ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.50785-50790, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SCREENING AND *IN-VITRO* ANTIBIOGRAM PATTERN OF ANTIBIOTICS AGAINST ENTEROTOXIC BACTERIA *STAPHYLOCOCCUS AUREUS* FROM THE MILK SAMPLES

Pavayee, P., *Umavathi, S., Thangam, Y., Madhiyazhagan, P., Umamaheshwari, S. Shenkani, K., Sowdeshwari, R., Syed Ali Fathima, K.M. and Sathish, V.

PG and Research Department of Zoology, J.K.K. Nataraja College of Arts and Science, Komarapalayam- 638 316, Namakkal (Dt), Tamil Nadu

ARTICLE INFO

Article History:
Received 07th February, 2017
Received in revised form
25th March, 2017
Accepted 26th April, 2017
Published online 23rd May, 2017

Key words:
Milk samples,
Staphylococcus aureus,
Antibiotic and resistance.

ABSTRACT

Staphylococcus aureus is a leading cause of food poisoning resulting from the consumption of contaminated food with Staphylococcal enterotoxins. Different food can act as a good medium for *S. aureus* such as milk and milk products. In the present study differential conventional methods were used to detect the *S. aureus* isolates from 30 samples of milk were collected from different regions of Tamil Nadu. Among that 23 samples showed positive results and 7 samples showed negative results for *S. aureus*. The bacterial isolates were tested with gram staining, motility test, indole test, catalase test, oxidase test, methyl red test, Voges-proskauer test, urease test etc. The *S. aureus* isolates were found different kind of antibiotic resistant against the antibiotics like Clindamicin, Methicillin, Nitrofurantoin, Pencillin and Tobramycin. The *S. aureus* isolates were analyzed by biofilm formation and coagulase negative test. Among the 23 isolates 52.1% showed positive result on biofilm formation and 39% showed coagulase negative. Based on the biofilm formation, methicillin susceptability, coagulase negative tests the 23 isolated were classified as A, B, C, D, E and F. The highest isolates was observed in biotype A (21.8%), followed by D (17.4%), G (13.04%) B, C, E, F (8.7%) respectively. Among these 13.04% of isolates were not comes under any one of these category. Our study result indicates that samples of examined raw milk contained *S. aureus*. The presence of *S. aureus* in milk represents a public health threat. Hence, there is an urgent need for more strict and hygienic preventive measures to reduce the bacterial contamination, so as to increase the wholesomeness and quality of these milk and milk based products for the good health of all consumers. It is essential to prevent risks of contamination of *S. aureus* from the point of production to the point of consumption of milk.

Citation: Pavayee, P., Umavathi, S., Thangam, Y., Madhiyazhagan, P., Umamaheshwari, S. Shenkani, K. et al. 2017. "Screening and *in-vitro* antibiogram pattern of antibiotics against enterotoxic bacteria *staphylococcus aureus* from the milk samples", *International Journal of Current Research*, 9, (05), 50785-50790.

INTRODUCTION

Food-borne diseases are infectious ant it is a toxic nature caused by bacteria were causing food poisoning depends on their capacity to produce toxins in the digestive tract or intoxication. Among the bacteria predominantly involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Different food can act as a good medium for *S. aureus* such as milk and its products, meat and meat products and ready-to-eat foods (Aydin *et al*., 2011). The presence of these pathogenic bacteria in milk emerged major public health concerns and it cause food borne diseases.

Corresponding author: Umavathi, S.,
PG and Research Department of Zoology, J.K.K. Nataraja College of Arts and Science, Komarapalayam- 638 316, Namakkal (Dt), Tamil Nadu

Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environmental, air, animals feed, equipment cleanliness, season soil, faeces and animal health. Differences in feeding and housing strategies of cows may influence the microbial quality of milk (Torkar and Teger, 2008). Rinsing of milking machine and milking equipment with unclear water may also be one of the reasons for the presence of a higher number of microorganisms including pathogens in raw milk (Bramley and McKinnon, 1990). Milk and its derivate are considered vehicles for *S. aureus* infection in humans (Zecconi and Hahn, 2000). *S. aureus* causes a wide variety of diseases in humans and animals, ranging in severity from a mild skin infection to more severe diseases, such as pneumonia and septicemia (Fagundes *et al.*, 2010). In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products (Jones *et al*., 2006). Although

pasteurization is likely to destroy all pathogens, there is concern when raw milk is consumed or when pasteurization is incomplete or faulty. *S. aureus* produces several staphylococcal virulence factors, including enterotoxins and other toxins, such as exfoliative toxin A and B, and Toxic Shock Syndrome Toxin (TSST-1) (Fagundes and Oliveira, 2004). These toxins are known to cause nausea, vomiting and abdominal cramps when ingested by human and are responsible for staphylococcal food poisoning outbreak (Kerouanton *et al*., 2007). *S. aureus* in raw milk generally comes from cows with mastitis from handlers or from deficient hygiene. A treatment efficacy of *S. aureus* mastitis is usually disappointing because the disease causes great damages in the udder cells and drugs are not able to penetrate to all infected sites (Nickerson, 1993). On the other hand, *S. aureus* suppresses phagocytosis and cell mediated immunity (Yancy *et al*., 1991) and produces an enzyme that inactivates most penicillin based treatments (De oliveira *et al*., 2000, Osteras *et al*., 1999). Once established, *S. aureus* usually does not respond to antibiotic treatment. Control of *S. aureus* can be achieved through the correct diagnosis, segregation of infected animals, dry cow therapy, treatment during lactation and culling program (Wilson *et al*., 1995). The results of the analysis by Zecconi and Piccinini (2002) showed that the incidence of new infection peaked in the first 30 days of lactation. Antibiotic treatment before calving applied to heifers and dry cow therapy to multiparous has been suggested to decrease infection rate after calving (Oliver *et al*., 1996).

This method could decrease the sensitivity of 184 bacteriological examinations after calving by lowering the concentration of bacteria in milk, as well as by inhibition of bacterial growth *in vitro*. On the other hand, the sensitivity of bacteriological tests depends on shedding pattern of *S. aureus* (Sears *et al*., 1990). Milk is a compulsory part of daily diet for human beings and also serves as a good medium for the growth of many microorganisms. The occurrence of these pathogenic bacteria in milk and milk products can cause severe health hazards to people as they are highly susceptible to variety of microorganism because of high nutritive value and complex chemical composition (Soomro *et al*., 2003). Many contaminants find their way to raw milk, from which they gain access to dairy products (Bhatia and Zahoor, 2007; Al-khatib and Al-Mitwalli, 2009). Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins preformed in the food (Loir *et al.,* 2003). Besides these, enterotoxins producing *S. aureus* are most dangerous and harmful for the human health. About 50 % strain of this organism are able to produce enterotoxins associated with food poisoning (Payne and Wood, 1974). Enterotoxins are highly thermostable in normal cooking and pasteurization cannot totally inactivate them, so they cause food poisoning (Nagarajappa *et al.,* 2012). Hence in the present study an attempt has been made to assess the diversity of enterotoxic bacteria from *S. aureus* from 30 local Cow milk samples.

MATERIALS AND METHODS

Collection of milk samples: Totally 30 milk samples were collected in sterilized milk collecting tubes and polyethylene bags and transported in an icebox to the laboratory.

Isolation and identification of *S. aureus:* The collected milk samples (0.01 mL) were inoculated into 10ml of peptone broth (HiMedia®, India) incubated at 37°C for overnight. The presumptive colonies of *S. aureus* were cultured with Mannitol Salt Agar (MSA) and repeatedly sub-cultured to get pure culture. These isolated pure culture were preserved for further bacterial identification. The pure culture were identified as *S. aureus* on the basis of Gram staining, colony morphology on Mannitol Salt Agar (MSA) and biochemical test (Thaker *et al*., 2013).

Antibiotic Susceptibility Tests: Antibiotic susceptibility tests were performed on Mueller-Hinton Agar (Himedia India) against Penicillin, Methicillin, Nitrofurantoin, Clindamycin and Tobramycin. Antibiotic discs were obtained from Himedia, India. Agar plates were evaluated after 18 hours of incubation at 37 °C. The inhibition zone was measured and compared with standard antibiotic chart.

Biofilm Formation: Vijayalakshmi *et al.* (2013) had described screening of slime formation by *Staphylococcus* isolates; which requires the use of a specially prepared solid medium-Brain Heart Infusion Broth (BHI) supplemented with 5% sucrose and Congo red. The medium were composed of BHI, sucrose, agar and Congo red stain. Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes, separately from other medium constituents and was then added when the agar had cooled to 55°C. The isolates were streaked to a length of 1.5 cm on Congo red plate and incubated at 37 C for 48 hours and the results were recorded as given below:

- Dry black, crystalline colonies: Strong positive
- Wet, black, non-crystalline colonies: Moderate positive
- Brownish black colonies: Weak positive
- pink colonies: Negative

Culture media

BHI - 37 gm
Sucrose - 50 gm
Agar- 10 gm
Congo red stain- 0.8 gm
Distilled water- 1000 ml

Coagulase negative test

The Coagulase negative test was followed by Adejuwon *et al*. (2011) method. There was clotting of human blood plasma in the test tube after 3 hrs. The controls gave no change when observed.

Biotyping of the MRSA isolates

All MRSA was characterized into 8 groups by biotyping. The biotyping was carried out with following characters such as Methicillin resistance, biofilm and coagulase negative (Coia *et al*., 1990).

RESULTS AND DISCUSSION

S. aureus is an important food pathogen in the world. In the present study on the basis of conventional methods 23 *S. aureus* isolates were identified from 30 milk samples as shown in table.1. Out of 30 samples (S-1 to S-30) *S. aureus* was obtained in 23 samples (76.7%) and 7 samples (S3, S14, S18, S22, S24 and S26) showed negative results for *S. aureus* (Fig.1). The isolates were identified as *S. aureus* on the basis

Gram staining, colony morphology on Mannital Salt Agar (MSA) and biochemical test. These findings were similar those reported by Jyothi Yadav *et al.* (2014), Purba Sarkar *et al.* (2014), Sudhasaravanan and Binukumari (2015). El-Jakee *et al. (*2013) investigated 250 food samples to detect the occurrence of enterotoxigenic *Staphylococcus aureus.* Out of 250 food samples 127 isolates were identified as *Staphylococcus* species (50.8%). The highest isolate rate was observed in raw milk samples (56%) followed by Yoghurt samples (22%) chicken products (6%) white soft cheese samples and pasteurized milk samples (4% each) then meat and meat products (6%). Park *et al*. (2007) analyzed 30,019 samples of raw milk in Korea and detected 109 (0.35%) samples contaminated with *Staphylococcus* and Daka *et al.* (2012) reported 40.6% in South Ethiopia. The difference in the prevalence rate was due to variation in the sanitary condition of udder, size of sampling and geographic region (Sadashiva and Kaliwal, 2013). *S. aureus* causes alimentary toxicosis and produces different extracellular products (Yang *et al*., 2011). Staphylococci food poisoning resulting from contaminated milk and dairy products, especially cheeses produced from raw milk in unclean conditions, causes staphylococcal intoxication (Can and Celik, 2012). Generally, five classical Staphylococci Enterotoxin (SE) SEA to SEE are recognized. It was shown that about 95% of staphylococcal food-poisoning outbreaks were caused by strains carrying the classical SE and the remaining 5% of outbreaks were associated with other identified (Wang *et al*., 2012).

Table 1. Biochemical result of *S.aureus*

S. No	Type of Biochemical test	Results
1	Glucose Fermentation	A+
2	Sucrose Fermentation	A+
3	Lactose Fermentation	A+
4	Maltose Fermentation	A+
5	Mannital Fermentation	A+
5	Triple Sugar Iron	A/A
6	Indole Production	-
7	Methyl Red	+
8	Voges Proskauer	+
9	Citrate Utilisation	-
10	Catalase Test	+
11	Oxidase Test	-
12	Gram Staining	+
13	Mannital Salt Agar	Golden yellow

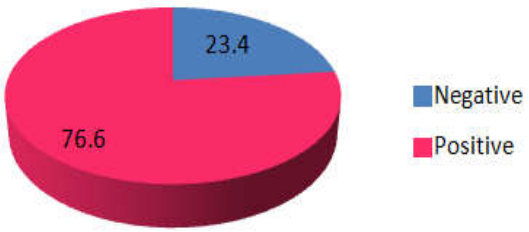

Fig.1. Prevalence of *S. aureus* in collected thirty *m*ilk Samples

In the present study *S. aureus* isolates were found different kind of resistant against the antibiotics Clindamicin, Methicillin, Nitrofurantoin, Penicillin and Tobramycin were represented in the table.2. In the present study 8 isolates of *S. aureus* (S1, S2, S4, S8, S10, S16, S22 and S23) showed 100% sensitivity against all antibiotics. Sample 4 and sample 8 showed least susceptibility (20%) against the antibiotics respectively. The *Staphylococcus aureus* showed 100% resistance against Pencillin followed by Methillin (69.5%), Nitrofurantion (56.5%) Clindamicin (52.1%) and Tobramycin (47.8%). Among the antibiotic patterns 21.73% of resistance was noticed against the Pencillin (Table.4). Association between Methicillin resistant and sensitive *Staphylococcus* were represented in graph.2. Thaker *et al*.(2013) reported the 100%resistant against Pencillin-G followed by Ampicillin (40%), Oxytetracycline and Oxacillin (20%) and Streptomycin and Gentamicin (10%). In our study we observed that Pencillin showed 100% resistance against the organisms tested. Similar types of resistance pattern also reported by Islam *et al*. (2007a 2007b),Jahan *et al.*2015), Thaker *et al*.(2013), Santos *et al.* (2014), Rubin *et al.(*2011). The discovery of a high percentage of resistance strains to Penicillin and Oxacillin has important implications for bovine mastitis control since these drugs represent the main antibiotics group recommended for staphylococcal mastitis treatment and the emergence of resistant strains in the dairy herds could be related to the emergence of virulence strains (Van der Mee-Marquet *et al*., 2004). Although the number of isolates could be considered as limited, our data indicate that bacterial antibiotic resistance could be low in the area, probably due to appropriate therapeutic managements.

Table.2. Antibiotic susceptibility of *S. aureus*

S. NO	Sample ID	Clinda micin (CD)	Methicillin (M)	Nitrofurantoin (NF)	Penicillin (P)	Tobramycin (TB)	% of Resistant
1	S1	R	R	R	R	R	100%
2	S2	R	R	R	R	R	100%
3	S4	S	I	I	R	S	20%
4	S5	R	R	R	R	R	100%
5	S6	S	R	S	R	R	60%
6	S7	I	I	R	R	S	40%
7	S8	S	S	S	R	S	20%
8	S9	S	I	R	R	S	40%
9	S10	R	R	R	R	R	100%
10	S11	S	R	S	R	R	60%
11	S12	R	R	R	R	R	100%
12	S13	S	R	R	R	S	60%
13	S15	S	S	I	R	S	20%
14	S16	R	R	S	R	I	60%
15	S17	S	S	S	R	S	20%
16	S19	R	R	R	R	R	100%
17	S20	S	S	I	R	S	20%
18	S21	S	R	S	R	I	40%
19	S23	R	R	R	R	S	80%
20	S25	R	R	R	R	S	80%
21	S27	R	R	I	R	R	80%
22	S28	R	R	R	R	R	100%
23	S30	R	R	R	R	R	100%
% of Resistant		52.1 %	69.5 %	56.5 %	100 %	47.8 %	

Table. 3. Antibiotic patterns of *S. aureus* screened form milk samples

S.No	Antibiotic pattern	No. of Isolates	Percentage
1	P	5/23	21.73
2	NF,P	2/23	8.6
3	M,P	1/23	4.3
4	M, P,TB	2/23	8.6
5	M,NF,P	1/23	4.3
6	CD,NF,P	1/23	4.3
7	CD,M,NF,P	2/23	8.6
8	CD,M,P,TB	1/23	4.3
9	CD,M,NF,P,TB	8/23	34.7

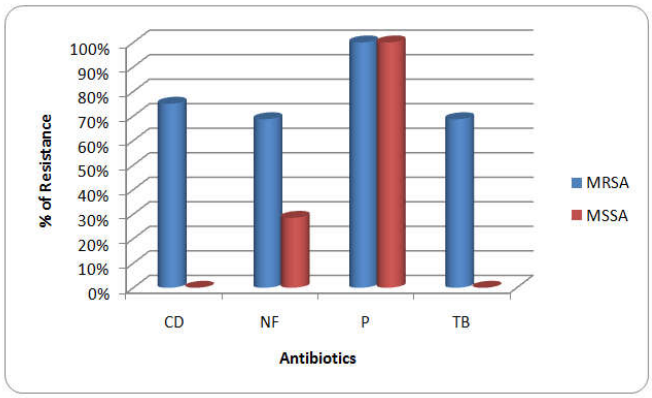

Fig.2. Association between MRSA-MSSA antibiotic activity

The Staphylococcus isolates virulence factor was analyzed by Biofilm formation and coagulase negative test. Among the 23 species of tested staphylococcus 52.1% showed positive result on biofilm formation and 39% showed coagulase negative (table5-7). The MRSA was reported with 25% and 17.3% with biofilm formation and coagulase negative test. Santos *et al*. (2014) classified the *Staphylococcus aureus* isolates as high, modulate and weak biofilm producers, based on OD540 results. Among them one was a high biofilm producer, other 10 were moderate biofilm producers while the 9 isolates produced weak biofilms. During intramammary inflammation (IMI), bacterial clusters may develop with the udder and biofilm structures, may facilitate bacterial adherence and colonization of the epithelium. In general all isolates could isolates establish biofilms in 24h.

Table 4. Determination of Biofilm producing and coagulase Negative isolates from Methicillin Resistance *S.aureus* (MSSA)

SAM-ID	MRSA		
	Bf	CoNS	%
S1	+	+	100
S2	+	-	50
S4	+	-	50
S5	+	-	50
S6	-	-	0
S7	+	-	50
S8	-	-	0
S9	-	-	0
S10	+	+	100
S11	+	+	100
S12	-	-	0
S13	-	-	0
S15	+	-	50
S16	-	+	50
S17	+	-	50
S19	-	-	0
S20	-	-	0
S21	+	-	50
S23	-	+	50
S25	-	+	100
S27	-	+	50
S28	+	+	100
S30	-	+	50

Bf- Biofilm, CoNS - *Coagulase negative*

Adhesion was a crucial early step for mammary gland infection but biofilm also may enable recurrent infections protecting bacterial cells from host defenses and the effects of antibiotics (Cucarella *et.al.*2004). In the present study 52.7% of *S. aureus* isolates obtained from 23 milk samples produced biofilm. Similar results were reported by Vasudevan *et al*.(2003). Fox (2014) reported that 68.6%, 41%, 95.7% and 94.7% respectively, of samples between biofilm- producing *S. aureus* isolated from milk. The results of the present study showed that the high frequency of isolation of *S. aureus* in milk, indicating the presence of bovine mastitis, tends to have higher biofilm production, suggesting a possible relationship between the occurrence of Bovine mastitis and their virulence. Some authors suggest that Staphylococcus ability to form biofilms increased ability to initiate and trigger persistent intramammary infections (Baselga *et al*. 1993; Cucarella *et al*., 2004; Melchior *et al.,* 2006).

Table 5. Determination of Biofilm and coagulase producing isolates from Methicillin sensitive *S.aureus* (MSSA)

Sample ID	MSSA		
	Bf	CoNS	%
S8	+	-	50
S15	-	-	0
S17	-	+	50
S18	-	-	0

Bf- Biofilm, CoNS - *Coagulase Negative*

Table 6. Association of virulence factors between MRSA and MSSA

S.No	Sample	Virulence Factor	
		Bf	CoNS
1	MRSA	52.1	39
2	MSSA	25	17.3

Table.7. Biofilm production, Coagulase negative test and Methicillin resitance of *S. aureus* isolates

SAM-ID				Bio typing
	Bf	CoNS	MRSA	
S1	+	+	R	A
S2	+	-	R	B
S4	+	-	I	C
S5	+	-	R	B
S6	-	-	R	D
S7	+	-	I	C
S8	-	-	S	E
S9	-	-	I	NO TYPE
S10	+	+	R	A
S11	+	+	R	A
S12	-	-	R	D
S13	-	-	R	D
S15	+	-	S	F
S16	-	+	R	G
S17	+	-	S	F
S19	-	-	R	D
S20	-	-	S	E
S21	+	-	R	NO TYPE
S23	-	+	R	G
S25	-	+	R	NO TYPE
S27	-	+	R	NO TYPE
S28	+	+	R	A
S30	-	+	R	G

Bf- Biofilm, CoNS – *Coagulase negative*

The ability of microbial adhesion and biofilm formation may occur as a result of deposition of micro-organisms on a surface of contact, where they attach and hygiene growing (Zottola, 1994).The main problem is recontamination of milk and thus there is a high microbial load in the product. Based on the biofilm formation, methicillin susceptability, coagulase negative tests the 23 isolated were classified as A, B, C, D, E, and F. The highest isolates was observed in biotype A (21.8%), followed by D (17.4%), G (13.04%) B, C, E, F (8.7%). Among these 13.04% of isolates were not comes under any one of these category (Table.7). Milk is normally sterile in the udder of the cow and buffalo provided they do not suffer from mastitis (udder infection). If they have mastitis, a large number

of generally gram positive bacteria such as *Streptococcus* and *Staphylococcus sp.* may be present in milk when it leaves the udder (Holm and Jespersen (2003). Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udder, milking equipments, milk handling technique and improper storage will increase the proportion of Gram-positive and Gram negative bacteria in the bulk tank milk (Bonfonth *et al.*, 2003).

Table.8. Biotyping of *S. aureus* isolates from milk samples

Biofilm	Methicilin susceptibility	Coagulase negative	Biotyping
+	+	+	A
+	-	+	B
+	I	-	C
-	R	-	D
-	S	-	E
+	S	-	F
-	R	+	G

Spans *et al*. (2012) observed that strains carrying one or more genes for production of enterotoxins and other virulence factors and some of the virulence factors investigated could be considered important determinants for the host pathogen relationship providing information that allows for tracing the most probable source of contamination. Ote *et al*.(2011) demonstrated a large variation in the presence of virulence genes in *S. aureus* isolates and the considerable diversity of strain populations capable of causing mastitis in cows. Moreover, the presence of isolates carrying genes coding for toxins involved in impotent human infections renders the milk of cows with mastitis a potential reservoir for these toxins and therefore a potential dander in human health, which underscores the importance of carefully scrutinizing raw milk for consumption and its processing. Zeccone *et al*. (2000) reported that the presence of subclinical mastitis showed the role of *Spa* and *Sej* gene as risk factors. An effective vaccine against bovine mastitis was not yet available and prevention and control of mastitis requires identification of the main antigenic determinants for the design and development of more efficient vaccines (Franco *et al*. 2008). *S. aureus* isolates from per acute and acute mastitis have been reported to produce large amount of beta toxin than *Staphylococcus aureus* isolated from chronic infections (Matsunaga *et al.* 1993). The mammary gland is the principle place of infection of *S. aureus* when the animal has mastitis, but studies carried out by Capurro *et al*.(2010) identified the skin of the shanks as being a significant reservoir due its anatomical position and other places such as Muzzle, grain and wounds, reinforcing the care to be taken during milking so as not disseminate the bacteria to other animals, compromising the quality of milk.

Food products serve not only as a source of nutrition but as substrates for the growth of micro-organisms. The growth of micro-organisms causes food spoilage. It may result in food borne-illness. In tropical countries raw milk and milk products are responsible for many outbreaks of gastrointestinal tract. It is also reported that immunocompromised individuals are prone to food-borne infection (Alte Kruse *et al*.,1994). Milk is a good substrate for *S. aureus* growth and enterotoxin production. In addition, enterotoxins retain their biological activity even after pasteurization (Asao *et al*.,2003). Imani fooladi *et al.*(2010) concluded that 32% of all dairy products were contaminated by *staphylococcus aureus. S. aureus* causes alimentary toxicosis and produces different extracellular products (Yang *et al.*, 2011). Generally five classical staphylococci enterotoxin (SE) SEA to SEE are recognized. It was show that about 95% of staphylococcal food poisoning outbreaks were the remaining 5% of outbreaks were associated with other identified (Wang *et al*., 2012).

REFERENCES

Al-khatib, l. A. and Al-Mitwalli, S. M. 2009. Microbiological quality and sample collection policy for dairy products in Ramallah and Al-Bireh district, Palestine. *East Mediterranean Heal.J*., 15: 709-716.

Aydin, A., K. Muratoglu, M. Sudagidan, K. Bosten, B. Okuklu and S. Harsa, 2011. Prevalence and antibiotic resistance of food borne *S. aureus* isolates in Turkey. *Food borne Path. Dis.,* 8 (1): 63-69.

Bhatia, A. and Zahoor, S. 2007. *Staphylococcus aureus* Enterotoxins: A Review. *J.Clin. & Diag. Res.,* 1: 188-197.

Bramely AJ and McKinnon CH. 1990. The Microbiology of Raw Milk. In: Dairy microbiology, I. Robinson RK (Ed.), London, NY, *Elsevier Applied Sci.* pp: 171.

Coia, J.E., Carter F.T., Baird D and Platt D.J. 1990**.** Characterization of methicillin- resistant *Staphylococcus aureus* by biotyping, immunoblotting and restriction enzyme fragmentation patterns. J. Med. Microbiol. 31: 125- 132.

De Oliveira A.P., Watts J.L., Salmon S.A., Aarestrup F.M.: Antimicrobial susceptibility of *Staphylococcus aureus* isolated from mastitis in Europe and the United States. *J. Dairy Sci*., 2000, 83, 855-862.

Fagundes, H. and Oliveira, C.A.F. 2004. Staphylococcus aureus intrammary infections and its implications in public health. *Cienc. Rural.,* 34: 1315-1320.

Fagundes, H., Barchesi, L., Filho, A. N., Ferreira, L. M. and Oliveira 2010. Occurrence of *S. aureus* in raw milk produced in dairy farms in Sao Paulo state, Brazil. *Braz. J. Microbial.,* 41: 376-380.

Jones, F.T., Creech, B.C., Erwin, Baird, G.S., Woron, A. M. and Schaffner, W. 2006. Family outbreaks of invasive community associated Methicillin resistant *S. aureus* infection. Clin. Infect. Dis., 42: 76-78.

Kerouanton, A., Hennekinne, J.A., Letertre, C., Petit, L., Chesneau, O., Brisabois, A., De Buyser, M.L. 2007. Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. Int. J. Food Microbial., 115: 369-375.

Loir YL, Baron, F, Gautier, M 2003. *Staphylococcus aureus* and Food foisoning. *Genet. Mol. Res.* 2: 63-76.

Nagarajappa, S., M.S. Thakur and H.K. Manonmani, 2012. Detection of enterotoxigenic *Staphylococci* by Loop-mediated isothermal amplification method. *J. Food. Safety,* 32: 59-65.

Nickerson P.C.: Eliminating chronic staphylococcus mastitis. Vet. Med., 1993, 88, 375-381.

Oliver S.P., Lewis M.J., Gillespie B.E., Dowlen H.H.: Strategies for the control of mastitis in heifers during the periparturient period. National Mastitis Conuncil Annual Meeting, Nashville TE, 1996, pp. 202-203.

Osteras O., Martin S.W., Edge V.L.: Possible risk factors associated with Penicillin- resistant strains of *Staphylococcus aureus* from bovine subclinical mastitis in early lactation. J. Dairy Sci., 1999, 82, 927-938.

Payne, D.N. and Wood, J.M. 1974. The incidence of enterotoxin production in strains of *Staphylococcus aureus* isolated from food. *J.Appl. Bacteriol*., 37(3): 319-325.

Sears P.M., Smith B.S., English P.B., Herer P.S., Gonzalez R.N.: Pattern of *Staphylococcus aureus* from bovine intramammary infections. J. Dairy Sci., 1990, 73, 2785-2789.

Soomro, A.H., Arain, M.A., Khaskheli, M. and Bhutto, B. (2003). Isolation of *Staphylococcus aureus* from milk products sold at sweet meat shops of Hyderabad. *Online J. Biol. Sci.,* 3(1):91-94.

Torkar, K.G. and Teger, S.G. 2008. The Microbiological Quality of Raw milk after introducing the two days milk collecting system. *African Journal of Nutrition.* 92(1): 61-74.

Van der Mee-Marquet N., Blanchard M., Domelier A. S., Quentin R. 2004.Virulence and antibiotic susceptibility of *Staphylococcus aureus* strains isolated from various origins. *Pathol Biol*. 52:579-583.

Vijayalakshmi, S.Lalith, D.Jegadeesshkumar, P.Rajeswari. 2013. Biotyping of coagulase negative and biofilm producing wound isolates of *Staphylococcus aureus. International Journal of Science Innovations and Discoveries*. 3(3): 376-385.

Wilson D.J, Gonzalez R.N., Sears P.M.: Segregation or use of separate milking units for cows infected with *Staphylococcus aureus* .- Effects on prevalence of infection and bulk tank somatic cell count . *J. Dairy Sci*., 1995, 78, 2083-2085.

Yancey R.J., Sanchez M.S., Rzepkowski R.A., Barnes R.E., Ford C.W.: Therapy of chronic mastitis. Flem. Vet. J., 1991, 62, 187-204.

Zecconi A., Piccinini R.: Control of *Staphylococcus aureus* infection – practical aspects and perspectives. Practical aspects of scientific examinations of reproduction system and mammary gland in animals. Wenecja 2002. Wyd. PIWet Pulawy, pp. 18-30.

Zecconi, A. and Hahn, G. 2000. *Staphylococcus aureus* in Raw Milk and Human Health Risk. *Bulletin of the International Dairy Federation,* 345, 15-18

****