

Study on the Prevalence, Incidence, Drug sensitivity and Extra Chromosomal Impact on Bovine Mastitis Pathogens

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Abstract: India is the highest milk producer in the world, but the per capita availability of milk still remains half of the world average. One of the reasons for low productivity is poor animal health, particularly mastitis, which is single largest problem in dairy animal in terms of economical losses in India. Mastitis destroys the milk secreting cells. Scar or connective tissue replaces the milk secreting tissue, resulting in a permanent loss of productive ability. Mastitis is a multi-etiological disease wherein about 95 % of reported cases are caused by *Staphylococcus*. In this study plasmid DNA of the strain was isolated and the antibiotic sensitivity was done for strain with plasmid and without plasmid in order to found genetic impact of bovine mastitis.

Keywords – antibiotic sensitivity, bovine mastitis, plasmid curing, pathogen, *Staphylococcus*.

I. Introduction

Mastitis is a multi-etiological disease of dairy animals, causing heavy economical losses to the dairy industry all over the globe. It affects 50-55% of the herd population and approximately 70% of all over avoidable losses incurred during milk production [1]. Mastitis also called Dairyman's grief in either the acute or the chronic form, remains a major cause of economic loss throughout the world. More exact definition of the type of mastitis depends on the identification of the causative agent whether it is physical or infectious [2]. Certain udder characteristics, breeds, teat injuries, poor hygiene, poor management, faulty milking machines, accumulation of milk and the presence of bacteria in or around the udder are all factors which predispose cows to mastitis [3]. About 90 % of mastitis cases in the national herd were caused by *Streptococcus agalactiae* and *Staphylococcus aureus* infections, which were spread from cow to cow at milking time [4].

Staphylococcus aureus is probably the most common etiological agent that causes contagious mastitis. An old adage holds that "once a Staph. Cow, always a Staph. Cow"[5]. Cows are most susceptible to infection during the transition from lactation involution and from involution to colostrogenesis[6]. Sub-clinical mastitis is found more prevalence in India varying from 10-50% in cows and 5-10% in buffaloes than clinical mastitis (1-10%). The incidence was highest in purebred Holsteins and Jerseys and lowest in local cattle and buffaloes [7].

II. Materials and Methods

A total of 13 dairy farms were visited and 40 lactating cows were examined for sub clinical mastitis. The presence of inflammatory signs in udder such as heat pain, swelling, edema and asymmetry of shape and size of quarters and physical changes in milk such as flakes/clots, discoloration and consistency were the criteria for the inclusion of the animals in study. All the dairy farms chosen in the study were situated 50km in and around Coimbatore.

Uniform breed of 40 cows were selected, out of which 11 affected cows were identified, milk samples collected, cooled and immediately transported to the laboratory within 2 hours. The microscopic, biochemical and enzymatic characterization studies were done for the confirmation of colonies. The culture was used for the antibiotic sensitivity test, plasmid DNA isolation and plasmid curing to study the drug sensitivity and extra chromosomal impact on bovine mastitis pathogens.

2.1 Isolation of Plasmid DNA

The plasmid DNA was isolated from the isolated culture by following Birnboim, H.C. and Doly, J. (1979) [8] method.

2.2 Plasmid Curing

Plasmid curing was carried out by following Silhavy, Berman and Enquist, (1984) [9] method.

2.3 Antibiotic Sensitivity Test

The antibiotic sensitivity tests were done by the disc diffusion method following Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M.(1996) [10] method.

III. Results and Discussion

3.1 Prevalence and incidence of bovine mastitis in Coimbatore district

A total of 28 pathogenic microorganisms were isolated from 11 milk samples collected in this study, which determines the prevalence of bacterial mastitis among the dairy cows of Coimbatore district. The pathogens isolated were listed (Table 1). The incidence of *S.aureus* (60.71%) was found to be the major causatives in the field, followed by strains of coagulase negative *staphylococci* (14.28%), *Escherichia coli* (10.71%), *streptococcus sp.*, (10.71%) and other unidentified species 1 (3.57%).

Table .1 Numbers and the prevalence of microorganism from bovine mastitis milk samples

Bacterial Strains	No of Isolates	Percentage (%)
<i>Staphylococcus aureus</i>	17	60.71
Coagulase negative <i>Staphylococcus</i>	4	14.28
<i>Escherichia coli</i>	3	10.71
<i>Sterptococcus sp</i>	3	10.71
Others	1	3.57

3.2 Isolation of Plasmid DNA

In this study the plasmid DNA was isolated from the *S.aureus* and visualized by agarose gel electrophoresis.

3.3 Antibiotic sensitivity test

The antibiotic sensitivity test was done for the sample strains with and without plasmid (Table 2). This study conclude that the plasmid strains shows high resistant where as plasmid cured strains shows high sensitivity to antibiotics, which proves that antibiotic resistance of the pathogen present in the plasmid DNA but not in a chromosomal DNA.

Table: 2 Antibiotic sensitivity tests with regards to presence or absence of plasmids

Antibiotics	<i>Staphylococcus aureus</i> with plasmid n=17				<i>Staphylococcus aureus</i> without plasmid n=17			
	Resistant		Sensitive		Resistant		Sensitive	
	n	%	n	%	n	%	n	%
Ampicillin	11	64.7	6	35.3	11	64.7	6	35.3
Amoxicillin	11	64.7	6	35.3	4	23.5	13	76.5
Chloraphenicol	2	11.8	15	88.2	3	17.6	14	82.4
Gentamycin	6	35.3	11	64.7	3	17.6	14	82.4
Vancomycin	12	70.6	5	29.4	12	70.6	5	29.4
Tetracycline	5	29.4	12	70.6	4	23.5	13	76.5
Ciprofloxacin	12	70.6	5	29.4	5	29.4	12	70.6
Ceftazidine	5	29.4	12	70.6	2	11.8	15	88.2
Cephalothin	11	64.7	6	35.3	3	17.6	14	82.4
Kanamycin	15	88.2	2	11.8	2	11.8	15	88.2
Streptomycin	6	35.3	11	64.7	1	5.9	16	94.1

IV. Conclusion

It has been reported in several studies that *S.aureus* is a major microorganism isolated from mastitis milk and infected mammary glands of cows. In our study, *S.aureus* was found to be the most prevalent pathogen (60.71%). Since the majority of the curd plasmid strains show increase in antibiotic

sensitivity than the strain with plasmid, hence it was found and that the antibiotic resistance is due to the genes present in the plasmid DNA

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