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 beta-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.

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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 soy broth (TSB) (Hi-Media) and incubated at 35°C for 24 h. Enriched samples were streaked onto Mannitol salt agar (Hi-Media) and incubated at 35°C 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 coagulase activity both by slide/tube coagulase test and using Vogel-Johnson 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 35°C 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 (CLSI) guidelines [12]. Oxacillin and cefoxitin resistant cultures were inoculated into TSB, incubated at 35°C 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 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 MgCl₂: 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 92°C for 3 min, 30 cycles of denaturation at 92°C for 1 min, annealing at 56°C for 1 min, elongation at 72°C for 1 min and final elongation at 72°C 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 MgCl₂: 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 95°C for 3 min, 30 cycles of denaturation at 95°C for 30 sec, annealing at 54°C for 30 sec, elongation at 72°C for 30 sec and final elongation at 72°C for 4 min.

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

<table>
<thead>
<tr>
<th>S. No</th>
<th>Primer</th>
<th>Sequence (5’-3’-)*</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>mecA</em>, F</td>
<td>TGGCTATCGTGTCAACATCG TCTGAACTTGTGAGCAGAG</td>
<td>310 bp</td>
<td>Vannuffel et al. [14],</td>
</tr>
<tr>
<td></td>
<td><em>mecA</em>, R</td>
<td>ACTTCAACACCTGTGCTTTC TGACCACCTTTATCGCAACC</td>
<td>173 bp</td>
<td>Martineau et al. [15],</td>
</tr>
</tbody>
</table>

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. [18] 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. Griffith 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 discrepancies between phenotypic and mecA detection of methicillin resistance in S. aureus have also been reported by other authors [20, 21].

![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](A). 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

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


