



Original Research

Prevalence, Isolation and Antibigram of Coliforms Isolated from Clinical Bovine Mastitis

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Abstract

The present study was conducted to test the prevalence of bovine mastitis caused by coliforms and to understand its antibiogram. A total of 173 milk samples were collected under sterile precautions from 168 cows presented to University Veterinary hospitals, Mannuthy and Kokkalai, University Livestock Farm, Mannuthy and other veterinary dispensaries in Thrissur district during the period from October 2017 to May 2018. Primary isolation on brain heart infusion agar and blood agar yielded 123 bacterial isolates. Out of that, 90 were Gram positive organisms (73.17 per cent) and 33 were Gram negative organisms (26.82 per cent). Based on the cultural characteristics on Mac conkey's agar and Eosin methylene blue agar and biochemical characterization yielded 26 coliform organisms (21.13 per cent) which includes 14 *Escherichia coli* (11.38 per cent), 10 *Klebsiella* spp. (8.9 per cent), 1 *Enterobacter* spp. (0.81 per cent) and 1 *Citrobacter* spp. (0.81 per cent). All the isolates were subjected to in vitro antibiotic sensitivity studies using disc diffusion technique as per standard protocol. Antibigram revealed that most coliforms were sensitive to ceftizoxime (53.84 per cent) followed by amoxicillin –sulbactam (42.30 per cent), ceftriaxone –tazobactam (34.61 per cent), cefoperazone sulbactam (26.92 per cent), ceftriaxone (15.38 per cent), tetracycline (15.38 per cent) and were resistant to chloramphenicol, gentamicin, co-trimoxazole and enrofloxacin. The present study revealed that prevalence of coliforms was comparatively lower than Gram positive organisms causing mastitis and ceftizoxime was found to be the most sensitive antibiotic against coliforms isolated from bovine mastitis.

Key words: Antibiogram, Bovine, Coliforms, Mastitis, Prevalence

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Introduction

Mastitis is defined as the inflammation of mammary gland parenchyma leading to physical and chemical changes in the milk and pathological changes in the glandular tissue. It is one of the most economically important diseases causing heavy loss to dairy farmers and the industry worldwide (Dua, 2001). Economic





consequences of mastitis either clinical or sub-clinical, include reduced and poorer milk quality, increased cost of medicines and veterinary care, increased labour cost and increased culling rate (Tiwari *et al.*, 2013). Clinical mastitis causes an annual economic loss of Rs. 3014.35 crores per year and the estimated loss of milk yield per cow per lactation may range from 100 to 500 kg as per the report of National Academy of Agricultural Sciences, India, 2013 (Hossain *et al.*, 2017)

Mastitis in dairy herds is basically classified into environmental and contagious mastitis and an increase in the incidence of environmental mastitis has been noticed recently. Primary environmental pathogens include coliforms viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Citrobacter* spp. and streptococcal species other than *Streptococcus agalactiae* which mostly causes clinical mastitis (Jones, 2006). Coliforms are the normal inhabitants of digestive tract, soil and manure and enter the mammary gland from the contaminated bedding. Rapid immune response occurs which leads to the release of lipopolysaccharide (LPS) endotoxin causing acute clinical mastitis characterised by high fever, udder inflammation, depressed appetite, dehydration, diarrhoea, decreased production and abnormal milk. Coliform mastitis results in a higher incidence of cow death or agalactia-related culling than other mastitis (Mbuk *et al.*, 2016)

Long term use of antibiotics in mastitis therapy has led to an increase in the occurrence of antibiotic resistant strains leading to treatment failure and the resistant strains entering the food chain (Collins *et al.*, 2010). Bacterial isolation and antibiotic sensitivity studies are always essential to chalk out suitable antibiotic therapy. Hence, the present study was conducted to test the prevalence of bovine mastitis caused by coliforms and to select a suitable antibiotic or antibacterial agent which may prove effective for its treatment as the injudicious and indiscriminate use of antibiotic and irrational treatment of bovine mastitis with various antibiotics resulted in emergence of drug resistant organisms.

Materials and Methods

A total of 173 milk samples were collected from 168 cows presented to University Veterinary Hospital, Mannuthy and Kokkalai, University Livestock Farm, Mannuthy and various veterinary dispensaries in Thrissur district during the period from October 2017 to May 2018. Few squirts of milk from each affected quarter were discarded and midstream milk was collected in a sterile vial and brought to the laboratory. The milk samples were streaked onto blood agar and brain heart infusion agar and incubated at 37°C for 24-48 hours. The colonies were stained by Gram's staining technique as recommended by the manufacturer's kit (Himedia). Those colonies, which revealed Gram negative bacilli were sub-cultured onto Mac Conkey's agar for differentiation of lactose fermentors and Eosin methylene blue medium for identification of *E.coli*. Based on the cultural characteristics produced on these media, the organisms were classified and subjected to further biochemical tests as per Barrow and Feltham (1993) and Quinn *et al.* (2013). Minimum inhibitory



concentration (MIC) values of the coliforms were analysed for ten different antimicrobials namely enrofloxacin (10µg), gentamicin (50µg), tetracycline (30µg), ceftizoxime (30µg), amoxicillin/sulbactam (30/15µg), ceftriaxone (30µg), co-trimoxazole (25µg), ceftriaxone-tazobactam (80/10µg) and cefoperazone- sulbactam (75/30µg) and chloramphenicol (30µg) (M/s HiMedia Laboratories Ltd., Mumbai). The disc diffusion method as described by Bauer *et al.* (1966) was performed and zone size interpretation was made as sensitive, moderately sensitive and resistant according to chart provided by the manufacturers.

Result and Discussion

A total of 123 bacterial isolates were obtained from 173 milk samples of 168 clinical cases of bovine mastitis. Out of the 123 isolates, 90 (73.17 per cent) were Gram-positive whereas 33 (26.82 per cent) were Gram negative. The prevalence of coliforms among total isolates was found to be 21.13 per cent (26 isolates), among which *Escherichia coli* was the predominant coliform, 14 isolates (11.38 per cent), followed by *Klebsiella* spp. 10 isolates (8.9 per cent), 1 *Enterobacter* spp. (0.81 per cent) and 1 *Citrobacter* spp. (0.81 per cent). Other than coliforms, non-lactose fermenters were also identified such as *Pseudomonas* spp. (2.43 per cent), *Shigella* spp. (1.62 per cent), and *Proteus* spp. (1.62 per cent). The frequency of isolation of coliforms from clinical mastitis cases is depicted in Table1.

Table 1: Frequency of isolation of coliforms from clinical mastitis milk samples

S. No.	Organism	No. of Isolates	Per cent
1	<i>Escherichia coli</i>	14	11.38
2	<i>Klebsiella</i> spp.	10	8.9
3	<i>Enterobacter</i> spp.	1	0.81
4	<i>Citrobacter</i> spp.	1	0.81
	Total	26	

Results of the present study, i.e. lower occurrence of Gram negative isolates when compared to Gram positive isolates was in accordance with Sumathi *et al.* (2008), Mohanty *et al.* (2013) where *Staphylococcus* spp. and *Streptococcus* spp. were the most frequent isolates when compared to *E. coli*. Prevalence of bovine mastitis caused by coliforms noted in this study was in agreement with Kateete *et al.* (2013) who observed as 25 per cent and Verma *et al.* (2018) who observed as 21.28 per cent. The comparatively lower incidence of coliforms despite its wide presence in the environment may be due to the inherent udder defense mechanisms such as opsonisation of these bacteria along with phagocytosis and killing by neutrophils, and lactoferrin via binding iron inhibits multiplication and establishment of coliform infection. Higher prevalence of coliforms than the present study was recorded by Rathish, (2014) and Chandrasekaran *et al.* (2015) with 29.43 per cent and 45.89 per cent respectively.

In vitro antibacterial sensitivity of coliforms revealed that all coliform isolates were sensitive to ceftizoxime (58.34 per cent), amoxicillin –sulbactam (42.30 per cent), ceftriaxone – tazobactam (34.61 per cent) followed by decrease in susceptibility to cefoperazone sulbactam (26.92 per cent), ceftriaxone (15.38 per cent), tetracycline (15.38 per cent) and resistant to chloramphenicol, gentamicin, co-trimaxazole and enrofloxacin (Table 2).

Table 2: *In vitro* sensitivity of individual coliforms from bovine mastitis to ten antibiotics

Bacterial Isolate	No. of isolates	No. (per cent) Sensitive to Each Antibiotic									
		EX	TE	CTR	CIT	COT	CFS	AMS	CZX	GE	C
<i>Escherichia coli</i>	14	1 (7.14 %)	2 (14.28 %)	3 (21.42%)	6 (42.85%)	1 (7.14%)	1 (7.14%)	4 (28.57%)	7 (50%)	2 (14.28%)	1 (7.14%)
<i>Klebsiella</i> spp.	10	NS	2 (20%)	1 (10%)	3 (30%)	1 (10%)	5 (50%)	5 (50%)	5 (50%)	NS	NS
<i>Enterobacter</i> spp.	1	NS	NS	NS	NS	NS	S	S (100%)	S(100%)	NS	NS
<i>Citrobacter</i> spp.	1	S	NS	NS	NS	NS	NS	S	S	NS	NS
Overall sensitivity to each antibiotic	26	2 (7.69%)	4 (15.38%)	4 (15.38%)	9 (34.61%)	2 (7.69%)	7 (26.92%)	11 (42.30%)	14 (58.34%)	2 (7.69 %)	1 (3.84 %)

Figures in parentheses indicate percentage of sensitive isolates. EX: Enrofloxacin TE: Tetracycline CTR: Ceftriaxone CIT: Ceftriaxone-Tazobactam COT: Cotrimoxazole CFS: Cefoperazone sulbactam AMS: Amoxicillin sulbactam CZX: Ceftizoxime GE: Gentamicin C: Chloramphenicol S - Sensitive NS - Not sensitive.

Antibiogram representation of a single coliform isolate is depicted in Plate 1. Higher sensitivity to ceftriaxone – tazobactam and amoxicillin – sulbactam moderate sensitivity to ceftizoxime was observed by Jhambh *et al.* (2012), Bhat *et al.* (2017).



Plate 1: Antibiogram representation of a single coliform isolate (Sensitive to CZX, AMS, CIT; Resistant to EX, TE, GEN, CFS)



Higher sensitivity to these drugs could be due to the reason that they are recently marketed drugs and injudicious use of these drugs also may lead to increased resistance. The results of the present study were not in agreement to works by Mohanty *et al.* (2013) in which *E.coli* was sensitive to chloramphenicol, enrofloxacin and gentamicin, Virpari *et al.* (2013) who showed 100 per cent sensitivity to co-trimoxazole followed by gentamicin, trimethoprim and doxycycline. Inappropriate and indiscriminate use of antibiotics for treating mastitis cases will eventually lead to development of resistance to antibiotics. Variation in regions, managemental practices and preference of antibiotics used by veterinarians may also lead to differences in the antibiogram results (Mohanty *et al.*, 2013).

Conclusion

The present study concluded that prevalence of coliforms was comparatively lower than Gram positive organisms and among the coliforms, *E.coli* was the most predominant. Ceftizoxime was found to be the most sensitive antibiotic for coliform mastitis. Increased antimicrobial resistance is a looming threat to the public health and hence a well-planned antimicrobial therapy strategy must be carried out according to antibiogram. Creating awareness among dairy farmers and veterinary practitioners is mandatory to prevent indiscriminate use of antibiotics and hence making them fall into resistant category. Accurate diagnosis and identification of organism along with appropriate antibiogram studies will help prevent economic loss due to mastitis.

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