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INCIDENCE AND ANTIBIOTIC SENSITIVITY OF ORGANISMS CAUSING MASTITIS IN BUFFALOES

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One hundred and fifty clinical cases of mastitic buffaloes which came to the university or civil veterinary clinics were studied. The incidence of *Staphylococcus aureus* was highest being 48.6 per cent, next in order were those due to mixed infections (staphylococcal and streptococcal) 22 per cent, then *Escherichia coli* 14.6 per cent, *Corynebacterium pyogenes* 5.3 per cent, *Streptococcus pyogenes* 3.3 per cent, *Streptococcus agalactiae* 2.6 per cent, *Mycobacterium tuberculosis* 1.3 per cent, and finally *Streptococcus zooepidemicus* 0.6 per cent.

Antibiotic sensitivity tests *in vitro* revealed that the bacteria sensitive to combiotics were 44 per cent, terramycin 32 per cent, chloramphenicol 10 per cent, streptomycin 10 per cent, achromycin 5 per cent whereas all were resistant to penicillin.

INTRODUCTION

Mastitis is a serious hazard in dairy industry. Unfortunately the disease is on the increase and is causing serious damage to milk industry. A variety of micro-organisms produce mastitis in cattle and buffaloes. Although a lot of work has been done on the incidence of mastitis in cows, however the position in buffaloes is not yet clear. The object of this study is to record the incidence of mastitis in buffaloes in Faisalabad area and to study the causal organisms associated with the disease. There is also need to determine the antibiotic sensitivity of the organisms isolated.

MATERIALS AND METHODS

This study was made on 150 cases of mastitic buffaloes brought to the University or Civil Veterinary Hospital. Milk sample from each affected udder was collected under sterile conditions. The udder was washed with acriflavine

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solution, dried, and then about 10 mls. of milk was collected in sterilised test tubes. Each sample was subjected to Hotis test and Strip cup test. Within an hour after collection, a loop full (0.01 ml.) was streaked on Staphylococcus medium No. 110 (Difco) and blood agar (Difco blood agar base to which 10 per cent defibrinated sheep blood was added), and incubated at 37°C under aerobic and anaerobic conditions. Growth was examined after 24 hours and then after 48 hours. Smears were prepared from the colonies obtained, stained with Gram's method, (Hucker's modification, Hucker and Conne 1923) and observed microscopically. The organisms were isolated by repeated sub-culturing on Nutrient agar (Difco). Pure cultures were subjected to the routine biochemical tests.

To determine the antibiotic sensitivity *in vitro*, paper diffusion test and plate method was used. Susceptibility test using discs was carried out on primary as well as secondary cultures. The procedure followed was similar to that of Bailey and Scott (1962). The antibiotics used were crystalline penicillin, combioties, streptomycin, chloramphenicol, achromycin and terramycin. The differences between sensitivity of different micro-organisms to various antibiotics were analysed statistically using analysis of variance technique. Comparison between the relative effectiveness of these antibiotics was determined by using Duncan's Multiple Range test. The most effective antibiotic *in vitro* was administered correspondingly *in vivo*. Each milk sample was cultured aerobically for bacterial growth after treatment as well.

Besides these tests, observations were made on seasonal variations, defect in management and husbandry, age, stage of lactation and environmental factors which may influence the onset of the disease. The course of the disease and its clinical manifestations were also recorded.

RESULTS

The present study on 150 samples from mastitic buffaloes revealed pure staphylococcal infection in 73 cases (48.6%), whereas *Streptococcus agalactiae* was isolated in 4 cases (2.6%) *Streptococcus zooepidemicus* from a single case (0.6%), *Streptococcus pyogenes* in 5 cases (3.3%), mixed infection due to Streptococci and Staphylococci occurred in 33 cases (22%). *E. coli* was in 22 cases (14.6%), *Corynebacterium pyogenes* in 8 cases (5.3%) and *Mycobacterium*

tuberculosis in 2 cases (1.3%). Two samples did not reveal any growth even on repeated culturing on both nutrient agar and blood agar (Table I).

Out of 73 strains of *Staphylococcus aureus* thus isolated 44 strains (60.2%) were highly sensitive to combiotics, 20 (27.3%) to terramycin, 5 (6.8%) to achromycin, and 4 (5.4%) to chloramphenicol. The differences in antibiotic sensitivity were significant (PL 0.01) as shown in Table II. Combiotics differed significantly in sensitivity from the rest of the antibiotics used. However, the effectiveness of terramycin, Streptomycin and Chloramphenicol were non significant. Similarly chloramphenicol had a similar action as Streptomycin and achromycin. All 4 strains (100%) of *Streptomycin agalactiae* were highly sensitive to combiotics whereas the single strain of *Streptococcus zooepidemicus* was inhibited by streptomycin. The antibiotic sensitivity differed significantly for *Streptococcus agalactiae* (PL 0.01) as given in Table II. Combiotics was significantly more effective than all the other antibiotics used. The differences between other antibiotics used were non-significant. Out of 4 strains of *Streptococcus pyogenes*, 2 strains (50%) were sensitive to terramycin, 1 (25%) to achromycin and 1 (25%) to chloramphenicol. The difference in antibiotic sensitivity was non-significant statistically (Table II). The 33 strains of mixed infections consisted of 10 (30.3%) combiotic-sensitive strains, 14 (12.7%) terramycin-sensitive strains 3 (9%) chloramphenicol-sensitive strains and 2 (6%) achromycin-sensitive strains. These differences were also significant statistically (Table II). The effectiveness of terramycin was similar to that of combiotics but different from other antibiotics used. The effect of combiotic was similar to achromycin and penicillin, but differed from chloramphenicol and Streptomycin. Out of 22 strains of *E. coli*, 4 (18.2%) were inhibited by streptomycin, 6 (27.2%) by combiotics, 10 (45.4%) by terramycin and 2 (9%) by chloramphenicol. These differences in antibiotic effectiveness were also highly significant (Table II). Terramycin combiotics and streptomycin were more effective than achromycin, chloramphenicol and penicillin, whereas all other differences were non-significant. Maximum zone of inhibition in case of *Corynebacterium pyogenes* was given by combiotics for 2 cases (25%) streptomycin for 2 cases (25%) whereas terramycin was effective for 3 cases (37.5%) and the last case (12.5%) was inhibited by achromycin. *Corynebacterium pyogenes* had significant differences for antibiotic sensitivity. Combiotics, terramycin and chloramphenicol were more effective than achromycin, streptomycin and penicillin. Other differences in antibiotic sensitivity were non-significant. Both the mastitic

cases which revealed cultures of *Mycobacterium tuberculosis* were highly resistant to all the antibiotics used in this study.

DISCUSSION

One hundred and fifty mastitic cases were selected for treatment in the present study. The sensitivity of the isolated organisms to different antibiotics was also tested *in vitro*.

Clinical mastitis in buffaloes due to *Staphylococcus aureus* was found to be 48.6 per cent. This is in total agreement with Bakhshi's (1963) and Kalra and Dhanda's (1964) findings of 49 and 50 per cent of infections due to *Staphylococcus aureus* respectively. This correlation in results may be attributed to the similarities in environmental conditions. However, a high percentage of *Staphylococcus aureus* (91.2) per cent was reported by Ghumman (1967) and 48 per cent by Ahmad (1968) which may be due to the use of different media for primary isolation and purification, or due to the chemotherapeutic use of penicillin which inhibits Streptococcal and enhances penicillin-resistant Staphylococcal growth. A significantly low percentage of Staphylococcal cases was reported by Stableforth (1952) who found 21.4 per cent *Staphylococcus aureus* out of 3810 clinical cases.

Mixed infections (Staphylococcal and Streptococcal) were 22 per cent which is in partial agreement with that of Afzal and Chaudhary (1971) who had reported it to be 16 per cent. The differences may be due to limited number of cases studied by the latter.

Infection due to *Escherichia coli* was 14.6 per cent which somewhat varies from that of Awan (1969) who isolated 41 (28 per cent) strains of *Escherichia coli* out of 146 strains.

Mastitic cases due to *Corynebacterium pyogenes* and *Mycobacterium tuberculosis* were 5.3 per cent and 1.3 per cent respectively. This is in close agreement with that of Afzal and Chaudhary (1971) who isolated them in the order of 8 per cent and 2 per cent.

The organisms isolated from buffaloes suffering from mastitis were subjected to *in vitro* antibiotic sensitivity test. Since 2 cases out of 150 did not

exhibit any growth, so only 148 cases were studied upon. It was observed that 44 (60.2 per cent) out of 73 Staphylococcal strains isolated were sensitive to combiotics, but none to penicillin. These findings are in contrast to that of Ahmad (1968) who recorded that penicillin inhibited 83.3 per cent *Staphylococcus aureus*. Twenty strains (27.2%) were sensitive to terramycin which is in partial agreement with that of Ahmad (1968) who reported 17 per cent sensitivity to terramycin. *Staphylococcus aureus* was 5.4% sensitive to chloramphenicol which is not in agreement with the report of Malik (1963) and Overgoor (1967). All except 5 strains (6.8 per cent) were resistant to achromycin. This resistance can be attributed to the *in vitro* treatment of mastitic cases in Lyallpur with achromycin or its allied preparation. Only a few strains were moderately sensitive to streptomycin while the rest were resistant. This is not in accordance with the results of Ahmad (1966) who revealed that dihydrostreptomycin had the highest inhibiting ability.

In case of streptococcal infections combiotics had the highest inhibiting power (inhibited 4 out of 9). Next in order was terramycin which inhibited 2 strains. Achromycin, chloramphenicol and streptomycin were all moderately effective whereas penicillin was totally ineffective (except in 1 case). This is contradictory to the findings of Jones (1966) who reported that penicillin was highly effective against streptococcal mastitis.

Mixed (streptococcal and staphylococcal) infections were more successfully eliminated by terramycin (42.7 per cent), less with combiotics (30 per cent) whereas the other drugs showed a negligible cure. The low percentage of sensitivity of these organisms to penicillin is probably due to the production of penicillase by them and also due to the emergence of resistant micro-organisms (Lowbury, 1962).

Three strains (37.6 per cent) of *Corynebacterium pyogenes* were highly sensitive to terramycin. These results correlate with those of Overgoor (1967) who claimed that *Corynebacterium pyogenes* was sensitive to terramycin and dihydrosteptomycin.

Table I. *Showing the percentage of isolates from mastitic cases.*

Organisms isolated	No. of cases	Percentage
Staphylococcus aureus	73	48.6
Streptococcus agalactiae	4	2.6
Streptococcus zooepidemicus	1	0.6
Streptococcus pyogenes	5	3.3
Staphylococco & Streptococci	33	22.0
E. coli	22	14.6
Corynebacterium pyogenes	8	5.3
Mycobacterium tuberculosis	2	1.3

Table II. *Analysis of variance for antibiotic sensitivity of various micro-organisms isolated from mastitic buffaloes.*

Source of variation	*Staphylococcus aureus*		*Streptococcus agalactiae*		*Streptococcus pyogenes*		*Streptococci* and *Staphylococci*		*Escherichia coli*		*Corynebacterium pyogenes*	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Between Antibiotics	5	22.54	5	5.22	5	1.60	5	10.09	5	8.41	5	6.034
Error (Within antibiotic)	432	1.20	18	0.42	18	1.14	192	1.21	126	1.034	42	1.304

NS

= Highly significant (PL 0.01)

NS = Non significant

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