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M Soma Sekhar
Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University (SVVU), Gannavaram, Andhra Pradesh, India

T Srinivasa Rao
Associate Professor and Head, Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

N Mohammad Sharif
Department of Veterinary Microbiology, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Correspondence
M Soma Sekhar
Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University (SVVU), Gannavaram, Andhra Pradesh, India

Nasal colonization of methicillin resistant *Staphylococcus aureus* (MRSA) among dogs and dog handlers in Andhra Pradesh, India

M Soma Sekhar, T Srinivasa Rao and N Mohammad Sharif

Abstract
The study was conducted to assess the nasal carriage rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among apparently healthy dogs (40), their owners (40) and veterinary students (40) attending canine wards in Andhra Pradesh. A total of 45 *S. aureus* isolates were recovered, of which 18 were found positive for coagulase enzyme production. Resistance to both oxacillin and cefoxitin was found to be high in coagulase positive *S. aureus* (CoPS) isolates of dog owners (15%) followed by dogs (12.5%) and veterinary students (10%). Bluish green colonies were observed on MeReSa CHROM agar supplemented with cefoxitin. Both *mecA* and *blaZ* genes were detected in 4 (10%), 4 (10%) and 6 (15%) of CoPS isolates of dogs, dog owners and veterinary students, respectively. Correlation between phenotypic resistance to oxacillin, cefoxitin and presence of *mecA* gene was not observed. The results suggest that healthy dogs may act as reservoirs of MRSA. Such carriage poses an underlying risk of infection, which should be considered during handling of healthy dogs by pet owners and veterinary personnel.

Keywords: CoPS, Dogs, MRSA, PCR, *mecA*, *blaZ*, Veterinary students

Introduction
Methicillin-resistant *Staphylococcus aureus* (MRSA) is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals [1]. Resistance to methicillin in *S. aureus* is due to the carriage of *mecA* gene on a mobile DNA element (staphylococcal chromosome mec, *SCCmec*) encoding the altered penicillin binding protein (PBP 2a) that shows low affinity for beta-lactam antibiotics [2]. Another mechanism of resistance to penicillin in *Staphylococci* is production of β-lactamase (encoded by *blaZ* gene) [2]. MRSA has been isolated from wide range of animal species, including cattle, buffaloes, horses, pigs, poultry, rabbits and exotic species both as a cause of infection and in healthy animals as a nasal carriage [3]. Healthy companion animals may be a reservoir of multidrug-resistant staphylococci, which may be transferred to owners and others who handle companion animals [4]. Recent studies revealed that healthy dogs and other companion animals also act as reservoir for MRSA [5]. Pet associated persons, pet breeders and veterinarians encompass the primary risk groups that may become colonized from MRSA of canine origin [6].
Data regarding molecular characterization of MRSA from dogs is not available in India, although epidemiological data regarding MRSA in hospitalized humans is available [7]. Keeping the above facts in view, the present study was carried out to assess the nasal colonization of MRSA among apparently healthy dogs, their corresponding owners and veterinary students attending canine wards in Andhra Pradesh, phenotypically by the detection of oxacillin and cefoxitin resistance followed by molecular confirmation by the detection of *mecA* gene (encodes for methicillin-resistance) and *blaZ* gene (encodes for beta-lactamase production) by polymerase chain reaction (PCR).

Materials and Methods
Bacterial reference strain
The reference strain of MRSA (ATCC 25923) was purchased from Hi-Media Laboratories (Mumbai) and maintained at the Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh.

Isolation and identification of *S. aureus*

A total of 120 nasal swab samples were collected from apparently healthy dogs (40), corresponding dog owners (40) and veterinary students (40) attending canine wards of Teaching Veterinary Clinical Complex (TVCC), NTR College of Veterinary Science (Gannavaram) and College of Veterinary Science (Tirupati), Andhra Pradesh. Nasal swabs were collected using sterile cotton swab and immediately transported to laboratory on ice and processed for isolation of *S. aureus* as per the method followed by Kateete *et al*. [8]. The nasal swabs were inoculated into 5 ml of Trypticase soya broth (TSB) (Hi-Media) and incubated at 35ºC for 24 h. Enriched samples were streaked onto Mannitol salt agar (Hi-Media) and incubated at 35ºC for 24 h. Plates with yellow colour colonies were selected and tested for Gram's staining (Gram positive cocci), catalase (positive), oxidase (negative), Voges-Proskauer (positive), haemolysis (positive) and coagualse activity both by slide/tube coagulase test and using Vogel-Johnson Agar [9].

Growth on MeReSa CHROM agar

Coagulase positive cultures were streaked onto the selective media, MeReSa (Methicillin Resistant *Staphylococcus aureus*) CHROM agar with cefoxitin supplement (0.4mg/1ml) and incubated at 35ºC for 20 h. Appearance of greenish blue colour colonies indicate presence of MRSA [10].

Oxacillin and cefoxitin sensitivity testing

Resistance against oxacillin (5µg/disc) and cefoxitin (30µg/disc) was studied by disc diffusion method [11]. Diameter of zone of inhibition was interpreted as per Clinical and Laboratory Standards Institute (CLSL) guidelines [12]. Oxacillin and cefoxitin resistant cultures were inoculated into TSB, incubated at 35ºC for 20 h and about 1.5 ml of culture was used for extraction of DNA by high salt method [13].

Detection of *mecA* gene

Detection of *mecA* gene was carried out the using primer combination given in Table 1, targeting *mecA* gene of *S. aureus* with 310 bp predicted amplicon size [14]. The PCR amplification was optimized in 25 µl PCR reaction mixture (containing 3.0 µl of DNA template; *Taq* buffer [10x] with $MgCl_2$ – 2.5 µl; dNTP mix [10mM] – 1.0 µl; forward primer [20 pmol/µl] – 1.0 µl; reverse primer [20 pmol/µl] – 1.0 µl; *Taq* DNA polymerase [1 U/µl] - 1 µl and nuclease free water – 15.5 µl) under the following optimized cycling conditions: initial denaturation at 92ºC for 3 min, 30 cycles of denaturation at 92ºC for 1 min, annealing at 56ºC for 1 min, elongation at 72ºC for 1 min and final elongation at 72ºC for 7 min.

Detection of *blaZ* gene

Detection of *blaZ* gene was carried out using the primer combination given in Table 1, targeting *blaZ* gene of *S. aureus* with 173 bp predicted amplicon size [15]. The PCR amplification was optimized in 25 µl PCR reaction mixture (containing 2.0 µl of DNA template; *Taq* buffer [10x] with $MgCl_2$ – 2.5 µl; dNTP mix [10mM] – 1.0 µl; forward primer [20 pmol/µl] – 1.0 µl; reverse primer [20 pmol/µl] – 1.0 µl; *Taq* DNA polymerase [1 U/µl] - 1 µl and nuclease free water – 14.5 µl) under the following optimized cycling conditions: initial denaturation at 95ºC for 3 min, 30 cycles of denaturation at 95ºC for 30 sec, annealing at 54ºC for 30 sec, elongation at 72ºC for 30 sec and final elongation at 72ºC for 4 min.

Table 1: Oligonucleotide primers used for the detection of *mecA* and *blaZ* genes

S. No	Primer	Sequence (5'-3')	Amplicon size	Reference
1.	*mecA*, F *mecA*, R	TGGCTATCGTGTCACAATCG CTGGAACTTGTTGAGCAGAG	310 bp	Vannuffel *et al.* [14].
2.	*blaZ*, F *blaZ*, R	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173 bp	Martineau *et al.* [15].

Results and Discussion

Nasal carriage of coagulase positive *S. aureus* (CoPS):

A total of 45 *S. aureus* isolates were recovered. Nasal carriage rate of *S. aureus* among dogs, dog owners and veterinary students was found to be 35, 40 and 37.5%, respectively (Table 2). *S. aureus* carriage rate detected in the present study was however lower than 55.1% reported for dogs in England [16]. In a study from Jordon, 12.7 and 10.0% carriage rate of *S. aureus* was reported from the nasal swabs of dogs and associated personnel [17]. Walther *et al.* [6] reported detection of *S. aureus* from 18.5% of the humans in contact with dogs in Berlin. Out of 45 *S. aureus* isolates, 18 isolates were found to be positive for coagulase production on both tube and slide coagulase tests. Nasal carriage of CoPS was found to be high in dog owners (17.5%) followed by dogs (15.0%) and veterinary students (12.5%) (Table 2). The present findings were in accordance with a study from England where 19.3% CoPS carriage rate was reported in dogs [16]. All the 18 CoPS isolates gave black colour colonies surrounded by yellow zone on Vogel-Johnson Agar supplemented with 1% Potassium Tellurite (Fig. 1A).

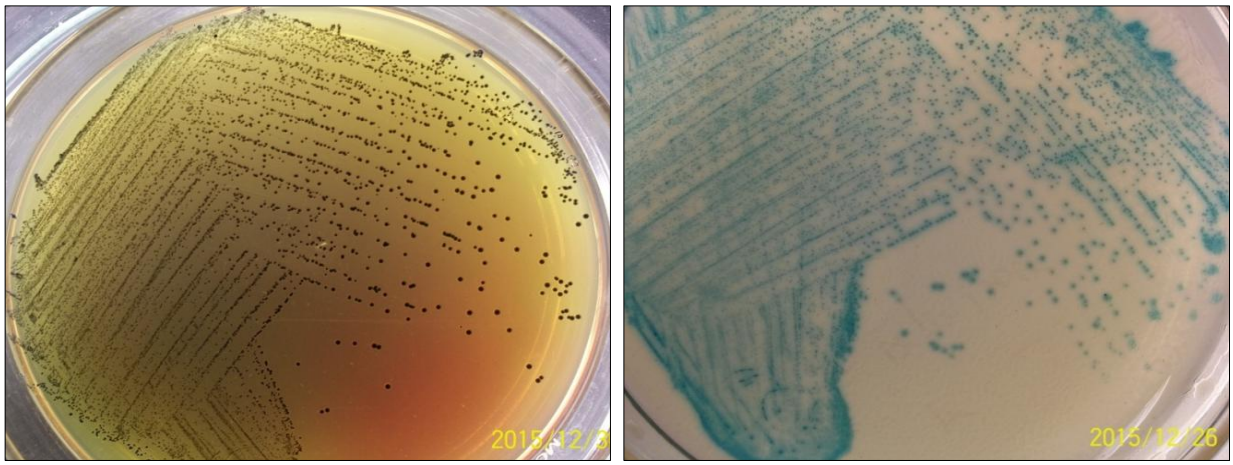
Phenotypic detection of MRSA

By disc diffusion test, 15 out of 18 CoPS isolates were found to be resistant to both oxacillin and cefoxitin. All the 15 CoPS isolates gave bluish-green colour colonies on MeReSa CHROM agar indicating methicillin resistance (Fig. 1B). Phenotypic resistance was found to be high in CoPS isolates of dog owners (15%) followed by dogs (12.5%) and veterinary students (10%) (Table 2). Phenotypic resistance in CoPS among dogs in this study was similar to an another study (14.8%) in England [16]. Kottler *et al.* [18] reported a lower level of resistance (5.6%) that the present study in people who had contact with dogs. Griffeth *et al.* [19] reported a higher level of methicillin resistance (41%) in healthy dogs from Philadelphia.

Molecular detection of MRSA

Accurate detection of MRSA is vital in the management of animals and humans with *S. aureus* infections. Although many phenotypic methods have been developed for phenotypic identification of MRSA, the current gold standard for MRSA detection is identification of *mecA* gene by PCR. In the present study, high incidence of *mecA* gene was detected in CoPS isolates of dog owners (15%) followed by

dogs (10%) and veterinary students (10%) (Table 2, Fig. 2). For further confirmation, when all the 14 CoPS isolates carrying *mecA* gene were subjected to PCR targeting *blaZ* gene, all 14 were found to be positive for *blaZ* gene (Table 2, Fig. 2). Correlation between the presence of *mecA* gene in phenotypically identified MRSA was not observed, as evidenced by the failure in the detection of *mecA* gene in a methicillin resistant CoPS isolate. Although *mecA* detection is regarded as the gold standard for MRSA detection, other non-*mecA*-dependent mechanisms may also contribute individually or in combination towards resistance in staphylococci strains. Hence the discrepancies observed between the conventional phenotypic detection of MRSA and the *mecA* detection might be attributed to the non-*mecA*-dependent methicillin resistance. Similar discripancies between phenotypic and *mecA* detection of methicillin resistance in *S. aureus* have also been reported by other authors [20, 21].



A B

Fig 1: (A). Selective isolation of coagulase positive *S. aureus* using Vogel-Johnson agar, with black colour colonies surrounded by yellow zone (B). Selective identification of MRSA using cefoxitin supplemented MeReSa CHROM agar, with greenish-blue colour colonies.

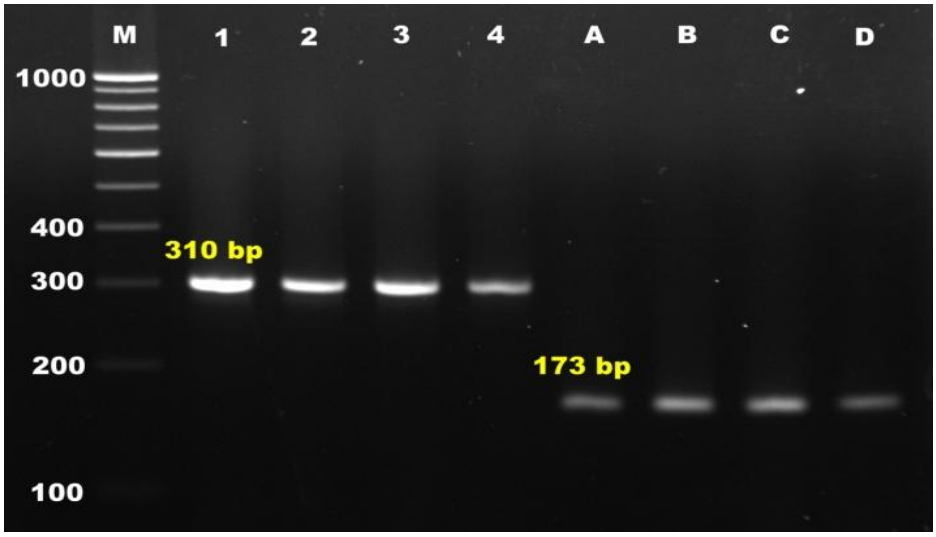


Fig 2: Amplification of *mecA* and *blaZ* genes. 100 bp DNA ladder (lane M); MRSA (ATCC 25923) positive for *mecA* gene, 310 bp (lane 1) and *blaZ* gene, 173 bp (lane A); MRSA isolates positive for *mecA* gene from dog (lane 2), dog owner (lane 3) and veterinary student (lane 4); MRSA isolates positive for *blaZ* gene from dog (lane B), dog owner (lane C) and veterinary student (lane D)

Table 2: Nasal carriage rates of CoPS and MRSA in dogs and dog handlers

Source	Nasal swab samples screened	Number positive for *S. aureus* (%)	Number positive for coagulase test (%)	Growth on Hicrome MeReSa agar (%)	Resistance to oxacillin and cefoxitin	Number positive for *mecA* gene (%)	Number positive for *blaZ* gene (%)
Dogs	40	14 (35.0%)	6 (15.0%)	5 (12.5%)	5 (12.5%)	4 (10.0%)	4 (10.0%)
Dog owners	40	16 (40.0%)	7 (17.5%)	6 (15.0%)	6 (15.0%)	6 (15.0%)	6 (15.0%)
Vety. Students	40	15 (37.5%)	5 (12.5%)	4 (10.0%)	4 (10.0%)	4 (10.0%)	4 (10.0%)
Total	120	45 (37.5%)	18 (15.0%)	15 (12.5%)	15 (12.5%)	14 (11.6%)	14 (11.6%)

Isolation of MRSA from canine samples was reported as early as 1972 [22], but it is only in the past 10 to 15 years, its clinical significance in veterinary medicine has increased. In the present study, the nasal carriage rate of MRSA in dogs and dog handlers was found to be 10% (4/40) and 12.5% (10/80). In a study from Jordon, MRSA colonization was reported to be 5.3% in healthy dogs and 5% in people who had contact with dogs [17]. The pathogen was isolated from only 3.5% of veterinary dermatology practice staff and 1.9% of their respective pets in another study [23], while Aklilu et al. [21] reported 23.3% prevalence rate of MRSA in Veterinary students from Malaysia. In the past few years, a number of studies have reported the incidence of MRSA in healthy dogs visiting the veterinary practice, with prevalences ranging from 0 – 3% [24-28] and even as high as 7.8% in a rescue kennel [29] have been reported, but many have involved a limited number of samples from dogs. The difference in MRSA nasal carriage rates can be attributed to difference in antibiotic use in animal husbandry and poor farm hygiene practices.

Conclusion

The current study concludes and confirms the presence of MRSA in the nasal cavities of dogs, dog owners and veterinary personnel strongly associated with dogs in Andhra Pradesh, India. Such carriage poses an underlying risk of infection, which should be considered during handling of healthy dogs by pet owners and veterinary personnel. No correlation was observed between phenotypic resistance to oxacillin, cefoxitin and presence of *mecA* gene.

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