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# DETECTION OF MASTITIS ORGANISMS IN UDDER SECRETIONS AND THEIR BEHAVIOUR TOWARDS VARIOUS ANTIBIOTICS

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860 samples of milk obtained from apparently healthy udder of cows belonging to organized farms and rural areas were examined by the Hotis-Miller, Card and Milk-ring tests. It is evident from the results that the Card test is the most suitable in the detection of sub-clinical form of mastitis. The percentage distribution of 350 bacterial isolates recovered from milk samples indicated that Staphylococci ranked the highest (42%), followed by *Coliforms* (22%), Gram-positive aerobic spore-formers (20%),*Streptococci* (12.57%), *Corynebacterium* (2%) and *Pseudomonas* (1.43%). The antibiotic sensitivity of the bacterial isolates revealed that Chloromycetin was most inhibitory.

## INTRODUCTION

Bovine mastitis continues to rank as one of the most costly diseases of dairy cattle in many parts of the world. The disease has also acquired importance in Bangladesh due to the establishment of plants and development of dairy industry for improving the quality and increasing the quantity of milk. In this country the incidence of mastitis has increased over the years in direct proportion to the increased production of milk by dairy cows. Since the disease is recognized as one of the most serious threats to the economic property of dairy industry, it is therefore a desideratum that the etiologic agents of mastitis be studied, so that the disease could be well embedded in a national programme on animal disease control. The present work reports the isolation and identification of mastitis organisms from the secretions of apparently healthy udders and the determination of antibiotic sensitivity of the isolates.

## MATERIALS AND METHODS

Milk samples were obtained after discarding first milk into a strip cup, by milking directly into a sterile sample bottle. All aseptic measures were taken to avoid any undue contamination from the udder by careful washing and disinfection of both the hands of the milker and of the udder. An amount of about 20 ml of milk was collected in a screw capped glass bottle from a single cow. Individual quarter samples from each animal were obtained and transported to the laboratory in ice packed condition within a period of 30 to 60 min. Milk samples from cows were collected from the following sources:

(i) Bangladesh Agricultural University Dairy Farm
(ii) Savar Dairy Farm, Dhaka
(iii) Domestic holdings of rural areas

The laboratory investigations conducted to determine the cases of subclinical mastitis and to detect the presence of mastitis organisms in udder secretions and their sensitivity to various antibiotics, when the cow and the milk showed no abnormality detectable by eye, are presented below:

(i) *The Hotis-Miller Test.* Milk samples collected from apparently normal udder were tested for subclinical form of mastitis due to *Streptococci,* particularly *Streptococcus agalactiae.* The test was performed by adding 0.5 ml of sterile 0.5% aqueous solution of bromocresol purple to 9.5 ml of milk. The milk was incubated at 37°c for 24 hr. The appearance of canary yellow colonies of bacterial growth alone the walls and in the bottom of the test tube indicates infection due to *Streptococcus agalactiae* i.e., a sub-clinical form of mastitis.

(ii) *The Card Test.* An indicator paper which serves for the rapid detection of secretory disturbances of the mammary gland is employed. By this method the first indication of a beginning of udder sickness can be evaluated. The udder test card is manufactured by Hauptner/ Solingen, West Germany. The Card test was performed in accordance with the instruction of the manufacturer.

(iii) *The Milk-Ring Test.* According to the technique of the test, glass agglutination tubes with an internal dia. of 8-10 mm were used. The test was performed by adding one drop (0.5 ml) of stained MRT antigen (Brucella anti-

gen) to 1 ml of milk sample taken in the agglutination tube. After mixing the contents, the tubes were incubated for 45 min. at 37°c. The positive reaction was marked by a distinct blue cream layer and white milk column; and the negative reaction was marked by a blue milk column and a white or slightly blue cream layer. The antigen was received from the Institute of Verterinary Medicine, Berlin.

(iv) *Isolation and Identification of organisms.* Isolations of mastitis organisms were normally carried out from individual quarter samples. A 4-mm loop was used to smear 0.01 ml of milk on to the surface of a previously prepared and well dried blood agar plate. When four-quarter samples from one animal were examined, these were streaked in separate section of one plate. The inoculated plates were incubated at 37° for 48 hr. and examined after 24 and 48 hr respectively. Since standard quantities were used for inoculation a comparison could be made of the numbers developing from individual quarter samples. The presence of any haemolytic colonies was also recorded.

The predominant organisms developing on the isolation plates were of utmost significance and presumed to be the causal organisms. For identification, the size and shape of representive colonies, haemolysis if any, pigmentation, Gram reaction and morphology were recorded. These observations were sufficient to indicate the probable genus of the organisms. Further confirmatory tests if required were carried out in accordance with Bergey's Manual of Determinative Bacteriology (1974), Cowan (1974) and Harrigan McCance (1976).

(v) *The Antibiotic Sensitivity Test.* The isolate strains of bacteria were subcultured into nutrient broth and incubated at 37° for 24 hr. An amount of 0.1 ml of culture was poured on to the surface of a previously poured and well dried nutrient agar plate. The culture was spread over the plate and allowed to dry. Individual antibiotic discs in conjunction with a suitable dispenser were used (BBL). After placing the discs at different positions in the agar plates, they were incubated at 37° for 24 hr. The presence of the zones of inhibition around the discs were recorded. Inhibition indicated sensitivity to a particular antibiotic.

## RESULTS AND DISCUSSION

Table I represents the result of laboratory macroscopic tests of 860 samples of milk from apparently healthy udder of cows belonging to organized farms and rural areas. It is evident from the result that the Card test is most suitable for the detection of sub-clinical form of mastitis. It has been found to be most sensitive of other laboratory tests performed to detect changes in the character of milk which follow microbial infection of the udder. This, on the other hand, is a simple and rapid test as well as economical and convenient to use. So it could be basically used as an indirect method of assessing potential infection of the udder.

It is interesting to note that the milk samples of cows of organized farms exhibited the presence of brucella agglutinin. The percentage incidence as revealed by MRT showed 11.15% in the Savar Dairy Farm, 2% in BAU dairy farm and 0% in domestic holdings of rural areas. All MRT positive samples were obtained from udder secretions of exotic bred and cross bred cows. The milk samples of native breed cows belonging to rural areas were negative to MRT, which led to the observation that local cows were either not exposed to brucella infection or they are naturally immune to the infection. The Hotis-Miller test was also found to be useful in deteching streptococcal mastitis. The result of this test reveals that the samples of udder secretions which show positive reactions, are also found positive by Card test. This means that the Card test like the Hotis-Miller test detects any abnormal changes in the character of milk that follow streptococcal invasion of the udder.

Table 2 represents the percentage distribution of 350 bacterial isolates of milk samples and the result of their antibiotic sensitivity. This test is important in the treatment of mastitis to determine which antibiotics are mostly likely to be effective in treatment. The bacterial isolates obtained from udder secretions exhibited a variety of mastitis organisms. Staphylococci ranked the highest in percentage. The recovery of Staphylococci and Streptococci from udder secretion calls for active consideration in respect of taking prophylactic measures with antibiotic therapy during the dry period. The distribution of these organisms in milk as observed indicate the subclinical form of mastitis. The presence of coliforms in milk stressed the need of hygienic and sanitary measures to be adopted in the dairy farm. The percentage of Gram-positive bacillus (aerobic spore-formers) in milk, although quite high, produced no priority to the ascription of any abnormality. These organisms are usually non-pathogenic and are common contaminants of milk which may get entry into milk in high percentage due to lack of proper hygienic management. *Corynebacterium* includes animal parasites and pathogens, plant pathogens and soil-dwelling (saprophytic) organisms. Few species of *Corynebacterium* were isolated from most freshly drawn milk samples, whether from udders which were normal or from those presenting a subclinical mastitis. The occurrence of *Pseudomonas* suggested the abnormal quality of milk which might have gained access from water contaminats.

Table 1. Results of laboratory macroscopic test of milk samples obtained from apparently healthy udder

| Laboratory test performed | Sources of milk samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Bangladesh agricultural university dairy farm | | | Savar dairy farm | | | Domestic holdings of rural areas | | |
| | Total number of samples examined | Number of positive samples | Percentage of incidence | Total number of samples examined | Number of positive samples | Percentage of incidence | Total number of samples examined | Number of positive samples | Percentage of incidence |
| Hotis and Miller Test | 250 | 10 | 4% | 260 | 16 | 6.15% | 350 | 11 | 3.14% |
| Card Test | 250 | 28 | 11.20% | 260 | 26 | 10% | 350 | 44 | 12.57% |
| Milk-Ring Test | 250 | 5 | 2% | 260 | 29 | 11.15% | 350 | 0 | 0% |

Table 2. Distribution of 350 bacterial isolates obtained from milk samples and their sensitivity of various antibioties

| Bacterial isolates | Number and (percentage) of bacterial isolates among total isolates recovered | Number of (percentage) of bacterial isolates among individual variety showing inhibition to antibiotic sensitivity test. | | | |
|---|---|---|---|---|---|
| | | Penicillin | Streptomycin | Terramycin | Choloromycetin |
| Staphylococci | 147(42%) | 66(44.89%) | 36(24.48%) | 110(74.82%) | 132(89.79%) |
| Streptococci | 44(12.57%) | 14(31.81%) | 8(18.18%) | 35(79.54%) | 39(88.63%) |
| Coliforms | 77(22%) | 30(38.96%) | 55(71.42%) | 61(79.22%) | 68(88.31%) |
| Gram positive Aerobic spore Forming bacilli | 70(20%) | 38(54.28%) | 8(11.42%) | 62(88.57%) | 64(91.42%) |
| Pseudomonas | 5(1.43%) | 2(40%) | 4(80%) | 4(80%) | 5(100%) |
| Corynebacterium | 7(2%) | 5(71.42%) | 3(42.85%) | 5(71.42%) | 7(100%) |

The antibiotic sensitivity of 350 isolates revealed that Chloromycetin was most effective. Out of the total 350 isolates, 155, 144, 277 and 315 isolates were sensitive respectively to Penicillin, Streptomycin, Terramycin and Chloromycetin antibiotics *in vitro*.

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