

Original Research Article

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Antimicrobial Susceptibility and Resistance Pattern of Mastitis Milk Samples from Chittoor District, Andhra Pradesh, India

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ABSTRACT

Mastitis has been a major problem to the dairy industry and pose main impact on the economy of dairy farmers. Antibiotic resistance is increasing day by day and has become a big challenge worldwide with public health significance. In the present study antimicrobial resistance and sensitivity pattern of mastitis milk samples were assessed during the period from August, 2015 to March, 2020 at State level diagnostic laboratory, Sri Venkateswara Veterinary University, Tirupati. A total of 2,488 milk samples were subjected for cultural isolation and *invitro* antibiotic sensitivity test with standard disc diffusion method (Bauer et al., 1966). Out of 2,488 milk samples on cultural isolation, 2,174 (87.38%) of gram positive and 314 (12.62%) of gram negative bacteria were identified. Among 2,174 gram positive bacteria, *Staphylococcus aureus* -2,133(98.11%) was predominant followed by Streptococci-20 (0.92%) and bacillus species -19 (0.87%). Similarly, among 314 gram negative bacteria, *E.coli*-255(81.20%) were major pathogens followed by Klebsiella-53 (16.88%) and *Pseudomonas* -14 (4.46%) were identified. *Invitro*, antibiotic sensitivity test results of gram positive bacterial isolates (2,174) showed resistance and sensitivity to Amikacin (90.4% ; 9.60%) followed by Kanamycin (88.4%;11.6%), Streptomycin (87.5% ; 12.50%), Tetracycline (79.6 ; 20.4%), Gentamycin (75.3% ; 24.7%), Pencillin (60.2% ; 39.8%),Ciprofloxacin (52.50 ; 45.7%), Enrofloxacin (48.9% ; 51.20), Ampicillin (45.1%;54.9%) and Amoxycillin (36.4% ; 63.6%) respectively. Whereas the isolates of gram negative bacteria revealed resistance and sensitivity to Kanamycin-78.71% ; 21.30% followed by Pencillin-76.81% ; 23.10%, amikacin-69.11% ; 30.90%), Tetracyclin (66.61% ; 33.40%), Streptomycin-(66.6%;33.40%), Ampicillin (64.70%;35.40), Gentamycin (51.0% ; 49.0%), Ciprofloxacin (42.0% ; 58.0%) and Enrofloxacin-(34.71%;65.30%) (109/314).

Keywords

Dairy industry-
Economy,
Antibiotics,
Resistance and
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*Staphylococcus
aureus* and *E.coli*

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Introduction

Mastitis is the most common and most economically significant disease affecting dairy cattle. It is the leading cause of antimicrobial use on dairy farms (Saini *et al.*,

2012). Dairy farming is major livelihood of rural farmers in Andhra Pradesh and facing great problem of incidence of mastitis in diary animals. A variety of bacteria can be isolated from bovine mastitis cases. *Staphylococcus aureus* and *Escherichia coli* are the most

common causes of contagious and environmental clinical as well as subclinical mastitis cases respectively (Barkema *et al.*, 1988). Mastitis adversely affects animal health, quality of milk and milk production affecting economy of the developed and developing countries causing huge economic losses (Sharma *et al.*, 2007). The main line of treatment of mastitis is the use of antibiotics as intramuscular infections or intra mammary infections (Kalmus *et al.*, (2014). In majority cases, treatment failure and recurrence of mastitis is due to indiscriminate use of antibiotics without testing for invitro sensitivity of causative organisms (Koch *et al.*, 2013). This practice not only causes economic losses to dairy farmers but also leads to antimicrobial resistance in animals and consequently affecting humans and as a source of food borne pathogen. Despite the best possible antimicrobial treatments, failures of bacteriological common, especially for *Staphylococcus aureus* mastitis, and antimicrobial resistance (AMR) is considered as one of the reasons for low cure rates (Barkema *et al.*, (2006). Multi-drug resistant bacteria are a persistent problem in modern healthcare, food safety and animal health. Therefore, present work was undertaken to study the antibiotic sensitivity and resistance pattern of common pathogens of mastitis cases in milch animals of Andhra Pradesh for therapeutic measure in control of mastitis.

Materials and Methods

Milk samples

A total of 2,488 Milk samples were collected aseptically and randomly from organized and unorganized sectors of milch animals in Chittoor District of Andhra Pradesh during the period from August, 2015 to March, 2020. Milk samples were collected in 10ml sterile tubes with strict aseptic precautions. The udder was washed with 1% potassium

permanganate solution and wiped with clean dry cloth or tissue paper. Teats and hands were disinfected with 70 percent alcohol and first few strippings's were discarded and finally last milk was collected into sterile tubes and transported immediately to laboratory on ice.

Culturing

The collected milk samples were inoculated into nutrient broth and streaked on to selective medium. Mannitol salt agar media, Edward's media, Eosine Methylene blue agar media and selective differential media of blood agar and macconkey's agar media to obtain pure cultures.

Identification of bacteria

Isolated cultures were identified by grams staining as per the method of Cruick shank *et al.*, (1970).

Bio-chemical confirmation

Cultural isolates were confirmed with bio-chemical test, catalase test, coagulase test, oxidase test, indole test, methyl red test, voges proskauer test and citrate test as per the protocols mentioned in the text book of Clinical Veterinary Microbiology by Markey *et al.*, 2013; Quin and Carter.

In-vitro antibiotic sensitivity test for bacterial isolates

All the isolated bacterial cultures (2,488) were subjected to antibiotic sensitivity test by standard disc diffusion technique as per the method of Bauer et al, 1966 using Muller Hinton agar media. The antibiotics discs were procured from Hi-Media laboratories private limited, Bombay, India. The sensitivity and resistance patterns were recorded with the zone of inhibition and compared with zone

size interpretative chart furnished by the manufacturer of the antibiotics (Hi-media).

Results and Discussion

A total of 2,488 milk samples were collected and processed for culturing as well as invitro susceptibility test to assess the pattern of antimicrobial susceptibility and resistance from Chittoor District, Andhra Pradesh during the period from August, 2015 to March, 2020 at State Level Diagnostic Laboratory, Tirupati. Out of 2,488 milk samples subjected for cultural isolation, 2,174 (87.38%) were of Gram positive and 314 (12.62%) of gram negative pathogens were identified. Among 2,174 gram positive bacterial pathogens, *S.aureus* -2,131(98.02%) was found to be more predominant bacteria followed by Streptococcus-20 (0.92%) and Bacillus species-20 (0.92%) (Table.1). Similarly, among 314 gram negative bacteria, *E.coli*-255 (81.20%) followed by *Klebseilla*-53 (16.88%) and *Pseudomonas*-14 (4.46%). However, out of 2,488 milk samples, 3 samples (0.14%) were found positive for *Candida* (Table.1).

During the period of work, invitro antibiotic sensitivity test results of gram positive bacterial isolates showed resistance to Amikacin -90.43% (1966/2174) followed by Kanamycin -88.36% (1921/2174), Streptomycin-87.49% (1902/2174), Tetracycline-79.62% (1731/2174), Gentamycin-75.30% (1637/2174), Pencillin-60.17% (1308/2174), Ciprofloxacin-52.48% (1141/2174), Enrofloxacin-48.80% (1061/2174), Ampicillin-45.08% (980/2174) and Amoxycillin-36.43% (792/2174) (Table.2 & Fig.1).

Similarly, antibiotic sensitivity pattern of gram positive bacterial isolates include higher sensitivity to Amoxycillin -63.57% (792/2174) followed by Ampicillin-54.92% (980/2174), Enrofloxacin-51.20% (1061/2174), Ciprofloxacin-47.52% (1141/2174),

Pencillin-39.83% (1308/ 2174), Gentamycin-24.70% (1637/2174), Tetracycline-20.38% (1731/2174), Streptomycin-12.51% (1902/2174), Kanamycin-11.64% (1921/2174) and Amikacin -9.57% (1966/2174) (Table-3 & Fig.2).

Whereas, gram negative bacterial isolates (314) revealed resistance to Kanamycin-78.66% (247/314) followed by Pencillin-76.75% (241/314), Amikacin-69.11% (217/314), Tetracycline-66.56% (209/314), Streptomycin-66.56% (209/314), ampicillin-64.65 (203/314), Gentamycin-50.96% (160/314), Amoxycillin-45.22% (142/314), Ciprofloxacin-42.04% (132/314) and enrofloxacin-34.71% (109/314) (Table-3 & fig.3) respectively.

But, gram negative bacterial isolates showed high sensitivity to enrofloxacin-65.20% (109/314), Ciprofloxacin-57.96% (132/314), Amoxycillin -54.78% (142/314), Gentamycin -49.04% (160/314), Ampicillin-35.35 % (203/314), Streptomycin-33.44% (209/ 314), Tetracycline -33.44% (209/314), Amikacin -30.89% (217/314), Pencillin-23.25% (241/314) and Kanamycin -21.34% (247/314) respectively (Table.3 & Fig.4) respectively.

During the study out of 2,488 milk samples, 2,174 (87.37%) gram positive bacteria and 314 (12.62%) gram negative bacteria were recorded on cultural isolation. But earlier Nesser *et al.*, 2006 reported higher 93% of gram positive bacteria and 30% of gram positive bacteria.

Whereas Keffe *et al.*, (2011) recorded 55% of gram positive and 11% of gram negative bacteria. *Staphylococcus aureus* is an important cause of contagious mastitis responsible for udder infections in dairy herds.

Table.1 Prevalence of various bacterial pathogens isolated from bovine clinical mastitis

S.No.	Year	Total milk samples screened/testes	Gram positive organisms				Fungi	Gram negative organisms			
			<i>S.aureus</i>	<i>Strepto coccus</i>	<i>Bacillus</i>	Total	<i>Candida</i>	<i>E.coli</i>	<i>Klebseilla</i>	<i>Pseudomonas</i>	Total
1.	2015-16	320	272	6	-	278	-	32	8	2	42
2.	2016-17	480	408	6	3	417	-	52	7	4	63
3.	2017-18	460	393	3	5	401	-	46	10	3	59
4.	2018-19	650	557	2	4	565	2	71	12	2	85
5.	2019-20	578	501	3	8	513	1	54	8	3	65
Grand total		2,488	2,131	20	20	2,174	3	255	53	14	314
		% Positivity	98.02	0.92	0.92	87.37	0.14	81.20	16.88	4.46	12.62

Table.2 Pattern of Antibiotic Resistance and Sensitivity against gram positive bacteria

S.no	Antibiotic	Gram positive bacteria (n=2,174)			
		No. Resistant	% Resistance	No. Sensitive	% Sensitivity
1.	Amikacin	1966	90.43	208	9.57
2.	Kanamycin	1921	88.36	253	11.64
3.	Streptomycin	1902	87.49	272	12.51
4.	Tetracyclin	1731	79.62	443	20.38
5.	Gentamycin	1637	75.30	537	24.70
6.	Pencillin	1308	60.17	866	39.83
7.	Ciprofloxacin	1141	52.48	1033	47.52
8.	Enrofloxacin	1061	48.80	1131	51.20
9.	Ampicillin	980	45.08	1194	54.92
10.	Amoxycillin	792	36.43	1382	63.57

Table.3 Pattern of Resistance and Sensitivity against gram Negative bacteria

S.no.	Antibiotic	Gram Negative bacteria (n=314)			
		No. Resistant	% Resistance	No. Sensitive	% Sensitivity
1.	Kanamycin	247	78.66	67	21.34
2.	Pencillin	241	76.75	73	23.25
3.	Amikacin	217	69.11	97	30.89
4.	Tetracycline	209	66.56	105	33.44
5.	Streptomycin	209	66.56	105	33.44
6.	Ampicillin	203	64.65	111	35.35
7.	Gentamycin	160	50.96	154	49.04
8.	Amoxycillin	142	45.22	172	54.78
9.	Ciprofloxacin	132	42.04	182	57.96
10.	Enrofloxacin	109	34.70	205	65.29

Fig.1 Antibiotic resistance pattern of gram positive bacteria

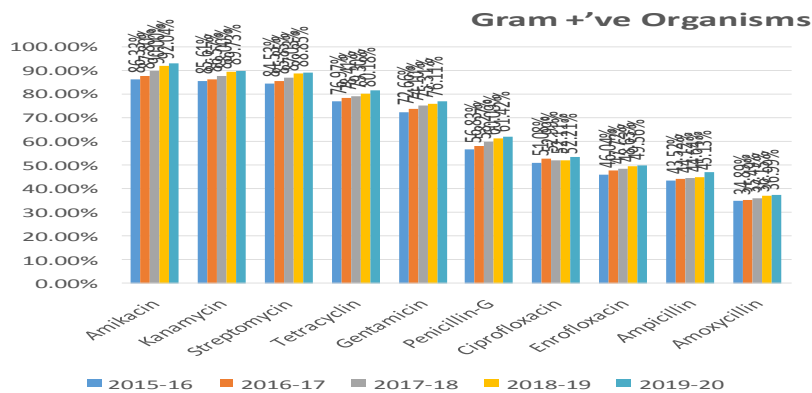


Fig.2 Antibiotic Sensitivity pattern of Gram Positive bacteria from 2015-16 to 2019-20

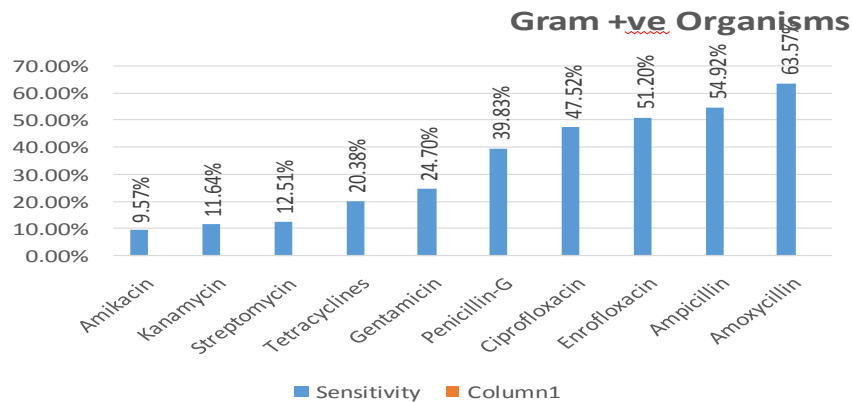


Fig.3 Antibiotic Resistance pattern of gram negative bacteria

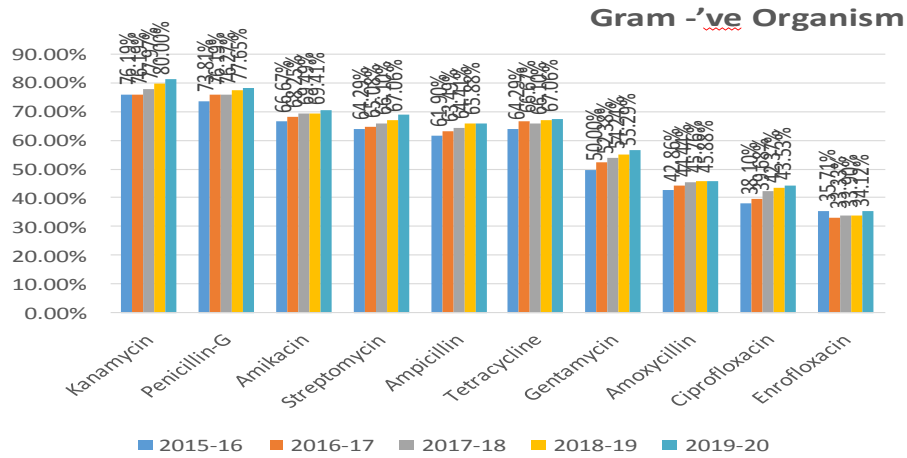
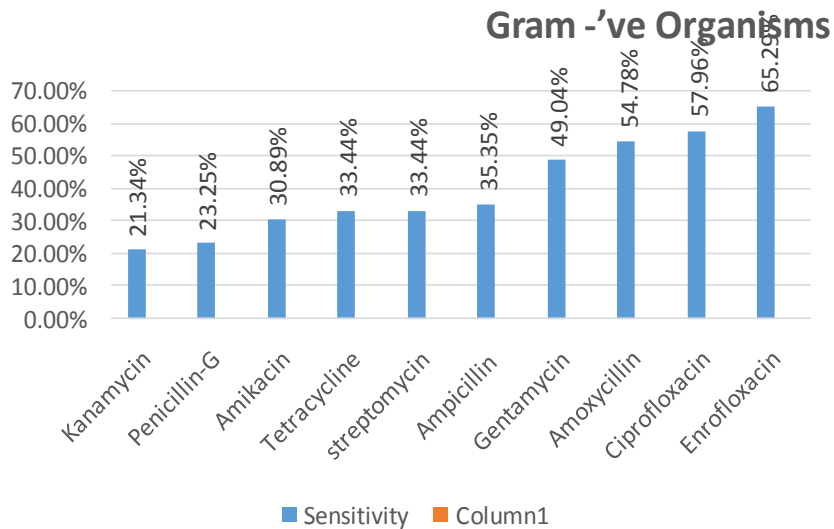


Fig.4 Antibiotic Sensitivity pattern of Gram negative bacteria from 2015- 16 to 2019-20



Staphylococcal species was the major pathogen causing mastitis with high incidence was reported during the study. Previously, similar reports were recorded by several workers from different parts of the country. (Sumathi *et al.*, (2008); Das and Joseph (2005); Sharma and Sindhu (2007); Bhanot *et al* (2012); Hawari and Dabas (2008); Tenhagen *et al.*, (2009); Nickerson (2009)

and Zutic *et al.*, (2012) etc. In the present study, among gram positive bacteria cultural isolation yielded (98.11% of Staphylococcus aureus as major causative gram positive bacteria followed by Streptococcus (0.92%) and bacillus species with 0.87%.

The higher incidence of staphylococcus during the study indicated the unhygienic

milking hands and practices as *Staphylococcus* mainly spread during milking via milkers hands Bradley (2002). Further, this might also be due to harbouring of *Staphylococci* on the skin of the udder, teat which acts as reservoir for the infection (Spencer and Lasmanis (1952); Davidson (1961). Distribution of mastitis pathogens changes overtime, therefore, bacteriological examination at herd level must be taken regularly to monitor udder health.

Antibiotic sensitivity profile will be helpful to recommend early therapy at the field level prior to availability of results of cultural isolation. During the study, gram positive bacteria showed higher resistance to Amikacin (90.43%) followed by Kanamycin (88.36%) and Streptomycin (87.49%) but showed high sensitivity to Amoxycillin (63.57%) followed by Ampicillin (54.92%) and Enrofloxacin (51.20%) respectively. Higher resistance to Amikacin followed by Kanamycin and streptomycin might be due to indiscriminate usage of these antibiotics in majority of the Districts of Andhra Pradesh in treating mastitis cases. This was in accordance with previous reports of Qayyum *et al.*, 2016; Hussain *et al* and Idriss *et al.*, 2014.

Among gram negative bacteria, *E.coli* was the major pathogen isolated (81.21) followed by *Klebsiella* (16.88%) and *Pseudomonas* (4.46%) and it could be due to damp environmental conditions in the dairy herds. Among gram negative bacteria, the prevalence of *E.coli* was indicator of poor hygienic practices in dairy environment, as these organisms originate from the cow's environment and infect the udder through teat canal. Contamination of end of the teat is a major predisposing factor in the development of environmental mastitis (Bradley, 2002.)

In the Current study, gram negative bacteria

showed higher resistance to Kanamycin, Pencillin, Amikacin, Streptomycin and Tetracyclins and high sensitivity to Enrofloxacin followed by Ciprofloxacin, Amoxycillin and Gentamycin respectively. Almost similar findings were reported by Chauhan *et al.*, (2016) & Arthanari Eswaran *et al.*, (2018) except Amoxycillin.

The mastitis bacteria showed less sensitivity to the commonly used antibiotics, due to the frequent use of the particular antibiotics in that particular geographical locations. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their in-effectiveness against mastitis bacteria (Harshit Verma *et al.*, 2018).

Antimicrobial resistance represents a serious problem in the treatment of infectious diseases including mastitis. In recent times, an increasing antimicrobial resistance rate has been recognized in *Staphylococcus aureus* from bovine mastitis (Saini *et al.*, 2012; Wang *et al.*, 2013.). Due to antimicrobial usage over many decades, multiple drug resistance among the mastitis causing agents is a major problem in controlling intra-mammary infections. This is generally attributed to indiscriminate and continuous use of antibacterial drugs without prior drug susceptibility testing or selection pressure of antimicrobials on pathogens or colonization of the mammary gland by resistant strains. Such antimicrobial resistant organisms can pose serious health related problems to animals as well as human beings.

In conclusion, the present work represents the data pertaining to the prevalence of mastitis causing pathogens of bacterial origin and the assessment of resistance as well as sensitivity of various antimicrobials used against mastitis of the different Districts (Geographical areas) of Andhra Pradesh. Gram positive organisms playing important role compared to the gram

negative bacteria in causing mastitis. Among gram positive bacteria staphylococcus aureus was the predominant pathogen followed by streptococcus and bacillus species where as among gram negative bacteria, *E.coli* was the predominant pathogen followed by Klebsiella and Pseudomonas species. Gram positive bacteria showed higher resistance to Amikacin (90.43%) followed by Kanamycin (88.41%) and streptomycin (87.49%) but amoxicillin (63.57%) followed by Ampicillin (54.90%) and Enrofloxacin (51.20%) showed higher sensitivity. Similarly, gram negative bacteria showed resistant to Kanamycin (78.66%) followed by Pencillin (76.81%) and Amikacin (69.10%) and Tetracyclin (66.60%) and higher sensitivity to Enrofloxacin (65.29%) followed by ciprofloxacin (57.46%) and Amoxicillin (54.88%) etc was observed. However, epidemiological surveillance and regular conduct of antibiotic sensitivity tests are of important to avoid antimicrobial resistance, as antibiotic therapy is playing major role in control of mastitis in milch animals to serve the farmers and to increase the socio economic status of the farmers.

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