Pakistan Vet. J., 24(4): 2004

BACTERIOLOGY OF MASTITIC MILK AND *IN VITRO* ANTIBIOGRAM OF THE ISOLATES

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ABSTRACT

Mastitis is one of the most costly diseases of lactating animals. Bacteria constitute the common etiological agents of mastitis. In the present study, bacteria associated with mastitis were investigated and their susceptibility to various antibiotics was tested. Milk samples collected from different sources were subjected to different screening tests (White Side Test, Surf Test and California Mastitis Test). The samples found positive were cultured on tryptose agar, MacConkey's agar, as well as blood agar, for bacterial isolation. The organism so isolated were identified and subjected to 12 commonly used antibiotics for determination of antibiogram. During the period of 52 months (July, 1997 to October, 2001), 6522 milk samples from cattle, buffaloes, sheep and goats were tested for mastitis, out of which 1512(23.18%) were found positive. Growth of different bacteria was yielded by 236(15.16%) out of the positive ones. Gentamicin, enrofloxacin, norfloxacin and kanamycin were found most effective drugs amongst the 12 antibiotics tested *in vitro*.

Key words: Mastitis, bacteriology, antibiogram.

INTRODUCTION

Mastitis is the most costly disease of dairy animals. This condition is widespread in dairy herds and is associated with a significant reduction in milk yield, increased costs of production and deteriorated milk quality. These costs are borne directly by milk producers and indirectly by the consumers of dairy products. The disease also results in partial or complete damage to udder tissues and decreases the productive life span of the animal. Mastitis is caused by many bacteria, which include the coliform group (specifically *Escherichia coli*, Enterobacter, Klebsiella species), Streptococci, Staphylococci, Corynebacteria, Pasteurella, Mycoplasma, Leptospira, Yarsinia, Mycobaceteria, Pseudomonas, Serratia, and other organisms like fungi, yeasts and virus (Kotowshi, 1988; Gonzalez *et al.*, 1980).

Little work has been carried out in our region, regarding the isolation and antibiogram of bacteria causing mastitis. This study was, therefore, conducted to achieve the following objectives:

1. To detect the mastitic milk by various indirect laboratory diagnostic tests for mastitis.
2. To isolate the prevailing causal bacterial organisms from mastitis positive milk.
3. To carry out *in vitro* sensitivity tests of the isolates to the commonly used antibiotics.

MATERIALS AND METHODS

Collection of milk samples

During a period of 52 months (July, 1997 to October, 2001), a total of 6522 milk samples were collected by the staff of Mastitis Section, or submitted by the farmers themselves to the Mastitis Section, Veterinary Research Institute, Peshawar. These samples were collected from dairy animals mainly cattle, buffaloes, sheep and goats, suspected for mastitis from government, private and commercial dairy farms in and around Peshawar.

Testing of milk samples for mastitis

All the milk samples were subjected to the following indirect field or animal side mastitis tests:

i. White Side Test (WST) was carried out according to the modified method of Murphy and Hanson (1941).
ii. California Mastitis Test (CMT) was performed according to Schneider and Japer (1964).
iii. Surf Field Mastitis Test (SFMT) for which the method of Muhammad *et al*. (1995) was followed.

Isolation and identification of bacteria

A total of 1512 mastitis positive milk samples were inoculated on to tryptose and MacConkey's agar plates, as well as blood agar, for bacterial isolation. The

inoculated plates were incubated aerobically at 37°C for 24-48 hours. The bacterial isolates were identified on the basis of their cultural, morphological characteristics and biochemical reactions (Hargital *et al.,* 1992).

In vitro antibiogram of isolates

All the bacteria isolated were tested *in vitro* for their sensitivity to 12 different antibiotics, commonly used in veterinary practice. These included Chloramphenicol, Cloxacillin, Cepharidine, Amoxicillin, Enrofloxacin, Gentamicin, Norfloxacin, Kanamycin, Fluemequin, Oxytetracycline, Penicillin and Streptomycin.

RESULTS AND DISCUSSION

Out of 6522 milk samples from different animal species, 1512 were found positive for mastitis. The overall occurrence of mastitis was 23.18% (Table 1). The yearwise occurrence of mastitis ranged from 16.61 to 50%, showing that the occurrence of mastitis varied in different years. These findings are in conformity with those of Hussain *et al*. (1984), who recorded 23.59 to 34.5% mastitis cases in milking cows. But our findings differ from Ikhwan *et al.* (1989), who reported overall occurrence of mastitis in buffaloes, cattle, sheep and goats as 9.53%. The test used in this study was White Side Test which is not very sensitive in detecting mastitis positive cases (Iqbal *et al.,* 2003). The incidence of mastitis in cows was 50.0, 29.1, 18.3, 27.8 and 18.8% respectively in 1997, 1998, 1999, 2000 and 2001. In buffaloes, it was 47.7, 18.2, 14.6, 19.8 and 23.2%, while in sheep and goats, it was 80.0, 26.7, 33.3, 57.1 and 42.0%, respectively in 5 successive years. The overall incidence of mastitis in cows, buffaloes, sheep and goats was 28.8, 24.7 and 47.8%, respectively (Table 2). This showed that the highest incidence was in sheep and goats, followed by cows and buffaloes. The highest incidence of mastitis in sheep and goats may be due to the reason that in these species the milk samples were collected from the animals having udder complaints. The incidence of mastitis in cattle was higher than buffaloes, which is in conformity with our previous findings (Iqbal *et al*., 1998; Rasool *et al.,* 1985).

In this study, out of a total of 570, 890 and 52 mastitis positive milk samples from buffaloes, cows, sheep and goats respectively 83(14.56%), 194(16.74%) and 4(7.69%) yielded bacterial growth on culture. Out of the total 1512 mastitis positive milk samples bacterial growth was obtained from 236(15.16%), while

Table 1: Year-wise occurrence of mastitis in farm animals

year	Total number of samples tested	No. of samples found positive	%ge positive
1997	276	138	50.00
1998	1316	316	24.01
1999	1174	195	16.61
2000	1788	444	21.83
2001	1968	419	21.29
Total	6522	1512	23.18

Table 2: Occurrence of mastitis in different animal species

year	Buffaloes		Cattle		Sheep/Goats	
	Total samples tested	+ive samples (%)	Total samples tested	+ive samples (%)	Total samples tested	+ive samples (%)
1997	65	47.7	206	50.0	05	80.0
1998	611	18.2	690	29.1	15	26.7
1999	594	14.6	568	18.3	12	33.3
2000	766	19.8	994	27.8	28	57.1
2001	816	23.2	1095	18.8	57	42.0
Total	2852	24.7	3553	28.8	117	47.8

Table 3: Bacterial growth obtained from milk samples of different species

Animal species	No. of milk samples cultured	No. of samples that yielded bacterial growth	+ive samples (%)
Buffaloes	570	83	14.56
Cattle	890	194	16.74
Sheep/Goats	52	04	7.69
Total	1512	236	15.16

the remaining 1276(84.40%) samples yielded no growth (Table 3).

The failure of negative finding of cultures in high percentage of samples may be due to premedication of the animals with antibiotics, non-bacterial causes and the type of media that did not support the growth of whole range of bacteria associated with mastitis. Arshad *et al*. (1998) obtained bacterial isolates from 90 (90%) of clinically mastitis positive milk samples. This is not comparable to our findings.

Table 4 indicates the relative occurrence of various bacteria isolated from buffaloes, cows, sheep and goats. The microorganisms isolated from buffaloes milk were 83 which comprised of 25(30.12%) *E. coli*, 14(16.87%) Staphylococci, 3(3.6%), Streptococci, 12(14.51%) Pseudomonas, 4(4.82%) Proteus, 1(1.20%) Pasteurella, 2(2.41%) Salmonella, 2(2.41%) Bacilus and 20 (24.09%) mixed growth. The overall percentage of microorganisms isolated from buffaloes milk samples was 35.17%.

Similarly, the milk samples of cows yielded 149 isolates, which exhibited 70(46.98%) *E. coli*, 20(13.42%) Staphylococi, 14(9.39%) Streptococci, 22(14.77%) Pseudomonas, 6(4.02%) Proteus, 3(2.01%) Pasteurella, 2(1.34%) Salmonella and 12(8.02%) mixed growth. The overall microflora percentage of microorganisms isolated from cow milk samples was 63.14

Likewise, the milk samples of the sheep and goats yielded growth of 4 different micro-organisms, which showed 1(25%) *E. coli*, 1(25%) Streptococci and 2(50%) mixed growth. The overall percentage of milk samples yielding growth was 1.69.

The overall relative percentage of *E. coli*, Staphylococci, Streptococci, Pseudomonas, Proteus, Pasteurella, Salmonella, Bacillus and mixed organisms were found to be 40.7, 14.41, 7.63, 14.41, 4.24, 1.69, 1.69, 0.85 and 14.41%, respectively. This shows the highest incidence of *E. coli* (40.7%), which is presumably due to the fact that *E. coli* is the commonest environmental contaminants, which is closely associated with hygiene. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. Moreover, the existence of high concentration of *E. coli* in milk also indicates the relatively poor quality of milk, related with sub-standard hygiene of farm management, milk collection and processing system. Staphylococci, Pseudomonas and mixed growth were the second after *E. coli*. Their presence was also an indication of sub-standard dairy farming.

Table 5 indicates the antibiotic sensitivity of the isolates. In this trial 12 available antibiotics were used. It is evident from this table that gentamicin, enrofloxacin, norfloxacin and kanamycin were found most effective drugs amongst the 12 antibiotics tested *in vitro*.

Table 4: Relative occurrence of various bacterial species isolated from mastitic milk samples

Bacteria	Buffaloes		Cattle		Sheep/Goats		Overall	
	No	%age	No	%age	No	%age	Total	%age
E. coli	25	30.12	70	46.98	1	25.0	96	40.7
Staphylococi	14	16.87	20	13.42	--	--	34	14.41
Streptococci	3	3.60	14	9.39	1	25.0	18	7.63
Pseudomonas	12	14.51	22	14.77	--	--	34	14.41
Proteus species	4	4.82	6	4.02	--	--	10	4.24
Pasteurella	1	1.20	3	2.01	--	--	4	1.69
Salmonella	2	2.41	2	1.34	--	--	4	1.69
Bacillus	2	2.41	--	--	--	--	2	0.85
Mixed growth	20	24.09	12	8.02	2	50.0	34	14.41
Total	83	35.17	149	63.14	4	1.69	236	--

Table 5: Antibiogram of mastitis-associated isolates

Name of antibiotics	No. of isolates sensitive	% sensitivity
Chloraphenicol	57	24.2
Cloxacillin	22	9.3
Cepharidine	23	9.8
Amoxicillin	38	16.1
Enrofloxacin	92	39.0
Gentamicin	93	39.4
Norfloxacin	83	35.2
Kanamycin	80	33.9
Flumequin	46	19.5
Oxytetracycline	35	14.8
Penicillin	19	8.1
Streptomycin	29	12.3

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