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Full Length Research Paper

Isolation and identification of *Staphylococcus aureus* from bovine and the detection of its coagulase gene (*coa*) using polymerase chain reaction (PCR)

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Two hundred and fifty different samples were collected from bovine and examined for the presence of staphylococcal bacteria. 189 isolates were able to grow on the mannitol salt agar (MSA), known as staphylococci. Coagulase test revealed that 165 isolates were able to produce this enzyme; 138 of these isolates were *Staphylococcus aureus which* appeared in 55.2% of the isolates. Deoxyribonuclase (DNAase), urase and beta haemolysis activities of the isolates were also investigated and it showed 90.69, 86.23, and 87.86% of the isolates respectively. An enzymatic examination of the isolates was combined in numerous tests like catalase test, coagulase test, non- producing oxidase, sugar fermentation, oxidative and fermentation test, liquefaction of gelatin and MR-VP test. The polymerase chain reaction (PCR) amplification of *coa* gene products of *S. aureus* showed the following: gene product of 500 bp (22.5%); 650 bp (15%); 800 and 850 bp (25% for each); and 600 bp (12.5%).

Key words: *Staphylococcus aureus*, bovine, coagulase, coa gene, polymerase chain reaction (PCR).

INTRODUCTION

Staphylococcus aureus can infect any part of the body; it causes some diseases in humans and animals, ranging from skin infection, food poisoning, brain abscesses and outbreak in post operative wound infection (Kenneth, 2008). In cows, it causes some important diseases; for example, mastitis (clinical and sub clinical) and respiratory tract infection, skin sepsis, tick pyemia in lamb and contagious skin necrosis (Kinight, 1999). *S. aureus* is one of the major causes of serious infections, passively colonizing human skin and nasal passages of healthy individuals; although this opportunistic pathogen colonizes without causing diseases (Kloos et al., 1992). One of the specific features of *S. aureus* is its ability to acquire resistance to antibiotics (Ohta et al., 2004). The coagulase protein is an important phenotypic determinant and is accepted as a major virulence factor of *S. aureus*. In coagulase-negative staphylococci (CoNS) originating from bovine mastitis, methicillin resistance is more common (Gindonis et al., 2013). The analysis of coagulase encoding *S. aureus* DNA *coa* gene has demonstrated variable sequences in the 3´ end coding region (Goh et al., 1992). This region contains a polymorphism repeat region that can be used to differentiate *S. aureus* isolates. This characteristic has

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Table 1. Numbers and percentages of bovine's samples that gave growth on MSA and positive coagulase result *(Staphylococcus)*.

Sample /No	Growth on MSA		Coagulae negative	Coagulase positive		Other coagulase +ve Staphylococcus		Suspected *S .aureus*	
	No.	%	No.	No.	%	No.	%	No.	%
Milk/70	44	62.9	4	40	90.1	6	8.6	34	48.6
Nasal Swabs/70	55	78.6	10	45	81.8	8	11.4	37	52.9
Vaginal Swabs/70	50	71.1	3	47	94	6	8.6	41	58.6
Urine/40	40	100	7	33	82.5	7	17.5	26	65
Total/250	189	75.6	24	165	87.3	6.75 ±0.957		34.5 ± 6.351	

X^2=20.06, P < 0.01.

been used to type *S. aureus* isolates of human and bovine origin (Guler et al., 2005). The aim of this study is to investigate the presence of virulence (coa) gene in *S. aureus* that is isolated from bovine.

MATERIALS AND METHODS

Samples collection

Two hundred and fifty samples were obtained from pathogenic and non pathogenic cases of bovine (70 milk sample from clinical and subclinical mastitis, 70 vaginal swabs, 70 nasal swab, and 40 urine sample) from AL- Tathamine Bovine Station in the Waste Province. Each sample was collected from different animals. The specimens were transported to the laboratory directly and inoculated onto plates of mannitol salt agar (MSA); they were incubated at 37°C for 24 h. All colonies from primary cultures were purified by subculturing onto MSA medium and incubating at 37°C for 24 to 48 h (Talan et al., 1989).

Biochemical testes

Different tests were performed for bacterial identification of *S. aureus*. The tests were catalase test; oxidase test; coagulase; clumping factor test; free coagulase test; Vogas- Proskauer test; ONPG ; Latex agglutination (MASTSTAPH); heamolysin production; NAase production test; urease test; O\F test; gelatin test; methyl red test and sugar fermentation test. The tests were done using the methods of Treagan and Pulliam (1982), Finegold and Baron (1986), Baron et al. *(*1994) and Macfaddin, (2000).

API Staph test was done for the conformity of the identification of isolates. Homogenous bacterial suspension was prepared with a turbidity equivalent to 0.5 McFarland stander.

Molecular study using polymerase chain reaction (PCR) technique

DNA kit was used for isolating DNA from bacterial cells based on the method of Sambrook et al. (1989) (Promega USA). Forty isolates from bovine (20 from milk, 10 from vaginal samples, 10 from nasal samples) were subjected to molecular study by using PCR technique. DNA from the 40 *S. aureus* isolates was extracted by purification using Promega kit to detect the presence of *coa* gene. The successful binding of gene appeared as clear band under U. V. light. The *coa* gene was studied according to the protocol of Hookey et al. (1998). Genomic DNA was amplified by using the primers given below:

5´-ATA GAG ATG CTG GTA CAG G-3´.
5´-GCT TCC GAT TGT TCG ATG C-3´.

PCR products were detected by electrophoresis on the agarose gel at 1%. 10 µl from PCR product was inoculated in each well from agarose gel. DNA ladder marker was used to measure the amplification molecular weight from PCR product compared to DNA marker. After 30 min examination was done under UV light (Sambrook et al., 1989).

RESULTS

The results showed that 189 out of 250 bovine samples (75.6%) gave positive result for *Staphylococcus* (able to grow on mannitol salt agar) and 165 isolates out of 189 (87.3%) were coagulase positive *Staphylococcus*. The urine samples revealed high percentage of isolation on MSA (100%), while nasal samples gave the lower percentage (62.9%). Coagulase results show that the high percentages of positive test were from vaginal isolates 47/50 (94%), while 45/55(81.8%) of nasal swabs isolates were coagulase positive. There were significant differences (P < 0.01) between numbers of isolation in different samples (Table 1).

Bacterial identification

Biochemical tests were used to confirm the identity of *S. aureus* isolates. *S. aureus* isolates were similar in some biochemical tests like catalase, oxidase, coagulase, O/F, ONPG, VP, MR, sugar fermentation, gelatin liquefaction, latex agglutination and API Staph. All tests were positive at 100%. On the other hand, *S. aureus* isolates differ in other tests like DNAase, urease and haemolysis, with average of 90.8, 87.23 and 87.86%, respectively. Most coagulase positive *S. aureus* isolates gave DNAase positive results. On the other hand, *S. aureus* isolates of bovine origin produced beta- haemolysis (Table 2).

Molecular genetics study results (PCR on *coa* gene)

The results of DNA amplification of *coa* gene in bovine

Table 2. Percentage of DNAase, Urease, Haemolysin reactions in the bovine *S. aureus* isolates.

Test	Milk	Nasal swabs	Vaginal swabs	Urine	Average	
DNAase	97.05% 33/34	91.89% 34/37	85.36% 35/41	88.46% 23/26	90.80%	X2= 3.19, P =0.363
Urease	91.17% 31/34	83.78% 31/37	85.36% 35/41	84.61% 22/26	87.23%	X2= 0.91, P = 0.808
Haemolysis	94.11% 32/34 X2=1. 063, P = 0.587	81.08% 30/37 X2=1.899, P = 0.386	87.80% 36/41 X2= 137, P =0.933	88.46% 23/26 X2=0.299, P =0.891	87.86%	X2= 2.81, P = 0.421

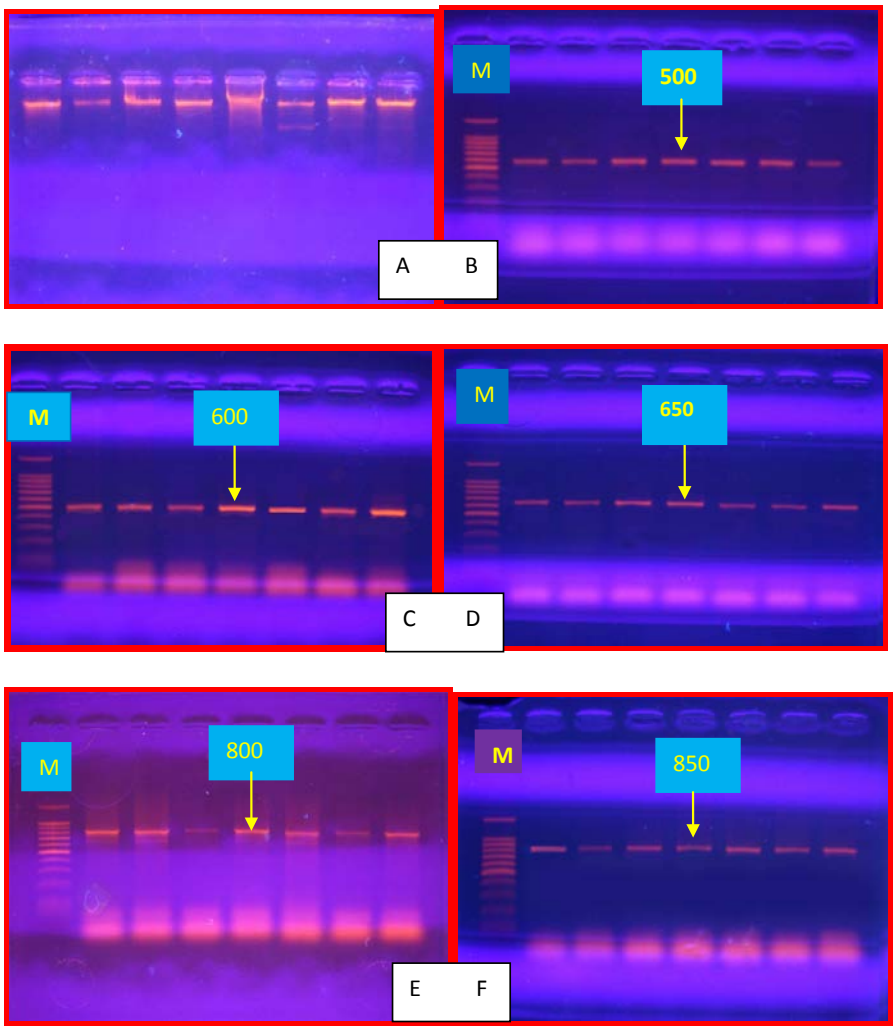

Figure 1. Electrophoresis in 1% agarose for *S. aureus* whole DNA (A) and *coa* gene showing different size, (B) 500bp; (C) 600 bp; (D) 650 bp; (E) 800 bp; (F) 850 bp; M= DNA ladder.

isolates revealed that PCR products were 500, 600, 650, 800, and 850 bp with 22.5, 12.5, 15, 25, and 25% respectively as a single band in 38/40 (95%) of isolates (Figure 1). From a total of forty studied isolates, there were significant differences (P< 0.01) between *coa* gene products in all the isolates (Table 3).

Table 3. Number and size product of *coa* gene in the bovine *S. aureus* isolates.

Sample	No. of isolates	Size of product bp					Total
		500	600	650	800	850	
Milk	20	9	0	1	10	0	20
Vaginal swabs	10	0	5	5	0	0	8
Nasal swabs	10	0	0	0	0	10	10
Total	40	9	5	6	10	10	38
Percentage		22.5%	12.5%	15%	25%	25%	95%

P > 0.05.

DISCUSSION

In this present study, different samples were collected from bovine (healthy and infected) for the isolation of *S. aureus.* This was done to study the properties of one of the most important pathogens in different samples of this microorganism responsible for economic loss and public health problems. This microorganism was isolated in 52.2% (milk, 48.57%; nasal swab, 52.85%; vaginal swab, 58.75% and urine, 52%). Kav et al. (2011) investigated the presence of *S. aureus* and staphylococcal enterotoxin (SE) genes in cheese samples. From a total of 127 cheese samples, 53 isolates (41.7% of the samples) were identified.

S. aureus isolates from bovine were similar in some biochemical tests results at 100%, and differ in other tests like, DNAase, Urease and heamolysis at 90.80, 87.23 and 87.86%, respectively. This result showed that *S. aureus* isolates from bovine are able to produce beta-haemolysin. Most of *S. aureus* isolates were able to produce urease enzyme at different percentages. Other study indicated that *S. aureus* strains were isolated in 481 (39%) samples. Of the 481 isolates of *S. aureus* tested, 255 (53%) were positive for one or more SE genes, and thirty-five different enterotoxin gene profiles were distinguished among the isolates; thus suggesting that the pathogenic potential of *S. aureus* may be of greater importance than previously thought (Bianchi et al., 2014).

Associations between bacterial genotype and outcome of bovine clinical *S. aureus* mastitis were investigated (Lundberg et al., 2014). The results of the presence of *coa* gene revealed that polymorphism phenomena of this gene resulted in different molecular weights of 500, 600, 650, 800 and 850 bp. The PCR test was used to detect the gene coded for producing coagulase enzyme that accounts for virulence of bacteria. *S. aureus* is able to produce different molecular weights found in the polymorphism phenomena and this enzyme is used to classify isolates depending on the different molecular weights in outbreak of epidemiological studies (Da Silva and Da Silva, 2005). Based on the detection of the most prevalent clones in a herd or region, appropriate antibiotic therapy and specific immunization can be used for the treatment and control of staphylococcal mastitis (Silveira et al., 2014). The prevalence of coagulase-positive staphylococci (CPS) was studied among 390 samples of ewe milk. Fifty-seven (14.85%) samples of tank milk and all samples (6) of silo milk gave a positive result. The detection of the coa gene from milk samples could help to assess the microbiological safety of raw milk intended for direct use in the dairy industry (Linage et al., 2012).

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Baron EJ, Peterson LR, Finegold SM (1994). Bailey and Scott's Diagnostic Microbiology 9th Ed. Mosby St. Louis.

Bianchi DM, Gallina S, Bellio A, Chiesa F, Civera T, Decastelli L (2014). Enterotoxin gene profiles of Staphylococcus aureus isolated from milk and dairy products in Italy. Lett. Appl. Microbiol. 58(2):190-196. http://dx.doi.org/10.1111/lam.12182

Da Silva ER, Da Silva N (2005). Coagulase gene typing of Staphylococcus aureus isolated from cows with mastitis in south-eastern Brazil. Can. J. Vet. Res. 69:260-264.

Finegold SM, Baron EJ (1986). Methods for testing antimicrobial effectiveness in Baily and Scott's diagnostic microbiology. 7th Ed. The C. V. Mos. By Co. West line. Industrial Drive, St., Louis, Missuri, USA.

Gindonis V, Taponen S, Myllyniemi AL, Pyörälä S, Nykäsenoja S, Salmenlinna S, Lindholm L, Rantala M (2013). Occurrence and characterization of methicillin-resistant staphylococci from bovine mastitis milk samples in Finland. Acta Vet Scand. 28:55:61.

Goh SH, Byrne SK, Zhang JL, Chow AW (1992). Molecular typing of Staphylococcus aureus on the basis of coagulase gene polymorphisms. J. Clin. Microbiol. 30:1642-1645.

Guler L, Ok Ü, Gündüz K, Gülcü, Y, Hadimli HH (2005). Antimicrobial susceptibility and coagulase gene typing of Staphylococcus aureus isolated from bovine clinical mastitis cases in Turkey. J. Dairy Sci. 88:3149-3154. http://dx.doi.org/10.3168/jds.S0022-0302(05)72998-2

Hookey JV, Richardson JF, Cookson BD (1998). Molecular typing of Staphylococcus aureus based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. J. Clin. Microbiol. 36:1083-1089.

Kav K, Col R, Ardic M (2011). Characterization of Staphylococcus aureus isolates from white-brined Urfa cheese. J Food Prot.74(11):1788-1796. http://dx.doi.org/10.4315/0362-028X.JFP-11-179

Kenneth T (2008). Staphylococcus aureus Text book of Bacteriology. University of Wisconsin-Medison. Department of Bacteriology.

Kinight C (1999). Oxytocin: An alternative to antibiotic for treating mastitis, Hannuch Research Insitute, Year book.

Kloos WE, Schleifer KH, Gotz F (1992). The genus Staphylococcus in the prokaryotes, 2nd ed, pp. 1369-1420.
Linage B, Rodríguez-Calleja JM, Otero A, García-López ML, Santos JA (2012). Characterization of coagulase-positive staphylococci isolated from tank and silo ewe milk. J. Dairy Sci. 95(4):1639-1644. http://dx.doi.org/10.3168/jds.2011-4734
Lundberg A, Aspán A, Nyman A, Unnerstad HE, Waller KP (2014). Associations between bacterial genotype and outcome of bovine clinical Staphylococcus aureus mastitis. Acta Vet. Scand. 8:56.
Macfaddin JF (2000). Biochemical tests for identification of medical bacteria. 3rd Ed. Lippincott Williams and Wilkins USA.
Ohta T, Hirakaw H, Morikaw K, Maruyama A, Inose Y, Yanashita A, Oshima K, Kuhara M, Hattori M, Hiramatsu K, Kuhara S, Hayash H (2004). Nucleotide substitutions in Staphylococcus strains, Mu50, Mu3, and N315. DNA Res. 11:51-56. http://dx.doi.org/10.1093/dnares/11.1.51
Sambrook J, Fritsch EF, Maniatis S (1989). Molecular cloning 2nd ed., Cold Spring Harbor Laboratory Press, N. Y.
Silveira-Filho VM, Luz IS, Campos AP, Silva WM, Barros MP, Medeiros ES, Freitas MF, Mota RA, Sena MJ, Leal-Balbino TC (2014). Antibiotic resistance and molecular analysis of Staphylococcus aureus isolated from cow's milk and dairy products in northeast Brazil. J. Food Prot. 77(4):583-591. http://dx.doi.org/10.4315/0362-028X.JFP-13-343
Talan DA, Staatz D, Staatz A, Goldstein E. JC, Singer K, Ocrturf GD (1989). Staphylococcus intermidius in canine gingival and canine-infected human wound infections: Laboratory characterization of newly recognized zoonotic pathogen. J. Clin. Microbiol. 27:78-81.
Treagan L, Pulliam L (1982). Medical microbiology procedures, W.B. Saunders Co. Philadelphia.