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Research Article

Identification of the *Staphylococcus* Species Which Cause Cattle Mastitis Using MALDI-TOF MS

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Abstract

The aim of this study was identification and antibiotic susceptibility of *Staphylococcus* species from mastitic milk, comparison of results by using commercial identification methods such as VITEK, MALDI-TOF MS analysis. A total of 100 milk samples taken in sterile conditions were investigated for the presence of *Staphylococcus* sp., which were found to have clinical mastitis problems in animals in the milk province of Aydın province and surrounding areas. Identification of *Staphylococcus* species was performed by VITEK 2, VITEK (MALDI-TOF) MS in addition to biochemical methods. *Staphylococcus* sp. was isolated from 34% of the milk samples. Seven (20.60%) of the isolates were *Staphylococcus aureus*, 10 (29.40%) were *Staphylococcus simulans*, 8 (23.50%) were *Staphylococcus epidermidis* and 4 (11.80%) *Staphylococcus saprophyticus*, 2 (5.90%) were *Staphylococcus chromogenes*, 2 (5.90%) were *Staphylococcus hyicus* and 1 (2.95%) were *Staphylococcus arlettae*. result of the antibiogram test, 34 *Staphylococcus* sp. isolates were sensitive to Cefoxitin, Ciprofloxacin, Vancomycin, Tigecycline and Fosfomycin in the ratio of 100% and resistant to Linezolide, Clindamycin and Tetracycline in the ratio of 85.2%, 100% and 82.4% respectively. In conclusion; the reliability of the MALDI-TOF MS proteomic biomass spectrophotometry method has been demonstrated to be verifiable and feasible.

Keywords: Mastitis; *S aureus*; *Coagulase Negative Staphylococcus* ; MALDI-TOF MS

Introduction

Mastitis is an important disease in terms of its prevalence and the economic losses it incurs due to the drop-in milk yield in the business. For this reason, it is important to prevent any damage that may be caused by early diagnosis of clinical mastitis, as in all diseases. Staphylococcal agents are the major bacterial agents causing bovine mastitis. These factors are spread widely, especially with staff and milking units. Staphylococci are predominant pathogens in mastitis cases of subclinical and clinical type. *Staphylococci* are able to adapt very rapidly to changes in environmental, host, mastitis control and care. *Staphylococcal* clinical cases are characterized by prolonged but relatively infrequent occurrence. Staphylococci are characterized by various pathogenic factors.

These factors are those that trigger damage to mammary tissues and neutralize antimicrobials that can escape immunity defences [1-3]. The virulence factors in the Staphylococci have been extensively researched. *Staphylococcus aureus* strains isolated from bovine mastitis cases include leukocidin, enterotoxin and coagulase in addition to alpha, beta, gamma, delta toxins. CNS strains also produce some toxins and enzymes (hemolysin, leukocidin, lipase, protease, DNase) that can be effective in disease formation. A large number of *Coagulase Negative Staphylococci* (CNS) isolated from mastitis cases show protease, DNase and lecithinase activity in excess, unlike the CNS isolated from normal cows. The isolation rate of *S. aureus* in previous studies performed in our country was 28.3% in Aydın, 40.1% in Afyon and 43% in Burdur [4-6].

Mastitis is an important mammary gland disease caused by microorganisms. The economic losses associated with mastitis are not only limited to a reduction in milk yield, but also include the treatment of the disease and the removal of diseased animals. In addition, deterioration in the quality of the milk, which is an important food source, causes negative results in terms of public health [4]. By means of MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization with Time-of-Flight Mass Spectrometry) method, a specific kind of fingerprints for microorganisms based on mass spectrometry are obtained from proteins specific for each organism and bacterial, fungal and virus identification can be performed [7]. The matrix is necessary for successful ionization of the material as it acts both as an environment in which ionization can occur and as a proton supplier for the ionization of the material.

The sample-matrix crystal on the surface of the metal plate is irradiated using a UV laser beam. Irradiation occurs briefly to avoid degradation, which may be caused by overheating [8]. Bacterial identification is the result of comparing the information obtained from the sample with the information in the database [9]. It has been reported that the correct identification rate of MALDI-TOF MS in species-based routine bacterial isolation varies between 84.1% and 95.2% [10,11]. The scope of this study was to determine isolation and antibiotic susceptibility of the most common *Staphylococcus* species in mastitis cases which are very important for milk production, to biotype and isolate the isolated strains by commercial identification methods such as VITEK and MALDI TOF MS.

Material and Methods

Sample collection

The study was carried out between March and August 2017 in dairy cattle breeding farms in private enterprises in Aydin province. One hundred milk samples from Holstein dairy cows which showed signs of clinical and subclinical mastitis in 8 farms with 25-250 head animal capacity were used. The animals chosen for sampling were in the lactation period, at 2-8 years of age, did not receive antibiotic treatment in one month of period. Adnan Menderes University Animal Experiments Local Ethics Committee was informed by ethics decision no. 64583101/2016/140 dated 25.08.2016 VII session.

Staphylococcus sp. isolation

Milk samples brought to the laboratory were directly inoculated in Mannitol Salt Agar (MSA). After 24 hours of incubation at 37 °C, the colonies were cultured in a blood agar containing 5% sheep blood. After 24 hours incubation, the biochemical characteristics of the strains evaluated as Gram positive cocci by Gram staining were evaluated by standard laboratory procedures. Round shaped bacteria with Gram (+) character were searched on microscope [12]. Identification of *Staphylococcus* sp. isolates was carried out by catalase, DNase and coagulase reactions, mannitol fermentation and bacitracin susceptibility assays.

Identification of *Staphylococcus* sp. with VITEK 2 compact®

In this study, VITEK GP (Gram Positive) identification cards were which included *Staphylococci* in test database. From 18-24h fresh cultures grown in 5% sheep blood agar, the suspension was prepared with sterile cotton swab sticks and with a density of 0.50-0.63 McFarland in 3ml sterile saline solution according to the manufacturer's instructions. In the McFarland measurement, the bioMérieux® brand DensiCHEK™ Plus model McFarland was used. The prepared bacterial suspension tubes were loaded with VITEK GP® cards and loaded onto the filler of the device by matching the card barcodes and sample names in accordance with the manufacturer's instructions. At the end of the filling process in the filler section, the cards were transferred to the incubation module of the device. After this transfer, the device again checks the entered sample names and card barcode's mappings and the filled cards are automatically transferred to the incubation module of the device. At the end of the incubation period the results were automatically printed by VITEK 2 Compact®.

Identification of *Staphylococcus* sp. with VITEK (MALDI-TOF) MS

In this study VITEK MS/IVD/V.3.0 (bioMérieux, France) database was used and all the operations performed in the direction of the manufacturer were carried out as follows. Bacterium agar subculture was obtained from our staphylococcal isolates determined as Gram positive cocci and catalase positive by preliminary tests. One colony from fresh colonies after 18-24 hours was taken with a 1µL aliquot and spread as a thin layer on VITEK MS slides. On the same slide, 34 isolates were prepared. The calibration curve, located in the middle of each 16 wells on the prepared slide, was spread in a thin layer of the *Escherichia coli* ATCC 8739 with mass spectrum profile for quality control and calibration purposes. A 1µl matrix solution of CHCA (α-cyano-4-hydroxycinnamic acid) was added to each of the prepared wells and allowed to dry in the chamber. After waiting for 2 minutes, the slide was inserted into the device and the operation started. After the vacuum and calibration process lasted about 10 minutes, the spectra of each of the isolates began to be taken when the device was first turned on. The total reporting time of our slide with 48 samples and 3 calibration points was measured as 57 minutes (about 1.7 Minutes per sample).

Antibiotic susceptibility test

VITEK AST-P641 cards with reference number 418591 were used for antibiogram. The antibiotics, concentrations and reported ranges of VITEK AST-P641 are given in Table 1. A total of 280µL bacterial suspension at 0.50-0.63 McFarland concentration prepared in the VITEK GP cards was transferred to another sterile 3mL saline solution and mixed homogenously by automatic pipetting. As it is in the process of identification with VITEK Cards, it was transferred to the incubator module after it was filled by the device first. Results were received by the device within 8 hours.

Table 1: The antibiotics, concentrations and reported ranges of VITEK AST-P641

Antimicrobial Agent	Concentration	Reported Ranges	
		≤	≥
Penicillin (P)	0.125, 0.25, 1, 2, 8, 64	0.12	8
Cefoxitin (OXSF)	6	NEG	POS
Ciprofloxacin (CIP)	1, 2, 4	0.5	8

Erythromycin (E)	1, 2, 4, 8	0.25	8
Clindamycin (CM)	0.06, 0.25, 1	0.125	4
Linezolid (LNZ)	0.5, 1, 2	0.5	8
Daptomycin (DAP)	0.5, 1, 2, 4, 16	0.12	8
Teicoplanin (TEC)	0.5, 2, 8, 32	0.5	32
Vancomycin (VA)	1, 2, 4, 8, 16	0.5	32
Tetracycline (TE)	0.5, 1, 2	1	16
Tigecycline (TGC)	0.25, 0.5, 1	0.12	2
Phosphomycin (FOS)	8, 32	8	128
Trimethoprim-sulfamethoxazole (SXT)	8/152, 16/304, 32/608	10 (0.5/9.5)	320(16/304)

Results

Isolation and identification

Of the 100 milk samples examined in our study, 34 (34.0%) of the samples were isolated as *Staphylococcus* sp. As a result of coagulase test, 7 (20.6%) isolates were *Coagulase Positive Staphylococci* and 27 (79.4%) were *Coagulase Negative Staphylococci* respectively. Seven (20.6%) isolates were identified as *Staphylococcus aureus*, 11 (32,3%) isolates as *Staphylococcus simulans* in, 8 (23.5%) isolates as *Staphylococcus epidermidis*, 4 (11.8%) isolates as *Staphylococcus saprophyticus*, 2 (5.9%) isolates as *Staphylococcus chromogenes*, and 2 isolates (5.90%) as *Staphylococcus hyicus* in conclusion of biochemical tests.

Table 2: The results of conventional methods, VITEK 2 Compact and MALDI-TOF MS identification

	Sample No.	Coagulase	*Staphylococcus* sp. identified by conventional methods	*Staphylococcus* sp. identified by VITEK 2 Compact	%	*Staphylococcus* sp. identified by VITEK MS (MALDI-TOF MS)	%
1	7	+	*S. aureus*	*S. aureus*	97,0%	*S. aureus*	99,9%
2	21	+	*S. aureus*	*S. aureus*	99,0%	*S. aureus*	99,9%
3	40	+	*S. aureus*	*S. aureus*	98,0%	*S. aureus*	99,9%
4	45	+	*S. aureus*	*S. aureus*	99,0%	*S. aureus*	99,9%
5	75	+	*S. aureus*	*S. aureus*	95,0%	*S. aureus*	99,9%
6	83	+	*S. aureus*	*S. aureus*	99,0%	*S. aureus*	99,9%
7	85	+	*S. aureus*	*S. aureus*	99,0%	*S. aureus*	99,9%
8	14	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
9	17	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
10	18	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
11	33	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
12	60	-	*S. simulans*	*S. simulans*	93,0%	*S. simulans*	99,9%
13	64	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
14	66	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
15	73	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
16	76	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
17	89	-	*S. simulans*	*S. simulans*	99,0%	*S. simulans*	99,9%
18	98	-	*S. simulans*	*S. simulans*	93,0%	*S. arlettae*	99,9%
19	15	-	*S. epidermidis*	*S. epidermidis*	99,0%	*S. epidermidis*	99,9%
20	19	-	*S. epidermidis*	*S. epidermidis*	95,0%	*S. epidermidis*	99,9%
21	23	-	*S. epidermidis*	*S. epidermidis*	97,0%	*S. epidermidis*	99,9%

22	42	-	*S. epidermidis*	*S. epidermidis*	99,0%	*S. epidermidis*	99,9%
23	46	-	*S. epidermidis*	*S. epidermidis*	99,0%	*S. epidermidis*	99,9%
24	55	-	*S. epidermidis*	*S. epidermidis*	98,0%	*S. epidermidis*	99,9%
25	62	-	*S. epidermidis*	*S. epidermidis*	97,0%	*S. epidermidis*	99,9%
26	87	-	*S. epidermidis*	*S. epidermidis*	99,0%	*S. epidermidis*	99,9%
27	8	-	*S. saprophyticus*	*S. saprophyticus*	97,0%	*S. saprophyticus*	99,9%
28	44	-	*S. saprophyticus*	*S. saprophyticus*	99,0%	*S. saprophyticus*	99,9%
29	92	-	*S. saprophyticus*	*S. saprophyticus*	99,0%	*S. saprophyticus*	99,9%
30	96	-	*S. saprophyticus*	*S. saprophyticus*	98,0%	*S. saprophyticus*	99,9%
31	67	-	*S. chromogenes*	*S. chromogenes*	99,0%	*S. chromogenes*	99,9%
32	77	-	*S. chromogenes*	*S. chromogenes*	97,0%	*S. chromogenes*	99,9%
33	69	-	*S. hyicus*	*S. hyicus*	99,0%	*S. hyicus*	99,9%
34	81	-	*S. hyicus*	*S. hyicus*	99,0%	*S. hyicus*	99,9%

As a result of identification with VITEK 2 Compact, 7 (20.6%) *Staphylococcus aureus*, 11 (32.3%) *Staphylococcus simulans*, 8 (23.5%) *Staphylococcus epidermidis*, 4 (11.8%) *Staphylococcus saprophyticus*, 2 (5.9%) *Staphylococcus chromogenes*, 2 (5.9%) *Staphylococcus hyicus* were identified. As a result of identification studies with MALDI-TOF MS device, 7 (20,60%) *Staphylococcus aureus*, 10(29,40%) *Staphylococcus simulans*, 8 (23,50%) *Staphylococcus epidermidis*, 4 (1,80%) *Staphylococcus saprophyticus*, 2 (5,90%) *Staphylococcus chromogenes*, 2 (5.90%) *Staphylococcus hyicus* and 1 (2.95%) *Staphylococcus arlettae* were identified. The results obtained with the VITEK 2 Compact in our study are in agreement with the results obtained with conventional methods. When the results of MALDI-TOF MS are examined, it is seen that 1 *Staphylococcus simulans* isolate is identified as *Staphylococcus arlettae* (Table 2).

Antibiogram

Staphylococcus strains which have been typed in our study were treated with VITEK AST-P641 reference no. 418591 and VITEK 2 Compact antibiogram device. The results of antibiograms evaluated according to CLSI (2017) standards of isolates are shown in Table 3 & 4. Identified 34 *Staphylococcus* sp. isolates were sensitive to Cefoxitin, Ciprofloxacin, Vancomycin, Tigecycline and Phosphomycin (100%) and Linezolide (85.2%); resistant to Clindamycin (100%) and Tetracycline (82.4%). *S. aureus* isolates were detected resistant to Cefoxitin, Vancomycin, Tigecycline, Phosphomycin and Clindamycin (100%) and Tetracycline (85.8%). It was determined that *Coagulase Negative Staphylococci* isolates were resistant to Cefoxitin, Vancomycin, Tigecycline and Fosfomycin (100%), Ciprofloxacin (92.5%), Linezolide (88.8%), Tetracycline and Trimetoprim/Sulfametoxazole (81.5%) and Clindamycin (100%). *S. simulans* isolates were resistant to *clindamycin* (100%), Teicoplanine (90%) and other 11 antibiotics (90%) used in this research. *S. arlettae* isolate was resistant to Clindamycin and Teicoplanin (100%), susceptible to other antibiotics. Clindamycin was found to be 100% resistant to *S. epidermidis* isolates were found resistant to Clindamycin (100%) and susceptible to the other 12 antibiotics (75-100%) used in this study. *S. saprophyticus* isolates were resistant to Clindamycin (100%) and susceptible to other 8 antibiotics (100%). *S. chromogenes* and *S. hyicus* isolates (n = 2) were found to be resistant to Clindamycin and Teicoplanin (100%).

Table 3: CLSI standard minimal inhibitory concentration (MIC) values (CLSI, 2017)

Antibiotics	S	I	R
Penicillin	≤0.12	-	≥0.25
Cefoxitin (OXSF)	≤4	-	≥8
Ciprofloxacin (CIP)	≤1	2	≥4
Erythromycin (E)	≤0.5	01-Apr	≥8
Clindamycin (CM)	≤0.5	01-Feb	≥4
Linezolid (LNZ)	≤4	-	≥8
Daptomycin (DAP)	≤1	-	-
Teicoplanin (TEC)	≤8	16	≥32
Vankomisin (VA) (S. aureus)	≤2	04-Aug	≥16
Vancomycin (VA) (KNS)	≤4	Aug-16	≥32
Tetracycline (TE)	≤4	8	≥16
Tigecycline (TGC)	≤0.5	-	-
Phosphomycin (FOS)	≤64	128	≥256
Trimethoprim-sulfamethoxazole (SXT)	≤2/38	-	≥4/76

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Table 4: The result of antibiogram in this study

Isolates	Antibiotics												
	P	OXFS	CIP	E	CM	LNZ	DAP	TEC	VA	TE	TGC	FOS	SXT
S. aureus (n=7)	%42.8 S		%57.2S	%57.2S		%57.2S	%57.2S	%14.2 S		%42.8 S	%100 S	%100 S	%57.2S
	%57.2 R		%42.8R	%42.8R	%100 R	%42.8R	%42.8R	%85.8R		%57.2 R			%42.8R
S. simulans (n=10)	%80.0 S		%100 S	%80.0 S		%90.0 S	%80.0 S	%10 S		%80.0 S	%100 S	%90.0 S	%80.0 S
	%20 .0 R			%20 .0 R	%100 R	%10 R	%20 .0 R	%90 R		%20 .0 R		%10 R	%20.0 R
S. arlettae (n=1)	%100 S		%100 S	%100 S		%100 S	%100 S			%100 S	%100 S	%100 S	%100 S
					%100 R			%100 R					
S. epidermidis (n=8)	%62.5 S		%87.5 S	%75.0 S		%87.5 S	%62.5 S	%37.5 S		%75.0 S	%100 S	%87.5 S	%75.0 S
	%37.5 R		%12.5 R	%25.0 R	%100 R	%12.5 R	%37.5 R	%62.5		%25.0 R		%12.5 R	%25.0 R
S. saprophyticus (n=4)	%75.0 S		%100 S	%75 S		%100 S	%75 S	%25.0 S		%100 S	%100 S	%100 S	%100 S
	%25.0 R			%25 R	%100 R		%25 R	%75.0 R					
S. chromogenes (n=2)	%50.0 S		%100 S	%50.0 S		%100 S	%50.0 S			%100 S	%100 S	%100 S	%100 S
	%50.0 R			%50.0 R	%100 R		%50.0 R	%100 R					
S. hyicus (n=2)	%50.0 S		%50.0 S	%50.0 S		%50.0 S	%50.0 S			%50.0 S	%100 S	%50.0 S	%50.0 S
	%50.0 R		%50.0 R	%50.0 R	%100 R	%50.0 R	%50.0 R	%100 R		%50.0 R		%50.0 R	%50.0 R

Discussion

S. aureus is the most common bacterial cause of chronic mastitis. Although some cattle catch clinical mastitis after calving, infection is usually subclinical and causes an increase in somatic cell counts without noticeable changes in the milk or mammary lobes. Once established, *S. aureus* infection does not respond to antibiotic therapy and infected cattle must be removed from the herd [13,14]. Vaious isolation rates in different studies for the identification of microorganisms that cause mastitis has been found in Turkey. Türütoğlu et al. [15] reported 28.1% *S. aureus* and 23.1% *S. epidermidis* in the Marmara region, Kuyucuoğlu and Uçar [5] detected 40.1% *S. aureus* in the Afyon region, Ergün et al. [16] found 42.4% CoNS and 25.1% *S. aureus*, Gürtürk et al. [17] have isolated 41% of *Staphylococcus* species in Van and its region. Yıldız [18] identified 6 (31.58%) *S. aureus*, 5 (26.32%) *S. epidermidis* in clinical mastitis milk samples.

In studies conducted abroad, Tenhagen et al. [19] identified 9.1% CoNS and 5.7% *S. aureus* in Germany, Gianneechini et al. [20] reported 62.8% *S. aureus* and 7.4% CoNS in Uruguay, Pitkälä et al. [21] identified 49.6% CoNS and 10.2% *S. aureus* in Finland, Workineh et al. [22] identified 40.5% *S. aureus* and 16.5% CoNS in Ethiopia. Lüthje and Schwarz (2006) analysed antimicrobial agents of *Coagulase Negative Staphylococci* and Macrolide and Linkosamide (ML) resistance for the control of pathogens associated with bovine mastitis. In total, 298 CNS isolates were collected from subclinical mastitis cattle in Germany between 2003 and 2005. *S. chromogenes* (99 isolates, 32.2%), *S. simulans* (69 isolates, 23.2%), *S. epidermidis* (35 isolates, 11.7%), *S. xylosus* and *S. haemolyticus*, 9.4%). In addition, *S. capricius*, *S. cohnii*, *S. hominis* (2 isolates from each) and single isolate *Staphylococcus caprae* (1 isolate), *S. sciuri* (8 isolates), *Staphylococcus equorum* (6 isolates), *S. saprophyticus*, *S. arlettae* and *Staphylococcus gallinarum* species were identified.

Pankaj et al. [23] investigated the etiologic factors of Murrah cows with mastitis. A total of 326 milk samples were taken from 82 healthy cattle. Analyses have revealed 44 organisms as a result. 15.90% of them were *Coagulase Positive Staphylococci*, 47.72% were *Coagulase Negative Staphylococci*, 25% were *Streptococcus dysgalactiae*, 9.09% were *Streptococcus agalactiae*, 2.27% were *Streptococcus uberis* and 13.63% *Staphylococcus* spp. and *Streptococcus* spp. *S. aureus* and *Staphylococcus haemolyticus* was detected as major isolates, followed by *S. epidermidis*, *S. simulans*, *S. hyicus*, *Staphylococcus pasteuria*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *S. arlettae* and *S. gallinarum* isolates.

The phenotypic differentiation of *Coagulase Positive Staphylococci* species presents a difficult situation due to the lack of specific biochemical markers. To overcome this problem, the use

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of automatized tools has become routine in human and veterinary microbiology. Nevertheless, phenotypic analyses are relatively time consuming and most importantly, difficult to analyse results [24]. In this study, identification of *S. aureus* and other coagulase negative species isolated by conventional and commercial biochemical identification methods was verified by VITEK 2, VITEK (MALDI TOF) MS analyses. Automated systems have proven to be an effective tool to distinguish Staphylococcal species.

β-lactam antibiotics have been used for many years to treat staphylococcal infections. The unconscious use of the β-lactam group of antibiotics has also led to the development of resistance to these antibiotics. When the literature data are examined; with regard to *Staphylococci* isolates, Penicillin resistance is in the ratio of 59-88% and Amoxicillin resistance is 29-78% [5,25,26] for Turkey's survey conducted in different regions. In this study, β-lactam group for Staphylococci isolated from mastitis milk were tested in antibiotic susceptibility tests; Penicillin is 35.3% resistant and Cefoxitin is 100% sensitive. In experiments with other antibiotic groups, strains were 100% sensitive Vancomycin, Tigecycline and Fosfomycin. The *Staphylococcus* strains isolated in our study showed 100% resistance to clindamycin. The high rate of Vancomycin susceptibility is considered due to the very limited use of vancomycin-containing preparations [27,28].

Conclusion

In addition to the standard biochemical procedures we use for identification in our study, the use of other automated systems has been shown to produce similar results compared to the factors isolated in mastitis studies in our country. Therefore, it has been demonstrated that the reliability of proteomic methods such as MALDI-TOF MS can be verified and applied. Thanks to our research, the isolation and identification of the pathogens in the clinic type mastitis cases, which are very important for milk production, with fast and reliable methods, will be shed light on future studies for the genetic and the peripheral of the disease. Future work may be effective in determining the diagnosis and treatment policies of the disease for our country. It should be taken into consideration that different antibiotic susceptibilities may also be detected in our studies when both antibiotic treatment and *S. aureus* and other Coagulase Negative *Staphylococci* species are present.

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