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Characterization of *Staphylococcus aureus* isolated from nasal discharge from pneumonic camels (*Camelus dromedarius*)

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Abstract. In the present investigation 15 *Staphylococcus aureus* isolates obtained from 46 nasal swabs from pneumonic camels were characterized for their sugar fermentation, coagulase production, hemolysis and antibiotic resistance properties. The isolates were grouped into seven fermentation types, human plasma was better coagulated than cattle plasma. Beta-haemolysis was produced by eight isolates whereas seven showed α-haemolysis on blood agar. All the isolates showed multiple antibiotic resistances as they were resistant to cefepime, cefotaxime, nalidixic acid and penicillin and all isolates were sensitive to ofloxacin, ciprofloxacin, imipenem, chloramphenicol, carbenicillin and gentamicin.
Key Words: *Staphylococcus aureus*, pneumonia, camel, antibiotic resistance.

Introduction. *Staphylococcus aureus* is a prominent bacterial pathogen associated with pneumonia in human and animals (Ragle et al 2010; Alhendi 1999; Rahimi & Alian 2013) though it also causes various other infections like mastitis, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and nosocomial infections in man and animals (Kluytmans et al 1997; Sousa & Lencastre 2004). Pulmonary diseases are among the emerging problems of camels that are causing considerable loss in production and death (Bekele 1999; Zubair et al 2004; Kane et al 2005). The pathogenicity of *S. aureus* is related to many virulence determinants making this organism capable to colonize and spread in tissues, evade phagocytosis, necrose tissue, haemolyse erythrocytes, etc. In addition, *S. aureus* is also endowed with property to acquire antibiotic resistance in short time which make it more potential threat (Bien et al 2011; Rathore & Kataria 2012).

The present study was carried out to characterize *S. aureus* from pneumonia in camel in terms of coagulase production, hemolysis, sugar fermentation and antibiotic resistance pattern.

Material and Method

Isolation and identification. A total of 46 samples of nasal discharge from pneumonic camels in and around Bikaner (Rajasthan, India) were collected aseptically with sterile absorbent swabs soaked in nutrient broth in winter season. The samples were inoculated on nutrient agar plates and then processed for isolation and identification of *S. aureus* (Cowan & Steel 1974; Quinn et al 1994). Out of the 46 samples 15 isolates of *S. aureus* were obtained on the basis of phenotypic and biochemical properties. These isolates were

further confirmed genotypically by ribotyping based on 16S-23S rRNA spacer region (Phuektes et al 2001) using following sequences for the two primers, Primer 1: 5'-TCTTCAGAAGATGCGGAATA-3' and Primer 2: 5'-TAAGTCAAACGTTAACATACG-3'.

Characterization. Coagulase activity of each isolate was tested through production of coagulase by tube coagulase test (Gillespie 1943) using sterile human and cattle plasma. Hemolysis activity of isolates was checked on sheep blood agar and sugar fermentation studies were carried out using 12 sugars namely mannitol, maltose, fructose, inositol, dulcitol, raffinose, arabinose, xylose, sucrose, dextrose, mannose, lactose (Cowan & Steel 1974). The antibiotic sensitivity test was carried out on Mueller-Hinton agar against 24 different antibiotics by disc diffusion method (Bauer et al 1966).

Results and Discussion. In the present investigation *S. aureus* were isolated and then identified by phenotypic and biochemical properties. Although *S. aureus* can be identified through primary and secondary biochemical tests but often cultural tests are cumbersome and time consuming. In addition, *S. aureus* has many colony variants (Sousa et al 2011; Qureshi & Kataria 2012). Hence, to overcome the limitations of cultural and biochemical methods, molecular typing has been described in order to obtain an accurate and rapid confirmation (Morandi et al 2009). All the 15 isolates obtained and identified were also genotypically confirmed on the basis of 16S-23S rRNA spacer region as they all produced a species-specific amplicon of 420 bp (Figure 1).

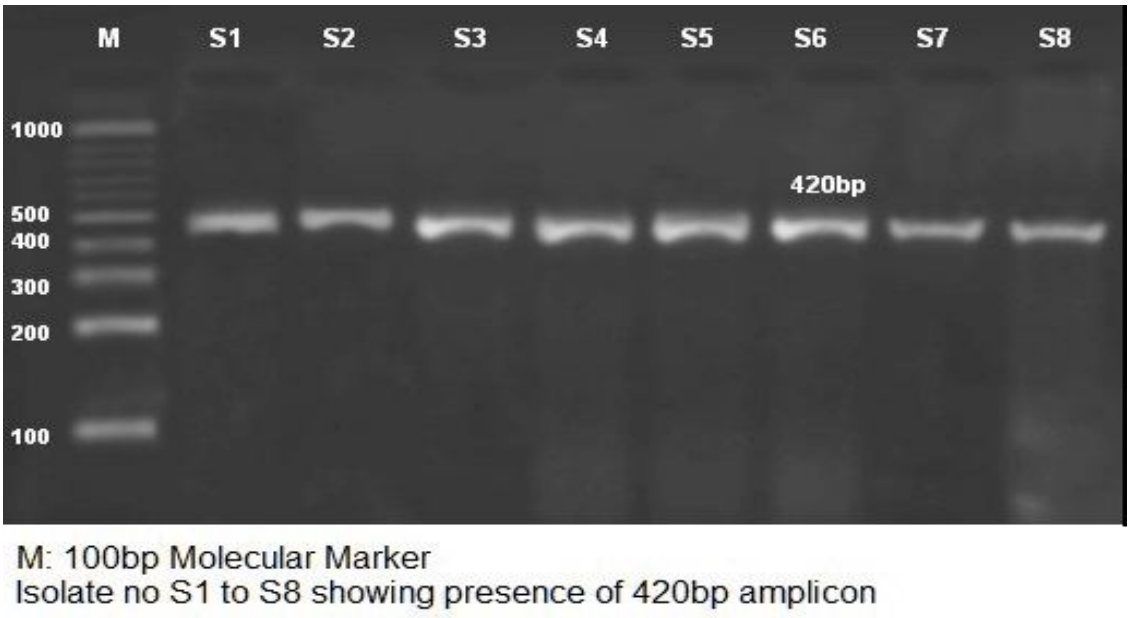

Figure 1. 16S-23S rRNA spacer region (ITS) based genotyping of *Staphylococcus aureus*.

Out of total 46 nasal discharge samples from pneumonic camels, 15 isolates were obtained suggesting 32.6% recovery of this organism. The recovery of *S. aureus* in the present study is similar to the report of Al-Doughaym et al (1999) who also obtained 34.1% *S. aureus* from nasal swabs from pneumonic camels. However, Abubakar et al (2010) recorded only 7% recovery from lung lesions of pneumonic camels.

All *S. aureus* isolates were coagulase positive in tube test using plasmas from cattle and human. Coagulase production is one of the important properties of *S. aureus* and is being used along with some other properties to identify this organism in some laboratories though coagulase negative isolates have also been reported (Sanjiv et al 2008; Momtaz et al 2010).

In a comparison of plasmas from human and cattle former was found superior in coagulation reaction than the cattle plasma. Similar results were documented by

Adesiyun & Shehu (1985) for isolates of food origin, Qureshi et al (2002) for isolates of camel origin and Upadhaya (2009) for isolates of cattle and goat origin.

All the 15 isolates showed hemolysis on sheep blood agar. Among these, only 12 isolates were β-haemolytic (partial haemolysis) of which four isolates later showed hot-cold lysis whereas three isolated were α-haemolytic (complete haemolysis). The hemolysis pattern observed in the present study was similar to that of Ariyanti et al (2011) who reported 100% isolates to be hemolytic and among those 18.18% were α-haemolytic. Our results are in complete agreement to those of Sanjiv & Kataria (2007), Upadhyay & Kataria (2010) who did not record presence of non-haemolytic *S. aureus* of mastitic milk origin. The results are also in complete agreement to those of Qureshi & Kataria (2012) who also reported all the 40 *S. aureus* isolates of camel wound origin to be haemolytic. Contrarily, Solanki & Kataria (2005) reported only 74% of the camel *S. aureus* isolates to be haemolytic. However, a variable percentage of haemolytic *S. aureus* of different origins have been reported by different researchers, 91.9% (El Jakee et al 2010), 42.87% (Coelho et al 2009) and 37.30% (Younis et al 2000).

Table 1

Antibiogram obtained for *Staphylococcus aureus* isolates from nasal discharge of pneumonic camels

No.	*Antibiogram disc*	*Conc. (mcg/disc)*	*Percent (%)*		
			Sensitive	*Intermediate*	*Resistant*
1	Carbenicillin	100	100	-	-
2	Chloramphenicol	30	100	-	-
3	Ciprofloxacin	5	100	-	-
4	Gentamicin	120	100	-	-
5	Imipenem	10	100	-	-
6	Ofloxacin	5	100	-	-
7	Ampicillin/Sulbactum	10/10	93.33	6.66	-
8	Co-trimoxazole	23.75/1.25	93.33	-	6.66
9	Nitrofurantoin	300	93.33	6.66	-
10	Colistin	10	80	13.33	6.66
11	Cephalothin	30	73.33	6.66	20
12	Clindamycin	2	60	40	-
13	Erythromycin	15	60	40	-
14	Neomycin	30	60	40	-
15	Oxacillin	1	60	-	40
16	Minocycline	30	53.33	46.66	-
17	Tetracycline	30	53.33	40	6.66
18	Rifampicin	5	46.66	20	33.33
19	Vancomycin	30	46.66	-	53.34
20	Ampicillin	10	6.66	-	93.33
21	Cefepime	30	-	-	100
22	Cefotaxime	30	-	-	100
23	Nalidixic Acid	30	-	-	100
24	Penicillin	10 units	-	-	100

Sugar fermentation is one of the important biochemical properties of *S. aureus* and different strains shows different fermentations patterns. In the present investigation also a wide variation was recorded as seven groups were obtained on the basis of fermentations of 12 sugars. All the isolates (100%) fermented fructose, dextrose, mannose, maltose and mannitol, 60% sucrose, 26.66% lactose, 13.33% xylose and none of the isolates fermented inositol, dulcitol, raffinose and arabinose. The sugar fermentation reactions in the present study were similar to those of Upadhaya (2009) who recorded 100% fermentation of mannitol, maltose, fructose, dextrose, and mannose. Sanjiv (2006) also reported similar observation for mannitol, maltose and dextrose.

Similarly Qureshi & Kataria (2012) also recorded fermentation of maltose, mannitol by all the camel *S. aureus* isolates in his study. In contrast to present study, Ajuwape & Aregbesola (2001) reported 100% fermentation for sucrose and dulcitol while similar results for mannitol fermentation for isolates of nasal swab samples of rabbits.

In antibiogram study, 100% isolates were resistant to cefepime, cefotaxime, nalidixic acid and penicillin, 93.33% to ampicillin, 53.34% to vancomycin, 40% to oxacillin, 33.33% to rifampicin and 20% isolates were resistant to cephalothin. The 100% isolates were sensitive to ciprofloxacin, carbenicillin, chloramphenicol, gentamicin, imipenem and ofloxacin followed by 93.33% to ampicillin/sulbactum co-trimoxazole and nitrofurantoin, 80% to colistin, 60% to clindamycin, neomycin and erythromycin and 53.33% isolates sensitive to minocycline and tetracycline (Table 1).

Similar to present study Al-Doughaym et al (1999) reported that, isolates obtained from nasal swab samples of pneumonic camels were 100% sensitive to gentamicin and 53.1% isolates were sensitive to tetracycline. Kataria (2008) found similar result for isolates of clinical cattle mastitis origin who reported that isolates were 100% resistant to penicillin, 100% sensitive to gentamicin, 96% sensitive to chloramphenicol 93% sensitive to ciprofloxacin and 95% sensitive to ofloxacin. This decreasing efficacy of antibiotics shows indiscriminate and unwise use of anitbiotics. In agreement to present study, Rathore & Kataria (2012) observed good efficacy for gentamicin, ofloxacin and co- trimoxazole and high resistance for nalidixic acid and ampicillin in the *S. aureus* isolates from camel skin wounds but in opposed to this study, isolates were found to be highly sensitivity for rifampicin, cephotoxime and vancomycin. Qureshi & Kataria (2004) also reported sensitivity pattern for *S. aureus* from camel skin wounds and abscesses and found similar effectiveness for gentamicin. Gitau et al (2011) also recorded highest sensitivity for gentamicin in a retrospective study on antimicrobial sensitivity to *S. aureus* from bovine mastitis.

Conclusions. The study revealed that *S. aureus* isolates obtained from nasal discharge of pneumonic camels showed variability in phenotypic characteristics (sugar fermentation, coagulase production and hemolytic activity). All isolates were confirmed positive in ribotyping method with highest resistance towards cefepime, cefotaxime, nalidixic acid and penicillin (100%) and most effective antibiotics recorded were ofloxacin, ciprofloxacin, imipenem, chloramphenicol, carbenicillin and gentamicin.

References

Abubakar M. S., Fatihu M. Y., Ibrahim N. D. G., Oladele S. B., Abubakar M. B., 2010 Camel pneumonia in Nigeria: Epidemiology and bacterial flora in normal and diseased lung. Afr J Microbiol Res 4(23):2479-2483.

Adesiyun A. A., Shehu L. M., 1985 Detection of staphylocoagulase using plasma from various animals. Vet Microbiol 10:387-392.

Ajuwape A. T. P., Aregbesola E. A., 2001 Biochemical characterization of staphylococci isolated from rabbits. Refu Vet 56(2):45-48.

Al-Doughaym A. M., Mustafa K. M., Mohamed G. E., 1999 Aetiological study on pneumonia in camel (*Camelus dromedaries*) and in vitro antibacterial sensitivity pattern of the isolates. Pak J Biol Sci 4:1102-1105.

Alhendi A. A. B., 1999 Nasal microflora of camels (*Camelus dromedarius*) under two different conditions. Pak Vet J 19(4):164-167.

Ariyanti D., Salasia S. I. O., Tato S., 2011 Characterization of haemolysin of *Staphylococcus aureus* isolated from food of animal origin. Indones J Biotechnol 16(1):32-37.

Bauer A. W., Kirly W. M. M., Sherris J. C., Turck M., 1966 Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493-496.

Bekele T., 1999 Studies on respiratory disease 'sonbobe' in camels in the eastern lowlands of Ethiopia. Trop Anim Health Prod 31(6):333-345.

Bien J., Sokolova O., Bozko P., 2011 Characterization of virulence factors of *Staphylococcus aureus*: novel function of known virulence vactors that are

implicated in activation of airway epithelial proinflammatory response. J Pathog Article ID 601905. DOI:10.4061/2011/601905.1-13.
Coelho S. M. O., Reinoso E., Pereira I. A., Soares L. C., Demo M., Bogni C., Souza M. M. S., 2009 Virulence factors and antimicrobial resistance of *Staphylococcus aureus* isolated from bovine mastitis in Rio de Janeiro. Presq Vet Bras 29:369-374.
Cowan S. T., Steel K. J., 1974 Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press, Cambridge.
El Jakee J., Nagwa Ata S., Gad El-Said W. A., Bakry M. A., Samy A. A., Khairy E. A., Elgabry E. A., 2010 Diversity of *Staphylococcus aureus* isolated from human and bovine estimated by PCR - gene analysis. J Am Sci 6(11):487-498.
Gillespie E. H., 1943 The routine use of coagulase test for Staphylococci. Mon Bull Emerg Publ Hlth Lab Serv 2:19.
Gitau G. K., Wabacha J. K., Mulei C. M., Ndurumo S., Nduhiu J. M., 2011 Isolation rates and antimicrobial sensitivity patterns of bovine mastitis pathogens in peri-urban area of Nairobi, Kabete, Kenya. Ethiop Vet J 15(1):1-13.
Kane Y., Kadja M. C., Bada-Alambedji R., Bezeid O. E., Akakpo J. A., Kaboret Y., 2005 Lung lesions and bacteria of the one humped camel (*Camelus dromedarius*) at Nouakchott slaughter house in Mauritania. Rev Elev Med Vet Pays Trop 58(3):164-171.
Kataria A. K., 2008 Evaluation of some antibiotics against *Staphylococcus aureus* milk isolates from mastitis cases in Rathi cattle. The Indian Cow (15):5-8
Kluytmans J., van Belkum A., Verbrugh H., 1997 Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10(3):505-520.
Momtaz H., Rahimi E., Tajbakhsh E., 2010 Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. Afr J Biotechnol 9(25):3753-3758.
Morandi S., Brasca M., Andrighetto C., Lombardi A., Lodi R., 2009 Phenotypic and genotypic characterization of *Staphylococcus aureus* strains from Italian dairy products. Int J Microbiol Article ID 501362, DOI:10.1155/2009/ 501362, pp. 1-7.
Phuektes P., Mansell P. D., Browning G. F., 2001 Multiplex polymerase chain reaction assay for simultaneous detection of *Staphylococcus aureus* and Streptococcal causes of bovine mastitis. J Dairy Sci 84:1140-1148.
Quinn P. J., Carter M. E., Markey B. K., Carter G. R., 1994 Clinical veterinary microbiology. Wolfe Publishing, London, England.
Qureshi S. D., Kataria A. K., 2012 Biotyping of *S. aureus* of camel (*Camelus dromedaries*) origin. Ruminant Science 1(1):23-25.
Qureshi S., Kataria A. K., 2004 In vitro evaluation of efficacy of some antibiotics against *S. aureus* and other bacterial microflora isolated from skin wounds and abscesses in camel. Journal of Camel Practice and Research 11(1):67-71.
Qureshi S., Kataria A. K., Gahlot T. K., 2002 Bacterial microflora associated with wounds and abscesses in camel (*Camelus dromedarius*) skin. Journal of Camel Practice and Research 9(2):129-134.
Ragle B. E., Karginov V. A., Wardenburg J. B., 2010 Prevention and treatment of *Staphylococcus aureus* pneumonia with a β-Cyclodextrin derivative. Antimicrob Agents Chemother 54(1):298–304.
Rahimi E., Alian F., 2013 Presence of enterotoxigenic *Staphylococcus aureus* in cow, camel, sheep, goat, and buffalo bulk tank milk. Veterinarski Arhiv 83(1):23-30.
Rathore P., Kataria A. K., 2012 Antimicrobial susceptibility profiling of *Staphylococcus aureus* of camel (*Camelus dromedarius*) skin origin. ABAH Bioflux 4(2):47-52.
Sanjiv K., 2006 Phenotypic and genotypic characterisation of *Staphylococcus aureus* isolated from clinical mastitis in indigenous and exotic cross-bred Cattle. M.V.Sc. thesis submitted to the Rajasthan Agriculture University, Bikaner, Rajasthan.
Sanjiv K., Kataria A. K., 2007 Typing and titration of haemolysins produced by *S. aureus* of cattle mastitis origin. Indian J Anim Hlth 46(1):51-55.

Sanjiv K., Kataria A. K., Sharma R., Singh G., 2008 Epidemiological typing of *Staphylococcus aureus* by DNA restriction fragment length polymorphism of coa gene. Veterinarski arhiv 78(1):31-38.
Solanki S., Kataria A. K., 2005 Toxigenic and coagulase types of *S. aureus* isolated from camel. National symposium and XII Annual Conference of IAAVR 2005 held at College of Veterinary and Animal Science, Bikaner pp. 129.
Sousa A. M., De Lencastre H., 2004 Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. FEMS Immunol Med Microbiol 40(2):101–111.
Sousa A. M., Machado I., Pereira M. O., 2011 Phenotypic switching: an opportunity to bacteria thrive. In: Science against microbial pathogens: communicating current research and technological advances. Méndez-Vilas A. (ed), pp. 252-262.
Upadhaya A., 2009 Characterization of *Staphylococcus aureus* with special reference to capsular polysaccharide genotyping isolated from clinically mastitic cattle and goats. M.V.Sc. thesis submitted to the Rajasthan Agriculture University, Bikaner, Rajasthan.
Upadhyay A., Kataria A. K., 2010 Haemolytic properties and titration of haemolysins of *Staphylococcus aureus* of milk origin from cattle and goat with clinical mastitis. Indian Journal of Veterinary Research 19(2):60-65.
Younis A., Leitner G., Heller D. E., Samra Z., Gadba R., Lubashevsky G., Chaffer M., Yadlin N., Winkler M., Saran A., 2000 Phenotypic characteristics of *Staphylococcus aureus* isolated from bovine mastitis in Israeli dairy herds. J Vet Med B Infect Dis Vet Public Health 47(8):591-597.
Zubair R., Khan A. M. Z., Sabri M. A., 2004 Pathology of Camel Lungs. J Camel Sci 1:103-106.

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