Prevalence and characterization of *Staphylococcus aureus* from raw milk in Mumbai region

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Abstract

Staphylococcus aureus is a vital pathogenic microorganism and can cause spoilage and contamination of milk samples that make it unsafe for drinking purposes. Such contaminated milk causes many infections in the human population like gastroenteritis, diarrhea, nausea, abdominal cramps and fever. The present study was planned to isolate and identify *S. aureus* from raw milk samples collected from the Mumbai region. A total of 106 raw milk samples were collected from cattle and buffalo during this study. These samples were processed for isolation and identification of *S. aureus* by conventional method and molecular characterization by PCR assay. Based on conventional method results, 45 isolates were identified as *Staphylococcus aureus*. The isolation rate of *Staphylococcus aureus* from raw milk of cattle and buffalo was found to be 42.45% per cent. Antibiotic sensitivity testing of *Staphylococcus aureus* showed that imipenem was the most sensitive (100%) antibiotic followed by amoxicillin/ clavulanic acid (91.11%), ceftriaxone (84.44%), tetracycline (80%), ciprofloxacin (77.77%) and amikacin (71.11%). The most resistant antibiotics against *Staphylococcus aureus* were amikacin (28.88%) followed by ciprofloxacin (22.22%), tetracycline (20%), ceftriaxone (15.55%) and amoxycillin/ clavulanic acid (8.88%). All the 45 isolates showed *Nuc* gene specific amplicon of 270 bp. The present study poses a risk to the milk consumers as the raw milk samples contain *S. aureus*; therefore avoiding consumption of raw milk is the best alternative to remain safe from gastrointestinal infections.

Key words: *Nuc* PCR assay, Raw milk, *Staphylococcus aureus*

Highlights

- The isolation rate of *Staphylococcus aureus* from raw milk of cattle and buffalo was found to be 42.45%.
- Antibiotic sensitivity testing of *Staphylococcus aureus* showed that imipenem is the most sensitive (100%) antibiotic followed by amoxicillin/ clavulanic acid (91.11%), ceftriaxone (84.44%), tetracycline (80%), ciprofloxacin (77.77%) and amikacin (71.11%).
- The most resistant antibiotics against *Staphylococcus aureus* are amikacin (28.88%) followed by ciprofloxacin (22.22%), tetracycline (20%), ceftriaxone (15.55%) and amoxycillin/ clavulanic acid (8.88%).
- Avoiding consumption of raw milk is the best alternative to remain safe from gastrointestinal infections.
- *Nuc* PCR has successfully amplified the 270 bp product indicating the *Staphylococcus aureus*.

INTRODUCTION

Milk is considered as complete food. Dairy foods are ideal foods for all age groups in both rural and urban people all around the world as these are rich sources of protein, vitamins and minerals which are essential for health of human body. At present, India ranks first in world in

terms of milk production with 16% of global production (FAO, 2014).

The milk samples serve as an excellent source of many pathogenic microorganisms that may cause illness to the human population. Hence such raw contaminated milk samples, if

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consumed by humans, may increase the risk of infections. So the quality of milk samples must be checked by microbiological analysis of milk before its consumption. Also all the hygienic precautions must be followed while collecting, processing and transporting milk samples from farm to the door of consumers (Soomro *et al.*, 2002).

Raw milk may be contaminated by pathogenic microorganisms including, *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Bacillus* spp., *Brucella* spp., *Salmonella* spp., yeasts and moulds. *Staphylococcus aureus* is the most common bacterial pathogen that causes contamination of raw milk samples, affecting the quality of milk samples. *S. aureus* is a facultative anaerobic gram-positive coccus, non-motile, catalase and coagulase positive bacteria found in water, dust and air. If such milk samples contaminated with *Staphylococcus aureus* are consumed by humans may cause severe gastrointestinal infections. Hence hygienic conditions at the farm level are an important factor of milk contamination. If milk handlers are not followed proper sanitary conditions at the farm level may cause *S. aureus* infection. Milk handlers must avoid coughing, sneezing while collecting milk or handling infected cows while milking healthy ones to prevent infections (Kadariya *et al.*, 2014; Abebe *et al.*, 2016).

S. aureus produces several toxins i.e haemolysin, leukotoxins, enterotoxins, exfoliative toxins, toxic shock syndrome toxins. Apart from that many enzymes and surface proteins are produced by *S. aureus* like coagulase, thermonuclease etc. These toxins when consumed by humans may cause severe gastrointestinal infections like nausea, vomiting, diarrhea, abdominal pain and toxic shock syndrome etc. Coagulase, enzyme produced by *S. aureus* is used as marker for the bacteria. The *Nuc* gene, which encodes the thermostable nuclease of *Staphylococcus*, is used for the identification of *S. aureus* isolates in many laboratories (Lachica *et al.*, 1971; Tucker, *et al.*, 1978; Kerouanton *et al.*, 2007; Fagundes *et al.*, 2010).

The quality of raw milk samples can be improved by adopting proper hygienic practices. The milking of animals must be done in a clean area free from dust and mud. The healthy and infected animals must be kept separately. The infected animals should not be mixed with healthy ones during the milking procedure. The milker should wash hands with soap and clean water before milking. The milker should wash the udder with clean and warm water, dry the udder with clean cloth, and use clean containers for milking. The milker should not cough or sneeze during milking. Also, milking of infected cows should be done separately, their milk should be discarded, and teat dip must be carried out to reduce the chances of contamination of raw milk samples (De Silva, 2016). The present study was planned to isolate and identify *S. aureus* from raw milk samples collected from the Mumbai region and detect *S. aureus* by *Nuc* PCR assay.

MATERIALS AND METHODS

A total of 106 raw milk samples of cattle and buffalo were collected from the Mumbai region during the present study, as shown in Table 1. The samples were transported to the laboratory in iceboxes and stored at 4°C till further processing. All samples were processed for isolation and identification of *Staphylococcus* spp. by conventional methods within 2 hrs of arrival in the laboratory and further subjected to PCR.

Isolation and identification of *Staphylococcus* spp.

Isolation: The milk samples were processed for isolation of *Staphylococcus* spp. using standard bacteriological procedures (OIE, 2004). The milk samples were centrifuged at 12000 rpm for 15 minutes and pellet was inoculated aseptically on the plates of nutrient Agar and Mannitol salt agar, and incubated at 37°C for 24 hrs in incubator. The cultures were observed regularly for the appearance of growth. The colonies suggestive of *Staphylococcus* spp. were checked for the morphology and direct microscopy by Gram's staining method. The

Table 1. Details of specimens collected from different sources

Sl.No	Name of the farm	Species	Number of milk samples collected for isolation
1	Narayan Dairy Farm, Dombivli	Cow	1
2	Durga Dairy Farm, Dombivli	Cow	1
3	Nanda Dairy, Dombivli	Cow	1
4	Yadav Dairy, Dombivli	Cow	1
5	Goshala, Dombivli	Cow	5
6	Kopargaon Dairy Farm	Cow	10
7	Ambernath Dairy Farm	Buffalo	20
8	Shubham Dairy Farm, Diva	Cow	5
9	Shri Radha Govind Goshala, Badlapur	Cow	5
10	Goshala Kalwa	Cow	5
11	Local vendor, Ambernath	Buffalo	7
12	Thane Dairy Farm	Buffalo	10
13	Gokul Dairy Farm, Thane	Cow	10
14	Titwala Dairy Farm	Buffalo	15
15	Kalyan Tabela	Buffalo	10
Total		106	

colony characters suggestive of *Staphylococcus* spp. were processed further for identification of the organism using standard bacteriological procedures (Edward and Ewing, 1972; Quinn, 1994; Agarwal *et al.*, 2010). The isolates showing gram's positive spherical shape arranged in irregular grape like clusters and showing golden yellow discoloration on MSA were considered as *Staphylococcus* spp. and further identified by biochemical studies.

Identification: The isolates were subjected further to different identification tests recommended (Cruickshank, 1975; OIE, 2009; Agarwal *et al.*, 2010) as described below.

Oxidase test- Standard oxidase discs (Hi Media Laboratories Ltd., Mumbai) were used to perform the test. The loopful of culture from single colony was touched on the disc. Immediate development of blue color was considered as positive test whereas in negative test no color change was observed.

Catalase test- This test was performed by taking 2-3 drops of 3 per cent H₂O₂ on clean grease-free glass slide and single colony was mixed with the help of a wire loop. Immediate production of gas bubbles was considered as

positive test.

Indole test- The test was done by inoculating the organisms in tryptone broth (Hi-media) and incubating at 37°C for 24 hrs followed by addition of 0.2 mL of Kovac's reagent from side of the test tube (Hi-media). Appearance of pink colored ring was considered as positive and yellow colored ring at the interface was considered as a negative test.

Methyl-Red (MR) test- The test was performed using Methyl red (MR) broth in which pure culture was inoculated. The tubes were incubated at 37°C for 48 hours. Thereafter 5 drops of methyl red indicator solution was added to the test tube. Positive results were indicated by bright red color whereas yellow color indicated negative results.

Voges-Proskauer (VP) test- The test was performed using Voges-Proskauer (VP) broth in which pure culture was inoculated. The tubes were incubated for 48 hours at 37°C. After incubation 3 mL of Barrit B reagent (alpha naphthol) and 1 mL of Barrit A (40% KOH) reagent was added. The tube was exposed to atmospheric oxygen for 10-15 minutes. Positive results were indicated by pink color in 2-5 mins and negative by yellow or copper color at the surface.

Citrate test- Simmon's citrate agar slants were inoculated from 18–24 hours old culture colony and incubated at 37°C and observed upto 7 days. A positive reaction was observed by color change from green to blue in the slant along with growth and negative showed no color change and no growth.

Urease test- Christensen's urea agar slants were inoculated with 18–24 hours old culture and incubated at 37°C and observed upto 7 days. A positive reaction was observed by development of pink color in the slant and no color change of the slant in negative reaction.

***In vitro* antimicrobial sensitivity test**

Antibiotic sensitivity test of *Staphylococcus* spp. was carried out by using Muller Hinton Agar (MHA) medium (Bauer *et al.*, 1966). Antibiotic disc of imipenem (IPM 10 mcg), ciprofloxacin (CIP 5 mcg), amoxicillin/clavulanic acid (AMC 10 mcg), ceftriaxone (CTR 30 mcg), tetracycline (TE 30 mcg) and amikacin (AK 30 mcg) from Hi media Mumbai were used. The antibiotics were graded as susceptible (S), intermediate (I) or resistant (R) based on diameter of zone of inhibition (millimetre) developed around the discs as per Clinical Laboratory Standards Institute (CLSI, 2018).

Characterization of *Staphylococcus aureus* by Nuc PCR assay

DNA extraction: DNA of the *S. aureus* isolates was extracted as per the method described by Wilson (2001) with some modifications.

The isolates were inoculated in BHI broth and done incubation at 37°C for overnight. The inoculum was then transferred to micro-centrifuge tube and done the centrifugation at 12,000 rpm for 10 min. Discard the supernatant and pellet was mixed with 200 µL of T.E. buffer and 50 µL of 50 mg/mL stock solution of lysozyme was added and mixed by vortexing. This mixture was incubated at 37°C for 2 hrs. A 30 µL of 10% SDS and 80 µL of 20 mg/mL stock of proteinase K were then added, mixed by vortexing and incubated in water bath at

65°C for 1 hr. On completion of incubation, added 100 µL 5M NaCl and mixed thoroughly by vortexing. Then added 80 µL of C-TAB/NaCl solution, mixed the suspension thoroughly and incubated at 65°C for half an hour in dry bath. An equal volume of phenol:chloroform (1:1) was added and vortexed for 2-3 min followed by centrifugation at 12,000 rpm for 10 min. Then the upper aqueous phase was transferred to a sterile microcentrifuge tube. An equal quantity of chloroform: isoamyl alcohol (24:1) was then added mixed thoroughly and done centrifugation at 12,000 rpm for 10 min. The upper aqueous layer was transferred to a fresh micro-centrifuge tube. Then precipitation of DNA was carried out using one third volume of 7.5 M ammonium acetate and double volume of chilled absolute ethanol. The tube containing DNA was stored at -20°C for overnight. Next day the centrifugation was done at 12,000 rpm for 15 min, the supernatant was carefully discarded leaving the DNA pellet at the bottom of the micro-centrifuged tube. The washing of pellet was done with 70% ethanol, was air dried and resuspended in 30 µL of sterile nuclease free water. The samples were incubated in water bath at 65°C for 2 hrs. The DNA isolated as above was stored at -20°C until further use.

Detection of *Staphylococcus aureus* using Nuc PCR: The Nuc PCR was carried out for detection of *Staphylococcus aureus* by using published oligonucleotide primer sequences Nuc F (5' - CGA-TTG-ATG-GTG-ATA-CGG-TT-3') and Nuc R (5'-ACG-CAA-GCC-TTG-ACG-AAC-TAA-AGC-3') manufactured and supplied by M/s Bangalore Genei, Bangalore (India). This PCR assay shows an amplification product of 270 bp region of Nuc genetic element of *Staphylococcus aureus*. A PCR assay targeting of Nuc gene was employed for identification of *Staphylococcus aureus* during present investigation as described by Ali *et al.* (2014).

The PCR standardized in a reaction volume of 25 µL, containing 10X PCR buffer, MgCl₂ (25 mM), dNTPs mix (10 mM), Primers Nuc- F

(10pM/μL) and Primer Nuc- R (10pM/μL), Template DNA and Taq DNA polymerase (5 U/μL). The reaction was standardized in a thermal cycler (Master Cycler, Eppendorf) with initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 65°C for 45 sec and 72°C for 45 sec. Final extension was carried out at 72°C for 5 min. The amplified product was electrophoresed on 2% agarose gel stained with ethidium bromide (EtBr) and the products were visualized and documented using Automatic Computerized Gel Documentation and Analysis System (Gel Doc EZ Imager, Bio-Rad) using UV light source. The size of PCR product was estimated with the help of Image Lab (Version 4.1) software available with the gel documentation system.

RESULTS

Isolation of *Staphylococcus* spp.: A total of 106 raw cattle and buffalo milk samples were processed for isolation of *Staphylococcus* spp. resulting in the recovery of 45 isolates (42.45%) of *Staphylococcus* spp. (Table 2).

All the isolates recovered were tentatively

identified as *Staphylococcus* spp. based on morphology, staining reaction in Gram's staining method and growth characteristics on Mannitol salt agar (MSA). Further the isolates showing gram positive cocci arranged in irregular grape like clusters (Plate 1) and colonies with golden yellow pigmentation on MSA (Plate 2) were identified as *Staphylococcus aureus* after results of biochemical tests. The isolates showed positive results by catalase (Plate 3), urease (Plate 4), MR (Plate 5), VP (Plate 6) and Citratetests (Plate 7) and were indole (Plate 8) and oxidase (Plate 9) negative (Table 3). The organisms were non-motile.

***In vitro* antimicrobial sensitivity test:** Results of antimicrobial sensitivity testing showed that all 45 *Staphylococcus aureus* isolates (100%) were sensitive to imipenem. Forty-one isolates (91.11%) were sensitive to amoxicillin/clavulanic acid. While thirty-eight isolates (84.44%) were sensitive to ceftriaxone. 36 of 45 *Staphylococcus aureus* isolates (80%) were sensitive to tetracycline, 35 (77.77%) to ciprofloxacin and 32 (71.11%) to amikacin. 13

Table 2. Isolation of *Staphylococcus aureus* from milk specimens

Sl. No	Name of the farm	No. of samples processed for isolation	No. of isolates recovered
1	Narayan Dairy Farm, Dombivli	1	1
2	Durga Dairy Farm, Dombivli	1	1
3	Nanda Dairy, Dombivli	1	1
4	Yadav Dairy, Dombivli	1	1
5	Goshala, Dombivli	5	None
6	Kopargaon Dairy Farm	10	10
7	Ambernath Dairy Farm	20	14
8	Shubham Dairy Farm, Diva	5	None
9	Shri Radha Govind Goshala, Badlapur	5	None
10	Goshala Kalwa	5	None
11	Local vendor, Ambarnath	7	None
12	Thane Dairy Farm	10	3
13	Gokul Dairy Farm, Thane	10	8
14	Titwala Dairy Farm	15	6
15	Kalyan Tabela	10	None
Total		106	45

Molecular Detection of *Staphylococcus aureus* from raw milk in Mumbai

Plate 1. Gram's staining

Plate 2. Colonies of *Staphylococcus aureus* isolated on Mannitol Salt Agar (MSA)

Plate 3. Catalase test

Plate 4. Urease test

Plate 5. Methyl red test

Molecular Detection of *Staphylococcus aureus* from raw milk in Mumbai

Plate 6. Voges-Proskauer test

Plate 7. Citrate test

Plate 8. Indole test

Plate 9. Oxidase test

Plate 10. Antibiotic sensitivity test results

Molecular Detection of *Staphylococcus aureus* from raw milk in Mumbai

[Lane L - 100bp Ladder, 1-5 Isolates, 6- Positive control, N-Negative Control]

Plate 11: Identification of *Staphylococcus aureus* by *Nuc* PCR Assay

[1. IMP- Imipenem 2. CIP- Ciprofloxacin 3. AMC- Amoxicillin + clavulanic acid 4. CTR- Ceftriaxone 5. TET- Tetracycline 6. AK- Amikacin]

Graph 1. In-vitro Antimicrobial sensitivity test results of *Staphylococcus aureus*

Table 3. Identification of *Staphylococcus aureus* isolates by biochemical tests

Sr. No.	Name of the test	<i>Staphylococcus aureus</i>
1	Catalase test	Positive
2	Urease test	Positive
3	Oxidase test	Negative
4	MR (Methyl Red)	Positive
5	VP (Voges Proskaur test)	Positive
6	Indole test	Negative
7	Nitrate reduction test	Positive
8	Citrate	Positive

Table 4. In-vitro antimicrobial sensitivity test results of *Staphylococcus aureus*

Antibiotics used	IMP	CIP	AMC	CTR	TET	AK
Resistance	None	10 (22.22%)	04 (8.88%)	07 (15.55%)	09 (20%)	13 (28.88%)
Sensitive	41 (91.11%)	32 (71.11%)	29 (64.44%)	34 (75.55%)	33 (73.33%)	17 (37.77%)
Intermediate	04 (8.88%)	03 (6.66%)	12 (26.66%)	04 (8.88%)	03 (6.66%)	15 (33.33%)
Total	45	45	45	45	45	45

1. IMP- Imipenem, 2. CIP- Ciprofloxacin, 3. AMC- Amoxicillin + clavulanic acid, 4. CTR- Ceftriaxone, 5. TET- Tetracycline, 6. AK- Amikacin

(28.88%) isolates were resistant to amikacin. 10 (22.22%) were resistant to ciprofloxacin, while 09(20%) were resistant to tetracycline. 04 (8.88%) of 33 isolates were resistant to amoxycillin/ clavulanic acid and 07 (15.55%) to ceftriaxone (Plate 10, Table 4 and Graph 1) Antimicrobial sensitivity testing of *Staphylococcus aureus* showed that imipenem was the most effective (100%) antibiotic followed by amoxicillin/ clavulanic acid (91.11%), ceftriaxone (84.44%), tetracycline (80%), ciprofloxacin (77.77%) and amikacin (71.11%). The most resistant antibiotics were amikacin (28.88%) followed by ciprofloxacin (22.22%), tetracycline (20%), ceftriaxone (15.55%) and amoxycillin/ clavulanic acid (8.88%).

Nuc PCR assay: The Nuc PCR assay using Nuc F/ Nuc R primers was employed for further confirming the identity of recovered isolates

during present study. All the 45 isolates of *Staphylococcus aureus* were included in PCR assay. All the 45 isolates showed Nuc gene specific amplification product of 270 bp (Plate 11) i.e all the 45 isolates carried Nuc gene. In the present study, Nuc PCR assay found to be effective in identification of *Staphylococcus aureus*.

DISCUSSION

The milk acts as an excellent source of many pathogenic and non pathogenic bacteria and may cause severe illness to the human. Hence such raw contaminated milk samples, if consumed by humans, might be hazardous. Also all the hygienic precautions must be followed while collecting, processing and transporting milk samples from farm to the door of consumers. The present study was planned to isolate and identify *S. aureus* from raw milk of cattle and buffalo and to characterize the isolates by NucPCR.

The isolation rate of *Staphylococcus aureus* from raw milk samples in our study was 42.45%. Similar studies were conducted out by different workers on isolation of *S. aureus* from raw milk samples. Pourhassan and Taravat (2011) collected 100 raw milk samples and cultured on suitable media for isolation of organisms by conventional methods. They found 52% prevalence of *Staphylococcus aureus* in milk samples. Mekonnen *et al.* (2018) observed that 39.5% of milk samples were contaminated with *Staphylococcus* species in Debre Zeit, Ethiopia. Daka and Yihdego (2012) isolated seventy-eight *Staphylococcus aureus* isolates and 48.75% isolation rate of *S. aureus* was noticed. In another study carried out in Bangladesh, out of 47 samples 12 (25.53%) isolates were confirmed as *S. aureus* (Jahan *et al.*, 2015). Tanzin *et al.* (2016) recovered 12 isolates (35.29%) of *S. aureus* from 34 milk samples of cattle and buffaloes. Bano *et al.* (2020) processed 200 raw milk samples of cow and buffaloes in Pakistan. They found that 45% *staphylococcus aureus* was recovered from raw milk samples.

The present study revealed that raw milk samples have *Staphylococcus aureus* which can be transmitted to human through milking and consumption of milk. The higher prevalence of *S. aureus* in raw bovine milk may attribute to the lack of hygiene and sanitation in the farm or their handlers. Milk can be contaminated by *Staphylococcus aureus* by infection of the mammary gland, by bad hygienic habits or direct contact of human such as handling equipment of milk during or after milking, coughing, sneezing, lack of washing hands etc. Dung, mud, the milk containers used for milk is also act as the source of many pathogenic bacteria. The most important contaminant is water sample which is added in the milk at farm to increase its quantity. Long duration for reaching the milk to the consumer level increases the chance of poliferation of bacteria due to elevation of temperature of milk during transport (Hassan *et al.*, 1978).

Results of *in vitro* antimicrobial sensitivity

testing are in agreement with Thaker *et al.* (2013) who reported the sensitivity of *S. aureus* isolates towards cephalothin, co-trimoxazole, cephalixin, methicillin, gentamicin, ciprofloxacin, oxacillin, streptomycin and ampicillin. Abo-shama (2014) recovered 136 isolates of *S. aureus* from 340 raw milk samples and performed antibiotic sensitivity test. These isolates showed sensitivity towards ciprofloxacin and tetracycline. Oliveira *et al.* (2011) isolated *staphylococcus* from buffalo milk samples and noticed that the organisms were sensitive to amoxycillin with clavulanic acid and resistant to ampicillin, penicillin and oxacillin.

The beta-lactam antibiotics have been widely used for treatment of *Staphylococcal* infections since many years. In the present study all the 45 *Staphylococcus aureus* isolates were sensitive to imipenem (100%) followed by ceftriaxone (84.44%). The tetracycline antibiotics are frequently used for treating infections caused by *S. aureus* in Iran (Eslampour *et al.*, 2009). In the present study, the sensitivity to tetracycline was determined in 20% of the *Staphylococcus aureus* isolates. These findings indicate that the antibiotic sensitivity testing should be recommended in infections caused by *Staphylococcus aureus*.

In the present study Nuc PCR found to be effective in detecting *Nuc* gene from *Staphylococcus aureus* isolates. Similar results were recorded (Wilson *et al.*, 1991; Brakstad *et al.*, 1992; Sahebnaasagh *et al.*, 2014).

According to Sahebnaasagh *et al.* (2014) compared *Nuc* PCR and 16S rRNA PCR for detection *Staphylococcus aureus*. They found that *Nuc* PCR is more specific than 16S rRNA PCR assay for detection of *Staphylococcus aureus*. Recently, findings of *Nuc* PCR assay were reported by Wilson *et al.* (1991) who used a primer set different from that used in the present study. Those investigators amplified a 450-bp segment of the gene. The studies were carried out by Brakstad *et al.* (1992) and it was observed that *Nuc* PCR has potential for the rapid diagnosis of *Staphylococcus aureus* infections. Akindolire *et al.* (2015) found that

the *Nuc* gene is more specific in *S. aureus* isolates than 16S rRNA PCR. The *Nuc* PCR assay for the detection of *Staphylococcus aureus* was 100 per cent specific and sensitive and the assay was particularly suitable for analysis of samples shipped or stored without refrigeration (Khan *et al.*, 1998). Further *Nuc* PCR assay was found to be very specific to detect *Staphylococcus aureus* (Kim *et al.*, 2001; Kuzma *et al.*, 2003; Kalorey *et al.*, 2007).

Forty five isolates of *Staphylococcus aureus* were recovered from 106 raw milk samples with an isolation rate of 42.45%. Imipenem and amoxicillin/ clavulanic acid were found to be more effective antibiotics while amikacin and ciprofloxacin were the least effective antibiotics against *Staphylococcus aureus* isolated from raw milk samples in the present study. The *Nuc* PCR assay was very effective in detecting *Staphylococcus aureus* isolates recovered from raw milk samples. Strict hygienic precautions must be followed to reduce contamination of raw milk samples at

farm level. Further elaborate studies are required to detect the contamination of raw milk samples at farm level and reduce its transmission to human population.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: VRP: Writing, original draft preparation; VDT: Writing, review and editing, conceptualization, data curation; SBM: Project administration, supervision; RSG: Visualization, investigation.

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