

Original Research Article

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Identification and Characterization of *Escherichia coli* Isolates in Bovine Mastitis Milk from Bhubaneswar, Odisha, India

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ABSTRACT

Escherichia coli is one of the most commonly infecting bacterial etiology of the Bovine mastitis. The coliform mastitis is the most difficult type to treat due to increase in antibiotic resistance and generally cause recurrent and chronic form of mastitis. Therefore this present study was done to determine the prevalence rate of *E. coli* from the bovine mastitis milk and its antibiogram status. 250 mastitis milk samples were collected from the bovines. 27.2% (n= 68) *E. coli* isolates were identified in the samples. The isolated *E. coli* also showed biofilm forming ability in 51 isolates using Congo red agar method. Gentamicin is most sensitive drugs against *E. coli* isolates whereas ampicillin and tetracycline showed highest resistance. For the biofilm forming isolates, gentamicin is most effective whereas ampicillin followed by tetracycline and ofloxacin showed higher ineffectiveness. It was evident that the sessile form of *E. coli* isolates are more resistant to antibiotics compared to their planktonic forms. The multidrug resistance in *E. coli* isolates was also reported.

Keywords

Escherichia coli,
Mastitis,
Antibiogram status
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Introduction

The mastitis in bovines is a predominant dairy disease. It results in major economic losses in dairy farms, caused by the decrease in milk yield, increased veterinary cost and increase in culling and mortality (Melchior *et al.*,

2006). *Staphylococci*, coliforms, *Enterococci* and *Streptococci* are frequently isolated from cows with mastitis (Smulski *et al.*, 2011). It affects the physical, chemical, biological, organo-leptic, bacteriological and other properties of the milk. The loss due to the decrease in milk production and other

associated losses are more severe in case of subclinical mastitis than those occurred due to clinical cases. Preventing subclinical mastitis in a dairy farm can reduce the losses in milk production. As the annual losses to dairy industry due to mastitis is documented around Rs 7,165 crore in India (Anonymous, 2011). The mastitis milk may accidentally contaminate bulk milk on mixing and enters the food chain which poses a great threat to public health (Hameed *et al.*, 2006).

Biofilm is a complex assemblage formed by single or more species of bacteria that is encased by an exopolysaccharides matrix (Zhu *et al.*, 2009). It is found that many pathogenic veterinary bacteria do exist predominantly as biofilm or sessile organisms within or around animal tissue. It is well recognized by many researchers that biofilm producing infections are extremely difficult to treat (Morck *et al.*, 1989; Sandholm *et al.*, 1990; Baselga *et al.*, 1993 and Potera, 1999). The biofilm formed has been also considered as a virulence factor contributing to persistent infection (Christensen *et al.*, 1982; Arciola *et al.*, 2001). Antibiotic therapy is the main treatment for control of bovine clinical mastitis. Indiscriminate and large scale use of antimicrobials in farm animals leads to considerable increase in antimicrobial-resistant strains of bacteria. The antibiotic resistant can increase the treatment cost and period of disease (Sawant *et al.*, 2007). Therefore, the aim of this study was to identify and characterize *E. coli* isolates associated with bovine mastitis milk from area in and around Bhubaneswar, Odisha.

Materials and Methods

In this study 250 mastitis milk samples collected from lactating cattle (n= 172) and buffaloes (n= 78) was used. All mastitis milk samples used for the study were referred by field clinicians to either Teaching Veterinary

Clinical Complex (TVCC), C.V.Sc.&A.H., O.U.A.T or directly received in the department of Veterinary Microbiology for the antimicrobial susceptibility testing. The current study was carried out in the department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar.

All the samples were processed according to the Collins and Lyne (1970) for isolation and identification of the *E. coli* using cultural and biochemical tests. The mastitis milk is inoculated in the brain heart infusion (BHI) broth for primary cultivation of pathogenic bacteria. The MacConkey lactose agar was used for the primary isolation and Eosin Methylene blue agar and biochemical test like indole test, methyl red reduction test, voges Proskauer test, citrate reduction test and triple sugar iron test was done to confirm *E.coli*. Then all *E. coli* isolates were screened for their biofilm producing ability by using Congo Red Agar method (Cruzado-Bravo *et al.*, 2019).

The ABST for the planktonic and biofilm forming *E. coli*(n=50) bacteria isolates was conducted as per Clinical & Laboratory Standards Institute (CLSI) guidelines (Bauer *et al.*, 1966; Wayne, 2009) with modification as mentioned below. For antibiotic sensitivity testing of biofilm forming isolates, 0.3% of bentonite clay was added to Mueller-Hinton Agar (MHA), whereas for planktonic isolates bentonite clay was not added as a substrate. The inoculum was prepared by diluting the isolated colony in normal saline with turbidity matched to 0.5 McFarland turbidity standard and swabbed evenly onto the surface of MHA for planktonic growth and MHA + 0.3% Bentonite clay for biofilm growth. Antibiotic discs were ampicillin (10 mcg), enrofloxacin (10 mcg), erythromycin (15 mcg), ciprofloxacin (5 mcg),

piperacillin/tazobactam (100/10 mcg), gentamicin (10 mcg), amoxicillin/ clavulanic acid (20/10 mcg), cefoperazone (75 mcg), chloramphenicol (30 mcg), doxycycline (10 mcg), ceftriaxone (10 mcg), nalidixic acid (30 mcg), ofloxacin (5 mcg), streptomycin (10 mcg) and tetracycline (30 mcg). All the antibiotic discs were procured from HiMedia, Mumbai. Antibiotic sensitivity test (ABST) plates were incubated for 18-24 hrs at 37 °C. The results of the ABST were interpreted according to guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2009).

Results and Discussion

All results of *E. coli* screening, their biochemical testing, detection of biofilm producing ability and antimicrobial susceptibility testing for bacterial isolates were studied. In this study, 68 *E. coli* isolates were isolated from the 250 bovine mastitis milk samples. Out of total *E. coli* isolates, 42.65% (n = 29) of *E. coli* were isolated from buffaloes milk sample and 57.35% (n= 39) were isolated from cattle milk samples. Gram's stain of *E. coli* shown in figure 1. The biochemical characteristics of *E. coli* tested were given in the table 1. On screening of the biofilm formation by *E. coli* isolates using the

Congo red agar method showed 75% (n= 51) of *E. coli* isolates as positive for biofilm production and 25% (n= 17) of *E. coli* isolates were found negative.

The antibiotic sensitivity test results for planktonic form of *E. coli* isolates using 15 antibiotics showed that the most sensitive antibiotic against *E. coli* is gentamicin followed by chloramphenicol, cefoperazone, ceftriaxone, ciprofloxacin, erythromycin, enrofloxacin, doxycycline, amoxiclav, nalidixic acid, ofloxacin and streptomycin. Whereas the most resistant drug against *E. coli* isolates is ampicillin and tetracycline. The results of antibiotic sensitivity test for sessile form of *E. coli* isolates showed gentamicin followed by erythromycin, ceftriaxone, cefoperazone and doxycycline as most effective antibiotic whereas the higher degree of resistance was shown against ampicillin followed by tetracycline and ofloxacin. The antibiotic sensitivity test results for the planktonic and the sessile form of *E. coli* isolates was given in table 2. The multidrug resistance was also shown by the bovine mastitis milk associated *E. coli* isolates which further increases in the case of biofilm formation. There is no specific resistance pattern for the multidrug resistance by these *E. coli* isolates.

Table.1 Biochemical characterization of *E. coli* isolated from mastitis milk

Test	Reaction	Positive
Gram stain	Gram negative bacilli	100%
Biochemical identification test		
Indole	Red ring	100%
Methyl red	Red colour	100%
Voges proskauer	Negative	0%
Simmon's citrate	Negative	0%
Catalase	Gas bubbles	100%
Triple sugar iron	A/A/±/H ₂ S-	100%

Table.2 Depicts the comparative percentage of susceptibility shown by both planktonic and biofilm forming bovine mastitis *E. coli* isolates (n= 50isolates), to different antibiotics

Antibiotics	Planktonic <i>E. coli</i> (n= 50) (%)			Sessile <i>E. coli</i> (n= 50) (%)		
	S	I	R	S	I	R
Ofloxacin	66	24	10	42	16	42
Ciprofloxacin	84	10	6	70	10	20
Gentamicin	96	4	0	88	8	4
Streptomycin	70	16	14	20	44	36
Tetracycline	58	12	30	24	28	48
Chloramphenicol	82	14	4	72	8	20
Amoxicillin/ Clavulanic acid	74	6	20	22	44	34
Enrofloxacin	70	14	16	64	8	28
Erythromycin	86	0	14	78	6	16
Ceftriaxone	82	8	10	78	6	16
Cefoperazone	86	10	4	80	4	16
Nalidixic acid	70	16	14	66	6	28
Ampicillin	54	14	32	8	26	66
Piperacillin/ Tazobactam	76	2	22	28	36	36
Doxycycline	84	0	16	76	6	18

Figure.1 *E. coli* on gram's staining

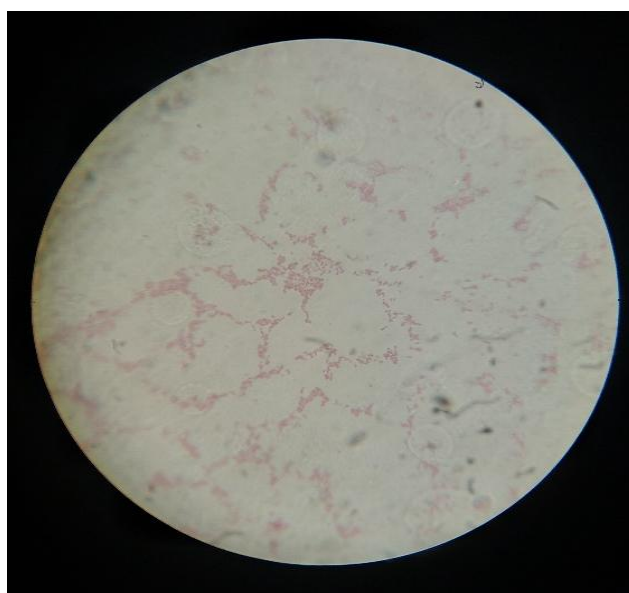
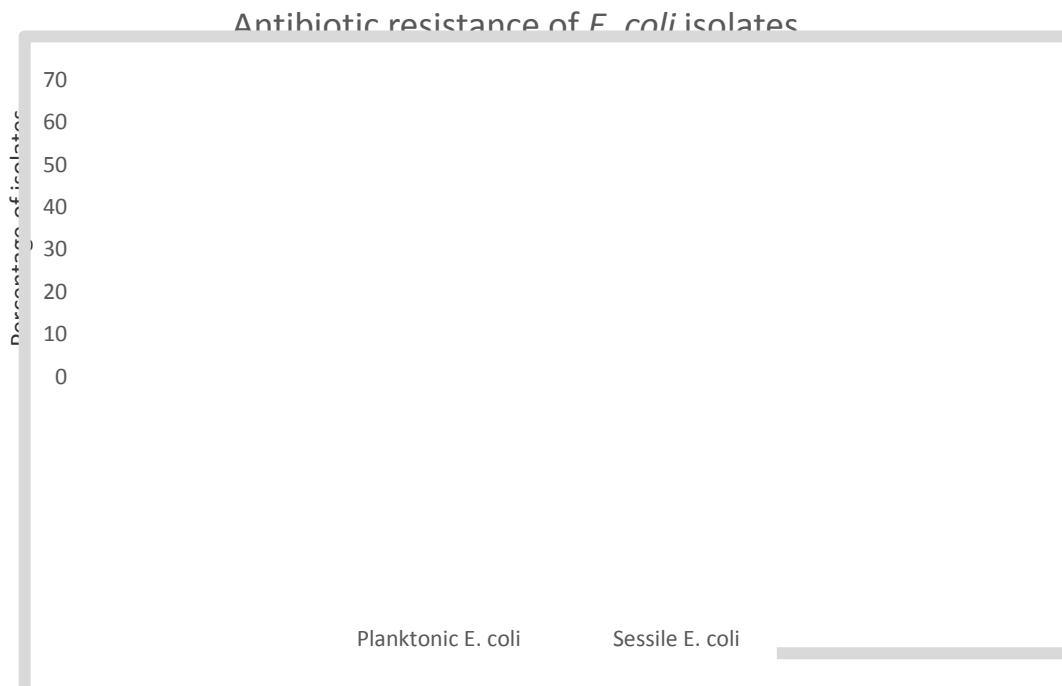


Figure.2 Chart depicting comparative antibiotic resistance between planktonic and sessile form of *E. coli* isolates of bovine mastitis milk



In the present study, the prevalence rate of the *E. coli* infection in 250 bovine mastitis milk samples was found to be 27.2% (n = 68). This isolation rate is in agreement with the findings of Momtaz *et al.*, (2012) with 27.23% and Rafik *et al.*, (2014) with 25.5% isolation rate of *E. coli*. The 75% (n = 51) of *E. coli* showed positive result to form biofilm on Congo red agar assay. Other researchers, like Parussolo *et al.*, (2019), Cengiz *et al.*, (2014) and Chen *et al.*, (2010) also found comparable results of biofilm formation by total *E. coli* from different sources to be around 67.52%, 67.8% and 76.56% positivity respectively.

On antibiotic sensitivity testing, the high susceptibility to gentamicin and chloramphenicol is in agreement with Charaya *et al.*, (2014). The high antibiotic resistance level of *E. coli* to ampicillin and tetracycline is in agreement with the finding of Zhao *et al.*, (2001), Lira *et al.*, (2004) and Rangel and Marin, (2009). The biofilm

producing bacterial isolates were said to be up to 1000 time more resistant to antimicrobial agents in comparison to their planktonic counterparts (Costerton *et al.*, 1999; Nickel *et al.*, 1985). Therefore the antibiogram of the biofilm producing *E. coli* isolates were also done. As mentioned before, the resistance of the biofilm forming *E. coli* isolates against the antibiotics was much higher compared to planktonic isolates also evident in this study. This finding was in line with the finding of Abdul-Ratha and Nadhom (2014).

The growing multidrug resistance against antimicrobials by the bacteria was a great concern threatening the health industry whether it is veterinary or human health. The extent of multidrug resistance is increased many fold by biofilm forming *E. coli* isolates compared to planktonic forms. All these biofilm forming of *E. coli* isolates were resistant to at least one antibiotic used for antibiotic susceptibility testing.

It is concluded, as bovine mastitis is a most prominent problem in dairy industry which is responsible for many herd level and foodborne public health outbreaks. The two main routes for foodborne human infection from dairy source are through either contamination by mastitis milk or faecal matter of bovine or ruminant sources. As the *E. coli* is one of the important pathogens for the bovine mastitis.

The higher incidence of mastitogenic *E. coli* isolates may be due to poor hygienic dairy practices, unsanitary conditions and no personal hygiene. As the *E. coli* causing mastitis are mainly from environmental sources as an indicator of faecal contamination.

In some recent studies, it has been found that the antibiotic resistance of *E. coli* bacteria in the sessile (i.e., biofilm forming) mode of growth contributes to the reoccurrence and chronicity of mastitis. It was found that the biofilm producing isolates are more resistant and can easily survive the effect of a particular antibiotic than their planktonic counterparts (Figure 2). Therefore, based on the present study we recommend relevant awareness programs on best dairy practice and milk handling should be organized for dairy farmers to control contamination of milk and milk products and there is a need for investigation of other sources of *E. coli* infection, incidence rate with respect to different seasons, cycle of lactation, age and geographical distribution in Bhubaneswar along with change in milk production of affected cattle.

Conflict of interest

There is no conflict of interest.

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