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Plasmid profiling with respect to identification of multidrug resistance in *Staphylococcus aureus* isolated from dairy products

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ABSTRACT

Present study to examine the Plasmid profiling with respect to identification of multidrug resistance in Staphylococcus aureus separated from dairy products. In this study S. aureus were separated from milk and its products on selective Mannitol Salt Agar (MSA) medium which was followed by gram staining and biochemical characterization. The gram staining results demonstrated that graphs like cluster of cell of S. aureus whereas biochemical test results also showed that positive for S. aureus. Antibiotic resistance was identified by disc diffusion method. The antibiotic resistant pattern of S. aureus isolate was tested against 8 commercially available antibiotics as Ampicillin, Gentamicin, Kanamycin, Ciprofloxacin, Cefotaxime, Bacitracin, Methicillin and Penicillin. Results showed that isolated organism showed resistance to Methicillin, Bacitracin and Penicillin. Intermediate level of resistance was recorded in Ciprofloxacin and Cefotaxime. Isolated S. aureus were sensitive for the antibiotics such as Ampicillin, Gentamicin and Kanamycin. The percentage occurrence of Multiple Antibiotic Resistance (MAR) index was the highest (70%) for the MAR index of 0.8. The plasmids were separated and bands (1500-100bp) were observed followed by electrophoresis. The antibiotic resistant genes was identified by PCR. The result showed that 7 isolated strains which carried on mec gene (1000bp-100bp). This mec gene was resistant to Methicillin, Penicillin and Bacitracin. Conclusion: Ampicillin, Gentamicin and Kanamycin were active against the isolated organisms which can be used for treatment of bovine mastitis in cattle.

Keywords: *Staphylococcus aureus*, Cattle, Plasmid, Antibiotic resistant

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INTRODUCTION

Milk was a nutritional food that is rich in carbohydrates, proteins, fats, vitamins and minerals. However, health risk to consumers can be associated with milk due to the presence of zoonotic pathogens and antimicrobial drug residues. The quality of milk may be lowered by a number of factors such as adulteration [1], contamination during and after milking and the presence of udder infection. Pathogenic organisms in milk can be derived from the cow itself, the human hand or the environment [2].

Mastitis is the inflammation of the mammary gland occurs often due to microorganisms that invade the udder, multiply and produce toxins that are harmful to the mammary tissue. Mastitis is characterized by physical, chemical and usually bacteriological changes in the milk and pathological changes in the glandular tissue of the udder and affects quality and quantity of milk [3]. Mastitis is one of the most crucial diseases of cattle and buffalo because it causes innumerable problems to milk production, milk processing and quality of milk & milk products which results in huge economic losses to the dairy industry. The physical, chemical, bacteriological and other qualities of milk are affected by mastitis. Mastitis as a dairy scourge represents an impediment to the development of dairy industry. In as much as, the milk of infected animals contains pathogenic organisms and their toxins, the disease is also important from consumers stand point [4]. Mastitis which is mostly caused by the interaction of multiple pathogenic agents

(primarily bacteria), can expose human beings to various organisms through infected milk, thus serving as a media for transmission of various zoonotic diseases like Tuberculosis, brucellosis, diphtheria, scarlet fever and Q fever [5].

Large number of microbes is causing the disease in dairy animals. Bacterial agents like *Staphylococcus spp., Streptococcus spp., Escherichia coli, Corynebacterium spp., Klebsiella spp., Pseudomonas spp*., Mycoplasmal agents, fungal agents, viral agents *etc.,* are responsible for the disease. About 95% of intramammary infections are caused by *Staphylococcus spp* and *Streptococcus spp*. The remaining 5% are caused by other organism. *S. aureus* is a major pathogen in dairy cattle mastitis [6]. Among the various causative agents, *S. aureus* is one of the most prevalent and contagious pathogens of intra-mammary infections in dairy cattle globally. Epidemiological studies revealed the transmission of *S. aureus* from cow to cow, the primary source of which is the milk from infected glands, and also from dairy cows to humans and humans to cows. It is causing both clinical and subclinical form of mastitis in cattle [7]. Mastitis is one of the major causes of antibiotic use in dairy cows [8].

Plasmid may contain resistance genes for single or multiple antimicrobial agents and may report to transfer these resistances from one bacterium to another. In many instances, resistance to antimicrobial agents in staphylococci has also been due to plasmids that carry the genetic determinants of resistance [9]. Plasmid profiles have been found useful in epidemiological surveillance of disease outbreaks and in tracing antibiotic resistance [10]. Plasmid profiles determination is a useful and the earliest DNA- based method applied to epidemiological studies [11]. Profile identification can be used as serotype – specific reference pattern for detecting certain strain with possible variation in plasmid content [12-13]. To investigate the prevalence of multi drug resistance *Staphylococcus spp*. Isolated from milk and milk products obtained from Koyambed in Chennai, including samples from cows with history of mastitis and to undertake plasmid profiling.

MATERIALS AND METHODS

Sample collection

A total of 10 milk and milk product samples were obtained from Koyambed in Chennai for the study. These samples were processed for the isolation of *Staphylococus spp.* These samples were streaked on a selective medium Mannitol Salt Agar (MSA) to get *Staphylococcal* colonies for further studies.

Sample processing

- 1g/1ml of each milk samples was weighed and mixed with sterile distilled water or saline. Then it was kept on a rotary shaker for 18-24 hrs.
- This process was done to enrich the isolates. Then the samples were serially diluted from 10^{-1} and 10^{-7}.

Enumeration of total bacterial population

Spread plate technique was employed to enumerate the *Staphylococus spp.* colony count on MSA media (Koch, 1994). Identification of *Staphylococcus species* (Barrow *et al.,* 2002). Gram staining [14].

Biochemical tests

The biochemical tests (Catalase test, Indole test, Kovac's reagent, Methyl red (MR) test, Voges proskauer (VP) test, Citrate test, Coagulase test and Urease test) carried out by the method of Oyekunle *et al.*,[24] Antibiotic susceptibility test for *staphylococcus spp.* Investigated by Kirby [15]. Isolation of plasmid DNA by the methold of Sambrook.. Plasmid profiling using agarose gel electrophoresis [16]. PCR - Polymerase Chain Reaction – Methicillin Resistant Gene[17].

RESULTS AND DISCUSSION

Mastitis is the inflammation of the mammary gland which is characterized by physical, chemical and microbiological alteration in milk. Mastitis is caused by *S. aureus* can result in long term infection and can become chronic, with low rate of cure and consequent loss of milk production. Mastitis can be cured by treatment with antibiotics after the identification of the causative agent. Antibiotic resistance is one of the important problems in the treatment of mastitis. Mastitis is caused by resistant bacteria. The determination of the antibiotics susceptibilities of pathogens causing mastitis is crucial importance for the treatment and control of dairy cattles.

For the present study "plasmid profiling of antibiotic resistant *Staphylococcus aureus* isolated from milk and milk products was performed. Ten samples were collected from different shops of Koyambed area in Chennai (Table:1, Figure 1)

Table 1: Samples collected from different area of Koyambed Chennai

S.NO	NAME OF THE SAMPLE	SAMPLE CODE
1.	MILK	MS1, MS2
2.	YOGHURT	YS1, YS2
3.	CHEESE	CS1, CS2
4.	BUTTER	BS1, BS2
5.	PANEER	PS1, PS2

Ten different milk samples were collected, two samples of each like Milk, Yoghurt, Butter, Cheese and Paneer.

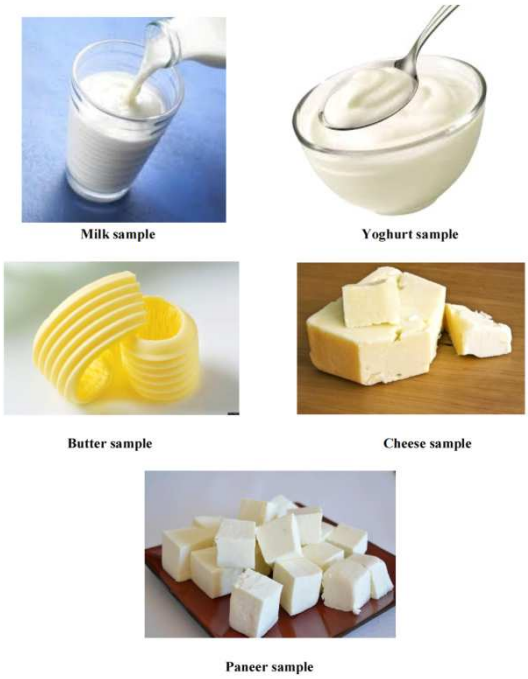
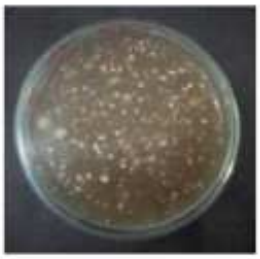


Figure 1: Milk and Milk products

Milk is the best media for the growth of many bacteria in which some of them are pathogenic. As we know fresh milk is enriched with pathogenic and non pathogenic *Staphylococcus* spp. which can be transmitted to human by milking and consumption of milk. *Staphylococcus* bacteria in the fresh raw milk. Because this *S. aureus* might be hazardous if proper boiling of milk is not done during consumption. It also causes disease if proper hygienic procedure is not maintained during milking. *Staphylococcus aureus* isolated from human and animal samples and Das [18] isolate and identify *Staphylococcus aureus* from laboratory animals and human and also determine antibiogram profile. Das [18] stated that the presence of strains assigned to this *Staphylococcus* spp. in bulk milk or in raw milk products could reflect human contamination.

The isolation, identification and antibiotic susceptibility characterization of *S, aureus* from milk obtained from dairy cattle. Our results indicate that 25 samples were positive for *s. aureus.* Based on observations made throughout the collection of samples, we therefore report that improper hygiene and poor farm management practices contribute to the presence of *S. aureus* in the milk [19]. *S. aureus* is a versatile microorganism that causes infection in different hosts. Moreover, this bacterium is one of the most important pathogens in the etiology of infectious mastitis in cows, goats, and sheep, causing chronic infection of the mammary tissue that is difficult to treat [20]. In the present study, *S. aureus* was identified in 20 (11.8%) milk samples collected from sheep with clinical mastitis.

This study revealed that milk and milk products may be contaminated with multiply resistant *S. aureus.* The high frequency of resistance observed with lincomycin(67.7%), Penicillin (66.7%) and cortimoxazole (51%) could be attributed to their use in treatment of diseases in animal and humans. Resistant bacteria may transfer resistance genes to other bacteria and become important in the spread of antibiotic resistance [21]. The collected samples were processed for the isolation of *Staphylococcus aureus* present in the sample. All the samples were serially diluted and performed pour plate technique on Mannitol salt agar (Figure:2).

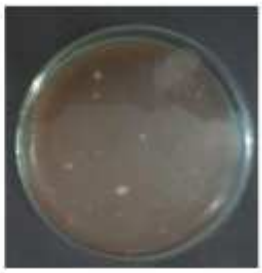


Milk sample: (MS1, MS2)

Yoghurt sample: (YS1,YS2)

Cheese sample: (CS1, CS2)

Butter sample: (BS1, BS2)



Paneer sample

Figure 2: *Staphylococcus aureus* isolated from milk and milk products on Mannitol Salt Agar.

Microbiological analysis of milk and milk products samples revealed that *Staphylococcus aureus* was the major part of bacterial flora in the samples like Milk, Yoghurt ,Cheese, Paneer and Butter. The overall 50% (*Staphylococcus aureus)* of incidence were found in Milk, Yoghurt, and Cheese products. *Staphylococcus aureus* is generally found in the intestinal warmed blood animals, and most of the strains do not affect the health of the host. According to the result highest *Staphylococcus aureus* contamination were recorded from all the samples. Our result showed that Milk and milk product samples were highly contaminated with *Staphylococcus aureus* and this study provided evidence that *Staphylococcus aureus* is frequently occurring organisms in Milk. The methods of production, transportation, handling and sales of Milk and entirely unhygienic. The isolated *Staphylococcus aureus* samples were inoculated into selective medium such as Mannitol salt agar. On mannitol salt agar, the colonies were observed as yellow colour colonies due to the fermentation of sugar lactose (Figure:3).

The isolated organism were streaked on Mannitol salt agar plates and incubated at 37 ^{0}C for 24 hrs. The growth of *S. aureus* on MSA were confirmed by the fermentation of mannitol with change of color of media and formation of yellow color colonies. Mannitol salt agar is a selective and differential media used to identify *Staphylococcus sp.* Only *S. aureus* can ferment mannitol after 24 hours of incubation and produce lactic acid as a result. This results showed that, 96% of isolates (90% clear positive, 6% weak positive) were positive for mannitol fermentation [22]. Mannitol salt agar is a selective and differential media used to identify *Staphylococcus sp.* Only *S.aureus* can ferment mannitol after 24 hours of incubation and produce lactic acid as a result. Out of our 100 isolates, 96% (90% clear positive, 6% weak positive) were positive for mannitol fermentation. Our results are in agreement with Arshad *et al*. [22] who reported that out of 90 bacterial isolates from bovine milk samples including 33 of staphylococci and 57 of other bacterial species, 23 were *S. aureus*, and all of these *S. aureus* isolates were able to ferment mannitol. Arshad *et al* [22] reported that all *S. aureus* isolates in their study were able to ferment mannitol while neither *S.*

hyicus nor S. intermedius were able to ferment mannitol. Arshad *et al* [22]did not state clearly for how long tested isolates in their study were incubated and it is known that *S. hyicus and S. intermedius* can ferment mannitol if incubated more than 24 hrs [23] .

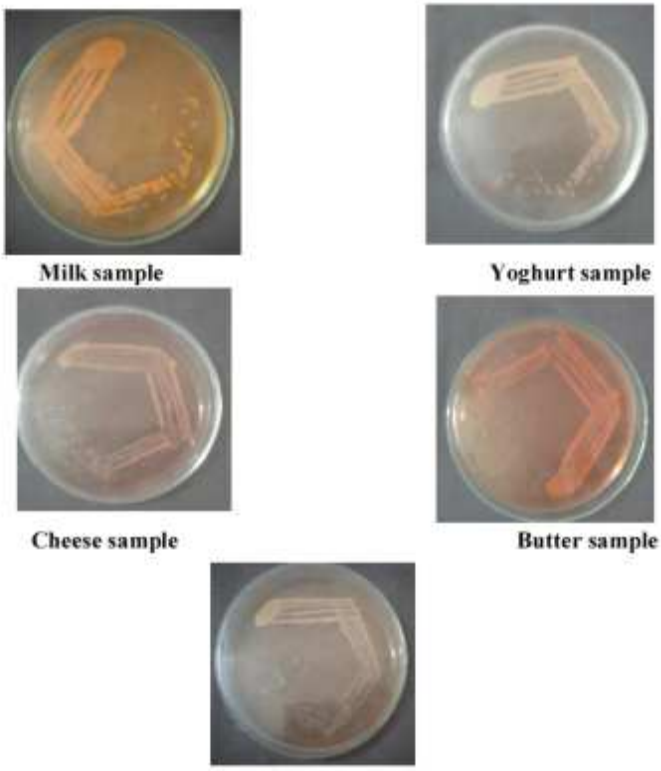


Figure 3: Yellow colour colonies were observed on the Mannitol salt agar medium

However, Oyekunle *et al*. [24] reported that a higher percentage (100%) of coagulase-negative isolates than coagulase-positive isolates (88.2%) of bovine *staphylococci* fermented mannitol. Thus, relying only on mannitol fermentation, when identifying *S.aureus*, could increase false positive results which will decrease specificity. Mannitol salt agar is a selective medium for *S. aureus* due to the high concentration of sodium chloride that the agar contains. Several biochemical test are also in place for confirmation of bacterial isolates in any selective media. During this study, all the colonies grown on MSA plate were subjected to gram staining and catalase test [25] .

Table 2: Gram staining and Biochemical tests for the identification of *S. aureus*

Biochemical test	MS1	MS2	YS1	YS2	CS1	CS2	BS1	BS2	PS1	PS2
Gram staining	+	+	+	+	+	+	+	+	+	+
Indole test	-	-	-	-	-	-	-	-	-	-
Methyl red test	+	+	+	+	+	+	+	+	+	+
Voges proskauer test	+	+	+	+	+	+	+	+	+	+
Citrate test	+	+	+	+	+	+	+	+	+	+
Coagulase test	+	+	+	+	+	+	+	+	+	+
Citrate test	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+

(+) – Positive, (-) - Negative

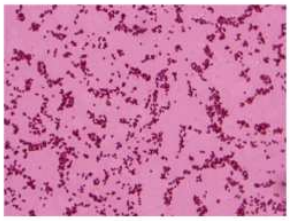


Figure 4: Identification of *Staphylocccus aureus* by Grams staining

All the *Staphylococcus aureus* were subcultured on Nutrient slant for further study. The *Staphylococcus aureus* samples were identified by conventional methods and compared with positive control *Staphylococcus aureus.* The Gram character, Biochemical test were observed and tabulated.(Table:3 Figure: 4 and 5).

The biochemical test results which were obtained for the isolates which contained *Staphylococcus spp.* are shown in Fig 5. Table 3 indicates the results for each biochemical test which indicate the presence of *Staphylococcal spp.* in the isolates.

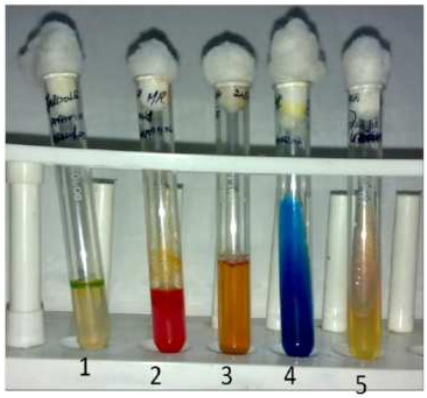


Figure 5: Identification of *Staphylococcus aureus* by Biochemical test results

1- INDOLE TEST (NEGATIVE)
2- METHYL RED TEST (POSITIVE)
3- VOGES TEST (POSITIVE)
4- CITRATE TEST (POSITIVE)
5- UREASE TEST (POSITIVE)

Figure 6: Coagulase test for *S. aureus*

Gram's stained smears from Mannitol Salt Agar medium culture were examined microscopically which revealed Gram positive, cocci arranged in grapes like clusters. In this study it was showed that in Gram's staining method it creates smooth, convex, lustrous, circular colonies reaching a size of 0.5-1.5µm in diameter and grown in an irregular three-dimensional bunch of grapes-like clusters of cells. Sushma *et al*.,[26], Alzbeta *et al*., [27] also recorded similar staining characteristics of *S. aureus.* The selected organism *S. aureus* gave positive result on catalase and coagulase test which were closely correlated with Sasidharan *et al*., [28].

Colonies that were successfully isolated on Mannitol salt agar and purified on trypic soy agar characterized by morphology, gram staining reaction, and biochemical test, such as, catalase and KOH test. These tests suggested high prevalence of *Staphylococcus* and *Streptococcus spp.* in many of the milk samples[29]

The isolated *Staphylococci* gave positive reaction in coagulase test indicated that the isolates were *S. aureus.* The positive result was confirmed by the formation of curd like clotting.

Figure 7: Catalase test for *S. aureus*

Catalase test was performed to differentiate *Staphylococci* (catalase producer) from Streptococci (non-catalase producer). Hydrogen peroxide was broken down into water and oxygen. Production of oxygen was indicated by bubble formation. All the isolates of *Staphylococcus aureus* were catalase positive. Whereas the negative control did not produce any bubble. The catalase test was done by slide method. Coagulase production is one of the most reliable criteria for *S.aureus* identification [30]. All our CPS isolates were confirmed as *S. aureus* based on results from PCR. Therefore, we concluded that the coagulase tube test is sufficient for the identification of *S. aureus* in milk samples. Similar results have been reported by Yazdankha et *al*. [31]. They reported that the coagulase tube test demonstrated 88·5% sensitivity and 100% specificity for the direct detection of *S. aureus* in milk samples Antibiotic resistance pattern of *Staphylococcus aureus* isolated from Milk and Milk product samples were observed and tabulated.(Table:4 Figure:8).

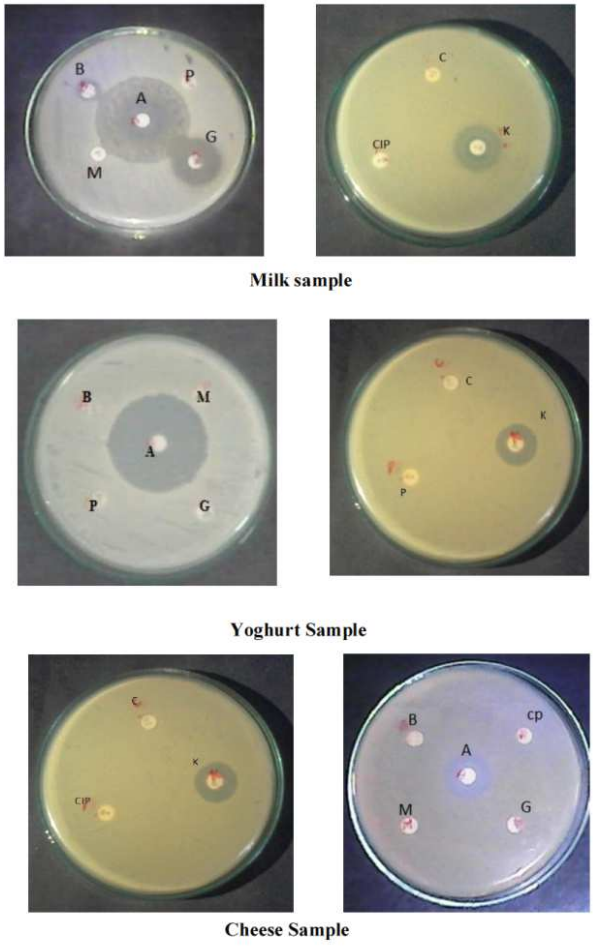


Figure 8: Antibiotic resistant test

Table 3: Antibiotic test and their concentration

S.NO	ANTIBIOTICS	SYMBOL	DISC CONTENT
1	Penicillin	P	10 Unit
2	Kanamycin	K	5µg
3	Gentamycin	GEN	30µg
4	Cefotaxime	CTX	5µg
5	Ciprofloxain	CIP	5µg
6	Ampicillin	AMP	10µg
7	Bacitracin	B	10Units
8	Methicillin	MET	5µg

Table 4: Zone of inhibition (mm) of antibiotic resistant in isolated *Staphylococcus aureus*

SAMPLES	P	K	G	CIP	CEF	A	B	M
MS1	-	**14**	**13**	-	**01**	**29**	**3**	-
MS2	-	**18**	**18**	-	**01**	**19**	-	-
YS1	-	**18**	**4.0**	-	-	**29**	-	-
YS2	-	**13**	**2.0**	**17**	**16**	**15**	-	-
CS1	-	**14**	**18**	**16**	**17**	**18**	-	-
CS2	-	**14**	**6**	**16**	**17**	**17**	-	-
BS1	-	**19**	**18**	-	-	**32**	-	-
BS2	-	**14**	**9**	**2**	**06**	**17**	-	-
PS1	-	**14**	**18**	-	-	**16**	-	--
PS2	-	**14**	**14**	-	-	**16**	-	-

Zone of inhibition were observed from the Disc diffusion method, this results were showed that antibiotic resistant pattern of isolated *S. aureus* from milk and milk product .

Table 5: Antibiotic resistant test for *Staphylococcus aureus* isolated from milk and milk products

SAMPLE	P	K	GEN	CIP	CEF	AMP	B	MET
S1	R	I	S	R	R	S	R	R
S2	R	S	S	R	R	R	R	R
S3	R	S	R	R	I	S	R	R
S4	R	R	R	I	I	R	R	R
S5	R	I	S	I	I	R	R	R
S6	R	R	R	I	I	R	R	R
S7	R	S	S	I	I	S	R	R
S8	R	I	R	R	I	R	R	R
S9	R	I	S	I	R	R	R	R
S10	R	R	S	I	R	R	R	R

R-RESISTANT; S-SENSITIVE' I-INTERMEDIATE

The results of antimicrobial resistant test showed that the isolated strains were resistant to Penicillin, Methicillin and Bacitracin and sensitive to the Ampicillin, Gentamicin and Kanamycin respectively.

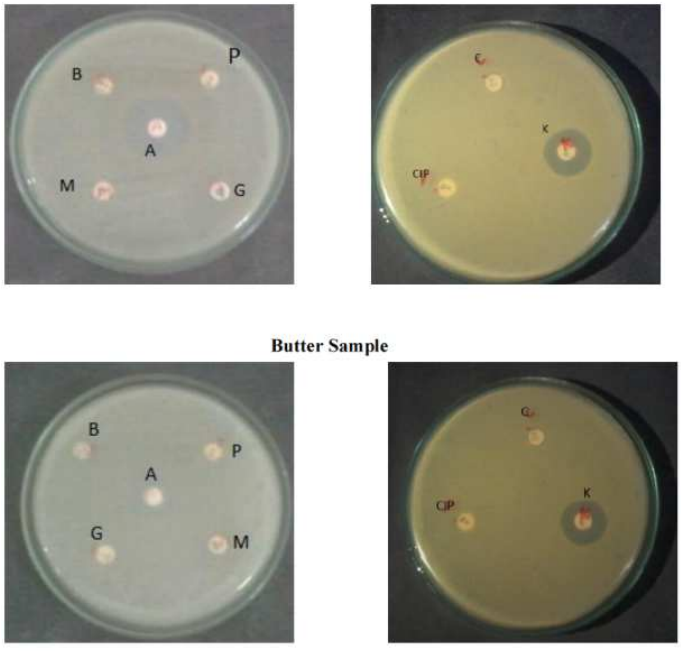


Figure 9: Antibiotic resistant test

Table 6: PERCENTAGE(%) OF ANTIBIOTIC RESISTANT OF *Staphylococcus aureus* ISOLATED FROM MILK AND MILK PRODUCTS

S.NO	ANTIBIOTICS	SYMBOL	TOTAL NUMBER OF ISOLATES N=5		
			RESISTANT	INTERMEDIATE	SENSITIVE
1.	Penicillin	P	100	-	-
2.	Kanamycin	KAN	13	22	65
3.	Gentamicin	GEN	41	-	59
4.	Cefotaxime	CTX	33	57	10
5.	Ciprofloxacin	CIP	10	67	13
6.	Ampicillin	AMP	10	-	90
7.	Bacitracin	B	100	-	-
8.	Methicillin	MET	100	-	-

Staphylococcus aureus exhibited 100% resistant against Penicillin, Methicillin, and Bacitracin. The intermediate level of resistant in *Staphylococcus aureus* exhibited by Ciprofloxacin and Cefotaxime (55%). The resistant of *Staphylococcus aureus* isolated from Milk samples (MS) were 69%. The resistant of *Staphylococcus aureus* isolated from Yoghurt and Cheese (YS, CS) were 60% the resistant of *Staphylococcus aureus* isolated from Cheese, and Paneer (CS,PS) were 50%. The intermediate level of *Staphylococcus aureus* isolated from Cheese and Butter (CS,BS) were 42%. The intermediate level of *Staphylococcus aureus* isolated from Yoghurt(YS) was 33%. The intermediate level of *Staphylococcus aureus* isolated from other samples (MS) were 25% and below. The susceptibility of *Staphylococcus aureus* isolated from all the samples were 40% and below.

The overall, 46 raw milk samples (13.2%) were found to be contaminated with *S. aureus*. Antibiotic susceptibilities of the isolates were determined against 11 antimicrobial drugs by the disk diffusion assay. Most of the isolates (82.6%) were resistant to one or more antimicrobial agent. Six isolates (13.0%) were resistant to single antibiotic and 16 isolates (34.8%) showed resistance to 2 antimicrobial agents. Multiresistance was found in 34.8% of *S. aureus* isolates. Resistance (resistance and intermediate resistance) to ampicillin was the most common finding (54.3%), followed by resistance to oxacillin (28.3%), tetracycline (26.1%), penicillin G (23.9%), erythromycin (23.9%), trimethoprim-sulfamethoxazole (17.4%) and cephalotin (2.2%). All isolates tested for antibiotic sensitivity were susceptible to methicillin, vancomycin, chloramphenicol and ciprofloxacin [32] .

S. aureus was resistant to multiple classes of antibiotics which can cause health problems. In the present study 115 raw milk samples were screened for incidence of *S. aureus* isolates exhibited multiple drug resistant. A total of 25 raw milk samples were found positive for the presence of *S. aureus* isolates from milk samples were found resistant to Nalidic acid, Cloxacilin, Erythromycin. On the other hand several isolates were found susceptible to the Ampicillin, Tetracycline, Oxacillin. The present study demonstrated that the resistant strains may be transferred to milk from infected udders poor form practices and due to poor handling during milking, it transmitted to the milk utensils, which can be the reason of infection in human beings [33].

Table 7: Mar Index of Antibiotic Resistance Pattern of *Staphylococcus Aureus* Isolated from Milk and Milk Products

SAMPLE	MAR INDEX	RESISTANCE PATTERN
S1	0.63	P-CIP-CEF-B-M
S2	0.75	P-CIP-CEF-A-B-M
S3	0.75	P-G-CIP-C-B-M
S4	0.75	P-K-G-A-B-M
S5	0.50	P-A-B-M
S6	0.75	P-K-G-A-B-M
S7	0.37	P-B-M
S8	0.87	P-G-CIP-CEF-A-B-M
S9	0.50	P-A-B-M
S10	0.50	P-K-B-M

MAR Index has been used as a indicator to identified high risk contamination potentially hazard to humans. The MAR index was performed for seven different strains of *Staphylococcus aureus* for eight different antibiotics. These results compared with standard ATCC -25923.(Table:7). In the study, the multiple antibiotic index (MARI) of *Staphylococcus aureus* ranged from 0.40 to 0.87 in which 28.5% of the isolates possess. MARI OF 0.66,42.8% of the isolated possess. MARI of 0.58 and the remainder 28.5% exhibit MARI of 0.50.(Table:6). This indicates that samples are luckily references. Three different sources of contamination which are high risk(MARI>0.2) sources.
Antibiotics still remain the mainstay for treating bacterial infection and alternative to the use of the anti microbial agents .Such as active and passive immune prophylaxis, non specific stimulation of the immune system, use of properties or competitive exclusion cannot effectively replace antimicrobial chemotherapy they may usually

represent additional preventive measure rather than real alternatives. Vaccines may be used for controlling bacteria that have high incidence of antibiotic resistance. In addition to developing new antibiotics it may be currently used antibiotics.

Plasmid profile Analysis

For plasmid profile, attempts were made to study plasmid profile of *Staphylococcus aureus* isolated from Milk and Milk products. Plasmid could be extracted from seven isolates and run it on Agarose gel electrophoresis (AGE). The results showed that all the isolates on lane 1 to 10 contains plasmids of different molecular weight ranging from 2000-100bp. Almost all the isolates exhibited plasmids,MS1,MS2,YS1,BS1,BS2 exhibited plasmids molecular weight 1500bp and 100bp, MS2 and BS2 exhibited 700bp, CS1,CS2,BS1 exhibited 600bp, YS1 exhibited 400bp,BS2 exhibited 150bp,PS1 exhibited 100bp.(Figure :14). It was confirmed that marker were encoded by the plasmid and it was observed that the banding pattern of plasmid were similar to that from lane1 to 10.

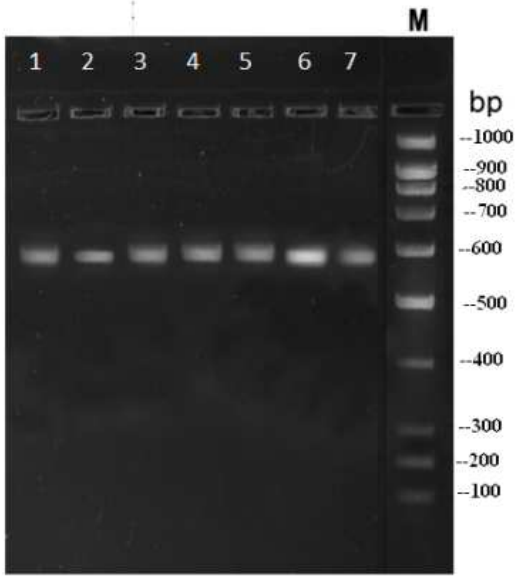


Fig 10: LM - lane mark is showed that positive for *S. aureus* strains (1500 – 100bp)

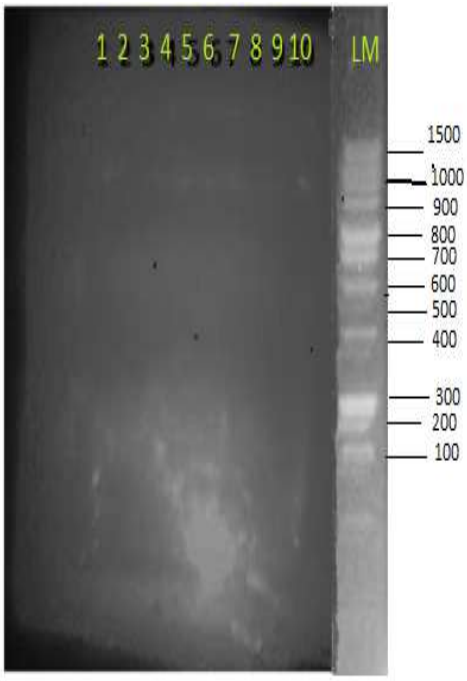


Fig 10: PCR product obtained with *mec* gene showing positive for *S. aureus*

The results plasmid profiling showed that isolated strains of *S. aureus.* the isolates from the various milk and milk product samples were determined for the presence of DNA plasmid using Agarose gel electrophoresis. It was observed that the isolates contained plasmids with molecular weights between 1500 – 100bp. This isolates plasmids showed that multiple antibiotic resistance pattern to most of the antibiotics. Plasmid profiles have been reported to be useful in tracing antibiotic resistance gene. However in this study, resistance to various amtimicrobial agents was not associated with presence of plasmids. This was because no particular molecular size plasmid could be associated with any particular antimicrobial resistance. Resistance was observed in isolates with various molecular size of plasmids. This could be attributed to the variety of sources milk samples. The Methicillin resistant gene has chromosomal locus, while tetracycline resistance observed in *S. aureus* strains was reported to be encoded by a 4.0 kb plasmid [34-35].

PCR Analysis

The results showed that the genomic level of optimized PCR assay was able to successfully amplify the target *mec* gene expected size 600 bp fragment from the genomic DNA of all isolated *S. aureus.* . Which revealed these *S. aureus* were resistant to methicillin antibiotic. PCR was done with different primers *mec* gene for the molecular identification of *Staphylococcus aureus*. PCR based molecular detection of these pathogens in the raw milk and milk products could be remarkably contribute to clarify the actual role in Staphylococcal food poisoning and other clinical symptoms associated with the consumption of milk and milk products. PCR could detect more specifically *S. aureus* than other method. It is considered that amplification of *mec* gene by PCR is useful tool for rapid identification of *S. aureus* by replacing the current biochemical phenotypic schemes which are time consuming [36] (Kim *et al.*, 2001). The results were found that all the isolated specific *S. aureus* gave positive results for *mec* gene that means these were *S. aureus* resistant to Methicillin, Penicillin and Bacitracin.

The PCR technique was shown to be efficient in typing the studied strains. All 122 isolates considered in this study were characterized by means PCR, a technique used by many to type *S. aureus* isolated from different food stuffs implicated in staphylococcal food poisoning, from individual quarter milk and human samples and from mastitis milk samples. The PCR analyse on all the isolates were carried out with the primers M13, AP4. PCR is a powerful tool, where DNA template and specific primers are used for molecular identification of any specific bacteria. Optimization of PCR is an important issue for any successful PCR run. During this study, the concentration of template DNA was optimized by running PCR with known positive samples. Five different concentration of sample (2,4,6,8 and 10µl of DNA in a 25µl reaction) were tested where strong single band was found at concentration of 2µl. For all subsequent reactions, similar template proportion used, According to PCRR results, 41.8% of coagulase positive *staphylococci* samples were identified [36].

CONCLUSION

Staphylococcus aureus is the main cause of bovine mastitis in dairy cattle. Mastitis is the inflammation of the mammary gland often due to microorganisms invade the udder, multiply and produce toxins that are harmful to the mammary tissue. Antibiotics are used to treat diseases of cattle and as well as used as preservatives for milk. Antibiotic resistant *S. aureus* isolates pose a severe challenge to both veterinary and health professions and dairy cattle producers because they have a negative impact on therapy. The aim of the study was to identify *S. aureus* from milk and milk products and screened for their antibiotic resistance gene followed by plasmid profiling.

In this study *S. aureus* were isolated from milk and milk products on selective Mannitol Salt Agar (MSA) medium which was followed by gram staining and biochemical characterization. The gram staining results showed that graphs like cluster of cell of *S. aureus* whereas biochemical test results also showed that positive for *S. aureus.* Antibiotic resistance were identified by disc diffusion method. The antibiotic resistant pattern of *S. aureus* isolates were tested against 8 commercially available antibiotic disc (Ampicillin, Gentamicin, Kanamycin, Ciprofloxacin, Cefotaxime, Bacitracin, Methicillin and Penicillin). The results showed that isolated organism showed resistance to Methicillin, Bacitracin and Penicillin. Intermediate level of resistance were recorded in Ciprofloxacin and Cefotaxime. Isolated *S. aureus* were sensitive for the antibiotics such as Ampicillin, Gentamicin and Kanamycin. The percentage occurrence of Multiple Antibiotic Resistance (MAR) index was the highest (70%) for the MAR index of 0.8. The plasmids were separated and bands (1500-100bp) were observed followed by electrophoresis. The antibiotic resistant genes were identified by PCR. The result showed that 7 isolated strains which carried on *mec gene* (1000bp-100bp). This *mec gene* were resistant to Methicillin, Penicillin and Bacitracin. It can be concluded that Ampicillin, Gentamicin and Kanamycin were active against the isolated organisms which can be used for treatment of bovine mastitis in cattle.

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