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Original Research Article

Antibiotic Sensitivity Pattern against *Staphylococcus aureus* from Raw Bovine Milk Samples, Thoothukudi District, Tamilnadu

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Abstract: The aim of this study was to isolate *Staphylococcus aureus* from samples of raw bovine milk obtained from different farms and to determine their antibiotic susceptibility patterns. A total of 144 milk samples were collected and screened for the presence of *S. aureus*. Antibiotic susceptibility was determined qualitatively using the Kirby-Bauer disc diffusion method. Piperacillin showed maximum resistance rate 62.90%, 61% isolates were resistant to Ceftazidime, 50% to Cephalothin, 48.38% to Gentamicin, 46.77% to Novobiocin, 43.54% to Amikacin and 40.32% to Cetoperazone. Only 9.67% of the isolates were found resistant to Erythromycin. *S. aureus* is normally resident in humans, therefore, the *S. aureus* present in cows may have resulted from transmission between the two species, emphasing the need to improve sanitary condition in the milking environment**.**
Keywords: Milk samples, antibiotics, disc diffusion method.

INTRODUCTION

Milk is an essential part of daily diet for the growing children and expectant mothers. Good quality milk meets the nutritional needs of the body better than any single food as it contains all essential food constituents. As a result of the presence of these nutrients, milk is an excellent culture medium for many kinds of micro-organisms. The presence and multiplication of these microorganisms in milk brings about changes in the properties of milk thus reducing it quality [1].

Raw Milk has a varied micro flora arising from several sources, such as exterior surfaces of the animal and the surface of milk handling equipment such as milking machines, pipelines and containers. Therefore, milk is susceptible to contamination by many pathogenic microorganisms, which result in infection [2]. Infection of the cow's udder has remained one of the major constraints in growth of dairy industry in India and abroad. The presence of pathogenic bacteria in milk often emerges as a major public health hazard, especially for the individuals drinking raw milk. Inappropriate therapeutic agents are frequently used to treat cattle diseases (like mastitis) without accurate diagnosis of causative agents in the herds such as checking the quality of milk and milk products. This leads to the emergence of multidrug resistant strains rendering the treatment ineffective [3].

Staphylococci are normal inhabitants of the skin and mucous membranes of animals and humans. Pathogenic strains are usually co-agulase positive and have been found to cause disease in their hosts throughout the world [4]. *Staphylococcus aureus* by far is the most frequent pathogen associated with outbreaks (85.5% of the outbreaks), followed by *Salmonella* (10.1%). Cooked food products and raw milk were most commonly contaminated with food borne pathogens and many of them were resistant to different antibiotics. Milk products are often contaminated with enterotoxigenic strains of *S. aureus* [1]. Presence of enterotoxigenic and antimicrobial resistant strains of *S. aureus* has become remarkably widespread in foods. This requires a better control of food contamination sources and distribution of antimicrobial-resistance organisms [5]. Bearing this in mind, this study was therefore aimed at; isolation and characterization of bacterial pathogen from raw milk samples and determination of the antibiotic susceptibility profiles of the isolates.

MATERIALS AND METHODS

Sample collection

Milk samples were collected from local farms in and around Coimbatore District, Tamilnadu, India. Samples of approximately 5- 10 ml of fresh milk were collected from milking containers and transferred into

sterile sample collection bottles. The samples were immediately transported on ice to the Microbiology Laboratory, RVS College of Arts and Science for analysis. Upon arrival in the Laboratory, samples were analyzed immediately.

Isolation of *S. aureus* from milk samples

Ten-fold serial dilutions were performed using 2% peptone water and aliquots of 100 µl from each dilution were spread plated onto Mannitol Salt Agar (MSA). The plates were incubated aerobically at 37 ºC for 24 h. Consequently, the *S. aureus* colonies that were golden yellow in colour from each MSA plates were further purified by subculturing onto MSA plates and the plates were incubated aerobically at 37 ºC for 24 h. These isolates were retained for further bacterial identification.

Antibiotic sensitivity profile

Antibiotic sensitivity screening of *S. aureus* was analyzed for fifteen different antimicrobial agents namely: Erythromycin (15 mcg), Ampicillin (10 mcg), Cefpodoxime (10mcg), Streptomycin (10 mcg), Levofloxacin (5 mcg), Amikacin (30 mcg), Ofloxacin (5 mcg), Tobramycin (10 mcg), Cephalothin (30 mcg), Novobiocin (30 mcg), Ceftazidime (30 mcg), Cefoperazone (75 mcg), Gentamicin (10 mcg), Cefixime (5 mcg) and Piperacillin (100 mcg).

Antibiotic Susceptibility

Antibiotic susceptibility tests were performed on all *S. aureus* isolates to determine their antibiotic-resistance profiles [6]. Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100 µL) from each isolate suspension was spread plated on Mueller Hinton agar. Susceptibilities of the isolates to a panel of fifteen different antibiotic discs (6 µm in diameter) were determined. Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37 ºC for 24 h. Inhibition zone diameters were measured and values obtained from the National Committee on Clinical Laboratory Standards were used to interpret the results obtained. *S. aureus* isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic.

RESULTS AND DISCUSSION

Antibiotic susceptibility is of great importance in monitoring food borne pathogens for their effective control in herd and in preservation of dairy products. Several animal pathogens can cause human disease and they are well known to be transmitted to humans through the consumption of raw milk. The high numbers of isolated microorganisms not only contaminate the milk, but also multiply and grow in the available media. This is due to the fact that milk is a good nutritive medium for the growth of microorganisms due to the impact of poor sanitary procedures [8], as well as lack of appropriate cooling facilities [7].

Table 1: Antibiotic sensitivity of *S. aureus* from milk samples

Antibiotic	Abb.	Disc Conc. (mcg)	Percentage susceptibility		
			Resistant	Mod. susceptible	Susceptible
Erythromycin	E	15	6 (9.67%)	2 (3.22%)	54 (87.09%)
Ampicillin	A	10	9 (14.5%)	5 (8.06%)	48 (77.41%)
Cefpodoxime	CEP	10	15 (24.19%)	11 (17.74%)	36 (58.06%)
Streptomycin	S	10	12 (19.35%)	6 (9.67%)	44 (70.96%)
Levofloxacin	LO	5	11 (17.74%)	5 (8.06%)	46 (74.19%)
Amikacin	AK	30	22 (35.48%)	9 (14.51%)	31 (50.05)
Ofloxacin	OF	5	17 (27.41%)	6 (9.67%)	39 (62.90%)
Tobramycin	TB	10	17 (27.41%)	9 (14.51%)	37 (59.67%)
Cephalothin	CH	30	31 (50.0%)	4 (6.45%)	27 (43.54%)
Novobiocin	NV	30	29 (46.77%)	8 (12.90%)	25 (40.32%)
Ceftazidime	CK	30	38 (61.29%)	6 (9.67%)	18 (29.03%)
Cefoperazone	CS	75	25 (40.32%)	18 (29.03%)	19 (30.64%)
Gentamicin	G	10	30 (48.38%)	11 (17.74%)	21 (33.87%)
Cefixime	CFX	5	27 (43.54%)	5 (8.06%)	30 (48.38%)
Piperacillin	PC	100	39 (62.90%)	7 (11.29%)	16 (25.80%)

A total of 144 milk samples were analyzed and were all positive for *S. aureus*. A total of 144 potential isolates were subcultured and further analyzed. However, only 62 isolates satisfied all the identification criteria and were used for subsequent analysis. These constituted a total 62 *S. aureus* isolates. All the 62 *S. aureus* isolates were subjected to antibiotic susceptibility tests. Fifteen antimicrobial agents, from different antibiotic classes were used. A summary of the percentage of *S. aureus* that were resistant to these antibiotics is provided in table 1 and fig1. According to the results of antibiotic sensitivity of *Staphylococcus aureus*, 87.09% organisms were susceptible to erythromycin. Only 3.22% was moderately susceptible

and 9.67% resistant to erythromycin. The susceptibility of the other antibiotics in decreasing order against *Staphylococcus aureus* was found to be ampicillin (77.41%), levofloxacin (74.19%), streptomycin (70.96%), Ofloxacin (62.90%), tobramycin (59.67%), cefpodoxime (58.06%), amikacin (50.05%), cefixime (48.38%), cephalothin (43.54%), novobiocin (40.32%),gentamycin (33.87%), cetoperazone (30.64%) and ceftazidime (29.03). Whereas resistance pattern of the peperacillin, ceftazidime, cephalothin, gentamycin, novobiocin, cefixime, cetoperazone, amikacin, ofloxacin & tobramycin, Cefpodoxime, Streptomycin, Levofloxacin and erythromycin was in decreasing order 62.90%, 61.29%,50.0%,48.38%, 47.77%, 43.54%,40.32%, 35,48%, 27.41% 24.19%,,19.35, 17.74 & 9.67% isolates respectively (Table 1).

Hassan *et al.* studied erythromycin and tetracycline as effective antibiotics against *Staphylococcus aureus.* Shoemaker and Yow also reported similar findings against *Staphylococcus aureus* isolates when large doses of erythromycin were given intravenously. The results of the present study are similar to those of above workers. Moreover, when low doses of antibiotics were used against bacteria, they inhibit the growth of susceptible bacteria and leave smaller number of already resistant bacteria, which thrive and grow. These bacteria spread their resistance traits to other previously non-resistant cells than eventually affecting other cells [9].

The present study put forth, shows that more efforts are needed to enhance and promote farms and sale points of milk by following confirmatory tests to check the microbial quality of the milk. Moreover, the concerned ministries should adopt a comprehensive strategy for ensuring a safe supply of good quality milk. These strategies should include promoting the knowledge of farmer's standard through training, extension programs, adoption of grading and quality testing of milk. Ultimately, the milk testing programs should become components of the quality process that should focus on production of high quality milk, not only at the preservation and supply level, but also at the production herd level.

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