Biological Forum – An International Journal **15(11): 82-87(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Molecular Characterization and Antimicrobial-resistant Pattern of *Staphylococcus* species Isolated from Pyoderma Cases in Dogs

Makwana P.M.[1]*, Parmar S.M.[2], Vala J.A.[2], Parasana D.K.[1], Patel D.R.[1], Kalyani I.H.[1] and Solanki J.B.[3]

[1]Department of Veterinary Microbiology, College of Veterinary Science & A.H., Kamdhenu University, Navsari (Gujarat), India.

[2]Department of Veterinary Clinical Complex, College of Veterinary Science & A.H., Kamdhenu University, Navsari (Gujarat), India.

[3]Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari (Gujarat), India.

(Corresponding author: Makwana P.M.)*

(Received: 06 September 2023; Revised: 27 September 2023; Accepted: 11 October 2023; Published: 15 November 2023)

(Published by Research Trend)

ABSTRACT: Pyoderma is one of the most common bacterial skin condition observed in dogs. *Staphylococcus* is considered as a predominant bacterial agent associated with bacterial dermatitis in dogs. A study was undertaken to detect involvement of *Staphylococcus* spp. from pyoderma cases in dogs and to determine *in vitro* antimicrobial resistant pattern of isolates. Total 37 samples from dogs showing pyoderma were aseptically collected for bacterial isolation. Out of 37 samples, 32 isolates were identified as *Staphylococcus* spp. (86.48 %) and one as *Streptococcus.* From 32 *Staphylococcus*, 13 isolates were identified as *Staphylococcus pseudintermedius* (40.63 %), 6 as *Staphylococcus schleiferi* subsp. *coagulans* (18.75 %), and 5 as *Staphylococcus aureus* (15.63 %) by PCR. Biochemically, 4 isolates characterized as *Staphylococcus hominis* (12.5 %), 3 as *Staphylococcus capitis* (9.38 %) and 1 isolate as *Staphylococcus gallinarum* (3.12 %). Antibiogram of *Staphylococcus* isolates showed highest resistance against clindamycin (59.38 %) and cefpodoxime (59.38 %) followed by oxacillin (50 %), neomycin (46.88 %), doxycycline (43.75 %), amoxyclav (34.38 %), enrofloxacin (31.25 %), amikacin (21.88 %) while cefalexin found as least resistant (18.75 %). Screening of methicillin resistant *Staphylococcus pseudintermedius* (MRSP) is necessary as it pose major challenge for antibiotic resistance. *S. pseudintermedius* can be transmitted from infected dogs to healthy dogs and humans; it needs attention for both the veterinary and human sector.

Keywords: Antimicrobial Resistance, Bacteria, Pyoderma, PCR, *Staphylococcus.*

INTRODUCTION

Dermatological conditions represent the majority of cases in small animal clinics, comprising more than 20 per cent of the case load (Nauriyal, 2007; Rakesh *et al*., 2023). Pyoderma is considered as one of the most frequently encountered dermatological disorders in dogs. The main clinical signs include circular alopecia, erythema, pustules, epidermal collarettes, crusts, desquamation and pruritus (Botoni *et al.*, 2016). Bacterial pyoderma is frequently observed clinical condition among canines and is also occurs as a consequences of underlying disorders such as hypersensitivity, ectoparasitic infestation and immunological disorders (Mason, 1991). Various bacterial agents are associated with bacterial pyoderma condition of dogs comprising *Staphylococcus* sp., *Streptococcus* sp., *Escherichia coli*, *Corynebacterium* sp., and others. Staphylococci constitute as significant opportunistic bacterial pathogens in dogs and widely reported worldwide pyoderma condition (Chaudhary *et al.*, 2019, Palomino-Farfán *et al*., 2021). *Staphylococcus aureus*, *S. pseudintermedius*, *S. schleiferi* sub spp. *schleiferi* and *S. schleiferi* sub spp. *coagulans* constitute the most frequent causes of canine Staphylococcal infections (Chaudhary *et al.*, 2021). Over 90% of pyoderma infections in dogs are caused by *Staphylococcus pseudintermedius*, while *Staphylococcus aureus* and *Staphylococcus coagulans* accounting for the remaining 10% of Pyoderma incidents, respectively (Andrade *et al.*, 2022).

Empirical diagnosis of pyoderma based on history, physical examination is followed by Gram staining and cultural isolation of causative agent from cutaneous lesions (Hariharan *et al*., 2014). Treatment of pyoderma mainly depends on antimicrobial use in small animal practice. Knowledge lacking of appropriate antibiotic drug in companion animals leads to emergence of resistance in these animals. Increase in resistance to commonly prescribed antimicrobial drugs is the cause of emergence and global spread of multidrug resistant (MDR) bacteria which has an especially detrimental effect on the management of these infections (Meroni *et al*., 2019). That is why bacterial culture and antimicrobial susceptibility testing from pyoderma

lesions is essential to treat the affected animal timely and appropriate treatment regimens.

MATERIAL AND METHODS

A. Sample collection

A total of 37 skin swabs from clinical cases of pyodermair respective of age, sex and breed from dogs were selected for the study. Dogs showing lesions like, pustules, scab, nodules, erythema, pruritus, alopeciapresented at Veterinary Clinical Complex, Veterinary College, Navsari, were aseptically collected by gentle rolling of the sterile cotton swab across the skin lesion and transported to the Microbiology department with ice packs.

B. Cultural isolation and identification of Staphylococcus spp.

For isolation and identification of *Staphylococcus* spp. skin samples were inoculated on 5 % blood agar and Brain heart infusion agar and incubated at 37° for 18-24 hrs. After incubation, colonies were observed for morphology, gram-stained and examined for microscopic appearance. After that, catalase, oxidase tests were carried out and colony was re-streaked onto Mannitol salt agar (HiMedia). Biochemically, sugar fermentation tests was performed for species identification.

C. Molecular detection of Staphylococcus species

The genomic DNA from cultures was extracted by manual method as per Chitra *et al.*, 2015 with slight modification. Briefly, Four to five colonies were suspended in 100 µl of sterile distilled water and boiled at 100°C for 10 min. Then the tubes were cooled immediately by placing them in deep freeze for 5 to 10 minutes. After that, tubes were centrifuged at 10000 rpm for 10 min, and the supernatant was used as template in the PCR reaction. For molecular confirmation universal primers targeting *Staphylococcus* spp., *S. pseudintermedius, S. aureus* and *S. Schleiferi* subsp. *coagulans* were used (Table 1). 25 µl reaction mixture was prepared containing 3.0 µl template DNA, 12.5 µl of 2x PCR master mix (Thermo Fisher Scientific, India), 1.0 µl of forward and reverse primer (10 pmol) and 7.5 µl sterile nuclease-free water. PCR cycling conditions were set as initial denaturation at 95ºC for 5 minute followed by 35 cycles of denaturation at 94ºC for 30 second, annealing at 54ºC for *Staphylococcus* spp. and *S. aureus*, 60ºC for *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* for 30 second, extension at 72ºC for 30 second with a final extension step at 72ºC for 10 minutes using thermal cycler (Applied Biosystems, USA). The PCR products were electrophoresed on 1.5 % agarose gel and visualized with UV Transilluminator (SynGene, UK).

D. Antimicrobial resistant pattern

Antibiotic resistant profile of *Staphylococcus* isolates performed according to Kirby- Bauer disk diffusion test using 9 antibiotics *viz.*, cefalexin, amoxyclav, clindamycin, enrofloxacin, doxycycline, cefpodoxime, amikacin, oxacillin and neomycin (HiMedia). Antibiotics were placed on Muller Hinton agar and incubated at 37ºC for 24 hrs. Zone of inhibition for each disc was measured and interpreted as susceptible, intermediate or resistant as per the clinical and laboratory standards institute (CLSI) guidelines.

RESULTS AND DISCUSSION

In the present study, dogs with pyoderma infection were of different age, breed and sex. Pustules, scab, nodules, erythema, pruritus, alopecia, crust and epidermal collarettes were noted on various body parts (Fig. 1). Similar types of lesions were reported in study by Borio *et al.* (2015); Parvathy *et al.* (2022). Sex wise, breed wise and age wise results are depicted in Table 2.

A. Sex wise occurrence of pyoderma cases

Study revealed higher infection of pyoderma in male (75.67 %) than female (24.33 %) dogs. The findings were in accordance with Curtseit *et al*. (2009); Sarma *et al.* (2013); Khurana *et al.* (2016); Khinchi *et al.* (2019); Chaudhry *et al.* (2019); Parvathy *et al.* (2022) who also reported higher occurrence of pyoderma in male dogs.

B. Breed wise occurrence of pyoderma cases

Highest occurrence of pyoderma was recorded in Labrador dogs (17 out of 37) 45.94 % followed by non descript breeds (18.91 %), German Shepherd (10.81 %), Pomeranianand Pug (8.10%), Golden retriever, Cocker Spanial and Lhasa Apso breed (2.70%) each. The breed predilection to dermatological problems varied with the breed composition of canine population in a particular region and popularity of individual breeds (Pocta and Svoboda 2007). This findings showed agreement with Khinchi *et al.* (2019); Chaudhary *et al.* (2019); Parvathy *et al.* (2022).

C. Age wise occurrence of pyoderma cases

Analysis of the percentage of dogs suffering from pyoderma in various age groups revealed higher infection rate in age group > 5 yrs -14 yrs (46.65 %) followed by age group of 1 yrs- 5.0 yrs (32.43 %) and least in < 1 year age group as 18.91 %. Similarly, Curtseit *et al.* (2009) recorded higher occurrence of pyoderma in dogs aged between 9-14 years. Contrary to this Parvathy *et al*. (2022) observed higher infection of pyoderma between the age group of 1-3 years followed by below 1 year and the least occurrence was in dogs above 6 years. Though there was no statistically significant difference was recorded among sex, breed and age.

D. Isolation of Staphylococcus from clinical samples

On BHI agar medium, whitish, yellow coloured, opaque, circular colonies (Fig. 2) while on blood agar haemolytic (Beta and double haemolysis) colonies (Fig. 3) were observed, which microscopically revealed gram positive cocci in cluster (Fig. 4). Further all the samples were catalase positive, oxidase negative and able to grow on mannitol salt agar (Fig. 5). Out of 37 skin swabs, 32 samples were identified as *Staphylococcus* spp. (86.48 %) indicated that *Staphylococcus* plays major role in pyoderma in dogs of this region. Similar to this others have also stated *Staphylococcus* as prime causative agent in pyoderma in dogs (Mark 2003; Chaudhary *et al.*, 2019; Palomino-Farfán *et al*., 2021).

Only one sample, on the gram staining basis identified as *Streptococcus.*

E. Identification of Staphylococcus spp. by biochemical and molecular method

After growth on selective media, for species identification all the isolates were subjected to molecular based characterization. *Staphylococcus*, isolates which were not specified by PCR was subjected for sugar fermentation test. Isolates of *S. aureus* were confirmed by both sugar fermentation (Fig. 6) and PCR technique.

From 32 *Staphylococcus*, predominant isolates were identified as *Staphylococcus pseudintermedius* (n=13, 40.63 %), followed by *Staphylococcus schleiferi* subsp. *coagulans* (n=6, 18.75 %) and 5 (15.63 %) as *Staphylococcus aureus* by polymerase chain reaction (Fig. 7). Biochemically, 4 (12.5 %) isolates characterized as *Staphylococcus hominis*,3 as *Staphylococcus capitis* (9.38%) and 1 (3.12 %) isolate as *Staphylococcus gallinarum* (Table 3).

Both *S. pseudintermedius* and *S. schleiferi* is an increasingly reported zoonotic pathogen, can cause opportunistic infections in humans and in dogs associated with pyoderma. In the present study *S. pseudintermedius* was reported as major *Staphylococcus* spp. amongst others. Similar to this previous authors have mentioned *S. pseudintermedius* as predominant isolate from canine pyoderma (Morris *et al*., 2006; Fazakerley *et al*., 2009; Kawakami *et al.*, 2010; Botoni *et al*., 2016). Similarly, many authors have described involvement of *S. schleiferi* subsp *coagulans* from canine pyoderma cases (Frank *et al.*, 2003, Kawakami *et al.*, 2010; Chaudhary *et al.*, 2019). Our study is in accordance with Hariharan *et al.* (2014) who reported *S. hominis* and *S. capitis* from canine pyoderma infection. Compared to other *Staphylococcus*, *S. hominis* and *S. capitis* were reported in limited studies, because both are opportunistic organisms in humans, may be transferred from their owner, exact reason of these bacteria in pyoderma infection in our study could not be explained.

F. Antibiotic resistant pattern of all Staphylococcus isolates

Among 32 *Staphylococcus* isolates, highest resistant was observed for cefpodoxime and clindamycin each 59.38 % followed by oxacillin (50 %), neomycin 46.88 %, doxycycline 43.75 %, amoxyclav 34.38 %, enrofloxacin 31.25%, amikacin 21.88 %and cefalexin 18.75 % (Table 4). Present study showed higher sensitivity of all the isolates for cephalexin (65.63 %) and amoxyclav (53.13 %). Similarly, Ravens *et al.* (2014); Rafatpanah *et al*. (2020) found higher susceptibility to cephalexin and amoxyclav for *Staphylococcus* isolates. Methicillin resistance was determined by oxacillin disk susceptibility that showed 50 % isolates are methicillin resistance. Methicillin-resistant *Staphylococcus* species are considered resistant to other β-lactams, cephalosporins, clindamycin and amoxycillin–clavulanate. Our study found agreement with this as isolates showed higher resistance to cefpodoxime, clindamycin and oxacillin. A low levels of antimicrobial resistance for cefalexin was recorded in this study, indicating prudent use of this antimicrobial in pyoderma cases in dogs.

Table 1: *Staphylococcus* Genus and species specific primer sequences.

	Primer (5'- 3')	Target	Product size	Reference
1.	GGCCGTGTTGAACGTGGTCAAATCA TIACCATTTCAGTACCTTCTGGTAA	*Staphylococcus* spp.	370 bp	Martineau *et al.* (2001)
2.	TGATGCAGCTTTTCCGTATG AAAGATGGGCAAGATGAACG	*S. pseudintermedius*	99 bp	González-Domínguez *et al.* (2020)
3.	GTGCTGGCATATGTATGGCAATTGT TACGCCGTTATCTGTTTGTGATGC	*S. aureus*	181 bp	Hegde *et al.* (2013)
4.	TTAAAACGACGGAAGGCAGT CCAATCATACGCACACGTTC	*S. schleiferi* subsp. *coagulans*	115 bp	González-Domínguez *et al.* (2020)

Table 2: Sex wise, age wise and breed wise cases of pyoderma recorded: (n =37).

Particulars of animals		Number of infected dogs	Per cent (%)
Sex	Male	28	75.67 ^{ns}
	Female	09	24.33 ^{ns}
Age	>5 & upto 14 yrs)	18	48.65 ^{ns}
	(1 yrs- 5.0 yrs)	12	32.43 ^{ns}
	< 1 year	07	18.92 ^{ns}
Breed	Labrador	17	45.95 ^{ns}
	ND (Non descript)	07	18.92 ^{ns}
	German Shepherd	04	10.81 ^{ns}
	Pomeranian	03	8.11 ^{ns}
	Golden retriever	01	2.70 ^{ns}
	Pug	03	8.11 ^{ns}
	Cocker spanial	01	2.70 ^{ns}
	Lhasa Apso	01	2.70 ^{ns}

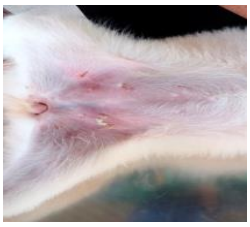
Table 3: Identification of various *Staphylococcus* species from Pyoderma cases.

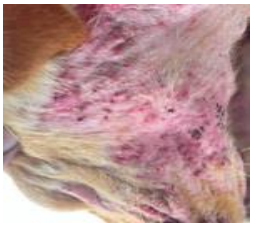
Sr. No.	Species of *Staphylococcus*	Biochemical analysis								No of (+ve) isolates	Percentage
		Lac	Ma	Mn	Su	TR	Xy	Cel	Raf		
1	*S. aureus*	+	+	+	+	+	-	+	-	5	15.63
2	*S. hominis*	+	+	-	+	+	-	-	-	4	12.5
3	*S. capitis*	-	-	+	+	+	-	-	-	3	9.38
4	*S. gallinarum*	+	+	+	+	+	+	+	-	1	3.12

Lac = Lactose, Ma =Maltose, Mn= Mannitol, Su = Sucrose, TR = Trehalose, Xy- Xylose, Cel- Cellobiose, Raf- Raffinose

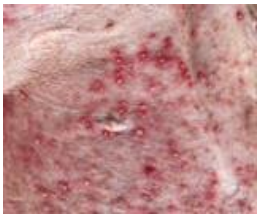
Table 4: Antibiogram of the *Staphylococcus* isolates (n=32).

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin (30 mcg)	10 (31.25)	15 (46.88)	07 (21.88)
Doxycycline (30 mcg)	13 (40.63)	05 (15.63)	14 (43.75)
Enrofloxacin (10 mcg)	14 (43.75)	08 (25.00)	10 (31.25)
Clindamycin (2 mcg)	05 (15.63)	08 (25.00)	19(59.38)
Cefpodoxime (10 mcg)	08 (25.0)	05 (15.63)	19 (59.38)
Amoxyclav (30 mcg 20/10)	17 (53.13)	04 (12.5)	11 (34.38)
Cefalexin (30 mcg)	21 (65.63)	05 (15.63)	06 (18.75)
Oxacillin (1 mcg)	11 (34.38)	05 (15.63)	16 (50.00)
Neomycin (10 mcg)	15 (46.88)	02 (6.25)	15 (46.88)


Lesions on ventral abdomen


Redness, pustules, alopecia on body


Scab formation, alopecia on face


Redness, pustules alopecia on abdomen


Pustules, erythema, alopecia on body


Pustule on ventral abdomen


Pustules, alopecia all over the body


Crust with scab on leg

Fig. 1. Various pyoderma lesions exhibited by affected animals.

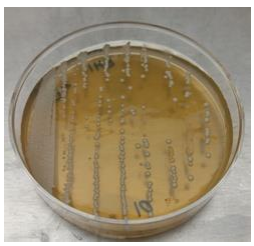


Fig. 2. whitish, opaque, circular colonies on BHI agar.


Fig. 3. Growth on blood agar (Haemolytic colonies).

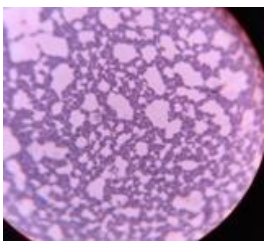


Fig. 4. Gram positive cocci in cluster.


Fig. 5. Growth on manitol salt agar (Yellow colonies shows fermentation of mannitol.

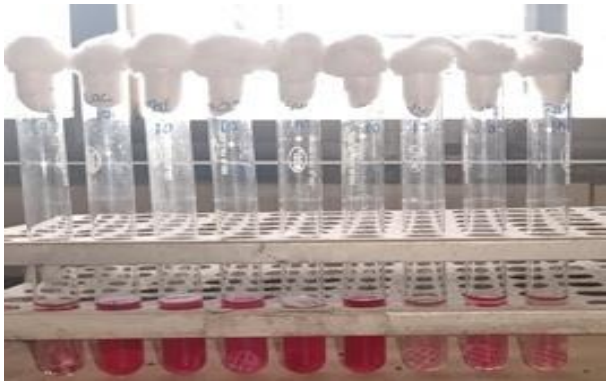
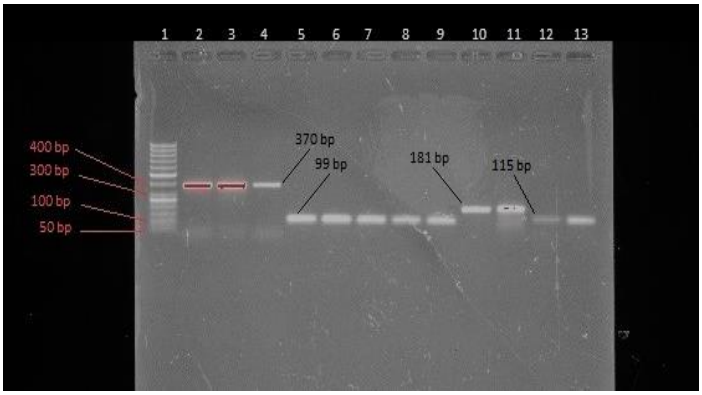


Fig. 6. Confirmation of *Staphylococcus aureus* by sugar fermentation test.



Lane 1: DNA marker (50 bp), Lane 2,3,4- *Staphylococcus* spp. (370 bp), Lane 5,6,7,8,9- *Staphylococcus pseudintermedius* (99 bp), Lane 10,11 – *Staphylococcus aureus* (181 bp), Lane 12,13- *Staphylococcus schleiferi* subsp. *coagulans*(115bp)

Fig. 7. Confirmation of various S*taphylococcus* spp. by PCR.

CONCLUSIONS

Based on our findings it can be concluded *Staphylococcus* spp. considered as the main bacterial agent in pyoderma condition of dogs of this region. *S. pseudintermedius, S. schleiferi* subsp *coagulans* and *S. aureus* represent as major causative agents. PCR technique considered as more precise and sensitive technique compared to biochemical methods but non specific isolates can be identified with the biochemical tests. Cefalexin appears appropriate choice of antibiotic against canine pyoderma caused by *Staphylococcus* for this region. However, bacterial culture and antimicrobial susceptibility testing should always be considered in context to antibiotic resistance and to check sensitivity pattern of particular bacterial agents of geographical area. Possibility of transfer of opportunistic staphylococci between animals and humans and vice versa should be considered and pet owners should be advised to maintain good hygiene practices.

FUTURE SCOPE

Emergence of Methicillin-resistant coagulase-positive staphylococci (MRCPS), such as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and methicillin-resistant *Staphylococcus schleiferi* (MRSS), from dogs, cats, and veterinarian have been reported, so further study is required for identification of these bacteria as they are considered as zoonotic and major threaten for treatment aspect.

Acknowledgement. The authors are highly thankful to Director of Research, KU and Principal Dr. V. B. Kharadi, Veterinary College, Kamdhenu University, Navsari for providing necessary funds for research work.

REFERENCES

Andrade, M., Oliveira, K., Morais, C., Abrantes, P., Pomba, C., Rosato, A. E., Couto, I. and 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.

Borio, S., Colombo, S., La Rosa, G., De Lucia, M., Damborg, P. and Guardabassi, L. (2015). Effectiveness of a combined (4% chlorhexidinedigluconate shampoo and solution) protocol in MRS and non-MRS canine superficial pyoderma: a randomized, blinded, antibiotic-controlled study. *Veterinary Dermatology*, *26*, 339-344.

Botoni, L. S., Scherer, C. B., Silva, R. O., Coura, F. M., Heinemann, M. B., Paes-Leme, F. O. and 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 Veterinária Brasileira*, *36*(12), 1178-1180.

Chaudhary, A. K., Kumar, A. and 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.

Chaudhary, S. S., Chauhan, H. C., Sharma, K. K., Patel, S. S., Mohapatra, S. K., Patel, A. C., Shrimali, M. D. and Chandel, B. S. (2021). Bacteriological and Molecular Identification and characterization of *Staphylococcus aureus* from different affections of canines. *Indian Journal of Animal Research*, *55*(4), 474-478.

Chitra, M. A., Jayanthy, C. and Nagarajan, B. (2015). Detection and sequence analysis of accessory gene regulator genes of *Staphylococcus pseudintermedius* isolates. *Veterinary world*, *8*(7), 902.

Curtseit, S., Ciobotaru, E., Militaru, M., Soare, T. and Dinescu, G. (2009). Diagnosis of pyoderma in dog. *Scientific Works-University of Agronomical Sciences and Veterinary Medicine, Bucharest Series C, Veterinary Medicine*, *55*(3), 80-87.

Fazakerley, J., Nuttall, T., Sales, D., Schmidt, V., Carter, S. D., Hart, C. A. and McEwan, N. A. (2009). Staphylococcal colonization of mucosal and lesion skin sites in atopic and healthy dogs. *Veterinary Dermatology*, *20*, 179-184.

Frank, L. A., Kania, S. A., Hnilica, K. A., Wilkes, R. P. and Bemis, D. A. (2003). Isolation of *Staphylococcus schleiferi* from dogs with pyoderma. *Journal of the American Veterinary Medical Association*, *222*(4), 451-454.

González-Domínguez, M. S., Carvajal, H. D., Calle-Echeverri, D. A. and Chinchilla-Cárdenas, D. (2020). Molecular detection and characterization of the *mecA* and *nuc* genes from *Staphylococcus* species (*S. aureus*, *S. pseudintermedius*, and *S. schleiferi*) isolated from dogs suffering superficial pyoderma and their antimicrobial resistance profiles. *Frontiers in Veterinary Science*, *7*, 376.

Hariharan, H., Gibson, K., Peterson, R., Frankie, M., Matthew, V., Daniels, J., Martin, N. A., Andrews, L., Paterson, T. and 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*.

Hegde, R., Isloor, S., Prabhu, K. N., Shome, B. R., Rathnamma, D., Suryanarayana, V. V. S. and Hegde, N. R. (2013). Incidence of subclinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. *Indian Journal of Microbiology*, *53*, 315-320.

Kawakami, T., Shibata, S., Murayama, N., Nagata, M., Nishifuji, K., Iwasaki, T. and Fukata, T. (2010). Antimicrobial Susceptibility and Methicillin Resistance in *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* subsp. *coagulans* isolated from dogs with pyoderma in Japan. *Journal of Veterinary Medical Science*, *72*(12), 1615–1619.

Khinchi, R. K., Sharma, S. K., Goklaney, D., Dhuria, R. K., Sharma, S. and Parashar, M. (2019). Superficial pyoderma in canines in southern part of Rajasthan-A detailed epidemiological study. *International Journal of Chemical Studies*, *7*(3), 2737-2740.

Khurana, R., Kumar, T., Agnihotri, D. and Sindhu, N. (2016). Dermatological disorders in Canines-A detailed epidemiological study. *Haryana Veterinarian*, *55*, 97-99.

Mark, C. (2003). Diagnosis and management of pyoderma in the dog. *In Practice*, *25*, 418-425.

Martineau, F., Picard, F. J., Ke, D., Paradis, S., Roy, P. H., Ouellette, M. and Bergeron, M. G. (2001). Development of a PCR assay for identification of staphylococci at genus and species levels. *Journal of Clinical Microbiology*, *39*(7), 2541-2547.

Mason, L. S. (1991). Canine pyoderma. *Journal of Small Animal Practice*, *32*, 381-386.

Meroni, G., Soares Filipe, J. F., Drago, L. and 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.

Morris, D. O., Rook, K. A., Shofer, F. S. and Rankin, S. C. (2006). Screening of *Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus schleiferi* isolates obtained from small companion animals for antimicrobial resistance: a retrospective review of 749 isolates (2003–04). *Veterinary Dermatology*, *17*(5), 332-337.

Nauriyal, D. S. (2007). Canine dermatosis associated with nutritional deficiencies, *IntasPolivet*, *8*, 471-478.

Palomino-Farfán, J. A., Vega, L. G. A., Espinoza. S. Y. C., Magallanes, S. G. and Moreno, J. J. S. (2021). Methicillin-resistant *Staphylococcus schleiferi subspecies coagulans* associated with otitis externa and pyoderma in dogs. *Open Veterinary Journal*, *11*(3), 364-369.

Parvathy, G. N., Deepa, P. M., Janus, A., Chintu, R. and Vijayakumar, K. (2022). Epidemiological study of bacterial dermatitis in dogs of Wayanad district. *Journal of Veterinary* and *Animal Sciences*, *53*(2), 143-147.

Pocta, S. and Svoboda, M. (2007). Incidence of canine hypersensitivity in the region of North-eastern Bohemia. *Acta Veterinarian*, *76*, 451-459.

Rafatpanah, S., Rad, M., Movassaghi, A. R. and 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.

Rakesh, S., Roy, K., Soni, A. K., Gupta, D., Tiwari, A., Singh, B., Pradhan, S., Pathak, S. K., Mahor, B.S and Karthik, I. (2023). Occurrence of Malassezia dermatitis in Dogs at Jabalpur (M.P.), *Biological Forum –An International Journal*, *15*(9), 01-05.

Ravens, P. A., Vogelnest, L. J., Ewen, E., Bosward, K. L. and 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.

Sarma, K., Mondal, D. B., Sarvanan, M., Kumar, M., Vijay Kumar, H. (2013). Incidence of dermatological disorders and its therapeutic management in canines. *Intaspolivet*, *14*(II), 186-192.

How to cite this article: Makwana P.M., Parmar S.M., Vala J.A., Parasana D.K., Patel D.R., Kalyani I.H. and Solanki J.B. (2023). Molecular Characterization and Antimicrobial-resistant Pattern of *Staphylococcus* species Isolated from Pyoderma Cases in Dogs. *Biological Forum – An International Journal, 15*(11): 82-87.