Molecular characterization of *Staphylococcus* spp. isolated from respiratory tract of apparently healthy and clinically sick sheep and goat in Nagpur, India

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**ABSTRACT**

Present study was undertaken to characterize *Staphylococcus* spp. associated with respiratory tract infection in small ruminants. A total of 139 bacterial isolates were recovered from clinically sick and apparently healthy small ruminants. The prevalence rate of *Staphylococcus* spp. in sick and healthy animals was 44.94 and 24% respectively. The sensitivity pattern of selected antibiotics was similar in both clinically sick and apparently healthy animals except streptomycin and penicillin-G. PCR assay was standardized for the detection of pathogenic genes of *Staphylococcus* spp. viz. clfA and spa. Among 34 random isolates selected, the prevalence rate of clfA and spa gene was 79.41 and 58.82% respectively indicating their pathogenic potential. In conclusion, *Staphylococcus* spp. was found to be one of the highly prevalent organism in respiratory tract infections.

**Key words:** ClfA, PCR, Small ruminant, Spa, *Staphylococcus*.

**INTRODUCTION**

Respiratory tract infection occur frequently in small ruminants. In many countries, respiratory diseases represent the most serious problem and can be an important cause of death and reduced productivity (Martin 1996). Respiratory diseases of small ruminants are multifactorial (Lacasta et al. 2008) and there are multiple etiological agents responsible for the respiratory disease complex. Of them, bacterial diseases are most important due to variable clinical manifestations, severity of diseases and re-emergence of strains resistant to a number of chemotherapeutic agents (Woldemeskel et al., 2002). This causes considerable financial losses to shepherds and goat keepers. Adverse weather conditions, stress, pregnancy, lactation, immune-suppression and old age of the animals favour the infection by normal inhabitants of the respiratory tract pathogens such as *Staphylococcus* spp. (Yesuf et al., 2012).

clfA and spa genes are associated with virulence of *Staphylococcus* spp. clfA gene codes for clumping factor which helps in infection process by facilitating bacterial binding via solubilised or immobilized fibrinogen. As fibrinogen plays significant role in platelet thrombus formation, it is likely that clfA gene may be involved in bacterial platelet interactions. Therefore, it is implicated as virulence factor (Stephan et al., 2001). spa gene codes for the IgG binding region of the protein A. Also it has the affinity with solubilised or immobilized von Willebrand factor (vWF) and identified as a novel Staphylococcal adhesin (Seki et al., 1998). In this context, the study was designed to screen the respiratory tract infection caused by *Staphylococcus* spp. by cultural and molecular technique.

**MATERIALS AND METHODS**

In the present study, nasal swabs (n=71) and tissue samples (lung=10 and tracheal swab=10) were collected from live and previously observed slaughtered sick animals respectively (n=91) from organized farms of MAFSU and nearby slaughter houses, Nagpur, India. Whereas, only nasal swabs (n=39) were collected from apparently healthy animals from organized farms of MAFSU, Nagpur, India during the period from January 2015 to March 2015. The samples were transported to the laboratory on ice.

**Cultural isolation:** Pieces of tissue samples were washed in sterilized saline and triturated separately in sterilized pestle and mortar under aseptic conditions. Bacterial isolation was done following standard technique by inoculating triturated tissue and nasal swab samples primarily on blood agar and then plates were incubated for 24-48 hrs at 37°C aerobically. After incubation, typical Staphylococcal colonies were studied. Preliminary morphological identification was based on Gram`s staining. Specific identification and biochemical characterization of the isolates was done as per the standard techniques described by Cruickshank et al., (1975) and Cowan and Steel, (1993).

**Biochemical characterization:** Primarily the isolates were characterized by KOH, Catalase, Oxidase and O-F tests performed as per Quinn et al., (1994). Following primary biochemical tests, the isolates were characterized by various

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secondary biochemical tests performed as per Cruickshank et al., (1975) and Cowan and steel, (1993) which included Indole test, Methyl-Red (MR) test, Voges-Proskauer (VP) test, Citrate utilization Test, Urease test, Nitrate reduction test and Carbohydrate fermentation test.

**In-vitro antimicrobial sensitivity assay**: The isolates were subjected to in-vitro antibiotic assay by disc diffusion method (Bauer et al., 1966) for sensitivity. Diameters of the clear zone of inhibition were measured and the interpretation of the results was made (HiMedia, India).

**DNA Extraction**: The extraction of bacterial DNA was carried out from the isolates using Phenol-chloroform method according to Wilson, (1987). Staphylococcal isolates were grown in 2 ml of BHI broth overnight at 37°C. DNA was purified and precipitated and kept at -20°C for 1 hr and later centrifuged at 10,000 rpm for 15 min. The pellet was washed in 70% alcohol and air dried and resuspended in 50 µl TE buffer. 3 µl of suspended pellet was used as the DNA template.

**PCR for amplification of associated virulence genes**: In the present study, the PCR was standardized for the detection of clfA and spa genes. PCR was carried out with 3 µl of template DNA, 1 µl each of forward and reverse primers, 12.5 µl of master mix and 7.5 µl of Nuclease Free Water in a total volume of 25 µl. The DNA was amplified by using the specific cycling conditions (Table 2). PCR products were separated in 1% agarose gel for 60 min at 80 V, stained with ethidium bromide (0.5µg/ml). At the end of electrophoresis, the gel was visualized under UV transilluminator for the amplicon of desired molecular weight and results were recorded using gel documentation system. Amplified genes were identified on the basis of fragment size shown in Table 1.

**RESULTS AND DISCUSSION**

A total of 139 bacterial isolates were recovered from 130 samples collected from clinically sick and apparently healthy small ruminants. Out of 139 bacterial isolates, 89 (64.03%) were from sick animals, while 50 (35.97%) were from nasal swabs of healthy animals. A total of 52 isolates of Staphylococcus spp. were recovered, of them 40 were isolated from sick and 12 were from healthy animals. The prevalence rate of Staphylococcus spp. in sick animals was 44.94%, on the other hand, in healthy animals it was 24%; which was in agreement with the findings of other (Megra et al., 2006).

### Table 1: The details of the primer sequences for amplification of clfA and spa genes of Staphylococcus spp.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>PCR product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>clfA Forward</td>
<td>GGC TTC AGT GCT TGT AGG</td>
<td>980</td>
<td>Stephan et al., (2001)</td>
</tr>
<tr>
<td>clfA Reverse</td>
<td>TTT TCA GGG TCA ATA TAA GC</td>
<td>920</td>
<td>Seki et al., (1998)</td>
</tr>
<tr>
<td>spa Forward</td>
<td>CAC CTG CTG CAA ATG CTG CG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spa Reverse</td>
<td>GGC TTG TTG TTG TCT TCC TC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Cycling conditions for PCR assay.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94°C</td>
<td>2 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>57°C</td>
<td>1 min</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>1.20 min</td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>10 min</td>
</tr>
<tr>
<td>End of the PCR cycles</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

In the present study, among the 40 isolates of Staphylococcus spp. obtained from diseased animals, 100% were sensitive to ampicillin and amoxicillin, 95% were sensitive to tetracycline and 92.5% to chloramphenicol. On the other hand, the isolates were resistant to streptomycin, penicillin-G erythromycin and amikacin. Moreover, 12 isolates of Staphylococcus spp. recovered from healthy animals were found sensitive to ampicillin (100%), amoxicillin (100%), tetracycline (91.67%), chloramphenicol (83.34%), streptomycin (75%) and penicillin-G (75%), while resistant to erythromycin and amikacin. From the sensitivity pattern it was indicated that Staphylococcus isolates from both sick and apparently healthy animals showed similar pattern of sensitivity towards antibiotics, however the isolates from sick animals were found resistant to streptomycin and penicillin-G, indicating more use of these antibiotics in the treatment of respiratory illnesses. Similar findings were recorded by Aher et al., (2012). In contrast, Asaduzzaman et al., (2013) observed that Staphylococcus spp. were highly sensitive to erythromycin and resistant to trimethoprim and metronidazole.

Out of 52 isolates of Staphylococcus spp., randomly 34 isolates were selected for detection of both clfA and spa genes. According to the present study, the prevalence rate of clfA gene of Staphylococcus spp. isolated from respiratory tract of small ruminants was 79.41% (Fig. 1). However the higher prevalence rate of 91.3% clfA gene was detected by Karahan et al., (2011) from bovine subclinical mastitis indicating that pathogens are having more coagulative ability to attach the respiratory tract and colonization. clfA gene codes for clumping factor, help in infection process by facilitating bacterial binding through immobilized fibrinogen. Therefore, it is implicated as virulence factor (Stephan et al., 2001). clfA gene has been shown to inhibit phagocytosis in the absence of fibrinogen and the inhibition is enhanced in the presence of fibrinogen.
In this current study, the prevalence rate of spa gene was 58.82% in sick animals (Fig. 2). However, low prevalence rate of spa gene was reported by Momtaz et al., (2010) and Aher et al., (2012) at the rate of 25.5% and 32.6% from bovine clinical and subclinical mastitis and respiratory tract of goats respectively.

spa gene codes for IgG binding region of the protein A and is known for the binding ability for its immunoglobulin Fc region. spa gene has affinity with solubilised or immobilized von Willebrand factor (vWF) and identified as a novel Staphylococcal adhesin (Seki et al., 1998).

The amplified potential of virulence genes viz., clfA and spa indicated the pathogenic potential of isolates from respiratory tract in the cases where the host goes immuno-compromised.

CONCLUSION

In conclusion, Staphylococcus spp. was found to be one of the highly prevalent organism recovered from respiratory tract infections. The presence of virulence marker genes of Staphylococcus spp. namely, clfA and spa indicates pathogenic potential of these organism and may indicate their correlation with respiratory infections. We therefore recommend a detailed study to look into the economic impact of these organisms.

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REFERENCES


