Study on drug susceptibility and bovine Hb using ELISA on bovine mastitis pathogens

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ABSTRACT:- Mastitis can be caused by a large variety of pathogens, some are infectious at the host level, and others are infectious at both host and population level. The aim of the present study was to to evaluate the drug sensitivity pattern of the isolated culture and Bovine milk Hp concentrations determination using a commercial bovine Hp ELISA kit. Milk samples were obtained with consent from the cows of different small holder dairy farms around Coimbatore District. The entire sample was collected according to the Scandinavian recommendation on examination of quarter milk samples and National Mastitis Council. The samples were cultured for the growth and isolation of microbes. Then the bacterial isolates subjected to biochemical tests for confirmation. The isolates were tested invitro for their antimicrobial susceptibility by agar disk diffusion method. Minimum inhibitory concentration (MIC) was analyzed for specific treatment and the Bovine Hp antibody used to determine the concentration of cell count presence in the milk sample.

Keywords:- Bovine mastitis, Bovine Hb, antibiotic resistance, MIC

I. INTRODUCTION

Mastitis is classified as a multi-etiological disease among the dairy animals which fetch considerable economic losses to the dairy industry throughout the world. Around 50 % of the herd populations of 70% of all available losses were observed in overall milk products (Sumathi et al., 2008).The factors which predispose cows to mastitis are presence ofbacteria in and around the udder, poor hygiene, accumulation of milk, improper milking machines, breeds and injuries in teat are the factors which predispose cows to mastitis (Amehet al., 1993 and Egwuet al., 1999). The udder infection can be contracted at different ages and also at different stages of the lactation cycle. The ability to overcome an infection after the established of the same also varies (Klastrupet al., 1987). A broad spectrum of pathogens are identified as causatives for mastitis, the infection level can be classified as in the host level, population level and in some microorganism it is at both the level.

II. MATERIALS AND METHODS

The selection of dairy herds and aseptic sampling procedures will be followed according to the method described by Barkemaet al., 1998.

2.1 Milk Sample Collection

Milk samples were obtained with consent from the cows of different small holder dairy farms around Coimbatore District (Table-2.1). The entire sample was collected according to the Scandinivian recommendation on examination of quarter milk samples and National Mastitis Council (Medison, Laboratory Handbook on Bovine Mastitis, NMC, 1999). The protocol was permitted by the owners of the dairy farms under investigation. All efforts were made to minimize animal suffering.

Table 2.1: Sampling places from Coimbatore Dist.

S.No	Sampling Place	Latitude and Longitude	No. of Sample (SD)
1	Kannampalayam	$11^{o}00'30.00''$N $77^{o}06'08.00''$E	12±0.8165
2	Vellalur	$10^{o}58'02.06''$N $77^{o}01'40.00''$E	10±0.5773
3	Kodangipalayam	$10^{o}47'27.06''$N $77^{o}02'08.08''$E	10±0.7071
4	Karadivavi	$10^{o}57'57.44''$N $77^{o}11'51.65''$E	15±1.2247
5	Pappampatti	$10^{o}57'34.30''$N $77^{o}06'06.38''$E	13±1.2583
6	Malumichampatti	$10^{o}54'06.32''$N $76^{o}59'45.55''$E	10±0.5773
7	Saravanampatti	$11^{o}05'24.00''$N $76^{o}59'52.00''$E	15±0.5477
8	Somanur	$11^{o}05'13.43''$N $77^{o}11'09.24''$E	12±1.1401
9	Kinthukadavu	$10^{o}49'00.00''$N $77^{o}01'00.00''$E	15±1.2247
10	Pollachi	$10^{o}39'17.52''$N $76^{o}59'22.27''$E	15±1.4142
11	Mettupalayam	$11^{o}17'57.14''$N $76^{o}55'29.26''$E	15±0.7071
12	Karamadai	$11^{o}14'31.68''$N $76^{o}57'10.09''$E	13±0.8165
		Total No. of Samples	**155±2.1087**

2.2 Identification of Bovine Mastitis Pathogen

The samples were cultured on Nutrient agar, Blood agar and MacConkey agar plates, supporting the growth of various types of bacteria for this study and isolation. The isolated bacteria were identified on the basis of their cultural and morphological characteristics. The pure cultures of bacterial isolates were obtained by sub culturing on differential and selective media. The bacterial isolates further subjected to biochemical tests for confirmation.

2.3 Antibiotics Sensitive Test

The isolates were tested invitro for their antimicrobial susceptibility by agar disk diffusion method in accordance with the standard in National Mastitis Council guidelines. Minimum inhibitory concentration (MIC) of 11 different antibiotics, ampicillin (10μg), Amoxicillin (10μg) Chloramphenicol (10μg), Gentamycin (10μg), Vancomycin (10μg), Tetracyclin (10μg), Ciprofloxacin (10μg), Ceftazidine (10μg), Cephalothin (10μg), Kanamycin (10μg), and streptomycin (10μg) was analyzed to suggest specific treatment.

2.4 Determination of milk Hp levels using ELISA

Freshly raw milk obtained from sampling places and immediately centrifuged. The top layer in the supernatant was carefully removed and remaining fractions (whey) used for Hp level analysis. Bovine milk Hp concentrations were determined using a commercial bovine Hp ELISA kit using ELISA,Thermo Fisher Scientific India Pvt. Ltd, India.(Chen et al.2006).

III.   RESULTS AND DISSCUSSION

A total of 155 milk samples were collected for clinical and subclinical cases of mastitis from twelve different places in and around Coimbatore district. From each place, minimum of ten and maximum of 15 milk samples were collected. In all the milk samples, we have identified the presence of different pathogenic bacterias such as 240 number of E.coli sp., 431 of Staphylococcus aureus, 157 of Streptococcus, 321 of Bacillus sp., and40 ofY.enterocolitica sp.(Table-3.2). Major bacterial isolates were Staphylococcus aureus (36.2%), Bacillus sp. (27.0%),E.coli (20.2%), Streptococcus sp. (13.2%), and Yerseniaenterocolitica (3.4%) (Table-3.3). The maximum bacterial load identified from Vellalur, Karadivavi, Saravanampatti and Karamadai area samples. The Haptoglobin (Hp) is an acute phase protein responsive to inflammation and infection. One of the major functions of Hp is to capture released hemoglobin during excessive hemolysis. Even though Bovine Hp is not abundantly expressed in normal plasma but it is considered to be one of the sensitive acute phase proteins during bacterial infection. The present study was confirming the Bovine Mastitis microbial load in quantitative and qualitative manner. The Bovine Hp antibody used to determine the concentration of cell count presence in the milk sample by using ELISA. The high level of cells count presence in Karamadai 2.568±0.275μg/ml, Saravanampatti 2.506±0.375μg/ml, Karadivavi 2.284±0.290μg/ml and Vellalur 2.106±0.313μg/ml.(Table-3.4) We find an antibiotic resistant and sensitive strains from the entire microbial load. The antibiotic activity of gentamycin shown sensitivity of 96.2% and streptomycin was 94.5%. The mastitis pathogenic organism is highly sensitive to Gentamycin and Streptomycin where as other antibiotics are more resistant to E.coli and S.aureus (Table-3.5).

Table 3.2: Number of Isolation of Pathogenic bacterial species from Mastitis Milk sample- Coimbatore Dist.

S.No	Sampling place (No.of Sample)	E.coli (SD)	Staphylococcus aureus (SD)	Streptococcus sp. (SD)	Bacillus sp. (SD)	Y.enterocolitica (SD)
1	Kannampalayam (12)	12±0.127	23±0.735	17±0.628	19±1.783	04±0.100
2	Vellalur (10)	18±0.352	48±1.008	14±0.332	38±1.654	07±0.121
3	Kodangipalayam (10)	19±0.120	33±1.112	11±0.197	27±1.931	Nil
4	Karadivavi (15)	25±0.187	52±0.937	14±0.188	40±2.101	09±0.137
5	Pappampatti (13)	22±0.410	25±1.203	10±0.273	25±1.772	03±0.100
6	Malumichampatti (10)	20±0.732	18±1.325	08±0.110	19±1.529	Nil
7	Saravanampatti (15)	23±0.198	65±1.098	16±0.784	34±1.782	06±0.112
8	Somanur (12)	20±0.110	27±0.999	19±0.931	27±1.900	04±0.120
9	Kinathukadavu (15)	17±0.387	21±0.774	12±0.320	18±1.287	02±0.100
10	Pollachi (15)	23±0.192	25±0.536	10±0.298	25±1.672	Nil
11	Mettupalayam (15)	20±0.112	31±0.839	14±0.349	22±1.375	Nil
12	Karamadai (13)	21±0.287	63±0.927	12±0.278	27±1.830	05±0.163
	Total	240	431	157	321	40

Table 3.3: Total Number and Percentage of Bacterial isolates.

S.No	Bacterial Species	No. of Isolates	% of isolates
1.	E.coli	240±3.384	20.2
2	Staphylococcus aureus	431±16.637	36.2
3	Streptococcus sp	157±3.203	13.2
4	Bacillus sp	321±7.262	27.0
5	Yerseniaenterocolitica	40±3.055	3.4
	Total	1189	100

Table-3.4: Haptoglobin (Hp) concentration of isolated Bovine milk samples.

S.No	Sampling Places	No. of Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total (S.D)
1	Kannampalayam	12	0.831	0.638	0.742	0.837	0.801	0.471	0.524	0.236	0.811	0.728	0.671	0.649	----	----	----	0.662 ±0.178
2	Vellalur	10	1.719	1.693	2.714	2.325	1.998	2.124	1.837	2.112	2.317	2.225	----	----	----	----	----	2.106±0.313
3	Kodangipalayam	10	0.237	0.315	0.216	0.299	0.312	0.327	0.286	0.274	0.301	0.278	----	----	----	----	----	0.285±0.035
4	Karadivavi	15	1.897	2.013	2.564	2.327	2.784	2.327	2.428	2.333	2.569	1.872	1.977	2.314	2.717	2.625	2.542	2.284±0.290
5	Pappampatti	13	0.542	0.616	0.471	0.529	0.587	0.611	0.627	0.588	0.421	0.439	0.498	0.52	0.399	----	----	0.537±0.070
6	Malumichampatti	10	0.322	0.299	0.271	0.289	0.311	0.302	0.277	0.278	0.314	0.289	----	----	----	----	----	0.295±0.017
7	Saravanampatti	15	1.924	1.735	2.901	2.873	2.801	2.542	2.421	2.338	2.367	2.593	2.709	2.872	2.638	2.479	2.118	2.506±0.375
8	Somanur	12	0.389	0.364	0.331	0.327	0.316	0.297	0.267	0.311	0.302	0.322	0.328	0.289	----	----	----	0.320±0.033
9	Kinathukadavu	15	0.225	0.229	0.283	0.255	0.267	0.291	0.283	0.311	0.221	0.197	0.273	0.299	0.275	0.301	0.269	0.261±0.036
10	Pollachi	15	0.544	0.521	0.632	0.598	0.562	0.611	0.618	0.615	0.471	0.479	0.328	0.379	0.428	0.473	0.459	0.530±0.099
11	Mettupalayam	15	0.225	0.274	0.293	0.428	0.399	0.421	0.401	0.297	0.283	0.294	0.422	0.385	0.368	0.399	0.343	0.344±0.072
12	Karamadai	13	1.987	2.342	2.934	2.991	2.732	2.543	2.463	2.725	2.577	2.632	2.578	2.314	2.714	2.634	2.581	2.568±0.275

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