DETECTION OF COAGULASE PRODUCTION VARIABILITY AMONG *STAPHYLOCOCCUS AUREUS* ISOLATES OF HUMAN AND ANIMAL ORIGINS

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ABSTRACT

Staphylococcus aureus is one of the most important pathogens causing various clinical and sub clinical infections in human and animals and is often associated with hospital acquired infections in hospitalized patients. This study was attempted to detect variations in coagulase reaction in relation varied *S. aureus* bacterial sources and varied plasma sources. Of the genotypically confirmed 157 *S. aureus* isolates from human and animal sources, 153 (97.4%) isolates were found to be coagulase producers while four (2.5%) isolates did not produce coagulase at 24 hour recording with any of the plasmas. Human plasma showed the best coagulation reaction followed by plasma from camel, poultry, sheep, goat and cattle in decreasing order. It was also observed from a particular species stronger coagulation reaction with plasma from same species. The analysis of coagulase reactions suggested that coagulation activity not only depended on source of bacterial isolate but also on source of plasma.

Key words: *Staphylococcus aureus*, coagulase, plasma, human and animals

Introduction

Staphylococcus aureus is associated with various clinical and sub clinical infections of human and animals. It secretes various exoenzymes such as staphylocoagulase or coagulase, aureolysin, V8 protease, hyaluronate lyase, lipase and staphopain enabling it to cause disease. Of these enzymes, coagulase is important virulent exoenzyme binds to prothrombin to form a complex called staphylothrombin. The protease activity of the thrombin complex is activated, resulting in the conversion of fibrinogen to fibrin. This is suggested to lead to dissemination of staphylococci into deeper and more remote tissues (Guggenberger *et al*., 2012).

Since, coagulase reaction is shown by almost all *S. aureus* strains with variabilities it would be an important simple and accurate tool for epidemiological typing of *S. aureus* from varied geographical regions and source of infections (da Silva and da Silva, 2005; Sanjiv *et al.,* 2008). It was reported that coagulation reaction having some correlation between source of bacterial isolate and source of test plasma (Upadhyay *et al.,* 2010; Yadav *et al.,* 2015).

Though the organism can be identified by conventional methods but because of its phenotypic variations, the molecular methods for its identification are preferred (Tenover *et al*., 1994). *Staphylococcus aureus* shows variable coagulase activity also thus, it's necessary to confirm by the molecular typing approaches (Yadav *et al.,* 2015). Straub *et al*. (1999) developed a PCR system that relied on one primer pair targeted against 23S rRNA based species specific probe, allowing specific detection of all strains of species. Thus, the present work was undertaken with the objectives of detection of variabilities for coagulase reaction among *S. aureus* isolates with different bacterial sources and plasma from different animal species and human.

One hundred and fifty seven isolates were obtained from human clinical infections (35), meat piece (20) clinical infections of horse (3), nasal swab of healthy pigs (2), diseased camels (8), sick dog (6), and unhealthy sheep (6) and from milk of mastitic buffalo (21), goat (28) and cattle (28). The isolates were genotypically confirmed (Fig. 1) by ribotyping method based on 23S rRNA gene identified (Straub *et al*., 1999) by using species-specific primer-1 (5'-ACGGAGTTACAAAGGACGAC-3') and primer-2 (5'-AGCTCAGCCTTAACGAGTAC-3') before subjected to proper test.

Test for free coagulase production

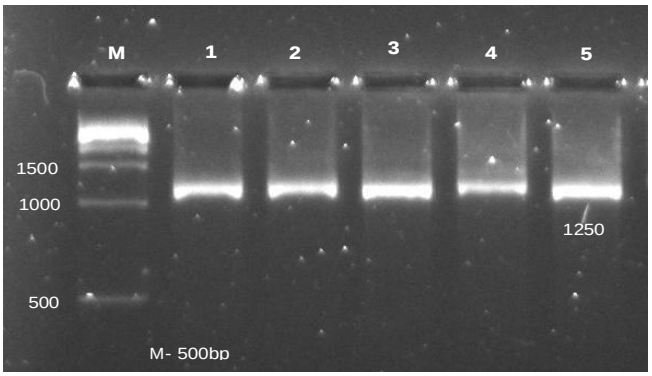

Fig. 1: 23S rrna gene based genotypic confirmation of *Staphylococcus aureus*

The test was carried out in sterile tubes using plasmas from six different animal species *viz*. cattle, sheep, goat, chicken, camel and human. The plasma was diluted to 1:10 in physiological saline solution and 0.5 ml of reconstituted plasma was taken in three serological tubes. A 0.1 ml of an overnight broth culture of the *S. aureus* was added to one tube and 0.1

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ml of broth culture of *S. epidermidis* was added to second tube (negative control) and remaining third un-inoculated tube was kept as control. The tubes were rotated gently to mix the contents and incubated in water bath at 37°C. By slowly tilting the tube at 90° angle, the tubes were examined at 5 h and 24 h for coagulase reaction. Clotting of plasma within 5 h was recorded as a positive for "free" coagulase enzyme. The results for coagulation of plasma from different animal species and human were also recorded for comparison for relative suitability of plasma in this test.

Results and Discussion

Coagulase is an extracellular protein encoded by *coa* gene and is considered as one of the important criteria not only for virulence but also for identification of this organism. In present investigation, production of coagulase was accessed in tube using plasma from six species of animals and human (Fig. 2). The purpose of incorporating different plasma was to access the suitability and interaction between source of isolates and plasma from a different species for use in tube coagulase test. In the present investigation out of 157 isolates, 148 (94.2%) isolates were found to be coagulase producers while nine (5.7%) isolates did not produce coagulase (H8, H48, Mt31, J4, G29, C12, C13, C15 and C50) at 5 h reading but of nine isolates four isolates (H8, H48, Mt31 and C50) showed positive reaction at 24 h. The isolate which did not give coagulase reaction with any of the plasmas was considered coagulase non- producers (Table 1).

Though, coagulase production has been considered to be important criterion in the identification of *S. aureus* but similar to present study, coagulase negative *S. aureus* isolates have also been reported by various workers. Jasper *et al*. (1985) isolated *S. aureus* from cows and found that 1-2% of the organisms did not produce coagulase. Singh *et al.* (2011) had reported coagulase production by only 78.5, 88.3 and 90.7% *S. aureus* isolates obtained from intramammary infections in Sahiwal cattle, Karan Fries cattle and Murrah buffalo, respectively.

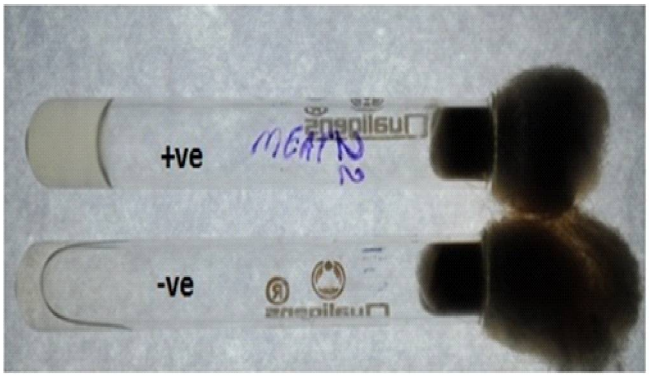

Fig. 2: Coagulation reaction of *Staphylococcus aureus* isolate

Coagulase negative *S. aureus* had also been reported by Citak *et al*. (2003), Wani and Bhatt (2003), Turkyilmaz and Kaya (2006), Sanjiv *et al.* (2008), Kateete *et al.* (2010), Oliveira *et al.* (2010) and Kenar *et al.* (2011), Abd El-Hamid and Bendary (2013) with variable percentage and from various sources.

In the present investigation, some of the isolates showed weak reaction even after 5 h of incubation but showed good reaction at 24 h. Rayman *et al.* (1975) and Turkyilmaz and Kaya (2006) also reported the coagulation of plasma after 24 h. Similarly, Boerlin *et al*. (2003) had also reported that 24 h incubation is necessary for the full sensitivity of coagulase test. They also recorded conversion of weak reactions into stronger one.

In the present study human plasma showed the best coagulation reaction followed by plasma from camel, poultry, sheep, goat and cattle in decreasing order for all studied isolates. The results suggested use of human plasma for the coagulase test for *S. aureus.* The analysis of reactions suggested that coagulation reaction may depends on both, source of isolate and source of plasma. In the present study, more positive reactions were recorded is isolates with plasma from same species of animal.

In our study human plasma was found superior than plasma from any other sources. Bhati (2013) and Kateete *et al.* (2010) also recorded human plasma to give the best coagulation. Our finding corroborated of Duthie and Lorenz (1952) who recorded that plasma of human contained relatively more amount of coagulase activator.

Our results except for dog plasma were also in accordance with those of Adesiyun and Shehu (1985) who evaluated effect of sources of *S. aureus* and plasmas and recorded that the value of plasmas in order of superiority was human and rabbit > pig > donkey > chicken > cattle > duck > goat > dog. Difference in coagulation of plasma from various species was also observed by Qureshi *et al.* (2002) who recorded that *S. aureus* isolates coagulated the plasma from rabbit, human, buffalo, horse, cattle, goat, camel and sheep in decreasing order of superiority the results were similar for human and other plasma but contrary for camel plasma. Similar to present investigation coagulase reaction with human plasma was more (91%) as compared to that with sheep plasma (81%) in identifying *S. aureus* (Kateete *et al.,* 2010).

In agreement to present investigation, Sharma *et al*. (2013) reported 15 (100%) coagulase producing *S. aureus* isolates from 46 nasal swabs of pneumonic camels. Similarly Yadav *et al.* (2015) also found 32 (100%) coagulase positive *S. aureus* from 89 mastitic milk samples of both cattle and buffalo. Strongest coagulation reaction recorded for human plasma followed by pig, rabbit, horse, bovine, chicken, and lamb in decreasing order. Khichar and Kataria (2015) characterize 28 *S. aureus* isolates obtained from 59 mastitis samples of Holstein-Friesian crossbred and Rathi cattle. All of the isolates produced coagulase and the overall strongest coagulation reaction in regards to early onset and firmness of clot was recorded with plasma from rabbit followed by buffalo, cattle, camel, human, goat, sheep, dog, horse, chicken and pig in decreasing order.

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References

Abd El-Hamid MI and Bendary MM (2015) Comparative phenotypic and genotypic discrimination of methicillin resistant and susceptible *Staphylococcus aureus* in Egypt. *Cell Mol. Biol.*

Table 1: Comparison of coagulation of plasmas from different species of animals and human by *Staphylococcus aureus* isolates

S. No.	Source of Isolate (Symbol)	Total No. of isolate	Total coagulase (%)		Plasma for coagulase production (%) at 5h.											
			P	N	Human		Cattle		Poultry		Sheep		Goat		Camel	
					P	N	P	N	P	N	P	N	P	N	P	N
1.	Human (H)	35	33 (94.3)	2 (5.7)	33 (94.3)	2 (5.7)	0 (0)	35 (100)	1 (2.9)	34 (97.1)	0 (0)	35 (100)	0 (0)	35 (100)	22 (62.9)	13 (37.1)
2.	Meat piece (Mt)	20	19 (95.0)	1 (5.0)	19 (95)	1 (5)	0 (0)	20 (100)	2 (10)	18 (90)	0 (0)	20 (100)	0 (0)	20 (100)	16 (80)	4 (20)
3.	Horse (Hrs)	3	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	3 (100)	3 (100)	0 (0)
4.	Pig (Pg)	2	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)	0 (0)
5.	Camel (J)	8	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	0 (0)	8 (100)	0 (0)	8 (100)	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)	7 (87.5)	1 (12.5)
6.	Dog (D)	6	6 (100)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	3 (50)	3 (50)	0 (0)	6 (100)	0 (0)	6 (100)	6 (100)	0 (0)
7.	Sheep (S)	6	6 (100)	0 (0)	6 (100)	0 (0)	1 (16.7)	5 (83.3)	6 (100)	0 (0)	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)	6 (100)	0 (0)
8.	Buffalo (B)	21	21 (100)	0 (0)	21 (100)	0 (0)	4 (19)	17 (81)	2 (9.5)	19 (90.5)	1 (4.7)	20 (95.2)	1 (4.8)	20 (95.2)	21 (100)	0 (0)
9.	Goat (G)	28	27 (96.4)	1 (3.6)	27 (96.4)	1 (3.6)	11 (39.2)	17 (60.8)	12 (42.9)	16 (57.1)	13 (46.4)	15 (53.6)	13 (46.4)	15 (53.6)	27 (96.4)	1 (3.5)
10.	Cattle (C)	28	24 (85.7)	4 (14.3)	24 (85.7)	4 (14.3)	12 (42.9)	16 (57.1)	19 (67.9)	9 (32.1)	12 (42.9)	16 (57.1)	11 (39.3)	17 (60.7)	23 (82.1)	5 (17.8)
Total		157 (100)	148 (94.2)	9 (5.7)[a]	148 (94.2)	9 (5.7)	28 (17.8)	129 (82.1)	45 (28.6)	112 (71.3)	29 (18.4)	128 (81.5)	28 (17.8)	129 (82.1)	133 (84.7)	24 (15.2)

Abbreviations:- P- Positive, N- Negative
Superscript:- a- Coagulase Negative (H8, H48, Mt31, J4, C12, C13, C15 and C50) at 5h but at 24h reading H8, H48, Mt31 and C50 showed positive reaction.

61(4): 101-12.

Adesiyun AA and Shehu LM (1985) Detection of staphylo-coagulase using plasmas from various animals.*Vet. Microbiol.* **10**(4): 387-392.

Bhati T (2013) Characterization of *Staphylococcus aureus* isolated from cattle with subclinical mastitis. M.V.Sc. Thesis submitted to Rajasthan University of Veterinary and Animal science, Bikaner.

Boerlin P, Kuhnert P, Hussy D and Schacllibaum M (2003) Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. *J. Clin. Microbiol.* **41**(2): 767-771.

Citak S, Varlik O and Gundogan N (2003) Slime production and DNase activity of Staphylococci isolated from raw milk. *J. Food Biochem.* **23**: 281–288.

da Silva ER and da Silva N (2005) Coagulase gene typing of *Staphylococcus aureus* isolated from cows with mastitis in southeastern Brazil. *Can. J. Vet. Res*. **69:** 260-264.

Duthie ES and Lorenz LL (1952) Staphylococcal Coagulase: Mode of action and antigenicity. *J. Gen. Microbiol.* **6:** 95-107.

Guggenberger C, Wolz C, Morrissey JA and Heesemann J (2012) Two distinct coagulase-dependent barriers protect *Staphylococcus aureus* from neutrophils in a three dimensional in vitro infection model. *PLoS Pathog.* **8:** e1002434.

Jasper DE, Infante F and Dellinger JD (1985) Relationships among the results of coagulase, staphylococcal toxin and thermonuclease tests on staphylococci from cow milk. *J. Clin. Microbiol*. **21**(4): 582-584.

Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML and Najjuka FC (2010) Identification of *Staphylococcus aureus*: DNase and mannitol salt agar improve the efficiency of the tube coagulase test. *Ann. Clin. Microbiol. Antimicrob.* **9**: 23.

Kenar B, Kuyucuoglu Y and Seker E (2011) Antibiotic susceptibility of coagulase-negative Staphylococci isolated from bovine subclinical mastitis in Turkey. *Pakistan Vet. J*. **32**(3): 390-393.

Khichar V and Kataria AK (2015) Characterization of *Staphylococcus aureus* of cattle clinical mastitis milk origin for coagulase and haemolysin properties. *Int. J. Vet. Sci. Res*. **1**(1): 1-5.

Oliveira A, de Lourdes M and Cunha RS (2010) Comparison of methods for the detection of biofilm production in coagulase-negative Staphylococci. *BMC Res. Notes*. **3**: 260.

Qureshi SD, Kataria AK and Gahlot TK (2002) Bacterial microflora associated with wounds and abscesses on camel (*Camelus dromedarius*) skin. *J. Camel Pract. Res*. **9(2):** 129 -134.

Rayman MK, Park CE, Philpott J and Todd ECD (1975) Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus aureus*. *Appl. Microbiol.* **29**(4): 451-454.

Sanjiv K, Kataria AK, Sharma R and Singh G (2008) Epidemiological typing of *Staphylococcus aureus* by DNA restriction fragment length polymorphism of *coa* gene. *Vet. Arhiv.* **78**(1): 31-38.

Sharma SK, Nathawat P, Bhati T, Mohammed N, Chaudhary S, Raj R, Solanki S, Kataria AK (2013) Characterization of *Staphylococcus aureus* isolated from nasal discharge from pneumonic camels (*Camelus dromedarius*). *ABAH Bioflux.* **5**(1): 38-43.

Singh RS, Kumar R and Yadav BR (2011) Distribution of pathogenic factors in *Staphylococcus aureus* strains isolated from intra-mammary infections in cattle and buffaloes. *Indian J. Biotech.***10**: 410-416.

Straub JA, Hertel C and Hammes WP (1999) A 23S rRNA target polymerase chain reaction based system for detection of *Staphylococcus aureus* in meat starter cultures and dairy products. *J. Food Prot.* **62**(10): 1150-1156.

Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, Goering R, Hancock G, Hebert GA, Hill B and Hollis R (1994) Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J. Clin. Microbiol.* **32**(2): 407-415.

Turkyilmaz S and Kaya O (2006) Determination of some virulence factors in *Staphylococcus* spp. isolated from various clinical samples. *Turk. J. Vet. Anim. Sci.* **30**: 127-132.

Upadhyay A, Kataria AK and Sharma R (2010) Coagulase gene-based typing of *Staphylococcus aureus* from mastitic cattle and goats from arid region in India. *Comp. Clin. Pathol.* DOI 10.1007/s00580-010-1141-z.

Wani SA and Bhat MA (2003) An epidemiological study on bovine mastitis in Kashmir valley. *Indian Vet. J.* **80**(9): 841-844.

Yadav R, Sharma SK, Yadav J, Nathawat P and Kataria AK (2015) Typing of *Staphylococcus aureus* obtained from mastitic milk of cattle and buffalo on the basis of coagulase (*coa*) gene RFLP patterns. *Israel J. Vet. Med.* **70**(4): 37-41.