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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(7): 662-666

www.thepharmajournal.com
Received: 19-05-2019
Accepted: 21-06-2019

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Isolation and identification of *Staphylococcus aureus* from contaminated wounds of dogs

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Abstract
The aim of this study was to isolate and identify *Staphylococcus aureus* from contaminated wounds of dogs. Total 24 swab samples were collected from the contaminated wounds of dogs. The collected swab samples were streaked on mannitol salt agar plates where after incubation the colour of mannitol salt agar was changed to metallic yellow colour which indicated the presence of *Staphylococcus* species. Further, biochemical characterization of isolated bacteria was done by Hi Staph Identification Kit, where 14 samples were biochemically positive out of 15 gram positive samples.

Keywords: *Staphylococcus aureus*, mannitol salt agar, Hi staph identification kit, biochemical characterization

Introduction
Staphylococcus aureus is both a commensal bacterium and a human pathogen. Approximately 30 per cent of the human population is colonized with *S. aureus*. Simultaneously, it is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary infections.
Microbial pathogens delay wound healing through several different mechanisms, such as persistent production of inflammatory mediators, metabolic wastes and toxins, and maintenance of the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals (Laato *et al*., 1988; Pope, 1993) [12]. In addition, bacteria compete with host cells for nutrients and oxygen necessary for wound healing (Rode heaver, 1997) [13]. *Staphylococcus aureus* is also the commonest wound pathogen reported.
To keep all these things in mind, in the present investigation, initially the swab samples were collected from contaminated wounds of dogs for isolation of *Staphylococcus aureus.* Further, these isolated organisms were identified by gram's staining and biochemical characterization.

Material and Methods
The proposed work was conducted in the Department of Veterinary Pharmacology and Toxicology in collaboration with Department of Veterinary Microbiology and Teaching Veterinary Clinical Complex (T.V.C.C), College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P).
The dogs presented with contaminated wound at TVCC, College of Veterinary Science and Animal Husbandry, Jabalpur were selected for the present study. The samples were collected from contaminated wound of dogs using sterile swab (Das, 2013) [4].

Isolation of bacteria
Collected samples were mixed with sterile nutrient broth taking all the aseptic measures and incubated overnight at 37 °C. The samples were then streaked on Mannitol salt agar plates and incubated for 24-48 hrs at 37 °C. All plates were examined for characteristic colony morphology of different bacteria. Single suspected colony from each sample was streaked on nutrient agar slants and was further identified by different phenotypic characteristics (Das, 2013) [4].

Microscopic identification
The bacteria isolated on mannitol salt agar were subjected to Gram's staining and the slide was viewed using a compound microscope under oil immersion (Das, 2013) [4].

Biochemical characterization
All isolates were biochemically characterized using Hi Staph™ Identification kit from HIMEDIA.

Results and Discussion
Isolation and Identification of *Staphylococcus aureus*
Isolation and identification of *Staphylococcus aureus* was done by collecting swab sample from contaminated wounds of dogs presented at TVCC, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P). Total 24 swab sample were collected from wounds of dogs.

Isolation of bacteria
Collected samples were inoculated in sterile nutrient broth taking all aseptic precautions and incubated overnight at 37°C. The samples were then streaked on Mannitol salt agar plates and incubated overnight at 37°C. The colour of Mannitol salt agar was changed to metallic yellow colour which indicated the presence of *Staphylococcus* species in the collected samples (Plate 01).

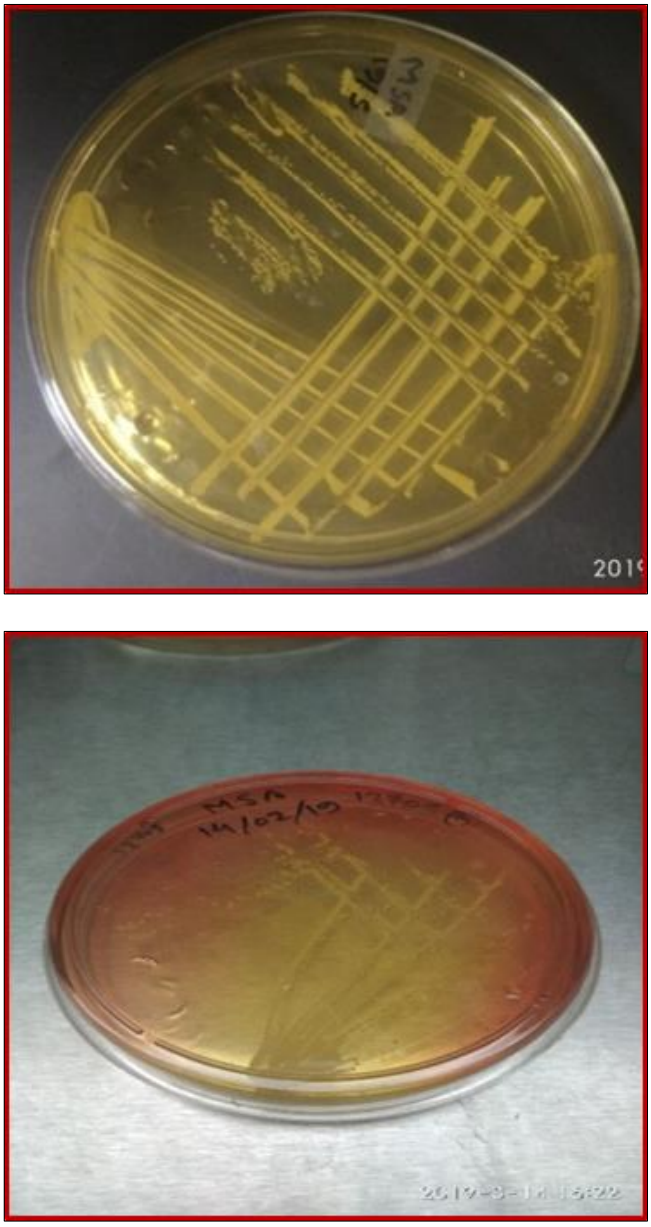

Fig 1: Growth of bacteria isolated from contaminated wounds of dogs on Mannitol salt agar plate

Identification of bacteria
A. Microscopic Identification:
Identification of bacteria was done as per the method described by Das (2013) [4] with slight modification. The bacteria isolated on mannitol salt agar were subjected for gram's staining which showed the presence of gram positive cocci. Out of 24 samples, 15 were positive for gram's staining (Table 01, Plate 02).

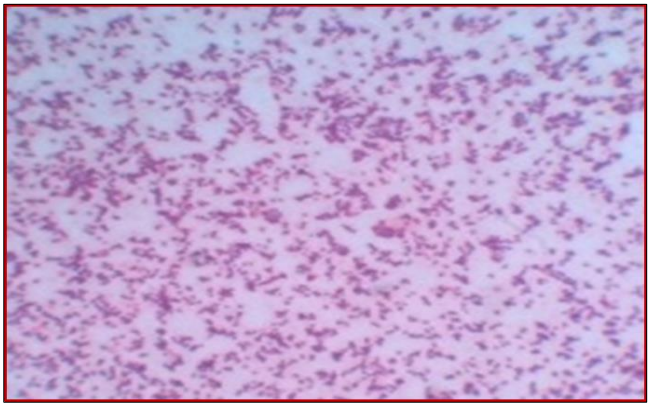

Fig 2: Gram positive cocci isolated from contaminated wounds of dogs

Table 1: Identification of bacteria by Gram's staining

Sample No.	Gram's Staining
1	Negative
2	Positive
3	Positive
4	Negative
5	Positive
6	Negative
7	Positive
8	Positive
9	Positive
10	Positive
11	Negative
12	Negative
13	Positive
14	Positive
15	Positive
16	Negative
17	Negative
18	Positive
19	Negative
20	Positive
21	Positive
22	Negative
23	Positive
24	Positive

B. Biochemical characterization
15 gram positive isolates were biochemically characterized by using Hi Staph identification kit. Fourteen isolates were positive for VP test, AP test, ONPG, Urease, Arginine, Mannitol, Lactose, Trehalose and Maltose but negative for Sucrose, Arabinose, and Rafinose. One isolate was found positive for only Arginine, Mannitol, and Lactose and negative for VP test, AP test, ONPG, Urease, Sucrose, Arabinose, Rafinose, Trehalose and Maltose (Table 02, Plate 03). These results indicate that out of 15 gram positive samples only 14 samples were biochemically positive for *Staphylococcus aureus* (Table 03). In the present study, *Staphylococcus aureus* was characterized by

Various biochemical tests. Jakee *et al.* (2008) [5] also characterized *Staphylococcus aureus* by various conventional biochemical tests. Kahsay *et al.* (2014) [6] isolated 39.7 per cent *Staphylococcus aureus* from surgical wounds of human beings. On the other hand, Kassam *et al.* (2017) [7] found that *Staphylococcus aureus* was the most common isolated bacteria from the wounds caused by trauma (40.0 per cent) and surgical site infection (20.6 per cent). *Staphylococcus aureus* and coagulase negative staph were the organisms which were abundantly present with 14.83 per cent and 10.43 per cent, respectively (Ayub *et al*., 2015) [1]. In present investigation also *Staphylococcus aureus* was isolated from 58.33 per cent of contaminated wound of dogs.

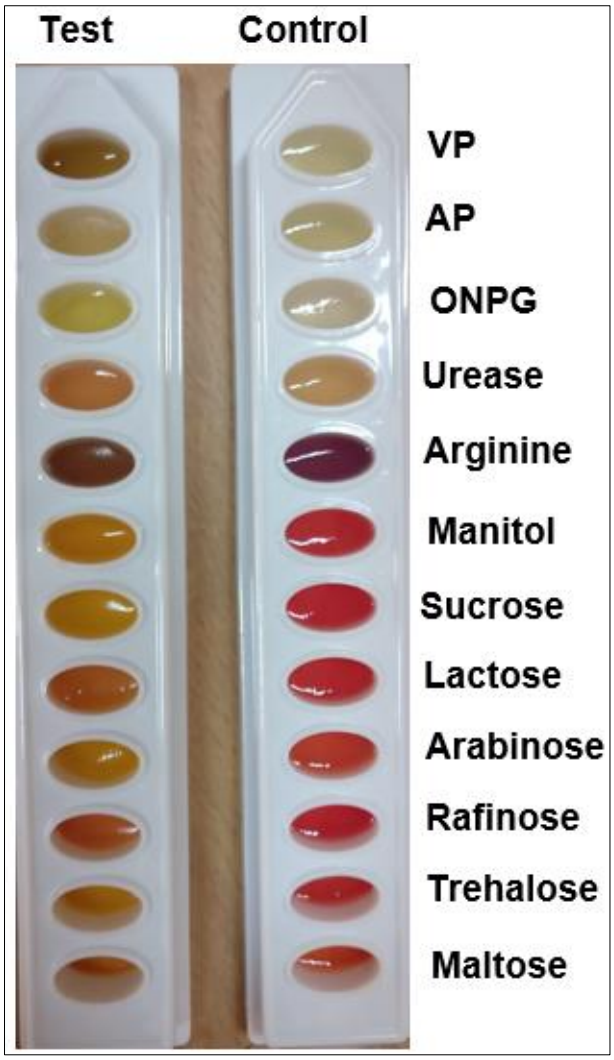

Fig 3: Biochemical test showing presence of *Staphylococcus aureus* in contaminated wounds of dogs

Table 2: Biochemical tests of bacteria isolated from contaminated wounds of dogs

Sample No.	VP test	AP test	ONPG	Urease	Arginine	Manitol	Sucrose	Lactose	Arabinose	Rafinose	Trehalose	Maltose
2	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
3	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
4	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
7	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
8	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
9	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
10	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
13	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative	Negative
14	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
15	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
18	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
20	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
21	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
23	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive
24	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive

Table 3: Identification of isolated bacteria by Gram's Staining and biochemical test

Sample. no	Gram's Staining	Biochemical test
1	Negative	Negative
2	Positive	Positive
3	Positive	Positive
4	Negative	Negative
5	Positive	Positive
6	Negative	Negative
7	Positive	Positive
8	Positive	Positive
9	Positive	Positive
10	Positive	Positive
11	Negative	Negative
12	Negative	Negative
13	Positive	Positive
14	Positive	Positive
15	Positive	Positive
16	Negative	Negative
17	Negative	Negative
18	Positive	Positive
19	Negative	Negative
20	Positive	Positive
21	Positive	Positive
22	Negative	Negative
23	Positive	Positive
24	Positive	Negative

Table 4: Percentage of gram positive and biochemically positive bacteria isolated from contaminated wounds of dogs

Particular	Number of samples	Percentage
Total number of samples	24	100
Gram positive samples	15	62.50
Biochemically positive samples	14	58.33

Wound infections are the commonest complications and one of the most frequently encountered. These infections complicate illness, because anxiety, increase patient discomfort and can lead to death (NINSS, 2002 & Cooper *et al.* 2003) [3]. The development of an infection will be influenced largely by the virulence of the organism and immunological status of the patient. Once a diagnosis of wound infection has been verified and antibiotic sensitivities detected, correct management regimens should be considered, with a high precession given to decreasing the chance of cross infection (Collier, 2001) [2]. Since wound colonization involves not only one type of potential pathogen but numerous types of microbes that can cause wound infections (Mousa, 1997) [9]. Although, it is a widespread opinion among practitioners that primary cause of delayed healing and infection in wounds, are aerobic or facultative pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *beta-hemolytic streptococci*. The incidence of occurrence of *S. aureus* is high in wounds therefore it is considered as the most problematic (Periti *et al*., 1998) [11]. The findings of present study are in agreement where, *Staphylococcus aureus* is the pathogen isolated from 58.33 per cent of contaminated wound of dogs.

Conclusion

Isolation and identification of bacteria was done from contaminated wounds of dogs were out of 24 swab samples collected 15 were gram positive. However, 14 samples were biochemically positive for *Staphyloccus aureus. Staphylococcus aureus* is the pathogen isolated from 58.33 per cent of contaminated wound of dogs.

References

1. Ayub M, Rizwan H, Siddique S, Maryam U. Isolation of Pathogens Causing Sepsis, Pus and Infected Wounds from Critical Care Unit: A Retrospective Study. Annals of Clinical and Laboratory Research. 2015; 3:4-5.
2. Collier MA. Ten-point assessment plan for wound management. Journal of Community Nursing. 2001; 16:22-26.
3. Cooper R, Kingsley A, White R. Wound Infection and Microbiology: Medical Communications (UK) Ltd for Johnson & Johnson Medical, 2003.
4. Das S. Occurrence and characterisation of bacterial dermatitis in dogs with special reference to therapeutic approaches using honey mvsc. Thesis (Department of Veterinary Medicine, Ethics and Jurisprudence). West Bengal University of Animal and Fishery Sciences Kolkata (WB), 2013.
5. Jakee J, Nagwa AS, Bakry M, Zouelfakar SA, Elgabry E, El-Said WAG *et al.* Characteristics of strains isolated *Staphylococcus aureus* from human and animal sources. American-Eurasian Journal of Agriculture and Environmental Sciences. 2008; 4(2):221-229.
6. Kahsay A, Mihret A, Abebe T, Andualem T. Isolation and antimicrobial susceptibility pattern of *Staphylococcus aureus* in patients with surgical site infection. Archives Public Health. 2014; 72(1):7-13.
7. Kassam NA, Damian DJ, Kajeguka D, Nyombi B, Kibiki GS. Spectrum and antibiogram of bacteria isolated from patients presenting with infected wounds. BMC Res Notes. 2017; 10:1-11.
8. Laato M, Niinikoski J, Lundberg C, Gerdin B. Inflammatory reaction in blood flow and experimental wounds inoculated with *Staphylococcus aureus*. European Surgical Research. 1988; 5(20):33-38.
9. Mousa HA. Aerobic, anaerobic and fungal burn wound infections. J Hosp Infect. 1997; 37:317-323.

10. NINSS. Surveillance of Surgical Site Infection in English Hospitals: a national surveillance and quality improvement programme. Public Health Laboratory Service, 2002.
11. Periti P, Tonelli F, Mini E. Selecting antibacterial agents for the control of surgical infection: mini-review. Journal of Chemotherapy. 1998; 10:83-90.
12. Pope ER. Current concepts of wound management. Veterinary Science Research Article. 1993; 2(5):43.
13. Rodeheaver GT. Wound cleansing, wound irrigation, wound disinfection in chronic wound. Journal of Health Management. 1997; 5(3):97-108.