

## Original Research Article

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## Incidence of Bovine Clinical Mastitis and Antibiogram of the Isolates in Odisha, India

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### ABSTRACT

The present study was taken up to evaluate the antibiogram against different mastitogenic pathogens isolated from the milk samples of clinical bovine mastitis cases. A total of 78 milk samples were collected from different dairy farmers of Nialiblock, Odisha and processed in Animal Disease Research Institute (ADRI), Phulnakhara, Cuttack for isolation, identification and antibiogram of pathogens. The samples were screened for mastitis by modified California mastitis test (MCMT). The milk samples were inoculated in BHI broth and incubated for 24-48 hours. Then a loopful of culture was streaked into EMB Agar, Mannitol Salt Agar and Blood Agar. The isolates were identified according to the cultural, morphological and biochemical characteristics as per standard methods. All the isolates were subjected to Kirby-Bauer disc diffusion methods using different antibiotics. Out of 78 samples, 73 samples were revealed to be positive for bacterial growth. Bacterial screening of samples revealed that 22 samples yielded pure culture and 51 samples yielded mixed cultures. The predominant bacterial isolates recovered were *Staphylococcus spp* (48.17%) followed by *E. coli* (27.73%), *Streptococcus spp* (12.41%), *Klebsiella spp* (9.48%) and *Bacillus spp* (2.19%). It was found that *Staphylococcus* is highly sensitive to Enrofloxacin and Gentamicin followed by Ceftriaxone/ Tazobactam and Ciprofloxacin. The study revealed that *E. coli* is highly sensitive to Chloramphenicol followed by Enrofloxacin, Ciprofloxacin and Gentamicin. The *Streptococcus spp* is found to be sensitive to Enrofloxacin followed by Ciprofloxacin and Gentamicin. All the isolates are found to be almost resistant against Penicillin G, Oxytetracycline, Ampicillin and Ampicillin/Cloxacillin.

#### Keywords

Bovine mastitis,  
MCMT,  
Antibiogram,  
*Staphylococcus*

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### Introduction

Mastitis is a multi-etiological complex disease, defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits *et al.*, 2000). It is a devastating

disease causing annual economic loss of Rs 6053.21 crores out of which, Rs 4365.32 crore (70-80%) has been attributed to subclinical mastitis (Dua *et al.*, 2001). Apart from the economic losses mastitis can have serious implications on public health. Infected milk is serving as a media for transmission of various zoonotic diseases like TB, Brucellosis, Diphtheria, Scarlet fever and Q fever

(Mahantesh *et al.*, 2011). About 137 species of microorganisms including bacteria, Yeast, fungi and algae are able to cause bovine mastitis. The majority of mastitis incidences are caused by only a few common bacterial pathogens like *Staphylococcus spp.* (*Staphylococcus aureus* and *Staphylococcus epidermidis*), *Streptococcus spp.* (*Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*), *Coliforms* (mainly *E. coli* and *Klebsiella pneumoniae*) and *Actinomyces pyogenes* (Sharma, 2010). Coagulase negative *Staphylococci* and *Corynebacterium bovis*, two other prevalent pathogens are described as minor pathogens.

Antimicrobials are routinely used for treatment of dairy cattle affected with clinical and subclinical infection (Aarestrup, 2005). The recovery rate is low due to continuous indiscriminate use of antibiotics defying the rational approach of selection of suitable antibiotics after doing culture and sensitivity test. The situation is further worsened by the approach of dairy farmers who prefer to take over the counter medicine without consulting a qualified veterinarian. Therefore the present study was undertaken to study the effectiveness of different antibiotics against mastitogenic isolates.

## **Materials and Methods**

The specimen for the present research work comprised of milk samples obtained from clinical cases of bovine mastitis from dairy farmers of Niali block of Cuttack district. The samples were processed in Animal Disease Research Institute (ADRI), Phulnakhara, Cuttack for isolation, identification and antibiogram of mastitis pathogens. All the samples were screened for mastitis by Modified California mastitis test (MCMT). Each sample was taken by means of a sterile inoculating loop and inoculated into liquid media (BHI Broth) followed by incubation for

24 to 48 hrs at 37<sup>0</sup>C. A loopfull of culture was streaked into Muller Hinton Agar (MHA), Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA) and Blood agar followed by 24 hr of incubation at 37<sup>0</sup>C.

The different colonies were observed and identified according to their colony characteristics and growth in selective media. The morphological characteristics were studied by examination of Gram stained smears. The isolates were identified according to the cultural, morphological and biochemical characteristics as per the methods of Cruickshank *et al.*, (1975).

All the isolates were subjected to *in vitro* antibiotic sensitivity test as per Kirby- Bauer disc diffusion methods using 14 no. of antibiotic discs procured from Hi-Media Pvt. Ltd. such as Ceftriaxone/Tazobactam (30/10mcg), Ceftriaxone (30mcg), cefoperazone (30mcg), ceftizoxime (30mcg), Ciprofloxacin (5mcg), cefuroxime (30mcg), Ampicillin/cloxacillin (10mcg), Gentamicin (10mcg), Amoxycillin/Sulbactam (30/15mcg), Chloramphenicol (30mcg), Enrofloxacin (10mcg), Penicillin G (10units), Oxytetracycline (30mcg) and Ampicillin (2mcg).

The inoculum was prepared by transferring 2 to 3 colonies from pure culture to BHI broth followed by incubation at 37<sup>0</sup>C for 6 to 8 hr until turbidity appears. The standardized inoculum was then swabbed on to the MHA plate. Then the antibiotic discs were then placed aseptically equidistant from each other on the surface of MHA plate. The plates were incubated at 37<sup>0</sup>C for 24 to 48 hrs. The diameter of zone of inhibition was measured and compared with the values listed in the standard chart provided by the manufacturer on the basis of which isolates were categorized as sensitive, intermediate and resistant to the antimicrobials.

## Results and Discussion

In this study, a total of 78 number of milk samples were examined out of which 73 samples were found to be positive and in 5 samples, no growth has been observed in liquid as well as solid media which may be due to prior medication within 72 hours before collection in udder. Bacteriological screening of samples revealed that out of 73 samples 22 samples yielded pure culture and remaining 51 samples yielded mixed culture. The pure culture revealed presence of *Staphylococcus*, *Streptococcus* and *E. coli* whereas mixed culture showed presence of *Klebsiella* and *Bacillus* in addition to the isolates of pure culture.

The predominant bacterial isolates recovered were *Staphylococcus spp* (48.17%) followed by *Escherichia Coli* (27.73%) followed by *Streptococcus spp* (12.41%), *Klebsiella Spp* (9.48%) and *Bacillus Spp* (2.19%). The frequency of isolation of different bacterial species is depicted in Table 1.

In the present study, prevalence of *Staphylococcus* is high followed by *E. coli* and *Streptococcus spp* which is in agreement with the earlier reports of Aarestrup *et al.*, (1995),

Sumathi *et al.*, (2008) and Junaidu *et al.*, (2011). *Staphylococci* are the most important and prevalent mastitogenic organisms globally including India. The prevalence of *Staphylococcus spp* may be due to ubiquitous nature of the organism and the drug resistance shown by them due to biofilm production. It may be associated with incomplete milking especially when the lesions in the udder are painful. The prevalence of *E. coli* is due to poor hygienic condition in the shed. The prevalence of *Streptococcus spp* may be due to poor dairy practice methods. The *Bacillus spp* is present in soil, water and manure. The bacteria infects animal via teat canal as reported by Mahantesh *et al.*, (2011).

### Antibiogram of the isolates

*Staphylococcus* is the most important and prevalent mastitogenic organism occurring globally including India (Sumathi *et al.*, 2008). In the present study, it was found that *Staphylococcus* is highly sensitive to Enrofloxacin and Gentamicin followed by Ceftriaxone/Tazobactam and Ciprofloxacin (Table 2). This is well in agreement with many earlier reports by Sumathi *et al.*, (2008), Kaliwal *et al.*, (2011) and Dhakal *et al.*, (2007).

**Table.1** Frequency of different mastitogenic isolates from clinical mastitis samples

S. No.	Name of the bacteria	Number of isolates	Percentage (%)
1	<i>Staphylococcus spp</i>	66	48.17
2	<i>Streptococcus spp</i>	17	12.41
3	<i>E. coli</i>	38	27.73
4	<i>Klebsiella spp</i>	13	9.48
5	<i>Bacillus spp</i>	3	2.19

**Table.2** Sensitivity pattern of microorganism isolated from clinical mastitis to various antimicrobial agents

Name of the antibiotics	<i>Staphylococcus</i>			<i>Streptococcus</i>			<i>E. coli</i>		
	S	I	R	S	I	R	S	I	R
Ampicillin	0	2	64	0	2	15	0	0	38
Ampicillin/Cloxacillin	0	4	62	0	3	14	0	0	38
Amoxicillin/Sulbactam	2	60	4	4	11	2	0	31	7
Chloramphenicol	56	8	2	10	6	1	36	1	1
Ciprofloxacin	58	8	0	15	2	0	32	3	3
Ceftriaxone	52	8	6	9	6	2	18	17	3
Ceftriaxone/Tazobactam	58	8	0	11	4	2	20	15	3
Cefoperazone	51	8	7	9	5	3	16	15	7
Cefuroxime	36	21	9	8	4	5	14	16	8
Ceftizoxime	40	18	8	8	4	5	13	14	11
Enrofloxacin	63	3	0	16	1	0	33	4	1
Gentamicin	60	5	1	12	3	2	27	9	2
Penicillin G	0	0	66	0	0	17	0	0	38
Oxytetracycline	0	5	61	0	3	14	0	6	32

The incidence of mastitis by *E. coli* may have been due to poor hygienic condition (Bradley, 2002). Current study revealed that *E. coli* is highly sensitive towards Chloramphenicol followed by Enrofloxacin, Ciprofloxacin and Gentamicin (Table 2). It is in accordance of the report by Mohanty *et al.*, (2013). *Streptococcus spp* in the current study was found to be sensitive to Enrofloxacin followed by Ciprofloxacin and Gentamicin. Jain *et al.*, (2012) also reported the similar result.

All the isolates are found to be almost resistant against Penicillin G, Oxytetracycline, Ampicillin and Ampicillin/Cloxacillin. The rate of Penicillin resistance (100%) in the current study is much higher than those reported by other workers like Rajala-Schultz *et al.*, (2009) and Kenar *et al.*, (2012), Mahami *et al.*, (2011) reported 100% resistance against Oxytetracycline which contradicts the current study. Resistance to Penicillin in *Staphylococcus* is associated with the production of Beta Lactamase and

low affinity penicillin binding protein Leelahapongsathan *et al.*, (2014). The overuse and improper selection of antimicrobial drugs to reduce infection of mammary glands may lead to lower production and development of resistant bacteria. In the current study the prevalence of *Staphylococcus* was found to be maximum followed by *E. coli* and *Streptococcus*. These three isolates showed high sensitivity to Enrofloxacin, Gentamicin and Ciprofloxacin. There is need to enhance the awareness of farmers to go for antibiotic sensitivity test while choosing the drug for mastitis.

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