DETECTION OF METHICILLIN-RESISTANT
Staphylococcus aureus IN NASAL CARRIAGE OF
BOVINES BY POLYMERASE CHAIN REACTION

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is an important human pathogen associated
with variety of nosocomial infections, which are extremely difficult to treat. It is also an emerging
concern in veterinary medicine where food animals may act as a possible source of human infection.
A study was designed to investigate the distribution of MRSA in nasal cavity of dairy cows. The nasal
swabs were collected from dairy cows (n=48) and enriched with Brain heart infusion broth with 10%
sodium chloride followed by direct plating on Baird-Parker agar (BP) at 37°C for 24-48 hours. Among
the 48 samples, 32 samples showed jet black colour colonies surrounded by a clear halo zone on BP
agar. The DNA was extracted from all the 32 presumptive isolates were screened for S. aureus by poly-
merase chain reaction targeting nuc gene. Out of that, S. aureus was detected in 25 isolates. All the
25 S. aureus were subjected to mecA gene to ascertain the presence of Methicillin resistance, in which
22 isolates showed positive for MRSA. Results of our study showed that presence of S. aureus in nasal
carryage of 52% of dairy cows among which 88% were positive for MRSA.

Key words: Methicillin-resistant Staphylococcus aureus (MRSA), nuc gene, mecA gene, Polymerase
chain reaction

Introduction

Staphylococcus aureus is a well-documented commensal organism encountered in wide
range of animal species, including dogs, cats, rabbits, horses, cattle, pigs, poultry and humans. It
has been implicated in wide variety of infections, ranging from superficial skin and soft tissue in-
fecions to life threatening septicaemia. Potential of this organism to acquire antibiotic resistance
warrants the need to identify antibiotic resistance pattern.
potential risks of carriage or disease in the general population (Graveland et al., 2011b; Vanderhaegen et al., 2010). Therefore, it is of importance to reduce LA-MRSA occurrence in food producing animals and consequently in occupationally exposed humans.

Methicillin resistance in S. aureus is conferred by the mecA gene, which encodes a modified penicillin-binding protein (PBP2a or PBP2β), that has low affinity for almost all β-lactam antibiotics (penicillins, cephalosporins, carbapenems). The mecA locus is a highly conserved gene that encodes PBP2a in resistant strains but is absent from susceptible ones, making it a useful molecular marker of β-lactam resistance (Pinho et al., 2001). Also, MRSA strains are often resistant to antimicrobials other than β-lactams, of which many members are widely used in both human and veterinary medicine (Lowy, 2003; Pinho et al., 2001). Thus the detection of the mecA gene using Polymerase Chain Reaction (PCR) can be used to identify MRSA. The present study was designed to envisage the prevalence of MRSA in nasal carriage of bovines by PCR targeting mecA gene.

**Materials and methods**

**Isolation of S. aureus:**

Nasal swabs were randomly collected from 48 dairy cows brought to the Madras Veterinary College Teaching Hospital, Chennai. Nasal swabs were inoculated into Brain heart infusion broth supplemented with 10% sodium chloride, and incubated at 37 °C for overnight. Baird-Parker (BP) agar medium supplemented with 5% Egg yolk tellurite was used for selective plating. The loopful of inoculum from the enrichment broth was streaked on BP agar medium and incubated at 37°C for 24-48 hours. The colonies showing characteristic appearance (circular, smooth, convex, jet black colour colonies surrounded by a clear halo zone) were selected and further confirmation by PCR.

**Fig 1:** Staphylococcus colonies on Baird-Parker (BP) agar medium

**DNA extraction:**

The DNA was extracted from the presumptive colonies by Alkanine lysis Polyethylene glycol (AL-PEG) method as outlined by Chomczynski and Rymaszewski (2006). In this method, loopful of presumptive colonies were dissolved in 100 μl of distilled water and 500 μl of AL-PEG reagent (60g PEG + 0.93ml 2M KOH + 39 ml water for 100ml AL-PEG reagent) was added and incubated in water bath at 60°C for 10 minutes. From this 2-3 μl of supernatant was used as a template for PCR.

Polymerase Chain Reaction (PCR) for detection of nuc and mecA gene from S. aureus:

The presumptive colonies from BP medium were screened for S. aureus by targeting the nuc gene and genotypic identification of MRSA by using mecA gene.

Target Primer Sequence 5'–3' Amplicon size (bp) Reference
Electrophoresis at 100 V for 30 min was performed to visualize the PCR amplicon on a 1.2% agarose gel containing 0.5 μg/ml ethidium bromide. The gels were visualised under UV trans-illuminator in gel documentation system. The size of the amplicon was determined by comparing it with a 100-bp DNA ladder.

Results and discussion

Out of the 48 nasal swabs samples from dairy cows, 32 samples showed jet black colour colonies surrounded by a clear halo zone on BP agar. Out of which, 25 were detected as S. aureus by using PCR targeting nuc gene. All the 25 S. aureus were subjected to mecA gene to ascertain the presence of Methillicin resistance, in which 22 isolates showed positive for mecA gene. Results of our study showed that nasal carriage of S. aureus and MRSA in dairy cows was 52% (25/48) and 48% (22/48), respectively. The distribution of MRSA isolates among the nasal S. aureus isolates was 88% (22/25) for dairy cows.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer Sequence 5'-3'</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuc-F</td>
<td>GTGCTGGCATATGTAACGCAATTGT</td>
<td>181</td>
<td>Hedge, 2013</td>
</tr>
<tr>
<td>nuc-R</td>
<td>TACGCCCTTAATCTGTTTGATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA-F</td>
<td>GAAATGACTGAACGTCCGATAA</td>
<td>310</td>
<td>Kobayashi et al, 1994</td>
</tr>
<tr>
<td>mecA-R</td>
<td>CCAATCCACATTGTTTGCTCAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR reaction carried out in a total volume of 25μl consisting of 12.5 μl Master mix, 1 μl forward primer, 1μl reverse primer, 8.5μl Nuclease free water and 2μl of DNA under the following conditions:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Cyclic condition</th>
<th>nuc gene</th>
<th></th>
<th>mecA gene</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
<td>Cycles</td>
<td>Temperature</td>
<td>Cycles</td>
</tr>
<tr>
<td>1)</td>
<td>Initial denaturation</td>
<td>94°C, 5 minutes</td>
<td>1</td>
<td>94°C, 5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>2)</td>
<td>Denaturation</td>
<td>94°C, 30 seconds</td>
<td></td>
<td>94°C, 30 seconds</td>
<td></td>
</tr>
<tr>
<td>3)</td>
<td>Annealing</td>
<td>54°C, 30 seconds</td>
<td>30</td>
<td>50°C, 40 seconds</td>
<td>25</td>
</tr>
<tr>
<td>4)</td>
<td>Extension</td>
<td>72°C, 30 seconds</td>
<td></td>
<td>72°C, 1 minute</td>
<td></td>
</tr>
<tr>
<td>5)</td>
<td>Final extension</td>
<td>72°C, 10 minutes</td>
<td>1</td>
<td>72°C, 5 minutes</td>
<td>1</td>
</tr>
</tbody>
</table>
Electrophoresis at 100 V for 30 min was performed to visualize the PCR amplicon on a 1.2% agarose gel containing 0.5 µg/ml ethidium bromide. The gels were visualised under UV trans-illuminator in gel documentation system. The size of the amplicon was determined by comparing it with a 100-bp DNA ladder.

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Fig 2: Agarose gel electrophoresis of PCR product amplified from nuc gene

Lane 1 - 100 bp Ladder
Lane 2 - Positive control
Lane 3 - Negative control
Lane 4 to 8 - nuc positive samples

181 bp

Fig 3: Agarose gel electrophoresis of PCR product amplified from mecA gene

Lane 1 to 4, 6 mecA positive samples
Lane 5 - mecA negative sample
Lane 7 - Positive control
Lane 8 - 100 bp Ladder

310 bp

Results of our study showed prevalence of MRSA was found to be 47.8% in nasal carriage of dairy cows whereas Spoehr et al. (2011) reported MRSA in (5/7) cows with 71 percent positivity and (4/7) calves with 57 percent positivity from nasal swabs in Germany. Variation in the prevalence of our study in comparison to other workers might be due of sample size, antibiotic use in animal husbandry and hygiene practices among the dairy cows.

The source of acquisition of MRSA in this study is uncertain but it may be due to contact with human or animal carriers. MRSA infected cattle acts as a reservoir and later transmit the infections to other animals and humans (Spoor et al., 2013). MRSA colonization in cattle may be an occupational risk to the people in close contact with MRSA infected cattle viz. veterinarians, farmers, milkers and people working at slaughterhouses (Paterson et al., 2012; Juhasz- Kaszanyitzky et al, 2007).

Conclusion MRSA has become established pathogen in human and various animal population. Irrational use of antibiotics in the treatment of human diseases and non-therapeutic use of antibiotics in animals may have played
a significant role in the emergence of resistant clones due to selection pressure. Such resistance may pose a great impact on public health if animal associated strains enter into the community and health care settings. Hence, strict regulations on the use of antibiotics in human medicine as well as in animal food production, strengthening surveillance and screening of MRSA in animal-population are required to effective infection control programme to limit the spread of drug resistant clones of S.aureus.

References


