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RESEARCH ARTICLE

ASSESSMENT OF VIRULENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* FROM DIFFERENT MILK SAMPLES

***Sangeetha, N., Thenmozhi, S. and Vijayalakshmi, P.**

Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam- 637 205, Tiruchengode, Namakkal District, Tamilnadu, India

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ABSTRACT

The study was carryout to evaluate the incidence and multidrug resistant of *Staphylococcus aureus* in different milk samples. *Staphylococcus aureus* is a major problem of public health which causes a number of human and animal diseases. The main source of infection is contaminated milk. Totally 50 raw and pasteurized milk samples were collected from three different sources such as Goat milk (15), Buffollow milk (25), and pasteurized milk (10). In order to isolate and identify the *Staphylococcus aureus* from these samples. The collected milk samples were cultured on nutrient agar; the presumptive *Staphylococcus* colonies were sub-cultured on Mannitol Salt Agar (MSA) and confirmed by using standard Bacteriological methods. Antimicrobial susceptibility pattern of *Staphylococcus aureus* was done by Kirby-Bauer disk diffusion method using eight antimicrobials. The prevalence of *S.aureus* was found to be 25 (50%) out of the total samples examined. In addition, the prevalence of *S.aureus* was 5 (33.3%) from Goat milk, 16 (64%) from Buffalo milk and 4 (40%) from pasteurized milk. *S.aureus* was more likely to occur in buffalo milk that were poorly managed and treated frequently with antimicrobials. Thus, out of a total of 25 isolates, high resistance rate was observed primarily to Methicillin 25 (100%) followed by Erythromycin15 (60%), Amoxycillin12 (48%), Vancomycin12 (48%), Gentamycin 12 (48%), Tetracycline 11 (44%), Ciprofloxacin 11 (44%), and low level of resistance to Kanamycin 8 (32%). *S.aureus* became almost resistant to β-lactams and Erythromycin. The virulence characters of *Staphylococcus aureus* were characterized phenotypically. The rate of positiveness for Protease, Lipase, β-hemolysis, β-lactamase and Slime formation were 48%, 60%, 60%, 44%, 48%.In this study we concluded that the hygiene of milk is poor and resistant strains have contaminated the milk probably during the process of milking and transportation.

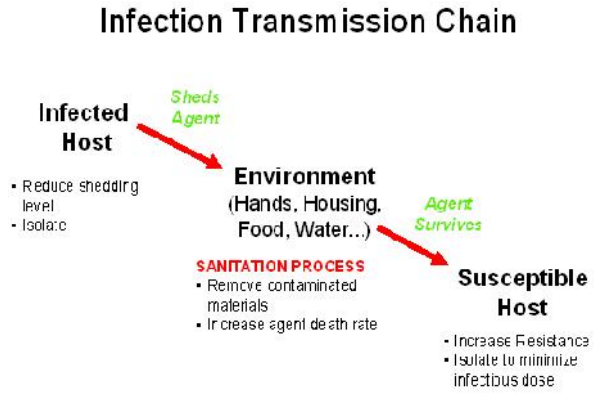
INTRODUCTION

Milk is an excellent bacteriological medium for a large number of microorganisms. When the milk is drawn from the udder of a healthy animal, it contains organisms that have entered the teat canal through its opening. They are mechanically flushed out during milking. The number ranged during milking between several hundreds to several thousands per millilitre. The source of contamination may be due to environment, milking utensils and the personals. A variety of diseases may be potentially transmitted through milk. The source of pathogenic agents occurring in milk may be either a cow, or a human, and it may be transmitted by both (Khan *et al.,* 2000). Milk is considered a good medium for the growth of microorganisms including *Staphylococcus aureus*). *S.aureus* is a facultative anaerobic Gram-positive coccus; it is non-motile, catalase and coagulase positive. Cells are spherical single orform grape-like clusters, the staphylococcal cell wall is resistant to lysozyme and sensitive to lysostaphin.

***Corresponding author: Sangeetha, N.** Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam- 637 205, Tiruchengode, Namakkal District, Tamilnadu, India.*

Staphylococcal food poisoning is a syndrome characterized by nausea, vomiting, diarrhea, general malaise and weakness. Such symptoms appear within 2-4 hours post-ingestion of contaminated food. Although the illness is seldom fatal, complications including dehydration and shock, can accompany severe attacks. *Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks. It is also a major causative pathogen of clinical or subclinical mastitis of dairy domestic ruminants (Le Loir *et al*., 2003). Milk and its products can harbor a variety of microorganism and can be important sources of food borne pathogens. The presence of these pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment or with excretions from the udder of infected animals (Oliver *et al.,* 2005). Mastitis is one of major cause of financial losses for dairy cattle formers. Staphylococcal species associated with bovine mastitis have been classified as coagulase - positive or coagulase- negative. Coagulase positive *Staphylococcus aureus* is considered a major cause of bovine mastitis. Bovine mastitis due to *Staphylococcus aureus* is generally chronic, not easily cured by antibiotic treatment and it represents a serious economic problem for milk producers and dairy industries. In

addition, the presence of *Staphylococcus aureus* in milk could also represent a serious public health problem because some strains have the ability to produce a variety of toxins. *Staphylococcus aureus* produces a broad spectrum of surface components and exotoxins, they are virulence factors involved in the pathogenesis of bovine mastitis as these toxins and products are Virulence factors involved in the pathogenesis of bovine mastitis as these toxins and products are injurious to milk producing cells of the mammary gland, impair glands and immune defense mechanisms, while they are capable to reside intracellular contributes the ability of *S. aureus* establish a chronic infection that can persist for the life of the animal (Taverna *et al.*, 2007).



S.aureus is historically one of the most important causes of subclinical mastitis and clinical mastitis that becomes chronic (Tenhagen *et al.*, 2009). Bovine mastitis is among the leading issues that cause serious economic losses in dairy industry throughout the world, resulting in reduced milk production, reduction of the quality of milk through contamination and due to treatment-associated hazards (Abeer *et al.*, 2010).Therefore, the identification of the *staphylococci* by their ability to produce coagulase is deemed the main criterion for differentiation of those with pathogenic potential from others. On the other hand, some coagulase-positive *staphylococci* (CoPS) other than *S. aureus* such as *S.intermedius* and *S. hyicus* could cause intramammary infections in cows (Akineden *et al.*, 2011). In that respect, the differentiation of the staphylococcal mastitis causative agents is important not only for the therapeutic approach and the remedial schemes, but also for the ability of some of them to produce a wide range of virulent factors (Rahimie *et al.*, 2012). There is a broad range of classic antigenic staphylococcal enterrotoxins (SE). The detection of (SE) is of public health significance and is epidemiologically essential, As the milk is the most important food in our country and the risk of poisoning from the ingestion of milk containing enterotoxin is well known (Terman *et al.*, 2013). The virulence of *Staphylococcus aureus* is considered to be the result of the coordinated activity of several secreted toxins and digestive enzymes as well as a large number of proteins on the bacterial surface that bind extra cellular matrix and plasma proteins. The resistance of the bacterial strains to β-lactam antibiotics is also under taken with special reference to the production of β-lactamases. It is believed that the results of this finding will not only add to the existing world data on bacterial resistance of food origin, but will sensitize the operators in this industry, policy markers and the regulatory agencies on the need to improve the quality of these products. The importance of Staphylococcal lipases, like other microbial lipases, results from their significance in bacterial lipid metabolism and their involvement in pathogenic processes. Most of the known Staphylococcal lipase is produced by pathogenic members of the genus, i.e., *Staphylococcus aureus* and *Staphylococcus epidermidis.* While it is possible that lipases might support the persistence of these strains in the fatty secretions in mammalian skin and thus have an indirect influence on their pathogenic potential, a direct involvement of lipases in pathogenesis remains to be demonstrated. *Staphylococcus aureus* produces lipases- producing *Staphylococcus aureus* cells by host granulocytes, this indicating a direct involvement of lipase in pathogenesis (Rollof *et al.*, 1988). β-hemolysin is one of a variety of diffusible substances produced by *Staphylococcus aureus* for specific characterization, isolation of this hemolysin in a pure and active form is essential. As a preliminary, isolation of a strain of *Staphylococcus aureus* producing large quantities of β-hemolysin alone, and a detailed survey of the conditions suitable for the invitro production and titration of β- hemolysin, are necessary. Biofilm are group of microorganisms attached to a surface and covered with by an exopolysaccharide matrix. Certain surface protein, extracellular proteins, capsular polysaccharides, adhesion are involved in regulation of biofilm production.

The most important microorganisms causing mastitis are Staphylococci, Streptococci and Coliform bacteria. The different diagnosis of clinical mastitis can be treated with various antibiotics. Antibiotics are used to treat diseases of cattle and as well as used as preservatives for milk. The indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective. Resistant bacteria occur in soil, water, plants and animals. The resistant bacteria present in environments are in contact with human beings and animals. It has been estimated that nearly equal tonnage of antimicrobial agents are used in man and in agriculture worldwide. When low doses of antibiotics are used, they inhibit the growth of susceptible bacteria while resistance bacteria thrive and grow such as in the presence of tetracycline (Eichner and Gravitz 1999). In the last few decades, Staphylococcal food poisoning has been reported as third cause of food-borne illnesses in the world. Among the foods implicated in Staphylo-coccal food poisoning, milk, dairy products and meats, particularly handled foods, play a vital role since entero-toxigenic strains of *S. aureus* have been commonly isolated in them (Ateba *et al.*, 2010).The essential goal of the present study was to determine the effect of Virulence factors and the multi-drug resistance patterens of *Staphylococcus aureus* in different milk samples.

Aim

To determinate the virulence distribution and antimicrobial susceptibility of *Staphylococcus aureus* from different milk samples.

Objectives

- The Goat milk, Buffalo milk and Pasteurized milk samples were collected from in and around the Namakkal district.

- To isolate and identify the *Staphylococcus aureus* by using the selective media and biochemical tests.
- To study the virulence factors like Protease, Lipase, Beta lactamase, Hemolysin, and Slime production.
- Then, to determine the multi drug resistant patterns of *Staphylococcus aureus*.

MATERIALS AND METHODS

Experimental Design

The different milk samples such as Goat milk, Buffalo milk, Pasteurized milk were collected from milk vendors in and around the Namakkal District. From these samples *Staphylococcus aureus* were isolated and identified by using standared biochemical tests. Then detect the virulence factors of *S.aureus* like Protease, Lipase, β-lactamase, Heamolysin and Slime production. And finally, detect their multi drug resistance.

Milk Sample size and sampling technique

A total of 50 three different milk samples were collected from milk vender in and around the Namakkal district during the period of March to April 2013. The samples were purchased and collected in the time between 7-9 am using sterile containers maintaining sterile conditions. The samples were immediately sent in an ice box to the laboratory. The microbial analysis was conducted within 1-3 h of collection and the samples were kept in the refrigerator at 4°C until microbial analysis was conducted.

Microbial analysis

Isolation and preservation

From each samples 10 ml of milk was mixed with 90ml of distilled water and homogenized in a flask for 5 min using shaker at 160 rpm. The homogenates were serially diluted (10^{-1}-10^{-5}) and loopful of sample were streaked on Nutrient agar plate. Then the isolated colonies were streaked on Mannitol Salt Agar (MSA) (Hi-Media, Mumbai, India) plate and incubated at 37°C for 24-48 h for the isolation of organisms. The selected colony was once again streaked on the selective media for the pure-culture isolation. Then the identified colonies were subsequently maintained in Nutrient agar slants at 4ºC (Alebel *Wubet et al*., 2013).

Morphological and Biochemical Tests

The presumptive isolates of *Staphylococcus* spp. were isolated from above method was identified as *Staphylococcus aureus* based on morphology, motility, Catalase, oxidase test and biochemical tests as recommended by using Bergey's manual of systematic Bacteriology.

Confirmative tests for *Staph.aureus* isolates

The following tests were performed for the identification of Staphylococcus aureus

- Gram's staining
- Catalase test
- Tube Coagulase test

Tube Coagulase Test

In this test one loopful (2-4 colonies) of *Staphylococcus aureus* culture was inoculated in to the plasma (from human blood) containing test tubes. The inoculated tube was incubated at 37°C for 4hrs. Then observed the clot formation at hourly intervals. The clot is formed after 4hrs of incubation it was considered as positive. If there is no clot in the test tubes after 4hrs it will be considered as negative. Then it should be left in overnight at room temperature and examined again for clot formation. Then record the results.

Disc Diffusion (Bauer-Kirby) Susceptibility Test

Antimicrobial susceptibility test was conducted on all isolated *S. aureus* isolates (n = 25) were isolated during the study. The isolates were tested for 8 antimicrobials using the Kirby-Bauer disk diffusion method (NCCLS, 1999). The following antimicrobial disks (Oxoid disks) with their corresponding concentration were used: Methicllin (30mcg), Vancomycin (30mcg) Erythromycin (10mcg) Gentamycin (30mcg) Kanamycin (30mcg) Amoxycline (30mcg) Tetracycline (10mcg) Ciprofloxacin (30mcg). Muller Hinton agar plates were prepared and sterilized at 121ºC for 15 minutes and swabed the cultures on the plates with sterile swab. Plates were left at room temperature to remove excess of moisture. With sterile forceps, different antibiotics were placed and kept at refrigerator for 30 minutes for pre- diffusion of the disc. Then the plates were incubated at 37ºC for 24 hours. Following incubation,the inhibition zone was reported as the diameter of the zone surrounding the individual disk in which bacterial growth was absent. Based on this, the isolates were defined as resistant, intermediate and susceptible are according to the guide lines of the National Committee for Clinical Laboratory Standards (NCCLS) for Gram positive bacteria.

Determination of virulence character

Phenotypic determination

Assay of Proteolytic Activity

Proteolytic activity was assayed by using the method of Anna Karlsoon *et al.* (2004). The overnight broth culture was spoted into 1% skim milk agar and incubated at 37°C for overnight. After incubation period, the clear zone of hydrolysis was observed. The presence of a transparent zone around the colonies indicated protease activity.

Determination of Lipolytic Activity

Lipolytic activity was determined by using the method of Neslihan Gundogan *et al.* (2010). Tributryin agar base (TAM) by adding Tributryin. The sterilized medium was poured into the petriplates. The isolates were spotted on the Tributryin agar medium and incubated at 37°C for 24hrs. After incubation, the

presence of a transparent zone around the colonies indicated the production of lipase enzyme.

Determination of Beta Lactamase Production

Beta lactamase production was assayed by using the method of Lateef (2004). Broth culture of the test organisms were spotted on to Muller-Hinton agar containing Penicillin and 1% starch and then incubated overnight at 37°C. After incubation, the plates were then flooded with freshly prepared Iodine solution. The presence of clear colorless zone around the bacterial growth was an indication of Beta lactamase.

Determination of Hemolysis

Hemolytic activity was determined by using the method of Riaz- ul haque *et al.* (2002). The hemolytic activities of all positive isolates were determined by blood agar assay. The nutrient agar was prepared, sterilized and cooled. 5ml of human blood was mixed and poured into the plates, After solidified, loopful of culture from all isolates were directly streaked on the plates and the plates were incubated at 37°C for 24 hours. The hemolytic activity was determined by the formation of alpha (or) beta (or) gamma-hemolysin around the colonies.

Determination of Slime Production

Slime production was assayed by using the method of Mathur *et al.* (2006). Broth culture of the test organisms were streaked on to brain heart infusion agar (BHI) Supplemented with 5% sucrose and congored. The congored was prepared as concentrated aqueous solution was autoclaved and added to the agar at 55°C and the plates were incubated at 37°C for 48 hours and the results were recorded. Black colonies were considered to be positive variants, while red colonies were considered to be negative.

RESULT AND DISCUSSION

Isolation of *Staphylococcus aureus* from three different milk samples

In this study, A total of 50 three different milk samples were examined, 5(33.3%) were positive for *S.aureus* in goat milk, 16 (64%) were positive for Buffaloes and 4(40%) for pasteurized milk. The highest isolation of *S.aureus* was from Buffaloes (64%) while the least was from Pasteurized milk (33.3%) Table 1.

Table 1. Prevalence of *Staphylococcus aureus* from different milk samples

S.No	Sample Name	No. Examined	No. Isolated (% Occurrence)
1	Goat milk	15	5 (33.3%)
2	Buffalo milk	25	16 (64%)
3	Pasteurized milk	10	4 (40%)
Total		50	25

Morphology and biochemical characteristics of bacterial isolates

In this study, A total total number of 25 possible *Staphylococcus spp*. Colonies from three different milk samples on the basis of morphological characters on the Nutrient agar and Mannitol Salt agar (MSA).These isolates were characterized with physiological and biochemical tests. This colony was Gram positive, non-motile, cocci (Grape like clusters), catalase and oxidase positive colonies were obtained. The colonies were large (2-4mm), Circular, Convex, Smooth, Shiny, Opaque and easily emulsifiable. Most strains produce golden yellow pigment, though some may be white (or) orange on Nutrient agar. The rounded and golder yellow colour colonies were observed from the Mannitol Salt Agar (MSA). After the application of other identification tests; tube coagulase test, out of 25 isolates 20 were confirmed as *S.aureus* and other 5 were coagulase negative *Staphylococcus aureus* Table 2, 3. And Fig 1, 2, 3.

Table 2. Morphology and Biochemical Characters of *Staphylococcus aureus* from Different milk samples

S.No	Tests	Results
1	Gram staining	Gram positive cocci
2	Motility	Non-motile
3	Catalase	+
4	Oxidase	+
5	Glucose	A^+G^-
6	Sucrose	A^+G^-
7	Lactose	A^+G^-
8	Maltose	A^+G^-
9	Mannitol	A^+G^-
10	Triple Sugar iron test	$A^+/A^+G^-,H_2S^-$
11	Indole test	-
12	Methyl Red test	+
13	Voges-Proskauer test	-
14	Citrate utilization test	-
15	Urease test	-
16	Nitrate reduction test	+
17	Gelatin hydrolysis test	+
18	Tube coagulase	+
	Growth on Nacl agar	
19	10%	+
20	15%	Weakly positive
	Growth at	
21	15%	+
22	45	Weakly positive
23	Alkaline phosphatase	+
24	Deoxyribonuclease (DNase agar)	+

A/Ak→Acid bud and alkaline slant H_2S→hydrogen sulfide, G^+→Gas production

Table 3. Comparative biochemical characteristics of *S.aureus* isolated from milk samples

Species	Gram's staining		Catalase test		Tube coagulase test	
	+ve	-ve	+ve	-ve	+ve	-ve
Staphylococcus aureus	25	-	25	-	20	5

Antibiotic susceptibility test

Antibiotic resistant bacteria pose a growing problem of concern; worldwide-mastitis is the most common cause for antibiotic use in dairy herds.

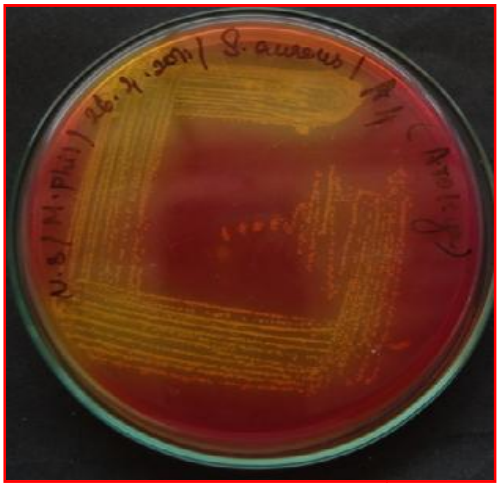


Fig.1. *S.aureus* on MSA

Fig. 2. Gram (+) Grape like clusters (Purple colour)

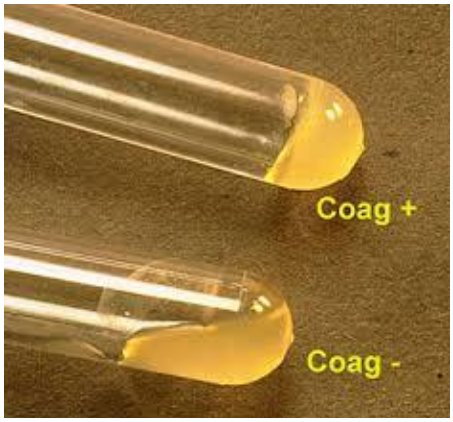


Fig.3. Tube Coagulase test

Effectiveness of current treatment and ability to control infectious diseases in both animals and humans may become hazardous. Susceptibility patterns of *Staphylococcus aureus* to antimicrobial agent have varied, but isolates were usually susceptible to Kanamycin, Ciprofloxacin, Vancomycin, Gentamycin. Invitro susceptibility of the *S.aureus* isolates to a varity of antibiotics shown in Table 4. These data revealed that to 100% of the *S. aureus* isolates sensitive to Vancomycin. 83.3% for Ciprofloxacin 48% for Gentamycin (Tandra chadha *et al.,* 2014). Among the 25 isolates of *S.aureus* 16 (64%) showed sensitive to Kanamycin, 12(48%) for Ciprofloxacin, 11(44%) for Vancomycin, Gentamycin and 9(36%), 8(32%) for Amoxycline, tetracycline. In resistance patterens all isolates of *S.aureus* showed 25(100%) resistant to Methicillin, 15(60%) for Erythromycin, 11(44%) for Ciprofloxacin, Vancomycin, Amoxycline. 10(40%) for Tetracycline, Gentamycin. In intermediate patterns showed 7(28%), 6(24%), 5(20%), 4(16%), 3(12%), 2(6%) for Tetracycline, Erythromycin, Amoxycline,Gentamycin,Kanamycin,Vancomycin,Ciprofloxacin.Intermediate patterns also considered as a resistant. This results is similar to the results obtained for *S.aureus* in Abebe Mekuria *et al*., 2013.

Characterization of virulence factors

Staphylococcus species secrets many extracellular active substances, such as coagulase, hemolysin, nuclease, phosphatase, lipase, proteases, fibrinolysin, enterotoxins and toxin shock syndrome toxin. These proteins are known as virulence factors that cause disease in animal and animals. Among the *Staphylococcus aureus* isolates obtained in the present study, 12 (48% of the isolates produced protease enzyme for the utilization of protein in medium. This results is similar to the results obtained from *S.aureus* Justyna Bien *et al.,* 2011). In this report, it was verified for the production of Lipase from among the 25 isolates, 15(60%) of isolates produce the Lipase production. Most of the known Staphylococcal lipases are produced by pathogenic members of the genus, i.e., *Staphylococcus aureus* and *S.epidermidis***.** Lipase interferes with the phagocytosis of the infectious lipase-producing *S. aureus* cells by host granulocytes, thus indicating a direct involvement of lipase in pathogenesis. The plasmid-mediated ß-lactamases in enteric bacteria have been studied and characterized extensively. Chromosomally mediated, inducible β-lactamases were recognized as the major mechanism of Antibiotic resistance in this report, it was verified for the production of Beta Lactamase from among the 25 isolates of *S. aureus*11(44%) produced the beta lactamase. In recent years, many isolates of *Staphylococcus aureus* have evolved resistance to both synthetic and traditional antimicrobial chemotherapy and their prevalence outside the hospital is of potential epidemiological threat (Kaplan *et al.* 2005) Recently large numbers of multidrug-resistant *Staphylococci* have been recovered from diverse environmental sources, such as drinking water supplies, foodstuffs, the mucosa of humans and farm animals and hospital environments. This increasing incidence of α- lactams and glycopeptides-resistant *Staphylococci* in the environment is of public health concern. (Hussein Abulreesh, 2011). ß-hemolysin is one of a variety of diffusible substances produced by *S.aureus* (Riaz-ul Haque *et al.,* 2004). Out of all the 25 isolates, 15 (60%) *Staphylococcus aureus* were β-hemolysis producer.

Slime production has been shown to play a major role in the pathogenesis of infection caused by *Staphylococcus aureus.* In our study out of all the 25% isolates, 12(48%) *S.aureus* were slime producers.The biofilm formation is the leading cause of the pathogenesis of *S. aureus* associated with biomaterial infections. In *S. aureus* polysaccharide intercellular adhesin (PIA) was encoded by *ica*A and *ica*D genes. Production of PIA

Table 4. Antibiotic sensitivity patterns of *Staphylococcus aureus* from different milk samples

Antibiotics (mcg)	Goat milk (5 isolates)						Buffaloe milk (16 isolates)						Pasteurized milk(4 isolates)					
	S		R		I		S		R		I		S		R		I	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Methicillin (30 mcg)	-	-	5	%	-	-	-	-	16	%	-	-	-	-	4	%	-	-
Amoxycline (30mcg)	2	40	2	40	1	20	5	31	7	44	4	25	2	50	2	50	-	-
Tetracycline (10mcg)	2	40	2	40	1	20	6	37	6	37	4	25	-	-	2	50	2	50
Ciprofloxacin (30 mcg)	1	20	3	60	1	20	10	62	6	37	-	-	1	25	2	50	1	25
Kanamycin (30mcg)	5	%	-	-	-	-	9	56	5	31	2	6	2	25	1	25	1	25
Vancomcin (30mcg)	3	60	1	20	1	20	5	31	9	56	2	6	3	75	1	25	-	-
Erythromycin (10mcg)	2	40	3	60	-	-	1	6	9	56	6	37	1	25	3	75	-	-
Gentamycin (30mcg)	4	80	1	20	-	-	7	44	7	44	2	6	-	-	2	50	2	50

S=Sensitive, R=Resistant, I=Intermediate, %=100

is currently responsible for staphylococcal biofilm development (Avinder Kumar *et al*., 2011). In this study, *S. aureus* strains isolated from auricular infection (n = 46) and *S. aureus* ATCC 25923 were phenotyped and genotyped. Slime production was assessed using Congo red agar plate assay. In order to determine the biofilm formation capacity at various pH levels of the studied *S. aureus* strains, micro titer plate assay was performed (Tarek Zmantar1 *et al.*, 2010).These results were showed in Table 5, Fig.4, 5, 6, 7, 8.

Table 5. Prevalence of Virulence factors producing *Staphylococcus aureus* from different Milk samples

S.No	Isolates	Protease	Lipase	β-Lactamase	β-Hemolysin	Slime
1	Gm1	+	-	+	+	+
2	Gm2	+	-	-	+	-
3	Gm3	-	+	-	+	+
4	Gm4	+	+	+	-	-
5	Gm5	-	-	+	+	+
6	Bm1	-	+	-	-	+
7	Bm2	+	+	+	+	-
8	Bm3	-	-	-	+	+
9	Bm5	-	+	+	-	+
10	Bm7	+	+	+	+	-
11	Bm8	+	-	-	+	+
12	Bm9	+	+	-	+	-
13	Bm11	-	+	-	+	+
14	Bm14	+	+	+	+	-
15	Bm15	-	-	-	-	-
16	Bm17	+	-	-	+	+
17	Bm18	+	-	+	-	-
18	Bm20	-	-	+	-	+
19	Bm21	-	+	-	+	-
20	Bm23	-	-	-	-	-
21	Bm25	-	+	+	+	-
22	Pm1	-	+	+	+	+
23	Pm2	+	+	-	-	+
24	Pm3	+	+	-	-	-
25	Pm4	-	+	-	-	-
%		48%	60%	44%	60%	48%

Gm - Goat milk, Bm – Buffalo milk, Pm –Pasteurized milk

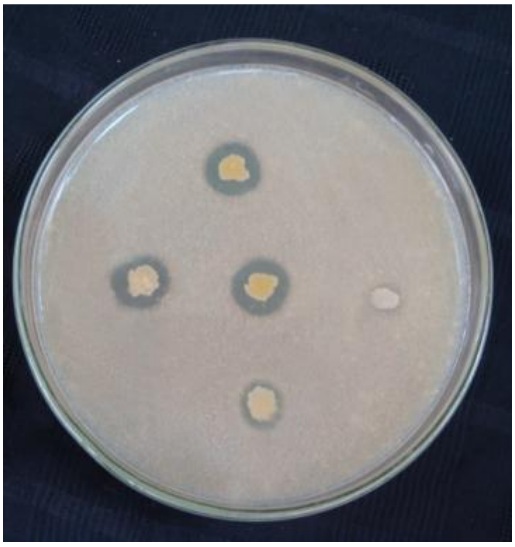


Fig. 4. Proteolytic activity

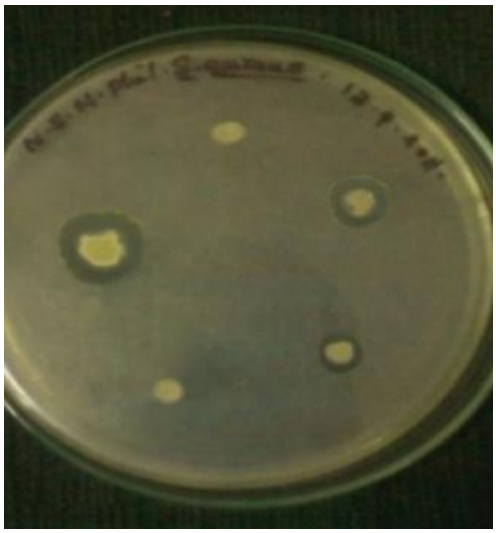


Fig.5. Lipolytic activity

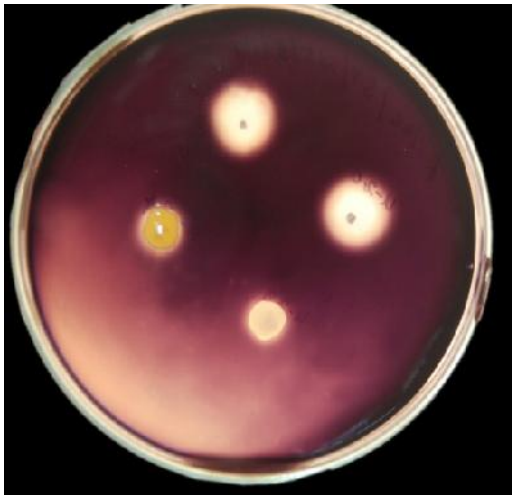


Fig.6. Beta-Lactamase activity

Fig.7. Slime production on CRA agar

Fig.8. Beta-haemolytic activity

Conclusion

Staphylococcus aureus control-choices

1. **Prevent spread**: Teat dip pre and post milking germicide
2. **Segregation**: Isolate infected cows (Mammals)
3. **Treatment**: Dry cow therapy
4. **Removal**: Cull chronic infections
5. **Vaccination**: Prevent new infection

On the basis of data obtained in the present study, conclusion may be drawn that microbial load and antibiotic resistance in milk distributed in Namakkal district in Tamil Nadu is increasing very fast. The main cause of microbial contamination of milk is due to milking from infected udders of the cows, unhygienic mechanical milking practices, unclean equipments or poor washing practices and improper storage conditions. Due to lack of awareness and negligence in this area, it is still observed. Improving animal health, reducing antimicrobial use in animal husbandry, implementation of regulations to restrict the use of antibiotics in animals, application of modern technologies may improve the current situation and establish India as largest and best quality milk producer in the world. The evolving threat of antimicrobial resistance and options for action is being released by WHO (World Health Organaization) that will extend our knowledge to understand the cause and solution of the problem.

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