Environment & Ecology 35 (4) : 2640—2643, October—December 2017
Website : environmentandecology. com ISSN 0970-0420

Isolation and Identification of *Escherichia coli* Isolates from Subclinical and Clinical Mastitis of Dairy Animals in and Around Mhow

Ravi Sikrodia, Daljeet Chabra
S.D. Audarya, Rakesh Sharda

Received 1 December 2016; Accepted 2 January 2017; Published online 20 January 2017

Abstract Mastitis is multifactorial disorder which affects high yielders most. Mastitis is simply inflammation of udder but several predisposing factors like age, breed, weather, immunological status of animal, body condition, sanitary condition and management practices are responsible for the development of disease. Mastitis pathogens including viral, bacterial and various fungal agents. Common bacterial pathogens are *Staphylococcus* spp., *Streptococcus* spp. and coliforms like *E. coli*. *Klebsiella* spp. and *Enterobacter* spp. *E. coli* is considered to be an opportunistic pathogen and originating from the environment is also one of the commonest cause of bovine clinical mastitis. This study aimed to isolate *E. coli* from milk collected from different dairy animals suspected for the mastitis in any subclinical or clinical form. Total of 80 bovine (cattle and buffalo) milk samples obtained aseptically from different dairy farms; in and around Mhow. California Mastitis Test was used to primary screening of the samples for subclinical or clinical form of the mastitis. Result of California Mastitis Test showed that out of total 80 milk samples (cattle and buffalo) 39 (48.75%) samples were found positive forming clumps of coagulation. Out of these 80 samples 26 (47.27%) out of 55 in cattle and 13 (52.00%) out of 25 in case of buffalo found positive in CMT. Out of total 39 samples 25 milk samples showed positive results for the presence of *Escherichia coli* after culturing. From these 25 samples, 18 (69.23%) out of 26 in cattle and 7 (53.84%) out of 13 milk samples found positive for the *E. coli* isolates. The results of the study showed that the *E. coli* is also very important pathogen responsible for clinical and subclinical mastitis in dairy animals other than staphylococcus and streptococcus.

Keywords Mastitis, *Escherichia coli*, Milk, Bovine.

Ravi Sikrodia*, S. D. Audarya, Rakesh Sharda
Assistant Professor,

Daljeet Chabra
Professor and Head,
Dept of Vety. Microbiology, College of Veterinary Science and AH, Mhow, NDVSU, India
e-mail : sikrodia1435@gmail.com
*Correspondence

Introduction

Mastitis is multifactorial disorder which affects high

yielders most [1]. Out of highly contagious disease such as FMD, HS and PPR, mastitis also causes irreparable economic losses to the farmers in terms of decreased production, milk quality, veterinary aid as well as loss of animals [2]. Mastitis is simply inflammation of udder but several predisposing factors like age, breed, weather, immunological status of animal, body condition, sanitary condition and management practices are responsible for the development of disease [3]. These factors provide the condition for various microorganisms to setup the disease. Mastitis pathogens include both contagious and noncontagious microorganism including viral, bacterial and various fungal agents. Common bacterial pathogens are *Staphylococcus* spp., *Streptococcus* spp. and coliforms like *E. coli, Klebsiella* spp. and *Enterobacter* spp [4]. Acute coliform mastitis is primarily caused by *E. coli* [5]. *E. coli* is considered to be an opportunistic pathogen and originating from the environment [6], is also one of the commonest cause of bovine clinical mastitis [7] and major problem in lactating dairy cow [8]. *Escherichia coli,* a lactose fermentating gram negative facultative aerobic rod bacteria associated with diarrhea, urinary tract infections, septicemia and mastitis, is a member of the coliform group of bacteria [9]. There are four main serotypes of *E. coli* identified based on antigens O, K, H and F. Serotype O, K and H are mainly associated with clinical cases of bovine mastitis. This study aimed to isolate *E. coli* from milk collected from different dairy animals suspected for the mastitis in any subclinical or clinical form.

(The authors are thankful to the Dean, College of Veterinary Science and AH, Mhow, and the Dept. of Veterinary Microbiology for the fund and infrastructure.)

Materials and Methods

The study was carried out in and around Mhow, district Indore, Madhya Pradesh. Mhow is a contonment in the indore district of MP state of India. It is located 23 Km. south of Indore city. It is 556 m (1824ft) from sea level and latitude 22.55° N and longitude 75.76° E. Total of 80 bovine (cattle and buffalo) milk samples obtained aseptically from different dairy farms; in and around Mhow. Approximately 10 ml milk were collected in sterile glass tubes and transported to laboratory in cool box for further diagnosis. Milk samples were collected irrespective of any clinical sign of mastitis. California Mastitis Test was used to primary screening of the samples for subclinical or clinical form of the mastitis, Table 1 [10]. Positive samples were cultured on media for isolation of *E. coli.* Nutrient broth, Nutrient agar, MacConkey agar and Eosine Methyline Blue media were used for primary culture and isolation of *E. coli.* Nutrient broth was inoculated with milk of samples found positive in CMT incubate for overnight at 37°C in the incubator. Overnight grown culture from Nutrient broth was used to inoculate the Nutrient agar and MacConkey agar plates, and incubate at 37°C in the incubator for overnight . Streak plate method was used to separate the single colony . Morphological characterstic of the isolated single colonies on Nutrient agar and MacConkey agar were noted and go for gram's staining. Colonies showing pink color on MacConkey agar and found rod shaped in the staining are inoculated on selective indicator EMB media and incu-

Table 1. Scores assigned to the various degree of coagulation.

Degree of coagulation	Assigned score	Observation after CMT
-Ve	0	When the mixture was watery with no clots
+1	1	When the mixture was slimy with no gel formation
+2	2	When the mixture was slightly watery with clumps of coagulated material
+3	3	When the background was definitely watery with larger clumps of coagulation than in 2

Table 2. Result of CMT test screened for clinical and subclinical mastitis.

Animal	Total number of sample	Positive in CMT (+2 or + 3 grade)	Percentage
Cattle	55	26	47.27%
Buffalo	25	13	52.00%
Total	80	39	48.75%

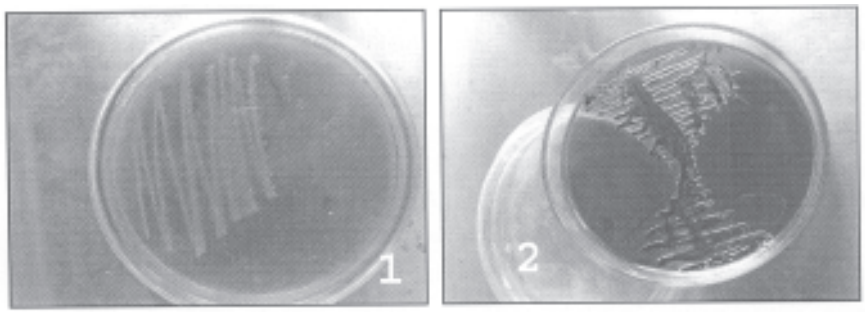

Fig. 1. Growth of *E. coli* on MacConkey agar. **Fig. 2.** Growth of *E. coli* on EMB media.

bate at 37°C in the incubator for overnight. Overnight grown culture showing metallic sheen were transferred in Nutrient agar slant for the biochemical test. Species identification carried out by the biochemical test kit Hi *E. coli*™ identification kit (HIMEDIA KB010). Culture from the slant inoculated in BHI broth and kept in incubator at 37°C for 5 h Hi *E. coli*™ Identification kit was inoculated with 50 µl of BHI broth growth and kept in incubator at 37°C for overnight.

Results and Discussion

The study animals were dairy cattle and buffalo at different age category. Raw milk samples were collected from dairy cattle and buffalo. Results of California Mastitis Test showed that out of total 80 milk samples (cattle and buffalo) 39 (48.75%) samples were found positive forming clumps of coagulation (Table 2). Out of these 80 samples 26 (47.27%) out of 55 in cattle and 13 (52.00%) out of 25 in case of buffalo found positive in CMT (Table 2). Out of total 39 samples 25 milk samples showed positive results for the presence of *Escherichia coli* afterculturing. From these 25samples, 18 (69.23%) out of 26 in cattle and 7 (53.84%) out of 13 milk samples found positive for the *E. coli* isolates (Table 3). The isolates were identified according to cultural, morphological and biochemical characterstics [11]. *E. coli* colonies showed different morphological characterstic on different media including Nutrient agar. MacConkey agar and EMB agar after incubation at 37°C for 24 h . On MacConkey agar colonies were appeared pink color because of acid production after lactose utilization (Fig. 1), while on EMB agar the visible colonies were appeared as green with metallic sheen Figure 2 [12]. Isolated bacteria were stained gram negative when stain and appeared rods under light microscope [13]. Biochemical test kit Hi *E. Coli*™ Identification kit result showed isolated bacteria was methyl red positive, voges-Proskauer's negative, citrate utilization negative, indole positive, glucorinidase positive, nitrate utilization positive, ONPG positive, Lysine utilization positive and vari-

Table 3. Result of isolation of *E. coli* on different culture media.

Sl. No.	Animal	Samples found positive in CMT	Sample (showed growth on media)	Positive samples on Nutrient agar	MacConkey agar	EMB agar
1	Cattle	26	18	18	18	18
2	Buffalo	13	7	7	7	7
	Total	39	25			

Table 4. Result of biochemical test of isolation of *E. coli* from milk.

Biochemical test	Result
Methyl red	(+) ve
Voges-Proskauer's	(-) ve
Citrate utilization	(-) ve
Indole	(+) ve
Glucoridase	(+) ve
Nitrate reductio	(+) ve
ONPG	(+) ve
Lysine utilization	(+) ve
Lactose	(+) ve
Glucose	(+) ve
Sucrose	(+) ve / (-) ve
Sorbitol	(+) ve

ous sugar lactose, glucose, sucrose and sorbitol were utilized by the isolates Table 4 [13].

The result of the study showed that the *E. coli* is also very important pathogen responsible for clinical and subclinical mastitis in dairy animals other than staphylococcus and streptococcus [14]. Incidence of *E. coli* mastitis may have ben due to poor hygienic condition. Animals may infect from environment and udder via teat canal [15] . Correct diagnosis of mastitis with isolation and identification of causative agent along with proper food and hygienic condition of the animal is very much necessary to prevent mastitis cases and economic losses.

References

1. Radostitis OM, Gay CC, Blood Dc, Funchcliff KW (2000) Veterinary medicine : A text book of the disease of cattle, sheep, goat, horse and pigs, 9th edn. WB Saunders, London.
2. Singh D, Kumar S, Singh B, Bardhan D (2014) Economic losses due to important diseases of bovines in central India. Vet World EISSN 2231—0916.
3. Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A, Duchateau L (2003) Severity of *E. coli* mastitis is mainly determined by cow factors. Vet Res 34 : 521—564.
4. Blood CD, Radostits OM (1985) A textbook of the diseases of cattle, sheep, pigs, goats and horses. Vet Med 7 edn. Oval R, London.
5. Wenz JR, Barrington GM, Garry FB, Ellis RP, Magnuson RJ (2006) *Escherichia coli* isolates, serotypes, genotypes and virulence genes and clinical coliform mastitis severity. J Dairy Sci 89 : 3408—3412.
6. Lehtolainen T, Pohjanvirta T, Pyorala S, Pelkonen S (2003) Association between virulence factors and clinical course of *Escherichia coli* mastitis. Acta Vet Scandinavica 44 : 203—205.
7. Green MJ, Green LE, Bradley AJ, Burton PR, Schukken YH, Medley GF (2005) Prevalence and associations between bacterial isolates from dry mammary glands of dairy cows. Vet Record 156 : 71—77.
8. Kobori D, Rigobelo EC, Macedo C, Marin JM, Avila FA (2004) Virulence properties of shiga toxin-producing *Escherichia coli* isolated from cases of bovine mastitis in Brazil. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux 57 : 15—20.
9. Hogan J, Smith KL (2003) Coliform mastitis. Vet Res 34 : 507—519.
10. Schalm OW, Carrol EJ, Jain AC (1917) Bovine Mastitis. Lea and Febiger Philadephia.
11. Cruckshank R, Duguid JP, Marmion BP, Swain RHA (1975) Medical microbiology Vol 2 12th edn. Cruchill Livngstone, New York, pp 31—57, pp 96—218.
12. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC (2004) Veterinary microbiology and microbial diseases text book .Printed and bound in Great Britain by International Ltd. Mosby. London, Padstow-Cornwall, pp 118—126.
13. Schulze J, Schiemann M, Sonnenborn U (2006) 120 years of *E. coli* its important in research and medicine . 6th edn. Alfred-Nissle-Gesellschaft, Germany, pp 11—13.
14. Jain B, Tewari A, Bhandari BB, Jhala MK (2012) Antibiotic resistance and virulence genes in streptococcus agalactiae isolated from cases of bovines subclinical mastitis. Vet Archiv 82 : 423—432.
15. Bradley AJ (2002) Bovine mastitis an evolving disease. Vet J 164 : 116—128.