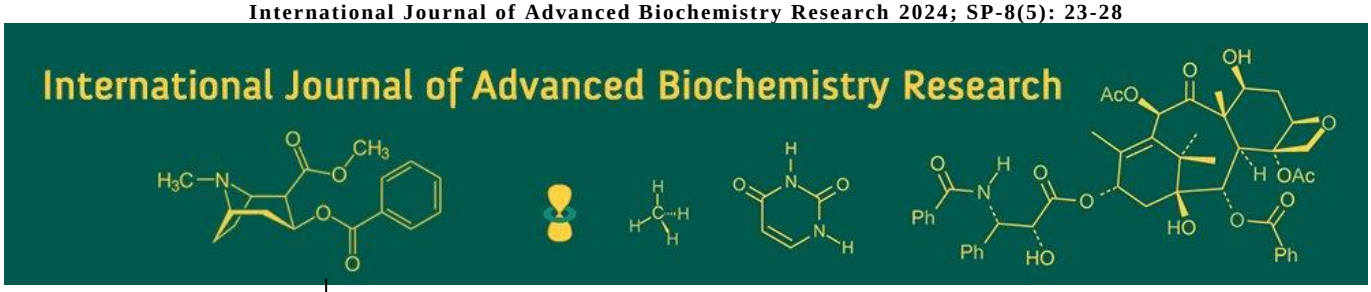

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(5): 23-28
www.biochemjournal.com
Received: 02-03-2024
Accepted: 06-04-2024

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Isolation and antibiogram profiling of *Staphylococcus* species in canine pyoderma

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DOI: https://doi.org/10.33545/26174693.2024.v8.i5Sa.1102

Abstract
Present study was conducted to study the isolation and antibiogram profiling of *Staphylococcus* species on 57 dogs diagnosed with canine pyoderma presented at the Veterinary Clinical Complex (VCC), Jabalpur (M.P.). The common clinical manifestations observed were pruritus, erythema, pustules, papules, crusts, epidermal collarettes and alopecia. The most prevalent clinical signs observed in canine pyoderma were pruritus (73.68%). The condition is diagnosed on the basis of clinical manifestations, isolation and identification of causative organisms by bacteriological cultural examination The antibiotic sensitivity test for *Staphylococcus* spp. revealed highest sensitivity for Linezolid 100 percent than Doxycycline hydrochloride 98.24 percent, Cefpodoxime 82.45 percent, Mupirocin 80.70 percent, Clindamycin 73.68 percent and Amoxycillin Clavulanic acid 77.19 percent.

Keywords: Canine pyoderma, *Staphylococcus* species, antibiotic sensitivity testing

1. Introduction
Canine pyoderma is one of the most common causes of dermatitis with worldwide occurrence in small animal practice. Pyoderma is more common in dogs due to certain characteristics of the 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. This may lead to a higher incidence of primary inflammatory disease that affects the first-line defences (Khinchi, 2019) [8]. Clinically the disease is characterized by primary skin lesions including papules, pustules, followed by secondary skin lesions crusting, epidermal collarettes, alopecia, scaling, erythema, pruritus, lichenification and hyperpigmentation (Reddy *et al*., 2016) [13]. Currently, the diagnosis of canine pyoderma is based on history and clinical observation of compatible clinical signs (Chaudhary *et al*., 2019) [3]. Antibiotic sensitivity test is necessary to avoid multidrug resistance in bacterial skin diseases. Multidrug-resistant poses a significant threat to both animal and human health. Therefore, utmost care must be taken when selecting antimicrobials, ensuring appropriate dosages and accurate treatment diagnosis.

2. Materials and Methods
2.1 Location and place of work
The study was undertaken for a period from August 2023 to January 2024. Dogs with clinical manifestations of different dermatological affliction brought to Veterinary Clinical Complex (VCC), College of Veterinary Science and Animal Husbandry Jabalpur (M.P.) were included in the study.

2.2 Collection of samples
The pus was collected with the help of a sterile cotton swab avoiding contact with the skin as per the method suggested by Shearer and Day (1997) [15] (Figure 01). The collected specimens were transported to the laboratory and processed as early as possible. The collected swabs were inoculated in trypton soya broth supplemented with 6.5% NaCl and were incubated at 37 °C for 24 hours.

2.2 Isolation and identification of *Staphylococcus* species: The gram-positive *Staphylococcus* organisms were tentatively identified using gram staining method according to Markey *et al.* (2012) [10]. The isolated organisms were streaked on sterile plates of nutrient agar and mannitol salt agar (MSA) and were incubated at 37 ˚C for 24 hrs and plates were observed for appearance of typical colonies of *Staphylococcus* spp. The isolates showing Gram positive cocci in clusters, suggestive for *Staphylococcus* spp.

2.3 Biochemical tests
The colonies showing typical characters suggestive of *Staphylococcus* spp. were further confirmed by various biochemical and sugar fermentation test *viz*. catalase test, tube coagulase test, oxidase test, mannitol fermentation test and haemolysis on blood agar.

2.4 Antibiotic sensitivity test
Antibiotic sensitivity test of *Staphylococcus* spp. were subjected by disc diffusion method as described by (CLSI, 2013) using following six antibiotics *viz*. Cefpodoxime (10 mcg), Clindamycin (02 mcg), Mupirocin (200 mcg), Amoxycillin clavulanic acid (30mcg), Doxycycline (30mcg), Linezolid (30 mcg) antibiotic discs from Hi Media.

3. Results and Discussion

3.1 Clinical manifestations
During this study a variety of clinical aberrations was observed among the affected dogs, pruritus was observed in 73.68 percent, followed by erythema 61.40 percent, crusts in 49.12 percent, papule in 45.61 percent, alopecia in 43.85 percent, pustule in 33.33 percent, scales 31.57 percent and epidermal collarette in 17.54 percent (Figure 02). The various clinical signs are summarized in table 01.
The clinical signs like alopecia, erythema, papule, pustules, crusts and scales might be caused by self-injuries due to sensation of pruritus and the release of proteolytic enzyme, histamine, leukotrienes and various peptidases. The present observations are in close agreement with the findings of other authors namely Reddy *et al*. (2016) [13], Haritha *et al*. (2022) [6] and Kumar *et al*. (2023) [9] who recorded alopecia, pruritus, erythema, papule, pustule, scale, crusts and epidermal collarette in dogs affected with Staphylococcal pyoderma. Haritha *et al*. (2022) [6] reported that the major clinical signs in Staphylococcal pyoderma was pruritus. Although, the results of the present study are in contrast to Dos Santos *et al*. (2021) [5] who reported the major clinical sign was alopecia in pyoderma affected dogs.

3.2 Impression smear examination
Glass slide impression smears were collected with the help of sterile cotton swab from pustule in 57 dogs affected with pyoderma. Microscopic examination of stained smear with gram staining under oil immersion revealed the presence of degenerated neutrophils and cocci (Figure 03). The above findings are in accordance with Bloom and Rosser (2001) [2] and Hillier *et al*. (2006) [7] who have also reported presence of neutrophils and coccoid bacteria in their study of impression smear examination in dogs affected with pyoderma.

3.3 Isolation and identification of bacteria on mannitol salt agar: A total of 57 *Staphylococcus* spp. isolates taken from skin sample of 57 dogs affected with pyoderma were streaked on nutrient agar (NA) and mannitol salt agar (MSA). Growth was observed on nutrient agar media with golden yellow colony (Figure 04) and on mannitol salt agar media (MSA) pink to yellow colour colony were evident (Figure 05). All the 57 isolates taken up for detailed study when stained with gram stain revealed the presence of violet colour i.e., in the form of bunch of cocci arrange in clusters (Figure 06). The above findings were in accordance with Ankita and Gandge (2018) [1] and Putriningsih *et al*. (2023) [11] who have also reported that *Staphylococcus* spp is the major pathogen found in cultural examination of isolated bacteria in dogs affected with pyoderma.

3.4 Catalase test
All the 57 isolates were positive for catalase test which was evident in the form of production of gas bubbles on addition of culture to 3 percent hydrogen peroxide which is due to release of nascent oxygen (Figure 07a).

3.5 Tube coagulase test
Production of coagulase by *Staphylococci* is an important indicator of pathogenicity. Coagulase is involved in conversion of fibrinogen to fibrin. The deposition of fibrin may shield *Staphylococci* from phagocytic cells. Out of 57 positive samples collected from skin, 52.63 percent (30) isolates were found coagulase positive *Staphylococcus* spp. As revealed by tube coagulase test and 47.36 percent (27) isolates were found to be coagulase negative *Staphylococcus* spp. as revealed by tube coagulase test. (Table 02 and Figure 07b).

3.6 Oxidase test
The isolates further examined for oxidase test to differentiate between gram negative organisms. All the isolates were negative for oxidase test which was evidenced by the development of colour form colourless to blue-violet colour on the oxidase disc in 5-10 seconds (Figure 07c).

3.7 Mannitol fermenting and non-fermenting
Out of 57 isolates of *Staphylococcus* spp. 34 were found to ferment mannitol which was observed in the form of yellow colour discolouration on mannitol salt agar MSA and the remaining 23 isolates were unable to ferment mannitol with the persistence or enhancement of pink color to the medium (Figure 08a and b).

3.8 Haemolysis production
The alpha (α) and beta (β) haemolysis were studied in sheep blood agar. While 42.11 percent (24 isolates) showed alpha haemolysis, 22.80 percent (13 isolates) showed beta haemolysis, 35.09 percent (20 isolates) showed alpha and beta haemolysis and none of the isolates showed gamma haemolysis (Table 03 and Figure 09).

3.9 Antibiotic sensitivity test
Antimicrobial resistance in isolated strains was detected using standard disk diffusion method i.e., Kirby-Bauer disk diffusion method according to (CLSI, 2013). All the 57 dogs affected with Staphylococcal pyoderma were tested for antibiotic sensitivity test for six antibiotics *viz*. Cefpodoxime, Clindamycin, Mupirocin, Amoxycillin clavulaunic acid, Doxycycline, Linazolid. The antibiotic sensitivity test for *Staphylococcus* spp. revealed highest

sensitivity for Linezolid as 100 percent than Doxycycline hydrochloride as 98.24 percent, Cefpodoxime as 82.45 percent, Mupirocin as 80.70 percent, Clindamycin as 73.68 percent and Amoxycillin Clavulanic acid as 77.19 percent. The results are shown in table 04. The above findings are in accordance with Shah *et al*. (2017b) who reported highest sensitivity for Linazolid, Amoxycillin and Clavulanic Acid and Clindamycin, Chaudhary *et al*. (2019) [3] reported highest sensitivity for Doxycycline and Reddy *et al*. (2011) [12] who reported higher sensitivity for Cefpodoxime in Staphylococcal pyoderma affected dogs.

Table 1: Clinical signs of canine pyoderma

Clinical signs	Frequency (n=57)	Percentage (%)
Pruritus	42	73.68
Alopecia	25	43.85
Erythema	35	61.40
Papule	26	45.61
Pustule	19	33.33
Scales	18	31.57
Crusts	28	49.12
Epidermal collarette	10	17.54

Table 2: Tube coagulase test of *Staphylococcus* spp

S. No.	Pathogen	Number of samples examined	Number of positive isolates	Percentage (%)
1.	Coagulase positive *Staphylococci*	57	30	52.63
2.	Coagulase negative *Staphylococci*	57	27	47.36

Table 3: Haemolysis pattern of *Staphylococcus* spp.

S. No.	Haemolysis	Type of Haemolysis	Number of isolates (n=57)	Percentage (%)
1.	Alpha (α)	Complete	24	42.11
2.	Beta (β)	Incomplete	13	22.80
3.	Alpha and beta	Become complete after further storage at 4 °C (hot-cold lysis)	20	35.09
4.	Gamma	None	-	-

Table 4: Antibiotic sensitivity test

Antibiotic disc	Symbol	Number of sensitive isolates (n=57)	Number of resistant isolates (n=57)	Sensitive (%)	Resistant (%)
Cefpodoxime	CPD	47	10	82.45	17.55
Clindamycin hydrochloride	CD	42	15	73.68	26.31
Mupirocin	MUP	46	11	80.70	19.3
Amoxicillin clavulanic acid	AMC	44	13	77.19	22.80
Doxycycline	DO	56	01	98.24	01.75
Linezolid	LZ	57	-	100	-

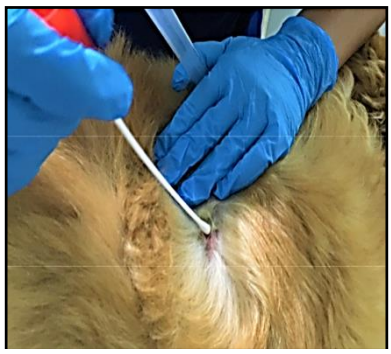
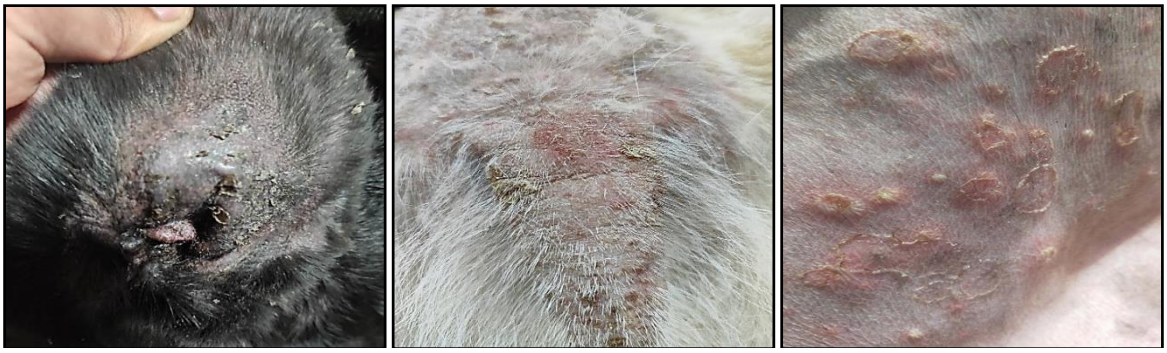


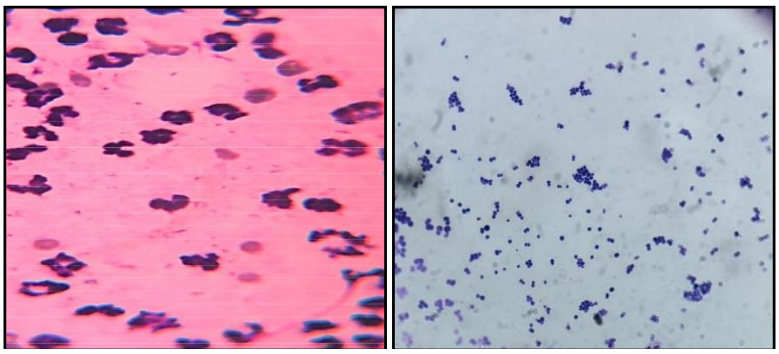
Fig 1: Sample collection using a sterile swab

(a) (b) (c)



(d) (e) (f)

Fig 2: Clinical signs of canine pyoderma in dogs
(a) Erythema (b) Papule (c) Pustule (d) Scales (e) Crusts (f) Epidermal collarettes



(a) (b)

Fig 3: Impression smear examination of isolated organism
(a) Degenerated neutrophils, (b) Microscopic examination of cotton swab smear showing cocci (Grams staining, x1000)

Fig 4: Growth of *Staphylococcus* spp. on nutrient agar (NA)

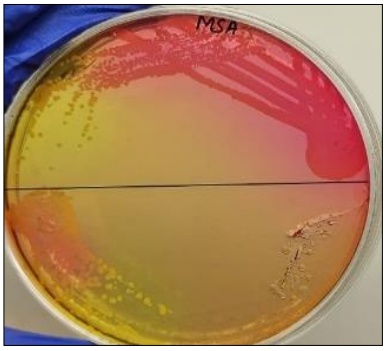


Fig 5: Growth of *Staphylococcus* spp. on mannitol salt agar (MSA) showing pink to yellow color colonies

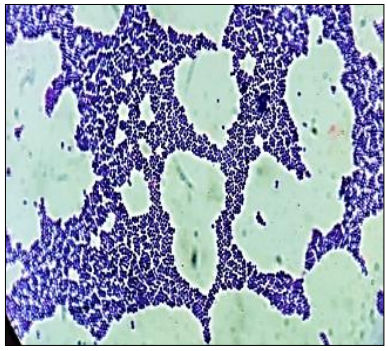
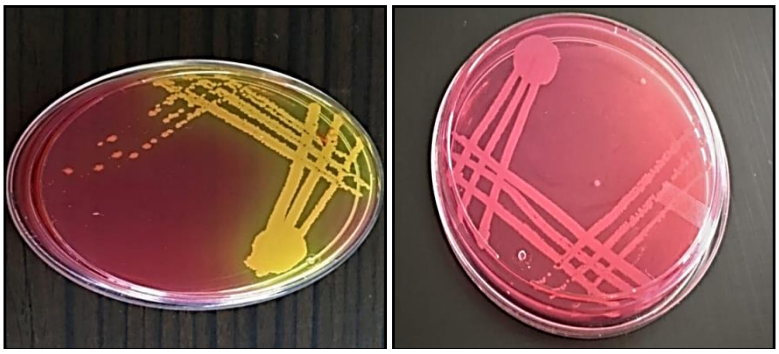


Fig 6: Microscopic identification of bacteria by gram staining x1000) showing clusters of gram positive *Staphylococcus* spp

(a) (b) (c)

Fig 7: Biochemical tests for *Staphylococcus* spp., (a) Catalase test positive, (b) Tube coagulase test positive, (c) Oxidase test negative



(a) (b)

Fig 8: Mannitol fermenting and mannitol non fermenting *Staphylococcus* spp., (a) Fermentation by *Staphylococcus* spp. on mannitol salt agar, (b) Non fermentation of mannitol salt agar by *Staphylococcus* spp.

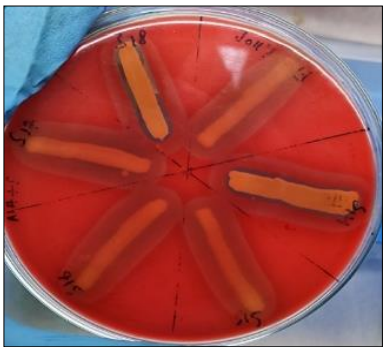


Fig 9: Alpha and beta haemolysis of *Staphylococcus* spp. on blood agar

4. Conclusion

Current epidemiologic trends on the prevalence of dog skin infections and our understanding of the precise causative agents may be crucial in controlling and preventing infections as well as modifying current treatment regimens. The major pathogen isolated and identified in canine pyoderma was *Staphylococcus* spp. The present study emphasizes the need for bacterial isolation with species identification and antimicrobial sensitivity test in order to careful selection of antibiotics based on results of susceptibility testing, to reduce the selection of multidrug resistant *Staphylococci*.

5. Future scope

Further scope of isolation and antibiogram profiling of *Staphylococcus* species in canine pyoderma involves advancement in genomic sequencing to better understand antibiotic resistant mechanisms, developments of targeted therapies based on individual bacterial profiles, and implementation of preventive strategies to reduce antibiotic resistance.

6. Acknowledgement

It is with great gratitude that the authors acknowledge the Dean, College of Veterinary Sciences and Animal Husbandry, NDVSU, Jabalpur for providing the necessary facilities for carrying out the study.

7. Disclosure statement

No potential conflict of interest was reported by the author(s).

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