Current trend of drug sensitivity in bovine mastitis

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Abstract

The study was conducted on 190 milk samples of bovine mastitis and 138 samples were confirmed positives for microorganisms. All the 138 samples were subjected to drug sensitivity test. The most effective antibiotic was enrofloxacin (91.67%) followed by ciprofloxacin (90.15%), amikacin (87.12%), ceftriaxone (84.10%), chloramphenicol (80.31%), cefotaxime (79.55%) and gentamicin (77.27%). Microorganisms were mostly resistant to drugs like streptomycin, penicillinG, ampicillin, cloxacillin, amoxycillin and neomycin in increasing order of resistance. Hence, it is suggested that the line of treatment should be based on antibiogram study of various isolates from bovine mastitis. Further, the selection of drugs after culture and sensitivity test should be based on their ability to cross blood tissue barrier or mammary parenchyma, lipophilicity and ability to work in alkaline pH.

Key words: bovine mastitis, drug sensitivity, microorganism, pH, lipid solubility.

Introduction

Mastitis is inflammation of the parenchyma of mammary gland, characterized by physical, chemical and bacteriological changes in milk and pathological changes in glandular tissues (16). An annual economic loss of over Rs. 6000 crore due to mastitis has been recorded, of this, Rs. 1700 crore are lost due to clinical mastitis and Rs. 4300 crore due to sub clinical mastitis (7). The situation has been compounded by the continued indiscriminate use of antibiotics, defying the rational approach of selection of suitable antibiotics after culture and sensitivity test of milk. This may be attributed to callous approach of the dairy farmers, who instead of consulting qualified veterinarian, prefer to take over the counter supply of medicine by the drug retailers. Veterinarians who do not capitalize on the available diagnostic tests are no less responsible for increase in the incidence of mastitis. Apart from antibiotic sensitivity testing, other parameters, which affect the efficacy of drugs in mastitis such as pH of milk, presence of lipid vacuole or reticulin fibres, are also important.

Therefore, the present study was undertaken to study drug sensitivity as well as change in pH, presence of lipid vacuole & reticulin in milk for selection of suitable drug for treatment of bovine mastitis.

Materials and methods

The specimen for the present research work comprised of milk samples obtained from clinical cases of bovine mastitis from dairy farms of Ranchi Veterinary College and Ranchi Agriculture College, Kanke, and private dairy farms of Ranchi. The samples so collected were subjected to isolation of microorganism, pH (pH paper of ranges 2- 10) of milk and microscopic examination. For microscopic examination of milk, Ten ml of properly mixed freshly collected milk samples were centrifuged at 1500 rpm for 5 min. Supernatant was discarded and smears were prepared in quadruplet from the sediment of each sample. Two smears each were stained by Papanicolaou technique (8) and Gomori's technique (6) and examined under oil immersion to determine the presence of degree of lipid vacuolation and reticulin.

All the isolates were subjected to in vitro drug sensitivity test as per method described by Bauer et al. (2). The antimicrobials commercially available in market as anti mastitis preparation like amoxycillin, penicillin, streptomycin, cloxacillin and neomycin were tested for their in vitro efficacy against various isolates. In addition, old and new generation antimicrobials like chloramphenicol, gentamicin, lincomycin, cephalexin, ciprofloxacin, enrofloxacin, ceftriaxone, cefotaxime, amikacin etc. were also tested (table 1). The antibiotic discs (Hi-Media, Mumbai, India) were placed on the surface of an agar plate previously seeded with a standard amount of the organism to be tested. The plates were incubated at 37oC for 18-24 hours. Subsequently, the plates were examined for the development of zone of inhibition around the discs.

Table1. Overall sensitivity pattern for different antibiotics in term of high to moderate and mild to resistant antibiotic sensitivity for selection of antibiotics for therapeutic use.

Sl. No.	List of the antibiotics with its MIC (g)	Bacterial Isolates			
		H, Mo		Mi, R	
		No.	%	No.	%
1.	Enrofloxacin (Ex, 10)	121	91.67	11	08.33
2.	Ciprofloxacin (Cf, 10)	119	90.15	13	09.85
3.	Amikacin (Ak,30)	115	87.12	17	12.88
4.	Ceftriaxone (Ci, 30)	111	84.10	21	15.90
5.	Chloramphenicol (C, 30)	106	80.31	26	19.70
6.	Cephotaxime (Ce, 30)	105	79.55	27	20.45
7.	Gentamicin (G, 10)	102	77.27	30	22.73
8.	Pefloxacin (Pf, 5)	86	65.15	46	34.85
9.	Cephalexin (Cp, 30)	84	63.63	48	36.36
10.	Neomycin (N, 30)	84	63.63	48	36.36
11.	Kanamycin(K, 30)	74	58.06	58	43.94
12.	Cloxacillin(Cx, 10)	60	45.45	72	54.54
13.	Amoxycillin (Am, 10)	66	50.00	66	50.00
14.	Lincomycin (L, 10)	66	50.00	66	50.00
15.	Ampicillin(A, 10)	46	34.85	86	65.15
16.	Penicillin G(PG, 10)	39	29.54	93	70.45
17.	Streptomycin(S, 10)	24	18.18	108	81.82

The diameter of the zone of inhibition was measured in mm and compared with the values listed in standard chart provided by the manufacturer, on the basis of which the isolates were categorized as resistant (R), mildly sensitive (Mi), moderately sensitive (Mo) or highly sensitive (H) to the antimicrobial contained in that particular disc.

Results and Discussion

Microorganisms could be isolated from 138 samples out of 190 and various isolates were identified as *Staphylococcus aureus,* coagulase negative *Staphylococcus spp*., *E. coli, Pseudomonas spp.*, *Streptococci spp., Klebsiella spp, Bacillus spp.*, Yeast. All the isolates obtained were subjected to antibiogram assay. Enrofloxacin (91.67%) was found to be most effective drug followed by ciprofloxacin (90.15%), amikacin (87.12%), ceftriaxone (84.10%), chloram-phenicol (80.31%) and cefotaxime (79.55%) (table 1). On the contrary, antibiotics showing higher rate of resistance patterns were streptomycin, penicillin G, ampicillin, cloxacillin, lincomycin, amoxicillin and kanamycin showing 81.81%, 70.45%, 65.15%, 54.54%, 50.50%, 50.50%, 43.94% resistance, respectively. This suggests that the antimicrobial agents commonly used in commercial preparation like amoxycillin, cloxacillin, lincomycin, ampicillin, penicillin and streptomycin if used indiscriminately, probably will further complicate mastitis if the causative agent happens to be resistant to these antimicrobials. Table 2 reveals that the Gram positive bacteria were relatively more sensitive than Gram negative ones to amikacin, cefotaxime, cephalexin, neomycin, lincomycin and amoxicillin. The efficacy of enrofloxacin was found to be equal for both the group of organisms. In accordance with our finding, higher sensitivity for enrofloxacin, ciprofloxacin, amikacin, ceftriaxone, chloramphenicol, cefotaxime and gentamicin has also been reported (4, 12, 9, 11, 1, 10, 19, 22).

In the present study, complete resistance to all the types of antimicrobial agents being tested characterized yeast mastitis. This is due to the fact that yeast has thick wall with starch on which antimicrobial has no effect. This finding is in accordance with the finding of *Chuang- ShihTe et al.* (5) who have also reported complete drug resistance to yeast. Drugs such as polyenes or imidazole can bind with sterol to damage the cell membrane function (14). Nystatin @ 10000 IU per ml has also been found to be effective against yeast (13). Some unconventional, nonantibiotic agents such as gastric paste have been found useful in treatment of yeast mastitis (20).

The result of silver impregnation staining (Gomori's method) of test sample for reticulin revealed that about 19.47% of total milk samples showed the presence of reticulin. About 57% of total reticulin positive milk samples were from mastitis caused by Gram positive organism, while 26 % samples were from yeast mastitis. Only 15 % of positive samples were observed in mastitis caused by Gram negative organisms. Additionally, Papanicolaou staining revealed a higher percentage of lipid vacuoles in staphylococcal and streptococcal mastitis followed by E. coli and pseudomonas mastitis.

Antibiotic sensitivity test is widely used clinical investigation being followed world wide in cases of

Table-2. Variation in sensitivity of different isolates to antimicrobial agents

Types of Isolates	No.	Pattern of Sensitivity	Ak	Am	A	Ci	Cp	Ce	C	Cf	Cx	Ex	G	K	L	N	Pf	PG	S
Staphylococcus	52	H Mo	90.39	55.77	36.94	80.77	69.23	78.81	80.77	92.31	53.84	98.08	76.92	53.84	51.92	75.00	55.77	32.69	13.46
aureus		Mi R	09.61	44.23	63.06	19.23	30.77	21.19	19.23	07.69	46.16	01.92	23.08	46.16	48.08	25.00	44.23	67.31	86.54
Staphylococcus	24	H Mo	83.33	41.67	41.67	75.00	75.00	79.16	87.50	83.34	41.67	83.34	100.00	50.00	62.50	70.83	66.67	29.16	08.33
spp.		Mi R	16.67	58.33	58.33	25.00	25.00	20.84	12.50	16.66	58.33	16.66	00.00	50.00	37.50	29.17	33.33	70.84	91.67
Streptococcus	11	H Mo	81.81	63.63	36.36	90.91	63.63	90.91	72.72	90.91	36.36	99.91	63.63	18.18	36.36	36.36	45.45	27.27	00.00
spp.		Mi R	18.19	36.36	63.64	09.09	27.27	09.09	27.28	09.09	63.64	09.09	36.37	81.82	63.64	63.64	54.55	63.64	100.00
E. coli	17	H Mo	76.46	35.28	23.52	88.22	64.70	76.48	94.12	94.12	29.40	94.12	82.34	58.81	23.52	23.52	64.69	23.52	17.64
		Mi R	23.54	64.72	76.48	11.78	35.30	23.52	05.88	05.88	70.60	05.88	17.66	41.19	76.48	76.48	35.31	76.48	82.36
Klebsiella spp.	03	H Mo	66.67	00.00	33.33	100.00	66.67	100.00	100.00	100.00	33.33	94.12	66.67	100.00	00.00	66.67	66.67	33.33	33.33
		Mi R	33.33	100.00	66.67	00.00	33.33	00.00	00.00	00.00	66.67	05.88	33.33	00.00	100.00	33.33	33.33	66.67	66.67
Pseudomonas	15	H Mo	93.33	26.66	20.00	73.34	13.33	60.00	13.33	86.68	26.67	100.00	66.67	53.33	46.66	73.33	80.00	20.00	20.00
spp.		Mi R	06.67	73.34	80.00	26.26	86.67	40.00	86.67	13.32	73.33	00.00	33.33	46.67	53.34	26.67	20.00	80.00	80.00
Bacillus spp.	01	H Mo	100.00	00.00	00.00	100.00	00.00	100.00	100.00	100.00	00.00	100.00	100.00	100.00	00.00	100.00	100.00	00.00	00.00
		Mi R	00.00	100.00	100.00	00.00	100.00	00.00	00.00	00.00	100.00	00.00	00.00	00.00	100.00	00.00	00.00	100.00	100.00
Mixed	09	H Mo	100.00	66.67	44.44	88.89	55.56	77.78	77.78	88.89	44.44	88.89	77.78	66.67	55.56	44.44	66.67	22.22	00.00
		Mi R	00.00	33.33	55.56	11.11	44.44	22.22	22.22	11.11	55.56	11.11	22.22	33.33	44.44	55.56	33.33	77.78	100.00
Yeast	06	H Mo	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
		Mi R	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

bovine mastitis with the sole purpose to select most appropriate antimicrobial agent for therapeutic use. However, it is difficult to judge the clinical efficacy of an antimicrobial agent solely on the basis of invitro test, as there are large variations in response among herds and within herds, due to type of organism involved, location of infected sites, degree of udder induration, physico-chemical properties and kinetic behaviour of antibiotics in udder and milk, site of injection and sensitivity of udder pathogens, lipid solubility and tissue protein binding of the drug, pH of milk and inflammatory exudates/ barrier at the site (21, 23, 24). Thus, following culture and sensitivity test the additional information on presence of fat globules, reticulin fibre obtained during cytological study as well as determination of the pH of milk sample could prove to be of vital importance in selection of medicines. Lipophilic drugs should be preferred when cytological studies show greater presence of lipid vacuolation. Drugs such as penicillinG, ampicillin, cephaloridine, aminoglycoside and polymixin have low solubility, hence will not prove to be effective when lipid content is high in milk. On the other hand, sulfa drugs, tylosin, erythromycin, oxytetracycline, doxycycline, chloramphenicol, fluroqulolone like ciprofloxacin, enrofloxacin and pefloxacin have moderate to high lipid solubility (17, 18, 25). These could prove to be more effective in lipid rich milk as detected during cytological study.

Similarly drug acting at higher pH such as amikacin, streptomycin, gentamicin and lincomycin should be preferred if the milk sample are showing high pH otherwise drug with better efficacy in acidic or neutral pH such as ampicillin, amoxycillin, cephalosporin, lincosamides, macrolides and chloramphenicol should be selected. (15).

Early detection of reticulin fibre in the milk by silver impregnation technique thus, can serve as an excellent marker for detection of onset of fibrosis in the udder. Consequently the information could be utilized in the positive cases for selection of antifibrotic therapeutic agents, which could be used in conjunction with antibacterial agents to annule the threat of onset of fibrosis, and thereby the curbing menace of chronic mastitis. Fibrinolytic agents such as hylase, streptokinase, streptodornase and hylurinidase (3) should be used as an adjunct therapy in those cases of bovine mastitis where the milk samples were found to be reticulin positive.

Thus, it could be concluded that the case of bovine mastitis should be handled carefully and reported comprehensively with respect to microscopic examination of milk with special emphasis on milk pH, clumping of cell population, lipid vacuolation and presence of reticulin. These preliminary tests should be followed by bacteriological examination and antibiotic sensitivity testing for judicious selection of drug in order to achieve maximum containment of bovine mastitis in field condition.

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