

IDENTIFICATION AND ANTIBIOTIC SENSITIVITY OF MICROBES ASSOCIATED WITH BUFFALO MASTITIS IN JABALPUR, MADHYA PRADESH, INDIA

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ABSTRACT

From 15 dairies located in different parts of Jabalpur city in Madhya Pradesh, 102 milk samples were collected from cases of clinical mastitis in buffaloes and 86 samples (84.31%) were found to be culturally positive for bacteria and yeasts. The identified bacteria belonged to seven genera while the yeast isolates belonged to two genera. The representative isolates from each genera were tested “*in vitro*” for their sensitivity towards eight antibacterial and two antifungal antibiotics. Ciprofloxacin was bactericidal towards the maximum number of isolates while streptomycin was the least bactericidal. Both Nystatin and Amphotericin B were inhibitory towards the yeasts; Nystatin, however, was found to be a more effective inhibitor.

INTRODUCTION

Milk is considered to be the cheapest and most readily available animal protein mastitis, which is an inflammation of the udder, leads to undesirable physical and chemical changes in milk. Due to it, productivity of the mammary glands is reduced by approximately 21% (Chakraborty, 1993).

In spite of the buffalo being the principal dairy animal in India, most of the work reported about mastitis deals with the problem in cows, while reports about buffalo mastitis are limited. Certain microbes associated with the problem could be of zoonotic importance.

The indiscriminate use of antibiotics has led to the development of resistant strains. This is a potential threat not only to animals, but also to humans who consume the milk of these animals.

It is therefore essential that the causative microbes be identified and their sensitivity towards the commonly used antibiotics be checked before being administered for therapy.

MATERIALS AND METHODS

Collection of Samples:

From cases of clinical mastitis in buffaloes, after mopping the affected quarters with 70 % ethyl alcohol, 102 milk samples were collected in sterile cotton plugged vials, and immediately brought to the laboratory in an ice container for processing.

Isolation of microbes:

A loopful of sample was streaked on to the surface of 5% sheep blood agar plates and incubated at 37 °C for 72 h. Growth from the appearing colonies was used for identification studies.

To detect presence of fungi, Sabouraud's dextrose agar (SDA) containing chloramphenicol, an antibacterial and SDA containing chloramphenicol and actidione, which inhibits saprophytic fungi were used (Emmons, 1977).

Identification:

The isolates were identified on the following basis

Cultural characteristics Besides the isolation media, growth characteristics of the microbes on blood agar, MacConkey agar, (Cruickshank, 1975) and Staib's niger seed medium (Staib, 1962) were noted.

Morphology: The morphology and staining reaction of isolates were observed after staining by Gram's method, negative staining and lactophenol cotton blue staining.

Motility was observed by the "Hanging drop" method (Cruickshank, 1975).

Biochemical tests : The tests included fermentation of various sugars, plasma coagulase, gelatin liquefaction, nitrate reduction. Tests for the enzymes desulfurase, dehydrogenase, tryptophanase, the methyl red, Voges Proskauer, citrate utilization and methylene blue reduction tests. The procedures followed were as described in Cruickshank, 1975.

Antibiogram : Sensitivity of the representative isolates towards eight antibacterial and two antifungal antibiotics was determined by the single disc diffusion method (Bauer *et al.* 1996). Commercially available antibiotic discs marketed by Hi-Media Laboratories were used. Three replications were maintained per antibiotic. The zones of inhibition which developed were measured in millimeters, and the average size determined. The cultures were categorized as sensitive or resistant according to the interpretative charts provided by the company.

Statistical analysis : To compare the effect of antibiotics on representative isolates the data were analysed by the single split plot design (Cochran and Coch, 1987).

RESULTS AND DISCUSSION

Microbial Isolates

Out of the 102 milk samples, eighty isolates belonging to seven genera of bacteria and six yeasts belonging to two genera were identified (Table 1).

In accordance with the present finding, Muhammad *et al.* (1997) reported a high percentage of *Staphylococci*, while Tijare (1997) isolated species of *Streptococci* and *Bacillus*. Dutta *et al.* (1995) found *Corynebacterium* sp. In samples of mastitis milk.

Among the Gram negative forms *E. Coli* and *Kl. Pneumoniae* have been reported from cases of bovine mastitis by Mitra *et al.* (1995) and Shirame (1996). *Ps. Aeruginosa* was reported by Singh and Bakshi (1982).

Presence of the yeasts *Cryptococcus neoformans* and *Trichosporon* sp. was reported by Daignault *et al.* (1997) and Chhabra *et al.* (1998), from cases of bovine mastitis.

Antibiogram

The antibiogram of representative isolates belonging to seven bacterial and two yeast genera was studied using eight antibacterial and two antifungal antibiotics.

Str. agalactiae was found resistant towards penicillin, while *Kl. pneumoniae* and *Ps. aeruginosa* showed resistance towards both penicillin and Erythromycin (Table 2). Six bacterial isolates were found to be sensitive in varying degrees towards all the tested antibiotics (Table 3). Chloramphenicol was the most effective inhibitor, while streptomycin and ampicillin were found to be least effective towards the six isolates.

Both Nystatin and Amphotericin B inhibited the yeasts *Cryptococcus neoformans* and *Trichosporon* sp. Nystatin however, produced a larger zone of inhibition in the case of both the yeasts (Table 4).

The resistance pattern of the isolated genera towards the tested antibiotics was more or less similar to the reports of Tijare (1997) and Quadri *et al.* (1986), while it differs with the results of Bakshi (1982) and Bagherwal and Skukla (1996). The difference could be due to constant or prolonged exposure of organisms to the antibiotics due to their prolonged use or underdosing.

CONCLUSION

Out of a total of 102 milk samples collected from cases of buffalo mastitis in Jabalpur, M.P. eighty bacteria belonging to seven genera and six yeast belonging to two genera were identified. The antibiogram of representative isolates belonging to the different genera was carried out against eight antibacterial and two antifungal antibiotics. Ciprofloxacin inhibited the growth of all isolates while *Str. agalactiae*, *Kl. pneumoniae* and *Ps.*

Table 1. Microorganisms isolated from cases of clinical mastitis in buffaloes.

S.No.	Isolates	Number and Percentage
1	<i>Staphylococcus spp.</i> (coagulase positive)	24(27.9%)
2	<i>Staphylococcus spp.</i> (coagulase negative)	14(16.28%)
3	<i>Streptococcus agalactiae</i>	6(6.98%)
4	<i>Corynebacterium bovis</i>	4(4.65%)
5	<i>Corynebacterium pyogenes</i>	5(5.81%)
6	<i>Bacillus cereus</i>	4(4.56%)
7	<i>Escherichia coli</i>	15(17.44%)
8	<i>Klebsiella pneumonie</i>	5(5.81%)
9	<i>Pseudomonas aeruginosa</i>	3 (3.49%)
10	<i>Trichisporon spp.</i>	3(3.49%)
11	<i>Cryptococcus neoformans</i>	3(3.49%)
	Total	86(100%)

Table 2. Antibiogram of *Strept. agalactiae*. *Kl. pneumoniae*. *Ps. aeruginosa*.

S. No	Organisma	Average zone of inhibition (in mm) of three replications							
		C	G	P	E	Cf	A	S	K
1	<i>Strept. agalactiae</i>	16.67	18.00	-	18.87	15.67	20.67	17.33	12.67
2	<i>Kl. pneumoniae</i>	19.67	18.00	-	-	32.23	23.33	17.67	13.33
3	<i>Ps. aeruginosa</i>	22.00	20.67	-	-	35.00	20.67	15.67	20.00

-No zone of inhibition

Table3. Antibioqram of bacterial isolates.

Organisms	Average zone of inhibition (in mm)						Mean
	<i>C. bovis</i>	<i>C. pyogens</i>	<i>Staph. spp. (coag. +ve)</i>	<i>Staph. spp. (coag. -ve)</i>	<i>E. coli</i>	<i>B. cereus</i>	
Ciprofloaxacin (Cf)	35.00 ^{ab}	35.33 ^a	28.33 ^{ab}	38.00 ^a	27.67 ^a	38.33 ^a	34.17 ^a
Penicillin (P)	23.67 ^a	25.33 ^{bc}	25.67 ^{bc}	35.00 ^{ab}	26.67 ^{ab}	34.00 ^b	28.39 ^b
Chloramphenicol (C)	32.00 ^d	27.33 ^b	30.67 ^a	24.33 ^d	24.33 ^{bc}	30.33 ^c	28.16 ^{bc}
Gentamycin (G)	28.00 ^c	24.00 ^{cd}	30.33 ^a	28.33 ^c	28.67 ^a	27.67 ^c	27.83 ^{bc}
Erythromycin (E)	35.33 ^a	21.67 ^d	22.00 ^d	32.33 ^b	21.33 ^d	29.33 ^c	27.00 ^c
Kanamycin (K)	22.00 ^d	21.67 ^d	24.00 ^{cd}	19.67 ^c	21.67 ^d	27.323 ^c	22.78 ^d
Ampicillin (A)	23.00 ^d	22.67 ^{cd}	24.67 ^{cd}	17.33 ^c	21.67 ^d	17.67 ^d	21.78 ^d
Streptomycin (S)	27/67 ^c	11.67 ^c	24.33 ^{cd}	11.67 ^f	23.33 ^{cd}	18.00 ^d	19.50 ^c
Mean	28.33 ^a	23.70 ^d	27.08 ^b	25.83 ^c	24.41 ^d	27.83 ^{ab}	

CD (P<0.05) for antibiotics = 1.304; CD (P<0.05) for organisms = 0.9248;

CD (P<0.05) for antibiotic X organisms = 3.193

Figures having same supercript do not differ significantly from each other

Table 4. Antibiogram of yeasts towards Nystatin and Amphotericin B.

Organisms	Nystatin Zones of Inhibition of Three replications (mm)			Avg. Zone of Inhibition (mm)	Amphotericin B Zones of Inhibition of three replications (mm)			Avg. Zone of Inhibition (mm)
	1	2	3		1	2	3	
<i>Cryptococcus neoformans</i>	18	16	3	17.00	10	12	10	10.67
<i>Trichosporon spp.</i>	18	20	20	19.33	11	12	11	11.33

aeruginosa were found resistant to Penicillin, and the latter two resistant to Penicillin as well as Erythromycin.

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