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STAPHYLOCOCCUS AUREUS ISOLATION FROM ANIMATE AND INANIMATE SAMPLES

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Abstract
Staphylococcus aureus is a common skin colonizer as well as opportunistic pathogen causing serious diseases including bacteremia, endocarditis and a number of different infections. So the aim of this study is to detect the presence of *Staphylococcus aureus* in animate and inanimate samples which can be achieved by the following, 40 samples were examined for presence of *S.aureus* on mannitol salt agar, the suspected colonies were identified by Gram staining. Molecular identification of *S.aureus* by polymerase chain reaction (PCR) through detection of 23SrRNA gene and *clfB* gene specific for *S.aureus*. This study showed that 25 out of 40 samples were appearing positive Staphylococci through conventional methods of isolation and were positive *S.aureus* according PCR technique. 18 out of 25 (72%) *S.aureus* isolates detected positive of *clfB* gene.
Keywords: *Staphylococcus aureus*, animate, inanimate and infection

1. INTRODUCTION

Staphylococcus aureus is a non-motile, non-spore forming, spherical gram positive, and facultative anaerobic firmicute bacterium of family Staphylococcaceae. *Staphylococcus aureus* commonly resides in the nasal passages, skin and mucous membranes of the animal and human. It is the most important pathogen of various sub clinical and clinical infections in the human and animals. It is most notorious for causing bacteremia in human with high mortality and morbidity as compared to other organisms. In the humans, it also cause serious infections, particularly in persons debilitated by traumatic injury, chronic illness, and immunosuppression. These infections include deep abscesses, pneumonia, osteomyelitis, furuncles, impetigo, phlebitis, endocarditis, boils, styes, meningitis, toxic shock syndrome and are often associated with hospitalized patients rather than healthy individuals in the worldwide communities (Carter *et al*., 1990). *Staphylococcus aureus* also known to cause variety of suppurative infections, septicemia and toxinoses in domestic birds and animals such as udder impetigo, mastitis, pyoderma, endometritis, dermatitis, cystits, arthritis, botryomycosis of mammary gland, bumble foot, scirrhous cord and tick pyaemia (Naber, 2009).

Staphylococcus aureus is unique with many typical phenotypic characteristics as rigid cell due to high content of teichoic acid helping in tolerance to high salt, drying and sucrose concentration enabling it to grow at a temperature range of 15 to 45ºC and at sodium chloride concentrations as high as 15%. This is grow in grape bunches clusters, pairs and occasionally in short chains that grow by aerobic respiration or by fermentation that yields principally lactic acid. However, the organism *S. aureus* was named so because of production of golden coloured colonies but production of variable pigments viz. pale yellow, mustard and white by this organism has also been discovered (Jonsson and Wadstrom, 1993, Khichar, 2011). The organism also indicate variations in coagulase activity, fermentation reactions haemolytic patterns, thermostable nuclease phenomena, DNase activity, slime production and biofilm formation (Yadav *et al.*, 2015a). Likewise, variations in other phenotypic properties have also been discovered by many workers.

2. Materials and method

a. Collection of samples

A total 40 samples of inanimate and animate collected from different sources *viz.* tea stalls, ATM, hostel and animate samples *viz.* nasal and saliva swab samples from camel and horse staphylococcus bacteria were collected from domestic area of Bikaner Rajasthan. Twenty five isolates were collected from variety of sources. The samples were collected in the morning aseptically and were immediately taken thereafter to the laboratory on ice for further processing. All experimental work was performed in the Department of Veterinary Microbiology and Animal Biotechnology, University of Veterinary and Animal Sciences, Bikaner.

b. Microbial media
Nutrient Agar, Nutrient Broth, Mannitol Salt Agar, Muller Hinton Agar were used at different steps.

c. Identification and characterization of isolated strains
Isolated strains were initially identified by Gram-staining as well as Mannitol fermentation testing.

d. Identification of the bacterial culture under microscope
Smears from the purified colonies were stained with Gram's stain and examined microscopically under oil immersion lens (Quinn *et al.* 2002). The typical colonies were showed gram-positive cocci occurring in bunched, grape like irregular clusters were taken as presumptive Staphylococcus species.

e. DNA Isolation
DNA isolation was carried out as per the method of Nachimuttu *et al.* (2001) with some modifications.

f. Molecular identification by PCR
In this study PCR technique was carried to confirm the presence of *S.aureus* by detection of 23S rRNA gene specific for Staphylococci and *clfB* gene specific for *S.aureus* from culture samples through the following steps (S. Nagar *et al.* 2017).

Gene	Primer sequence (5'-3')	Size	Reference
23S rRNA	F-5'-ACG GAG TTA CAA AGG ACG AC-3' R-5'-AGC TCA GCC TTA ACG AGT AC-3'	1250 bp	(Straub *et al.*, 1999)
clfB	F-5'-ACA TCA GTA ATA GTA GGG GGC AAC-3' R-5'-TTC GCA CTG TTT GTG TTT GCA C-3'	205bp	(Tristan *et al.*, 2003)

Table 1: Primers (Merk) used for PCR

Cycles	Step	Temperature (ºC)	Time	No. of cycles
1.	Initial Denaturation	94	3 min.	30
2.	Denaturation	94	1 min	
	Annealing	49-55	1 min.	
	Extension	72	1 min.	
3.	Final extension	72	7 min.	

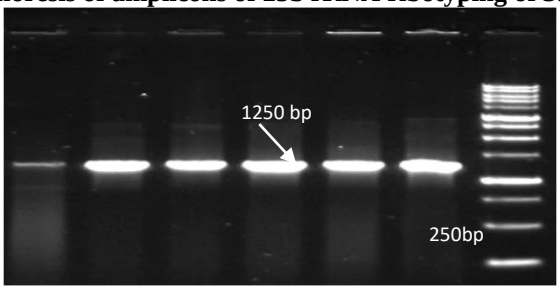
Table 2: Cycling conditions of the primers during PCR

For PCR amplification, using specific primers for gene as shown in Table 1. A simplex reaction mixture 25µl contained 1µl of Forward primers, 1µl of Reverse primers for each gene separately; 12.5µl Go Taq® Green Master Mix, 2X, 3µl Template DNA and 7.5µl nuclease free water. The tubes were subjected to the Veriti Thermal Cycler (Applied biosystem) with programme described in table 2.Amplified products were separated in 1.2% agarose gels prepared in 1x TBE buffer containing 0.5 µg/ml of ethidium bromide and 250 and 100 bp ladder was used as molecular marker. The amplification products were electrophoresed for 50-60 min at 100 V. The gel was then visualized under gel documentation system (ENDURO GDS).

3. Result and Discussion
Isolation and identification of Staphylococcus isolates from animate and inanimate samples. According to conventional method of identification through culture on mannitol salt agar and microscopic identification, they were 25 samples positive for *S.aureus* (inanimate isolates 4 from Tea Stall, 3 from Bank ATM and 3 from Hostel and animate isolates 6 from camel and 9 from horse). They have yellow colonies on mannitol salt agar microscopically appear gram positive cocci, arranged in clusters, non-spore forming bacteria.

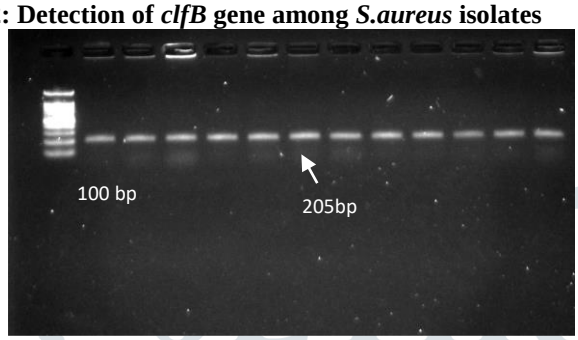
A. Molecular identification of *staphylococci*
All 25 isolates were examined by PCR using 23S rRNA gene specific for genus Staphylococcus and *clfB* gene specific for *S.aureus*. 18 out of 25 isolates were positive Staphylococci as shown in Figure 1, and all of them were *S.aureus* according *clfB* gene result as show in Figure 2.

Fig 1: Agarose gel electrophoresis of amplicons of 23S rRNA ribotyping of *S. aureus* isolated from various sources



Molecular ladder -250bp Product size-1250

Fig 2: Detection of *clfB* gene among *S.aureus* isolates



Molecular ladder-100bp Product size-205 bp

A total 25 (62.5%) *S.aureus* isolates were detected from 40 samples of inanimates and animates, all the isolates were mannitol fermenter gram positive cocci arranged in clusters. The colour of the colonies of *S.aureus* is due to a pigment namely staphyloxanthin which has been considered as important factor in alleviating stress to the organisms. And nearly similar finding to that assumed by who isolated *S.aureus* (Quinn *et al*., 1994; Adisiyun *et al*., 1999; Salasia *et al*. (2004). A variation in the colony pigmentation of *S. aureus* from skin wounds in camel was also reported by Qureshi and Kataria (2012). Similarly Islam *et al*. (2007) observed 28 *S.aureus* of bovine origin. Sanjiv *et al*. (2008) found 95.23% *S. aureus* from bovine mastitis milk origin. In the other study of El-Jakee *et al*. (2010) isolated *S. aureus* from mastitic cows and buffalo and Khichar (2011) also studied variation in the pigment production by *S. aureus* isolates of bovine mastitic origin.

In this study the 25 *S.aureus* isolates were conducted for PCR showing positive using 23SrRNA gene and of the total 25 isolates *clfB* gene were detected in 18 (72%) isolates with single amplicon of 205 bp. PCR is highly sensitive while conventional method is less sensitive as a result some microorganism give positive reaction by culture but give negative by PCR (S. Nagar *et al*. 2017).

4. Conclusion

In the present study describe the infection through animate and inanimate sources which could create health hazard when they are ingested, or they come in contact with the human skin. This study highlights the need for the public awareness to hygiene following the use of public facilities. Therefore, this study can form a ground work for epidemiologists and with the emergence of global infectious diseases like Covid-19 and SARS a lot of supermarkets have been implementing measures of hygiene by providing disinfectants at entry and at several critical contamination points such as ATM, Tea stalls and other public places. This could be taken as a step forward to minimize hand contamination. Such approaches should be undertaken in parallel with community education for hygienic standards, respiratory etiquette and hand washing.

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