RESEARCH ARTICLE

# Isolation and Molecular Characterization of Methicillin-Resistant *Staphylococcus pseudintermedius* in dogs

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## Abstract

Isolation and Molecular Characterization of Methicillin-Resistant *Staphylococcus pseudintermedius* (MRSP) in dogs were carried out. A total of 25 ear swabs were collected aseptically from both diseased (otitis externa) and apparently healthy dogs (for vaccination) presented to teaching Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry.

On analysis of 25 ear swab samples, a total of 14 (56%) *Staphylococcus pseudintermedius (S. pseudintermedius*) isolates were recovered, 6 (24%) isolates from healthy, and 8 (32%) isolates from diseased dogs. Among the 14 *S. pseudintermedius* isolates, eight isolates for *mecA,* which is 21.40% (3/14), and 35.70% (5/14) of MRSP isolates were detected in healthy and diseased dogs, respectively. The present study highlighted the presence of M*RSP* in dogs in Puducherry region.

**Keywords:** Dogs, Methicillin-resistance, *mecA* gene, Public health, *Staphylococcus pseudintermedius*.

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## Introduction

*S. pseudintermedius* is the major pathogenic species of dogs involved in a wide variety of skin and soft tissue infections. It is a novel coagulase-positive staphylococcal species found on the skin or in the mouth, nose, or intestinal tract of 50% or more of healthy dogs and a smaller percentage of healthy cats. Typically it causes no problems at all, but it is an opportunistic pathogen and a leading cause of skin and ear infections in dogs (Paul *et al.,* 2012).

In recent years, Methicillin-resistance has emerged as an important problem in both animals and human beings. MRSP and *Staphylococcus aureus* (MRSA) are common multidrug-resistant bacteria in pet animals. Especially there is an increasing number of infections reported worldwide in dogs and cats caused by MRSP (Hanselman *et al.,* 2008). MRSP is a form of *S. pseudintermedius* that is highly resistant to many antibiotics, including most of the drugs that are commonly used to treat bacterial infections in dogs and cats. Since 2006, there has been a significant emergence of MRSP, mainly due to clonal spread. Methicillin-resistance is mediated by the *mecA* gene, which encodes the penicillin-binding protein (PBP) that has a low affinity for all β-lactam antimicrobials. Transmission of MRSP from healthy and sick pets to owners probably occurs regularly, although MRSP is not a normal bacterial inhabitant of people and is not well adapted to cause disease in humans. But this has raised the serious concern of emerging antimicrobial resistance and infections that are difficult to cure with available antimicrobials both in animals and humans.

The prevalence of MRSP from ear infections of the dog in India has not been studied extensively earlier. Although there are reports of phenotypic and genotypic detection of MRSP in dogs from other countries, only a few studies were conducted in dogs from India. Hence, the present study was aimed to isolate MRSP from both healthy dogs and dogs with common ear infections in Puducherry.

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## Materials and Methods

### Collection and processing of samples

A total of 25 ear swabs were collected aseptically from both diseased (otitis externa). Apparently, healthy dogs (for vaccination) presented to Teaching Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry. These samples were transported to the Department of Veterinary Microbiology, RIVER, Puducherry under cold chain for the isolation of *MRSP.*

The samples were collected with the prior consent from the corresponding owners.

### Isolation and Identification of *Staphylococcus pseudintermedius*

After overnight incubation in 7.5% NaCl-Luria broth, the inoculums were streaked onto Mannitol Salt agar plates and incubated at 37°C overnight for the selective isolation of *Staphylococcus*. The isolated Mannitol positive yellowish colonies were subjected to Gram's staining and conventional biochemical methods for species-level identification. The biochemically confirmed *S. pseudintermedius* isolates were further subjected to molecular screening using Polymerase Chain Reaction (PCR).

### Molecular Screening of *Staphylococcus pseudintermedius*

A single colony from Mannitol Salt agar plates was inoculated into Luria broth and incubated at 37°C for overnight. Then the DNA was extracted by a chemical method using Triton X as described by Sowmya *et al*. (2012). *S. pseudintermedius* isolates were confirmed by using species-specific primer by targeting the *pse* gene described in Table 1. The amplification process requires the following major steps: Template DNA was initially denatured at 95°C for 5 mins followed by 35 cycles of denaturation at 95°C for 30 secs, annealing at 56°C for 45 secs and extension at 72°C for 1 min 30 secs and a final extension at 72°C for 10 mins. The PCR products were analyzed by 1.5% agarose gel electrophoresis, visualized under UV transilluminator, and the gel pictures were documented. The PCR product, which yielded 926 bp amplicon, was considered positive for *S. pseudintermedius*.

**Fig. 1:** Mannitol positive *Staphylococcus* isolates on Mannitol salt agar

### MALDI-TOF analysis of *S. pseudintermedius*

Randomly selected one positive isolate was subjected to matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik GmbH, Germany) and further confirmed as *S. pseudintermedius*, the bacterial culture was grown overnight at 37°C on brain heart infusion agar. Cells of a whole colony were transferred from the plate onto the MALDI steel plate. Each sample was overlaid with 2 µL of matrix solution (saturated solution of α-cyno-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid) and air-dried at room temperature. Later the spectra were acquired by using automated data analysis (MALDI BioTyper 1.1 software with default settings). From the generated peak, the bacterial species were identified directly using the integrated pattern matching algorithm of the software.

### Molecular screening of Methicillin-resistant *Staphylococcus pseudintermedius*

*S. pseudintermedius* positive isolates were further screened for the presence of *the mecA* gene using specific primers (Table 1). The amplification process requires the following major steps: template DNA was initially denatured at 94°C for 5 mins followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 mins and a final extension at 72°C for 10 mins. The PCR products were analyzed by 1.5% agarose gel electrophoresis, visualized under UV transilluminator, and the gel pictures were documented. The PCR product, which yielded 162 bp amplicon, was considered positive for methicillin-resistant *S. pseudintermedius*.

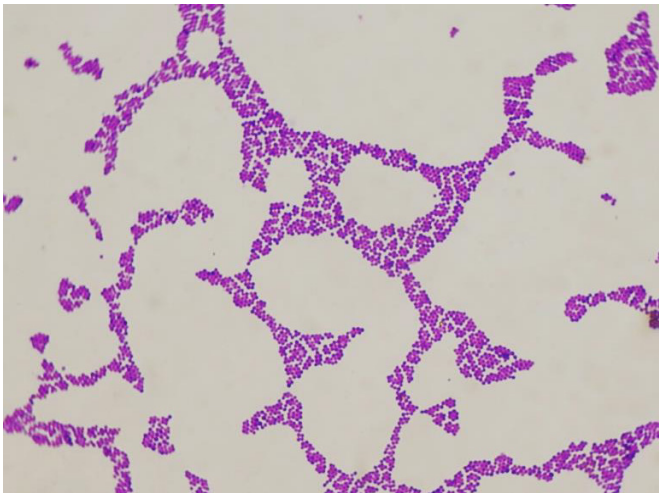

**Fig. 2:** Gram's staining of Mannitol positive *Staphylococcus* isolates showing Gram-positive cocci arranged in clusters

**Table 1:** Details of the Primers used in this study

| *S.No* | *Primer* | *Sequence* | *Amplicon size* | *Reference* |
|---|---|---|---|---|
| 1 | *pse* | F5′- TRGGCAGTAGGATTCGTTAA-3′<br>R5′- CTTTTGTGCTYCMTTTTGG -3′ | 926 bp | (Chrobak *et al*., 2011) |
| 2 | *mecA* | F5′-TCCAGATTACAACTTCACCAGG-3′ R5′-CCACTTCATATCTTGTAACG -3′ | 162 bp | (Oliveira and de Lencastre, 2002) |

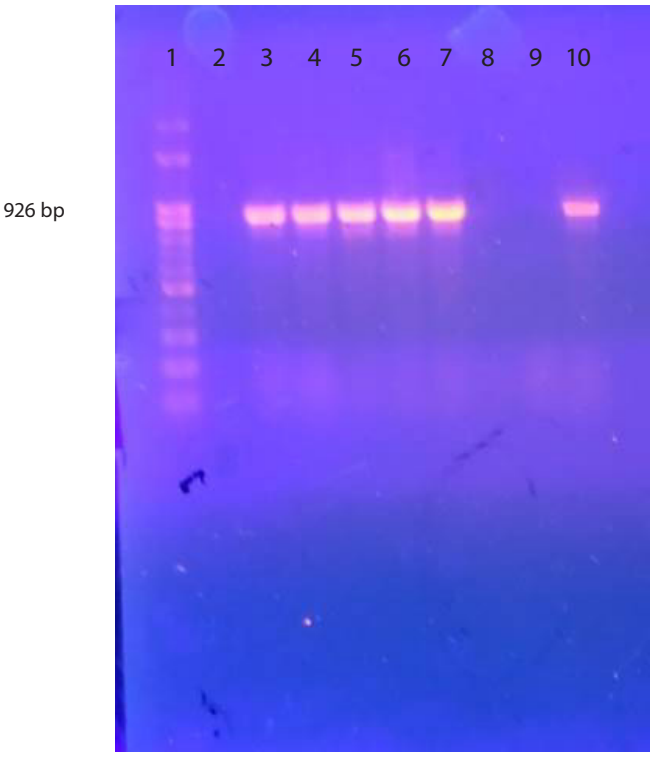


**Fig. 3:** Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 926 bp for the *pse* gene of *Staphylococcus pseudintermedius* isolates. Lane 1: 100 bp ladder; Lane 2 and 8,9: Negative control and Negative isolates; Lane 3 & 4,5,6,7,10: positive control and positive for *S.pseudintermedius* isolates, respectively.

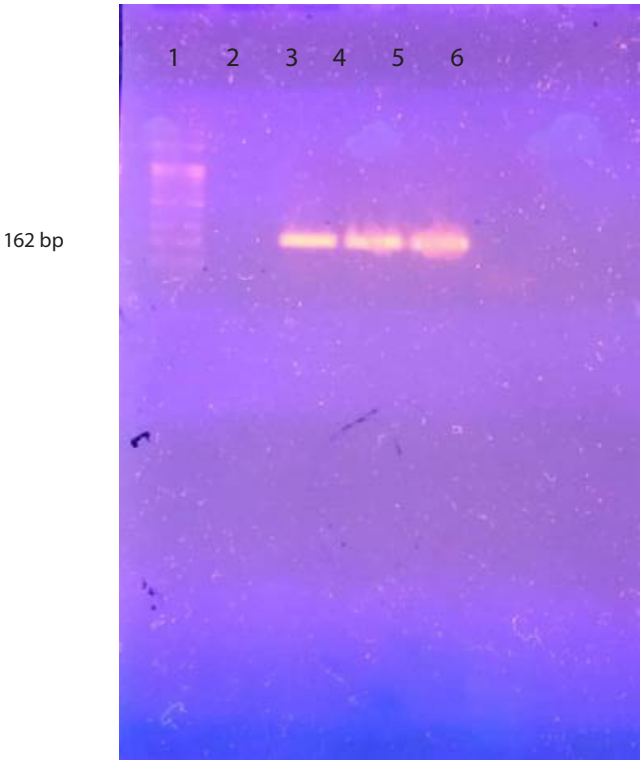


**Fig. 4:** Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 162 bp for the *mecA* gene of *Staphylococcus pseudintermedius* isolates. Lane 1: 100 bp ladder; Lane 2: Negative control; Lane 3,4 and 5: positive for *mecA* gene of *S. pseudintermedius* isolates; Lane 6: negative for *mecA* gene of SP isolates.

## Results and Discussion

On analysis of 25 ear swab samples, Staphylococcus isolates were identified phenotypically by cultural and biochemical tests (Figs. 1 and 2) followed by PCR targeting *S. pseudintermedius* thermonuclease (*pse*) gene yielded 926bp amplicon in 14 (56%) isolates (Fig. 3), of which 6 (24%) isolates were from healthy dogs and 8 (32%) isolates were from diseased dogs.

A similar kind of study in Japan showed a higher rate of incidence (76%) of *S. pseudintermedius* in pyoderma cases in dogs (Onuma *et al*., 2011). (Yoon *et al*.,2010) reported an incidence rate of 61% in the South Korea region. Almost similar rate of isolation of 59% was noticed in Chennai from both healthy and diseased dogs by targeting *S. pseudintermedius* thermonuclease gene by using their designed set of primers (Chitra *et al*., 2016), 52% from both healthy and diseased dogs in Poland by (Garbacz *et al*.,2013) and 55% from healthy dogs in Tunisia by (Gharsa *et al*.,2013). However, a lower isolation rate of 40% was observed in Guide dog school in Finland Gronthal *et al*.,(2015), 16 % from healthy and diseased dogs in South China (Feng *et al*., 2012) and 26.5 % from pyoderma cases in North China (Wang *et al*., 2012).

Of the 14 *S. pseudintermedius* isolates, eight isolates (57%) were also found to be positive for *mecA* (Fig. 4). In healthy and diseased dogs, respectively, 21.40% (3/14) and 35.70% (5/14) methicillin resistance *S. pseudintermedius* isolates were detected. One randomly selected isolate was also confirmed as *S. pseudintermedius* by MALDI-TOF MS method with an identification score of 1.832, based on comparing and analyzing the generated spectra in the automated data analysis (MALDI BioTyper 1.1 software).

MRSP has emerged as a significant pet animal health problem in veterinary medicine, and resistant is mediated by *mecA* gene that encodes the production of a modified penicillin-binding protein. In the present study also, a high prevalence (57%) of MRSP was noticed. In another study, (Chitra *et al*.,2015) reported 28% MRSP strains in different clinical specimens such as ear swabs, skin swabs, pus samples of dogs in Chennai (India). The frequency of MRSP was 57% in the present study, which was comparatively higher than the occurrence of MRSP from healthy dogs of about 2.6% in Norway (Kjellman *et al*.,2015) and 24.14% from West Indies (Hariharan *et al*., 2014).

## Conclusion

The present study highlighted the presence of MRSP in dogs in Puducherry, indicating a serious public health concern. The incidence of methicillin-resistance might probably

be the result of indiscriminate use of antibiotics in canine practice. The molecular characterization of *mecA* genes will help in better understanding methicillin-resistant *S. pseudintermedius* infection in pets, which will create awareness to formulate the preventive strategies.

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