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Study on biofilm characterization and its association with antibacterial resistance in *Staphylococcus pseudintermedius* isolated from skin infections of dogs

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Abstract

Dermatological ailments are a major concern in the pet population, especially canines and *S. pseudintermedius* is a major bacterial pathogen involved. The ability to form biofilms, together with increasing AMR pose major hindrances to the successful treatment of infections associated with *S. pseudintermedius*. In this study, we evaluated the AMR profiles and the potential to produce biofilms in 50 *S. pseudintermedius* isolates of canine origin from India. All the isolates were susceptible to amikacin. However, high percentages of resistance were observed to penicillin (78%) and ciprofloxacin (86%). Furthermore, 74% of the isolates showed MDR pattern. 80% of the isolates were weak or moderate biofilm producers. No significant association was observed in *S. pseudintermedius* AMR pattern and biofilm.

Keywords: *Staphylococcus pseudintermedius*, biofilm, AMR, canine

Introduction

Canine skin, due to exposure to allergens, parasites, microbes, irritants, etc. is highly susceptible to dermatological disorders. Further, other factors such as breed predisposition, immune status, and underlying ailment also contribute to many skin diseases, including pyoderma and atopic dermatitis (Zahri *et al.*, 2024) [14]. Thus, skin diseases constitute a major portion of the health problems encountered in companion animals. In the infectious category, bacterial pathogens contribute significantly to canine skin diseases.

In addition to obligate pathogens, resident bacteria of the normal canine skin flora can also act as opportunistic pathogens, especially when dogs' immune status is compromised. Among these, *S. pseudintermedius* is a major commensal inhabitant of canines that causes a wide spectrum of clinical diseases in dogs. Although it is a commensal organism, it has a repertoire of resistance genes and frequently exhibits MDR characteristics, thereby prolonging treatment regimens. Further, rising pet ownership and close contact with dogs pose a significant risk of zoonotic transmission of MDR *S. pseudintermedius* to humans (Guimaraes *et al.*, 2023; Somayaji *et al.*, 2016; Moses *et al.*, 2023) [4, 15, 16].

In addition to antimicrobial resistance phenotype, *S. pseudintermedius* isolates also exhibit biofilm formation, which hampers successful treatment. Biofilms are aggregates of microbial communities protected by an outer polymeric layer that is largely impermeable to antibiotics. Within the biofilm, bacterial communities communicate through quorum sensing and genetic exchange, disseminating resistance genes. The biofilms have the potential to enhance the tolerance of bacterial pathogens to antimicrobial substances up to 1,000-fold, thereby facilitating the selection and persistence of MDR bacteria (Nahum *et al.*, 2025) [10].

With this background, the current study was conducted to understand the AMR pattern, biofilm-forming potential, and association between these two phenotypes in *S. pseudintermedius* isolated from dogs.

Methods

S. pseudintermedius isolates

Fifty isolates recovered from dogs with clinical dermatological conditions from Andhra Pradesh, India, were used in the study. The isolates were previously characterized using conventional laboratory and molecular techniques.

Biofilm characterization

The isolates were tested for their biofilm using the microtiter plate method (Stempelova *et al.*, 2022) [12]. The optical density (OD) at $\lambda = 570$ nm was measured using a microplate reader. Biofilm was measured using the OD and the cut-off value (OD_c). OD_c is 3 SD above the mean optical density value of the negative control. The OD value for all isolates was calculated as the average OD minus the OD_c value. The isolates were then categorized as weak (OD_c < OD $\leq$ 2×OD_c), moderate (2×OD_c < OD $\leq$ 4×OD_c), and strong (OD > 4×OD_c) biofilm producers.

Antimicrobial Susceptibility Testing (AST)

The AST profiles of the *S. pseudintermedius* isolates were assessed using the disc diffusion assay (Kirby-Bauer method) in accordance with CLSI guidelines (CLSI, 2023) [3]. Briefly, single colonies of the *S. pseudintermedius* isolates were inoculated into BHI broth and incubated (for 24 hours at 37 °C). Further, inoculum was prepared by adjusting the culture turbidity to 0.5 McFarland standards. Using a sterile cotton-tipped applicator, the inoculum was plated onto Mueller-Hinton agar plates to form bacterial lawns. Once the plates were dry, Penicillin (P), Ampicillin (AMP), Amoxicillin-clavulanic acid (AMC), Ceftriaxone (CTR), Gentamicin (GEN), Amikacin (AK), Ciprofloxacin (CIP), Enrofloxacin (EX), and Tetracycline (TE) discs were placed on the bacterial lawns using sterile forceps, followed by incubation for 24 h at 37 °C. The Zone of Inhibition (ZOI) was measured and recorded in millimeters. Afterwards, isolates were categorized as resistant or susceptible as per the CLSI guidelines.

Statistical Analysis

The association between the AMR profile and biofilm production potential was assessed by performing Fisher's Exact Test. The strength of the association, where applicable, was estimated using the odds ratio with 95% confidence intervals. A *p*-value of < 0.05 was considered statistically significant. The analysis was conducted using IBM SPSS v.30.

Results

Biofilm Production Assay

Forty isolates produced biofilm, with 11 being moderate and 29 weak biofilm producers (Table 1; Figure 1). No isolate showed the strong biofilm-forming phenotype.

Antimicrobial Susceptibility Testing

Based on ZOI values obtained in the disc diffusion assay, the susceptibility of the *S. pseudintermedius* isolates was determined (Table 1&2; Figure 2). Amikacin was showed to be effective *in vitro*, as 100% susceptibility was observed. High levels of resistance were detected to ciprofloxacin (86%) and penicillins (78%). MDR was observed in 74% of the isolates.

Association between biofilm and AMR

Among the 50 isolates studied, 37 (74.0%) were multidrug-resistant (MDR) and 13 (26.0%) were non-MDR. Based on biofilm production strength, the MDR group comprised 22 (59.5%) weak biofilm producers, 8 (21.6%) moderate biofilm producers, 7 (18.9%) non-biofilm producers, and no strong biofilm producers. Similarly, among the non-MDR isolates, 7 (53.8%) were weak producers, 3 (23.1%) were moderate producers, and 3 (23.1%) were non producers, while none showed strong biofilm formation. Weak biofilm production was the predominant phenotype in both MDR and non-MDR isolates. The association between biofilm and AMR was investigated using Fisher's Exact Test and odds ratio analysis. Statistical analysis revealed no significant relationship between biofilm and resistance to the evaluated antimicrobial agents, namely penicillin, ampicillin, amoxicillin-clavulanic acid, ceftriaxone, enrofloxacin, ciprofloxacin, gentamicin, amikacin, and tetracycline (*p* > 0.05). Moreover, the 95% CI for all odds ratios included one, indicating no significant difference in AMR profiles of biofilm-producing and non-biofilm-producing isolates (Table 3).

Discussion

Staphylococcus pseudintermedius is a commensal bacterium in canine skin microbiota, yet it is a frequent opportunistic pathogen in skin infections. Antimicrobial resistance, frequently MDR patterns, and biofilm-forming capability are significant challenges in treating *S. pseudintermedius* associated dermatological conditions in dogs. Biofilm formation will increase the tolerance of the isolates to various commercially available antibiotics and the selection pressure on resistant isolates. In present study, evaluation of association between biofilm and AMR patterns among 50 *S. pseudintermedius* isolates were done. The isolates examined demonstrated AST patterns, with many showing resistance to commonly available drugs used to treat infections. In agreement with this, Kim *et al.* (2025) [6] reported 78.5% resistance to penicillin in the *S. pseudintermedius* population recovered from canines. In addition, Teixeira *et al.*, 2023 [13] reported a 76% rate of Penicillin resistance in *S. pseudintermedius* isolates, further confirming our findings. Apart from this, the AST pattern observed for other antibiotics was also in agreement with previous studies. Arefin *et al.*, 2025 [1] reported that 60% of isolates were resistant to erythromycin, ampicillin, and ciprofloxacin. Lord *et al.* (2022) [7] reported that the majority of isolates were ampicillin resistant (84.3%), while substantial proportions were gentamicin (27.0%) and tetracycline (47.9%) resistant. Similar to these findings, Morais *et al.* (2023) detected high resistance rates to penicillin (85.2%), tetracycline (55.5%), erythromycin (36.8%), clindamycin (36.8%), trimethoprim-sulfamethoxazole (30.3%), gentamicin (25.2%), and fluoroquinolones (25.2%). However, among all the drugs tested, amikacin was consistent with high effectivity *in vitro*, with 100% susceptibility (Calabro *et al.*, 2024) [2].

The biofilm production potential of *S. pseudintermedius* was evaluated using a microtitre plate method, and 80% of the isolates were weak or moderate producers of biofilm. Although no strong producers of biofilm were detected, the high potential for biofilm formation among *S. pseudintermedius* isolates poses a significant challenge to the management of canine skin ailments. However, the association between AMR and biofilm formation is yet to be fully elucidated. In this study, biofilm production was

observed in 30 MDR and 10 non-MDR isolates. However, no significant association between biofilm production and AMR could be detected. In conjunction with this finding, Stefanetti *et al.* (2017) ^[11] also reported no significant correlation between AMR and biofilm production in *S. pseudintermedius* isolates. However, in contrast, certain studies have reported a positive relation between these two phenotypes. Meroni *et al.*, 2019 ^[8] reported that MDR isolates of *S. pseudintermedius* exhibits greater biofilm production potential on comparison to the susceptible isolates. Similarly, Jantorn *et al.*, 2021 ^[5] observed that strong biofilm production is frequent in MDR isolates, whereas moderate biofilm production is common in susceptible isolates. The contrasting significance of the associations reported in these studies shows that biofilm production in bacteria is regulated by many factors,

including the genetic repertoire and prevailing environmental conditions. There is a limited possibility of categorizing an isolate as a weak-, moderate-, and strong biofilm producer based on its AMR profile, though further studies are warranted. Further, the high frequency of weak biofilm producers and low number of non-biofilm producers may have limited the subtle differences detection and correlation between AMR and biofilm.

To conclude, the study detected a high level of MDR among the *S. pseudintermedius* isolates of canine origin. Most of the isolates were biofilm producers, though no strong biofilm producers were detected. No significant association could be detected between AMR and biofilm production in *S. pseudintermedius*. Further studies involving a larger number of isolates are warranted to unravel the correlation between AMR and biofilm in *S. pseudintermedius*.

Table 1: AMR and Biofilm profiles of *S. pseudintermedius* isolates used in this study

Isolate ID	P	AMP	AMC	CTR	EX	CIP	GEN	AK	TE	BIOFILM
V1	R	R	S	S	R	R	R	R	R	Producer
V2	S	S	S	S	S	S	R	R	R	Producer
V3	S	S	S	S	S	S	S	R	S	Producer
V4	R	S	S	S	R	R	S	S	S	Producer
V5	R	R	R	R	R	R	S	S	R	Producer
V6	R	R	R	R	S	S	S	S	S	Producer
V8	S	S	S	S	S	R	R	R	R	Producer
V11	R	R	R	R	R	R	R	R	R	Producer
V12	R	R	R	R	R	R	S	S	R	Producer
V13	S	S	R	S	S	S	S	S	S	Non-Producer
V14	S	R	S	S	R	R	R	R	R	Producer
V15	R	R	R	R	R	R	R	S	R	Producer
V19	R	R	S	S	S	R	S	S	R	Producer
V20	R	R	S	S	S	R	S	S	S	Non-Producer
V22	R	R	R	S	R	R	R	S	R	Producer
V23	S	R	R	S	R	R	S	S	R	Producer
V24	R	R	R	S	R	R	R	R	R	Producer
V26	R	R	S	S	R	R	R	R	R	Producer
V27	R	R	S	S	R	R	S	R	R	Producer
V28	R	R	R	S	R	R	S	S	R	Producer
V29	R	R	R	R	R	R	R	R	R	Producer
V30	R	R	R	S	S	R	S	S	S	Producer
V31	R	S	S	S	R	R	S	S	R	Producer
V32	R	R	S	R	S	R	R	S	R	Producer
V33	R	R	R	S	S	R	R	S	R	Producer
V34	S	S	S	S	S	R	S	R	S	Producer
V35	R	R	R	R	R	R	R	R	R	Non-Producer
V36	S	S	S	S	S	S	S	S	S	Producer
V37	R	S	R	R	R	R	R	R	R	Producer
V38	R	R	S	R	R	R	R	R	R	Non-Producer
V39	R	R	R	S	R	R	S	R	S	Producer
V40	R	R	R	S	S	S	S	S	S	Producer
V42	R	R	R	R	R	R	R	R	R	Non-Producer
V43	R	R	R	S	R	R	S	S	R	Producer
V44	R	R	R	R	R	R	R	R	R	Producer
V45	R	R	R	R	R	R	S	S	R	Non-Producer
V46	R	R	R	R	R	R	R	R	R	Producer
V47	R	R	R	S	S	R	R	S	S	Producer
V48	R	R	R	S	R	R	S	S	R	Producer
V49	R	R	S	S	R	R	S	S	R	Producer
V51	S	S	S	S	S	S	S	R	S	Producer
V52	R	R	R	R	R	R	R	R	R	Producer
V53	R	R	R	S	R	R	R	R	R	Non-Producer
V54	R	R	S	S	R	R	S	S	R	Non-Producer
V55	R	R	R	R	S	R	R	R	S	Producer
V57	R	R	R	R	R	R	S	R	R	Non-Producer
V58	S	S	S	S	R	R	S	S	S	Non-Producer
V59	R	R	S	S	R	R	S	S	S	Producer
V60	S	S	S	S	S	R	S	S	S	Producer
V61	R	R	R	S	R	R	S	S	R	Producer

R: Resistant; S: Sensitive

Table 2: Antimicrobial resistance profile of *S. pseudintermedius* isolates

Antimicrobial agent	Resistant, n (%)	Susceptible, n (%)
Penicillin	39 (78%)	11 (22%)
Ampicillin	38 (76%)	12 (24%)
Amoxicillin-clavulanic acid	29 (58%)	21 (42%)
Ceftriaxone	17 (34%)	33 (66%)
Enrofloxacin	33 (66%)	17 (34%)
Ciprofloxacin	43 (86%)	7 (14%)
Gentamicin	22 (44%)	28 (56%)
Amikacin	23 (46%)	27 (54%)
Tetracycline	34 (68%)	16 (32%)

Table 3: Association between biofilm production and antimicrobial resistance

Antibiotic	OR	95% CI	Fisher's Exact p-value
Penicillin	0.861	0.154-4.799	1.000
Ampicillin	0.750	0.136-4.133	1.000
Amoxicillin-clavulanic acid	0.902	0.220-3.702	1.000
Ceftriaxone	0.429	0.104-1.759	0.277
Enrofloxacin	0.417	0.078-2.228	0.461
Ciprofloxacin	0.630	0.067-5.919	1.000
Gentamicin	1.227	0.300-5.028	1.000
Amikacin	0.818	0.204-3.276	1.000
Tetracycline	0.890	0.197-4.012	1.000

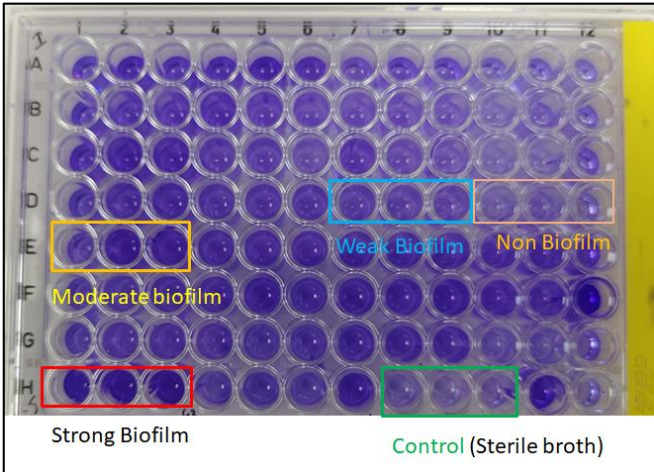


Fig 1: Microtitre plate method showing intensity of dye for biofilm formation ability of *S. pseudintermedius* isolates

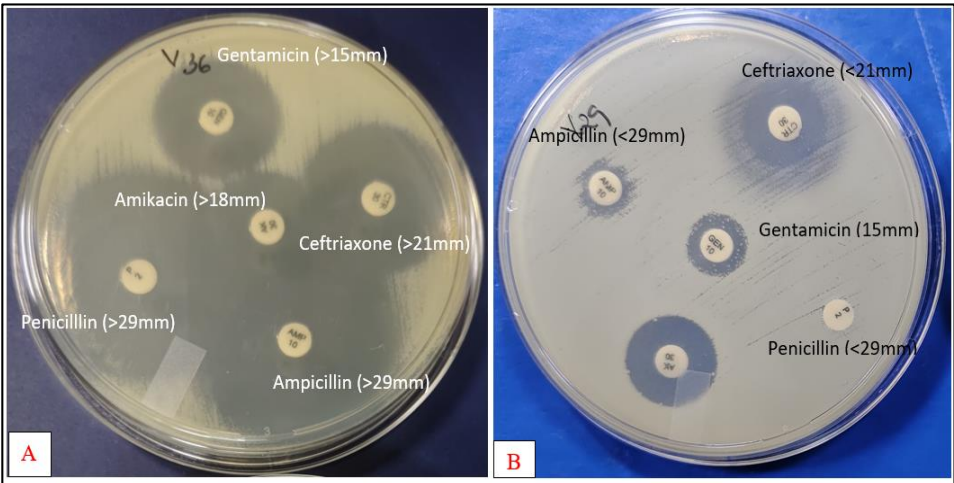


Fig 2: (A) Isolate showing sensitive pattern (B) Isolate showing resistant pattern

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