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Multidrug-Resistant Profile of Methicillin-Resistant *Staphylococcus pseudintermedius* in Dogs with Pyoderma in Lima, Peru

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ABSTRACT

Methicillin resistance is a major global health concern, and recently, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has emerged as a significant issue in veterinary medicine, with the potential to spread to humans. The present study aimed to analyze *Staphylococcus pseudintermedius* (*S. pseudintermedius*) strains isolated from 585 canine pyoderma samples from 2016 to 2024 at the Faculty of Veterinary Medicine, National University of San Marcos, Peru. The *mec*A gene was detected by conventional polymerase chain reaction, and antimicrobial susceptibility test included seven antibiotic families, including tetracyclines, sulfonamides, aminoglycosides, second-generation cephalosporins, penicillins, fluoroquinolones, and lincosamides. Out of 585 samples, 143 (24.4%) were positive for the *mecA* gene, confirming the presence of MRSP. Among these strains, 107 (74.8%) were multidrug resistant (MDR), and 119 (83.2%) exhibited phenotypic oxacillin resistance. The highest resistance rates of the *S. pseudintermedius* carrying the *mec*A gene were observed for lincosamides (86.0%), cephalosporins (78.3%), and sulfonamides (76.9%), whereas resistance to amoxicillin-clavulanate was the lowest (6.3%). In addition, two isolates were resistant to all the antibiotic families evaluated. These findings underscored the increasing prevalence of MRSP and MDR *S. pseudintermedius*, highlighting the need for improved antibiotic protocols in veterinary medicine to mitigate resistance and ensure effective treatment strategies for canine pyoderma.

Keywords: Dog, Methicillin, Resistance, *Staphylococcus pseudintermedius*

INTRODUCTION

Canine pyoderma, one of the most frequently diagnosed dermatological disorders in veterinary medicine, is commonly associated with *Staphylococcus pseudintermedius* (*S. pseudintermedius*), a coagulase-positive bacterium belonging to the *S. intermedius* group (Bannoehr and Guardabassi, 2012). *Staphylococcus pseudintermedius* is an opportunistic pathogen commonly implicated in canine skin infections. Several studies have reported that *S. pseudintermedius* represents 70-90% of bacterial isolates from dogs with pyoderma and other conditions, including otitis externa, otitis media, and postoperative or wound infections (Cheung et al., 2024). Additionally, *S. pseudintermedius* can occur in dogs with genetic, dermatological**,** or immunological factors that predispose certain breeds, including the West Highland White Terrier, Shar Pei, Boxer, English and French Bulldog, Labrador Retriever, Golden Retriever, Dachshund, and German Shepherd to skin disorders, creating an environment in which *S. pseudintermedius* can overgrow (Bannoehr and Guardabassi, 2012). In recent years, concerns about multidrug-resistant (MDR) strains, especially methicillin-resistant *S. pseudintermedius* (MRSP), have grown due to their resistance to first-line antimicrobials such as macrolides, tetracyclines, aminoglycosides, and fluoroquinolones. Limited treatment options are further constrained by co-resistance in MRSP, caused by the presence of multiple mobile genetic elements, such as *SCCmec* and *Tn5405*-like structures, which carry distinct resistance genes (Nocera and De Martino, 2024). In the Netherlands, the proportion of MRSP isolates increased from 0.9% in 2004 to 7% in 2013. Meanwhile, in Tennessee, USA, out of 6453 *S. pseudintermedius* isolates, 45.5% exhibited MDR, and 30.8% were methicillin-resistant (Lord et al., 2022; Nocera and De Martino, 2024) .

The emergence of MRSP is mainly driven by carrying the *mecA* gene, which encodes an alternative penicillin-binding protein (PBP2a) characterized by a reduced affinity for β-lactam antibiotics (Murakami et al., 1991). This resistance mechanism, first identified in *Staphylococcus aureus* (*S. aureus*) by Murakami et al. (1991), has since been acquired by coagulase-positive staphylococci of animal origin, contributing to the worldwide emergence of MRSP in veterinary clinical settings (Bannoehr and Guardabassi, 2012). The *mec*A-positive strains are often associated with resistance to multiple classes of antibiotics, such as macrolides, tetracyclines, aminoglycosides, and fluoroquinolones, making their management in veterinary practice a significant challenge (Nocera and De Martino, 2024).

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In Peru, veterinary studies on MDR staphylococcal infections remain limited. Reports on MRSP prevalence in canine pyoderma are especially rare, despite the critical implications for animal welfare, such as prolonged suffering, increased treatment failure, and the potential of zoonotic transmission. The present study aimed to evaluate the prevalence of MRSP among *S. pseudintermedius* isolates from dogs with canine pyodermas in Peru and assess their resistance profiles to seven commonly used families of antibiotics. Additionally, some of these affected dogs demonstrated mixed infections, with fungal agents identified and investigated to understand potential coinfections that could complicate treatment outcomes.

MATERIALS AND METHODS

Ethical approval

No approval from research ethics committees was required for the present study, as procedures were conducted using microbial culture collections from the Bacteriology and Mycology Lab at the School of Veterinary Medicine, National University of San Marcos, Peru. No animals were involved in the present study.

Study design

The present study was conducted as an observational study between January 2016 and December 2024 at the School of Veterinary Medicine, National University of San Marcos, Lima, Peru. Samples from dogs with pyoderma were collected by veterinarians using sterile swabs, which were then placed into tubes containing Stuart transport medium (Merck Millipore, Germany). Swabs were inoculated onto trypticase soy agar (Merck Millipore, Germany) and incubated at 37°C for 24-48 hours. Subsequently, biochemical characterization, including coagulase, catalase, and carbohydrate fermentation tests, were performed. Out of 585 canine pyoderma samples, *S. pseudintermedius* was identified in 86.92% of isolates using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), following the protocol described by Alvarez et al. (2020), in which the PCR amplicons were digested with the restriction enzyme MboI (5 U). These *S. pseudintermedius* isolates were then screened for the *mecA* gene using conventional PCR with primers described by Murakami et al. (1991). The isolates were stored at -20°C in 20% (v/v) glycerol (Merck Millipore, Germany) for long-term preservation (Miyamoto-Shinohara et al., 2008) during the study period at the Bacteriology and Mycology Lab of the Faculty of Veterinary Medicine at The National University of San Marcos, Peru. The mean age of the affected dogs was 5.36 years (ranged from 0.3 to 13.5). In terms of sex distribution, 51.1% (n = 299) of the dogs were male, and 48.9% were female (n = 286).

Conventional PCR was performed based on the method used by Murakami et al. (1991), with slight modifications to detect the *mecA* gene in 585 samples of *S. pseudintermedius* isolates using the primers previously reported by Murakami et al. (1991); forward 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and reverse 5'-AGT TCT GCA GTA CCG GAT TTG C-3', at a concentration of 0.2 µM each. The PCR was carried out using the DreamTaq PCR Master Mix (Thermo Fisher Scientific, USA) in a total volume of 30 µL. The thermocycling conditions consisted of an initial denaturation at 94°C for five minutes, followed by 30 cycles of denaturation at 94°C for 60 seconds, hybridization at 50°C for 30 seconds, and elongation at 72°C for 60 seconds, with a final elongation at 72°C for five minutes. *Staphylococcus aureus* ATCC 43300 was used as a positive control, and the Negative control was *Escherichia coli* ATCC 8739. An electrophoresis gel with agarose 1.5% (Sigma-Aldrich, USA) in Tris-borate-EDTA buffer (Sigma-Aldrich, USA) was prepared to evaluate the PCR products (533 bp), which were visualized under a blue light transilluminator.

All *mecA*-positive *S. pseudintermedius* isolates, along with the MRSA control strain *S. aureus* ATCC 43300, were tested for oxacillin susceptibility using the Kirby–Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI M100, 2025). For *S. pseudintermedius*, oxacillin disk diffusion was the recommended phenotypic method for detecting methicillin resistance, as specified in CLSI guidelines. In addition, seven families of antibiotics, including tetracycline (doxycycline and oxytetracycline), sulfonamide (sulfamethoxazole), aminoglycoside (gentamycin and neomycin), second-generation cephalosporin (cephalexin), penicillins (oxacillin and acid clavulanate amoxicillin), fluoroquinolone (enrofloxacin and ciprofloxacin), and lincosamide (clindamycin and/or lincomycin), were selected to determinate their resistance and classify as MDR those that exhibit resistance to three or more families of antibiotics (Magiorakos et al., 2012).

Fungal identification as a co-infection was determined in hair and scale samples from the affected skin area. Placing the collected material directly onto a fungal culture medium in a dermatophyte test medium (Merck Millipore, Germany) and sabouraud agar (Merck Millipore, Germany) at 25°C for 5-14 days. Macroscopic analysis, including colony morphology, colony color, growth rate, pigmentation, topography, and margin, and microscopic analysis (Zeiss, Germany) at 40× magnification was conducted using the Scotch tape impression technique, followed by staining with methylene blue (Merck Millipore, Germany). Hyphae, conidiophores or sporangiophores, conidia or spores, and

specialized structures, such as phialides, vesicles, chlamydospores, ascus/asci, and basidia, were identified to determine the genus and species of the fungus (Hayden et al, 2018).

Data analysis

Descriptive statistics were applied to report the proportion of *mecA*-positive samples and the distribution of antibiotic susceptibility and resistance rates.

RESULTS

The *mecA* gene, which indicates methicillin resistance, was identified in 143 out of 585 *S. pseudintermedius* isolates examined, resulting in a prevalence rate of 24.4%. All *mecA*-positive isolates were therefore classified as MRSP (Figure 1). Among these *mec*A-positive isolates, 83% (n = 119) exhibited phenotypic resistance to oxacillin, and ~75% (n = 107) were classified as MDR. These strains presented the highest resistance to lincosamides (86%), followed by cephalosporins (78.3%), sulfonamides (76.9%), fluoroquinolones (71.3%), aminoglycosides (55.2%), and tetracyclines (37.8%). The combination of amoxicillin and clavulanate resulted in the lowest resistance (6.3%). Notably, in the present study, two isolates exhibited resistance to all seven antimicrobial families, including Pandrug-resistant (PDR) strains.

A subset of 98 dogs with *mec*A-positive isolates had clinical suspicion of external mycoses. Among 98 dogs, six were positive for *Microsporum canis* (*M. canis*), and four were positive for *Malassezia pachydermatis* (*M. pachydermatis*).

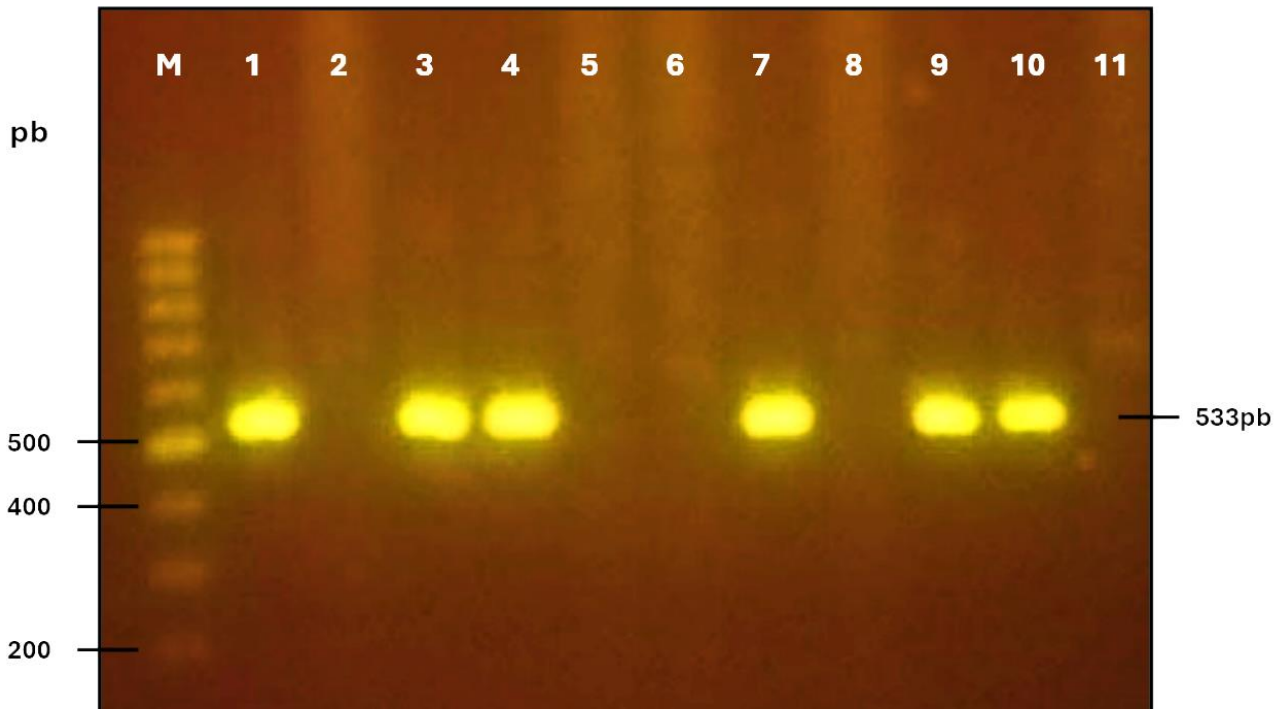

Figure 1. Agarose gel electrophoresis of the PCR products to detect the *mecA* gene in *Staphylococcus pseudintermedius* isolated from pyoderma in dogs. Lane M: Molecular weight marker 100 bp DNA ladder, Lane 1: Positive control, *Staphylococcus aureus* ATCC 43300, Lane 2: Negative control, *Escherichia coli* ATCC 8739, Lanes 3, 4, 7, 9, and 10: Positive samples at 533 bp, Lanes 5, 6, 8, and 11: Negative samples. The image was provided by the authors of the present study.

DISCUSSION

The detection of the *mec*A gene in 24.4% of isolates confirmed the presence of MRSP. This prevalence is higher than the 14.06% reported by Guimarães et al. (2023) in samples of pyoderma and otitis from dogs. Differences in sample origin may explain the lower rate observed in other studies, as *S. pseudintermedius* isolated from otitis cases often exhibited lower virulence and antimicrobial resistance profiles, with other *Staphylococcus* species, such as *S. schleiferi*, playing a more prominent role.

The current findings aligned with those of Ruzauskas et al. (2016), who reported a prevalence of 29%, and Lord et al. (2022), with a prevalence of 30.8% in dog samples. However, the prevalence of MRSP remains notably lower than

the 59% reported by Lynch and Helbig (2021), who recovered the isolates from dogs with chronic infections that had been previously diagnosed as pyoderma, having had major exposure to antimicrobials, which could explain the higher rate of resistance compared to the current study. Although humans are not the natural host of *S. pseudintermedius*, Guimarães et al. (2023) demonstrated that among nine MRSP strains isolated from canine pyoderma, two were closely related to strains found in their owners, highlighting potential zoonotic concerns.

The present findings emphasized the increasing threat posed by MRSP in veterinary medicine. Notably, ~75% of the *mecA*-positive isolates were MDR, exhibiting resistance to broad-spectrum antimicrobials such as macrolides, aminoglycosides, cephalosporins, fluoroquinolones, and lincosamides, medicines commonly used to treat severe or refractory infections (Nocera and De Martino, 2024). The present findings highlighted a crucial public health concern regarding the overuse of antibiotics in veterinary practice. This pattern aligns with the findings of Palomino-Farfán et al. (2021), who reported comparably higher rates of *mec*A-positive strains in South American canine populations. The role of MDR staphylococci in complicating routine veterinary treatments, as noted by Lynch and Helbig (2021), remains a considerable issue. Resistance to oxacillin was observed in 83.2% of the *mec*A-positive isolates, supporting its reliability as a phenotypic marker for MRSP detection, as outlined in the CLSI M100 guidelines (CLSI, 2025). In particular, two isolates demonstrated resistance to all seven antibiotic families tested, raising concerns about the limited treatment options available. The observed resistance patterns suggested potential clonal dissemination of highly resistant strains within veterinary hospital settings, a phenomenon previously reported in hospital-associated MRSP outbreaks (Lord et al., 2022).

Furthermore, concurrent mycoses were identified in ten dogs with *mec*A-positive isolates, with *M. canis* and *M. pachydermatis*. Mixed infections can amplify inflammatory responses, prolong wound healing, and increase the risk of developing chronic or recurrent dermatologic conditions. Dinkova and Rusenova (2025) highlighted the clinical relevance of co-infections involving *M. pachydermatis* and bacteria, noting that staphylococci are the bacterial pathogens most frequently associated with these skin disorders. Moreover, the administration of antibiotics without concurrent antifungal therapy in instances of mixed infection presents a risk of promoting microbial imbalance, which may facilitate fungal overgrowth and the development of multidrug-resistant bacteria such as MRSP. This finding highlighted the clinical necessity for effective topical agents with dual activity. Supporting these findings, Oliveira et al. (2018) demonstrated that a honey-based gel exhibited *in vitro* efficacy against primary canine pathogens, including *S. pseudintermedius* and *M. pachydermatis*, thereby indicating its potential as a single-agent therapeutic option for such cases.

The high MDR rate and the presence of PDR isolates in the present study highlighted the urgent need for enhanced responsible antibiotic use in veterinary practice in Peru. Empirical therapy for MRSP canine pyoderma should be guided by susceptibility testing, as demonstrated in the present study, to reduce the risk of promoting further antimicrobial resistance (Nocera and De Martino, 2024). Additionally, the detection of concurrent fungal infections necessitates a multidisciplinary approach to managing canine pyoderma, including the use of antifungal therapy when indicated.

CONCLUSION

The present study highlighted the significant presence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in canine isolates, with 24.4% of the canine isolates testing positive for the *mec*A gene and 75% of the canine isolates classified as multidrug resistant (MDR). The high MDR rate highlighted the growing concern regarding antibiotic overuse in veterinary medicine, particularly as two isolates of the 143 MRSP exhibited resistance to all seven antimicrobial families tested. The present findings underscore the urgent need for enhanced antibiotic protocols to prevent the spread of MRSP and MDR strains in Lima, Peru. Further studies are needed to investigate the molecular epidemiology of MRSP in Peru, including potential zoonotic transmission routes, given the close contact between dogs and humans in many households. In addition, surveillance programs aimed at monitoring antibiotic resistance trends in veterinary pathogens are essential for developing evidence-based treatment guidelines.

DECLARATIONS

Authors' contributions

All authors contributed to the conception, methodology, and design of the study. Material preparation and the original draft were carried out by Juan Tomás and Néstor Rivas. Writing, review, and editing were performed by Joel Palomino, Juan Siuce, and Luis Alvarez. Joel Palomino and Juan Siuce served as supervisors for the present study. All authors reviewed and approved the final edition of the manuscript.

Availability of data and materials

The data supporting the present findings are available upon reasonable request to the corresponding author.

Competing interests

The authors declared that there are no conflicts of interest.

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Ethical considerations

The present study was originally written by the authors and has not been published elsewhere. The authors checked the text of the article for plagiarism index and confirmed that the text of the article is written based on their original scientific results. The authors have not used AI in generating data and conducting the present study.

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