Indian J. Vet. Med. Vol. 40, No. 1, 2020 pp. 35-38

Prevalence of microorganisms causing mastitis in dairy animals and their antibiotic resistance

Teerath Raj Kulaste[1], Mudit Chandra[1], Gurpreet Kaur[1], Deepti Narang[1], D.K. Gupta[2], A.K. Arora[1]*
[1]Department of Veterinary Microbiology, [2]Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

Bovine mastitis, defined as inflammation of the mammary gland, can have an infectious or non-infectious etiology (Bradley 2002). It is characterized by physical and chemical changes in milk and pathological changes in glandular tissues of the udder that affects the quality and quantity of milk (Radostitis *et al.,* 2000, Sharma *et al.,* 2012). Mastitis is a very common problem affecting dairy animals in India and throughout the world (Miller *et al.,* 1993). There is a reduction in milk production, reduced milk quality leading to increased cost of production due to increased labor, treatment costs and disease transmission (Seegers *et al.,* 2003, Gröhn *et al.,* 2004, Pinzón-Sánchez and Ruegg 2011). It affects animal's health and carries public health concerns because of presence of antibiotic residues in the dairy and livestock products. A wide variety of pathogens could be involved in mastitis thus making it a multifactorial disease (Watts 1988). They are classified either 'contagious' or 'environmental' (Blowey and Edmondson, 1995). Contagious bacteria spread from an infected animal to healthy animals mainly at the time of milking through contaminated hands, towels, or the milking machine. Mainly contagious bacteria include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Corynebacterium bovis*, *Mycoplasma* spp. etc. while environmental contamination that is mostly influenced by management practices such as animal bedding, soil, manure, etc. which include *Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus licheniformis*, *Pasteurella* spp., Coagulase-negative Staphylococci, *Streptococcus faecalis* and various fungi including yeast. Antibiotic resistance is described as the ability of microorganisms to withstand the effects of antibiotic. This widespread use of antibiotics and intense human intervention has imposed a strong selective pressure that has contributed to the emergence of antibiotic resistant microorganisms. Keeping this in view, the present study was envisaged with the following objective to isolate mastitis causing microorganisms from mastitic animals and to study their antibiotic sensitivity pattern.

A total of fifty milk samples from suspected mastitis animals were collected from the Veterinary Clinical Complex, GADVASU, Ludhiana and also from the nearby dairy farms in and around Ludhiana. These samples were collected aseptically in a 50 ml sterile sample collection tube after discarding first few streaks of milk. These samples were kept on ice and transferred immediately to the laboratory. On the same day, these samples were processed and subjected to bacterial isolation, identification, and culture sensitivity test.

The milk samples were inoculated on Brain Heart Infusion (BHI) Agar, Eosin Methylene Blue (EMB) Agar, MacConkey's Lactose (MLA) Agar, Baird Parker Agar (BPA) supplemented with egg-yolk tellurite emulsion, Edward's medium and Blood Agar (BA). Later, these inoculated plates were incubated at 37ºC for 16-24 hours. The isolated bacterial colonies were subjected to Gram's staining for identification and subjected to various biochemical tests for confirmation.

All the isolates obtained were tested for sensitivity to various antibiotics as per the disc diffusion methods of Bauer *et al.* (1966). Fifteen different antibiotics were used viz., chloramphenicol (30 mcg), erythromycin (15 mcg), tetracycline (30 mcg), amoxycillin (10 mcg), co-trimoxazole (25 mcg), ciprofloxacin (5 mcg), gentamicin (10 mcg), cephalexin (30 mcg), ofloxacin (5 mcg), sparfloxacin (5 mcg), gatifloxacin (5 mcg), teicoplanin (30 mcg), azithromycin (15 mcg), vancomycin (30 mcg) and doxycycline (30 mcg). The antibiotic sensitivity test was performed on Muller Hinton Agar. Overnight grown culture of individual bacteria in LB broth was uniformly spread onto a Muller Hinton Agar plate with the help of a sterilized cotton swab. The discs were placed and incubated for 16-24 hours. After incubation, the zone of sensitivity was measured using a ruler and the diameter was recorded in millimeters (mm). All the isolates were classified as sensitive and resistant on the basis of zone of inhibition as per the standard guidelines of CLSI

*Corresponding Author: drmuditchandra@gmail.com

Standards, 2018.

Out of these fifty milk samples, forty samples yielded bacterial growth and a total of fifty-four bacteria were isolated. The bacteria predominantly isolated were *Staphylococcus* spp. 59.25% (32), *Streptococcus* spp. 7.4% (4), *Klebsiella* spp. 14.81 % (8) and *E. coli* 18.51 % (10). Out of these forty animals twenty six animals were harboring infection from a single bacterium whereas fourteen animals had mixed infection (more than one bacterium) as indicated by the presence of these organisms in the milk collected from these animals. Out of these single bacterium causing infection, predominant bacteria isolated were *Staphylococcus* spp. 19 (73.07%), *Streptococcus* spp. 4 (15.38%), *Klebsiella* spp. 2 (7.69%) and *E. coli* 1 (3.84%). Among mixed infection *Staphylococcus* spp. and *E. coli* were isolated from 8 (57.14 %) samples, *Staphylococcus* spp. and *Klebsiella* spp. from 5 (35.71%) and *E. coli* and *Klebsiella* spp. from 1 (7.14%) (Table 1).

Table 1: Organisms isolated from mastitic milk from individual animal

S. No.	Organisms	Number (%)
Single bacterial infection		
1.	*Staphylococcus* spp.	19 (73.07%)
2.	*Streptococcus* spp.	4 (15.38%)
3.	*Klebsiella* spp.	2 (7.69%)
4.	E. coli	1 (3.84%)
	Total	26
Mixed bacterial infection		
1.	*Staphylococcus* spp. + *E. coli*	8 (57.14 %)
2.	*Staphylococcus* spp.+ *Klebsiella* spp.	5 (35.71%)
3.	*E. coli* + *Klebsiella* spp.	1 (7.14%)
	Total	14

Upon analysis of the data, it was revealed that there was a total of 32 *Staphylococcus* spp., 4 *Streptococcus* spp., 8 *Klebsiella* spp. and 10 *E. coli* isolated from all the samples on the basis of their cultural, morphological and biochemical characters. Out of all the organisms isolated, *Staphylococcus* spp. was the predominant bacterium followed by *E. coli*, *Klebsiella* spp. and *Streptococcus* spp. The presence of *Staphylococcus* and *Streptococcus* organisms in mastitic milk is a common finding which has been observed by various workers. Fujikura and Shibata (1965) on bacteriological examination revealed that 72.45 of samples showed the presence of *S. aureus*, coagulase negative *Staphylococcus* (CoNS), *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa*, *Bacillus cereus* and members of *Enterobacteriaca*e. Similarly, isolation of *Staphylococcus* has been reported by Kour *et al.* (2018) and the main reason for it is due to the fact that the principal reservoirs of *Staphylococcus* spp. are the skin of the udder and milk of the infected gland. Similarly, Singh *et al.* (2018) studied the prevalence of microorganisms and the highest prevalence was of *Streptococcus agalactiae* and *Staphylococcus aureus* followed by *E. coli* and *Klebsiella pneumoniae*.

Antibiotic sensitivity results of *Staphylococcus* spp. showed that they were sensitive to sparfloxacin (96.88%) followed by tetracycline, gentamicin, vancomycin, doxycycline (90.63%), co-trimoxazole, erythromycin, cephalexin, gatifloxacin, teicoplanin, azithromycin (87.5%), ciprofloxacin, chloramphenicol and ofloxacin (84.38%) each and resistant to amoxicillin (68.75%). In a study by Unakal and Kaliwal (2010) highest numbers of *Staphylococcus aureus* isolates were susceptible to ceftriaxone, ciprofloxacin, erythromycin and gentamicin whereas the lowest susceptibility was shown in penicillin which is similar to the results of the present study in which we too observed sensitivity to gentamicin and quinolone group of antibiotics. Thaker *et al.* (2013) determined antibiogram pattern of *S. aureus* isolates by using disc diffusion method and reported sensitivity towards cephalothin, co-trimoxazole, cephalexin and methicillin followed by gentamicin, ciprofloxacin, oxacillin, streptomycin and ampicillin and resistance to penicillin-G followed by ampicillin, oxytetracycline, oxacillin, streptomycin and gentamicin which too is similar to the results of this study. In a study by Prabhu *et al.* (2013), *S. aureus* was susceptible to chloramphenicol (100%) followed by enrofloxacin (97.14%), kanamycin (85.75%), streptomycin (82.85%), cefalexin (74.28%) and gentamicin (65.71%). In contrast, isolates were resistant to tetracycline (74.28%), penicillin (71.42%) and ampicillin (45.71%). Ampicillin is resistant in the present study too thus the results are somewhat relevant with the present study. In another study by Bansal *et al.* (2015) antibiotic susceptibility of coagulase-negative Staphylococci (CoNS) revealed susceptibility to chloramphenicol (98.3%), gentamicin (93.1%), streptomycin (91.4%), linezolid (91.4%), ceftixozime (87.9%), cloxacillin (86.2%), clotrimazole (86.2%), bacitracin (86.2%), enrofloxacin (84.5%), and ceftrioxone+tazobactum (70.7%), while resistance was observed against amoxicillin (77.6%), penicillin (75.9%), ampicillin (74.1%) and cefoperazone (51.7%) which is again similar to the findings of the present study (Table 2).

Antibiotic sensitivity results of *Streptococcus* spp. showed that they were sensitive to vancomycin, azithromycin, teicoplanin, gatifloxacin, sparfloxacin, ofloxacin, cephalexin, gentamicin, ciprofloxacin and cotrimoxazole (100%) each whereas resistant to amoxicillin and doxycycline (100%) followed by tetracycline and erythromycin (75%). In a study by Krishnaveni *et al.* (2014), it was observed that *S. agalactiae* isolates revealed highest sensitivity to ampicillin with salbactam (94%) followed by chloramphenicol (81%), gentamicin (69%), ampicillin (50%), penicillin G (50%) and were resistant to oxacillin (100%) which is partially correlated with the results of present study (Table 2).

Antibiotic sensitivity results of *Klebsiella* spp. showed that they were sensitive to chloramphenicol, azithromycin and sparfloxacin (100%) each, followed by ciprofloxacin, gentamycin, ofloxacin and gatifloxacin (87.5%), cotrimoxazole, erythromycin, cephalexin, vancomycin, doxycycline (75%) each and tetracycline (62.5%) and resistant to amoxicillin (87.5%), followed by teicoplanin (50%). Kour (2016) reported antibiotic sensitivity testing on the isolated *Klebsiella* spp. that there was sensitivity to chloramphenicol, co-trimoxazole, gentamicin, ciprofloxacin, doxycycline and resistance to methicillin, penicillin, teicoplanin, azithromycin, vancomycin, amoxicillin which is similar to the findings of the present study (Table 2).

Antibiotic sensitivity results of *E. coli* showed that *E. coli* were sensitive to gentamicin and chloramphenicol (100%), followed by tetracycline, ciprofloxacin, erythromycin, sparfloxacin, gatifloxacin, azithromycin (90%), co-trimoxazole, ofloxacin (80%) and doxycyclin (70%) whereas resistant to amoxicillin (80%), followed by teicoplanin (60%), cephalexin and vancomycin (50%). Bouari *et al.* (2016) evaluated the in vitro antimicrobial susceptibility of bacteria isolated from milk from 204 milk samples and reported good sensitivity to enrofloxacin, masti discs and amoxicillin and clavulanic Acid whereas resistance was observed for penicillin and tetracycline. The results of the present study do not correlate with these as the antibiotics used in the present study were different than those used by Virpari *et al.* (2013). Further, Kour (2016) reported sensitivity to chloramphenicol (93.33%), ciprofloxacin and sparfloxacin (60%) each and resistance to amoxicillin and teicoplanin (93.33%) each and penicillin (86.67%) which is similar to the findings of this study (Table 2).

Table 2: Antibiotic sensitivity pattern of Individual isolates

Sr. No.	Antibiotic	Concentration	*Staphylococcus* spp.		*Streptococcus* spp.		*Klebsiella* spp.		*E. coli*	
			% S	% R	% S	% R	% S	% R	% S	% R
1	Chloramphenicol (C)	30 mcg	84.38	15.62	100	0	100	0	100	0
2	Ertyhromycin (E)	15 mcg	87.5	12.5	75	25	75	25	90	10
3	Tetracycline (TE)	30 mcg	90.63	9.37	75	25	62.5	37.5	90	10
4	Amoxicillin (AMX)	10 mcg	31.25	68.75	0	100	12.5	87.5	20	80
5	Co-trimoxazole (COT)	25 mcg	87.5	12.5	100	0	75	25	80	20
6	Ciprofloxacin (CIP)	5 mcg	84.38	15.62	100	0	87.5	12.5	90	10
7	Gentamicin (GEN)	10 mcg	90.63	9.37	100	0	87.5	12.5	100	0
8	Cephalexin (CN)	30 mcg	87.5	12.5	100	0	75	25	50	50
9	Ofloxacin (OF)	5 mcg	84.38	15.62	100	0	87.5	12.5	80	20
10	Sparfloxacin (SPX)	5 mcg	96.88	3.12	100	0	100	0	90	10
11	Gatifloxacin (GAT)	5 mcg	87.5	12.5	100	0	87.5	12.5	90	10
12	Teicoplanin (TEI)	30 mcg	87.5	12.5	100	0	50	50	40	60
13	Azithromycin (AZM)	15 mcg	87.5	12.5	100	0	100	0	90	10
14	Vancomycin (VA)	30 mcg	90.63	9.37	100	0	75	25	50	50
15	Doxycycline (DO)	30 mcg	90.63	9.37	0	100	75	25	20	30

Thus, from the present study it could be concluded that most of the infection was due to presence of single microorganism (twenty six) in comparison to mixed infection and *Staphylococcus* spp. was the most predominant organism that was isolated. Also, there is a presence of multidrug resistant bacteria causing mastitis in dairy animals.

Acknowledgement

The authors are grateful to University Grant Commission (UGC) India for the financial support. The authors are also thankful to the Director Research, GADVASU for providing the necessary laboratory facilities.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Bauer, A. W., Kirby, W. M., Sherirs, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by standardized single disk method. *Am. J. Clin. Pathol.*: **45:** 433-96.

Blowey, R., W. and Edmondson, P., W. 1995. *Mastitis Control in Dairy Herds*. Farming Press, Ipswich, pp 29.

Bouari, C., Nadas, G., Chirila, F., Rapuntean, S., Catoi, C., Tabaran, F., Gal, A., Taulescu, M., and Fit, N. 2016. Prevalence and antimicrobial susceptibility profiles of pathogen isolated from bovine mastitis milk in Transylvania Romania. *Bull UASVM Vet Med.*: *73:* 2.

Bradley, A. 2002. Bovine mastitis: an evolving disease. *Vet. J.*: **2:** 116-28.

CLSI, 2018. Performance standards for antimicrobial disc susceptibility tests, Approved Standards, 13th Edn.

Fujikura, P. and Shibata, S. 1965. Bovine Mastitis incidences in rural areas of Japan. *J. Dairy Sci.*: **5:** 65-72.

Gröhn, Y. T., Wilson, D. J., González, R. N., Hertl, J. A., Schulte, H., Bennett, G. and Schukken, Y. H. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.*: **87:** 3358-74.

Kour, G., Chandra, M., Kaur, G., Narang, D., Gupta, D. K., and Arora, A. K. 2018. Genotypic and phenotypic correlation of antimicrobial resistance in bacterial isolates from mastitic milk. *Vet. Immunol. Biotechnol.*: **1(2):** 24-30.

Kour, G. 2016. Studies on antibiotic resistance genes among bacterial isolates causing mastitis in dairy animals. M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Krishnaveni, N., Isloor, S. K., Suryanarayana, V. V. S., Rathnamma, D., Veeregowda, B. M., Nagaraja, C. S., and Sundareshan, S. 2014. Antibiogram profile of Group B Streptococci isolated from bovine mastitis cases. *Vet. Clin. Sci.*: **2:** 10-15.

Miller, R. H., Paape, M. J., Fulton, L. A., and Schutz, M. M. 1993. The relationship of milk somatic cell count to milk yields for Holstein heifers after first calving. *J. Dairy Sci.*: **76:** 728-33.

Pinzón-Sánchez, C., and Ruegg, P. L. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. *J. Dairy Sci.*: **9:** 3397-410.

Radostitis, O. M., Gay, C. C., Blood, D. C. and Hinchcliff, K. W. 2000. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 9th Edition Mastitis, W.B. Saunders, Philadelphia, USA, pp, 603-86.

Seegers, H., Fourichon, C., F. Beaudeau, F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res.*: **34:** 475-91.

Sharma, N., Srivastava, A. K., Bacic, G., Jeong, D. K., and Sharma, R. K. 2012. *Bovine Mastitis*. 1st edn., Satish Serial Publishing House, Delhi, India, pp, 231-312.

Singh, K. V., Chandra, M., Kaur, G., Narang, D., Gupta, D. K., Arora, A. K., and Sharma, N. S. 2018. Development of a multiplex PCR for identification of mastitis causing organisms. *J. Dairy Sci.*: **72: 2:** 177-182.

Thaker, H. C., Brahmbhatt, M. N., and Nayak, J. B. 2013. Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat. *Vet. World*: **6:** 10-13.

Unakal, C. G. and Kaliwal, B. B. 2010. Prevalence and antibiotic susceptibility of *Staphylococcus aureus* from bovine mastitis. *Vet. World*: **3:** 65-67.

Virpari, P. K., Nayak, J. B., Brahmbhatt, M. N., and Thaker, H. C. 2013. Study on isolation, molecular detection of virulence gene and antibiotic sensitivity pattern of Escherichia coli isolated from milk and milk products. *Vet. World*: **6:** 541-45.

Watts, J. L.1988. Etiological agents of bovine mastitis. *Vet. Microbiol.*: **16:** 41-66.

Received : 19.04.2020
Accepted : 24.06.2020