PREVALENCE OF Staphylococcus aureus AND METHICILLIN-RESISTANT STAPHYLOCOCCUS SPP. IN PET DOGS

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ABSTRACT

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically in humans. Methicillin-resistant Staphylococcus (MRS) strains have acquired a gene that makes them resistant to all beta-lactam antibiotics. The present study was carried out to find out the prevalence of Staphylococcus aureus in pet dogs as well as MRS. A total of 55 nasal swab samples were collected from pet dogs attending Madras Veterinary teaching hospital. The samples were processed by standard conventional procedures for isolation of the organism and molecular characterization of the isolates was done by using thermonuclease gene for Staphylococcus aureus and for methicillin resistant staphylococcus spp. meca gene was used. Out of fifty five samples screened 33 showed colonies characteristic of staphylococcus species on Baird parker agar plates. Molecular characterization of this isolates by thermonuclease (nuC) gene showed that 28(84%) isolates were PCR positives for Staphylococcus aureus and for meca gene about 26(74%) of the isolates were positive. The prevalence of Staphylococcus aureus and MRS highlights the possibility of zoonotic transmission to humans who are in contact with pet dogs.

Keywords: Methicillin-resistant Staphylococcus(MRS), Staphylococcus aureus, Prevalence, Zoonotic

Introduction

Staphylococcus aureus is a normal inhabitant in wide range of animals and humans and causes nosocomial and community onset infections. The possibility that dogs and cats could act as the source for zoonotic staphylococcal infections in humans was suggested many years ago (Mann, 1959). Antibiotic resistance is common phenomena encountered with S. aureus and methicillin-resistant Staphylococcus aureus (MRSA) is emerging as a pathogen of public health importance with zoonotic potential. MRSA has been found in a variety of domestic species including Dogs (Van Duijkeren et al., 2004; Walther et al., 2008) and it is found to be resistant to virtually all available beta-lactam antibiotics which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin and cephalosporins). Many reports worldwide suggests colonisation and transmission of S. aureus, including MRSA, between owners and their dogs (Köldler et al., 2008; Loeffler et al., 2005; Malik et al. (2006).

Considering the above points in mind the present study was designed to find out the prevalence of Staphylococcus aureus and methicillin resistant staphylococcus spp. in pet dogs.

Materials and methods

Collection of Samples:
Nasal swabs from pet dogs attending to Madras Veterinary teaching hospital were collected. A total of 55 nasal swabs were collected under sterile condition and were immediately brought to laboratory for further processing.

**Isolation of Staphylococcus species:**

Nasal swabs were inoculated into sterile brain heart infusion (BHI)broth with 10% sodium chloride and incubated at 37°C for overnight for propagation of Staphylococcus species. Selective plating was done by transferring a loopful of overnight grown inoculum on Baird parker agar media plates containing (5%egg-yolk emulsion and 3.5%potassium tellurite) and were incubated at 37°C for 24-48h to identify characteristic colonies as shown in figure 1.

Fig 1:Characteristic colonies in BP agar medium (circular, smooth, convex, moist, gray black to jet black, frequently with light coloured margin, surrounded by opaque zone and frequently with outer clear zone.

**DNA extraction:**

DNA extraction was done by using conventional method about 100µl of millipore water was taken in eppendorf tube and typical characteristic colony was selected and mixed with the water and incubated for 95°C for 10 minutes (Taydeet al.,2012).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>NAME</th>
<th>SEQUENCE 5'-3'</th>
<th>bp</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>nuc A-F</td>
<td>GCGATTGATGGGTGATACGGTT</td>
<td>267</td>
<td>Brakstad et al., 1992</td>
</tr>
<tr>
<td>2</td>
<td>nuc A-R</td>
<td>AGCCAAGCCTTGGACAACGAAAGGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>mec A-F</td>
<td>GAAATGACTGAACGTCGATAA</td>
<td>310</td>
<td>Kobayashi et al., 1994</td>
</tr>
<tr>
<td>4</td>
<td>mec A-R</td>
<td>CCAATTCCACATTGTTTCGCTAAGA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cycling conditions**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Initial denaturation</th>
<th>denaturation</th>
<th>annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuc A-F</td>
<td>94°C,5min</td>
<td>94°C,30s</td>
<td>55°C,30s</td>
<td>72°C,1min</td>
<td>72°C,5min</td>
</tr>
<tr>
<td>nuc A-R</td>
<td>Repeated for 30 cycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mec A-F</td>
<td>94°C,5min</td>
<td>94°C,30s</td>
<td>50°C,40s</td>
<td>72°C,1min</td>
<td>72°C,5min</td>
</tr>
<tr>
<td>mec A-R</td>
<td>Repeated for 25cycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Polymerase chain reaction:**
All the 33 isolates were subjected to PCR targeting nuc gene and mecA gene to identify S.aureus and Methicillin resistant staphylococcus spp. As shown in Table 1.

Table 1 PRIMERS AND THEIR CYCLIC CONDITIONS

PCR was performed in a 25 µl reaction mixture which includes 12.5 µl master mix(AMPLIQON),10pM concentration of each primer and 2.5 µl of DNA template and remaining volume was adjusted using nuclease free water. PCR Product were subjected to gel electrophoresis (1.5% agarose with 0.8µg/ml ethidium bromide) and the results were documented using gel documentation system (Biorad).

Results and discussion

Out of fifty five samples screened 33 showed colonies characteristic of staphylococcus species on Baird parker agar plates. Molecular characterization of this isolates by thermonuclease (nucA) gene showed that 28(84%) were PCR positives for Staphylococcus aureus and for mecA gene about 26(78%) isolates were found to methicillin resistant (Fig 2&3).

Human associated with canines are at great risk of S. aureus transmission in comparison with people associated with bovines and the possible causes for S. aureus transmission may be due to frequent contact with canines.(Fitzgerald, 2014). Brakstadlt et al., (1992) used nuc gene for identification of S. aureus and for our study we have also used nuc gene as a marker to identify S.aureus. Methicillin resistant S. aureus strains, because of their high mortality have become a major concern worldwide (Hookeyt et al., 1998). Hata et al (2010) reported that PCR based mecA gene amplification confirmed more than 99% of MRSA isolates. Several reports have documented an apparent increase in the number of MRSA infections in companion animals in recent years (Boaget et al., 2004; O'Mahony et al., 2005). Our results are in accordance with the results of various authors who has also reported the presence of methicillin resistance with Staphylococcus spp. The colonization of this antibiotic resistant organism in pet animals impose major risk in both animal and humans. (Loeffler et al., 2010).

Conclusion

This study report about the increase prevalence of methicillin resistant isolates from canine nasal region warrants the need for appropriate control strategies for effective screening by molecular methods and containment of this pathogen which
will aid in effective disease management.

References


