International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 7, Issue 3, 2015, pp.-648-655.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *Staphylococcus aureus* FROM GOATS, PIGS AND THEIR HANDLERS

REDDY M.S.[1], BABU A.J.[2]*, RAMYA P.[2] AND SWETHA C.S.[2]

[1]Veterinary Assistant Surgeon, AP, India.
[2]Department of Veterinary Public Health & Epidemiology, College of Veterinary Science, Proddatur - 516 360, AP, India.
*Corresponding Author: Email- drjagadeeshvet@yahoo.co.uk

Received: May 14, 2015; Revised: June 08, 2015; Accepted: September 12, 2015

Abstract- All the nasal swabs collected from the domestic animals like goat and pigs and from animal handlers were used for isolation of *Staphylococcus aureus*. From a total of 339 samples, 292 isolates were subjected to Gram's staining and found purple coloured cocci in clusters. Among the 339 isolates 101 isolates were confirmed as pathogenic *Staphylococcus aureus* by a positive coagulase test. The biochemical tests like IMViC tests, urease test, oxidase test, nitrate reduction test and catalase tests, confirmed the presence of *Staphylococcus aureus*. DNase test revealed the characteristic blue to purple coloured colonies with clear zones around them. On blood agar plates the isolates produced β haemolysis. Two sets of primers derived from *nuc* gene and *mecA* genes were used for the identification of *Staphylococcus aureus* and its methicillin resistance respectively for the PCR assay. Out of 151 goat samples 115 (76.15%) were positive for *Staphylococcus aureus* by cultural methods and 112 (74.1%) were positive by PCR method. Out of 112 PCR positives none were found positive for MRSA by PCR. Out of 102 pig samples 96 (94.1%) were positive for *Staphylococcus aureus* by cultural methods and 96 (94.1%) were positive by PCR method. Out of 96 PCR positives one isolate was found positive for *mecA* by PCR which accounts to 0.98% over the total number of samples and 1.04% over the positive samples for *Staphylococcus aureus* by PCR. Out of 86 human samples 81 (94.18%) were positive for *Staphylococcus aureus* by cultural methods and 80 (98.76%) were positive by PCR method. Out of 80 PCR positives none was found positive for *mecA* by PCR.

Keywords- Methicillin resistant *Staphylococcus aureus*, goats, pigs, humans and PCR

Citation: Reddy M.S., et al. (2015) Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* from Goats, Pigs and their Handlers. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 7, Issue 3, pp.-648-655.

Introduction

There has been an increased concern throughout the world about the pathogenic micro organisms which are resistant to commonly used antibiotics for their control. Among different multi drug resistant pathogenic micro organisms *Staphylococcus aureus* is one of the most important bacteria, particularly its methicillin resistant strains. The antibiotic methicillin was introduced in to medical practice in 1960s, and now resistant strains of *Staphylococcus aureus* were found in human population throughout the world [1]. Now MRSA has become a global health problem and there is a significant increase in both morbidity and mortality in humans throughout the world [2].

In the recent years increased reports are there regarding the occurrence of MRSA in livestock and companion animals and this has become an emerging problem in veterinary practice. The first case of MRSA was reported in the year 1972 from the Belgian cows affected with mastitis [3]. Since that time a large number of reports were there about the clinical cases of MRSA in dogs, cats, horses, pigs and other animal species [4,5]. Detection of MRSA in animals has been reported by various scientists in the previous studies [6]. Documentation is also there regarding the prevalence of MRSA in farm or domestic animals like goats, sheep, cattle, horses and further in different companion animals such as dogs and cats [6,7] and revealing the fact that MRSA has emerged as a potential zoonotic pathogen. These studies of MRSA among the domestic and companion animals have raised the curtains for extensive further studies to address the issue of MRSA colonization and transmission to human beings particularly those who are in close contact with the animals [8].

Methicillin is grouped under narrow spectrum beta-lactamase resistant penicillin's. The mechanism of action is by interfering primarily with the synthesis of bacterial cell wall and will be responsible for binding of methicillin to penicillin binding proteins (PBPs) [6]. *Staphylococcus aureus* has the ability to develop resistance to any antibiotic that comes in to clinical use. Methicillin resistance to *Staphylococcus aureus* is due to the acquisition of the *mecA* gene that encodes a new protein designated as PBP 2a which belongs to a family of enzymes necessary in building the bacterial cell wall. PBP 2a has a very low affinity for beta-lactams [9]. The *mecA* gene is placed on a mobile genetic element, which is called as staphylococcal cassette chromosome *mec (scc mec)* inserted in the staphylococcal chromosome upstream *orf X* [10]. Different types of *scc mec*

can be distinguished on the basis of different key elements present, that are the *mec* gene complex, comprising *mecA* and its regulatory genes *mec I* and *mec R1* and the *ccr* genes complex comprising to different *ccr* recombinases that are responsible for the mobility of the element [11].

MRSA is developed by the introduction of a *mecA* carrying element in a methicillin susceptible *Staphylococcus aureus* [12]. The origin of *mecA* has long been searched for [13] and found a *mecA* homolog with 80% identity to the *Staphylococcus aureus* gene in *Staphylococcus sciuri,* a methicillin susceptible staphylococcus of rodents and other primitive mammals. Another homolog of *mecA* with 91% identity with *Staphylococcus aureus mecA* was found in *staphylococci* isolated from horses and specifically in *Staphylococcus vitulinus* [14]. As per the recent data available *Staphylococcus fleurettii* belonging to *Staphylococcus sciuri* group, could be the origin of *mecA* as this species contains *mecA* and the chromosomal locus surrounding *mecA* that are almost identical to the corresponding sequence of *scc mec* [15]. As per these studies carried out it indicate that animal staphylococci are considered to be the origin and reservoir of *mecA.*

There has been a number of reports stating that animals may serve as reservoirs for MRSA infection of humans. In the last two decades, new generations of MRSA have emerged with the ability to transfer to human beings and food producing animals. There is a risk of transfer of MRSA in food animals to humans. The potential of MRSA to become a dangerous zoonotic pathogen could affect the epidemiology of MRSA in humans. As the prevalence of MRSA in animals is continuous to rise, there is an inherent risk for new MRSA clones to evolve secondary to horizontal gene transfer and host selection pressure and then spread to human hosts. Thus the presence of MRSA in animals is a concern not only to veterinarians and animal health care workers but also to public health.

Materials & Methods

The specimens selected for this study were nasal secretions. These secretions were collected by using sterile cotton swabs. Cotton swabs were sterilized in hot air oven at a temperature of 160ºC for 1 hour. A cotton tipped dry swab was inserted into the anterior nares of animals and human beings and rubbed gently against the mucosa for approximately 5 seconds and it was placed in normal saline. A total of 339 nasal swabs from anterior nare of animals and human beings were collected aseptically in sterile normal saline tubes. The collected specimens were processed within 2 to 24 hours of collection. The source and number of samples collected in this study are given in [Table-1].

Table 1- Source and number of samples collected

Species	Source of the samples	Number collected	Total
Goats	Kanigiri Mandal, Prakasam District, Andhra Pradesh	54	151
	Goat farm, College of Veterinary Science, Tirupati	20	
	Private goat farm, Thondawada, Chittoor, AP	19	
	Naidupet Mandal, Nellore District, Andhra Pradesh	58	
Pigs	AICRP on pigs, College of Veterinary Science, Tirupati	54	102
	Private pig farm, Tiruchanooru, Chittoor, AP	48	
Humans	Goat handlers	74	86
	Pig handlers of AICRP on pigs, College of Veterinary Science, Tirupati	12	
Grand Total			339

For the isolation and identification of *Staphylococcus aureus* tryptic soy broth was used for enrichment of inoculum. Baird Parker agar supplemented with 5% sterile egg yolk tellurite suspension was used for isolation of *Staphylococcus aureus*. During isolation cotton tipped dry swabs were inserted into the anterior nares and rubbed gently against the mucosa for approximately 5 seconds and they were placed in normal saline tubes. Loop full of inoculum from the tubes was transferred to tryptic soy broth tubes and the tubes were incubated at 37ºC for 24 hours. After incubation a loop full of inoculum from tryptic soy broth tubes was streaked over Baird Parker agar with egg yolk tellurite plates and the plates were incubated at 37ºC for 48 hours.

Identification of Staphylococcus aureus was done by taking a smear which was prepared from the growth on Baird Parker agar and it was stained with Grams method of staining. Gram positive bacteria were identified up to genus level as staphylococcus based on morphology. All the isolates were identified up to species level based on biochemical and sugar fermentation tests as per the methods described by Barrow and Felthan [16] and Bergeys manual of systemic bacteriology [17]. For confirmation of *Staphylococcus aureus*, the biochemical tests conducted were tube coagulase test, catalase test, DNase test, nitrate reduction test, oxidase test, urease test and IMViC tests, the sugars used for sugar fermentation tests were raffinose, sucrose, maltose, d-mannitol and d-mannose and further blood agar plate test was also conducted. For the preservation of the isolates a loop full of the isolated organism was added to the sterile tryptone soya glycerol broth vials and mixed well in vortex mixer. The vials were then labelled and stored at -20ºC.

Polymerase Chain Reaction

The reference strain for Methicillin Resistant *Staphylococcus aureus* ATCC 33591 was obtained from Deapartment of Veterinary Public Health & Epidemiology, NTR College of Veterinary Science, Gannavaram, Andhrapradesh.

Preparation template DNA from staphylococcus strains was carried out as per Lee [18] with minor modifications. Culture grown in 1.5ml of tryptic soya broth at 35ºC for 18- 20 hours was harvested and centrifuged at 12000 rpm for 10minutes. The pellet was washed twice with 1ml of sterile PBS, and re suspended in 100 µl of nuclease free water and boiled for 15min in a boiling water bath then it was subjected for snap chilling on ice for 20 min. The microfuse tube was centrifuged at 12000 rpm for 10 min at 4ºC and the supernatant was used as the template for duplex PCR assay for detection of *mecA* gene and *nuc* genes.

The primers used in the study were custom synthesized by M/s Eurofins Genomics, Banglore (India).The details of the primers are given in [Table-2].

A duplex PCR assay was developed through synthesis of specific primers targeting *nuc* gene (*S. aureus* species specific) and *mecA* gene (a determinant of methicillin resistance) was used in our study according to Zhang et al [19] with slight modifications. After rapid DNA extraction, 5 µl of bacterial DNA was added to a 25µl PCR mixture containing 50 mM KCl, 20mMTris-HCl (pH 8.4), 2.5mM MgCl2, 0.2mM each deoxynucleoside triphosphate (dATP, dUTP, dGTP, and dCTP) (Thermo, USA), 0.12 µl *mecA* primers, 0.04M each *nuc* primer, and 1.0 U of *Taq*DNA polymerase (Thermo, USA) [Table-3]. Amplification was performed by using a Thermal cycler (Corbett Research, Germany). Amplification was carried out at an initial denaturation of 94°C for 3 min followed by a cyclic denatura-

tion at 94°C for 30 seconds, 55°C for 35 seconds and 72°C for 1 min for 35 cycles. Final extension step carried out at 72°C for 10 min [Table-4]. On completion of the reaction, tubes with PCR products were held at 4ºC until further analysis/confirmation.

Table 2- Details of oligonucleotide primers used in this study

Primer	Target gene	Primer sequence (5^1 -3^1)	Expected amplicon size (bp)	Reference
Species specific primer for *S.aureus*	nuc gene	GCG ATT GAT GGT GAT AGG GTT AGC CAA GCC TTG ACG AAC TAA AGC	270	Brakstad et al [56]
Primer for methicillin resistance	mec A gene	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	533	Mo & Wang [62]

Table 3- Components of reaction mixture

SNo	Name of the reagent	Quantity µl
1	10 X PCR buffer	2.5
2	d NTP mix	0.2
3	Primer - F(10 p.mol)	2
4	Primer - R(10 p.mol)	2
5	Taq DNA polymerase (1 unit/µl)	0.3
6	Magnesium Chloride	1
7	Template DNA	2

Table 4- Cyclic conditions used for duplex PCR assay

SNo	Step	Temperature (ºC)	Duration	No of cycles
1	Initial denaturation	94ºC	3 min	1
2	Final denaturation	94ºC	30 sec	
3	Annealing	55ºC	35 sec	35
4	Initial extension	72ºC	1 min	
5	Final extension	72ºC	10 min	1
6	Hold	4ºC	10 min	---------

DNA amplified by PCR was subjected to 1.5% agarose gel electrophoresis as described by Sombrook and Russel [20]. Agarose gel (1.5%) was prepared by boiling agarose in an appropriate volume of 1 X TAE buffer. After cooling for about 3 minutes, ethidium bromide was added to the agarose solution to a final concentration of 0.5 µl / ml. The molten agarose was poured in to a gel casting tray fitted with acrylic comb was kept undisturbed till the gel has solidified. Once the gel is solidified a few ml of 1X TBE was added, comb was removed carefully and the tray containing the gel was then placed in a submarine horizontal electrophoresis unit filled with 1X TAE buffer upto a level of 1mm above the gel surface.

About 5 µl of each PCR product was mixed with 2 µl of bromophenol blue (6X) loading dye and loaded into each well. Electrophoresis was performed at 5v/cm and the motility was monitored by the migration of the dye. After sufficient migration, the gel was observed under UV transillumination using Alpha innotech gel documentation system to visualize the bands. The PCR product size was determined by comparing with a standard molecular weight marker.

Results

A total of 339 samples from different sources viz: goats (151), pigs (102), and humans(86) who were in close association with domestic and pet animals, and the particulars of the samples which were positive for *Staphylococcus aureus* were given in [Table-6]. All the 292 isolates were subjected to Gram's staining and found purple coloured cocci in clusters. Among the total isolates 101 isolates were confirmed as pathogenic *Staphylococcus aureus* by a positive coagulase test as shown in [Table-5].

The biochemical reactions of all the isolates were given in [Table-6]. The isolates were subjected to the biochemical tests like IMViC tests, urease test, oxidase test, nitrate reduction test, DNase test, blood agar plate test and catalase tests.

Table 5- Prevalence of *S. aureus* from various sources

SNo	Source	No. of samples screened	No. of samples positive for S. aureus	No. of samples positive for coagulase test
1	Goats	151	115 (76.15%)	61 (40.39%)
2	Pigs	102	96 (94.10%)	23 (22.54%)
3	Humans (Associated with animals)	86	81 (94.18%)	17 (19.76%)
	Total	339	292 (86.13%)	101 (29.79%)

Table 6- Results of the confirmation tests for *S. aureus*

SNo	Biochemical Test	Number of isolates positive for the biochemical and other tests		
		Goats (n-151)	Pigs (n-102)	Humans (n-140)
1	Gram's staining	115	96	81
2	Indole test	0	0	0
3	Methyl red test	115	96	81
4	Voges-proskauer test	115	96	81
5	Citrate utilization test	0	0	0
6	Urease test	115	96	81
7	Oxidase test	0	0	0
8	Nitrate reduction test	115	96	81
9	Catalase test	115	96	81
10	Coagulase test	61	23	17
11	DNase test	62	23	17
12	Blood agar plate test	115	96	81

All the isolates were negative for Indole and Citrate utilization tests. Whereas all the isolates produced bright red colour in methyl red test and red colour in Voges - proskauer test. All the biochemical reactions confirmed the presence of *Staphylococcus aureus.* Further all the isolates were subjected to urease test, oxidase test, nitrate reduction test and catalase test. The results revealed that all the isolates were positive for urease test, catalase test and nitrate reduction tests, on the other hand all of them were negative for oxidase test. The sterilized plates of DNase test agar base were streaked with the inoculums took from positive Baird Parker agar plates. The plates were incubated at 35-37ºC for 18-24 hrs and observed for blue to purple colored colonies with clear zones around the colonies. For further confirmation of *Staphylococcus aureus*, all the isolates were streaked on blood agar plates and incubated at 37ºC for 24 hours. The results revealed that all the isolates produced β haemolysis on blood agar plates.

For the phenotypic detection of MRSA, all the *S. aureus* isolates from different sourses were streaked on Hi-crome MeReSa agar

plates and the plates were incubated at 37ºC for 24 hr. The results revealed that 1 isolates (goats-0, pigs-1, humans-0) were grown as bluish-green coloured colonies.

Initial experiments to optimize PCR reaction conditions for *Staphylococcus aureus* template involved the empirical variation of annealing temperature (53ºC - 66ºC), concentration of primer (5 - 15 p mol), Magnesium chloride (1 mM - 3 mM), template volume (2µl - 8 µl) and the cycling conditions. Optimal results were obtained using 5 µl of bacterial lysate or 20ng of diluted DNA as template in a reaction mixture consisting of 2.5 µl of 10X assay buffer for *Taq* polymerase containing 1.5 mM Magnesium chloride, 1 µl of dNTP mix, 1 µl (4 p mol / µl) of each primer and 0.3 u / µl of *Taq* DNA polymerase in a final reaction volume made upto 25 µl with molecular grade water.

Initial denaturation at 94ºC for 3 minutes followed by 35 cycles each of denaturation at 94ºC for 30 seconds, annealing at 55ºC for 35 seconds and extension at 72ºC for 1 minutes with a final extension period of 10 minutes at 72ºC was found to be optimum for obtaining the desired PCR amplification of 270 bp from *nuc* gene and 533 bp from *mecA* gene of *Staphylococcus aureus*. Electrophoretic analysis of the PCR product revealed the specific amplification of a 533bp fragment without the presence of any spurious product.

The results of the samples by PCR method are, given and the same were described in discussion [Table-7], [Fig-1],[Fig-2],[Fig-3].

Table 7- Screening and comparison of culture method/ duplex PCR for detection of *S. aureus* and MRSA

SNo	Source	No. of samples screened	No. of *S. aureus* by culture method	Duplex PCR for nuc and mecA genes	
				Nuc	mecA
1	Goats	151	115 (76.15%)	112 (74.1%)	0(0%)
2	Pigs	102	96 (94.1%)	96 (94.1%)	1 (0.98%)
4	Humans	86	81(94.18%)	80 (98.76%)	0 (0%)
Grand total		339	292 (86.13%)	288(84.95%)	1 (0.29%)

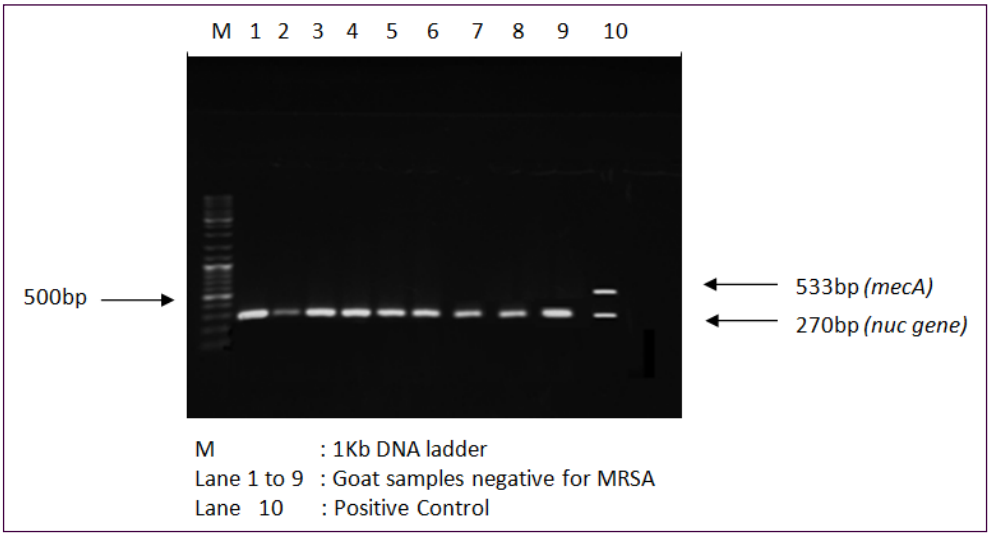

Fig. 1- Detection of mecA and nuc gene from Goat samples through Duplex PCR

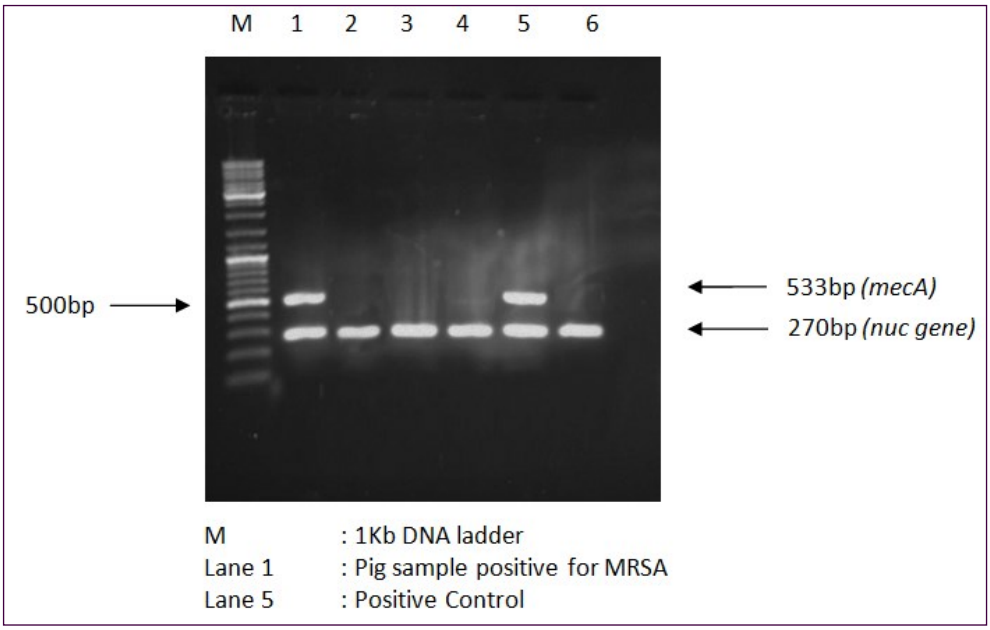

Fig. 2- Detection of mecA and nuc gene from Pig samples through Duplex PCR

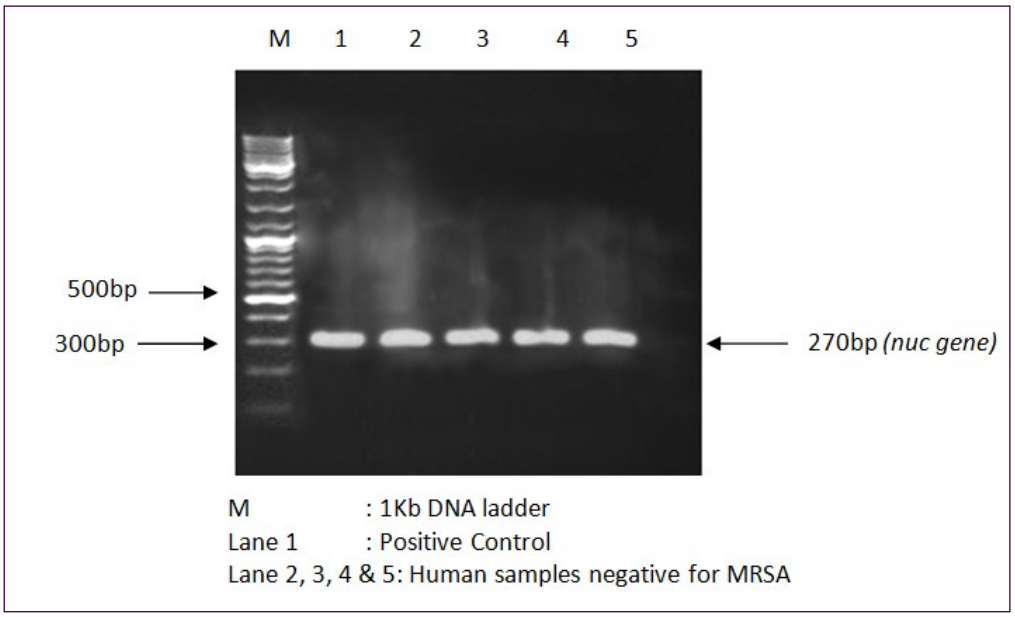

Fig. 3- Detection of mecA and nuc gene from Human samples through Duplex PCR

Discussion

Staphylococcus aureus causes severe animal diseases such as suppurative diseases, mastitis, arthritis and urinary infections that are associated with various virulent factors such as the production of extracellular toxins and enzymes. For humans this organism is an important cause of food poisoning, pneumonia, post operative wound infections and nosocomial bacteraemia. Human isolates of *S. aureus*, unlike animal isolates, are frequently resistant to the penicillinase resistant penicillins [21]. An organism exhibiting this type of resistance is referred to as Methicillin (oxacillin) Resistant *Staphylococcus aureus* (MRSA). Such organisms are also frequently resistant to most of the commonly used antimicrobial agents including the aminoglycosides, macrolides, chloromphenicol, tetracyclines and fluroquinolones [22]. In addition MRSA strains should be considered to be resistant to all cephalosporins, cephems and other β- lactams such as ampicillin, sublactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactum and the carbapenems, regardless of the *in-vitro* test results obtained with those agents [23].

The emergence of MRSA poses a serious public health threat. First described as a cause of nosocomial infection in hospital settings, now MRSA has gained attention as community pathogen [24]. Studies have shown that the epidemiology of MRSA over different parts of India is not uniform. Some studies have reported comparable prevalence: 38.56% in Delhi [25], 31.1% in a multicenter study in Tamilnadu [26], and 39.50% in South Gujarat [27]. In contrast, other studies have reported entirely different prevalence: 24% in Vellore [28], 80.89% in Indore [29], 52.9% in Assam [30], 19.56% in Nagpur [31], and 24% in Chandhigarh [32]. Although it's extremely difficult to explain these conflicting data with regards to both time and place of study, the variation is probably due to differential clonal expansion and drug pressure in community. In recent years, MRSA has been increasingly reported as emerging problem in veterinary medicine. MRSA has been isolated from cattle, dogs, cats, pigs, horses and poultry worldwide [33].

To date the only standardized means of identifying methicillin resistance in the clinical microbiology laboratories are susceptibility tests such as disk diffusion, agar or broth dilution and agar screen methods [23]. The performance of these tests has been erratic because many factors such as inoculums size, incubation time and temperature, pH of the medium, salt concentration of the medium and exposure to β-lactam antibiotics influence the phenotypic expression of resistance [34].Taking into account of these factors the empirical approach of most clinicians has been to view all levels of methicillin resistance as being equivalent to intrinsic high level resistant [35]. Because intrinsic resistance of both *S. aureus* and Coagulase negative *staphylococcus* appears almost exclusively to be due to PBP2a production, techniques have been developed to identify the *mecA* genetic determinant that encodes for this protein. These assays utilize PCR techniques [36]. These techniques show a high degree of correlation among susceptibility tests and allow accurate classification of not only highly resistant but also border line resistant strains [37]. As PCR relies on the detection of specific gene fragments, it can be applied in mixed microbial culture, avoiding problems which may arise by using other biochemical and morphological tests [38]. Similarly Cuny et al [39] utilized the PCR technique for the detection of staphylococcal species from food and clinical samples and to detect *mecA* gene which encodes for methicillin resistance also.

Several workers have used PCR with varied success for detection of MRSA from clinical samples using specific gene primers for targeting. Of the specific gene sequences *nuc* and *mecA* genes have been most frequently targeted for PCR based detection of *staphylococcus* and its methicillin resistance respectively. The *nuc* gene has been designated as species specific gene for *Staphylococcus aureus,* because *S. aureus* strains produce an extra cellular thermostable nuclease (thermonuclease, Tnase) with a frequency similar to that as which they produce coagulase enzyme [40]. The Tnase protein has been well characterized and its gene the *nuc* gene has been cloned and sequenced [41]. Thus the *nuc* gene which encodes the thermostable nuclease that is highly specific for *Staphylococcus aureus* [42]. Similar to this study Gao et al [45], Nasreen et al [43], Kateete et al [44], Padmapriya et al [46] and Chikkala et al [47] worked on the detection of *S. aureus* by PCR amplification of the *nuc* gene and reported that the PCR amplification of *nuc* gene

has potential for the rapid diagnosis of *S. aureus* including specimens from patients with ongoing antimicrobial therapy.

The *mecA* gene has been designated as the gene for methicilslin resistance of *S. aureus*, because MRSA produce a novel penicillin binding protein (PBP) in addition to the usual PBPs. This is the primary mechanism of *staphylococcal* methicillin resistance and is referred to as intrinsic resistance [48]. PBP2a has a low affinity for β -lactam antibiotics and is thought to function in their presence to confer resistance to the bacteria. MRCoNS also become resistant by acquisition of PBP2a encoding gene *mecA* [49]. *mecA* is a chromosomally derived gene that has been cloned and sequenced [50]. It has a very high level of homology in MRSA and MRCoNS and is absent from methicillin susceptible staphylococci isolates [51]. Additionally the *mecA* gene is virtually identical in all staphylococcal strains and thus is a useful molecular marker of methicillin resistance [52]. *mecA* primers were used by Pantosti et al [9], Tsubakishita et al [15] and Renato et al [53] stated that the most reliable procedure for detecting the MRSA remains the PCR amplification of the *mecA* gene.

The PCR procedure using *nuc* and *mecA* derived primers were standardized by optimizing the annealing temperature, primer concentration, $MgCl_2$ concentration, template volume and cyclic conditions. The specific PCR product of 270bp for *Staphylococcus aureus* (*nuc*) and 533bp for methicillin resistance (*mecA*) were stored at -20ºC, as it was observed that storage at a temperature of 4ºC for a longer period resulted in the degradation of the product. This degradation might be due to action of thermostable endogenous nuclease as reported by Gibson and McKee [54].

Nucleic acid amplification by PCR has applications in many fields of biology and medicine including the detection of viruses, bacteria and other infectious agent [55]. In the present study a oligonucleotide primer set was used which encodes the TNase produced by the bacteria. Primers were selected on the basis of published nucleotide sequence of the 270bp *nuc* gene [56]. The primers were synthesised by Eurofins Biolabs, Banglore, through High Salt Purification method which confirms the *Staphylococcus aureus* at species level. These results substantiate those obtained by other methodological approaches followed by Brakstad et al [56] from clinical specimens, Costa et al [57] by using Real-Time PCR, Kim et al [58] and Jian Gao et al [45] in *S. aureus* isolated from bovine milk, Asad Khan et al [59] in *S. aureus* isolated from hospital personnel, Saiful et al [60] in Malaysian clinical isolates of *S. aureus*, Biswajit Saha et al [42] in *S. aureus* isolates from Kolkata, Nasreen et al [43] in clinical isolates of *S. aureus,* David Kateete et al [44] in clinical isolates of *S. aureus* while comparing the PCR isolates with isolates confirmed by conventional tests, Padmapriya et al [46] in blood isolates of *S. aureus* by using Genexpert FB catridge system and Rosy Chikkala et al [47] in clinical isolates of *S. aureus* while comparing the sensitivity of *femA* and *nuc* gene for detection of *S. aureus* and all of them suggested that the *nuc* gene is unique to identify the bacteria at the species.

Current methods for identification of methicillin resistance in isolates based on conventional methods detect phenotypic expression rather than the presence of *mecA* gene and their results depend on numerous variables, specially requiring isolated colonies from an overnight subculture on solid media from the positive clinical samples and automated systems have excellent specificity but often lack sensitivity in detecting methicillin resistan *staphylococci*, particulary coagulase negative strains [61]. The use of PCR for the detection of *mecA* has been described previously. In the present study a oligonucleotide primer set was used which recognized the sequence of *S. aureus mecA* gene, which encodes the methicillin resistance of *S. aureus*. Primers were selected on the basis of the published nucleotide sequence of the 533bp *mecA* gene [62].

Out of 151 isolates from goats 115 (76.15%) were positive for *S. aureus* by cultural method and 112 (74.10%) by PCR method. Out of 112 PCR positives none was found positive for MRSA by PCR. Salvator Virdis et al [63] reported almost similar results obtained in this study, Vyletelova et al [64] also reported that no *mecA* gene was detected from *S. aureus* isolated from goats. Whereas Alharbi [65] found that out of 118 isolates 103 were positive for *mecA* from clinical isolates from goats, Birgit Strommenger et al [66] have identified 28 oxacillin resistant strains from 30 staphylococci isolates and reported that all carried *mecA* gene and Ariyo Oludotum Soyege et al [67] reported that out of 120 staphylococci isolates 32 (26%) were susceptible for methicillin and vancomycin and among them 10% of the isolates were positive for *mecA* gene.

Out of 102 *S. aureus* isolated from pigs 96 (94.10%) were positive for *S. aureus* by cultural methods and 96 (94.10%) samples by PCR method. Out of 96 PCR positives 1 sample was positive for *mecA* gene by PCR which accounts to 0.98% over total number of samples and 1.04% of the positive samples for *S. aureus* by PCR. Very high incidence of MRSA in pigs was reported than the present study by Zhang et al [19] who reported that out of 42 *S. aureus* isolates from pig tonsils 18 (42.85%) were methicillin resistant by detecting *mecA* gene, Pawel Tulinski et al [68] reported that 44 *mecA* positive staphylococci were isolated from 65 staphylococci isolates of these 33 (75%) were from nose swabs of pigs, Park et al [69] reported that from a total of 176 isolates the presence of *mecA* gene was identified in 63 (35.79%) staphylococci isolates, Boris Habrun et al [70] found that 8 (25%) out of 32 *S. aureus* isolates from pig farm facilities were the carriers of *mecA* gene, Milenko Zutic et al [71] from Serbia reported that MRSA was detected from one pig with endometritis and Conter et al [72] reported that among 51 staphylococci isolates 49 (96%) were carried *mecA* gene.

Out of 86 *S. aureus* isolated from humans 81 (94.18%) were positive for *S. aureus* by cultural methods and 17 (19.76%) samples by PCR method. Out of 86 PCR positives no sample was positive for *mecA* gene by PCR. A relatively high percentage of MRSA was reported from humans than the present study by Islam *et al* [73] who reported that out of 94 clinical strains of *S. aureus* 255 of human clinical isolates were positive for *mecA* in Bangladesh, Khulai Manal et al [74] reported that 39 (92.85%) out of 42 MRSA isolates were positive for *mecA* gene, Wulf et al [75] found that 4.6% of the participants at a livestock conference in Netherlands were nasal carriers of MRSA, Hanselman et al [76] found that 16% of nasal carriers were large animal vets whereas only 4.4% of those working with small animals were positive and none of the research workers were positive for MRSA, Baptiste et al [77] reported that in Northern England 11 of 67 (16%) horses were MRSA positive whereas their attendants were negative for MRSA and Eva Juhasz-Kaszanyitzky et al [78] found that in 3 tonsils samples of people who were in contact with cows 1 was positive for *mecA* gene. In the present investigation no MRSA was found from the human isolates, but there are number of references which indicates the presence of MRSA in humans who are associated with animals.

Conclusion

In the present investigation no MRSA was identified either in goats or in humans who were closely associated with these species whereas pigs have shown one sample positive for MRSA. The percentage of MRSA that was observed in samples collected from pigs was negligible. Based on this report we can not come to a conclusion regarding the zoonotic significance of MRSA as it was proved by number of scientists that there was a transfer of MRSA from animals to humans. Although it is extreemely difficult to explain these conflicting data with regards to both time and place of study, the variation is propably due to differential clonal expression and drug pressure in community.

Conflicts of Interest: None declared.

References

[1] Grundmann H., Aires-de-Sousa M., Boyce J. & Tiemersma E. (2006) *Lancet*, 368, 874-885.

[2] Van Loo I.H., Diederen B.M., Savelkoul P.H., Woudenberg J.H., Roosendaal R., van Belkum A., Lemmens-den Toom N., Verhulst C., van Keulen P.H. & Kluytmans J.A. (2007) *Emerg. Infect. Dis*., 13(11), 1753-1755.

[3] Devriese L.A. & Hommez, J. (1975) *Research in Veterinary Science*, 19, 23-27.

[4] Abraham J., Morris D., Griffeth G., Shofer F. & Rankin S. (2007) *Vet Dermatol*, 18, 252-259.

[5] Kottler S., Middleton J.R., Perry J., Weese J.S. & Cohn L.A. (2010) *J. Vet. Intern. Med.*, 24, 132-39.

[6] Walther B., Wieler L.H., Friedrich A.W., Hanssen A.M., Kohn B., Brunnberg L. & Lubke-Becker A. (2008) *Vet Microbiol*., 127(1-2), 171-178.

[7] Saleha A. & Zunita Z. (2010) *J. Anim. Vet. Adv.*, 9(7), 1094-1098.

[8] Khanna T., Friendship R., Dewey C. & Weese J. (2010) *Vet. Microbiol*., 128, 298-303.

[9] Pantosti A., Sanchini A. & Monaco M. (2007) *Future Microbiol*., 2(3), 323-334.

[10] Katayama Y., Ito T. & Hiramatsu K. (2000) *Antimicrob. Agents Chemother.*, 44, 1549-1555.

[11] Ma X.X., Ito T., Tiensasitorn C., Jamklang M., Chongtrakool P., Boyle-Vavra S., Daum R.S. & Hiramatsu K. (2002) *Antimicrob. Agents Chemother*., 46, 1147-1152.

[12] Enright M.C., Robinson D.A., Randle G., Feil E.J., Grundmann H. & Spratt B.G. (2002) *Proceedings of the National Academy of Science*, 99, 7687-7692.

[13] Couto I., Wu S.W., Tomasz A. & de Lencastre H. (2003) *J. Bacteriol.*, 185, 645-653.

[14] Schnellmann C., Gerber V., Rossano A., Jaquier V., Panchaud Y., Doherr M.G., Thomann A., Straub R. & Perreten V. (2006) *J. Clin. Microbiol*., 44(12), 4444-4454.

[15] Tsubakishita S., Kuwahara-Arai K., Baba T. & Hiramatsu K. (2010) *Antimicrobial Agents and Chemotherapy*, 54(4), 1469-1475.

[16] Barrow G.I. & Feltham R.K. (1993) *Cowan and Steel's Manual for the Identification of Medical Bacteria.*, 3rd ed., Cambridge University Press, London.

[17] Garrity G. (2012) *Bergey's Manual of Systematic Bacteriology: Volume One: The Archaea and the Deeply Branching and Phototrophic Bacteria*, Springer Science & Business Media.

[18] Lee J.H. (2003) *Appl. Environ. Microbiol*, 69, 6489-6494.

[19] Zhang K., Sparling J., Chow B.L., Elsayed S., Hussain Z., Church D.L., Gregson D.B., Louie T. & Conly J.M. (2004) *J. Clin. Microbiol*, 42, 4947-4955.

[20] Sambrook J. & Russell D.W. (2001) *Molecular cloning: A laboratory manual*, 3rd ed., Cold Spring Harbor Laboratory Press, New York.

[21] Kloos W.E. & Bannerman T.L. (1995) *Staphylococcus and Micrococcus*. Manual of Clinical Microbiology, ASM press, Washington DC, 282-298.

[22] Mandell G., Douglas J. & Bennett R. (1995) *Principles and practices of infectious diseases*, 4th ed., Churchill Livingstone, Edinburgh, United Kingdom.

[23] National Committee for Clinical Laboratory Standards. (2001) *Performance standards for antimicrobial susceptibility testing*, Eleventh informational supplement, Wayne, Pa, M100-S11.

[24] Said-Salim B., Mathema B. & Kreiswirth B.N. (2003) *Infect Control Hosp. Epidemiol*., 24, 451-455.

[25] Mohanty S., Kapil A. & Dhawan B. (2004) *Indian J. Med. Microbiol.*, 58, 10-15.

[26] Rajaduraipandi K., Mani K.R. & Panneerselvam K. (2006) *Ind. J. Med. Microbiol*., 24, 34-38.

[27] Mulla S., Patel M. & Shah L. (2007) *Indian J. Crit. Care Med*., 11, 99-101.

[28] Pulimood T.B., Lalitha M.K. & Jesudason M.V. (1996) Ind. J. Med. Res., 103, 212-215.

[29] Verma S., Joshi S. & Chitnis V. (2000) *Indian J. Med. Sci*., 54, 535-540.

[30] Assadullah S., Kakru D.K. & Thoker M.A. (2003) *Indian J. Med. Microbiol*., 21, 196-198.

[31] Tahnkiwale S.S., Roy S. & Jalgaonkar S.V. (2002) *Ind. J. Med. Sci*., 56, 330-334.

[32] Mehta M., Dutta P. & Gupta V. (2007) *Indian J. Plast Surg*., 40, 25-28.

[33] Leonard F.C. & Markey B.K. (2008) *Vet. J*., 175, 27-36.

[34] Madiraju M.V.V.S., Brunner D.P. & Wilkinson B.J. (1987) *Antimicrob. Agents Chemother*., 35, 575-578.

[35] McDougal L.K. & Thornsberry C. (1986) *J. Clin. Microbiol.*, 23, 832-839.

[36] Tokue Y., Shoji K., Satoh Watanabe A. & Motomiya M. (1992) *Antimicrob. Agents Chemother*., 36, 6-9.

[37] Gerberding J.L., Mlick C., Liu H.H. & Chambers H.F. (1991) *Antimicrob. Agents Chemother.*, 35, 2574-2579.

[38] Catarame T.M.G., O'Hanlon K.A., McDowell D.A., Blair I.S. & Duffy G. (2005) *J. Food. Saf*., 26, 1-15.

[39] Cuny C., Friedrich A., Kozytska S., Layer F., Nübel U., Ohlsen K., Strommenger B., Walther B., Wieler L. & Witte W. (2010) *Int. J. Med. Microbiol*., 300(2-3), 109-117.

[40] Madison B.M. & Baselski V.S. (1993) *J. Clin. Microbiol*., 18, 722-724.

[41]Tucker P.W., Hazen E.E. & Cotton F.A. (1978) *Mol. Cell Biochem*., 22, 67-77.

[42]Saha B., Singh A.K., Ghosh A. & Bal M. (2008) *Journal of Medical Microbiology*, 57(1), 72-79.

[43]Nasreen O. Musa., Kamal Elton., Frank Gessler., Helge Bohnel., Abdulkhalig Babiker., Muna O. Ethaj. & Suleiman M. Sanousi. (2009) Characterization of some *Staphylococcus aureus* sub species anaerobicus by *spa* and *coa* genes. J. Animal and Veterinary Advances, 8(11), 2272-2275.

[44]Kateete D.P., Kimani C.N., Katabazi F.A., Okeng A., Okee M.S., Nanteza A., Joloba M.L. & Najjuka F.C. (2010) *Annals of Clinical Microbiology and Antimicrobials*, 9(1), 23.

[45]Gao J., Ferreri M., Liu X.Q., Chen L.B., Su J.L. & Han B. (2011) *Journal of Veterinary Diagnostic Investigation*, 23(5), 894-901.

[46]Banada P.P., Chakravorty S., Shah D., Burday M., Mazzella F.M. & Alland D. (2012) *PloS one*, 7(2), e31126.

[47]Chikkala R., George N.O., Ratnakar K.S., Iyer R.N. & Sritharan V. (2012) *Advances in Infectious Diseases*, 2(03), 82-88.

[48]Hartman B.J. & Tomas A. (1984) *J. Bacteriol*., 158, 513-516.

[49]Chambers H.F. (1987) *Antimicrob Agents Chemother.*, 31, 1919 -1924

[50]Matsuhashi M., Song M.D., Ishino F., Wachi M., Doi M., Inoue M., Ubukata K., Yamashita N. & Konno M. (1986) *J. Bacteriol*., 167, 975-980.

[51]Predari S.C., Ligazzi M. & Fontana R. (1991) *Antimicrobial Agents and Chemotherapy*, 35, 2568-2573.

[52]Archer G.A. & Climo M.W. (1994) *Antimicrobial Agents and Chemotherapy*, 38, 2231-2237.

[53]Renato P.M., Ariel E.S., Everlon C.R., Ana C.O.D., Manoel V.F.L. & Fernando A. (2010) *African Journal of Microbiology Research*, 4(18), 1879-1883.

[54]Gibson J.B. & Mckee R.A. (1993) *Lett. Appl. Microbiol.*, 16, 59-61.

[55]Thiele D. (1990) *Bacteriol., Parasitenkd. Infektionskr. Hyg. Abt.*, 273, 434-454.

[56]Brakstad O.G., Aasbakk K. & Maeland J.A. (1992) *Journal of Clinical Microbiology*, 30(7), 1654-1660.

[57]Costa A.M., Kay L. & Palladino S. (2005) *Diagn. Microbiol. Infect. Dis.*, 51(1), 7-13.

[58]Kim C.H., Khan M., Morin D.E., Hurley W.L., Tripathy D.N., Kehrli Jr. M., Olouch A.O. & Kakoma L. (2001) *J. Dairy. Sci.*, 4 (1), 74-83.

[59]Khan A.U., Sultan A., Tyagi A., Zahoor S., Akram M., Kaur S., Shahid M. & Vaishnavi C.V. (2007) *The Journal of Infection in Developing Countries*, 1(03), 289-295.

[60]Saiful A.J., Mastura M., Zarinel S., Muzarrah M.I., Shuhaimi M. & Ali A.M. (2006) *World J. Microbiol. Biotechnol.*, 22, 1289-1294.

[61]Christensen G.D., Bisno A.L., Parisi J.T., Mc Laughlin B., Hester M.G. & Luther R.W. (1982) *Ann. Intern. Med.*, 96, 1-10.

[62]Mo L. & Wang Q. (1997) *Int. J. Infect. Dis*., 2, 15-20.

[63]Virdis S., Scarano C., Cossu F., Spanu V., Spanu C., & De Santis E.P.L. (2010) *Veterinary Medicine International*, doi: 10.4661/2010/517060.

[64]Vyletělová M., Hanuš O., Karpíšková R. & Šťástková Z. (2014) *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 59(3), 209-214.

[65]Alharbi Khaled B. (2014) *Int. J. Food. Agri. and Vet. Sciences*, 4 (1), 81-92.

[66]Birgit S., Guido W. & Wolfgang W. (2003) *J. Clin. Microbiol*., 41 (9), 4089-4094.

[67]Soyege A.O., Ajayi A.O., Ngwenya E., Basson A.K. & Okoh A.I. (2014) *Jundishapur Journal of Microbiology*, 7(4), e9310.

[68]Tulinski P., Fluit A.C., Wagenaar J.A., Mevius D., van de Vijver L. & Duim B. (2012) *Applied and Environmental Microbiology*, 78(2), 299-304.

[69]Park J., Friendship R.M., Weese J.S., Poljak Z. & Dewey C.E. (2013) *BMC Veterinary Research*, 9(1), 211.

[70]Habrun B., Račić I., Kompes G., Beck R., Benić M. & Cvetnić Ž. (2012) *Rad Hrvatske akademije znanosti i umjetnosti. Medicinske znanosti*, (511= 37), 97-103.

[71]Zutic M., Cirkovic I., Pavlovic L., Asanin J., Jovanovic S., Zutic J. & Asanin R. (2013) *Acta Veterinaria Brno*, 81(3), 225-227.

[72]Conter M., Di ciccio P., Zanardi E., Ghidini S., Borracci G., Vergara A. & Lanieri A. (2012) *Italian J. Food Safety*, 1(3), 234-239.

[73]Islam M.A., Alam M.M., Uddin M.S., Kobayashi N. & Ahmed M.V. (2011) *Bangl. J. Vet. Med*., 9(2), 161-166.

[74]Al-Khulaifi M.M., Amin A.N.M. & Al Salamah A.A. (2009) *Saudi Journal of Biological Sciences*, 16(1), 37-49.

[75]Wulf M., Van Nes A. & Eikelemboom-Boskamp A. (2006) *Emerging Infectious Diseases*, 12, 1939-1941.

[76]Hanselman B.A., Kuth S.A. & Rousseau J. (2006) *Emerging Infectious Diseases*, 12, 1933-1938.

[77]Baptiste K.E., Williams K., Williams N.J., Wattret A., Clegg P.D., Dawson S., Corkill J.E., O'Neill T. & Hart C.A. (2005) *Emerging Infectious Diseases*, 11, 1942-1944.

[78]Juhász-Kaszanyitzky É., Jánosi S., Somogyi P., Dán Á., vanderGraaf van Bloois L., Van Duijkeren E. & Wagenaar J.A. (2007) *Emerging Infectious Diseases*, 13(4), 630-632.