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Bovine clinical mastitis and antibiotic resistance pattern in Tirupur district

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Abstract
The present investigation was undertaken to monitor antimicrobial sensitivity pattern of bacteria causing bovine clinical mastitis from 2015 to 2017 in Tirupur district. Out of 105 mastitis milk samples examined, 85 bacterial isolates were obtained. The isolates were identified according to the cultural, morphological and biochemical characteristics as per standard methods. All the isolates were subjected to antimicrobial sensitivity testing by Kirby-Bauer disc diffusion methods using different antibiotics. The predominant mastitis causing pathogens were *Escherichia coli* (40%) followed by *Staphylococcus aureus*, (27%), *Klebsiella pneumoinae* (20%) and *Streptococcus dysgalactiae* (13%). The highest and consistent sensitivity was recorded against Enrofloxacin (95.3%) followed by Gentamicin (92.1%) and Ciprofloxacin (89.2%) throughout the study period. The antibiotic resistance was recorded against Streptomycin, Amoxycillin, Penicillin, Oxytetracyclin, Chloramphenicol, Amoxyclav and Ceftriaxone (90.8% to 49.8%). The isolates showed high resistance towards *Streptomycin* (90.8%), Amoxycillin (81.6%) followed by Penicillin (76%), Oxytetracyclin (60.7%), Chloramphenicol (54.2%) and Amoxyclav (53.2%). The improper selection of antimicrobial drugs and overdose to reduce infection of mammary glands might lead to development of resistant bacteria. The present study showed that routine investigation, in-vitro antimicrobial sensitivity testing and recording of epidemiology of bovine mastitis should be carried out to decide the appropriate antimicrobial drug for successful treatment of mastitis and thereby preventing the development of antimicrobial resistance.

Keywords: Antibiogram, clinical mastitis, bovine, antibiotic resistance

1. Introduction
Milk production in India is characterized by small marginal rural milk producers scattered all over the country accounting for about 70 per cent of the production. Dairy farming is one of the major enterprises for livelihood of rural farmers in Tirupur district. One of the main impacts on the economy of dairy farming is mastitis as these rural farmers often face a great setback due to high prevalence and incidence of mastitis in milch animals. Most common pathogens that caused mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis* and *E. coli*. The main line of treatment of mastitis is the use of antibiotic as intra muscular injections or intramammary infusions (Kalmus *et al.*, 2014) [5]. In most cases treatment failure and recurrence of mastitis is due to indiscriminate use of antibiotics without testing *in vitro* sensitivity (Koch *et al.*, 2013) [6]. This practice not only causes economic losses to dairy farmers but also leads to antimicrobial resistance in animals and consequently affecting humans also as a source of food borne pathogen. Limited information regarding the prevalence, etiology of bovine clinical mastitis and antimicrobial sensitivity pattern in this region was available. Keeping the above facts in view, the present investigation was undertaken to monitor antimicrobial resistance trends at Tirupur district in bovines affected with bovine mastitis. The data generated can be used for proper therapeutic, preventive, and control measures for bovine mastitis. The data would also be helpful for the field veterinarian in the early treatment and also to aid in developing the antimicrobial usage policy.

2. Materials and Methods
The culture and antibiogram was conducted on the milk samples collected from animals with clinical mastitis. The study comprised of 105 dairy animals including cross bred and indigenous cattle and buffaloes over the period of three years in Tirupur district. The detailed history of the case, age, lactation, breed, clinical signs and quality of milk were recorded separately. The milk samples collected in 10 mL aseptic containers were transported to the VUTRC laboratory in ice box and processed immediately.

The milk samples were inoculated into nutrient broth and streaked on to blood agar and Mac Conkey's agar plates and subsequently inoculated on selective media for obtaining pure culture of bacteria. These bacterial isolates were subjected to antibiogram using Mueller-Hinton agar and readymade antibiotic discs (M/s. Hi-media Laboratories, Mumbai) as given in the Table No. 2 and following standard disc diffusion method (Bauer *et al*., 1966) [2]. The sensitivity and resistance patterns were recorded with the zone of inhibition and then compared with the zone diameter interpretation chart provided by the manufacturer of the antibiotic discs.

3. Results and Discussion

A total of 85 bacterial isolates were obtained from the milk samples during the study period 2015 to 2017 were shown in Table 1. The predominant mastitis causing pathogens were *Escherichia coli* (40%) followed by *Staphylococcus aureus*, (27%), *Klebsiella pneumoniae* (20 %) and *Streptococcus dysgalactiae* (13%).

Table 1. Prevalence of bacterial pathogens isolated from bovine clinical mastitis (n=85)

S. No.	Isolates	No. of positive samples	Percentage (%)
1	*Escherichia coli*	34	40
2	*Staphylococcus aureus*	23	27
3	*Klebsiella pneumoinae*	17	20
4	*Streptococcus* dysgalactiae	11	13
	Total	85	100

Table 2: Sensitivity pattern of bacterial isolates towards the commonly used antibiotics in bovine mastitis (n=85)

Antibiotics (µg /disc)	No. of sensitive samples	Per cent (%)
Gentamicin G^{10}	78	92.1
Enrofloxacin Ex^{10}	81	95.3
Oxytetracycline O^{30}	33	39.3
Streptomycin S^{10}	8	9.2
Ciprofloxacin CIP^{30}	76	89.2
Amoxycillin AMX^{10}	16	18.4
Amoxyclav AMC^{30}	40	46.8
Penicillin- G P^{10}	20	24.0
Ceftriaxone CTR^{30}	43	50.2
Chloramphenicol C^{30}	39	45.8

The percentage of antimicrobial sensitivity of mastitis causing bacteria to the commonly used antibiotics was shown in Table 2. Maximum and consistent sensitivity was recorded against Enrofloxacin (95.3%) followed by Gentamicin (92.1%), Ciprofloxacin (89.2%) throughout the study period of three years. Similar to the present findings, Singh *et al.* (2018)[10] concluded that the most frequently isolated organisms from clinical bovine mastitis were *Streptococcus dysgalactiae*, *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumoniae* and further reported that the third generation fluoroquinolones were the most sensitive drugs.

Current study revealed that *E. coli* showed more sensitivity to Enrofloxacin and Gentamicin (100%) followed by Ciprofloxacin (87 %). These findings were in accordance with the results of Chauhan *et al.* (2016) [3], reported that *E.coli* isolated from bovine clinical mastitis showed the highest sensitivity to Gentamicin and Enrofloxacin. Vinod kumar *et al.* (2016) [12] also found that Ciprofloxacin was most effective against bovine mastitis. In general all the isolates showed maximum sensitivity towards Enrofloxacin, Gentamicin and Ciprofloxacin (Table 3). The isolates were found to be almost resistant to Streptomycin, Amoxycillin, Penicillin, Oxytetracyclin, Chloramphenicol and Amoxyclav (90.8% to 53.2%). Sudheer *et al.* (2017) [11] reported that long term use of Penicillin's in Proddatur region of YSR Kadapa district has led to emergence of resistance in bacteria isolated form bovine mastitis.

Table 3: Sensitivity (S) and resistant (R) pattern showed by different bacteria isolated from bovine clinical mastitis to antibiotics

	E. coli (n=34)		*Staphy. spp* (n=23)		*Strept. spp* (n=17)		*Kleb. spp* (n=11)	
Antibiotics (µg /disc)	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Gentamicin G^{10}	34	0	20	3	14	3	10	1
Enrofloxacin Ex^{10}	34	0	20	3	15	2	11	0
Oxytetracycline O^{30}	13	21	6	17	4	13	7	4
Streptomycin S^{10}	0	34	3	20	5	12	1	10
Ciprofloxacin CIP^{30}	30	4	23	0	16	1	8	3
Amoxycillin AMX^{10}	6	28	4	19	2	15	3	8
Amoxyclav AMC^{30}	14	20	11	12	6	11	7	4
Penicillin- G P^{10}	0	34	14	8	9	8	0	11
Ceftriaxone CTR^{30}	23	11	14	9	9	8	0	11
Chloramphenicol C^{30}	11	23	16	7	10	7	3	8

The most common disease of dairy cows is mastitis and is the most common reason for the use of antibiotics (Pol and Ruegg, 2007; Saini *et al*., 2012) [8, 9]. Current findings were in accordance with the observations of Moges *et al.* (2011) [7], reported that extensive, indiscriminate use of streptomycin for treating mastitis might have led to the development of high resistance. Repeated therapeutic use and indiscriminate usage of antibiotics results in the development of antibiotic resistance. Many times mastitis treatments are administered simply based on observation of inflammation and are difficult to justify. In recent times, both clinical and sub-clinical mastitis are treated with new higher antibiotics.

The emergence of antibacterial resistance is of immediate concern in veterinary medicine as a potential health risk for

human beings as food borne pathogens. Antibiotic sensitivity test plays a key role in successful treatment of bovine mastitis. Anakalo *et al.* (2004) [1] suggested that systematic recording and maintenance of the epidemiological findings on bovine mastitis including type of infection; antimicrobial resistance and therapeutic patterns would provide useful management information to dairy farmers and field veterinarians. Sudheer *et al.* (2017) [11] emphasized that regular screening of milk samples is necessary to select an effective antibiotic for treatment and control multi-drug resistance.

4. Conclusion

The most prevalent mastitis causing pathogens were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Streptococcus dysgalactiae*. The antibiotics Enrofloxacin, Gentamicin and Ciprofloxacin were effective against pathogens causing bovine mastitis. The isolates were resistant to Streptomycin, Amoxycillin, Penicillin, Oxytetracyclin, Chloramphenicol and Amoxyclav. Thus it could be concluded that routine investigation, in-vitro antimicrobial sensitivity testing and recording of epidemiology of bovine mastitis should be carried out to decide the appropriate antimicrobial drug for successful treatment of mastitis and to limit the indiscriminate use of antibiotics and to avoid the development of antimicrobial resistance in organisms.

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