International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

Prevalence Study of Biofilm Marker *ica* D Gene among Coagulase Positive *Staphylococcus* of Mastitic Origin

Shubhangi Warke[1], Prashant Sonkusale[2], Sumedha S. Boabde[3]
[1],[3]Department of Veterinary Microbiology and Animal Biotechnology T & R cell
[2] Department of Veterinary Pathology, Nagpur Veterinary College , Maharashtra Animal & Fishery Sciences University, Seminary Hills, Nagpur-440006, India

ABSTRACT: Bovine Mastitis is an inflammation of the mammary gland, usually due to microbial infection, which causes economic loss to dairy producer every year. Staphylococcus is one of the most common pathogens responsible for contagious mastitis in bovines. The aim of this study was to identify coagulase positive Staphylococci isolated from bovine subclinical mastitis. *In vitro* biofilm forming ability of the isolates, an important virulence factor of mastitogen and prevalence of biofilm marker *ica*D gene among the isolates. In the present study, 70 isolates were biochemically identified as *S. aureus*. Biochemically characterized strains of *S. aureus* were screened for biofilm formation. Phenotypic detection of biofilm formation of *S.aureus* were done by two methods, namely, Congo Red Agar (CRA) method and Tube Method (TM). The frequency of biofilm formation in *S. aureus* strains was 28.57 per cent on CRA plates while 71.42 per cent were biofilm non-producers. The frequency of biofilm formation in *S. aureus* strains was 38.57 per cent in total as shown by tube method while 61.42 per cent were biofilm non-producers. Employing PCR, molecular detection technique of *ica*D gene was carried out. In this study, 72.85 per cent *S. aureus* strains were found to be positive for *ica*D gene which produced an amplicon of 381bp.

KEYWORDS: biofilm, coagulase, CRA, *ica* D, mastitis, PIA

INTRODUCTION

The dairy sector in India has grown substantially over the years, making India the world's largest producer of milk, In India, 85% of total milk produced is handled by the noncommercial sector [1]. *Staphylococcus aureus* is the bacterium responsible for various diseases in animals and humans [2]. *Staphylococcus aureus* (*S. aureus*) is a significant cause of mastitis in ruminants such as cows, sheep, and goats worldwide [3]. *Staphylococcus aureus* is a highly prevalent cause of mastitis in dairy herds worldwide [4].

Subclinical mastitis (SCM) with no sign of inflammation is more severe than clinical forms and is responsible for a huge loss to the dairy industry [5]. Preventive strategies could be better implemented by understanding the prevalence, genetic patterns, and the presence of enterotoxin and bioflm-producing genes along with the antibiotic susceptibility of this organism [2].

Bioflm-producing *S.aureus* strains, especially the enterotoxigenic and antibiotic-resistant ones, are the major cause of several persistent bacterial diseases in the livestock sector, including mastitis [6]. The ability to form biofilm is a trait associated with bacterial virulence and many chronic bacterial infections [7]. During biofilm formation, initially, the bacteria adhere to each other by polysaccharide intercellular adhesion (PIA) and then propagate. The PIA is under the control of the *ica*ADBC operon, and the strains possessing this gene cluster have been reported as strong biofilm producers. Several genes are involved in the production and maintenance of biofilms by staphylococci, of which the most widely studied are the *ica*A and *ica*D (intercellular adhesion A and B) genes responsible for the production of polysaccharide intercellular adhesion (PIA) that includes N-acetyl glucosamine as a primary component of the exopolysaccharide matrix surrounding the bacterial cells within the biofilm [8].

The formation of biofilm also provides a shielding effect to the bacterium from antibiotic attacks [2]. The production of biofilms is an important factor and makes these bacteria resistant to antimicrobial therapy [9].

Therefore, this study aimed to assess the prevalence and identify Coagulase Positive Staphylococci isolated from bovine subclinical mastitis. *In vitro* biofilm forming ability of the isolates and prevalence of biofilm marker *ica*D gene among the isolates.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

MATERIAL AND METHODS

1. Identification of coagulase positive Staphylococcus (CPS)

The isolates were subjected to Gram's method to study morphology and staining reaction. Cultures of Gram positive cocci typical to that of Staphylococcus were selected for further characterization. Identification of CPS was carried out as per the procedure of Cowan and Steel (1970) [10] and Cruickshank *et al.* (1975) [11]. The isolates were further processed for Biochemical tests for the identification and characterization of CPS. The Gram-positive cocci cultures were tested for oxidation fermentation (OF) test as per the procedure of Baker and Breach (1980) [12]. The OF positive cultures i.e. Staphylococci were further subjected for coagulase and Sugar fermentation and biochemical tests in order to identify *S.aureus*. All the tests were performed as per Sperber and Tatini (1975) [13].

Phenotypic characterization of CPS for biofilm formation: *In vitro* qualitative biofilm formation was assessed by following methods:

A. Congo Red Agar (CRA) method:

Qualitative detection of biofilm production in CPS strains was performed by cultivation on CRA as described by Vasudevan *et.al.* (2003) [14]. The medium composed of brain heart infusion (BHI) broth (37gms/L), sucrose (36gms/L), agar powder (30gms/L) and Congo red dye (0.8gms/L). CRA plates were kept at room temperature overnight for sterility test. Inoculated CRA plates were incubated at 37˚C for 24 hrs followed by subsequent storage at room temperature. Colonies on CRA were observed at 48 and 72 hrs. Along with CPS test strains, known biofilm positive strains served as control.

Strains producing black colonies with a rough, dry and crystalline consistency were considered biofilm producer. Strains producing red colonies with rough, dry and crystalline consistency or smooth colonies were classified as biofilm non-producer.

B. Tube Method (TM):

A qualitative assessment of biofilm formation was determined as described by Christensen *et. al*., (1982) [15] with some modifications. Briefly, 5mL trypticase soya broth (TSB) with 0.25 per cent glucose was inoculated with loopful of broth culture and incubated by 24 hrs at 37˚C. the tubes were decanted and washed for three times with sterile phosphate buffer saline (PBS, pH 7.4) and dried. After drying, tubes were stained with 0.1 per cent safranin. Excess stain was removed and tubes were washed with sterile distilled water. Tubes were dried in an inverted position and observed for biofilm formation. Biofilm formation was considered positive when a visible stained film lined the wall and bottom of the tube. Ring formation at the liquid interface was not considered an indicative of biofilm formation. Tubes were examined and the amount of biofilm formation was scored as strong, moderate and weak.

Genotypic identification of *ica*D gene in *S. aureus* by Polymerase Chain Reaction

The primers for the detection of intercellular adhesion gene, *ica*D used in this study were synthesized from Genetix, Biomed Asia Pvt. Ltd. New Delhi. The details of the primer sequence are shown in Table 1.

Table 1: Details of primers for amplification of intercellular adhesion genes of *S. aureus*.

Primer Name	Primer Sequence		Product Size	Reference
*ica*D	Forward	5'-AAA CGT AAG AGA GGT GG-3'	381 bp	Vasudevan *et al.* (2003)[14]
	Reverse	5'-GGC AAT ATG ATC AAG ATA C-3'		

Preparation of the Genomic DNA: The *S.aureus* isolates were grown in 2mL of BHI broth overnight at 37ºC. Cultures were harvested by centrifugation (8000g for 10 min) and suspended in 400 µL of TE solution (10mM Tris HCl; 1mM EDTA, pH8.0). Bacteria were lysed by addition of 10 µL of proteinase K (20mg/mL) and 100 µL of 10% SDS, followed by incubation of 1 hr. The cell wall debris, denatured proteins, polysaccharides and polymeric matrix were eliminated by precipitation with addition of 80 µL of 5M NaCl and 64 µL of CTAB solution (10% CTAB in 0.7 M NaCl) and incubation at 65ºC for 10 min. DNA was purified by two extractions with phenol:chloroform (1:1) and chloroform:isoamylalcohol (24:1). DNA was precipitated by adding 100 µL of isopropanol and kept at -20ºC for 30 min or overnight and later centrifuged at 8000g for 15 min. The pellet was washed

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

in ethanol (80%) and air dried to remove alcohol and resuspended in 50 µL TE buffer. Two µL of suspended pellet was used as the template.

Single-plex PCR targeting the intercellular adhesion gene *ica*D of *S. aureus*.

The PCR was standardized for the detection of *ica*D gene. The methodology described by Vasudevan *et.al.* (2003)[14] was suitably modified. The standardized PCR protocol was used for screening of all the *S. aureus* strains for *ica*D gene.

PCR was set for 25 µL reaction volume for the detection of *ica*D gene in *S. aureus* strain. Reaction mixture for PCR was optimized as follows- 2.5 µL of 10x PCR buffer with 15mM MgCl2, 0.75 µL of dNTP mix (10mM with a final concentration of 0.2 mM), 1 µL of forward and reverse primer of each set (final concentration 0.1 µL each), 1 µL of Taq DNA polymerase, 2 µL of template DNA and sterilized milliQ water to make up the reaction volume.

PCR tube (0.2mL) containing the reaction mixture was flash spun on a micro centrifuge (Remi, C 24) to get reactants at the bottom. The reaction was performed in Px2 Thermal cycler (Thermal electronic corporation, USA) with a pre-heated lid. The cycling conditions included an initial denaturation at 94ºC for 3 min followed by 40 cycles each of 45 seconds denaturation at 92ºC, 45 seconds annealing at 49ºC and 1 min extension at 72ºC. After the reaction, PCR products were kept at -20ºC until further analysis by agarose gel electrophoresis.

Comparative evaluation of methods employed for detection of biofilm formation in *S. aureus*

Sensitivity and specificity of various tests namely Congo Red Agar (CRA) method and Tube Method (TM) for detection of biofilm formation by strains of *S. aureus* from bovine SCM was worked out as per the method described by Thapliyal and Mishra (1996)[16]. PCR for the detection of *ica*D genes was considered as golden standard test for comparison.

RESULTS

Worldwide, mastitis is the most common infectious disease affecting dairy cows and the most economically important disease of the dairy industry [17]. The first step in pathogenesis is adhesion of microorganisms to the extra-cellular matrix promoting colonization to host tissue. Another step-in colonization is the formation of biofilm [18].

In the present study, 70 isolates were biochemically identified as *S. aureus* were screened for biofilm formation.

Phenotypic characterization of *S. aureus* for biofilm formation:

Congo Red Agar (CRA) method:

Phenotypic detection of biofilm formation by *S. aureus* isolates was evaluated by culturing on CRA plates. In the present study, strains producing black rough colonies on CRA were considered as biofilm producing strains as per Vasudevan *et al.* (2003) [14]. Whereas, black smooth, red rough, red smooth and white smooth colonies were classified as non-biofilm producing strains.

The colony morphology of *S. aureus* strains observed in present investigation are shown in Figure 1 and results of biofilm production by CRA method is presented in Table 2.

Table 2: *In vitro* biofilm production in *S. aureus*

Staphylococcus aureus	
Biofilm positive (CR+ve)	Biofilm negative (CR-ve)
20 (28.57%)	50 (71.42%)

Tube Method (TM):

Phenotypic detection of biofilm formation was assessed by Tube method as described by Christensen *et al.*, (1982) [15] with some modifications. By Tube Method, a visible stained film lined the inner wall and the bottom of the tube was considered as positive for biofilm formation. Ring formation at the liquid interface was not taken as an indicative of biofilm formation. The amount of biofilm formation was scored as strong, moderate and weak. (Table 3; Figure 2)

The frequency of biofilm formation in *S. aureus* strains was 28.57 per cent on CRA plates while 71.42 per cent were biofilm non-producers. Likewise, Vasudevan *et al.* (2003) [14], screened 35 isolates out of which 24 (68.57%) *S. aureus* were biofilm producing. Yazdani *et al.* (2006) [19] also recorded 52 % biofilm positive *S. aureus* isolates by CRA method. Dhanawade *et al.* (2009) [20] examined a total of 102 *S. aureus* isolates of bovine subclinical mastitis cases of which biofilm producing strains

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

detected by Congo red agar (CRA) method were 48.03 per cent. Salina *et al.*, 2020 [21], studied biofilm production in *Staphylococcus aureus* from subclinical and clinical bovine mastitis and reported that total of 25 (25%) *S. aureus* isolates produced biofilms on CRA.

Tube Method (TM):

Table 3: Biofilm producing *S. aureus* strains as detected by Tube Method

Biofilm non-producer	Biofilm producer		
	Strong	Moderate	Weak
43 (61.42%)	10 (14.2%)	6 (8.5%)	11 (15.71%)

The frequency of biofilm formation in *S. aureus* strains was 38.57 per cent in total as shown by tube method while 61.42 per cent were biofilm non-producers. The present results of biofilm formation by Tube Method are in agreement to those of Dhanwade *et al.*, (2009) [20] who observed 36.27 per cent biofilm production by Tube Method.

Genotypic characterization of *S. aureus* strains:

The present study was undertaken to detect the biofilm marker *ica*D gene among Coagulase positive *Staphylococcus* of mastitic origin. The PCR was standardized for detection of *ica*D gene in *S. aureus*. The primer set for *ica*D gene allowed positive amplification of 381 bp product represented by a single band in the corresponding region of the 100 bp DNA ladder. (Fig.3). The result of biofilm formation of *S. aureus* strains of mastitic origin by PCR is shown in Table 4.

Table 4: *S. aureus* strains detected a *ica*D positive by Polymerase Chain Reaction

Positive for *ica*D gene	51 (72.8%)
Negative for *ica*D gene	19 (27.1%)
Total samples	70

Overall by PCR, 51 (72.8%) *S. aureus* strains were observed positive for *ica*D gene and 19 (27.1%) strains were negative. Vasudevan *et al.* (2003)[14] reported 100 per cent prevalence of *ica*AD genes. Likewise Yazdani *et al.* (2006) [19] also reported similar results. Ciftci *et al.* (2009) [22] reported 64.40 per cent strains positive for *ica*D gene. In order to isolate and identify *S. aureus*, 1555 milk samples were collected from 401 cows, located in different regions of the Republic of Serbia. Using the conventional microbiological methods 100 isolates were characterized as coagulase-positive staphylococci. The presence of *ica*D gene alone was confirmed in 40.9% of cases totaling *ica*D positive isolates to 65.9% [23]. Comparison of methods for the detection of biofilm formation by *Staphylococcus aureus* isolated from bovine subclinical mastitis was studied and reported ninety strains (95%) were positive by PCR for both *ica*A and *ica*D genes [24].

Staphylococcus aureus isolated from bovine subclinical mastitis, the results revealed that 85% of the isolates tested produced slime on the Congo red agar and 95.7% of the isolates carried the *ica*D genes. The study recorded that 215 *S. aureus* strains collected from human and dairy cow's infections and the most prevalent gene was *ica*D found in 88.4% of the isolates [25].

The distribution of the frequencies of *icaD* gene in *S. aureus* isolates was 83%.(Salina *et al* 2020) [21]. The prevalence and characteristics of staphylococcal biofilm formation in raw milk samples was studied and reported the *ica*D was found in both CoNS strains (21.4%) and *S. aureus* (100%) [26]Over all biofilm formation by *S. aureus* by different methods is presented in Table 5.

Table 5: Overall Biofilm formation in mastitic *S. aureus* as seen by different methods

Test	Total samples	No. of positive strains	Percentage
Congo Red Agar (CRA) method	70	20	28.57
Tube Method (TM)	70	27	38.57
Polymerase Chain Reaction (PCR)	70	51	72.85

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

CONCLUSION

The present study was undertaken for the evaluation of biofilm formation among Coagulase positive *Staphylococcus* of mastitic origin and also to study the prevalence of *ica*D gene in *S. aureus* recovered from bovine subclinical mastitis. Bacterial cultures of mastitic origin were taken from the Department of Veterinary Microbiology and Animal Biotechnology, Nagpur Veterinary College, Nagpur. The cultures were identified as CPS and characterized as *S. aureus*. This study was performed to generate useful information for field veterinarians and policy makers to form strategies for controlling *S. aureus*-mediated infections and ultimately improving the quality of milk.

Phenotypic detection of biofilm formation of *S. aureus* was done by two methods, namely, Congo Red Agar (CRA) method and Tube Method (TM). In present study, by CRA method 28.57 per cent and by TM 38.57 per cent of *S. aureus* of mastitic origin were found to be biofilm producer. Employing PCR molecular detection of *ica*D gene was carried. In this study, 72.85 per cent *S. aureus* strains were found to be positive for *ica*D gene which produced an amplicon of 381bp. It seems that the biofilm in *Staphylococcus aureus* has a high phenotypic expression, and the high ability of this bacterium to form a biofilm can be particularly important in the emergence of infections and the creation of multiple antibiotic strains.

ACKNOWLEDGMENT

The author thanks to Department of Science & Technology, New Delhi for Financial Assistant and Associate Dean, Nagpur Veterinary College, Nagpur for the facilities and cooperation extended for this work.

Conflict of Interest: The authors declare that there are no conflicts of interest.

REFERENCE

1. FAO. A report on milk and milk products. Food and agricultural organization, United States. 2013. www. milkproduction.com/Library/Editorial-articles/ FAO-Food-Outlook-Nov-2013—Milk-and-Milk-products/
2. Sharma V, Sharma S, Dahiya DK, Khan A, Mathur M, Sharma A. Coagulase gene polymorphism, enterotoxigenecity, biofilm production, and antibiotic resistance in Staphylococcus aureus isolated from bovine raw milk in North West India. Ann Clin Microbiol Antimicrob. 2017. 20;16(1):65.
3. Keane, O. M. ,Symposium review: Intramammary infections—Major pathogens and strain-associated complexity. J. Dairy Sci. , 2019; 102, 4713–4726.
4. Åvall-Jääskeläinen S., Koort J., Simojoki H., Taponen S., Genomic analysis of Staphylococcus aureus isolates associated with peracute non-gangrenous or gangrenous Mastitis and comparison with other mastitis-associated Staphylococcus aureus isolates. Front Microbiol. 2021 Jul 8;12:688819
5. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res. 2003; 34(5):475–91.
6. Fox L., Zadoks R., Gaskins C..Bioflm production by Staphylococcus aureus associated with intramammary infection. Vet Microbiol. 2005; 107(3):295–9.
7. Jamal A, David B: Biofilm formation by enterococci. J Med Microbiol 2007, 56:1581-88.
8. Tang J., Chen J., Li. H., Zeng P, Li J., Characterization of adhesin genes, staphylococcal nuclease, hemolysis, and bioflm formation among Staphylococcus aureus strains isolated from different sources. Foodborne Pathog Dis. 2013; 10(9):757–63.
9. Hamad P.A. Phenotypic and Molecular Detection of Biofilm Formation in Methicillin-Resistant Staphylococcus aureus Isolated from Different Clinical Sources in Erbil City. Mediterr J Hematol Infect Dis. 2023;1;15(1):e2023016.
10. Cowan, S.T. and K.J. Steel (1970). Manual for the identification of Medical Bacteria. The Syndics of the Cambridge Univ. Press, Bentley House 200, Euston Road, London,
11. Cruickshank, R., J.P. Duguid, B.P. Marmoin and R.H.A. Swain (1975). Medical Microbiology: 2, 12th edn. Churchill
12. Baker, F. J and Breach. M. R. (1980): Medical Microbiology Techniques (1st ed.). Butter worths and co. (publishers) ltd. London. Pp 547.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

13. Sperber W.H., Tatini S.R., Interpretation of the tube coagulase test for identification of Staphylococcus aureus. Appl Microbiol. 1975; 29(4):502-5.
14. Vasudevan, P.; Nair, M.K.M.; Annamalai, T.; Venkitanarayanan, K.S. Phenotypic and genotypic characterization of bovine mastitis isolates of Staphylococcus aureus for biofilm formation. Vet. Microbiol., 2003 ; .92, 179-185.
15. Christensen G.D., Bisno A.L., Simpsom W.A., Beachey E.H., Adherence of slime producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun 1982, 37:318-326.
16. Thapliyal D.C. and Mishra D.S., Fundamentals of animal hygiene and epidemiology, 1996 .1st edn. International Book Distributing. Co, Charbagh, 92 .
17. Gillespie B.E. and Oliver S.P.. Simultaneous detection of mastitis pathogens, Staphylococcus aureus, Streptococcus uberis, and Streptococcus agalactiae by multiplex real-time polymerase chain reaction. J Dairy Sci. 2005 .,88(10):3510-8.
18. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penadés J.R (2001) Bap a Staphylococcus aureus surface protein involved in biofilm formation. J Bacteriol .183:2888-2896.
19. Yazdani R., M. Oshaghi, A. Havayi, E. Pishva, R. Salehi, M. Sadeghizadeh, Detection of icaAD gene and biofilm formation in Staphylococcus aureus isolates from wound infections Iranian J Publ Health, 2006; 35 (2) : 25-28
20. Dhanawade, N.B., Kalorey, D.R., Srinivasan, R. Detection of intercellular adhesion genes and biofilm production in Staphylococcus aureus isolated from bovine subclinical mastitis. Vet Res Commun .2010; 34, 81–89 .
21. Salina. A., F.F. Guimarães, V.B. Richini Pereira, B.D Menozzi, V.L.M. Rall, H. Langoni. Detection of icaA, icaD, and bap genes and biofilm production in Staphylococcus aureus and non-aureus staphylococci isolated from subclinical and clinical bovine mastitis Arq. Bras. Med. Vet. Zootec., 2020; 72(3): 1034-1038.
22. Ciftci A., Findik A., Onuk E.E., Savasan S. Detection of methicillin resistance and slime factor production of Staphylococcus aureus in bovine mastitis. Braz J Microbiol 2009; 40:254- 61.
23. Branko S., Vlado T., Dragan V., Neđeljko K. , Mirjana D, Jasna D., Vera K.,. Detection of icaA and icaD genes of Staphylococcus aureus isolated in cases of bovine mastitis in the Republic of Serbia.. Acta Veterinaria-Beograd , 2017; 67 (2), 168-177.
24. Melo P.C., Ferreira L.M., FilhoA.N., ZafalonL.F., Vicente H.G., Souza V., Comparison of methods for the detection of biofilm formation by Staphylococcus aureus isolated from bovine subclinical mastitis, Brazilian Journal of Microbiology , 2013; 44(1): 119-124.
25. Khoramian B., Jabalameli F., Niasari-Naslaji A. , Taherikalani M., Emaneini M., Comparison of virulence factors and biofilm formation among Staphylococcus aureus strains isolated from human and bovine infections, Microbial Pathogenesis 2015; 88: 73-77.
26. Gajewska J. and Wierzchowska W.C., Biofilm Formation Ability and Presence of Adhesion Genes among Coagulase-Negative and Coagulase-Positive Staphylococci Isolates from Raw Cow's Milk,; Pathogens .2020 ; 9: 654.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org

Biofilm non producer on Congo Red Agar

Biofilm producer on Congo Red Agar

Figure 1: Colony morphology of *S. aureus* originated from bovine subclinical mastitis on CRA plate

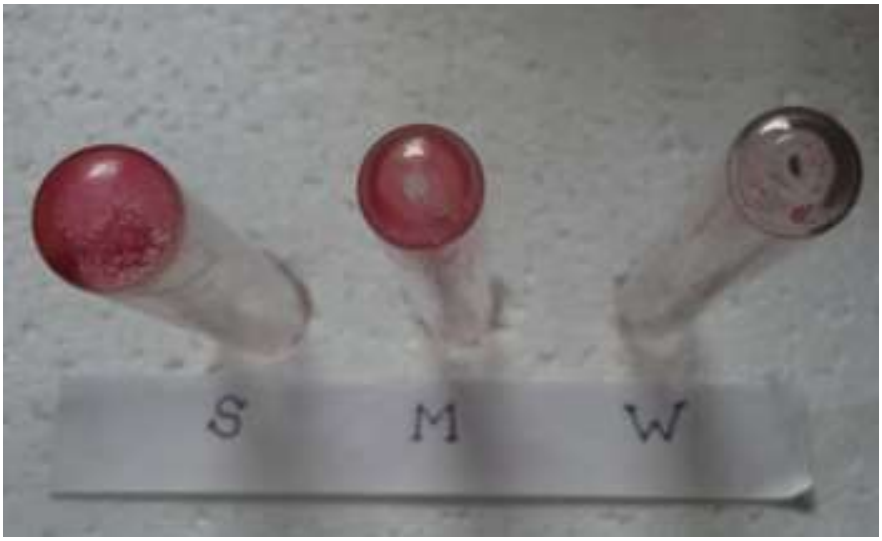
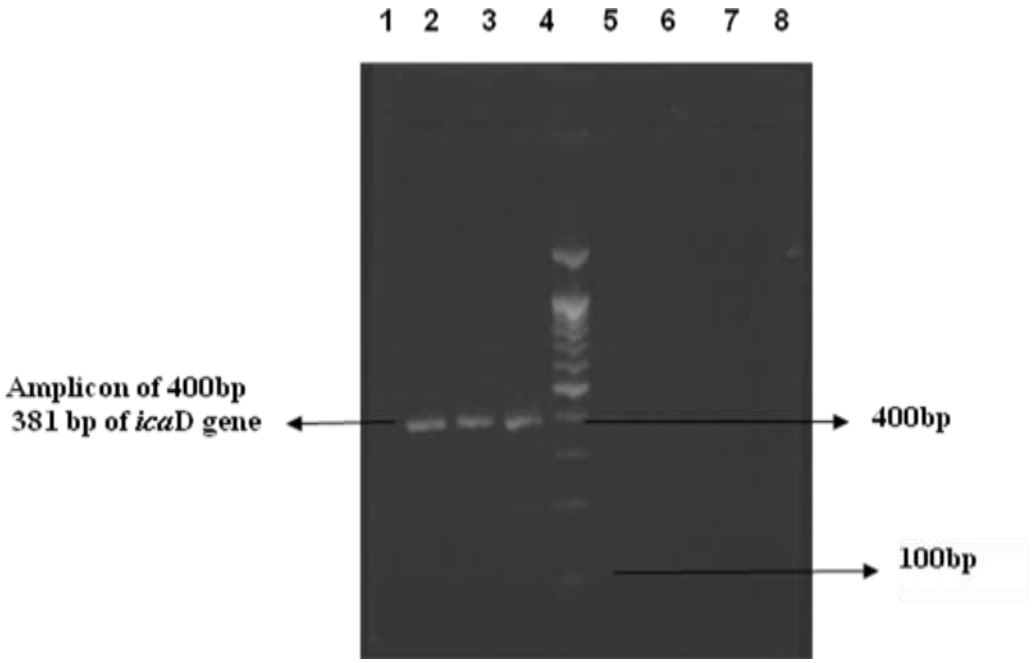


Figure 2: Screening of biofilm producing *S.aureus* of bovine subclinical mastitis by TM method (S : Strong ; M : Moderate ; W : Weak)

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 06 June 2023
DOI: 10.47191/ijcsrr/V6-i6-07, Impact Factor: 6.789
IJCSRR @ 2023
www.ijcsrr.org



Lane 1, 2, 3: Positive sample of *ica*D gene
Lane 4: DNA ladder
Lane 6,7,8: Negative sample of *ica*D gene
Figure 3 : Agarose gel electrophoresis of PCR amplified product of *ica*D gene with amplification of 381 bp product with 100 bp DNA ladder.

Cite this Article: Shubhangi Warke, Prashant Sonkusale, Sumedha S. Boabde (2023). Prevalence Study of Biofilm Marker ica D Gene among Coagulase Positive Staphylococcus of Mastitic Origin. International Journal of Current Science Research and Review, 6(6), 3140-3147