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BACTERIOLOGICAL STUDIES ON MILK WITH PARTICULAR REFERENCE TO PUBLIC HEALTH.

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Several different organisms were isolated from the milk sampled from the city of Lyallpur. All samples were negative for *Brucella abortus*. All the mastitic milk samples examined revealed the presence of a great number of mononuclear cells, few scattered neutrophils and a large number of disintegrated leucocytic cells.

INTRODUCTION

Milk is an important human food and is taken by the people all over the world. Almost all the countries of the world have faced various disease problems at one time or another through the agency of this human diet. As compared with advanced countries of the world, hygienic conditions are very poor in our country and chances of contamination of milk with different pathogenic organisms are greater. This study was therefore designed to know the various types of organisms present in milk available for human consumption.

REVIEW OF LITERATURE

Rice and Markley (1924) examined the milk of a number of cows and noted variation in acidities from 0.086 to 0.229 per cent and in pH values from 6.432 to 6.893. They also found that high acid milk contained more nutrients and was particularly high in phosphates. They further noted that dilution of milk with water causes decrease in its acidity due to hydrolysis of both phosphates and caseinates.

According to Petersen (1939), mastitic milk usually turns alkaline in its reaction. Colostrum milk and milk retained in the udder for 2 or more days is also alkaline. Malek and Gibson (1948a) examined milk samples particularly for streptococci. These samples were plated in duplicate on meat infusion agar containing 1 per cent tryptone and 0.25 per cent glucose. The streptococcal species isolated were bovine mastitis-organisms, *Streptococcus bovis* and *Streptococcus thermophilus*, *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus faecalis* and its variants and hetero-fermentative streptococci. The same authors (1948 b) isolated staphylococci

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from raw milk samples. The species of micrococci were *Micrococcus lutexts* and *M. varians*

Gibson and Malek (1957) made a bacteriological examination of various classes of milk and studied their growth potentials under different conditions. The organisms isolated were streptococci, staphylococci, micrococci, diptheroids, *Bacillus subtilis* and various other species of the genus bacillus.

According to Smith (1959) diseases of the udder, such as mastitis usually decrease casein content and curd tension, decrease lactose content and increase chlorides of milk. There is a drop in Vitamin C content of milk and a shift towards more alkaline reaction, which in most instances is probably due to greater permeability of cell walls to the bicarbonates.

Kalara and Dhanda (1964) reported incidence of mastitis in cows and buffaloes in North West India. The overall incidence of clinical mastitis was 10.23 per cent in cows and 11.08 per cent in buffaloes in urban area compared with 7.55 per cent and 8.8 per cent, respectively in rural areas. Staphylococci were the chief cause of clinical mastitis in both animals. In latent infections staphylococci were equally important. In Streptococcal infections *Str. agalactiae* and *Str. dysgalactiae* were almost equally distributed.

Ghumman (1967) studied the aetiology of mastitis in buffaloes in Lyallpur district. One hundred and twenty five samples were examined. *Staphylococcus aureus* was isolated from 114, *E. coli* from 2 and *Pseudomonas aeruginosa* and *Str. agalactiae* from each one sample of milk. No organisms could be detected in 7 milk samples.

Thompson and Houston (1967) studied an incidence of bovine mastitis in dairy herds in two districts in Victoria The two regions were denoted as A and B. Clinical and sub clinical mastitis due to pathogenic streptococci was found in 29 per cent of cows in A region, and 49 per cent of cows in B region. Mastitis due to *Staphylococcus aureus* was found in 41 per cent of cows in A region and 32 per cent of cows in B region.

Naqvi (1972) studied the microflora of 150 composite samples of milk routinely supplied in market in Lyallpur city. The organisms isolated were *Escherichia coli*, 99 strains; *Aerobacter aerogenes*, 72 strains; *Staphylococcus aureus* 137 strains; *Bacillus cereus* 127 strains; and *Bacillus megagerium*, 13 strains. The media used for primary isolation of organisms were milk agar and desoxycholate agar.

MATERIALS AND METHODS

A total of 65 market milk samples were collected from various shops in the Lyallpur city, 25 mastitic milk samples and samples from 10 animals suspected of *Mycobacterium tuberculosis* infection were collected from the University Veterinary Hospital and the Civil Veterinary Hospital, Lyallpur. All samples were tested with haematoxylin stained brucella antigen for presence of *Brucella abortus* agglutinins in milk.

All samples were centrifuged at 3000 R.P.M. for 30 minutes and cultured on blood agar at 37C° for 24 hours for primary isolation of organisms. Samples suspected for *Mycobacterium tuberculosis* infection were centrifuged at 3000 R. P. M. for 30 minutes and sediment cultured on Lowenstein Jensen Medium, blood agar and incubated at 37C° for 6 weeks and 24 hours respectively.

One ml of sediment from each milk sample was also injected into two guinea pigs intraperitoneally. After 3 weeks, the animals were tested with tuberculin. The pH of milk was determined by Backman pH meter. The cellular picture of mastitic milk samples was seen by staining smears with Giemsa' method of staining and examining under the microscope.

All organisms isolated were subjected to *in vitro* sensitivity tests to streptomycin, penicillin, chloramphenicol and erythromycin antibiotic discs.

RESULTS

The biochemical reactions of the organisms other than the species of the genus streptococcus are shown in Table 1. The growth on various media and biochemical reactions of various species of the genus streptococcus are shown in Table 2.

The organisms isolated from 65 normal milk samples were *Staphylococcus aureus*, 20 strains; *Staphylococcus epidermidis*, 38 strains; Bacillus cereus, 25 strains; *Escherichia coli*, 35 strains; *Aerobacter aerogenes*; 25 strains; *Bacillus megaterium*, 6 strains; *Streptococcus pyogenes* 10 strains; *Streptococcus faecalis*, 15 strains; and *Streptococcus lactis*, 20 strains.

Three out of 25 mastitic milk samples did not show any growth. The organisms isolated from the remaining 22 samples were *Staphylococcus aureus*, 19 strains; *Streptococcus agalactiae*, 10 strains; *Streptococcus dysgalactiae*, 2 strains; and *Escherichia coli*, 4 strains.

All the milk samples were negative for *Brucella abortus*.

TABLE 1. *Biochemical reactions of the organisms other than the species of the genus streptococcus, isolated from 100 milk samples.*

Organisms	No. of strains	Glycerol	Glucose	Maltose	Lactose	Mannitol	Motility	Litmus milk	Coagulaseest	Catalase test	Indole production	Methyl red test	Voges Proskauer test
Staphylococcus aureus	42	l	l	l	l	l		AC	+	+	—	+	+
Staphylococcus epidermidis	46	l	l	l	±	±		A	—	+	—	+	+
Escherichia coli	43	(+)	(+)	(+)	(+)	(+)	+	AC		+	+	+	—
Aerobacter aerogenes	25	(+)	(+)	(+)	(+)	(+)	—	AC		+	—	—	+
Bacillus cereus	28	l	l	l	—	l	+	P		+	—	—	+
Bacillus megaterium	6	l	l	l	—	l	+	P		+	—	+	—

l = Acid without gas (+)= Acid and gas + = Posive or negative — = Negative C= Clotting P= Peptonization

Cultural and animal inoculation tests performed for ten milk samples suspected for *Mycobacterium tuberculosis* infection revealed that they were negative for these organisms. The other organisms isolated from these samples were *Staphylococcus aureus*, 3 strains; *Staphylococcus epidermidis*, 8 strains; *Escherichia coli*, 4 strains; *Streptococcus agalactiae*, 6 strains; and *Bacillus cereus*, 3 strains.

pH of Milk: The pH of the 65 market milk samples determined by Backman pH meter was found to be ranging from 6.5 to 6.9, while pH of the mastitic milk samples and samples suspected for *Mycobacterium tuberculosis* infection ranged from 6.7 to 7.4.

Cellular Picture of Mastitic Milk Samples: All the mastitic milk samples examined revealed the presence of a great number of mononuclear cells, few scattered neutrophils, and a large number of disintegrated leucocytic cells.

Antibiotic Sensitivity test. The maximum inhibitory zones were observed with streptomycin and chloramphenicol. Erythromycin produced comparable inhibitory zones but of slightly smaller diameter. Penicillin was found to be less effective in inhibiting the growth of the organisms.

TABLE 2. *Growth on various media and biochemical*

Organism	No. of strains	Growth on blood agar	Growth in broth at pH 9.6	Growth in broth containing 6.5% NaCl
Streptococcus pyogenes	10	beta haemolytic	—	—
Streptococcus agalactiae	16	beta haemolytic	—	—
Streptococcus dysgalactiae	2	beta haemolytic	—	—
Streptococcus faecalis	15	alpha haemolytic	+	+
Streptococcus lactis	20	alpha and non haemolytic	—	—

DISCUSSION

The organisms isolated from 65 market milk samples were *Staphylococcus aureus*, 20 strains; *Staphylococcus epidermidis*, 38 strains; *Escherichia coli*, 35 strains *Aerobacter aerogenes* 25 strains; *Bacillus cereus*, 25 strains; *Bacillus megaterium*, 6 strains; *Streptococcus pyogenes*, 10 strains; *Streptococcus faecalis*, 15 strains; and *Streptococcus lactis*,, 20 strains. The presence of these organisms in milk has been reported by Naqvi (1972), Malek and Gibson (1948a), Malek and Gibson (1948b) and Gibson and Malek (1957).

From 25 mastitic milk samples the organisms isolated were *Staphylococcus aureus*, 19 strains; *Streptococcus agalactiae*, 10 strains; *Streptococcus dysgalactiae*, 2 strains; and *Escherichia coli*, 4 strains. These findings are in agreement with Ghumman (1967), Kalara and Dhanda (1964) and Thompson and Houston (1967). The increase in number of udder infections with *Staphylococcus aureus* could be attributed to introduction of antibiotic therapy. Studies have shown that many strains which were antibiotic sensitive initially have become resistant to antibiotics.

pH of market milk ranged from 6.5 to 6.9. These findings are in accordance with Rice and Markley (1924). pH of mastitic milk was found to be slightly alkaline. These findings are in agreement with Petersen (1939) and Smith (1959).

Reactions of different species of the genus streptococcus.

Growth on blood agar containing 40% bile.	Growth in milk containing 0.1% Methylene blue	Maltose	Lactose	Sucrose	Mannitol	Glycerol	Catalase	Litmus milk
—	—	+	+	+	—	—	—	A
+	—	+	—	—	—	—	—	AC
—	—	+	—	—	—	—	—	AC
+	+	—	—	—	—	—	—	ACR
+	+	+	—	—	—	—	—	ACR

Penicillin was found to be less effective as compared with streptomycin, chloramphenicol and erythromycin. These findings are in partial agreement with Naqvi (1972).

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