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Antibiogram pattern against Bovine mastitis in YSR district of Andhra Pradesh, India

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ABSTRACT

Bovine mastitis is economically important disease. Improper use of Antimicrobial agent for treating the condition will result in bacterial resistance and further economic losses. In the present study, the antibiotic sensitivity pattern was assessed in clinical bovine mastitis samples. The study was done during the period of April 2008 to March 2013 in various divisions of YSR district in Andhra Pradesh. A total of 257 subclinical mastitis samples were subjected to antibiotic sensitivity test by using various locally available antibacterial agents. Standard disc diffusion method was used to classify the bacterium involved as susceptible; intermediately susceptible; or resistant. From the study, the highest sensitivity was observed with ciprofloxacin, followed by chloramphenicol, enrofloxacin, amikacin and ofloxacin.

Keywords: Antimicrobial Susceptibility Test (AST), Bovine mastitis, Disc diffusion method

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INTRODUCTION

Bovine mastitis creates considerable animal welfare problems and economical losses in the dairy industry. Most of the mastitis cases are of infectious in origin; the others include trauma, physical injury and hyperactive lymphocytes (Bradely, 2002)[1]. The clinical form of the disease shows inflammation whereas subclinical form exhibits deterioration in the quality and quantity of milk. Most clinical cases are acute, while the subclinical forms are chronic (Barkema *et al*, 2006)[2]. The clinical form is further classified as mild, moderate or severe and it can turn fatal if untreated (Eberhart, 1984)[3]. Despite extensive research on preventive strategies, control programmes and treatment regimens during the last few decades (Eberhart, 1986, Ziv, 1992, Dupreez, 2000)[4-6], still it is considered to be the most important production disease in cattle.

The economic losses are due to improper use of antimicrobial agent against the disease, which can be minimized by conducting antimicrobial susceptibility test (AST) before treatment. Rational and correct use of antibiotics requires geographical understanding of common pathogens and their drug resistance or susceptibility patterns. Now a days, antimicrobial drug resistance is an important problem and due to wide variations in bacterial drug resistance, results of studies and reports in one region at a particular time were not applicable to other areas and even at other time period. They are related with species, environment and management practices. Keeping in view, the present study was conducted on the antibiogram pattern of bovine mastitis in different divisions of YSR district in Andhra Pradesh at Animal Disease Diagnostic Laboratory, Kadapa.

MATERIALS AND METHOD

Samples

A total of 257 bovine subclinical mastitis milk samples were collected from different divisions in YSR district of Andhra Pradesh during the period of April 2008 to March 2013. The per cent sensitivity against each antimicrobial agent was calculated year wise.

Antibiotics

Various classes of antibacterial agents used in the study are as follows

1. Cell wall synthesis inhibitors - Ampicillin, pencillin, cloxacillin, ceftriaxone, amoxicillin and amoxyclav.
2. Protein synthesis inhibitors - Oxytetracycline, tetracycline, streptomycin, amikacin, gentamicin, doxycycline and chloramphenicol
3. Nucleic acid synthesis inhibitors - Ciprofloxacin, ofloxacin, norfloxacin, nitrofurantoin, enrofloxacin and furazolidone and

4. Antimetabolites - Co-trimoxazole.

Antimicrobial Susceptibility Test (AST)

Disc diffusion method

This method entails the placing of discs impregnated with Antimicrobial agents onto an agar plate seeded with the bacterium to be tested. The Antimicrobial agents diffuse into the agar creating a zone saturated with the agent, in which an organism susceptible to that agent will not grow. The edges of the zone are the point of minimum inhibitory concentration (MIC). This method is easy to perform, reproducible and low cost to run. The method should only be used for the rapidly growing pathogens. The standard developed for testing of bacteria isolated from animals is described in document M31-A3 (CLSI, 2008)[7].

Procedure

The collected milk samples were inoculated into nutrient broth and incubated at 37^0C for 24 h to obtain the culture. A sterile- non-toxic swab was dipped into the broth culture and excess fluid was expressed by pressing and rotating firmly against the inside of the tube above the fluid level. The swab was streaked in three directions over the entire surface of the Mueller-Hinton agar plate with the objective of obtaining a uniform inoculum. The inoculated plates were allowed to stand for 3 to 5 minutes, but less than 15 minutes for absorption of the excess moisture from the inoculum. The antibiotic discs were placed at least 24 mm apart (centre to centre) gently using sterile forceps with a maximum of six discs per standard 90 mm Petri dish. The plates were inverted and placed in a 37^0 C incubator and kept for 24 h. After 24 h incubation at 37^0C , the diameters of the zones of inhibition were measured by using a Vernier caliper. An interpretation of the diameter of the inhibition was made with reference to available interpretation tables made available by CLSI (2008) document M21-A3. Based on the tables, the bacterium was classified as susceptible, intermediate susceptible or resistant to each antimicrobial agent used in the test.

Susceptible: The infection may respond to the treatment at the normal dosage.

Intermediate: This category may be used when the pathogen may be invited by attainable concentration of the antimicrobial agent, provided a higher dosage is used or the pathogen is in a certain body site, such as urine, where the drug is physiologically concentrated. Classification of a result as intermediate can be used for technical reasons only.

Resistant: The bacterium is not inhibited by the usually achievable systemic concentrations of the antimicrobial agent and efficacy has not been reliable in clinical studies.

Statistical analysis:

The percentage sensitivity was calculated for each antibiotic used in the study from the total number of sensitive samples and total samples studied

The division wise incidence of mastitis in YSR district and statistical analysis of highest sensitive antibacterial agent in various divisions was calculated by multiple comparisons for proportions by Marasculio Procedure using Microsoft Excel 2013.

RESULTS AND DISCUSSION

During 2008-09, ciprofloxacin was highly sensitive followed by, ofloxacin and enrofloxacin (Table 1). During 2009-10 and 2010-11 ciprofloxacin was the sensitive antibiotic followed by enrofloxacin and chloramphenicol. During 2011-12, ciprofloxacin (or) chloramphenicol was the first choice antibiotic, followed by enrofloxacin and amikacin. During 2012-13, chloramphenicol was highly sensitive followed by ciprofloxacin and enrofloxacin. The results of the five year study showed that ciprofloxacin (32.29%) was the highly sensitive antibiotic followed by chloramphenicol (24.12%), enrofloxacin **(**22.56%), amikacin (16.73%) and ofloxacin (16.34%) against bovine mastitis cases in YSR district (Figure 1)**.** Other authors also reported similar findings (Awandkar *et al*. (2009);Ranjan *et al.,(*2010)][8,9]**.** In this district, ciprofloxacin showed the highest sensitivity in mastitis cases during the last five years when compared to other antibiotics used in this study.

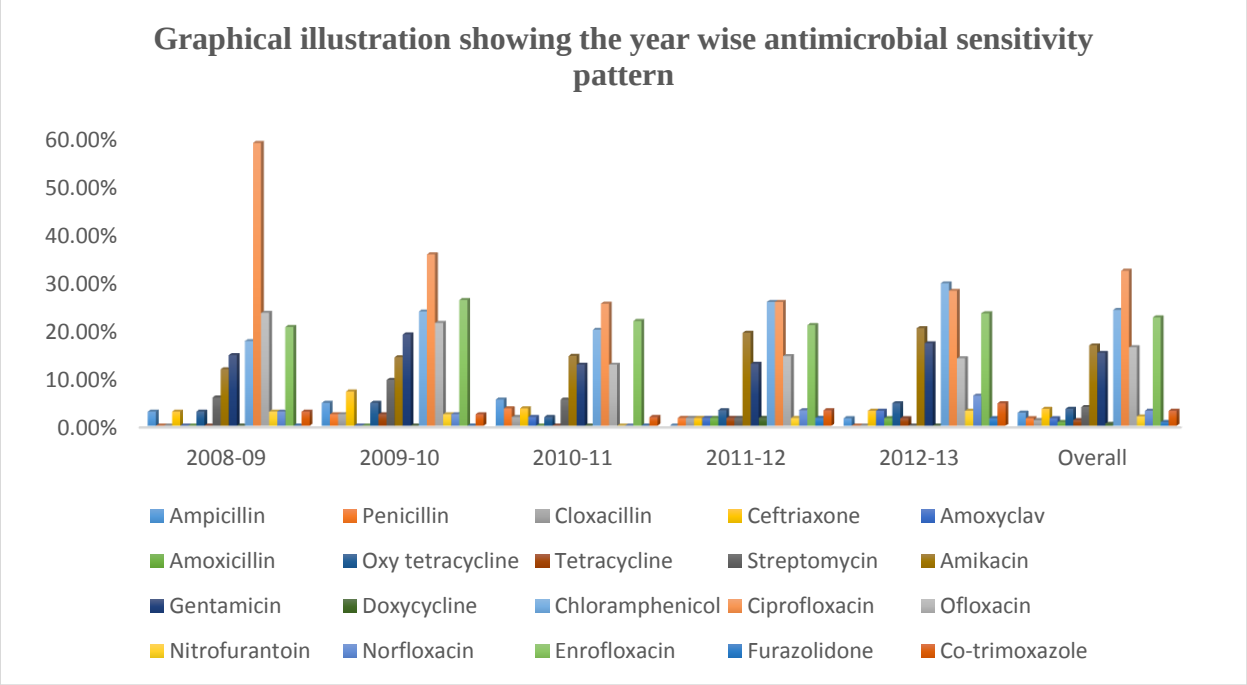

Figure 1: Graphical illustration showing the year wise antimicrobial sensitivity pattern

The study showed that, Rayachoty division had the highest (38.91%) incidence of mastitis in the YSR district (Table 2). This might be due to the fact that most of the animals are crossbred high

yielders in this division and these crossbred animals are more prone to the mastitis agents, when compared to native breeds [Markey *et al.*,2013][10].

The highest sensitive antibiotic in Kadapa division was Amikacin and Ciprofloxacin (Table 3) and in Rayachoty division was ciprofloxacin (Table 3).

Table 1: Antimicrobial susceptibility test results expressed in percent of the samples showing sensitivity.

Name of the antibiotic used in AST	2008-09	2009-10	2010-11	2011-12	201-13	Total
Ampicillin	2.94 (1/34)	4.76(2/42)	5.45(3/55)	0.00(0/62)	1.56(1/64)	2.72(7/257)
Penicillin	0.00 (0/34)	2.38(1/42)	3.63(2/55)	1.61(1/62)	0.00(0/64)	1.55(4/257)
Cloxacillin	0.00(0/34)	2.38(1/42)	1.81(1/55)	1.61(1/62)	0.00(0/64)	1.16(3/257)
Ceftriaxone	2.94(1/34)	7.14(3/42)	3.63(2/55)	1.61(1/62)	3.12(2/64)	3.50(9/257)
Amoxyclav	0.00(0/34)	0.00(0/42)	1.81(1/55)	1.61(1/62)	3.12(2/64)	1.55(4/257)
Amoxicillin	0.00(0/34)	0.00(0/42)	0.00(0/55)	1.61(1/62)	1.56(1/64)	0.77(2/257)
Oxytetracyclin	2.94(1/34)	4.76(2/42)	1.81(1/55)	3.22(2/62)	4.68(3/64)	3.50(9/257)
Tetracyclin	0.00(0/34)	2.38(1/42)	0.00(0/55)	1.61(1/62)	1.56(1/64)	1.16(3/257)
Streptomycin	5.88(2/34)	9.52(4/42)	5.45(3/55)	1.61(1/62)	0.00(0/64)	3.89(10/257)
Amikacin	11.76(4/34)	14.28(6/42)	14.54(8/55)	19.35(12/62)	20.31(13/64)	16.73(43/257)
Getamicin	14.70(5/34)	19.04(8/42)	12.72(7/55)	12.90(8/62)	17.18(11/64)	15.17(39/257)
Doxycycline	0.00(0/34)	0.00(0/42)	0.00(0/55)	1.61(1/62)	0.00(0/64)	0.38(1/257)
Chloramphenicol	17.64(6/34)	23.80(10/42)	20.00(11/55)	25.80(16/62)	29.68(19/64)	24.12(62/257)
Ciprofloxacin	58.82(20/34)	35.71(15/42)	25.45(14/55)	25.80(16/62)	28.12(18/64)	32.29(83/257)
Oflaxacin	23.52(8/34)	21.42(9/42)	12.72(7/55)	14.51(9/62)	14.06(9/64)	16.34(42/257)
Nitrofurantoin	2.94(1/34)	2.38(1/42)	0.00(0/55)	1.61(1/62)	3.12(2/64)	1.94(5/257)
Norfloxacin	2.94(1/34)	2.38(1/42)	0.00(0/55)	3.22(2/62)	6.25(4/64)	3.11(8/257)
Enrofloxacin	20.58(7/34)	26.19(11/42)	21.81(12/55)	20.96(13/62)	23.43(15/64)	22.56(58/257)
Furazolidone	0.00(0/34)	0.00(0/42)	0.00(0/55)	1.61(1/62)	1.56(1/257)	0.77(2/257)
Co-trimoxazole	2.94(1/34)	2.38(1/42)	1.81(1/55)	3.22(2/62)	4.68(3/64)	3.11(8/257)

Values in parentheses indicate number of susceptible / total number of samples.

Table 2: No of cases of mastitis in various divisions during the study period

Year	Kadapa	Rayachoty	Rajampeta	Jammalamadugu	Pulivendula	Total
2008-09	08	12	06	05	03	13.23^{a} (34/257)
2009-10	09	16	07	08	02	16.34^{ab} (42/257)
2010-11	11	21	07	11	05	21.40^{ab} (55/257)
2011-12	13	25	09	11	04	24.12^{a} (62/257)
2012-13	12	26	08	14	04	24.90^{a} (64/257)
	20.62^{b} (53/257)	38.91^{c} (100/257)	14.40^{ab} (37/257)	19.07^{b} (49/257)	7.00^{a} (18/257)	

Values are percentages with actual proportion in parenthesis.

Marasculio Procedure using Microsoft Excel 2013.

Percentages with different superscripts in a column are significantly different ($P<0.05$)

Table 3: Sensitivity of isolates (%) to various antibiotics division wise in YSR district during the study period

Name of the highest sensitive antibiotic used	Kadapa	Rayachoty	Rajampeta	Jammalamadugu	Pulivendula
Ciprofloxacin	28.30 (15/53)[b]	35.78 (39/109)[b]	25.00 (11/44)[a]	23.33 (14/60)[a]	18.18 (4/22)[a]
Chloramphenicol	18.87 (10/53)[ab]	17.43 (19/109)[a]	27.27 (12/44)[a]	23.33 (14/60)[a]	13.64 (3/22)[a]
Enrofloxacin	5.66 (3/53)[a]	10.09 (11/109)[a]	25.00 (11/44)[a]	18.33 (11/60)[a]	27.27 (6/22)[a]
Amikacin	30.19 (16/53)[b]	22.02 (24/109)[ab]	15.91 (7/44)[a]	18.33 (11/60)[a]	18.18 (4/22)[a]
Ofloxacin	16.98 (9/53)[a]	14.68 (16/109)[a]	6.82 (3/44)[a]	16.67 (10/60)[a]	22.73 (5/22)[a]

Values are percentages with actual proportion in parenthesis

Marasculio Procedure using Microsoft Excel 2013

Percentages with different superscripts are significantly different ($P<0.05$)

CONCLUSION

It is concluded that the *in vitro* results indicate ciprofloxacin as the most effective antibiotic against bovine mastitis followed by Chloramphenicol, Enrofloxacin, Amikacin and Ofloxacin respectively in YSR district.

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