Isolation, identification and antibiogram study of *Escherichia coli* from the cases of mastitis

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Abstract

Context: Mastitis is the devastating diseases among the dairy farms in Bangladesh.

Objectives: To isolate, identify and antibiogram study of *Escherichia coli* from the cases of mastitis among suspected dairy farm.

Materials and methods: The study was conducted to isolate, identify and antibiogram study of significant bacterial pathogens causing mastitis in dairy cow. The study was conducted on 50 suspected dairy cows from 5 commercial dairy farms and Upazilla veterinary hospitals during the period from March, 2011 to August, 2011 of Gaibandha district under the Department of Microbiology Hajee Mohammed Danesh Science and Technology University, Dinajpur, Bangladesh.

Results: Result found on milk samples from different sources were 30% in positive for *Escherichia coli*, 20% were found to be positive for Staphylococcus *species.* 20% for *Streptococcus species*, 14% were *Corynebacterium species* and16% were undefined species of mixed bacteria. Results of the antibiograms among various isolates of *Escherichia coli* were found to be highly sensitive (+++) to Chloramphenicol (70%), Kanamycin (70%) and Nitrofurantoin (90%).

Conclusion: Among the isolated bacterial pathogen *Escherichia coli* plays significant role (30%) on mastitis infection and it is highly sensitive to Nitrofurantoin, Chloramphenicol and Kanamycin. As Nitrofurantoin has the huge public health hazard, so drug of choice can be the Chloramphenicol and Kanamycin against *Escherichia coli.*

Key wards: Mastitis, bacterial pathogen, dairy farm, antibiogram, drug of choice.

Introduction

Bangladesh is an agricultural country. Livestock plays one of the important sources of our national economy. Commercially milk production plays an important role to alleviate poverty and to improve the economic status of the people through self-employment. The total cattle population of the country is about 24.50 million, which is about 1.79% of the world and 5.47% of Asian cattle population (FAO, 2004). In the last 10 years the cattle population has increased by 0.30% in contrast with 0.40% of the world. Number of cattle per livestock household is 3.50 (Saadullah and Hossain, 2000) and that of 0.94 for all household (BBS, 2002). There is no statistics of cattle yet according to type or variety in the country. Total milk production of cattle is 78200 MT (SAIC 2003).There are about 24 million cows present in our Bangladesh (DLS, 2008-09). The number of milking cow in Bangladesh is 3.75 million, which is 35.00% of the total population of Bangladesh (DLS, 1998). The annual milk production in Bangladesh is nearly 1.62 million metric ton which is very low in respect of our demand which is nearly 9 million metric ton (DLS, 1998). Domestic and small cow farming have occupied important source of income of our rural peoples. The domestic house holders, small farmers however are facing a great problem with the diseases of udder of their animals and this has become a threat to their economy. Inflammation of udder or mastitis needs to be thoroughly studied with respect to the etiologic agents and holistic approaches.

Bovine mastitis (both clinical and subclinical) is mainly caused by bacterial infection in the udder (Abdella, 1996) but some determinant factors such as inadequate hygiene and management practices, faulty technique of milking, high milking machine pulsation with vacuolization, pendulous udder with long teats, larger size of teat orifice in high yielding cows, traumatic injury etc. may play an important role and lead to incitedeveloping infection of mastitis.pathogens from the milk (Handique *et al.,*1988). Many bacteria have been implicated as causes of mastitis such as *Escherichia coli*, *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus pyogens, and Staphylococcus aureus*. (Kalra *et al.,*1962).

There are two main source of infection in mastitis, the infected udder and environment. In dairy cattle the important infections are those which persist readily in the udder, especially *Escherichia coli*, *Streptococcus agalactiae* and Staphylococcus aureus.

The organisms of Escherichia coli are divided into pathogenic and non pathogenic based on their ability to cause of disease. Pathogenicity of Escherichia coli. Strains are due to the presence of one or more virulence factor including invasiveness factors invasions, heat labile, heat stable, enterotoxigens, verotoxigens and colonization factors or adhesions (Smith and Haibs, 1967).

Pathogenic *Escherichia coli* are divided into two types namely as enteropathogenic *Escherichia coli* and Uropathogenic *Escherichia coli.* Further pathogenic *Escherichia coli* are grouped into enterotoxigenic *Escherichia coli* (ETEC). Enteropathogenic *Escherichia coli* (EPEC), enteroinvasive *Escherichia coli* (EIEC), enteroaggregative *Escherichia coli* (EAggEC), enterohaemorrhagic *Escherichia coli* (EHEC). Arter (1986).

The severity, frequency and economic impact of mastitis depend upon preventive and management approaches. Sub-optimal management such as improper milking hygiene, poor house hygiene and lack of use of post milking teat dipping (Mdegela *et al.,*2004), poor nutrition and various diseases adversely affect the productive efficiency of dairy animals (Ali *et al.,* 2008). There exists a distinct difference in incidence and the patterns of causative agents from place to place, herd to herd and even time to time.

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In the diagnosis and control of mastitis, laboratory procedures are of value in the diagnosis of milk samples for cells, bacteria and chemical changes and for testing the sensitivity of bacteria to speciesecific drugs. Culturing of milk samples is a standard method of examination for mastitis. In mastitis control, the costs of bacteriological culture can be greatly reduced by screening the cows with an indirect test first and then culturing the positive reactors (Samad, 2008). The chemotherapeutic usefulness of antimicrobial drugs in treating infectious mastitis of domestic animals has been well documented (Samad, 2008; Einstein *et al.,*1994). The disease occurs in the cow, buffalo, ewe, doe, and sow and rarely in the mare (Verma, 1994). From the above, somatic cell count (SCC) is a measure that is widely used to assess mammary health (Smith, 2002). Milk SCC includes all types of cells: polymorpho nuclear leukocytes (PMN), macrophages and lymphocytes. An increase in SCC is due largely to an increase in PMN. The primary function of PMN is ingestion and destruction of invading microorganisms as well as secretion of inflammatory regulators (Kelly *et al.,* 2000). Some researchers consider the PMN count as an earlier and more specific indicator than SCC (Kitchen, 1981; O'Sullivan *et al.,* 1992).

Various indirect tests have been described (Schalm *et al.,* 1971) to detect the SCC but each test has some limitations as it fails to detect at all stages of mastitis. The somatic cell count and bacterial load Count (BLC) are two methods accepted reliably for detecting the early infection (Schalm *et al.,* 1971). In addition, some indirect tests like White Side Test (WST) and California Mastitis Test (CMT) have been developed for rapid screening the infection in the udder (Schalm *et al.* 1971; Rasool *et al.,* 1985; Shukla and Superkar 1984; Ali *et al.,* 1989; Guha *et al.,* 1989).

Rapid detection of udder infection is basic requirement for the outcome of treatment to prevent the loss of milk production as well as species spread of infection. Considering the above facts, the present research works have been undertaken with following objectives:

- To study the prevalence of Clinical and sub clinical mastitis of cow in Gaibandha district
- To isolate and identify the *Escherichia coli* associated with Clinical and sub clinical mastitis in cow.

Materials and methods

The study was carried out during the period from March, 2011 to August, 2011 in the department of Microbiology Hajee Mohammed Danesh Science and Technology University, Dinajpur, Bangladesh. The detailed outline of the materials and methods are given below:

Husbandry and management system

The cows are managed under intensive husbandry system with semi paca floor in dairy farm and in local farms the animals are reared by traditional system. They are often provided with some green grass in addition to natural pasture and concentrate diet and are kept together in common shed. In local farms, they are gazed at day time and kept in kacha floor as in traditional housing system.

Survey design and sampling

A cross sectional observational study was conducted in cross-breed dairy farm, indigenous lactating cow and various dairy cows of different house in Palashbari, Sadullapur, Gobindogonj Upazila of Gaibandha district.

Selection of experimental animals

Clinically affected and sub clinically suspected Mastitis cow were selected from the commercial dairy farm, Veterinary hospital and lactating cow of different farmer of the study area were selected for the study. Samples collected from healthy and infected teat from the diseased cow. Samples were also collected from apparently healthy dairy cow.

Collection of milk sample

Milk samples were collected by the soaking the teat with Povidone Iodine and drying off by tissue paper, one to two drops of milk was discarded and then 2-3 ml of milk was taken from each quarter into labeled sterilized test tubes with rubber cap\screw cap or cotton Plaque.

Results and Discussions

A total of 50 milk samples were collected from different area of Gaibandha district (Table 01) in sterilized test tube following aseptic measures. Out of 50 milk samples 29 were collected from clinically mastitis infected cows, 11 milk samples were collected from sub-clinically suspected cows and the rest 10 milk samples were collected from healthy cows.

Of the samples tested, 18.04% and 22.03% animals, and 15.05% and 16.81% quarter wise were found to be positive for sub clinical mastitis by using California Mastitis Test (CMT) and Somatic Cell Count (SCC) respectively. Prevalence was found by CMT compared to SCC. The CMT result was somewhat lower than the report of Schmidt *et al.,*(2009) who reported 22.50%. The result of Somatic Cell Count (SCC) revealed the prevalence of sub clinical mastitis 16.81% in the present study which some what lower than the results of Min *et al.,* (2007) who reported 19-30% positive result.

The highest prevalence (80.00%) of sub-clinical mastitis was recorded at 2-3 years aged group and lowest prevalence (6.25%) of SCM at 5-6 years aged group. The highest prevalence (66.66%) of SCM was found at lactation whereas the lowest prevalence (4.76%) of SCM at the 5th or 6th lactation period. These findings support the report of Neelesh-Sharma *et al.,* (2007) who reported the highest prevalence of SCM during the 1st lactation and at 2 to 3 years of age in cows. An obvious trend of increasing prevalence of SCM to the stage of lactation observed in this study. Highest prevalence (50.00%) of SCM was found at early lactation stage whereas lowest prevalence (23.68%) of SCM was found at mid lactation stage (4-8 weeks). These observations were also support to the report of Neelesh-Sharma *et al.,* (2007) who observed the infection rate higher during the 1st lactation followed by early and mid-stage of lactation.

The bacteria isolated from the positive cases in this study were *Escherichia coli*. *Staphylococcus aureus*, *Staphylococcus epidermidis, Bacillus subtilis* and Gram negative rods. Aydn *et al.,* (2007) and Kostelic *et al.,*(2009) also isolated these bacteria from milk samples originating from sub clinical mastitis of cow. Among 50 milk samples, 15 (30.00%) were found positive for *Escherichia coli*, 10 (20.00 %) were found to be positive for Staphylococcus *species.*, 10(20.00%) for *Streptococcus*

species, 7 (14.00%) for *Corynaebacterium species*, and8 (16.00%) were undefined species. of mixed bacteria. Cultural examination revealed that *Staphylococcus* species as the predominant organisms followed by other bacteria. These findings are in agreement with the earlier report that made by Mhase *et al.,* (2007) in which they reported *Staphylococcus aureus* as the highest (34.38%) frequency, followed by *Bacillus* in12.50 % *Escherichia coli* (25.00%) in cow.

The frequency distribution of different species of bacterial isolates in different milk samples were found variable. Results of the present study indicate that all the four different types of bacteria were not present in the same milk sample collected from sub clinical mastiticcows milk.

The antibiograms of various isolates of *Escherichia coli* were found to be highly sensitive (+++) to Chloramphenicol (70.00%), Kanamycin (70.00%) and Nitrofurantoin (90.00%), moderately sensitive (++) to Erythromycin (50.00%), Less sensitive (+) to nalidaxic acid (35.00%), Cephalexin (35.00%), Amoxycilline (35.00%) and resistant to Cloxacilin, Ampicillin, Cephradin.

The results of isolation, identification, biochemical test, frequency distribution and antibiotic sensitivity of the bacteria isolated from milk of sub clinical mastitis of cow in the present study, indicated that the microbial factors play an important role for the development of caprine sub clinical mastitis. Detailed further epidemiological study about the extrinsic and intrinsic factors, which might have direct or indirect influence on the development of sub clinical mastitis in association with microbs are necessary.

Frequency of bacteria isolated from the milk of clinically mastitis affected cows:

The result of frequency of different bacterial isolates are presented in Table-1. A total of 50 milk samples were examined for the isolation of bacteria.

Table 1. Frequency of isolates in milk samples.

Name of isolated organisms	No. of isolated organisms	Percentage (%) of isolated organisms
Escherichia coli	15	30
Staphylococcus species.	10	20
Streptococcus species.	10	20
Corynebacterium species.	7	14
Unidentified	8	16
Total	50	100

Results of cultural examination

Nutrient broth: The organisms of the milk sample produced turbidity in nutrient broth.

Nutrient agar: After incubation on nutrient agar organisms were produced circular, smooth and colorless colonies.

Salmonella-Shigella agar: The organisms of the milk sample produced slight pinkish smooth colonies were tentatively choosen to be *Escherichia coli.*

MacConkey (MC) agar: After overnight incubation on MacConkey agar suspected organisms were produced bright pink colonies. (Table 4)

Eosine methylene blue (EMB) agar: The greenish-black colonies with metallic sheen on Eosine methylene blue (EMB) agar were presumptively selected *Escherichia coli.* (Table 4).

Staphylococcus 110 agar medium: *Escherichia coli* did not produce colonies where as staphylococcus produce whitish colonies.

Results of Gram's stain: In Gram's staining under microscope the organisms revealed Gram-negative, pink color, small rod shaped arranged as single or paired.

Table 2. Results of cultural, morphological and motility characteristics of the isolates of *Escherichia coli.*

Source of sample	Colonies characteristics			Staining characteristics	Motility
	SS agar	MC agar	EMB agar		
Milk sample of SCDF, Palashbari, Gaibandha.	Slight pinkish smooth colonies.	Bright pink color smooth transperent colonies.	Greenish black colonies with metallic sheen.	Pink short rod, gram negative bacilli.	+
Milk sample of HDF, Sadullapur, Gaibandha.	Slight pinkish smooth colonies.	Bright pink color smooth transperent colonies.	Greenish black colonies with metallic sheen.	Pink short rod, gram negative bacilli.	+
Milk sample of SDF, Gobindogonj, Gaibandha.	Slight pinkish smooth colonies.	Bright pink color smooth transparent colonies.	Greenish black colonies with metallic sheen.	Pink short rod, gram negative bacilli.	+
Milk sample of PTVH, Gaibandha.	Slight pinkish smooth colonies.	Bright pink color smooth transperent colonies.	Greenish black colonies with metallic sheen.	Pink short rod, gram negative bacilli.	+
Milk sample of StDF, Palashbari.	Slight pinkish smooth colonies.	Bright pink color smooth transperent colonies.	Greenish black colonies with metallic sheen.	Pink short rod, gram negative bacilli.	+

SS = Salmonella-shigella; MC = MacConkey; EMB = Eosine-methylene blue; + = Positive. **SCDF =** Shilpi commercial dairy farm,

HDF = Hossain dairy farm, **SDF =** Saha dairy farm,

PTVH = Palashbari Thana Veterinary Hospital,**StDF =** Sattar dairy farm,

Table 3. Results of biochemical characteristics of *Escherichia coli*.

Different biochemical tests	Result
. Fermentation reaction with five basic sugars	
Dextrose	+
Sucrose	+
Fructose	+
Maltose	+
Mannitol	+
Indole	+
MR	+
VP	_
MIU	+
TSI	+

+ = Positive, - = Negative, MR = Methyl red, VP= Vogesproskaur, MIU = Motility Indole Urease , TSI = Triple sugar Iron.

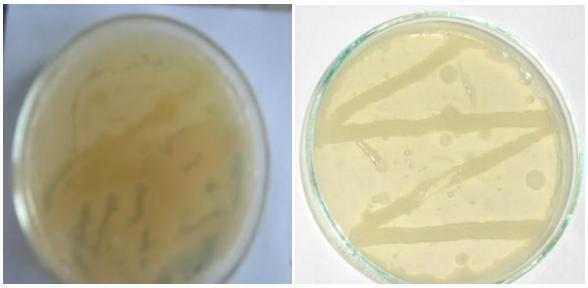


Plate 01-02: Growth of the organisms on Nutrient agar plates showing smooth, colorless colonies.

Plate 03: The organisms of the milk sample produced golden yellow color colonies on Staphylococcus 110 agar media.

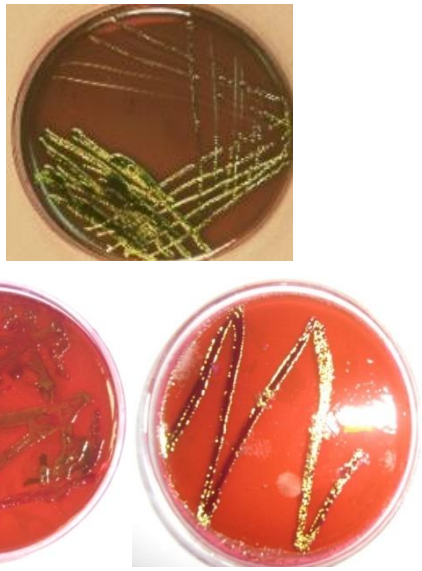


Plate 04-06: Growth of *Escherichia coli* on EMB agar showing greenish black colonies with metallic sheen.

Plate 07: Growth of *Escherichia coli* on MacConkey agar showing bright pink color colonies.

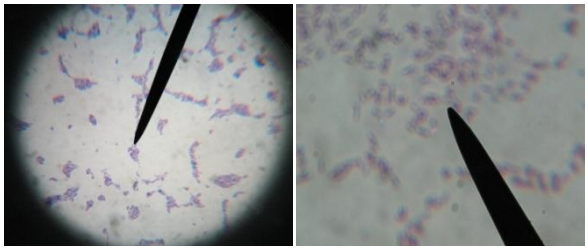


Plate 08-09: Gram's staining of *Escherichia coli* showing gram negative, pink color, short rod shaped organismss which are arranged singly in pairs or in short chains.

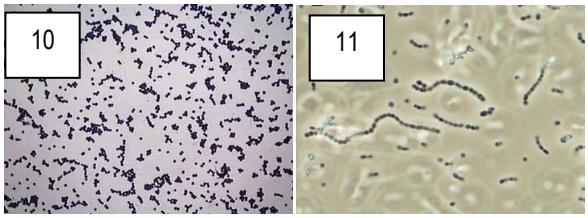


Plate 10-11: *Staphylococcus*species. Showing Gram positive (violet color)speciesherical cocci and arrenged in groups or grap like cluster at 100X (Gram's staining) and 11 *Streptococcus*species. Showing Gram positive (violet color) speciesherical or ovoid and chain formed arrenged in pairs at 100X (Gram's staining).

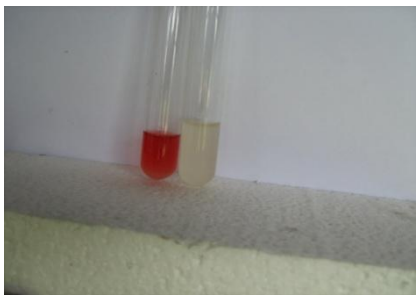
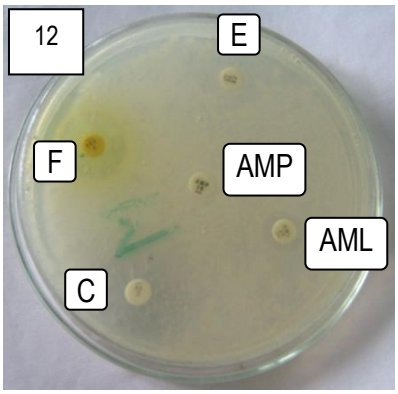
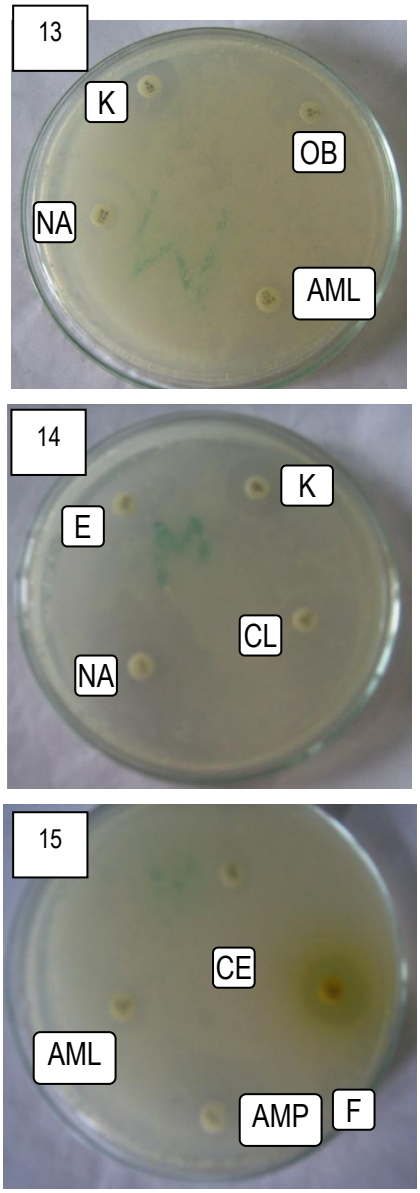


Plate 12: *Escherichia coli* showing positive results with bright red color in methyl red (MR) test (left) and Control (right).





Plates 13-15: Multi drug sensitivity and resistant of *Escherichia coli* are showing high sensitivity to C, F, K; Moderate sensitivity to E; Less sensitivity to NA, CL, AML and resistant to CE, AMP, OB on nutrient agar plate.

Legends: Amoxycillin = AML, Cephradin = CE, Chloramphenicol = C, Cephalexin = CL, Kanamycin = K, Nalidaxicacid = NA, Nitrofurantoin = F, Cloxacilin = OB, Ampicillin = AMP and Erythromycin = E .

Conclusion

- The major bacteria isolated from the milk samples of mastitis infected cows were *Escherichia Coli Staphylococcus* species, *Streptococcus species, Corynebacterium species,* and other undefined species of Gram positive and Gram-negative bacteria.
- Among the isolated bacterial pathogen *Escherichia coli* plays significant role (30%) on mastitis infection.
- *Escherichia coli* is highly sensitive to Nitrofurantoin, chloramphenicol and Kanamycin. As Nitrofurantoin has the huge public health hazard, so drug of choice can be the chloramphenicol and Kanamycin against *Escherichia coli.*

Authors contribution

Successfully isolate the *Escherichia coli* from mastitis infected milk and find out the drug of choice for the treatment against Escherichia coli infected cases.

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