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Isolation and Biochemical Characterization of *Escherichia coli* from Bovine Mastitic Milk

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A B S T R A C T

Mastitis is an inflammation of udder which is mainly caused by coliforms and other microorganism. It is a most economically important disease in bovines. In the present study a total number of 100 bovine mastitis milk samples were collected. All the samples were inoculated into enrichment broth which was further cultured on Mac-Conkey plate. Out of these 42 samples that were showing lactose fermentation were further streaked on EMB selective agar plates. Colonies showing metallic sheen were processed for biochemical analysis. Based on the morphological, cultural and biochemical characterization indicated that 40 isolates were *E. coli*. *E. coli* is one of the most important bacterium of coliform group that causes mastitis in bovine.

Introduction

Mastitis is the most economically significant 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*, *Yersinia*, *Mycobacteria*, *Psuedomonas*, *Serratia* and other organisms like fungi, yeasts and virus (Burns *et al.*, 1996).

Escherichia coli (*E. coli*) is a Gram negative bacteria belonging to Family Enterobacteriacea. *E. coli* is a rod-shaped member of the coliform group, can be

distinguished from most other coliforms by its ability to ferment lactose at 44°C, and by its growth and color reaction on certain types of culture media. It is the lactose fermenter and produces pink colonies in Macconkey agar. When cultured on an EMB plate, a positive result for *E.coli* is a metallic green media with dark purple colonies (Kavitha and Devasena, 2013).

The aim of this study was to isolate and biochemical characterization of *Escherichia coli* from bovine mastitic milk.

Materials and Methods

Collection of samples

A total of 100 bovine mastitis milk samples were collected from the dairy herd farms in and around Bidar. All the samples were subjected to bacteriological isolation.

Isolation of *E. coli*

Inoculation into broth

In the laboratory the milk sample was inoculated into tryptose soy broth under sterile condition. After inoculation, the test tube was incubated at 37°C for overnight. (Cruickshank *et al.,* 1975) The suspected colonies were transformed onto MacConkey's agar and EMB agar. The inoculated plates were incubated for 24-48 hours at 37°C. Suspected colonies were confirmed primarily as Gram-negative rods (Cruickshank *et al.,* 1975).

Identification of *E. coli*

The pure colonies subjected for biochemical test using Indole test, Methyl red test Sugar fermentation reaction, Citrate utilization test and Voges-Proskauer test (Kavitha and Devasena, 2013).

Results and Discussion

100 Milk samples were collected from mastitis affected bovine. The samples were inoculated in Triptose soya broth, After an incubation period of 4-6 hours, the samples were streaked on differential media namely Mac Conkey agar and Eosine Methylene blue agar.

Eosin Methylene Blue agar (EMB)

Eosin Methylene Blue (EMB) Agar is used for identification of microorganism. *E. coli* produced Metallic sheen colour colonies in Eosin Methylene Blue (EMB) Agar. In the present study 42 isolates which produced Metallic Sheen colour colonies indicating the presence of *E. coli* (Fig. 1).

MacConkey agar

Mac conkey agar is used to isolate coliform. If the organism is coliform they 100 Mastitis milk samples collected, 42 samples were produced ink colour colony indicating the presence of coliform (Fig. 2).

Gram staining

Gram staining is used for identification of Gram positive and Gram negative organism. *E.coli* is a gram negative rod Shaped bacterium. In the present study the 42 isolates were subjected to gram staining all the isolates were found to be gram negative rods.

Biochemical characterization

All suspected colonies of *E.coli* on the basis of cultural and morphological properties were subjected to biochemical tests (Fig. 3 to 6). Indole test, Methyl red test Sugar fermentation reaction, Citrate utilization test and Voges-Proskauer test. 42 isolates were confirmed to be *E. coli* after the biochemical results.

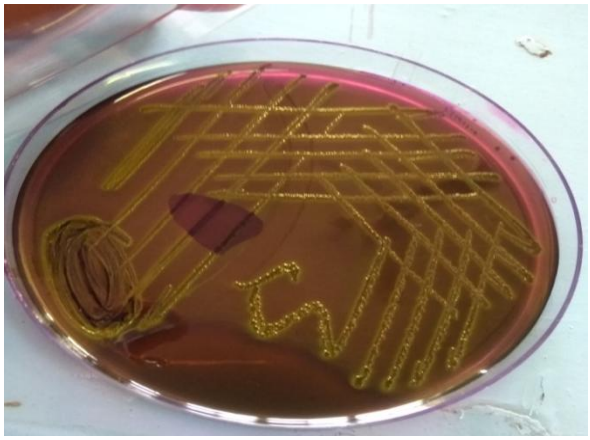

Fig.1 Eosine Methylene blue agar

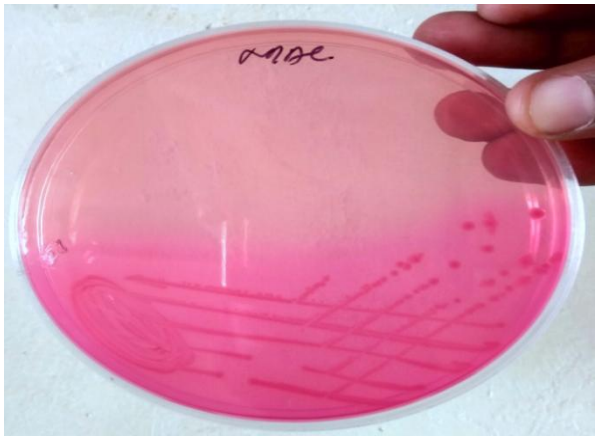

Fig.2 MacConkey agar

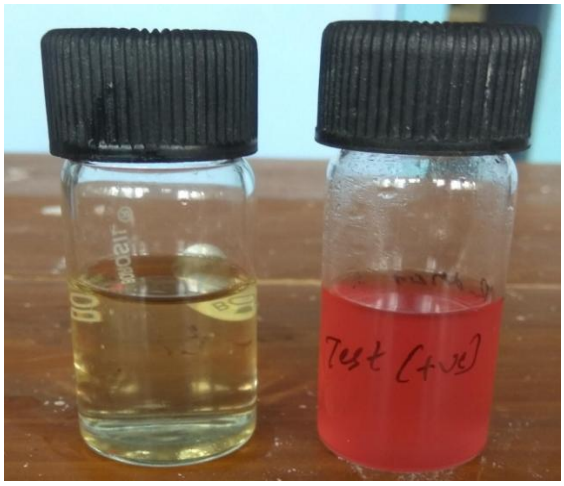

Fig.3 Methyle red test

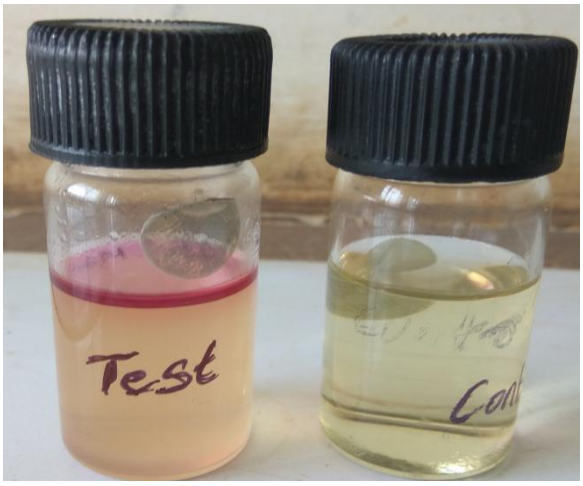

Fig.4 Indole test

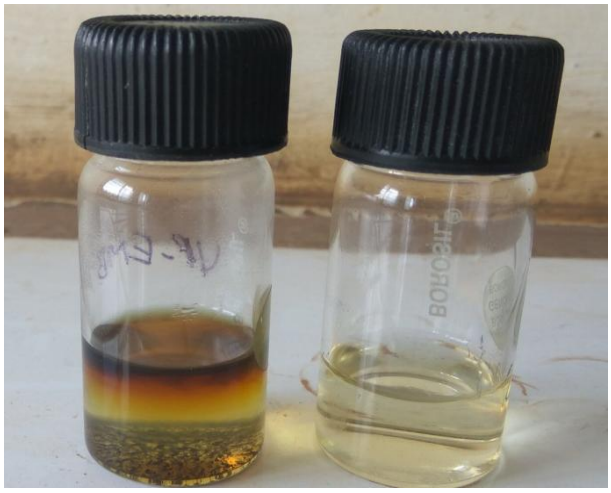

Fig.5. Voges-Proskauer test

Fig.6 Citrate utilization test

Therefore the basis of cultural characteristics and biochemical reaction 40 were confirmed to be *E.coli* (Kavitha and Devasena, 2013).

To conclude, the Coliform mastitis is caused by a group of coliform organisms. *E.coli* is one of the most important bacterium of coliform group that causes mastitis in bovine. *E.coli* isolates were isolated by culturing in Eosine Methylene blue agar and in Mac Conkey agar. The isolates were confirmed by different Biochemical Tests.

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