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Research Article

CLONAL SPREAD AND ANTIMICROBIAL RESISTANCE PATTERNS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN CANINE PYODERMA SAMPLES FROM INDIA

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ABSTRACT: Canine pyoderma is most commonly caused by the bacterium *Staphylococcus pseudintermedius*. Methicillin-resistant *S. pseudintermedius* (MRSP) is a major health risk in dogs. It can cause various diseases, including systemic infections in the urinary, respiratory, and reproductive tracts and topical infections such as canine pyoderma and otitis externa. The present study was conducted to screen and effectively analyze MRSP in suspected pyoderma cases and determine the prevalence of MRSP in India by conducting phylogenetic analysis. In the present study, a total of 30 skin swab samples were collected from dogs suspected of *Staphylococcus* pyoderma to characterize and identify resistant genes. Molecular characterization was performed by amplifying the target pse gene. Further, the mecA gene was amplified and sequenced to understand the incidence of Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and also to analyze their population genomic diversity. Among 30 samples, 17 were found positive for *Staphylococcus pseudintermedius*, of which, 6 samples tested positive for MRSP by the amplification of the mecA gene. Antibiotic sensitivity tests were carried out to study the resistance profile against MRSP. The phylogenetic analysis of the isolated strains of this study revealed the spread of the conserved mecA gene among *Staphylococcus pseudintermedius* in India.

Keywords: Canine, Methicillin-resistant, pse gene, mecA gene, *Staphylococcus pseudintermedius.*

INTRODUCTION

Canine pyoderma is a prominent infection in dogs. caused by *Staphylococcus* which is naturally present in canine's skin. The disease is characterized by pus-filled lesions due to secondary bacterial infections triggered by the exponential growth of normal resident or transient flora [1] and also infections like food allergens, environmental allergens, or poor managemental practices. It is more often reported in dogs than cats [2, 3, 4]. The clinical signs include excessive itching, licking, or chewing. The skin will appear crusty or moist and the fur of the canine would be patchy with peeling.

Pyoderma is caused by three major species of *Staphylococcus* namely *Staphylococcus aureus*, *Staphylococcus intermedius*, and *Staphylococcus pseudintermedius* [5, 6]. Methicillin resistance has emerged in some species of *Staphylococcus* and poses a serious concern for animals and human beings. Methicillin-resistant *Staphylococcus pseudointermedius* (MRSP) has increased significantly since 2006 [7]. In pets, multidrug-resistant bacteria including *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudointermedius* (MRSP) are frequently found. Methicillin-resistant illnesses caused by *Staphylococcus pseudintermedius* (MRSP) in particular are becoming

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more common in dogs and cats around the world [8]. *S. pseudintermedius* is a member of the *Staphylococcus intermedius* group (SIG), which includes *S. intermedius*, *S. delphini*, and *S. cornubiensis* [9,10]. It is hard to distinguish organisms of the SIG group from the *Staphylococcus aureus* complex (which includes *S. aureus*, *S. argenteus*, and *S. schweitzeri*) biochemically.

A strain of *Staphylococcus pseudintermedius* known as MRSP is extremely resistant to a wide range of antibiotics, including the majority of those that are frequently prescribed for treating bacterial infections in cats and dogs. The mecA gene, which produces the penicillin-binding protein (PBP), which has a low affinity for all β-lactam antibiotics, is the mediator of methicillin resistance [11]. The clonal spread of MRSP has been reported in previous studies and is considered a serious zoonotic issue. The ability of MRSP to spread clonally has made continuous surveillance, and controlled antibiotic use a serious concern [8, 9]. However, in India, especially in the veterinary field, very limited work has been carried out on MRSP in pyoderma cases. In this context, the purpose of this investigation is to identify any methicillin-resistant *Staphylococcus* species that may exist in the suspected pyoderma cases and further, characterization, antibiotic resistance profiling, and phylogenetic analysis of methicillin - resistant *Staphylococcus pseudintermedius* (MRSP) were conducted to determine its spread in India since it plays a vital role in veterinary and public health.

MATERIALS AND METHODS

Sample collection

Canine pyoderma - suspected skin swab samples were collected from the Dermatology ward of the small animal unit of Madras Veterinary College teaching hospital, Chennai - 600 007 for a period of 6 months from November 2022 to April 2023. A 75 mm sterile cotton swab (Himedia®) was rubbed on the lesion site of the skin and replaced in the sterile tube after labelling. The swab samples were then transferred to the laboratory for processing. The collected swab samples were inoculated in sterile phosphate-buffered saline (1X PBS) to maintain a constant pH and stable environment for bacterial growth.

Culture and identification

About 50 µL of swab sample immersed in 1X PBS was inoculated in sterile Brain Heart Infusion (BHI) agar followed by Mueller-Hinton (MH) agar (Himedia®) and kept for overnight incubation at 37ºC. 10% NaCl was added to BHI broth, which was subsequently enhanced for eight to ten hours at 37°C. The enriched samples were streaked over Baird Parker agar (Himedia, India), and suspicious colonies were detected by Gram's staining, the Catalase test, Mannitol fermentation, coagulase, and thermonuclease tests [12].

DNA extraction

Staphylococcal genomic DNA was extracted with a Qiagen genomic-DNA purification kit according to the instructions of the manufacturer [13]. Briefly, 5 ml of culture was taken and centrifuged at 7,500 rpm for 10 min to collect the cell pellet. The DNA was extracted in the column provided and eluted with 30 µL Nuclease free water (NFW) and stored at –20°C until further use.

Detection and molecular characterization of *Staphylococcus pseudintermedius*

The pse gene of *Staphylococcus pseudintermedius* was amplified for molecular characterization. The *Staphylococcus pseudintermedius* gene-specific pse forward primer 5'- TRGGCAGTAGGATTCGTTAA-3' and reverse primer 5'- CTTTTGTGCTYCMTTTTGG -3' were self-designed retrieving sequences from NCBI and using Beacon Designer™ application. The reaction mixture consisted of 12.5 µL of 1X master mix (Biolabs, USA), 1µL of forward and reverse primer each, 9µL of nuclease-free water, and 1.5µL of DNA template. The PCR condition was initial denaturation for 4 mins at 94ºC, followed by 30 cycles of denaturation for 1 min at 94ºC, annealing at 56°C for 45 secs, extension at 72ºC for 1min, and final extension at 72ºC for 4 mins (Applied Biosystems Veriti™ 96-Well Thermal Cycler) [14]. 1.5% agarose gel electrophoresis was used to analyze the amplified products (926 bp).

Screening of Methicillin-resistant gene (mecA)

All the positive sample for *Staphylococcus* sp. was subjected to screening of Methicillin resistance by amplification of mecA gene to study the incidence of MRSP. The mecA gene - specific primers were designed with Beacon Designer™ application, forward primer 5'-AATGCTCAAATTTCAAACAAAAA-3' and reverse primer 5'- CACTTTCAACATACAATGAAAATGAA -3'. The following condition was optimized for the PCR cycle. The reaction mixture contained 12.5µL of master mix (Biolabs, USA), 1µL of each forward and reverse primer, 9µL of nuclease-free water, and 1.5µL of DNA template. The PCR condition was initial denaturation at 94ºC for 4 mins, followed by 30 cycles of

denaturation at 94ºC for 1 min, annealing at 54°C for 1 min, extension at 72ºC for 1 min, and final extension at 72ºC for 4mins. Further, the PCR products (600 bp) were subjected to gel electrophoresis on 1.5% Ultra-Pure™ Agarose gel containing ethidium bromide (5µg/mL) for 50 mins at a constant current of 100V. The PCR product that resulted was observed using a UV transilluminator and captured.

Sequence analysis and phylogenetic tree construction

The mecA gene PCR products were purified using a Qiagen PCR gel purification kit and sequencing was performed at M/s. Eurofins Sequencing Services, Chennai, India. Following submission to the NCBI, the nucleotide sequence data were subjected to BLAST analysis (www.ncbi.nlm.nih.gov), assembled and examined using the Seqman and MegAlign programs of the Lasergene package (version 7.1.0) (DNA Star Inc. Madison, WI). The data were also subjected to BLAST analysis (www.ncbi.nlm.nih.gov) [15]. Nucleotide sequence alignment was performed by the ClustalW method with MEGA software version 11. Phylogenetic analysis of the mecA sequence was performed using the Maximum likelihood method with 100 bootstrap replications.

Antimicrobial susceptibility test

The test for antimicrobial susceptibility was conducted using the (MRSP) *Staphylococcus pseudintermedius* isolates (n = 6) using the Kirby-Bauer disk diffusion method as per guidelines of the Clinical and Laboratory Standards Institute [12]. Following turbidity correction to the 0.5 McFarland standard, each bacterial suspension was inoculated onto a Mueller-Hinton agar plate (MHA; Himedia, India). Using sterile forceps, a commercial antimicrobial-impregnated disc (Himedia, India) was then placed over the agar surface and incubated for 24 hours at 37°C. A total of 9 antimicrobials (Cefotaxime/ Clavulanic Acid, Ciprofloxacin, Tetracycline, Gentamicin, Doxycycline, Ampicillin, Amoxicillin, Enrofloxacin, Streptomycin) were tested. After incubation, the inhibitory zones' width was calculated in millimeters using a measuring scale across the discs' center. The European Committee on Antimicrobial Susceptibility Testing [17] and the Clinical and Laboratory Standards Institute [16] established the criteria that were used to interpret the results. According to the test results, the bacteria were classified as resistant, intermediate, or sensitive to each antimicrobial agent.

RESULTS AND DISCUSSION

Isolation and identification of *Staphylococcus* sp.

Staphylococcus pseudintermedius is a pathogen that commonly affects canine and feline and is occasionally associated with human diseases brought on by dogs and cats. It has become the most significant bacterial pathogen causing dermatitis issues in dogs and cats [18]. In the present research, seventeen samples were determined to be positive for *Staphylococcus*. Staphylococcal colonies (small, creamy grey to white, round colonies with a smooth margin) from Baird Parker agar plates (Fig. 1), were purple after gram staining denoting their gram-positive nature. They were found in clusters with typical spherical shapes upon morphological observations. They were positive for biochemical tests namely coagulase and catalase tests.

Molecular confirmation and characterisation of *Staphylococcus pseudintermedius* by PCR

The DNA was extracted from all the positive samples suspected of Staphylococci by staining and biochemical tests. Further molecular characterization was done by amplifying the pse gene and the mecA gene was amplified with a product size of 660 bp (Fig 2 and Fig. 3). Among 30 samples, seventeen were found to be positive for *Staphylococcus pseudintermedius* and six samples were found to possess the mecA gene responsible for antimicrobial resistance. The mecA gene was sequenced for the six isolates and submitted to GenBank with the accession numbers OR349235-40 respectively. The methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in the swabs collected from veterinary staff in a UK hospital as stated by Loeffler [4]. This study ascertains the spread of MRS (methicillin-resistant *Staphylococcus*) to humans which could lead to deleterious health effects in the human population. A similar outcome was reported in a study focused on the isolation and characterization of *Staphylococcus* spp. from Veterinary hospital samples performed in Zambia and Lithuania [19, 20]. The presence of MRSP strain was reported in dogs from the Puducherry region upon analysis of 25 ear swab samples collected from both healthy and diseased dogs [21]. Similarly in a study, 28% of MRSP strain was detected by the presence of mecA gene in 91 samples collected from Chennai [22]. The presence of positive MRSP in the clinical samples suggests the increased prevalence of MRSP in pyoderma animals and elevates the concern for the spread of resistant forms causing laborious treatment regimes.

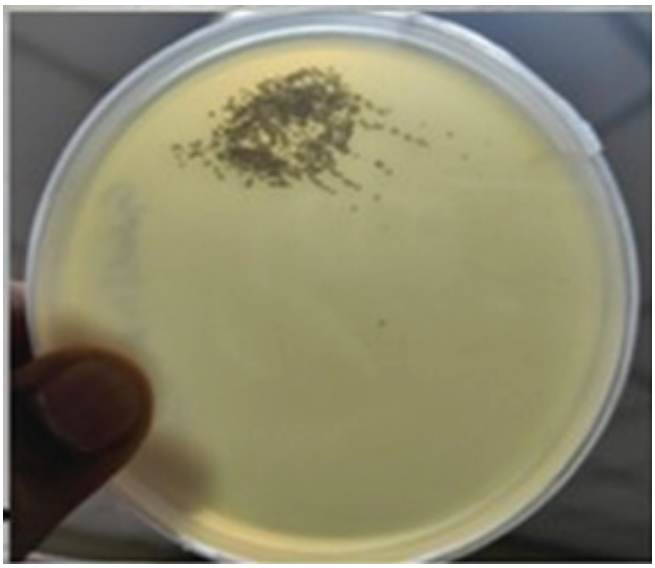

Fig. 1. Isolation of single colony of *Staphylococcus* sp.

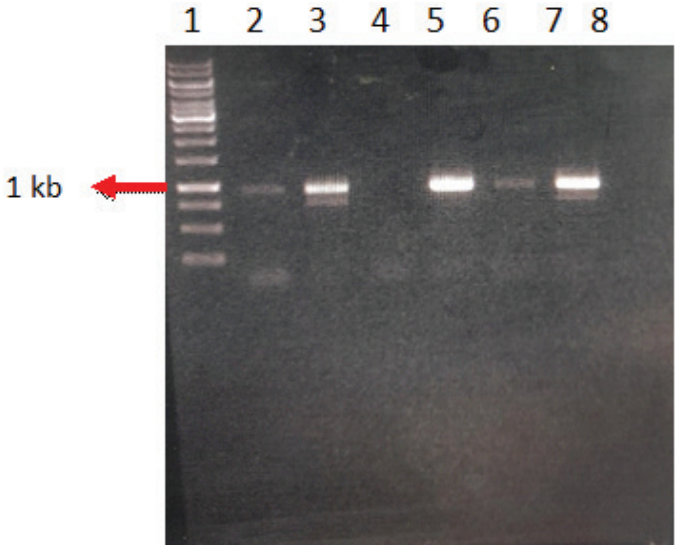

Fig. 2.Identification of *Staphylococcus pseudintermedius* by targeting species specific pse gene from suspected cases. (Lane 1: 1kb Ladder; lane 2-6: samples; lane 7: positive control; lane 8: negative control).

Antimicrobial susceptibility test

The antimicrobial sensitivity of *Staphylococcus pseudointermedius* displayed distinct zones of inhibition for the antibiotic discs used from different class of antibiotics which were commonly used against canine dermatitis namely Cefotaxime/ Clavulanic acid, Ciprofloxacin, Tetracycline, Gentamicin, Doxycycline, Ampicillin, Amoxicillin, Enrofloxacin and Streptomycin (Fig 4). The zone of inhibition given by antimicrobial susceptibility tests shows whether they are susceptive, intermediate, or resistive to the antibiotics. Except for one isolate all other five isolates of *S. pseudintermedius* were found to be resistant to a minimum of two of the nine antibiotics tested. Five isolates showed resistance against Cefotaxime and four isolates were found to be resistant to doxycycline. Only one of the isolated *S. pseudintermedius* was resistant to streptomycin. Three isolates showed intermediate response against gentamicin. Four isolates were susceptible to ampicillin,

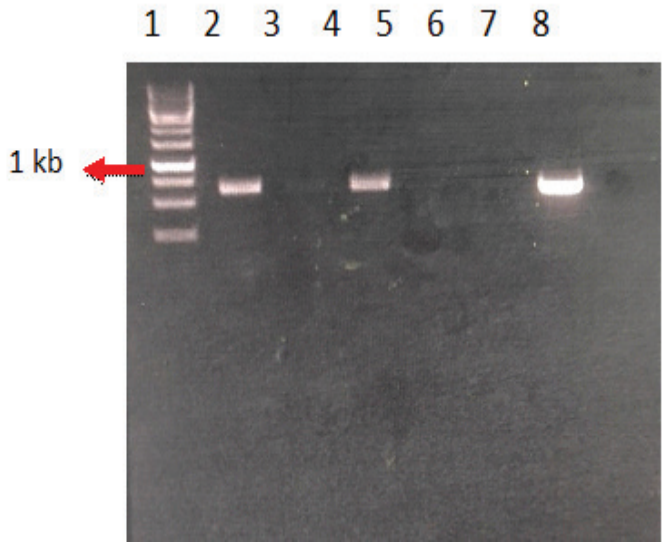

Fig. 3. Amplification of mecA gene of Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). (Lane 1: 1kb Ladder; lane 2-6: samples; lane 7: positive control; lane 8: negative control).

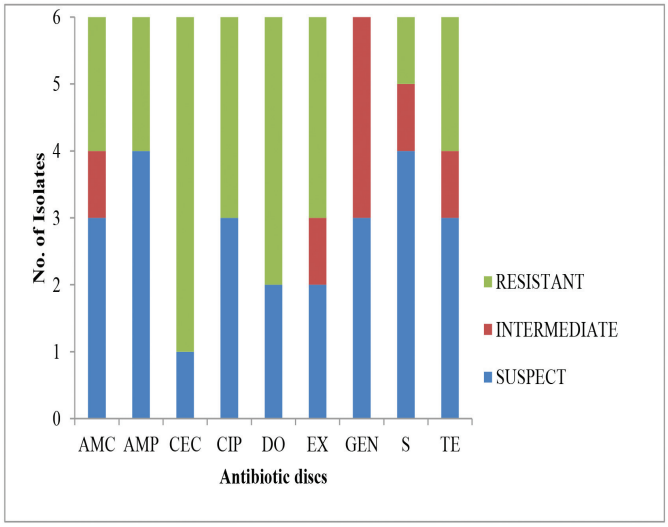

Fig. 4. Antibiotic susceptibility test profile of all 6 isolated *Staphylococcus pseudintermedius*. (AMC: Amoxicillin, AMP: Ampicillin, CEC: Cefotaxime/Clavulanic Acid, CIP: Ciprofloxacin, DO: Doxycycline, EX: Enrofloxacin, GEN: Gentamicin, S: Streptomycin, TE: Tetracycline).

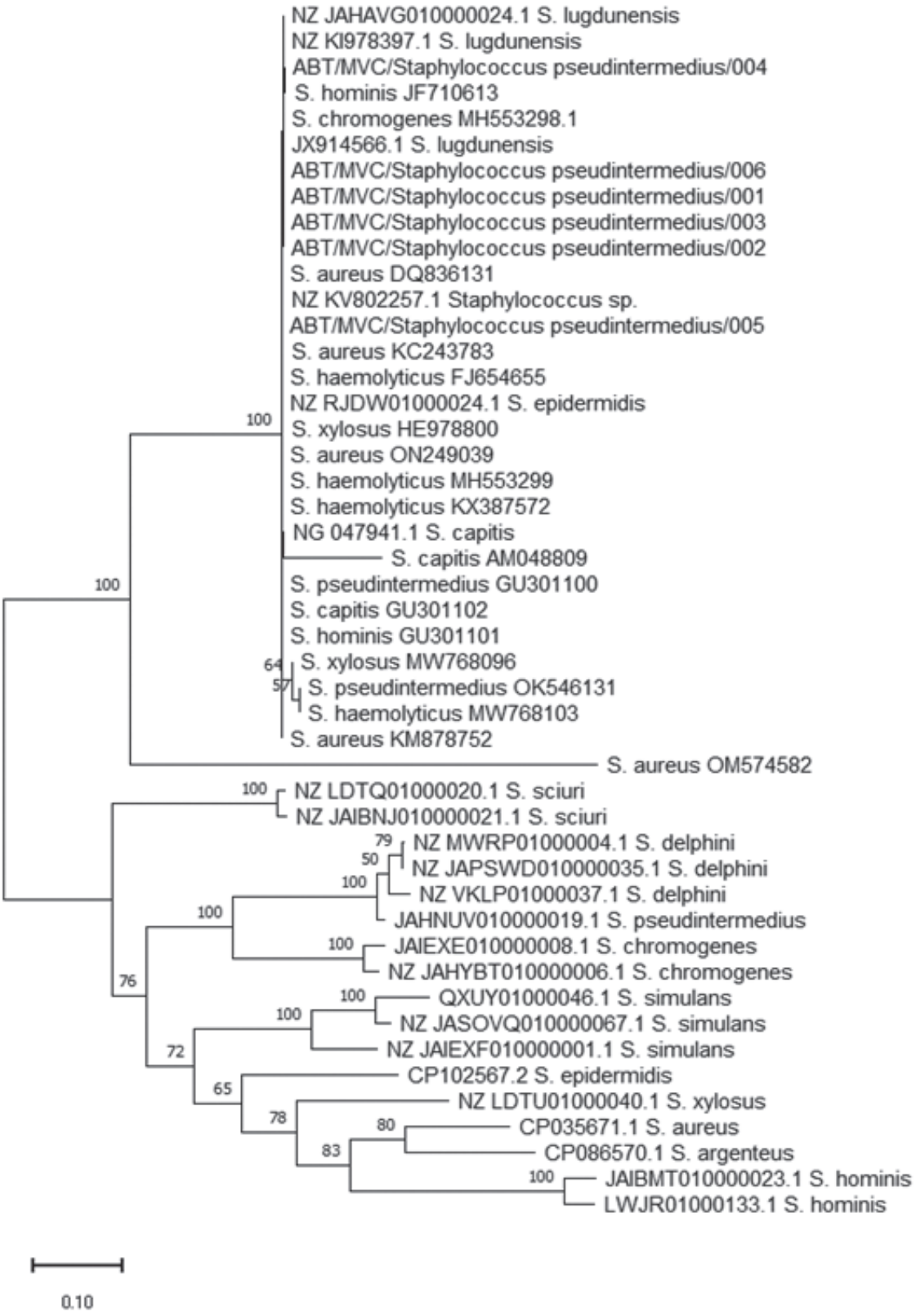

Fig. 5. Phylogenetic tree analysis of *Staphylococcus* sp. from the reference sequence available in NCBI database. The phylogenetic tree was constructed using MEGA 11 software by maximum likelihood method with 100 bootstraps. (All *S. pseudintermedius* from the present study form a single cluster which indicates that they belong to a single clonal spread of MRSP in India).

and streptomycin. In the present study, no isolates revealed resistance against gentamicin. The results reveal that *S. pseudintermedius* is resistant to a wide range of antibiotics that are regularly used to treat bacterial infections. Similar to this report Grönthal [23] studied the susceptibility of *Staphylococcus pseudintermedius* isolated from clinical samples to different antibiotics and stated that the isolated strains were 14% resistant to oxacillin (methicillin). Anandachitra [22] also studied the antibiotic susceptibility of MRSP strains and reported that higher susceptibility was determined against tetracycline and ciprofloxacin and the isolates were highly resistant to erythromycin and sulphamethoxazole. In a study by Feng *et al.* [24] it was reported that a high number of the isolated *Staphylococcus pseudintermedius* in China were found to be resistant to penicillin and erythromycin followed by ciprofloxacin and enrofloxacin, present work revealed that most of the isolates were found to be resistant to ciprofloxacin. *Staphylococcus pseudintermedius* isolates from Korea were found to be resistant to penicillin, erythromycin, and tetracycline [13]. The variations in reported resistance rates to various antimicrobials across different geographical regions attributed to the investigation period and antimicrobial susceptibility testing methods.

Phylogenetic analysis

Phylogenetic analysis of mecA gene sequence of isolates with GenBank accession number OR349235-40 was found in the same clade along with other *Staphylococcus* variants or species. These six strains form a clade with *Staphylococcus aureus*, *S. lugdunensis*, *S. haemolyticus*, *S. captis*, and *S. hominis* found in other states within India and also from other countries. The phylogenetic tree of *Staphylococcus pseudintermedius* is given in Fig 5. It was also reported that more number MRSP positive was obtained from clinical dog samples. The incidence of the mecA gene in the isolated Staphylococcal strains and their resistance to (streptomycin, doxycycline, ciprofloxacin, cefotaxime, enrofloxacin, ampicillin, amoxicillin, and streptomycin) antibiotics denotes the occurrence of multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) in the canine pyoderma cases in India. The phylogenetic analysis also revealed the similarity between the mecA gene of the isolated *S. pseudintermedius* with Staphylococcal species isolated from different parts of India including Andhra Pradesh, Karnataka, Pune, and Madurai, and also with Staphylococcal species identified across the world from Taiwan, USA, Turkey, Switzerland, and China. The widespread distribution of evolutionarily dominant antibiotic-resistant genes among microbial populations is supported by the occurrence of the mecA gene among Staphylococcal strains across the Indian subcontinent. The presence of all the isolated MRSP in a single cluster denotes a single lineage of MRSP in the Indian sub-continent. The extensive spread of MRSP may lead to the transfer of multidrug - resistant *S. pseudintermedius* to clinicians which would lead to kindling one health approach in order to protect both humans and animals from antibiotic-resistant strains. Whereas, in a study by Perreten [25] two major clonal lineages North American and European were reported in Europe, the USA, and Canada. MRSP was isolated from clinical dog and cat samples in Japan and the presence of mecA was confirmed, and clonal spread analysis was carried out and it was described that all the MRSP isolated belonged to the single clonal lineage in a study by Bardiau [26]. Similar studies concerning the spread of methicillin-resistant *Staphylococcus pseudintermedius* were described from different regions of the world Norway, Zambia, and Lithuania [19, 20, 27]. Cengiz [28] has studied the occurrence of MRSP in samples from dogs and cats and has also analyzed the similarity in the MRSP strains between animals and humans. Therefore, these studies on the presence and expansion of multi-drug-resistant strains call for efficient strategies to combat the adverse outcomes.

The results of the phylogenetic analysis revealed that the MRSP strains isolated from the samples collected at the Clinics of the Madras Veterinary College were closely related to isolates identified from various other geographical regions across India. This close genetic relationship among the MRSP strains suggests that these bacteria are not confined to a single area but are being transmitted and circulated throughout the country. This widespread distribution poses a significant threat to animal health and potentially to public health, as it indicates the possibility of a more extensive and uncontrolled spread of these multiresistant bacteria. The circulation of MRSP strains across different regions underscores the need for a coordinated national approach to monitoring, controlling, and preventing the spread of this pathogen, which could otherwise have serious implications for both veterinary and human medicine, particularly in terms of treatment options and the economic impact on the livestock industry.

CONCLUSION

This study highlights a serious public health risk by focusing on the prevalence and transmission of Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in dogs that may be suffering from pyoderma in India. About 50% (n=17) of the collected samples were positive for *Staphylococcus pseudintermedius* of which about 35% (n=6) were identified to be MRSP by the amplification of mecA gene. The phylogenetic analysis also revealed that genetically similar MRSP strains are prevalent across the nation denoting clonal spread of MRSP. These results emphasize the possibility that the veterinary personnel treating the animal with MRSP would either get infected or become a carrier and spread it to other animals. The identification of mecA genes at the molecular level will aid in the development of preventive treatment plans for pet infections caused by methicillin-resistant *Staphylococcus pseudointermedius*.

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REFERENCES

1. Gross TL, Ihrke PJ, Walder EJ, Affolter VK. Skin diseases of the dog and cat: clinical and histopathologic diagnosis. 2008; John Wiley & Sons.

2. Leib MS, Monroe WE. Practical small animal internal medicine. 1997. ISBN 10: 0721648398, ISBN 13: 9780721648392, Saunders.

3. Bemis DA, Jones RD, Frank LA, Kania SA. Evaluation of susceptibility test breakpoints used to predict meca-mediated resistance in *Staphylococcus pseudintevmedius* isolated from dogs. J Vet Diagnost Invest. 2009; 21(1):53-58.

4. Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, *et al.* Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. J Antimicrob Chemother. 2005; 56(4):692-697.

5. Morris DO, Boston RC, O'Shea K, Rankin SC. The prevalence of carriage of meticillin-resistant *Staphylococci* by veterinary dermatology practice staff and their respective pets. Vet Dermatol. 2010; 21(4):400-407.

6. Bajwa J. Canine superficial pyoderma and therapeutic considerations. Canadian Vet J. 2016; 57(2):204.

7. Windahl U, Reimegård E, Holst BS, Egenvall A, Fernström L, *et al.* Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs - a longitudinal study. BMC Vet Res. 2012; 8:1-8.

8. Hanselman BA, Kruth S, Weese JS. Methicillin-resistant Staphylococcal colonization in dogs entering a Veterinary teaching hospital. Vet Microbiol. 2008; 126(1-3):277-281.

9. Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, *et al.* Public health risk of antimicrobial resistance transfer from companion animals. J Antimicrob Chemother. 2017; 72(4):957-968.

10. Bibby HL, Brown KL. Identification of *Staphylococcus pseudintermedius* isolates from wound cultures by matrix-assisted laser desorption ionization - time of flight mass spectrometry improves accuracy of susceptibility reporting at an increase in cost. J Clinic Microbiol. 2021; 59(11):110-128.

11. Weese JS, van Duijkeren E. Methicillin - resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol. 2010; 140(3-4):418-429.

12. Cruickshank R, Duguid JP, Marmion BP, Swain RH. Medical microbiology: the pratice of medical microbiology. 1975; 587-587.

13. Ruscher C, Lübke-Becker A, Wleklinski CG, Soba A, Wieler LH, Walther B. Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. Vet Microbiol. 2009; 136(1-2):197-201.

14. Chrobak D, Kizerwetter-Swida M, Rzewuska M, Moodley A, Guardabassi L, Binek M. Molecular characterization of *Staphylococcus pseudintermedius* strains isolated from clinical samples of animal origin. Folia Microbiologica. 2011; 56:415-422.

15. Fazakerley J, Williams N, Carter S, McEwan N, Nuttall T. Heterogeneity of *Staphylococcus pseudintermedius* isolates from atopic and healthy dogs. Vet Dermatol. 2010; 21(6):578-585.

16. Performance Standards for Antimicrobial Susceptibility Testing (CLSI M100), 34th edn., 2020; Clinical and laboratory standards institute.

17. EUCAST T. European committee on antimicrobial susceptibility testing. 2020; Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 10.0.

18. Duim B, Verstappen KM, Broens EM, Laarhoven LM, Van Duijkeren E, *et al.* Changes in the population of methicillin-resistant *Staphylococcus pseudintermedius* and

dissemination of antimicrobial-resistant phenotypes in the Netherlands. J Clinical Microbiol. 2016; 54(2):283-288.

19. Youn JH, Park YH, Hang'ombe B, Sugimoto C. Prevalence and characterization of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from companion animals and environment in the veterinary teaching hospital in Zambia, Africa. Comparati Immunol, Microbiol Infectious Dis. 2014; 37(2):123-130.

20. Ruzauskas M, Couto N, Pavilonis A, Klimiene I, Siugzdiniene R, *et al.* Characterization of *Staphylococcus pseudintermedius* isolated from diseased dogs in Lithuania. 2016; Polish J Vet Sci. 19(1):7-14, DOI: 10.1515/pjvs-2016-0002.

21.Tamilarasu S, Jayalaskhmi V, Srinivasan VV, Barbuddhe SB, Antony PX, Mukhopadhyaya HK. Isolation and molecular characterization of methicillin resistant *Staphylococcus pseudintermedius* in dogs. Indian J Vet Sci Biotechnol. 2020; 15(4):71-74.

22. AnandaChitra M, Jayanthy C, Nagarajan B. Virulence genes detection and antimicrobial susceptibility of *Staphylococcus pseudintermedius* isolates from canine skin infection in Chennai, India. Proc Nat Acad Sci, India, Section B: Biologic Sci. 2018; 88:355-361.

23. Grönthal T, Eklund M, Thomson K, Piiparinen H, Sironen T, Rantala M. Antimicrobial resistance in *Staphylococcus pseudintermedius* and the molecular epidemiology of methicillin-resistant *S. pseudintermedius* in small animals in Finland. J Antimicrob Chemother. 2017; 72(4):1021-1030.

24. Feng Y, Tian W, Lin D, Luo Q, Zhou Y, *et al.* Prevalence and characterization of methicillin - resistant *Staphylococcus pseudintermedius* in pets from South China. Vet Microbiol. 2012; 160(3-4):517-524.

25. Perreten V, Kadlec K, Schwarz S, Andersson GU, Finn M, *et al.* Clonal spread of methicillin - resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother. 2010; 65(6):1145-1154.

26. Bardiau M, Yamazaki K, Ote I, Misawa N, Mainil JG. Characterization of methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs and cats. Microbiol Immunol. 2013; 57(7):496-501.

27. Osland AM, Vestby LK, Fanuelsen H, Slettemeås JS, Sunde M. Clonal diversity and biofilm-forming ability of methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother. 2012; 67(4):841-848.

28. Cengiz S, Okur S, Oz C, Turgut F, Gumurcinler B, *et. al.* Prevalence and clonal diversity of methicillin-resistant *Staphylococcus aureus* and methicillin - resistant *Staphylococcus pseudintermedius* isolated from dogs and cats with eye discharge. Acta Microbiologica et Immunologica Hungarica. 2023; 70(2):134-141.

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