ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY OF  
*STAPHYLOCCUS SCHLEIFERI SUBSP. SCHLEIFERI* FROM CANINE  
PYODERMA AND OTITIS IN CHENNAI  

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**ABSTRACT**  

Coagulase negative Staphylococci were considered as commensal  
organisms or contaminants with limited pathogenic potential. However, they  
are becoming more frequently associated with nosocomial infections in human  
and skin infections in animals. In this study, we attempted to isolate and identify  
the Staphylococci species that causes dermatological infections in dogs. Out  
of 35 isolates, 27 were identified as *S. pseudintermedius* and 3 as  
*S. schleiferi subsp. schleiferi*. Among three *S. schleiferi* isolates, 2 were closely  
related to *S. schleiferi* ATCC strain in pta gene sequences. Methicillin resistant  
gene (*mecA*) was not detected in these isolates. All three isolates were  
susceptible to oxacillin, amikacin, gentamicin and tetracycline, intermediate  
susceptibility to ceftriaxone and resistant to Penicillin, ampicillin,  
sulphadiazine. *S. schleiferi* is an emerging cause of infections in veterinary  
patients. None of the isolate produced biofilm in microtitre tissue culture plate  
adherence method. This is the first report of isolation and identification of  
*S. schleiferi* from canine skin infections from India. Further studies are needed  
for early detection and virulence factors of *S. schleiferi* in the pathogenesis of  
pyoderma and otitis in dogs.  

**Keywords:**  
*S. schleiferi*, skin infections, dog, antibiogram  

**INTRODUCTION**  

*Staphylococcus* species are the normal  
skin microflora of man and animals. *Staphylococcus pseudintermedius*, coagulase  
positive staphylococci, is the most common pathogenic organisms isolated from skin  
infections in dogs (Bannoehr et al., 2007). Whereas, the ubiquitous nature of coagulase  
negative staphylococci (CNS) enhances the  
opportunities for infection and they are  
generally regarded as susceptible to many antimicrobials. Among them, *Staphylococcus  
schleiferi subsp. schleiferi* has been frequently isolated from healthy and diseased skin of dogs  
(Cain, 2013). The purpose of this study was to  
ascertain the prevalence of *S. schleiferi* from  
otitis and pyoderma of dogs in Chennai and  
their susceptibility to various antimicrobials  
used for treatment.
MATERIALS AND METHODS

Bacterial Isolates: A sterile cotton swab was used to sample canine otitis and pyoderma cases and swabs were inoculated by streaking on mannitol salt agar plates. A single representative colony of each sample was propagated by streaking it on nutrient agar plate and subjected for Gram’s staining, catalase tests and other biochemical tests.

DNA extraction: Four to five colonies were suspended in PBS and centrifuged at 6000 rpm for 10 min. The pellet was suspended in 100 µl of sterile distilled water and boiled at 100°C for 10 min. Then the tubes were cooled immediately by placing them on ice. Later, they were centrifuged at 10000 rpm for 10 min and the supernatant was used as template in nucleic acid amplification reaction.

Species identification by PCR and DNA Sequencing analysis: Primers for staphylococcal phosphate acetyltransferase (pta) gene published by Bannoehr et al (2009) were used in PCR and the sequences are: ptaF 5’-AAAGAC AAA CTT TCA GGTAA-3’ and pta R 5’- GCATAAA CAAGC ATT GTA CCG-3’. Methicillin resistance (mecA) gene was detected using the following primers: F 5’- CAAA ACTACGGTAA ACATTGAC CGC-3’ and R 5’- GCCTATCTCATATGCTGTTCC-3’. PCR was performed in a reaction volume of 10 µl containing approximately 100 ng of genomic DNA, 5 pmol of each primer and 2 x master mix (Ampliqon, Denmark). Cycling conditions were 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C (pta) or 60°C (mecA) for 30 sec, extension at 72°C for 30 sec and a final extension cycle of 5 min at 72°C. PCR products were loaded on a 1.5% agarose gel for electrophoresis, visualized with ethidium bromide and documented. Purification of amplicon was done using PCR purification kit (Real Biotech Corporation, Taiwan) as per the manufacturer’s instruction. DNA sequencing of purified nucleotides was done with automated high throughput nucleic acid sequencer of Applied Biosystems3500 (USA). Homology searches were performed with the NCBI database and BLAST. Alignment and phylogenetic tree analysis were carried out using Mega5.2 software.

Antimicrobial Susceptibility test: Antimicrobial susceptibility test was performed by disk diffusion method on Mueller-Hinton agar and classified as Sensitive, Intermediate and Resistant based on CLSI guidelines. Penicillin G10, Ampicillin 10mcg, Amoxyclav 30mcg, Cephotaxime30mcg, ceftriaxone 30mcg, Ciprofloxacin 5mcg, Enrofloxacin 10mcg, Erythromycin 15mcg, Oxacillin 1mcg, Oxytetracycline 30mcg, Streptomycin 25mcg, Gentamicin 10mcg, Amikacin 30mcg and sulpha diazine 100mcg used in this study were procured from HiMedia Pvt Ltd, India.

Biofilm formation: Biofilm formation was carried out as per the method described by Hassan et al. (2011) using a quantitative spectrophotometric microliter plate assay. Briefly, organisms isolated from fresh agar plates were inoculated in 5 mL of tryptic soy broth and was incubated at 37°C overnight. After incubation, the culture was diluted (1:100 dilution) with fresh broth with 1% glucose. 200 µL of the diluted cultures was added in triplet to 96 well flat bottom microliter plates with sterile broth as negative control. The plates were incubated at 37°C for 24 hours. After incubation, contents of each well were removed by gentle tapping. The wells were washed with...
0.2 mL of phosphate buffer saline (pH 7.2) twice. Biofilm formed by bacteria adherent to the well surface were fixed by 2% sodium acetate. Then staining was done using 0.2 ml of crystal violet (0.1%) for 15 min. Excess stain was removed by using deionized water and plates were kept for drying. 200 µL of 95% ethanol was added to the wells and OD of stained adherent biofilm was obtained by using micro ELISA auto reader at wavelength 570 nm. The interpretation of biofilm production was done based on the OD value as given below:

<table>
<thead>
<tr>
<th>OD VALUE</th>
<th>BIOFILM PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD of isolate ≤ OD of control (ODc)</td>
<td>No biofilm formation</td>
</tr>
<tr>
<td>ODc &lt; OD of isolate</td>
<td>Weak biofilm formation</td>
</tr>
<tr>
<td>2 ODc &lt; OD of isolate</td>
<td>Moderate biofilm formation</td>
</tr>
<tr>
<td>4 ODc &lt; OD of isolate</td>
<td>High biofilm formation</td>
</tr>
</tbody>
</table>

Nucleotide sequence accession number: The GenBank accession numbers for nucleotide sequence of pta gene of three *Staphylococcus schleiferisubschleiferi* were KJ146046, KJ146051 and KJ146052.

RESULTS AND DISCUSSION

Totally 35 skin and ear swabs were taken from pyoderma and otitis cases presented in the Teaching Hospital of Madras Veterinary College, Chennai. *Staphylococci* were isolated from all the above cases and out of 35 isolates, 27 (77%) were identified as *Staphylococcus pseudintermedius* by biochemical and molecular methods. Three isolates (9%) were identified and confirmed as *Staphylococcus schleiferisubschleiferi* by biochemical tests and by sequencing of pta gene. Other staphylococcal isolates were identified as *S. aureus* (3%), *S. intermedius* (3%), *S. simulans* (3%) and 2 unspeciated staphylococci (6%). The amplified partial pta gene length was 320 bp as shown in Fig.1. Phylogenetic tree of pta gene of all three isolates of *S. schleiferisubschleiferi* along with ATCC 43808 *Staphylococcus schleiferi* subsp. *schleiferi* strain was shown in the Fig.2. It was found that two isolates 139781 & 108576 were closely related with the ATCC strain but the isolate 100437 was distant from other isolates and ATCC strain.

*S. schleiferisubschleiferi* was first reported by Freney et al. in 1988 as a new species isolated from human clinical specimens. The isolation of *S. schleiferi subsp coagulans* was from canine otitis case in 1990 (Igimi et al., 1990). Genetically, coagulase positive and coagulase negative *S. schleiferi* appear similar in pulsed-field gel electrophoresis (PFGE) and are likely variations of coagulase-producing strains within one species (Cain et al., 2011a). Historically, *S. schleiferi* has been considered as an opportunistic pathogen and susceptible to many antimicrobials. The isolation of this pathogen has been reported in recurring pyoderma cases, dogs with both otitis and pyoderma, and from apparently healthy dogs. *Staphylococcus schleiferi* is very similar in appearance with other species of staphylococci. It is particularly difficult to differentiate it from *S. aureus* because both species present a very similar morphology. It is actually believed that this is the reason why pathogenic activity by *Staphylococcus schleiferi* has been underreported and maybe some infections that were believed to be caused by *S. aureus* could...
actually be blamed on *S. schleiferi* (Kluytmans, 2001).

The frequency of isolation of coagulase negative schleiferi subspecies is more than the coagulase positive subspecies. In this study also, three coagulase negative *S. schleiferi* (i.e. *S. schleiferi* subsp. *schleiferi*) were isolated. Frank *et al.* (2003) isolated 9 coagulase negative and 6 coagulase positive *S. schleiferi* from total of 54 canine pyoderma cases. Hariharan *et al.* (2014) isolated only one *S. schleiferi* subsp. *coagulans* out of 66 pyoderma cases in Greneda, West Indies. Now, *S. schleiferi* is considered as an emerging pathogen of canine pyoderma infections and emergence of methicillin resistant *S. schleiferi* is a cause for concern of small animal dermatologists. The present study also confirms that next to *S. pseudintermedius, S. schleiferi* is the major opportunistic skin pathogen of canine.

All three isolates of *S. schleiferi* exhibited different susceptibility pattern against various antimicrobial agents which was presented in Table 1. All three isolates were susceptible to oxacillin disk and *mecA* gene was not detected in any of the isolates. All the three isolates were susceptible to amikacin, gentamicin and oxytetracycline, showed intermediate susceptibility to ceftriaxone and resistant to Penicillin, ampicillin and sulphadiazine. Two isolates were susceptible to amoxyclav, ciprofloxacin, enrofloxacin and streptomycin and showed intermediate susceptibility to cefotaxime. Susceptible, intermediate and resistant pattern was observed for erythromycin in each one isolate. More coagulase-negative *S. schleiferi* isolates were identified with *mecA* gene-mediated resistance to methicillin, compared with coagulase-positive *S. schleiferi* isolates. Among methicillin- resistant isolates, decreased susceptibility to erythromycin or fluoroquinolones has been observed (Cain *et al.*, 2011b).

Virulence factors of *S. schleiferi* have not been studied in detail and genome of *S. schleiferi* has also not yet sequenced at present. Like *S. aureus*, *S. schleiferi* may produce beta-hemolysin and exoenzymes, such as lipase, potentially associated with virulence (Yamashita *et al.*, 2005). It also shows adherence to glass, which may be relevant as a marker of propensity for infection of indwelling devices. But none of the strain in our study produced strong biofilm in microtitre plate adherence test.

This study is a preliminary step with the initiation to isolate and explore the involvement of other staphylococcal species in the skin infections of dogs in Chennai, India. With the best of our knowledge, this is the first study to isolate and characterize *S. schleiferi* species from canine in India. However further studies are required for early detection of this particular pathogenic species in the clinical specimens.

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Fig. 1: Agarose gel electrophoresis showing the amplicon of *pta* gene of *S. schleiferi* subsp. *schleiferi* isolates

Lane MM: 100 bp DNA ladder; Lane 1 & 2: Negative isolates; Lane 3, 4 & 5: Amplicons of *pta* gene of *S. schleiferi* isolates

Fig. 2: Phylogenetic tree of *pta* gene of *S. schleiferi* subsp. *schleiferi* isolates

Table 1: Susceptibility pattern of *S. schleiferi* subsp. *schleiferi* against various antimicrobial agents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antimicrobial agents</th>
<th>SSS 100437</th>
<th>SSS 139781</th>
<th>SSS 108576</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Penicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2.</td>
<td>Ampicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3.</td>
<td>Amoxyclov</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>4.</td>
<td>Cefotaxime</td>
<td>R</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>5.</td>
<td>Ceftriaxone</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>6.</td>
<td>Oxacillin</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>7.</td>
<td>Streptomycin</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>8.</td>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>9.</td>
<td>Amikacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>10.</td>
<td>Sulphadiazine</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>11.</td>
<td>Oxytetracycline</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>12.</td>
<td>Ciprofloxacin</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>13.</td>
<td>Enrofloxacin</td>
<td>S</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>14.</td>
<td>Erythromycin</td>
<td>R</td>
<td>S</td>
<td>I</td>
</tr>
</tbody>
</table>
REFERENCES


