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Isolation and Assessment of Antibiotic Response Pattern of Heat Resistant *Staphylococcus aureus* from Milk

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A B S T R A C T

Staphylococcus aureus, is ranked as the third most important cause of food borne illnesses in the world. This organism is considered as a pathogen of great concern due to various factors like high frequency of acquisition of antibiotic resistance, ability to cause a diverse array of life threatening infections and capacity to adapt to different environmental conditions. In the current study *Staphylococcus aureus* cultures were isolated from milk heat treated at processes severer than pasteurization (72^0C for either two or three minutes) and were evaluated for their response pattern towards ten antibiotics. Five typical colonies were selected from Baird-Parker agar and confirmed as *S. aureus* through biochemical and molecular level tests. All the five isolates obtained were found to be sensitive/intermediate to three cell wall synthesis inhibitors: amoxycillin, ceflazidime, cefepime and two protein synthesis inhibitors: erythromycin, and tetracycline. Three isolates each were found to be resistant to the cell wall synthesis inhibitor ampicillin, and the protein synthesis inhibitor, gentamicin. Further, minimum inhibitory concentrations of three antibiotics, ampicillin, erythromycin and tetracycline for the isolates were determined. The values ranged from 0.001µg/ml to 4µg/ml, 0.001µg/ml to 8µg/ml and 0.1µg/ml to 8µg/ml for ampicillin, erythromycin and tetracycline respectively. The high heat resistance and antibiotic resistance exhibited by *S aureus* cultures isolated from milk necessitate further studies to delineate their mechanisms of resistance. The study also underscores the need for re-considering the current time temperature combinations recommended for ensuring safe milk.

Introduction

Milk, one of the most nutritionally complete foods, adds high-quality protein, fat, milk sugar, essential minerals, and vitamins to our diet. The nutritional components that make milk an important part of the human diet also support the growth of the microorganisms. So this nutrient rich medium could act as vehicles of disease transmission and thus is associated with disease outbreaks of major proportion. Milk and its derivatives are considered as a major source of *Staphylococcus aureus* associated diseases in human beings (Zecconi and Piccinini, 1998). *S. aureus* get access to milk either by direct excretion from udders with clinical or subclinical *Staphylococcal* mastitis or by contamination from the

environment during handling of raw milk (Scherrer *et al.,* 2004; Jorgensen *et al.,* 2005). Staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. They are mesophils with optimum growth temperature in the range from 37°C to 40 °C. Temperatures higher than 46°C are not acceptable for majority of the strains, but some may grow up to 50 °C (Medveďová and Valík, 2012). However there are reports of isolation of heat resistant *S aureus* from different food samples (Beena *et al.,* 2014; Montanari *et al.,* 2015; Yaniarti *et al.,* 2017). Pasteurization, the process of heating every particle of milk to at least 63^{o}C for 30min or 72^{o}C for 15sec or to any temperature-time combination which is equally efficient in approved and properly operated equipment is one of the major thermal processes the dairy industry relies on to assure the safety and quality of the milk. So evidences of any pathogens capable of surviving this process are of great public health relevance. Considering the public health threat posed by the prevalence of multiple drug resistance in heat resistant strains of *S aureus*, it was felt appropriate to carry out a study to evaluate the antibiotic resistance pattern in heat resistant *S aureus* from milk.

Materials and Methods

Isolation, physiological, biochemical and molecular level identification of selected *Staphylococcus aureus* isolates

Raw milk samples were subjected to a heat treatment of 72^{0}C for two or three minutes and cooled immediately. A temperature control was kept along with the sample to ensure that heat treatment of samples was proper Appropriate dilutions of the heat treated samples were pour plated on Baird-Parker agar (HiMedia, Mumbai) and incubated at 37^{o}C for 48 h. Five typical colonies (shiny black with an opaque ring, surrounded by a clear halo) were randomly selected from the plate and subjected to preliminary identification tests: Gram staining and catalase test. The isolates were stored in nutrient agar slants at 4^{0}C and activated in nutrient broth to carry out further studies. Carbohydrate fermentation (Barrow and Feltham, 1993) and coagulase tests (tube method, Coagulase plasma, HiMedia, Mumbai, as per manufacturer's directions) were also done as part of biochemical identification and characterization.

For the molecular level identification of the isolates, DNA of 24 h old cultures were extracted using the HiPurA Bacterial Genomic DNA Purification kit (Hi Media, Mumbai) as per the manufacturer's instructions. Fragments of the genes of interest were amplified using standard PCR protocol using the primers – Forward primer (Sa442-1, 5'-AAT CTT TGTCGG TAC ACG ATA TTC TTC ACG-3') and Reverse primer (Sa442-2, 5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA -3'). The100µl reaction mixture comprised of 2 µl each of 0.4µM primers (Sigma, USA), 10 µl of 25 mM $MgCl_2$ (Sigma, USA), 10 µl of PCR 10X assay buffer (GeNei), 10µl of dNTPs (10mM), 0.6µl of 0.05 units/ ml of Taq DNA polymerase (Sigma, USA), 59.4 µl of nuclease free water and 6 µl of template DNA sample. Amplification was carried out in a thermal cycler (BIO RAD T100) as follows: initial denaturation at 95 °C for 1 min, followed by 35 cycles each consisting of denaturation at 95 °C for 1 min, annealing at 56^{o}C for 30 sec, extension at 72 °C for 45 sec, followed by a final five -minute extension step at 72 °C. 10 µl of the reaction products were resolved by electrophoresis in a 1.5% agarose gel containing 0.5µg/ml of ethidium bromide in 1X TAE buffer (Tris Acetate EDTA, HiMedia, Mumbai) buffer at 50 V. A 100 bp DNA ladder (200pg/µl, HiMedia, Mumbai)

was included as a reference. The gel was observed and photographed in the Gel documentation system (G-BOX, Syngene).

Assessment of antibiotic sensitivity of the selected isolates

Disc diffusion assay

The susceptibility of the isolates to different antibiotics was determined as per the methods of Bauer *et al.*, (1959). For this Mueller Hinton agar (MHA, HiMedia, Mumbai) plates were prepared and active cultures of isolates were swabbed over the surface of the plate. Nine different antibiotic discs (Ampicillin (10 µg), Amoxicillin (10 µg), Amoxyclav (30 µg), Ceflazidime (30 µg), cefixime (30 µg), Cefepime (30 µg), Oxacillin (5 µg), Erythromycin (15 µg), Gentamicin (50 µg), Tetracycline (30 µg) and Linezolid (30 µg) (HiMedia, Mumbai) were placed over the surface of the inoculated plate and incubated at 37^0C for 24 hours. Diameter of the zone of clearance developed around the discs were measured and recorded. The isolates were graded as either resistant/sensitive based on the antibiotic sensitivity chart provided by the supplier (HiMedia, Mumbai).

Determination of minimum inhibitory concentration of selected antibiotics

The minimum inhibitory concentration (MIC) for four different antibiotics (Ampicillin, Erythromycin and Tetracycline) for the isolates were determined using EZY MIC™ and HiComb™ MIC strips (HiMedia, Mumbai) as per the manufacturer's instructions. For this active culture of isolates were inoculated at 1% level to nutrient broth and incubated at 37^0C until an optical density of 0.08 at 620 nm was obtained. These O.D. adjusted active cultures were swabbed on pre-prepared MHA plates, the antibiotic strips were then placed on the plates and incubated at 37^0C for 24 hours and read the MIC as the concentration where the ellipse intersects the MIC scale on the strip. Resistance/sensitivity was determined based on the interpretive data provided by the manufacturer of the strips.

Results and Discussion

Isolation of *S. aureus* from heat treated milk

On pour plating of milk heat treated at 72^0C for two or three minutes on Baird Parker agar medium typical colonies of *S. aureus* appeared as black and shiny with narrow white margins and surrounded by clear zone extending into the opaque medium **(**Figure 1**)**. From the typical colonies, five were randomly selected (Table 1) and subjected to various tests to further characterize them.

Physiological, biochemical and molecular level characterization of selected isolates

Primary identification of the isolates was carried out by Gram staining and catalase test. *Staphylococcus aureus* are gram positive cocci which are catalase positive (Barrow and Feltham, 1993). All the isolates obtained in this work were found to be catalase positive, Gram positive cocci in clusters (Fig. 2). When streaked on Mannitol salt agar (MSA) all of them formed small yellow colonies and were presumptively identified as *S aureus* as the growth and production of yellow colonies in MSA is regarded as a presumptive tool in the identification of *S. aureus* (Fig. 3, Shittu *et al.*, 2006).

Development of yellow colonies was considered as a characteristic for the differentiation of coagulase-positive staphylococci from coagulase-negative staphylococci (CNS, Duguid, 1989) earlier, but now it is known that some CNS can also produce yellow colonies on MSA (Zadik *et al.*, 2001; Shittu *et al.*, 2006).

Carbohydrate fermentation pattern of all the five isolates were in agreement with that given in Bergey's manual (Schleifer and Bell, 2009) in terms of their ability to ferment maltose, mannitol, fructose, sucrose, trehalose (Table 2).

Fermentation pattern of the sugars xylose, raffinose and mannose varied in between strains as well as from the Bergey's manual. Surprisingly none of the isolates could ferment cellobiose, though it is fermentable as per Bergey's manual. Such variations in biochemical reactions of *S. aureus* isolates are reported by El-Hadedy and Abu El-Nour (2012). All the isolates were found to be coagulase positive (Table 2) on performing the tube test indicating the presence of unbound extracellular coagulase.

Agarose gel electrophoresis of the PCR products revealed the presence of bands slightly higher than 100 bp size (Fig. 4). This is in agreement with Singh and Prakash (2012) who reported 108bp sized PCR products for *S.aureus* when species specific primers as that in current study were used.

Isolation and confirmation of the presence of *S aureus* in milk subjected to heat treatments exceeding that of pasteurization raises an alarm. As observed in the current study other studies also reported the isolation of heat resistant *S. aureus* from food samples (Parente and Mazzatura 1991; Nema *et al.,* 2007; Dewanti-Hariyadi *et al.,* 2011).

Assessment of antibiotic sensitivity of the selected isolates

Disc diffusion assay

In general, differences were observed among isolates in their antibiotic sensitivity or resistance patterns (Fig. 5 and Table 4). Isolate 1 was found to be sensitive to all tested antibiotics except linezolid, one of the protein synthesis inhibitors tested. Isolate 3 being resistant to four antibiotics (ampicillin, amoxyclav, gentamicin and linezolid) was identified as the most resistant isolate and could be categorized as a multi-drug resistant *S aureus* (resistance to three or more antibiotics, Chandrasekharan *et al.,* 2014). Isolate 4 was found to be resistant to only one antibiotic, ampicillin.

Two isolates, namely isolates 2 and 5 exhibited resistance to two antibiotics. All the isolates were sensitive or intermediate to the antibiotics amoxicillin, ceftazidime (a third-generation cephalosporin), cefapime (a fourth-generation cephalosporin), erythromycin (a macrolide antibiotic) and tetracycline (Table 3).

It is noteworthy that two out of the five isolates were resistant to linezolid, an anti-MRSA (Methicillin resistant *S. aureus*) drug approved as an alternative to vancomycin for infections caused by MRSA.

This observation is markedly different from the earlier report of sensitivity of all *Staphylococcus aureus* isolates to linezolid (Niveditha and Sujatha, 2015).

Out of the six cell wall synthesis inhibiting and four protein synthesis inhibiting antibiotics tested, highest number of resistant isolates (three each) were obtained for ampicillin and gentamicin, a protein synthesis inhibiting aminoglycoside.

Considering that the isolates were obtained from milk, the comparatively high resistance observed against these antibiotics could be due to the facts that *Staphylococcus aureus* is a major pathogen associated with bovine clinical and subclinical mastitis (Bhatt *et al.,* 2011; Verma *et al.,* 2018) and these drugs are commonly used for mastitis therapy (De Oliveira *et al.,* 2000; Manimaran *et al.,* 2014)

Fig.1 *S. aureus* colonies on Baird Parker agar

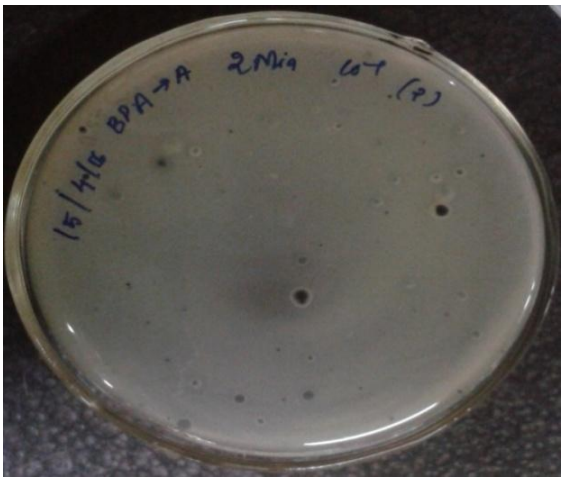

Fig.2 Microscopic appearance of Gram stained *S. aureus*

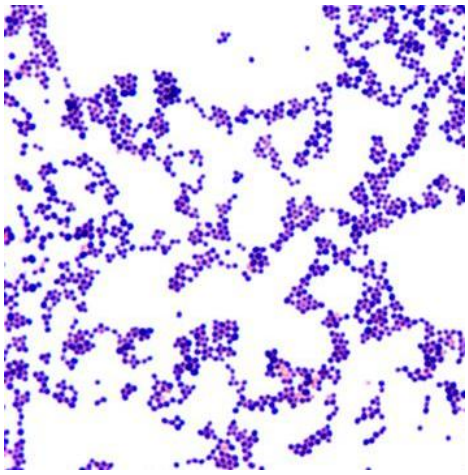

Fig.3 Growth of *Staphylococcus aureus* isolates on mannitol salt agar medium

Fig.4 Electrophoretic profile of PCR products of *S. aureus* isolates amplified by the species specific primers Sa442-1 and Sa442-2.Lane 1- 100bp marker, Lanes 2-6-Isolates 1-5

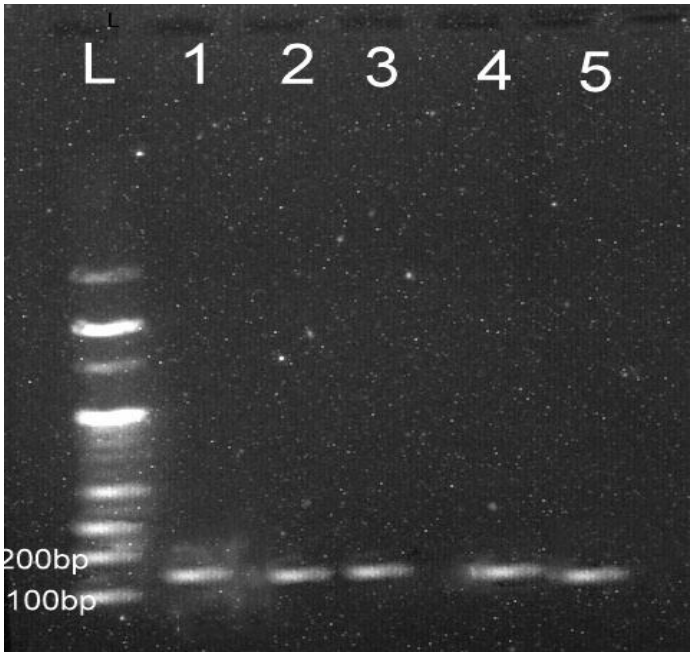

Fig.5 Disc diffusion assay for antibiotic susceptibility testing

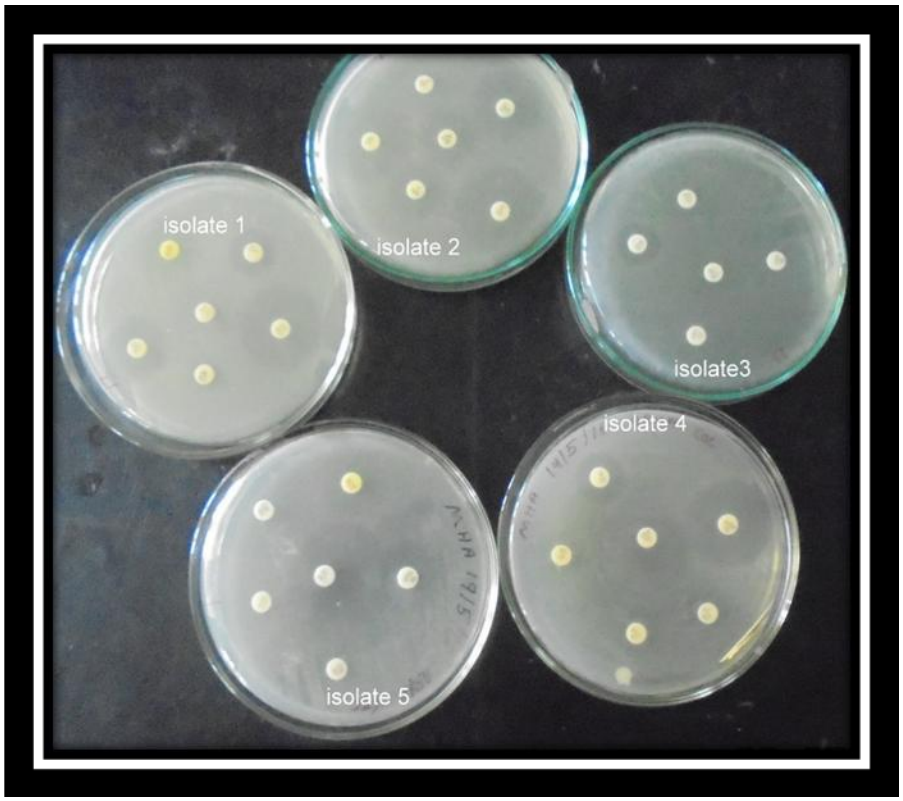

Fig.6 Determination of MIC values using MIC strips

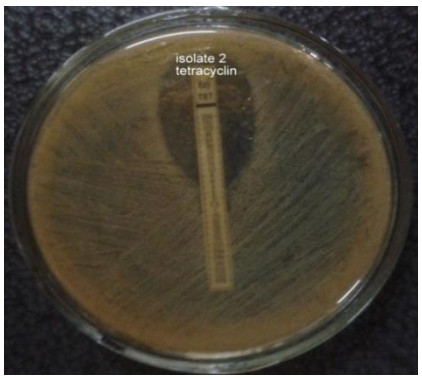

Table.1 Details of isolates selected

Isolate no:	Source of isolation
Isolate 1	Milk heat treated at 72^0C/ 2 minutes
Isolate 2	Milk heat treated at 72^0C/ 2 minutes
Isolate 3	Milk heat treated at 72^0C/ 2 minutes
Isolate 4	Milk heat treated at 72^0C/ 3 minutes
Isolate 5	Milk heat treated at 72^0C/ 3 minutes

Table.2 Carbohydrate fermentation by *Staphylococcus aureus* isolates

Test	Bergey's manual	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Catalase test	+	+	+	+	+	+
Carbohydrate fermentation						
Lactose	+	-	-	+	-	+
Maltose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+
Xylose	_	+	-	-	-	+
Cellobiose	_	+	+	+	+	+
Raffinose	_	+	-	+	-	+
Mannose	+	+	-	+	-	+
Coagulase test	+/	+	+	+	+	+

+ - Positive reaction - Negative reaction

Table.3 Resistance / Sensitivity of the isolates to the antibiotic tested

a. Cell wall synthesis inhibiting antibiotics								
Sl no:	**Antibiotic tested**	**Antibiotic content/disc µg**	**Isolate 1**	**Isolate 2**	**Isolate 3**	**Isolate 4**	**Isolate 5**	**Number of resistant isolates**
1	Ampicillin	10	S	R	R	R	S	3
2	Amoxicillin	10	S	S	S	S	S	0
3	Amoxyclav	30	S	S	R	S	S	1
4	Ceftazidime	30	S	I	I	I	S	0
5	Cefepime	30	S	S	I	I	S	0
6	Oxacillin	5	S	S	S	S	R	1
	Number of antibiotics to which the isolate was resistant		0	1	2	1	1	
b. Protein Synthesis Inhibitory Antibiotics								
1	Erythromycin	15	I	I	S	S	I	0
2	Gentamicin	50	S	R	R	S	R	3
3	Tetracycline	30	S	I	I	I	I	0
4	Linezolid	30	R	S	R	S	S	2
	Number of antibiotics to which the isolate was resistant		1	1	2	0	1	

R- Resistant, I – Intermediate, S-Sensitive

Table.4 MICs (µg/ml) of selected antibiotics (determined using E-strips) for *S aureus* isolates and their resistance/ sensitivity (*Alphabets in brackets are indicative of: R- Resistant, I – Intermediate, S-Sensitive)*

Sl no:	Antibiotic tested	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
1	Ampicillin	0.001(S)	3 (R)	4(R)	0.001(S)	0.01(S)
2	Erythromycin	0.001(S)	8 (R)	2(S)	0.01(S)	0.001(S)
3	Tetracycline	3(S)	2(S)	0.1(S)	8(I)	6 (I)

Determination of minimum inhibitory concentration of selected antibiotics

On determining the minimum inhibitory concentration of ampicillin, erythromycin and tetracycline for the isolates, marked differences were observed in their values for different isolates (Table 5). In the case of ampicillin, the minimum inhibitory concentrations ranged from 0.001 to 4µg/ml. For isolates 2 and 3, the resistance patterns obtained based on MIC values was in agreement with that obtained for disc diffusion assay. However in the case of Isolate 4, there was difference between the results obtained for disc diffusion and MIC determination assays which needs to be further investigated. The widest range of MIC values was obtained for erythromycin: varying from 0.001 to 8µg/ml. Different from the 'intermediate resistant' grading obtained for isolate 2 based on the disc diffusion assay, it was graded as resistant on the basis of MIC value of erythromycin whereas for all the other isolates the observations were agreeing with each other. In the case of the other protein synthesis inhibitor tested, tetracycline the MIC values ranged from 0.1 to 8 µg/ml, the highest MIC value being similar to that obtained for erythromycin (Fig. 6). As observed in the disc diffusion assay none of the isolates was found to be resistant to this antibiotic based on their MIC values. So in general, irrespective of some variations between the resistance /sensitivity pattern derived based on zone of inhibition (disc diffusion assay) and minimum inhibitory concentrations, majority of the observations were comparable with each other. Previous studies also reported different levels of agreement between E-test and disk diffusion in determining antimicrobial sensitivity depending on the types of specific organisms and antibiotics used in the studies (Erfani *et al.,* 2011; Khalili *et al.,* 2012). Such variations are also attributed to the type of antibiotic discs used due to differences in quality of discs from different manufacturers. However this does not stand in the current study as both were from the same manufacturers. So the differences observed could be attributed to the differences in the isolates.

Based on their MIC values three of the isolates, namely 1, 4 and 5 were not found to sensitive/intermediate to all the tested antibiotics. Isolate 3 was found to be resistant to ampicillin and isolate 2 to ampicillin and erythromycin.

Exposures to deleterious stresses force the stressed bacteria to undergo phenotypic and genotypic adaptations in order to reduce the impact of these stresses. Some of these adaptation mechanisms are found to impart cross protection against a range of apparently unrelated challenges including resistance to antibiotics. However no such cross protection effects between heat and antibiotic resistances were observed in the current study. Results of this work is in agreement with the reports of absence of any interrelationship between antibiotic resistance and heat resistance in *L monocytogenes* (Walsh *et al.,* 2001) and also between laboratory acquired antibiotic resistant mutation and heat resistance in *Salmonella* strains (Walsh *et al.,* 2005).

Antibiotic resistance in bacteria is a critical problem that is globally recognized. Indiscriminate use of antibiotics/antimicrobial agents for prophylactic and therapeutic purposes is identified as major reasons for this threat. Due to their unique ability to quickly develop resistance to new antibiotics *Staphylococcus aureus* is widely recognized as the best example for the adaptive evolution of bacteria in this antibiotic era. Current study reports the presence of *S aureus* in milk even after a heat treatment of 72^0C for 3 minutes. This observation itself is of great relevance

due to the risk associated with their ability to surive pasteurization, a process highly relied upon to safeguard the health of consumers. The resistance exhibited by the isolates against multiple antibiotics makes the situation still graver and also reemphasize the need for adoption of good agricultural /hygienic practices at all levels of production and processing to safeguard the health of consumers.

References

Barrow, C. I., and Feltham, R. K. A. 1993. Cowan and steel's; Manual for the identification of medical bacteria. 3rd edition. Cambridge University Press. pp. 68-76.

Bauer, A. W., Perry, D. M. and Kirby, W. M. 1959. Single-disk antibiotic-sensitivity testing of staphylococci; an analysis of technique and results. AMA Arch. Int. Medicine 104(2):208–216.

Beena, A.K., James, L. and Jisna, T. 2014. Antibiotic response pattern of *Staphylococcus aureus* isolated from pasteurized milk and the milk sweet-Pedha. Int. J. Agri. Food Sci. Tech. 5(1):53-60.

Bhatt, V.D., M.S. Patel, C.G. Joshi and A. Kunjadia, 2011. Identification and antibiogram of microbes associated with bovine mastitis. Anim. Biotechnol., 22 (3): 163-169.

Chandrasekaran, D., Venkatesan, P., Tirumurugaan, K.G., Nambi, A.P., Thirunavukkarasu, P.S., Kumanan, K., Vairamuthu, S. and Ramesh S. 2014. Pattern of antibiotic resistant mastitis in dairy cows, Vet. World 7(6): 389-394.

De Oliveira, A.P., Watts, J.L., Salmon, S.A. and Aarestrup, F.M. 2000.Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. J. Dairy Sci. 83(4):855–862.

Dewanti-Hariyadi, R., Hadiyanto, J. and Purnomo, E. K. 2011. Thermal resistance of local isolates of *S. aureus.* As. J. Food Ag- Ind. 4(4):213-221.

Duguid, J. P. 1989. Staphylococcus: cluster-forming Gram-positive cocci. In: Colle, J. G, Duguid, J.P., Fraser, A.G. and Marmion, B.P. (Ed.) Mackie and McCartney Practical Medical Microbiology, Thirteenth ed. Churchill Livingstone. New York, pp. 303–316.

El-Hadedy, D. and Abu El-Nour, S.A. 2012. Identification of *Staphylococcus aureus* and *Escherichia coli* isolated from Egyptian food by conventional and molecular methods. J. Genet. Eng. Biotechnol.10:129-135.

Erfani, Y., Rasti, A., Mirsalehian, A., Mirafshar S. M. and Ownegh, V. 2011. E-test versus disk diffusion method in determining multidrug resistant strains of *Escherichia coli* in urinary tract infection. Afr. J. Microbiol. Res. 5 (6): 608-611.

Jørgensen, H. J., Mørk, T., Høga°sen, H. R. and Rørvik, L.M.2005.Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. J. Appl. Microbiol. 99 (1): 58–166.

Khalili, H., Soltani, R., Negahban, S., Abdollahi, A. and Gholami, K. 2012. Reliability of Disk diffusion test results for the antimicrobial susceptibility testing of nosocomial Gram-positive Microorganisms: Is E-test Method Better? Iran J. of Pharm. Res., 11 (2): 559-563.

Lopez, C.C., Vannini, L., Lanciotti, R. and Guerozoni, M.E. 1998. Microbiological quality of filled Pasta in relation to the nature of heat treatment. J. Food Prot., 61 (8):994-999.

Manimaran, A., Layek, S.S., Kumaresan, A., Prasad, S., Sreela, L., Boopathi, V., Kumar. K. and Venkatasubramanian, V. 2014. Estimation of antimicrobial drug

usage for Treatment of clinical mastitis cases in organized dairy farm. Ind. J. Vet & Anim. Sci. Res. 43 (2) 140 – 150.

McMahon, M. A.S., Xu, J., Moore, J.E., Blair, I.S. and McDowell, D.A.2007. Environmental stress and antibiotic resistance in food-related pathogens. Appl. Environ. Microbiol. 73(1):211-217.

Medveďová, A. and Valík, *L. 2012. Staphylococcus aureus*: Characterisation and Quantitative Growth Description in Milk and Artisanal Raw Milk Cheese Production.In: Structure and Function of Food Engineering. Eissa, A.A. (Ed.) IntechOpen, Pp. 73. DOI: 10.5772/48175.

Montanari, C., Serrazanetti, D. I., Felis, G., Torriani, S., Tabanelli, G., Lanciotti, R., Gardini, F. 2015. New insights in thermal resistance of staphylococcal strains belonging to the species *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* and *Staphylococcus aureus.* Food Control. 50:605-612.

Nema, V., Agrawa, L R. and Ajay, K. G. 2007. Isolation and characterization of heat resistant enterotoxigenic *Staphylococcus aureus* from a food poisoning outbreak in Indian subcontinent. Int. J. Food Microbiol. 117(1): 29–35

Niveditha, N. and Sujatha, S.2015. Worrisome trends in rising minimum inhibitory concentration values of antibiotics against methicillin resistant *Staphylococcus aureus*-Insights from a tertiary care center, South India. Braz. J. Infect. Dis., 19(6):585-589.

Parente, E. and Mazzatura, A. 1991. Growth and heat resistance of *Staphylococcus aureus* in goat milk. Ital. J. Food Sci., 1: 27-37.

Scherrer, D., Corti, S., Muehlherr, J. E., Zweifel, C. and Stephan, R. 2004. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. Vet. Microbiol.101 (2):101-7.

Schleifer, K. and Bell, J.A. 2009. Family VIII Staphylocccaceae fam.nov. In: De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W, Rainey, F.A., Schleifer K. and Whitman, W.B. (Ed.), Bergey's Manual of Systematic Bacteriology - The Firmicutes,Volume Three. Second ed. Springer, New York, p. 394.

Shittu, A., Lin, J., Morrison D. and Kolawole, D. 2006. Identification and molecular characterization of mannitol salt positive, coagulase-negative staphylococci from nasal samples of medical personnel and students. J. Med. Microbiol. 55(3):317-324.

Singh, P and Prakash, A. 2012. Species Specific PCR Based Rapid Detection of *Staphylococcus aureus* from Cottage Cheese, and its Sensitivity against Antibiotics and natural products. Internet J. Food Saf. 14:.89-92

Venkatesan, P., Tirumurugaan, K.G., Nambi, A.P., Thirunavukkarasu, P.S., Kumanan, K., Vairamuthu, S. and Ramesh, S. 2014. Pattern of antibiotic resistant mastitis in dairy cows. Vet. World 7(6): 389-394

Verma, H., Rawat, S., Sharma, N., Jaiswal, V. and Singh, R. 2018. Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut. J. Entomol. Zool. Stud. 6(1): 706-709.

Walsh, C., Duffy, G., Sheridan, J.J., Fanning, S., Blair I.S. and McDowell, D.A. 2005. Thermal resistance of antibiotic resistant and antibiotic sensitive

Salmonella spp, on chicken meat. J. Food Saf. 25(4): 288-302.

Walsh, C., Sheridan, J., Duffy, G., Blair I.S., McDowell, D.A. and Harrington, D. 2001. Thermal resistance of wild-type and antibiotic-resistant *Listeria monocytogenes* in meat and potato substrates. J. Appl. Micro. 90 (4):555-560.

Yaniarti, M.N., Amarantini, C. and Budiarso, T.Y. 2017. The effect of temperature and Pasteurization time on Staphylococcus aureus isolates from dairy products.AIP Conference Proceedings 1908, 050003 (2017); doi: 10.1063/1.5012727

Zadik, P. M., Davies, S., Whittaker, S. and Mason, C. 2001. Evaluation of a new selective medium for methicillin-resistant *Staphylococcus aureus*. J Med. Microbiol. 50 (5): 476–479

Zecconi, A. and Piccinini, R. 1998. *Staphylococcus aureus*: a problem for Italian dairy herds. Bull. Int. Dairy Fed., 330: 25-26.

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