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Antibiogram of bovine mastitis milk samples in Proddatur region of YSR Kadapa district

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Abstract
In India dairy sector is the largest contributor to the agriculture Gross Domestic Product (GDP). In terms of output, milk is now the single largest agricultural commodity in India. India ranks first in milk production accounting for 18.5% world production achieving a annual growth rate of 6.25% contributing about 500,000 crores. Mastitis is one of the most important infections of dairy animals affecting quality and quantity of milk, drug costs and discarded milk. In the dairy industry, antibiotic treatment is the key strategy for treatment of bovine mastitis. However indiscriminate use of antimicrobials results in development of multidrug resistance in microbes and the appearance of resistant bacteria in food chain. Therefore, there is an urgent need to find effective tool to apply specific antibiotic which can effectively prevent and control bovine mastitis. With this objective in the present study antibiotic sensitivity test is carried out for mastitis milk samples to treat mastitis at early stage and to control development of drug resistance in microbes.

Keywords: Kirby-Bauer disc diffusion, Muller-Hinton agar, Multidrug resistance

Introduction
Mastitis is considered to be one of the most common and substantial production diseases of dairy livestock worldwide (Ruegg & Erskine, 2015). Mastitis is the inflammation of the mammary gland. It may be infectious or non-infectious in origin. Based on source of infection Contagious mastitis is primarily caused by the bacteria which reside in udder and environmental mastitis is caused by bacteria present in environment (NMC, 2011; Ranjan *et al.*, 2011) [5, 7]. Because of multiple aetiology mastitis remained as a big challenge to veterinarians all over the world (Vashney *et al*., 2012) [8]. There are two forms of mastitis viz., clinical and sub clinical mastitis. Clinical mastitis is characterised by detectable changes in milk and udder which affects both quality and quantity milk. Subclinical mastitis is difficult to diagnose as there are no visible changes in milk (Hamadani *et al*., 2013) [2]. Because of indiscriminate usage of antibiotics, the bacteria are gaining antibiotic resistance (Koch *et al*., 2013) [3] which became a major problem in controlling mastitis. Antibiotic residues and multi drug resistance pathogens in milk posing a challenging to human life. In order to control mastitis antimicrobial sensitivity test is recommended by OIE. The present study was aimed to identify antimicrobial susceptibility and resistance pattern in mastitis milk sample which is useful for both treatment and controlling the origin of drug resistant pathogens.

Materials and Methods
A total of fifty milk samples were collected from bovines which were reported to TVCC with apparent clinical signs of mastitis following standard aseptic procedures. Teats and hands were disinfected with alcohol and the milk was collected in sterile tubes after discarding the first few strippings (Marimuthu *et al.*, 2014) [4]. The samples were screened using Kirby-Bauer disc diffusion method according to guide lines of Clinical and Laboratory standards Institute.

Kirby-Bauer disc diffusion method: A loop full of all milk samples was inoculated separately in to nutrient broth and incubated at 37 ^{0}C for 4-6 hours. After incubation broth was centrifuged at 3000 rpm for4- 5 min. Later the supernatant was discarded, pellet was suspended in Phosphate buffer saline (PBS, pH 7.2) and turbidity was adjusted to 0.5 scale McFarland turbidity standard ($1x10^6$-$5x10^6$ cells per/ml) to obtain semi confluent growth. Finally antibiotic susceptibility test is performed on Mueller-Hinton agar plates as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) against different antimicrobials.

The antimicrobialdiscs (Himedia) namely Amoxyclav (30 µg), Bacitracin (10units), Doxycycline Hcl (30µg), Ciprofloxacillin (5µg), Tetracycline (30µg), streptomycin (10 µg), pencillin G (10 units), gentamycin (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), and cefoxitin (30 µg) were used in present study. Finally all the results were compared with standards provided by manufacture to ascertain pattern of drug sensitivity and resistance against mastitis causing bacteria in and around proddatur region of YSR kadapa district.

Results

The antimicrobial sensitivity test against mastitis milk revealed resistance to multiple antibiotics. The patterns of sensitivity and resistance were expressed in percentage. The degree of sensitivity was determined on the basis of zone of inhibition. Among the Fifty milk samples 10% found to be resistant against all antimicrobials included in the study. Milk samples are highly resistant to Doxycycline (92%), Pencillin (84%), Amikacin (82%), Gentamycin (81%), Amoxyclav (78%), streptomycin (72%), Cefotaxime (68%), Bacitracin (62%), Cefotriaxone (56%) (Fig:1 &Tab-1). Milk samples are sensitive towards Ciprofloxacin (78%), Tetracyclines (44%), Cefotriaxone (44%), Bacitracin (38%), Cefotaxime (36%), streptomycin (28%), and Amoxyclav (22%) (Fig: 1&Tab-1).

Discussion

Mastitis is the most costliest disease affecting dairy animals throughout the world. Indiscriminate use of antibiotics developed multi drug resistance in majority of bacteria involved in mastitis (Waller *et al*., 2011) [11]. Early treatment of mastitis with effective antibiotics significantly limits the severity of mastitis, economic loss and development of antimicrobial resistance. Antibiotic sensitivity test is done and based on Zone of inhibiton. Majority of them showed resistance to Pencillin, Amikacin, Gentamycin, Amoxyclav, streptomycin, and third generation cephalosporin's which is in concordance with reports of few researchers (vinod kumar singh *et al* 2017). susceptibility to ciprofloxacin, Tetracyclines, Streptomycin and bacitracin (Vinod Kumar *et al* 2016) [9, 10]**.** Patterns of resistance and susceptibility is not correlated to many researchers. vinodkumar *et al* reported that tetracyclines are less effective but in present study it is second effective antibiotic. Sudhakar *et al* (*loc cit*) observed high sensitivity to gentamycin followed by ciprofloxacin, but in present study gentamycin is less effective. Long term use of penicillin's, amikacin, gentamycin and cephalosporin's in this region led to emergence of resistance. Susceptibility to ciprofloxacin, tetracycline, and streptomycin may be due to their low usage for treatment of mastitis

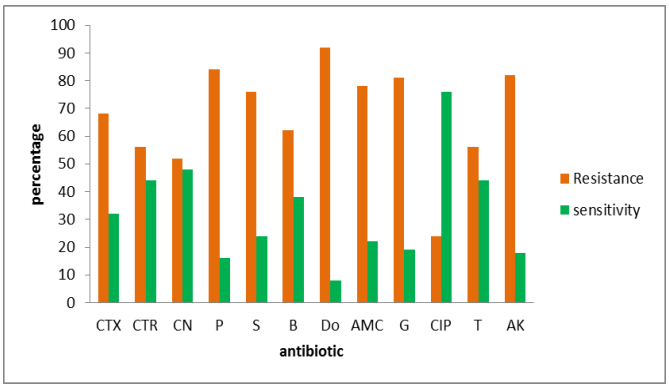

Fig 1: Percentage of antibiotic resistance and sensitivity

Table 1: percentage of antibiotic resistance and sensitivity

S. No	Antibiotic	Resistance (%)	Sensitivity (%)
1	CTX	68	32
2	CTR	56	44
3	CN	52	48
4	P	84	16
5	S	76	24
6	B	62	38
7	D	92	8
8	O	78	22
9	AMC	81	19
10	CIP	24	76
11	T	56	44
12	AK	82	18

Conclusion

Antimicrobial resistance is one of the biggest threats to both public and animal health. Apart from the risk of therapy failure, bacteria gradually become resistant to routinely used antibiotics. So regular screening of samples is necessary to choose a effective antibiotic to treat and control multi-drug resistance. Prevention of mastitis is also important to reduce the usage of antimicrobial substances and thereby reduce the risk of development of drug resistance.

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