 30 sec, Extension at 72 °C for 60 sec and Final extension at 72 °C for 4 min for different primer pairs described by Boye *et al.*, (Boye *et al.*, 2007). The reference strain of *E. coli* (MTCC-522) strain was used as SCC*mec* types negative.

PCR products (5 µL) were analyzed by electrophoresis on agarose 1.5% w/v gel followed by staining with ethidium bromide. The SCC*mec* type was determined on the basis of the band pattern obtained (Table 3).

Isolates with no visible bands, or with a band pattern that was not in agreement with one of the five predicted band patterns, were classified as non typeable (NT).

Results and Discussion

m-PCR for the detection of MRSA from animal and human isolates

The 202 animal samples (milk, pus/exudate) and 100 human nasal swabs were processed for isolation of bacteria as per standard procedures (Quinn *et al.*, 1994). Out of total 202 animal and 100 human samples collected 86 (42.57%) and 62 (62%) isolates, respectively were identified as *Staphylococcus* spp. based on phenotypic, biochemical growth patterns (Result not shown) and molecular characterization.

The m-PCR was carried out for detail classification and characterization of presumably identified staphylococci. Out of total 202 animal samples, 86 isolates were identified as staphylococci based on amplification of *16S rRNA* gene. Of these, 74 isolates were identified as Coagulase Negative Staphylococci (CoNS) based on amplification of only *16S rRNA* gene and 12 isolates were identified as Coagulase Positive Staphylococci (CoPS) based on amplification of both *16S rRNA* and *Coa* gene. Based on amplification of three genes *16S rRNA*, *Coa* and *mecA*, 8 isolates were identified as Methicillin-resistant Coagulase Negative Staphylococci (MRCoNS) and 1 isolates were Methicillin-Resistant Coagulase Positive Staphylococci (MRCoPS), whereas only one isolate was identified (based on all four gene amplification pattern) as Methicillin-Resistant Coagulase Negative *Staphylococcus aureus* (MRCoNSA) from the animal samples (Table 4). Based on amplification of genes *16S rRNA* and *mecA* gene, 9 isolates were identified as positive for Methicillin-Resistant Staphylococci (MRS) (Figure 1).

The 100 samples were collected from the human nasal swabs which had remained in contact with these animals. Of these, 62

isolates were identified as *Staphylococcus* based on amplification of *16S rRNA* gene and 50 isolates were identified as Coagulase Negative Staphylococci (CoNS) based on amplification of only *16S rRNA* gene and 12 isolates were identified as Coagulase Positive Staphylococci (CoPS) based on amplification of both *16S rRNA* and *Coa* gene. Based on amplification of three genes *16S rRNA*, *Coa* and *mecA*, 18 isolates were identified as Methicillin-Resistant Coagulase Negative Staphylococci (MRCoNS) and 2 isolates were identified as Methicillin-Resistant Coagulase Positive Staphylococci (MRCoPS), whereas only 2 isolates were identified (based on all four gene amplification pattern) as MRSA. Of this 2 isolates, one isolate was Methicillin-Resistant Coagulase Negative *Staphylococcus aureus* (MRCoNSA) and one isolate was Methicillin-Resistant Coagulase Positive *Staphylococcus aureus* (MRCoPSA) from the human samples (Table 4). Based on amplification of genes *16S rRNA* and *mecA* gene, 20 isolates were identified MRS positive.

Molecular characterization of major SCC*mec* types among MRS from animal and humans

The occurrence of staphylococcal cassette chromosome *mec* types (SCC*mec* types) were investigated from MRS isolates obtained from animal and human samples. Out of total 133 and 102 *Staphylococcus* spp. isolates (47 and 40 departmental staphylococci isolates from animal and human were included, respectively), 16 and 40 isolates from animal and human were identified as Methicillin-resistant staphylococci, respectively. Of these 16 animal MRS isolates, 14 isolates having one of the SCC*mec* types (SCC*mec* type I, 2; SCC*mec* type II, 0; SCC*mec* type III, 1; SCC*mec* type IV, 5; SCC*mec* type V, 6), whereas 2 isolates were untypable, whereas from 40 human MRS isolates, 28 isolates having one of the SCC*mec* types (SCC*mec* type I, 7; SCC*mec* type II, 0; SCC*mec* type III, 3; SCC*mec* type IV, 9; SCC*mec* type V, 9), whereas 12 isolates were Untypable (Table 5 and Figure 2).

During the current study, 18.75% (3/16) isolates were classified as hospital associated methicillin-resistant staphylococci (HA-MRS) (SCC*mec* type I, 2; SCC*mec* type III, 1), whereas 68.75% (11/16) isolates were classified as community associated methicillin resistant staphylococci (CA-MRS) (SCC*mec* type IV, 5; SCC*mec* type V, 6) and 12.5% (2/16) isolates remained untypable from animal MRS isolates.

Table.1 Nucleotide sequences of primers used for m-PCR along with their product size and references

Sr. No.	Name of primer	Primer sequence (5'-- 3')	Product size	Reference
1	TStaG422F1 TStaG765R1	5'- GGC CGT GTT GAA CGT GGT CAA ATC A-3' 5'- TIA CCA TTT CAG TAC CTT CTG GTA A-3'	370 bp	(Martineau*et al.*, 2001)
2	Nuc F Nuc R	5`- GCG ATT GAT GGT GAT ACG GTT -3` 5`- ACG CAA GCC TTG ACG AAC TAA AGC -3`	280 bp	(Brakstad*et al.*, 1992)
3	Coa F1 Coa R1	5'- GTA GAT TGG GCA ATT ACA TTT TGG AGG -3' 5'- CGC ATC AGC TTT GTT ATC CCA TGT A -3'	117 bp	(Moon *et al.*, 2007)
4	mecA F1 mecA R1	5'- 'GAGT TGT AGT TGT CGG GTT TGG-3' 5'- ' GGC CAA TTC CAC ATT GTT TC-3'	454 bp	(Malik *et al.*, 2006)

Table.2 Nucleotide sequences of primers used for the identification of SCC*mec* types of MRS

Sr. No.	Name of primer	Primer sequence (5'-- 3')	Product size	Reference
1	β F1 α 3 R1	5'- ATTGCCTTGATAATAGCCYTCT -3' 5'- TAAAGGCATCAATGCACAAACACT-3'	937 bp	(Ito *et al.*, 2001)
2	ccrCF1 ccrCR1	5'- CGTCTATTACAAGATGTTAAGGATAAT-3' 5'- CCTTTATAGACTGGATTATTCAAAATAT-3'	518 bp	(Ito *et al.*, 2004)
3	1272F1 1272R1	5'- GCCACTCATAACATATGGAA-3' 5'- CATCCGAGTGAAACCCAAA-3'	415 bp	(Boye *et al.*, 2007)
4	5RmecAF1 5R431R1	5'- TATACCAAACCCGACAACTAC-3' 5'- CGGCTACAGTGATAACATCC-3'	359 bp	(Boye *et al.*, 2007)

Table.3 Primers used in SCC*mec* typing PCR and the resulting gel band patterns of SCC*mec* types I-V

Sr. No.	Name of primer	Product size	Target Gene	SCC*mec* type				
				I	**II**	**III**	**IV**	**V**
1	β F1 α 3 R1	937 bp	ccrA2-B		√		√	
2	ccrCF1 ccrCR1	518 bp	ccrC			√		√
3	1272F1 1272R1	415 bp	IS1272	√			√	
4	5RmecAF1 5R431R1	359 bp	*mecA*–IS431					√

√-Desired amplification of gene.

Table.4 Coagulase Negative (MRCoNS) and coagulase positive (MRCoPS) MRS isolates from animal ad humans

Sample origin	No. of samples collected	No. of sample found positive for *Staphylococcus* spp. No. (%)	Methicillin-Resistant *Staphylococcus* (out of positive *Staphylococcus*) No. (%)	No. (%) Methicillin-Resistant *Staphylococcus*	
				MRCoNS No. (%)	**MRCoPS No. (%)**
Animals (Milk+Pus)	202	86 (42.57)	9 (10.47)	8 (88.89)	1 (11.11)
Human (Nasal swabs)	100	62 (62)	20 (32.26)	18 (90)	2 (10)

MRCoNS – Methicillin-Resistant Coagulase Negative *Staphylococcus*
MRCoPS – Methicillin-Resistant Coagulase Positive *Staphylococcus*

Table.5 Occurrence and correlation between SCC*mec* type isolates based on HA-MRS and CA-MRS among animal and human MRS isolates

	MRS isolates from Animal			MRS isolates from Human		
SCC*mec* types of MRS	**Positive for SCC*mec* type No. (%)**	**MRS type**	**No. (%) Positive for HA-MRS/ CA-MRS**	**Positive for SCC*mec* type (No.)**	**MRS type**	**No. (%) Positive for HA-MRS/ CA-MRS**
SCC*mec* type I	2 (12.5)	HA-MRS	**3 (18.75)**	7 (17.5)	HA-MRS	**10 (25)**
SCC*mec* type II	0	-		0	-	
SCC*mec* type III	1 (6.25)	HA-MRS		3 (7.5)	HA-MRS	
SCC*mec* type IV	5 (31.25)	CA-MRS	**11 (68.75)**	9 (22.5)	CA-MRS	**18 (45)**
SCC*mec* type V	6 (37.5)	CA-MRS		9 (22.5)	CA-MRS	
UT	2 (12.5)	-	**2 (12.5)**	12(30)	-	**12 (30)**
Total	**16**			**40**		

Figure.1 PCR amplicons of Methicillin-resistant coagulase negative/positive *Staphylococcus aureus* by mPCR (*16S rRNA* gene = 370bp, *MecA* = 454bp, *Coa*= 280bp and *Nuc* = 117bp) from animal isolates

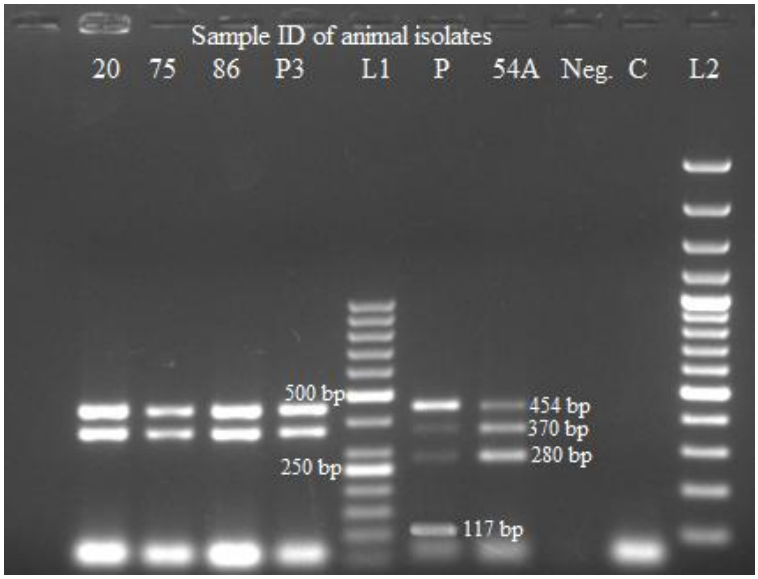
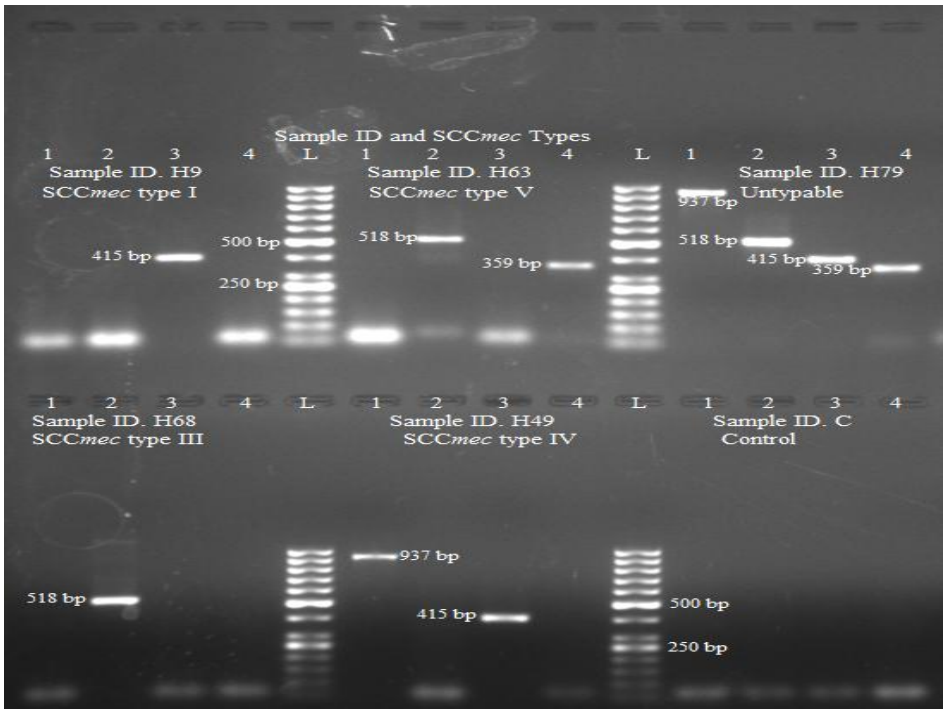


Figure.2 SCC*mec* type specific PCR amplification products (SCC*mec* type I-V) from human MRS isolates



Similarly, study revealed 25% (10/40) isolates were classified as hospital associated methicillin resistant staphylococci (HA-MRS) (SCC*mec* type I, 7; SCC*mec* type III, 3), whereas 45% (18/40) isolates were classified as community associated methicillin resistant staphylococci (CA-MRS) (SCC*mec* type IV, 9; SCC*mec* type V, 9) isolates and 30% (12/40) isolates remained untypable from human MRS isolates. The overall percentage of CA-MRS (63.04%) was higher as compare to HA-MRS (28.26%) among both the species (Figure 2).

In many countries, the presence of MRSA in animal and human is of veterinary and public health concern. Nasal carriage has an important role in the epidemiology and pathogenesis of MRSA infection in human and animal (Kluytmans *et al.*, 1997). Several reports showed the prevalence of MRSA in cattle with mastitis (Bhagat *et al.*, 2017). In a study made in farm animals, the nasal MRSA prevalence was found to be 0.3 % and 1 % in 400 cattle and 300 calves, respectively (Huber *et al.*, 2010) which was lower as compared to present findings. In comparison to current finding, higher rates of MRSA were reported by, Erdem and Turkyilmaz (Erdem and Turkyilmaz, 2013) reported 7.14% (4/56) bovine and 17.64% (6/34) human nasal isolates were positive for MRSA. In another study from Turkey, Methicillin resistant was detected in 26.8 % (15/56) and 62.8 % (22/35) of bovine nasal and human nasal isolates, respectively (Inegol and Turkyilmaz, 2012). Garipcin and Seker, (Garipcin and Seker, 2015) revealed the nasal carriage rates of MRSA in 150 humans and 250 cattle at the rate of 8.7 % and 1.2 % respectively.

Amplification of all these primers in accordance with presence of respective genes gives identification of presence of Methicillin-resistant Coagulase Negative Staphylococci (MRCoNS) or Methicillin-

Resistant Coagulase Positive Staphylococci (MRCoPS). Similar to present study Nimavat, (Nimavat, 2015) and Hetal, (Hetal, 2016) applied multiplex PCR assay using same primer pairs used in this study and characterized the staphylococci as similar fashion as characterized in this study.

The study revealed higher percentage of CA-MRSA isolates as compare to HA-MRSA in animal as well as in human, indicating emergence of CA-MRSA strains in this geographical area. Inegol and Turkyilmaz (Inegol and Turkyilmaz, 2012) revealed higher rate of (67.80%) isolates including type II and III were hospital acquired (HA-MRS) whereas 32.20% isolates including type IV and V were community acquired (CA-MRS), which was lower as compared to present finding, but they had reported similarity of SCC*mec* types between bovine and human isolates, suggestive of evidence of transmission from animals to humans, or vice versa. Bhutia *et al.,* (Bhutia *et al.,* 2015) reported 25.33% (38/150) isolates met the definition of CA-MRSA and 10% (15/150) of HA-MRSA, which was lower as compare to present study.

During the study, the isolates from animal (Milk, pus/exudate) yielded various SCC*mec* type (SCC*mec* type I, III, IV and V), similarly the same SCC*mec* types were isolated from the human nasal swab of labors/worker/personnel who remain in close contact with this animals. During the current study, the same SCC*mec* types prevail in animaland human beings attributed the transmission of MRS from animal to human or vice versa indicating potential zoonotic pathogen prevalence in farm animal and their workers.

Simultaneously, we found that the MRSA strains harboured the high and variable prevalence of SCC*mec* alleles which is an additional pathogenic factor for infections. SCC*mec* IV and V were the most prevalent alleles in our findings. The CA-MRSA is mainly considered by SCC*mec* IV and V alleles, while HA-MRSA are recognized by SCC*mec* I, II and III alleles (De Lencastre, 2007). During the current study, MRS strains were mainly associated with CA-MRSA. In other hand, detection of SCC*mec* types IV and V suggested the emergence of CA-MRSA strains in this geographical area and occurrence of SCC*mec* I and II alleles indicated a possible transmission of MRSA from humans to animals. Further studies are needed to establish clonal relation of MRS from animaland farm workers with advance molecular techniques (Pulsed field gel electrophoresis, Staphylococcal Protein A analysis, multilocus sequence typing). The result of these studies may shed light on the clonility and transmission of resistance strain between human and animal. High prevalence of SCC*mec* types IV and V were also detected by various authors (Havaei *et al.,* 2015).

MRSA infected cattle acts as a reservoir and later transmit the infections to other animals and humans. MRSA colonization in cattle may be an occupational risk to the people in close contact with MRSA infected cattle such as veterinarians, farmers, milkers and people working at slaughterhouses. Transmission of animal MRSA to veterinary personnel has been found and it is more common for large animal personnel than small animal personnel (Wulf *et al.,* 2008).

Although, MRSA has been reported as transmissible diseases of zoonosis as well as humanosis importance, the direction and routes of transmission are superficially understood. Some authors have reported bidirectional transmission of MRSA (Ferreira *et al.,* 2011). Animal to human transmission occurs through direct contact, environmental contamination and through handling of

infected animal product, whereas human to animal transmission is still unclear (Weese, 2010).

The similar SCC*mec* types in both the groups (Animal and human) suggest co-circulation of MRS isolates between human and animal population which was indication of possible lateral gene transfer between the staphylococcal isolates in this region. Most of the reported SCC*mec* types (except SCC*mec* type II) were found in this region. Higher number of CA-MRS associated types IV, V and a marked number of these in animal isolates suggested the possible transfer of resistance from human to animal isolates.

References

American Veterinary Medical Association (AVMA). 2014. MRSA and animals FAQ. Available online at https://www.avma.org/KB/Resources/FAQs/Pages/MRS A-HHP-FAQs.aspx.

Bhagat, A. G., H.N. Kher, A.I. Dadawala, H.C. Chauhan, B.K. Patel, M.D. Shrimali and B. S. Chandel. 2017. Identification and characterization of Methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis. Int. J. Curr. Microbiol. App. Sci, 6(2): 223-230.

Bhutia, K. O., Singh, T. S. K., Adhikari, L. and Biswas, S. 2015. Molecular characterization of community- & hospital-acquired methicillin-resistant & methicillin-sensitive *Staphylococcus aureus* isolates in Sikkim. Indian J. Med. Res, 142: 330-335.

Boye, K., Bartels, M. D., Andersen, I. S., Moller, J. A. and Westh. H. 2007. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I–V. Clin. Microbiol. Infect, 13: 725–727.

Brakstad, O. G. Aasbakk, K and Maeland, J. A. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J. Clin. Microbiol, 30(7):1654-1660.

Chambers, H. F. and Deleo, F. R. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol, 7: 629-641.

De Lencastre, H., Oliveira, D. and Tomasz, A. 2007. Antibiotic resistant *Staphylococcus aureus*: A paradigm of adaptive power. Curr. Opin. Microbiol, 13: 971–979.

Erdem, Z. and Turkyilmaz, S. 2013. Molecular typing of methicillin resistant *Staphylococcus aureus* strains isolated from cows and farm workers. Kafkas Univ. Vet. Fak. Derg, 19: 963-968.

European Food Safety Authority (EFSA). 2009. Assessment of the Public Health significance of methicillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. Scientific Opinion of the Panel on Biological Hazards. EFSAJ,93:1-73.

Garipcin, M. and Seker, E. 2015. Nasal carriage of methicillin-resistant *Staphylococcus aureus* in cattle and farm workers in Turkey. Vet. Arhiv, 85:117-129.

Havaei, S. A., Assadbeigi, B., Esfahani, B. N., Hoseini, N. S., Rezaei, N. and Havaei, S. R. 2015. Detection of *mecA* and enterotoxin genes in *Staphylococcus aureus* isolates associated with bovine mastitis and characterization of Staphylococcal cassette chromosome *mec* (SCC*mec*) in MRSA strains. Iranian journal of microbiology, 3: 161-167.

Hetal, J. 2016. Optimization of real time PCR for detection of methicillin resistant staphylococci and molecular characterization of *mecA* gene. M. V. Sc. (Veterinary Microbiology) Thesis (Unpublished). Junagadh Agricultural

University, Junagadh.

Huber, H., Koller, S., Giezendanner, N., Stephan, R. and Zweifel, C. 2010.Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland. Euro Surveill, 15(16): 19542.

Inegol, E. and S. Turkyilmaz, S. 2012. Determination of SCC*mec* types in methicillin resistant Staphylococci isolated from cows and farm workers. *Ankara Univ*. Vet. Fak. Derg,59: 89-93.

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C. and Hiramatsu. K. 2001. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother, 45: 1323–1336.

Ito, T., Ma, X. X., Takeuchi, F., Okuma, K., Yuzawa, H. and Hiramatsu, K. 2004. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother, 48: 2637–2651.

Jakee, J. K., Aref, N. E., Gomaa, A., Hariri, M. D., Galal, H. M., Omar, S.A. and Samir, A. 2013. Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: An environmental hazard. Int. J. Vet. Sci. Med, 1: 74–78.

Kluytmans, J., Belkum, A. V. And Verbrugh, H. 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev, 10: 505-520.

Malik, S., Peng, H. and Barton, M. 2006. Partial nucleotide sequencing of the *mecA* genes of *Staphylococcus aureus* isolates from dogs and cats. J. Clin. Microbiol, 44: 413–416.

Martineau, F., Picard, F. J., Ke, D., Paradis, S., Roy, P. H., Ouellette, M. and Bergeron, M.G. 2001. Development of a PCR assay for identification of *staphylococc*i at genus and species levels. J. Clin. Microbiol, 39:2541–2547.

Moon, J. S., Lee, A. R., Kang, H. M., Lee, E. S., Joo, Y. S., Park, Y. H., Kim, M. N. and Koo, H. C. 2007. Antibiogram and coagulase diversity in staphylococcal enterotoxin-producing *Staphylococcus aureus* from bovine mastitis. J. Dairy Sci, 90: 1716-1724.

Naimi, T. S., LeDell, K. H., Como-Sabetti, K., Borchardt, S. M., Boxrud, D. J., Etienne, J., Johnson, S. K., Vandenesch, F. and Fridkin, S. 2003. Comparison of community- and health care associated methicillin-resistant *Staphylococcus aureus* infection. JAMA, 290: 2976–2984.

Nimavat, V. R. 2015. Optimization of multiplex PCR for identification of MRSA from bovine milk samples & its comparison with conventional diagnostic methods. M. V. Sc. (Veterinary) Thesis (Unpublished). Junagadh Agricultural University, Junagadh.

Parayre S., Falentin H., Madec M. N., Sivieri K., Le Dizes A. S., Sohier D. andLortal S. 2007. DNA extraction method and optimization of PCR-temporal temperature gel electrophoresis to identify the predominant high and low GC-content bacteria from dairy products. J. Microbiol. Methods, 69:431–441.

Peacock, S. J., de Silva I. and Lowy F. D. 2001. What determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol, 9 (12): 605–610.

Quinn, P. J., Carter, M. E., Markey, B. K. and

Carter, G. R. 1994. Enterobacteriaceae. In: Quinn, P. J.; Carter, M. E.; Markey, B. K. and Carter, G. R. (eds) Clinical Veterinary Microbiology. Mosby-Year Book Europe, London, pp. 209–236

Shore, A. Rossney, A. S. Keane, C. T. Enright, M. C. and Coleman, D. C. 2005. Seven novel variants of the staphylococcal chromosomal cassette *mec* in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. Antimicrob. Agents Chemother, 49: 2070-2083.

Weese, J. S. 2010. Methicillin-Resistant *Staphylococcus aureus* in Animals. ILARJ, 51: 233-244.

Wulf, M. W., Sorum, M., VanNes, A., Skov, R., Melchers, W. J. and 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.

Zhang, K. McClure, J. A. Elsayed, S. and Conly, J. M. 2009. Novel staphylococcal cassette chromosome *mec* type, tentatively designated type VIII, harboring class A*mec* and type 4 *ccr* gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother, 53: 531–540.

How to cite this article:

Ghodasara, S.N., J.H. Purohit, A.R. Baria, A.S. Patel, B.B. Javia, D.B. Barad, J.A. Kathiria and Bhadania, A.R. 2019. Molecular Characterization of Major Types of Staphylococcal Cassette Chromosomemec (SCCmec) in Methicillin-Resistant Staphylococci (MRS) from Animal and Human Origin. *Int.J.Curr.Microbiol.App.Sci.* 8(10): 916-927.
doi: https://doi.org/10.20546/ijcmas.2019.810.107