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Molecular Characterization of *Staphylococcus aureus* of Camel (*Camelus dromedarius*) Skin Origin

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A B S T R A C T

In the present study, a total of 95 skin samples were collected from various districts of Rajasthan. Out of 95 skin samples, 67 samples were positive for dermatitis and 49 samples showed presence of different Gram's positive bacteria after growth on Mannitol salt agar medium. Out of 49 Gram's positive bacteria, only 30 (61.22 per cent) isolates could be confirmed as *S. aureus* through 23rRNA based ribotyping with an amplicon of 1250 bp.

Introduction

Staphylococcus aureus is gram positive, ubiquitous, pathogenic bacteria found mainly in nose and skin of animals. It is known to cause variety of suppurative infections and has always been a major cause of wounds and abscesses and mastitis in animals.

Over the past few decades the organism has come up as a leading cause of hospital and community acquired infection in human subjects causing endocarditis, deep-seated abscesses and bacteremia leading to toxic shock syndrome. The disease caused by *S. aureus* is not always fatal but an indirect great economical loss is incurred due to reduced working efficiency. The abscesses and wounds caused by *S. aureus* spread rapidly over the body surfaces and become very difficult to manage making animal useless for any economical purpose. The camel has a low susceptibility to diseases but skin involvements like contagious skin necrosis, dermatitis, wounds, abscesses or similar problems were commonly observed in camels

(Rutter and Mack, 1963; Semushkin, 1968; Edlesten and Pegram, 1974; Domenech *et al.*, 1977).

Materials and Methods

Bacteriological sampling, isolation and identification of Staphylococcus aureus

Bacteriological sampling in cases of superficial lesions

Top of pustule/papule or crust was lifted with a sterile 25 gauge needle. A swab was gently pressed on the exposed surface and allowed time for the contents to be absorbed. Then, swab was placed in sterilized nutrient broth medium, for further processing.

Bacteriological sampling in cases of deep lesions

The surface of the affected area was clipped and disinfected with 70 percent ethanol and allowed to dry. The area was then gently squeezed to express exudates and then sampled with swab.

Ajayi *et al.,* (2013) reported that samples were inoculated on Mannitol Salt agar (MSA) and incubated at 37^0C for 24h and 48h. Colonies which appeared yellow on MSA were subjected for further tests. Examination indicated that the organism was Gram positive after the Grams staining technique and that they appeared in clusters and spherical (coccoid) in shape under the microscope.

Genotypic confirmation of organisms by ribotyping

The DNA was isolated as per the method described by Nachimuttu *et al.,* (2001) with some modification. In brief, the overnight grown bacterial culture was pelleted, washed twice with five hundred µLPBS and centrifuged 5000rpm for 5Min and resuspended in 1 ml of Tris-EDTA (TE) solution. One hundred µl of lysozyme solution (conc. 3 mg/ ml) was added and mixture was then incubated at 37°C in water bath for 15 min. One hundred µl of 10% SDS solution and 2 µl of proteinase K (10 mg/ml) solution was added, incubated at 60°C in water bath for 1 h with gentle mixing at 10 min intervals, then 0.75 ml of DNA extraction buffer was added and further incubated for 30 min at 60°C in water bath. An amount of 0.5 ml of penol: chloroform: isoamyl alcohol mixture (25:24:1) was added to the DNA preparation and mixed gently for about 10-15 min, mixture centrifuged at 15000 rpm for 15 min at 20°C and upper aqueous phase containing DNA was transferred to another tube. To this mixture 0.5 ml of cold isopropanol was added and the tube was replaced on ice for 15 min and then centrifuged at 15000 rpm for 10 min at 20°C. The supernatant was discarded and the pellet was dissolved in 0.5 ml of cold 70% ethanol and centrifuged at 10000 rpm for 10 min at 20°C. Supernatant was discarded and the tubes were inverted on a filter paper for 5 min and then left at room temperature for overnight to allow evaporation of alcohol. Next morning the pellet was redissolved in 50 µl of TE buffer and left for 24 h for dissolution of the pellet.

The ribotyping based on 23S rRNA gene was carried out as per the method described by Straub *et al.,* (1999) using Primer-1 (5'-ACGGAGTTACAAAGGACGAC-3') and Primer-2 (5'-AGCTCAGCCTTAACGAGT AC-3').

A total volume of 30 µl reaction mixture for PCR was prepared by mixing 1.0 µl primer-1 (10 pM/µl), 1.0 µl primer-2 (10 pM/µl), 1.0 µl dNTP (10 mM), 5.0 µl 10x Taq buffer A containing, 3.0µl $Mgcl_2$, 0.2µlTaq DNA polymerase(5U/ µl), 11.3 µl deionised water and 2.5 µl DNA (25 ng/µl).

Table.1 Site wise number of isolates used for 23SrRNA based ribotyping

S. No.	Site	No. of sample	No. of isolates obtained (code numbers)
1.	Neck	3	2(1, 3)
2	Chest pad	4	3(7, 15, 24)
3.	Tail	10	6(2, 9, 23, 30, 39, 43)
4.	Abdomen	3	1 (35)
5.	Nostril	4	2 (27, 41)
6.	Hump	10	6 (5, 19, 25, 37, 40, 45)
7.	Fore Legs	7	4(4, 11, 33, 43)
8	Hind Legs	8	6(6, 8, 13, 17, 22, 31)

Table.2 Isolates confined to be *S. aureus* based on 23SrRNA based ribotyping

S. No	Gene type	Isolate numbers	Total isolates	Amplicon Size(bp)
1	23SrRNA	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 17, 19, 22, 23, 24, 25, 27, 30, 31, 33, 35, 37, 39, 40, 41, 43, 45, 48	30	1250

Fig.1 Photograph showing yellow colonies of *S. aureus* in Mannitol Salt Agar

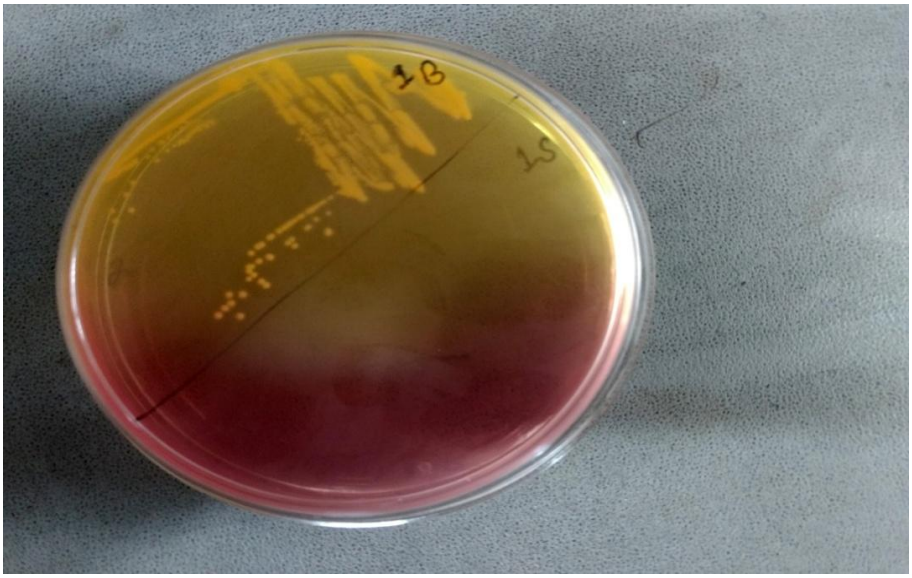


Fig.2 Photograph showing *S. aureus* grape cluster like morphology on Gram stain. 1000X

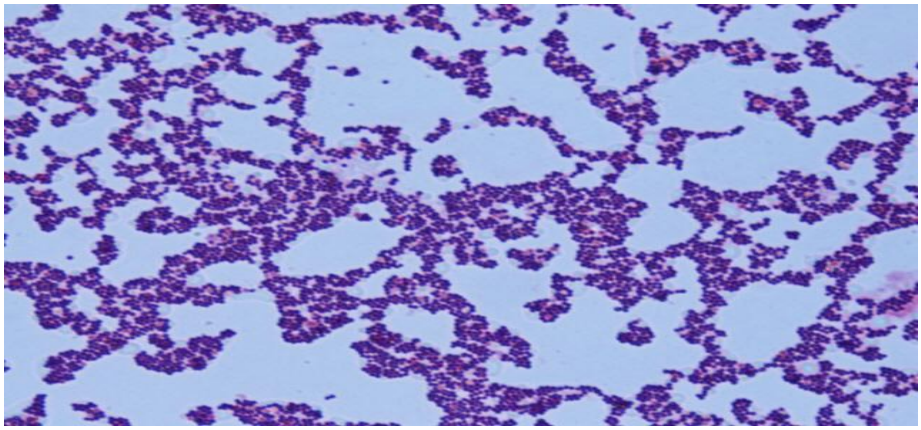


Fig.3 23SrRNA ribotypic *of S. aureus* obtained from camel skin lesions
M-Molecular marker (250bp)

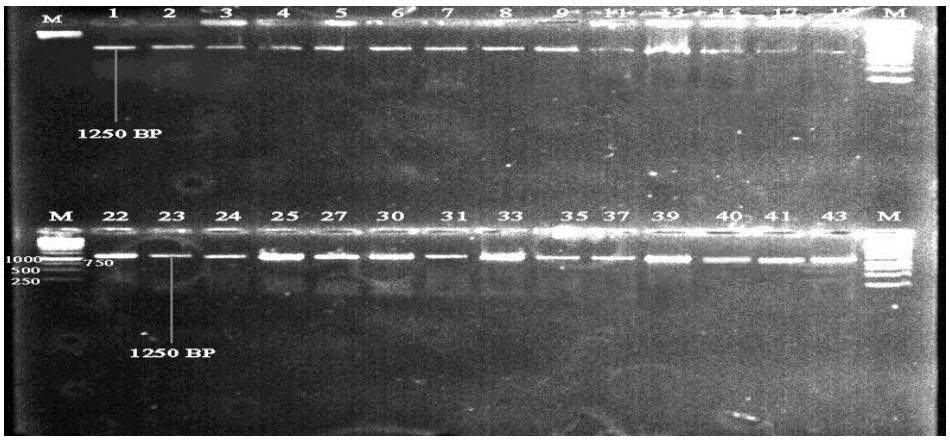
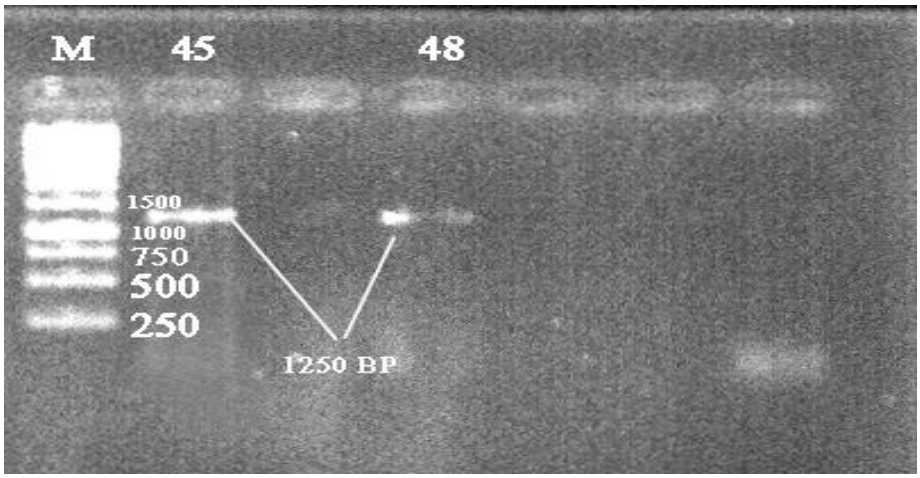


Fig.4 23SrRNA ribotypic *of S. aureus* obtained from camel skin lesions
M-Molecular marker (250bp)



The denaturation, primer annealing and primer extension was carried out at 94°C, 55°C and at 72°C, respectively, in each cycle and the time given for denaturation, primer annealing and primer extension for cycle 1 was 300 sec, 30sec and 75 sec, respectively; for cycle 2-37 it was 40sec, 60 sec and 75 sec, respectively; and for cycle 38 it was 60 sec, 60 sec and 180 sec, respectively. The PCR products, after addition of 2 µl of trekking dye were resolved in 1.0 % agarose gels prepared in 1.0 x TBE buffer containing 0.5 µg/ml of ethidium bromide and 250bp DNA ladder was used as molecular marker. The amplification products were electrophoresed for 1h 30 min at 100 V. The gel was then visualized under U.V. transilluminator

Results and Discussion

In the present investigation, out of 95 skin samples of camel, 49 samples showed presence of different Gram's positive bacteria after growth on Mannitol salt agar medium(Figs. 1 and 2). Out of 49 Gram's positive bacteria, only 30 isolates could be confirmed as *S. aureus* through 23rRNA based ribotyping with an amplicon of 1250 bp. (Table 1 and 2, Figs. 3 and 4).

In our study, the overall *S. aureus* prevalence was recorded as 61.22%. Similar genotypic method of *S. aureus* identification have been used by Rathore and Kataria (2012), Ariyanti *et al.,* (2011), Dhirenda (2016) for *S. aureus* isolates from elsewhere.

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