Original Research Article

Antibiotic Resistance Profile of *Escherichia coli* from Bovine Mastitic Milk Samples of Bikaner Region

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A B S T R A C T

Bovine mastitis is a large-scale infectious disease of the mammary gland with significant impact on the economy of milk production along with alterations in its quality. A total of 100 mastitis suspected milk samples were collected from different organized dairy farms of Bikaner city and were evaluated for isolation, identification and antibiotic sensitivity pattern of *Escherichia coli*. Out of 100 mastitis suspected milk samples, 24 samples were positive for *Escherichia coli* (24.0%). The antimicrobial resistance profile of the tested *Escherichia coli* isolates to 16 different antibiotics revealed that all 24 isolates were sensitive to gentamicin, tetracycline and chloramphenicol (100%) whereas, resistant to ampicillin and penicillin-G (100%).

Introduction

Bovine mastitis is a large-scale infectious disease of the mammary gland with significant impact on the economy of milk production along with alterations in its quality (impaired nutritive and technological properties of milk), fertility disorders and even systemic diseases. Moreover, causative agents of mastitis with zoonotic potential may represent a health risk for human populations via the food chain. Mastitis can appear in a clinical and subclinical form, the latter being commonly found in most herds (Gruet *et al.,* 2001; Bradley 2002; Halasa *et al.,* 2007; Malinowski and Gajewski, 2010; Le Marechal *et al.,* 2011; Awale *et al.,* 2012).

Different microorganisms can make access to milk and milk products, among them *Escherichia coli* used as marker organisms to check fecal contamination of milk and commonest pathogen in environmental mastitis resulting in sudden onset of fever, loss of appetite, diarrhea, toxaemia and the infected quarter show swelling, pain with discharge of watery or bloody milk or milk with large thick clots. Although, majority of obtained *E. coli* strains from milk products are non-virulent, but highly pathogenic strains

were also reported from many milk born outbreaks that have lethal effects on host (Diliello, 1982; Benkerroum *et al.,* 2004).

The presences of pathogenic bacteria in milk often emerge as a major public health concern, especially for those individuals who drink raw milk. Initial bacterial contamination of milk may occur from milking animal itself, through shedding of microorganisms colonize on its teats canal or an infected udder (clinical and subclinical mastitis) or it gets contaminated later on at various stages from the animal skin, handling persons, equipments used, through extraneous dirt or use of unclean water (Banwart, 1989; Hayes *et al.,* 2001), therefore the microbial content of milk is a major feature in determining its quality.

Antimicrobials are routinely used for treatment of dairy cattle affected with clinical and subclinical infections (Aarestrup, 2005). Antimicrobial resistance of mastitis pathogens to multiple drugs has been reported worldwide (Waller *et al.,* 2011; Oliver and Muranda, 2012; Chaudhary and Payasi, 2013).

This is because of indiscriminate use of the antibiotics by farmers, thereby rendering them ineffective and leading to permanent loss of the mammary tissues. Antibiotic susceptibility testing results, provided by a diagnostic laboratory, may serve two purposes:

To confirm the efficacy of the antibiotic being administered.

To modify therapy if results demonstrate the antibiotic failed against a pathogen.

Antibiotic susceptibility testing results provided before starting treatment may help dairies institute a more judicious therapy, especially for subclinical mastitis.

Materials and Methods

The present study was conducted at the Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India.

A total of 100 mastitis suspected milk samples were collected for the present study from Veterinary clinical complex, Rajasthan University of Veterinary and Animal Sciences, Bikaner, and different organized dairy farms of Bikaner. Samples were collected in sterilized test tubes and immediately brought to the laboratory under cold conditions. The samples were subjected to cultural examination using standard laboratory procedures. Each of the milk samples were transferred to 10 ml of nutrient broth and incubated at 37°C for 22-24 hours to resuscitate the organisms. Then each sample was streaked on Nutrient agar plates in primary, secondary, and tertiary fashion in order to obtain isolated colonies of bacteria. These petri plates were incubated for 24 hr at 37^0C. After 24 hr of incubation these isolated colonies were cultured on eosin methylene blue agar (EMB) plates for isolation of *Escherichia coli*. The growth was examined for the colonial morphology and pigmentation, and different types of colonies were sub-cultured on separate nutrient agar plates in order to obtain a pure culture.

The confirmation of the isolates as *Escherichia coli* were done using Gram's staining, Catalase test, Coagulase test, Oxidase test and a set of 12 biochemical tests provided in HiEcoliTM Identification Kit (HiMedia, Mumbai) for *Escherichia coli*.

Antibiotic sensitivity test (ABST) was conducted as per the procedure prescribed by Kirby *et al.,* (1966). Spread the pure culture on a specific agar medium (Mueller-Hinton).

Apply filter paper discs containing standardized quantities of antimicrobial drug onto the agar surface. These petri plates were incubated for 24 hr at 37°C. To conduct antibiotic sensitivity test, sixteen antibiotic discs were selected viz., ampicillin, chloramphenicol, tetracycline, penicillin G, streptomycin, sulphatriad, ceftriaxone, ciprofloxacin, cotrimaxazole, trimethroprim, clindamycin, erythromycin, gentamicin, levofloxacin, vancomycin and lincomycin.

Results and Discussion

Out of 100 mastitic milk samples 24 (24%) were found positive for *Escherichia coli.* Balakrishnan *et al.,* (2004) found 27.5% sample positive for *Escherichia coli* which is slightly higher than present investigation. Das and Joseph (2005) isolated 17.44% samples positive for *Escherichia coli* while Ataybi *et al.,* (2006) found 10.16% from the farms around Tehran which is lower than present study.

Sumathi *et al.,* (2008) observed 20.0% sample positive for *Escherichia coli* which is slightly lower than present observation. Anjum *et al.,* (2010) also observed lower prevalence than present study which is 11.11%. Cervinkova *et al.,* (2013) isolated 6.60% samples positive for *Escherichia coli* whereas, Mohanty *et al.,* (2013) found 21% samples positive for *Escherichia coli* from various regions of Bhubaneswar and areas around which are also lower than present study. Chandrasekaran *et al.,* (2014) isolated 45.89% *Escherichia coli* in Tamil Nadu while Sharma *et al.,* (2014) found 35.63% sample positive from raw milk samples supplied in the Jaipur city of Rajasthan which are higher than present investigation. Idriss *et al.,* (2014) found 12.82% samples positive for *Escherichia coli* whereas, Singh *et al.,* (2016) isolated 16.66% samples positive from different area of Rewa (M.P.) which are also lower than present

results. Tanzin *et al.,* (2016) reported 25% samples positive for *Escherichia coli* which is almost similar to present investigation. Navaneethan *et al.,* (2017) found 10.57% *Escherichia coli,* out of 104 milk samples collected from TVCC, VCRI, Namakkal which is also lower than present study. Jayaweera *et al.,* (2018) reported 41.9% samples positive for *Escherichia coli* from dairy farms in Nuwera Eliya District, Sri Lanka which is higher than present observations while Armanullah *et al.,* (2018) isolated 19.05% *Escherichia coli* which is lower than present study.

In the present study all the isolates that were gram negative, oxidase negative, catalase positive. All *Escherichia coli* strains gave green metallic sheen on EMB agar. For confirmation of *Escherichia coli* HiE.coli ^{TM} commercial kits were used. The isolates showing positive reaction for the Methyl red test, Indole test, Glucuronidase test, Nitrate reduction test, ONPG test, Lysine utilization test, Lactose, Glucose, Sucrose and Sorbitol sugar tests while negative with Voges Proskauer's test and Citrate utilization test were confirmed as *Escherichia coli*.

Variability in the results might be occurred due to presence of different types of strains of *Escherichia coli*.

The antimicrobial resistance profile of the tested isolates *Escherichia coli* to different antibiotics revealed that all 24 isolates were sensitive to Gentamicin, tetracycline, chloramphenicol (100%), 23 isolates were sensitive to ceftriaxone (95.83%), 22 isolates were sensitive to streptomycin (91.67%), 21 isolates were sensitive to sulphatriad, 18 isolates were sensitive to ciprofloxacin (75.00%), 15 isolates were sensitive to cotrimoxazole (62.50%) and 13 isolates were sensitive to trimethoprim (54.17%).

In the present study it was also reported that all 24 isolates were found resistant to ampicillin and penicillin-G (100%), 22 isolates were found resistant to vancomycin (91.67%). 20 isolates were found resistant to levofloxacin (83.33%), 18 isolates were resistant to lincomycin (75.00%), 16 isolates were found resistant to clindamycin (66.67%) and 14 isolates were found resistant to erythromycin (58.33%).

Cortis *et al.,* (2003) reported 20% *Escherichia coli* resistance to ampicillin while Sharma *et al.,* (2014) and Armanullah *et al.,* (2018) reported 80.76% and 41.23%, respectively which are lower than present study. Mohanty *et al.,* (2013) and Idriss *et al.,* (2014) found 96.66% and 96.00% *Escherichia coli* isolates resistant to Penicillin which is almost similar to present investigation. Sharma *et al.,* (2014) reported lower results which are 77.19% and Chandrasekaran *et al.,* (2014) also showed lower results (60.5%) of Penicillin resistant *E. coli* isolates of mastitis in Tamil Nadu. In the present study *E. coli* isolates showed 0% resistance towards tetracycline whereas,

Sharma *et al.,* (2014) reported 33.34% of *Escherichia coli* resistance which is higher than present observation. Mohanty *et al.,* (2013), Sharma *et al.,* (2014) and Idriss *et al.,* (2014) found 50%, 19.30% and 10% *Escherichia coli* isolates resistant to Streptomycin, respectively. Mohanty *et al.,* (2013) found similar result which is 100% *Escherichia coli* isolates were sensitive to Chloramphenicol while Jayaweera *et al.,* (2018) reported 90% sensitivity towards Chloramphenicol which is slightly lower than present observation. Sharma *et al.,* (2014) reported 85.96% *Escherichia coli* isolates sensitive towards Chloramphenicol which is also lower than present investigation (100.00%). Rangel and Marin (2009), Sharma *et al.,* (2014) and Armanullah *et al.,* (2018) reported 68.8%, 71.93% and 83.33% sensitivity towards Co-trimoxazole which are higher than present results (62.50%). Idriss *et al.,* (2014) reported that 96% *E. coli* were resistant to Lincomycin which is higher than present results (75.00%) (Table 1; Fig. 1 and 2).

Table.1 Results of biochemical tests for *Escherichia coli* obtained from Hi*E.coli* ™ commercial kits

S. No.	TEST	POSITIVE		NEGATIVE	
		Number	**%**	**Number**	**%**
1.	Methyl red	22/24	91.67%	02/24	08.33%
2.	Voges proskauer's	2/24	08.33%	22/24	91.67%
3.	Citrate utilization	05/24	20.83%	19/24	79.17%
4.	Indole	24/24	100.00%	-	-
5.	Glucuronidase	23/24	95.83%	1/24	04.17%
6.	Nitrate reduction	24/24	100.00%	-	-
7.	ONPG	23/24	95.83%	1/24	04.17%
8.	Lysine utilization	21/24	87.50%	3/24	12.50%
9.	Lactose	23/24	95.83%	1/24	04.17%
10.	Glucose	24/24	100.00%	-	-
11.	Sucrose	23/24	95.83%	1/24	04.17%
12.	Sorbitol	18/24	75.00%	6/24	25.00%

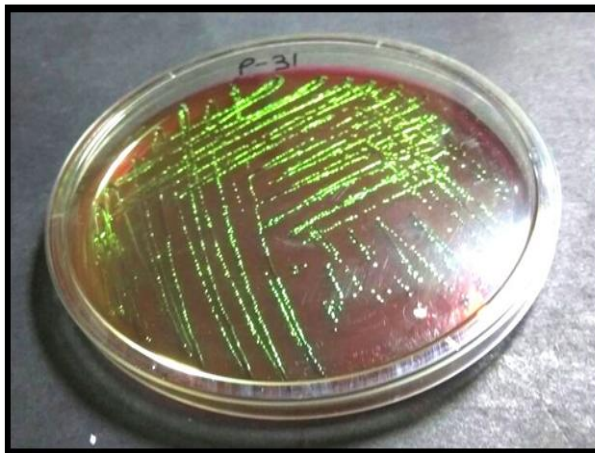**Fig.1** Isolation of *Escherichia coli* on EMB agar from mastitic milk samples

Fig.2 Antibiotic sensitivity test conducted on *Escherichia coli* isolates

In the present investigation levofloxacin showed 20% resistance whereas, Mohanty *et al.,* (2013) found 0% resistance. Chandrasekaran *et al.,* (2014) reported 56.30% gentamicin resistant *E. coli* isolates of mastitis in Tamil Nadu which is very much lower than present investigation while Armanullah *et al.,* (2018) showed 83.33% sensitivity towards gentamicin which is also lower than present result. Jayaweera *et al.,* (2018) reported 30% *Escherichia coli* isolates were resistant to Ciprofloxacin which is nearby to present investigation (25.00%) whereas, Mohanty *et al.,* (2013) and Armanullah *et al.,* (2018) found 13.33% and 16.67% *Escherichia coli* isolates resistant to Ciprofloxacin which is lower to present results. In the present investigation 95.83% *E. coli* isolates were sensitive towards Ceftriaxone while Rangel and Marin (2009) reported 82.20% resistant *E. coli* isolates from bovine mastitic milk which is just opposite to present investigation. Chandrasekaran *et al.,* (2014) studied the prevalence of drug

resistant *E. coli* isolates of mastitis in Tamil Nadu and reported 86.60% sensitive to Ceftriaxone which is almost similar to present investigation.

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