RESEARCH ARTICLE

Molecular Detection and Antibiogram of Bacterial Isolates from Canine Pyoderma Cases

Chaithanya Milandahalli[1], Ramesh Poojary Thimmaiah[2], Chandrashekar Kodavenakapalli Munishamy[3]*, Lathamani Venkatapura Shekharaiah[2], Anjan Kumar Keeramande Ramakrishna[4], Renukaradhya Gudepalya Jayanna[5]

Abstract

Canine pyoderma is one of the most common causes of dermatitis with worldwide occurrence in small animal practice. This study was carried out to identify bacterial isolates by simplex, multiplex PCR and to know its antibiotic susceptibility profile from canine pyoderma cases. *Staphylococcus intermedius* (n=17, 53%) was the major species identified followed by *Staphylococcus schleiferi* (n=10, 32%), *Staphylococcus aureus* (n=6, 15%), *Pseudomonas aeruginosa* (n=5, 12.5%) and *Escherichia coli* (n=3, 7.5%) by PCR. Antibiogram profile of *Staphylococcus* isolates showed sensitive to enrofloxacin (84.37%), ceftriaxone (81.25%), ceftriaxone/tazobactam (62.5%), whereas *P. aueroginosa* was sensitive to meropenem (100%), ciprofloxacin (100%), ceftriaxone/tazobactam (80%) and *E. coli* was sensitive to meropenem (100%), ceftriaxone/tazobactam (100%), ampicillin/sulbactam (100%). All isolates of this study were resistant to pencillin G, clindamycin (*P. aueroginosa and E. coli*), co-trimoxazole (*Staphyloccous* spp. & *P. aueroginosa*) and streptomycin (*E. coli*) indicating frequent and indiscriminate use of these antimicrobials in pyoderma treatment.

Key Words: Antibiotic susceptibility, Canine pyoderma, Meropenem, PCR, Penicillin G, *Staphylococcus.*

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.4.10

Introduction

Pyoderma is considered as one of the most frequently encountered dermatological disorders in dogs due to certain characteristics of dog's skin like thin stratum corneum with less lipid material and unprotected hair follicles that are at increased risk for bacterial invasion and subsequent colonization and overgrowth. The main clinical signs include circular alopecia, erythema, pustules, epidermal collarettes, crusts, desquamation and pruritus (Botoni *et al*., 2016). Common bacterial species associated with canine pyoderma are *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Corynebacterium* spp., and others. Among them majority of pyoderma infections are caused by *Staphylococcus* spp. especially *Staphylococcus pseudintermedius* followed by *Staphylococcus aureus* and *Staphylococcus coagulans* (Andrade *et al*., 2022). The diagnosis is mainly based on history, clinical examination and other occasionally used confirmative assays like cultural isolation and molecular identification (Hariharan *et al*., 2014). The indiscriminate and frequent use of common antibiotics to treat pyoderma leads to multi-drug resistance in dogs (Meroni *et al*., 2019), which can be avoided by performing antibiotic sensitivity test to choose an appropriate antibiotic for treatment in non-responsive pyoderma cases. In view of the above, the facts of canine skin, causative agents and bacterial resistance towards antibiotics, this study was conducted to identify the bacteria by polymerase chain reaction and to know its antibiogram profile from canine pyoderma cases.

[1]Department of Animal Husbandry and Veterinary Services, Government of Karnataka, India

[2]Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru-560024, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

[3]Department of Veterinary Microbiology, Veterinary College, Gadag-582101, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

[4]Department of Veterinary Pathology, Veterinary College, Hassan-573202 Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

[5]Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Gadag-582101, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

Corresponding Author: Dr. K.M. Chandrashekar, Department of Veterinary Microbiology, Veterinary College, Gadag-582101, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India. e-mail: dr.chandruvk112@gmail.com

How to cite this article: Chaithanya M., Ramesh P.T., Chandrashekar K. M., Lathamani V., Anjan kumar K. R., & Renukaradhya G. J. (2024). Molecular Detection and Antibiogram of Bacterial Isolates from Canine Pyoderma Cases. Ind J Vet Sci and Biotech. 20(3), 45-49.

Source of support: Nil

Conflict of interest: The authors declare that there is no conflict of interest.

Submitted 01/04/2024 **Accepted** 30/04/2024 **Published** 10/07/2024

Materials and Methods

Source and Selection of Animals

Dogs presented to small animal outpatient section of Veterinary College Hospital, Hebbal, Bangalore, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), and different Veterinary Hospitals of Bangalore city, with various dermatological disorders especially those suggestive of pyoderma were considered for the study. Forty dogs were selected for the study after a detailed history of recurrent pyoderma and thorough physical examination for clinical signs. A total of ten apparently healthy dogs which were presented for general check up and vaccination were randomly selected and considered as control group.

Sample Collection and Processing

Skin swab samples were collected aseptically from all the selected dogs by using sterile swabs (M/S HiMedia Pvt Ltd, Mumbai) and inoculated into brain heart infusion broth (BHIB) and incubated overnight at 37°C. After incubation, a loopful of culture from BHIB was streaked onto brain heart infusion agar (BHIA) and incubated overnight at 37°C to isolate organisms. Different bacterial species were initially identified based on Gram's staining technique and colony morphology.

Molecular Detection by Polymerase Chain Reaction

The isolated bacterial colonies suggestive of *Staphylococcus* spp., *Pseudomonas* spp. and *E. coli* based on staining and colony morphology were subjected to PCR for confirmation of organisms. Genomic DNA was extracted from the isolates by using DNA extraction kit (DNeasy Blood and Tissue Kit Qiagen, USA). Genomic DNA of *Staphylococcus* spp. was subjected for multiple PCR to identify *Staphylococcus aureus* (*S. aureus*), *Staphyloccous intermedius* (*S. intermedius*), *Staphylococcus schleiferi* (*S. schleiferi*) and *S. pseudintermedius* (*S. pseudintermedius*) as described by Sasaki *et al*. (2010). Primer details and cycling conditions used in multiplex PCR were as mentioned in the Table 1.

The genomic DNA of *Pseudomonas* spp. and *E. coli* were subjected for species specific simplex PCR as described by Degi *et al*. (2021) and Bej *et al.* (1991) for identification of *Pseudomonas aueroginosa* and *E. coli* with primers and cycling conditions as mentioned in the Table 2. After PCR, the products were subjected to electrophoresis and examined under the gel documentation system.

Antibiogram

Antimicrobial sensitivity test was performed by Kirby Bauer disc diffusion method (Bauer *et al*., 1966) on Muller Hinton agar (Himedia, India) based on standards of the Clinical and Laboratory Standards Institute (CLSI, 2020). The different isolates were tested for their susceptibility to different antibiotics, *viz*., amoxicillin-clavulanic acid (AMC 30 µg), ampicillin (AMP 25 µg), ampicillin/sulbactam (A/S 10/10 µg), amikacin (AK 30 µg), azithromycin (AZM 30 µg), ceftriaxone (CTR 30/10 µg), ceftriaxone/tazobactam (CIT 30/10 µg), cefixime (CFM µg), Cefpodoxime (CEP µg), cephalexin (CN 30 µg), chloramphenical (C 30 µg), ciprofloxacin (CIP 5 µg), co-trimoxazole (COT 25 µg), clindamycin (CD 10 µg), doxycycline (DO 10 µg), enrofloxacin (EN 10 µg), gentamicin (GEN 10 µg), meropenem (MRP 10 µg), streptomycin (S 10 µg) and pencillin G (P 10 units) (Himedia, India). *Escherichia coli* ATCC 25922 was used as a quality control strain. The zone of inhibition was interpreted based on the CLSI guidelines by using antibiotic zone measuring scale in mm and inferred as sensitive & resistant.

Table 1: Primers for identification of *Staphylococcus* spp. by multiplex PCR

Species	Primer	Nucleotide Sequence (5'-3')	Amplicon (bp)	Cycling conditions
S. aureus	au-F3 au-nucR	TCGCTTGCTATGATTGTGG GCCAATGTTCTACCATAGC	359	One cycle at 95° C for 10 min; 30 cycles at 95° C for 30 sec, 56° C for 1 min, 72° C for 1 min; One cycle at 72°C for 10 min
S. intermedius	in-F in-R3	CATGTCATATTATTGCGAATGA AGGACCATCACCATTGACATATTGAAACC	430	
S. schleiferi	sch-F sch-R	AATGGCTACAATGATAATCACTAA CATATCTGTCTTTCGGCGCG	526	
S. pseudo-intermedius	pse-F2 pse-R5	TRGGCAGTAGGATTCGTTAA CTTTTGTGCTYCMTTTTGG	926	

Table 2: Primers for identification of *P. aueroginosa* and *E. coli* by simplex PCR

Species	Primer	Sequence(5'-3')	Amplicon (bp)	Cycling conditions
P. aeruginosa	PA-SS-F PA-SS-R	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	1124	95° C for 5 min; 32 cycles of 95° C for 1 min, 55° C for 1 min, 72° C for 1 min; 72°C for 10 min
E. coli	*UidA F* *UidA R*	AAAACGGCAAGAAAAAGCAG ACGCGTGGTTAACAGTCTTGCG	147	94° C for 5 min; 35 cycles of 95° C for 30 sec, 57° C for 30 sec, 72° C for 30 sec; 72°C for 5 min

Results and Discussion

All forty samples showed positive results for growth of microorganisms in BHIB and BHIA. Thirty two bacterial colonies showed grayish white to golden yellow colour, revealed Gram positive cocci in cluster in Gram's staining suggestive of *Staphyloccous* spp (80 %). Five bacterial colonies which were mucoid on BHIA showed bluish green pigmentation and appeared as Gram negative rods was assumed as *Pseudomonas aeruginosa* (12.5%). Three bacterial colonies which were Gram negative coccobacilli in Gram's staining and after subculturing on eosin methylene blue agar showed metallic sheen growth was suggestive of *Echerichia coli* (7.5%).

The products of multiplex PCR for identification of *Staphylococcus* spp. revealed the amplicon size of 430 bp, 526 bp and 359 bp, which were specific for *Staphylococcus intermedius* (n=17, 53%), *Staphylococcus schleiferi* (n=10, 32%) and *Staphylococcus aureus* (n= 6, 15%)(Fig. 1). Makwana *et al.* (2023) also identified 32 *Staphylococcus* spp. by PCR, out of which six were *S. schleiferi* and five were *S. aureus,* which was in accordance with findings of this study.

The predominance of Gram positive *Staphylococcus* spp. (32/40, 80%) in this study was in accordance with the findings of Naveena *et al.* (2023), Khinchi *et al.* (2022) and Marco-Fuertes *et al.* (2024). Even though the main causative agent of canine pyodemra is known to be *Staphylococcus pseudintermedius* (*S. pesudintermedius*), but in this study none of the sample revealed the presence of *S. pesudintermedius*. However, *S. intermedius* was isolated as a major pathogen of pyoderma which was in line with the reports of Reddy *et al.* (2014), Ankita and Gandge (2018), and Khinchi *et al.* (2022). The simplex PCR yielded amplicon of 1124 bp and 147 bp which was confirmative for *P. aeruginosa* (n=5, 12.5%) *and E. coli* (n=3, 7.5%)(Fig. 2, 3). Presence of Gram negative bacteria was considered as transient organisms due to secondary invasion.

The isolates of *Staphylococcus* spp. were sensitive to enrofloxacin (84.37%) followed by ceftriaxone (81.25%), ceftriaxone-tazobactam (62.5%), amoxicillin-clavulanic acid (59.37%), cefixime (50%), cephalexin (50%), ampicillin (40.62%), clindamycin (40.62%), cefpodoxime (40.62%), ciprofloxacin (34.37%), azithromycin (21.87%), gentamycin (21.87%), doxycycline (9.37%), co-trimoxazole (3.12%), and pencillin G (3.12%). Similar to this study, the resistance of *Staphylococcus* isolates against clindamycin (59.38%) and cefpodoxime (59.83%) was showed by Makwana *et al.* (2023). On the contrary Ravens *et al.* (2014), Rafatpanah *et al.* (2020) and Makwana *et al.* (2023) showed highest susceptibility to cephalexin and amoxicillin-clavulanate.

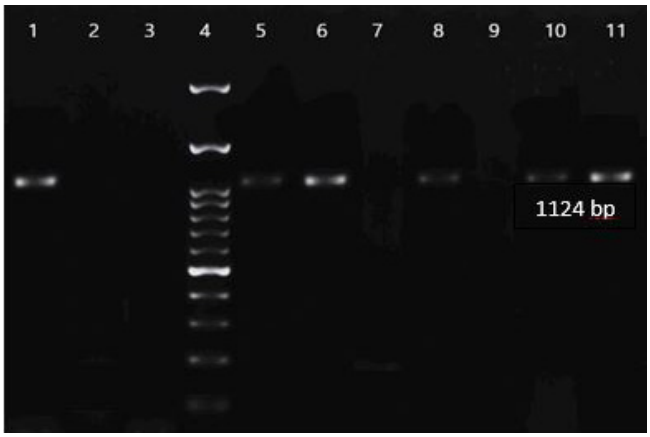

Fig. 2: Simplex PCR for identification of *Pseudomonas aeruginosa* (Lane 1: Positive control, Lane 2: Negative control, Lane 3: Non template control, Lane 4: 100bp DNA ladder, Lane 5, 6, 8, 10, 11: *P. aeruginosa* which corresponded to 1124 bp amplicon)

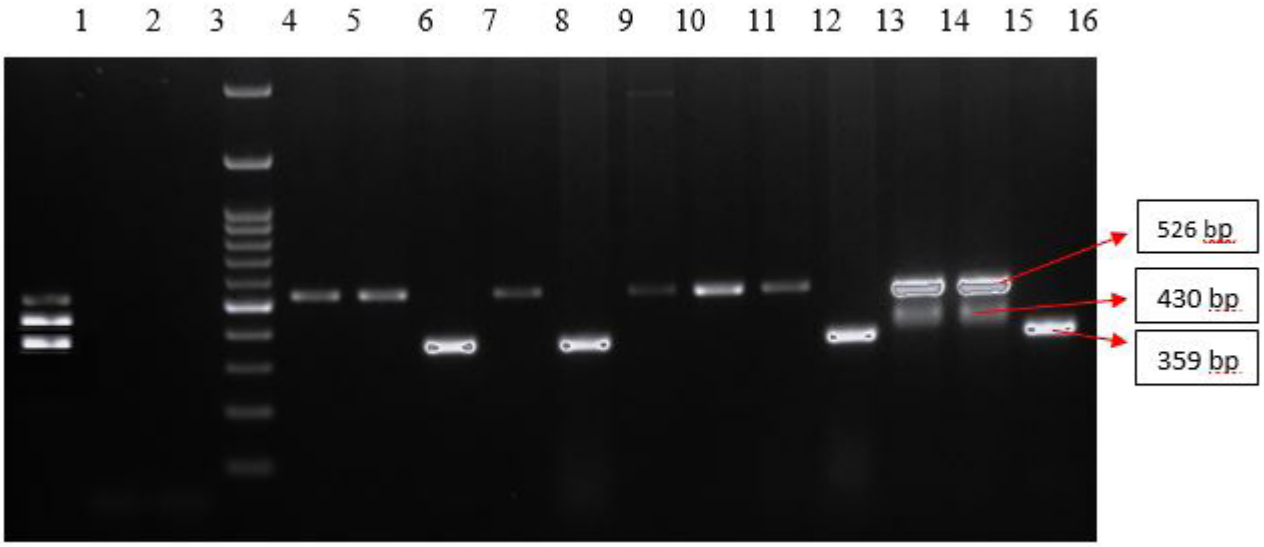

Fig. 1: Multiplex PCR for identification of *Staphylococcus* spp. (Lane 1: Positive control, Lane 2: Negative control, Lane 3: NTC (Non template control), Lane 4: 100bp DNA ladder, Lane 5, 6, 8, 10, 11, 12, 14, 15: S. schleiferi which corresponded to 526 bp amplicon, Lane 7, 9, 13, 16: S. aureus which corresponded to 359 bp amplicon, Lane 14, 15: S. intermedius which corresponded to 430bp amplicon)

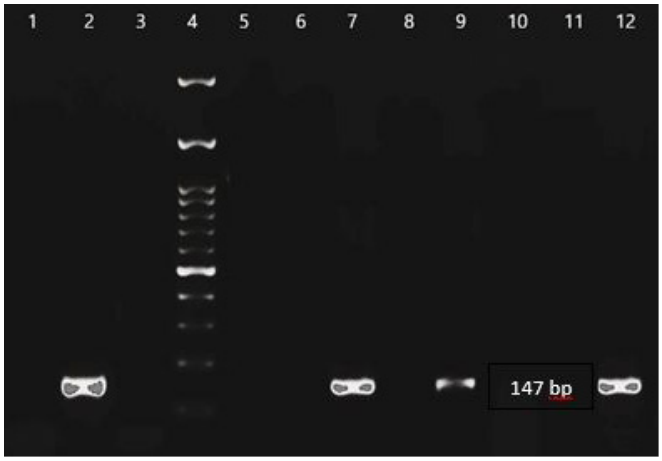

Fig. 3: Simplex PCR for identification of *E. coli*
(Lane 1: NTC - Non template control, Lane 2: Positive control, Lane 3: Negative control, Lane 4: 100bp DNA ladder, Lane 7, 9, 12: *E. coli* which corresponded to 147 bp amplicon)

The isolates of *S. intermedius* were sensitive to cefpodoxime (88.23%) followed by enrofloxacin (82.35%), amoxicillin-clavulanic acid (58.82%), cephadroxil (52.94%), cephalexin (52.94%), cefixime (41.17%), ciprofloxacin (41.17%), amoxicillin-sulbactum (35.29%), amikacin (23.52%), chloramphenicol (17.64%) and resistant to azithromycin, gentamicin and pencillin G. The susceptibility results of *S. intermedius* were in contrary with the reports of Ankita and Gandge (2018); Chaudhary *et al.* (2019) and Khinchi *et al.* (2022).

The isolates of *Staphylococcus schleiferi* showed sensitive to ciprofloxacin (80%), enrofloxacin (70%), cephalexin (70%), ceftriaxone-tazobactam (60%), cefpodoxime (60%), cephadroxil (50%), amoxicillin-sulbactum (40%), amoxicillin-clavulanic acid (40%) and resistant to chloramphenicol, doxycycline, pencillin and amikacin.

The isolates of *Staphylococcus aureus* were sensitive to enrofloxacin (85%), ceftriaxone (80%), amoxicillin-clavulanic acid (60%), cefixime (60%), cefalexin (60%), clindamycin (40%), cefpodoxime (40%), ampicillin (40%), ciprofloxacin (20%), gentamycin (20%), azithromycin (20%) and resistant to co-trimoxazole, doxycycline and pencillin G. The findings of Ankita and Gandge (2018) and Khinchi *et al.* (2022) were in contrast to the above results.

The isolates of *P. aueroginosa* were sensitive to ciprofloxacin (100%), meropenem (100%), ceftriaxone-tazobactam (80%), chloramphenicol (60%), enrofloxacin (60%), amoxicillin-clavulanic acid (40%), azithromycin (20%) and resistant to clindamycin (100%), co-trimoxazole (100%) and pencillin G (100%). These findings were not in agreement with Khinchi *et al.* (2022). In contrast to our findings, Degi *et al.* (2021) showed higher sensitivity to azithromycin (41.37%) and lower sensitivity to meropenem (25.86%), ciprofloxacin (17.24%), which may be attributed to the high sample size.

The isolates of *E. coli* were sensitive to ceftriaxone-tazobactam (100%), ampicillin/sulbactam (100%), meropenem (100%), amikacin (66.66%), enrofloxacin (66.66%), ciprofloxacin (33.33%), azithromycin (33.33%), gentamycin (33.33%) and resistant to clindamycin (100%), streptomycin (100%), pencillin G (100%). These findings were in agreement with Reddy *et al.* (2011), who reported that *E. coli* isolates were sensitive to ciprofloxacin and enrofloxacin, whereas Khinchi *et al.* (2022) showed sensitivity to enrofloxacin (100%) and gentamicin (66.67%). A high level of antimicrobial resistance for penicillin G by all isolates was recorded in this study, indicating frequent and indiscriminate use of this antimicrobial in pyoderma cases in dogs. Geographical region wise selection of appropriate antimicrobial agents should be prioritized based on antibiotic susceptibility pattern to decrease the antimicrobial resistance and for better therapeutic outcome.

Conclusions

This study concluded *Staphylococcus* spp. especially *S. intermedius* as a major pathogen associated with canine pyoderma. PCR can be considered as a quick and sensitive technique for detection of causative agents of skin infections after preliminary genus level identification based on Gram's staining and colony morphology. Based on antibiotic susceptibility test, ceftriaxone, enrofloxacin and meropenem, ceftriaxone/tazobactum were considered as choice of antibiotics against canine pyoderma caused by *Staphylococcus* spp. and *P. aueroginosa, E. coli*. In context of rising antimicrobial resistance in organisms antimicrobial susceptibility testing should always be considered to check sensitivity pattern of bacterial agents prior to treatment of pyoderma.

Acknowledfgement

The authors are thankful to the Dean, Veterinary College, Bengaluru, KVAFSU, for his constant support and permission to conduct this research work.

Referrences

Andrade, M., Oliveira, K., Morais, C., Abrantes, P., Pomba, C., Rosato, A.E., Couto, I., & Costa, S.S. (2022). Virulence potential of biofilm-producing *Staphylococcus pseudintermedius*, *Staphylococcus aureus* and *Staphylococcus coagulans* causing skin infections in companion animals. *Antibiotics, 11*(10), 1339.

Ankita, & Gandge, R.S. (2018). Prevalence and antibiotic susceptibility pattern of *Staphylococcus* species in canine skin infection. *International Journal of Current Microbiology and Applied Sciences*, *7*(6), 2305-2313.

Bauer, A.W., Kirby, W.M., Sherris, J.C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 493-496.

Bej, A.K., DiCesare, J.L., Haff, L., & Atlas, R.M. (1991). Detection of *E. coli* and *Shigella* spp. in water by using the polymerase chain Reaction and gene probes for uid. *Applied Environmental Microbiology*, *57*(4), 1013-1017.

Botoni, L.S., Scherer, C.B., Silva, R.O., Coura, F.M., Heinemann, M.B., Paes-Leme, F.O., & Costa-Val, A.P. (2016). Prevalence and *in vitro* susceptibility of methicillin-resistant *Staphylococcus*

pseudintermedius (MRSP) from skin and nostrils of dogs with superficial pyoderma. *Pesquisa Veterinaria Brasileira*, *36*(12), 1178-1180.

Chaudhary, A.K., Kumar, A., & Shrivastva, M. (2019). Study on prevalence and resistance patterns of bacterial pathogens isolated from canine pyoderma. *International Journal of Current Microbiology and Applied Sciences, 8*(1), 2305-2311.

CLSI (Clinical and laboratory Standards Institute) (2020), Performance standards for antimicrobial susceptibility testing, M100S, 30th ED., CLSI Vol.-40, N0. 1, Jan-2020, CLSI, Wayne, PA 19087 USA.

Degi, J., Motco, O.A., Degi, D.M., Suici, T., Mares, M., Imre, K., & Cristina, R.T. (2021). Antibiotic susceptibility profile of *Pseudomonas aeruginosa* canine isolates from a multicentric study in Romania. *Antibiotics*, *10*(1), 846.

Hariharan, H., Gibson, K., Peterson, R., Frankie, M., Matthew, V., Daniels, J., Martin, N.A., Andrews, L., Paterson, T., & Sharma, R.N. (2014). *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi Subspecies coagulans* from canine pyoderma cases in Grenada, West Indies, and their susceptibility to beta-lactam drugs. *Veterinary Medicine International, 2014,* 1-7.

Khinchi, R.K., Gaurav, A, Manju, Sharma, S.K., & Solanki, S. (2022). Antibiotic susceptibility pattern of bacterial pathogens isolated from canine superficial pyoderma. *Current Journal of Applied Sciences and Technology, 41*(43), 17-22.

Makwana, P.M., Parmar, S.M., Vala, J.A., Parasana, D.K., Patel, D.R., Kalyani, I.H., & Solanki, J.B. (2023). Molecular characterization and antimicrobial-resistant pattern of *Staphylococcus* species isolated from pyoderma cases in dogs. *Biological Forum, 15*(11), 82-87.

Marco-Fuertes, A., Marin, C., Gimeno-Cardona, C., Artal-Munoz, V., Vega, S., & Montoro-Dasi, L. (2024). Multidrug resistant commensal and infection causing *Staphylococcus* spp. isolated from companion animals in the Valencia Region. *Veterinary Science, 11*(2), 54.

Meroni, G., Filipe, J.F., Drago, L., & Martino, P.A. (2019). Investigation on antibiotic-resistance, biofilm formation and virulence factors in multidrug resistant and non multi drug resistant *Staphylococcus pseudintermedius*. *Microorganisms, 7*(12), 702.

Naveena, A., Kavitha, K.L., Kumar, N.V., & Babu, A.J. (2023). Clinical epidemiology and cutaneous microbiota associated with canine pyoderma. *Haryana Veterinarian*, *62*(SI), 26-30.

Rafatpanah, S., Rad, M., Movassaghi, A.R., & Khoshnegah, J. (2020). Clinical, bacteriological and histopathological aspects of first-time pyoderma in a population of Iranian domestic dogs: a retrospective study. *Iranian Journal of Veterinary Research, 21*(2), 130-135.

Ravens, P.A., Vogelnest, L.J., Ewen, E., Bosward, K.L., & Norris, J.M. (2014). Canine superficial bacterial pyoderma: Evaluation of skin surface sampling methods and antimicrobial susceptibility of causal *Staphylococcus* isolates. *Australian Veterinary Journal, 92*(5), 149-155.

Reddy, B.S., Kumari, K.N., Pramila, D.R., & Sivajothi, S. (2014). Isolation of bacteria in dogs with recurrent pyoderma in Andhra Pradesh. *Journal of Life Sciences*, *4*(3), 15-18.

Reddy, B.S., Kumari, K.N., Rao, V.V., & Rayulu, V.C. (2011). Cultural isolates and the pattern of antimicrobial sensitivity of whole cultures from recurrent pyoderma in dogs. *Indian Journal of Field Veterinarian, 7*(1), 40-42.

Sasaki, T., Tsubakishita, S., Tanaka, Y., Sakusabe, A., Ohtsuka, M., Hirotaki, S., Kawakami, T., Fukata, T., & Hiramatsu, K. (2010). Multiplex-PCR method for species identification of coagulase-positive staphylococci. *Journal of Clinical Microbiology*, *48*(3), 765-769.