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PREVALENCE AND IN- VITRO ANTIBIOGRAM PATTERN OF DIFFERENT ANTIBIOTICS AGAINST MAJOR BOVINE MASTITIS PATHOGENS

M. JAGADEESWARI[1], DR.N.HEMASHENPAGAM[2], R.DHANABALAN[1], K. MEKALA[1]

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1. Department of Microbiology, Rathnavel Subramaniam College of Arts & Science, Coimbatore, Tamilnadu, India.
2. Department of Microbiology, Hindusthan College of Arts &Science, Coimbatore, Tamil Nadu, India.

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Corresponding Author
Mrs. M. Jagadeeswari

Abstract

Dairy milk samples from normal and mastitis cows were collected from Coimbatore district and subjected to bacterial isolation and antimicrobial susceptibility test by disc diffusion method. Staphylococcus aureuswas found to be the predominant isolate causing 61.11 % of clinical and subclinical mastitis cases, followed by coagulase negative Staphylococci .S.aureus shows high rate of beta lactamase activity and resistant to beta-lactam antibiotics like Ampicillin, amoxicillin, Methicillin, Co-trimoxazole, Vancomycin and Cephotaxime. Gentamycin was found to be the most effective antibiotic in treating bovine mastitis cows.

Introduction:

Bovine mastitis is a parenchymal inflammation of the mammary gland that affects large number of dairy cattle throughout the world. The infection cause heavy economic loss to the dairy industry due to reduced milk yield and also affects the quality of the milk. Losses due to subclinical mastitis are more severe than those due to clinical mastitis. It is multi-etiological disease caused by both contagious and environmental pathogens. The antibiotic treatment may help in minimizing the losses and sometimes paves to drug resistance.

The objective of thepresent study wasto isolate mastitis causatives from normal, subclinical and clinical bovine mastitis dairy herds from Coimbatore district and to study the antibiogram pattern of *Staphylococcus aureus* and Coagulase negative *S.aureus* (CNS) isolated.

Materials and Method:

Selection of sampling area and dairy herds:

In the present study, thirty two villages around south zone of Coimbatore district were selected as sampling area. Small and large holder dairy farms were used for sampling. The cow breeds in the dairy farms selected were Jersey, Holstein Friesian, Zebu and Cross breeds.

Milk Sampling:

Prior to milk sampling, the previous antibiotic treatment and mastitis history of the cows in the herds were collected from the dairy owners. The physical appearance of the udder in selected cows were carefully monitored by palpation and noticed for any injuries in the teat and udder skin. Five ml screw capped polystyrene containers (Hi media) were used for sampling. The containers were labeled accordingly to identify the cows for the normal, clinical and subclinical mastitis status. The teats were cleansed with cotton, drenched with nonirritantchlorohexidine and few ml of milk samples were drawn out freely from the teat and followed by collection of 4 ml milk in the container. The samples were stored in the ice kit and transported to the microbiology laboratory. About 260 clinical mastitis and 750 normal milk samples were collected from the cow breeds selected and were analyzed for the presence of bacterial pathogens.

Microbiological analysis:

In the laboratory, the milk samples were shifted to room temperature for ten minutes and processed. In brief, one ml sample was twofold serially diluted and a loopful of each dilution was inoculated on blood agar and MacConkey agar (Hi media) plates, which were divided into four sections, a total of 6-8 lines were made in one agar section by turning the loop once between the streaking lines. Each milk sample (10µl) was thus streaked in one section of the plate as a line culture. Samples were cultured under aerobic conditions for 24 - 48 hrs at 37^{o}C and examined for bacterial growth. When growth was not observed after 48 hour incubation, the milk sample was inoculated into an enriched trypticase soya broth. In the cases of mixed growth, a new sample was taken and re-examined. Samples with unspecified mixed cultures were considered contaminated and excluded from subsequent analysis. Pure cultures were further examined for identification by cultural, staining and biochemical characteristics according to standard microbiological technique. Staphylococci were identified by gram staining reaction, growth characteristics on mannitol salt agar and blood agar, catalase, coagulase and gelatin liquefaction tests. Streptococci were identified by gram staining reaction, growth characteristics on blood agar medium, catalase and esculin hydrolysis. Gram negative isolates were identified by gram staining reaction, growth characteristics on MacConkey agar medium, sugar fermentation tests, IMViC test, urease, catalase and oxidase test (Quinn, 1999).

Antibiotic Sensitivity testing:

Antibiotic sensitivity was determined for 168 *S.aureus* and 52 CNS strains by disk diffusion method (Bauer *et al.,* 1966) on Muller Hinton agar plates. An overnight broth culture of milk isolated *Staphylococcus aureus* and CNS strains was standardized by McFarland' method (NCCLS, 1991). In brief, an aliquot (0.2 ml) of McFarland's standardized tube no.0.5 pure culture of each isolate previously incubated at 37^{o} Cfor 6- 8 hours in trypticase soya broth was transferred on to the Mueller Hinton agar and lawn culture was prepared using sterile cotton swabs. After drying for 2-3 minutes, the antibiotic discs were placed on the agar plates. The

plates were incubated at 37^{o} C for 24 hours. The zone of growth inhibition for each antibiotic discwas measured in mm. The sensitivity or resistances to the antibiotics were determined by comparing with standard chart (Hi Media).

Antibiotics used:

The antibiotic discs used in the study were Amoxicillin Ac (20mcg), Ampicillin A (10 mcg), CarbencillinCb (100mcg), Cefuroxime Ce (10mcg), CephalothinCh (30 mcg), CefotaximeCe (10mcg), Chloramphenicol C (30 mcg), Ciprofloxacin Cf (30 mcg), Clindamycin Cd (10mcg), CloxacillinCx (5 mcg), Co-trimoxazole Co (25 mcg), Erythromycin E (10 mcg), Gentamycin G (10 mcg), Kanamycin K (30 mcg), Methicillin M (30 mcg), NorfloxacinNx (10 mcg), Ofloxacin Of (5mcg), Penicillin P (10 units), Streptomycin S (25 mcg), Tetracycline T (10mcg), and VancomycinVa (10 mcg).

Testing for Beta lactamase activity:

Beta lactamase production was used to differentiate the beta lactamase producing staphylococci from other Staphylococci. The penicillin resistant strains were considered as beta lactamase producers. The strains susceptible to penicillin and its derivatives were classified as non-betalactamase producing Staphylococci.(Anon 1995, Jones and Health 1985).

Results and Discussion:

About 360 pathogenic bacteria (5 genera) were isolated from 1010 milk samples collected from normal and clinical mastitis cases in the study, which determined the prevalence of bacterial mastitis among the dairy cows of 32 farms in Coimbatore district. When the normal milk samples were confirmed with bacterial isolates it was called as subclinical status.The *S.aureus* was found to be the dominating pathogen(220 isolates)accounting for 61.1 % in the samples in selected province. The number of isolates and specificpathogens are listed in the Table: **1.**It was found that the major causative agent of bovine mastitis in the selected villages of Coimbatore district was found to be the *S.aureus*, followed by strains of coagulase negative S*taphylococci*, *Escherichia coli, Streptococcus sp.,Proteus sp.,* and *Pseudomonas sp.,***Table 2**

A total of 168*S.aureus* strains (Jersey - 55, HF -55, Zebu – 4 and cross breed – 54) and

52 strains of coagulase negative Staphylococci (Jersey – 10, HF – 15, Zebu – 3 and cross breed – 24) were tested for antibiotic susceptibility test by Kirby- Bauer method.(Table.3 and Table.4).Out of 168 *S.aureus* isolates, 110 (65.48%) were positive and 58 (34.52 %) were negative Beta-lactamase strains respectively. Among 52 CNS isolates 31 were positive and 21 were found to be Beta-lactamase negative respectively (Table 5).

Farming and dairy maintenance are the major economy in the villages of Coimbatore, Tamilnadu. The daily income of farmers always depends on their healthy cows with high yield. The present study was undertaken to investigate actual status in dairy farms for their sterile milk production. In the study both normal and clinical mastitis milk samples were collected for the analysis along with the information of previous mastitis history. All the breeds were monitored and samples were processed with care. The results revealed that the major pathogen was *Staphylococcus aureus*, followed by CNS, *E.coli, Strptococcus sp., proteus sp*., and *Pseudomonas sp*. The normal milk sample when found to be microbial positive was considered as subclinical in the study.It has been reported in many studies that *S.aureus* is a major bacteria isolated from mastitis milk (Francis and Carroll 1986, NazerandTavakoli 1994, Waage et al.1999, Watts 1988).

In our study also, *S aureus* was found to be the most prevalent pathogen. Many reported the presence of Beta-lactamase activity in *S.aureus* at different rates ranging between 23% and 64%. In our study, 65.48 % of *S aureus* isolates and 59.62 % of coagulase negative staphylococci revealed Beta-lactamase activity. The resistance/susceptibility of beta-lactamase producing Staphylococci to Beta-lactam antibiotics has been investigated by several research teams (Craven *et al.,* 1986; De Oliveira *et al.,* 2000; Francis and carol, 1986;Hadimli*et al.,* 2001; Jones and Heath 1985;Sezen*et al.,* 1986;Waats and Salmon 1997) reported penicillin was the most affective antibiotic. Most of the studies worldwide revealed the occurrence of penicillin resistance among S.aureus and CNS isolated from bovine mastitis cases. In our study, S.aureus and CNS show resistant to penicillin, 80% and 83% respectively. A similar report was observed in Denmark

36% (Aarestrupet al., 1995) and in Finland 37% (Myllys et al 1998) for CNS.S.aureus and CNS exhibit greater sensitivity to gentamycin and was similar to the results obtained for S.aureus in

Argentina (Gentilini*et al.,* 2000)

To conclude, *S.aureus* is the most prevalent pathogen causing mastitis in cows in the Coimbatore province. Gentamycin was found to be the most effective antibiotic. High rate of beta-lactamase activity was found in the Staphylococcal isolates which are responsible for the development of β-lactam resistance emergence among the dairy herds.

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Table 1 Number of clinical and subclinical bacterial isolates from the milk samples.

Cow breeds	Clinical		Sub clinical		Total	
	Sample No.	Isolates	Sample No.	Isolates	Sample No.	Isolates
Jersey	58	54	102	36	160	90
Holstein Friesian	80	76	123	51	203	127
Zebu	12	8	25	3	37	11
Total	260	240	750	120	1010	360

Table 2 Bacterial isolates from clinical and subclinical milk samples.

Bacterial isolates	Jersy		Holstein Friesian		Zebu		Cross breed		Total	%
	Clinical (58)	Sub Clinical (102)	Clinical (80)	Sub Clinical (123)	Clinical (12)	Sub Clinical (25)	Clinical (110)	Sub Clinical (500)	(1010)	
S. aureus	45	20	57	30	3	1	52	12	220	61.11
CNS	3	8	7	8	2	1	18	7	54	15.00
Streptococci	2	-	3	-	1	-	20	5	31	08.61
E.coli	3	8	8	13	2	1	11	6	52	14.44
Proteus sp.,	-	-	1	-	-	-	1	-	2	0.56
Pseudomonas sp.,	-	-	-	-	1	-	-	-	1	0.28

Table.3. Susceptibility of *S.aureus* from Clinical and Subclinical Bovine Mastitis milk samples to commercially available Antibiotics:

Antibiotics	*Staphylococcus aureus*							
	Beta lactamase positive(110)				Beta lactamase negative (58)			
	Resistant		Sensitive		Resistant		Sensitive	
	No	%	No	%	No	%	No	%
Amoxicillin	78	70.91	32	29.09	8	13.79	50	86.21
Ampicillin	82	74.55	28	25.45	5	8.62	53	91.38
Carbencillin	44	40.0	66	60.00	3	5.17	55	94.83
Cefuroxime	47	42.73	63	57.27	4	6.9	54	93.1
Cephalothin	51	46.36	59	53.64	14	24.14	44	75.86
Cefotaxime	72	65.45	38	34.55	15	25.86	43	74.14
Chloramphenicol	45	40.91	65	59.09	3	5.17	55	94.83
Ciprofloxacin	27	24.55	83	75.45	2	3.45	56	96.55
Clindamycin	61	55.45	49	44.55	9	15.52	49	84.48
Cloxacillin	50	45.45	60	54.55	7	12.07	51	87.93
Co-trimoxazole	43	39.09	67	60.91	2	3.45	56	96.55
Erythromycin	31	28.18	79	71.82	4	6.9	54	93.1
Gentamycin	21	19.09	89	80.91	1	1.72	57	98.28
Kanamycin	32	29.09	78	70.91	6	10.34	52	89.66
Methicillin	92	83.64	18	16.36	20	34.48	38	65.52
Norfloxacin	48	43.64	62	56.36	9	15.52	49	84.48
Ofloxacin	30	27.27	80	72.72	2	3.45	56	96.55
Penicillin	88	80.00	22	20.00	18	13.79	40	68.97
Streptomycin	30	27.27	80	72.73	16	27.59	42	72.41
Tetracycline	22	20.00	88	80.00	12	20.69	46	79.31
Vancomycin	39	35.45	71	64.55	5	8.62	53	91.38

Table 4. Susceptibility of Coagulase negative Staphylococci from Clinical and Subclinical Bovine Mastitis Milk samples to commercially available Antibiotics:

Antibiotics	Coagulase negative *Staphylococci*							
	Beta lactamase positive (31)				Beta lactamase negative (21)			
	Resistant		Sensitive		Resistant		Sensitive	
	No	%	No	%	No	%	No	%
Amoxicillin	15	48.39	16	51.61	15	71.43	6	28.57
Ampicillin	2	6.45	29	93.55	6	28.57	15	71.43
Carbencillin	21	67.79	10	32.26	8	38.1	13	61.9
Cefuroxime	13	41.94	18	58.06	9	42.86	12	57.14
Cephalothin	12	38.71	19	61.29	8	38.7	13	61.90
Cefotaxime	7	22.58	24	77.42	4	19.05	17	80.95
Chloramphenicol	14	45.16	17	54.84	3	14.29	18	85.71
Ciprofloxacin	7	22.58	14	45.16	2	9.52	19	90.48
Clindamycin	6	19.35	25	80.65	3	14.29	18	85.71
Cloxacillin	3	9.68	28	90.32	6	28.57	15	71.43
Co-trimoxazole	10	32.26	21	67.74	7	33.33	14	66.66
Erythromycin	13	9.68	28	90.32	5	23.81	16	76.19
Gentamycin	1	3.23	30	96.77	1	4.76	20	95.24
Kanamycin	20	64.52	11	35.48	18	85.71	3	14.29
Methicillin	10	32.26	21	67.74	13	61.9	8	38.1
Norfloxacin	7	22.58	24	77.42	17	80.95	4	19.05
Ofloxacin	4	12.9	27	87.1	4	19.05	17	80.95
Penicillin	26	83.87	5	16.13	12	57.14	9	42.86
Streptomycin	20	64.52	11	35.48	10	47.62	11	52.38
Tetracycline	10	32.26	21	67.74	9	42.86	12	57.14
Vancomycin	5	16.13	26	83.87	3	14.29	18	85.71

Table 5 Beta-lactamase test results for *S aureus*. and Coagulase negative Staphylococci.

Cow breeds	*S. aureus* (168)				Coagulase negative staphylococci (52)			
	Beta-lactamase				Beta- lactamase			
	positive	%	Negative	%	positive	%	Negative	%
Jersey	35	63.4	20	36.36	8	25.81	2	9.52
Holstein Friesian	40	72.72	15	27.27	10	32.26	5	23.81
Zebu	3	0.75	1	0.25	2	6.45	1	4.76
Cross breed	32	59.26	22	40.74	11	35.48	13	61.9
Total	110	65.48	58	34.52	31	59.62	21	40.38

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