



## **Status Of Methicillin Resistant *Staphylococcus Aureus* In Mastitic Cows Of Middle Gangetic Plains Of Eastern Uttar Pradesh**

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### **ABSTRACT**

*Mastitis is a disease complex that ensues as a sequel to interplay between microorganisms, environment and the managerial practices. Different microorganisms are associated with mastitis. Amongst all, Staphylococcus aureus is a major cause of bovine mastitis causing substantial economic losses in the dairy industry world-wide. In the present scenario, Methicillin-resistant Staphylococcus aureus (MRSA) holds importance and it has emerged as one of the most challenging task for veterinarians. The most effective antibiotic against S. aureus in this region against subclinical mastitis is Tetracycline followed by Ofloxacin, Gentamicin and Methicillin.*

**Key words:** Mastitis, *Staphylococcus aureus*, MRSA

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### **INTRODUCTION**

Mastitis is a disease complex that ensues as a sequel to interplay between microorganisms, environment and the managerial practices. It is a persistent enigmatic problem affecting the dairy herds and it is difficult to locate a cow that has not been affected with mastitis in her life time, even if maintained under best of the best managerial practices.

It is also a common cause of mastitis in dairy cows (Virgin *et al.*, 2009), a primary reason for antibiotic use on farms. Different microorganisms are associated with mastitis. Amongst all, *Staphylococcus aureus* is a major cause of bovine mastitis causing substantial economic losses in the dairy industry worldwide (Saei *et al.*, 2009). *Staphylococcus aureus* is an important opportunistic pathogen both in humans and in dairy cattle. *Staphylococci* are widespread in nature and occupy variety of niches. As a result of their ubiquity and adaptability, they are a

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major group of bacteria inhabiting the skin, skin gland and mucous membrane of humans, other mammals, and birds. *S. aureus* bovine mastitis is a common reason for therapeutic and/or prophylactic use of antibiotics on dairy farms.

In the present scenario, Methicillin-resistant *Staphylococcus aureus* (MRSA) holds importance and it has emerged as one of the most challenging task for veterinarians.

### **Methicillin-resistant *Staphylococcus aureus* (MRSA)**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult to treat infections in humans (Bagcigil *et al.*, 2007). It is also called multi drug resistant *Staphylococcus aureus* and Oxacillin-resistant *Staphylococcus aureus* (ORSA). Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged globally as a significant public health/antimicrobial resistance problem both in human and veterinary medicine. It has been well known as a nosocomial agent, later also as a community-associated pathogen. In recent years, so called non-typable (NT) MRSA (also called Livestock-associated MRSA, LA-MRSA) has become an additional focus of concern.

Till date no reports are available on MRSA and Multiple Drug Resistance (MDR) in bovines of middle gangetic plains of eastern Uttar Pradesh. So present study was designed in order to determine the status

of MRSA and resistance pattern against commonly used antibiotics in cows of middle gangetic plains of eastern Uttar Pradesh.

## MATERIALS AND METHOD

A total of 60 milk samples were collected from the animals present in the Sultanpur and Faizabad district. Two blocks from each district were selected and from each block three villages were chosen. From each village 5 samples were collected. In this way a total of 60 milk samples were collected from the cows.

### Clinical inspection of the udder

Udders of the cows were examined by visual inspection and palpation for the presence of any lesion, pain, heat and swelling. In addition, milk from each quarter was withdrawn and checked for any change in colour and consistency.

### Collection of milk sample

Milk samples were collected from Sultanpur and Faizabad districts of middle gangetic plains of eastern Uttar Pradesh. A total of 60 milk samples were collected from the animals present in the Sultanpur and Faizabad district. The affected quarter was washed with tap water, dried properly and teat and udder was sterilized with cotton soaked with 70% ethyl alcohol. Approximately 10 ml of milk was then collected aseptically from a mastitic water into sterile bottle after discarding the first 3 to 4 milking streams. Sample from each quarter were transported on ice to the bacteriology laboratory of department of veterinary microbiology and were immediately cultured or stored at 4°C for a maximum of 24 hrs until cultured on standard bacteriological media.

### Study of methicillin-resistant

The *Staphylococcus aureus* isolates were tested for antibiogram including resistance to methicillin using modified Kirby-Bauer disk diffusion method using standard antibiotics discs (Hi-Media) on Mueller Hinton Agar (MHA) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines using the criteria of standard zone sizes of inhibition to define sensitivity or resistance (CLSI 2005). Methicillin resistance was recorded by using Oxacillin disc (1 mcg) (Hi-Media). Also Oxacillin Ezy Strip (Hi-Media) were used for further confirmation of MRSA.

## RESULTS AND DISCUSSION

The present investigation was planned to study the antibiogram pattern of *S. aureus* with special reference to MRSA in cows of middle gangetic plains of eastern Uttar Pradesh and in order to find out the multi drug resistance pattern of *S. aureus* in the zone of study.

Out of 60 milk samples, 40 samples (66.66%) were found positive for mastitis due to *S. aureus*. Highest resistant was reported against Cefotaxime with resistance in 40 isolates (100%). Thirty five isolates (87.5%) were resistant to Ampicillin followed by resistance to Ceftazidime-Tazobactam (30 isolates, 75%), oxacillin (50%, 20 isolates), Cloxacillin (37.5%, 15 isolates) and Gentamicin, Ofloxacin and Methicillin (5 isolates (12.5%) of each).

On antibiogram mapping it was found that the most effective antibiotic against *S. aureus* in this region was Tetracycline with a sensitivity of 100% i.e. none of the isolates have shown resistance against it which was followed by Ofloxacin, Methicilline and Gentamicin (**Table 01**).

**Table 1. Resistant pattern in mastitis in cows**

S. No.	Antibiotic disc	Code	No. of susceptible isolates (%)	No. of resistant isolates (%)
1.	Methicillin	MET	35 (87.5%)	5 (12.5%)
2.	Oxacillin	OX	20 (50%)	20 (50%)
3.	Ceftazidime Tazobactam	CAT	10 (25%)	30 (75%)
4.	Cefotaxime	CTX	0 (0%)	40 (100%)
5.	Ampicillin	AMP	5 (12.5%)	35 (87.5%)
6.	Tetracycline	TE	40 (100%)	0 (0%)
7.	Gentamicin	GEN	35 (87.5%)	27 (12.5%)
8.	Ofloxacin	OF	35 (87.5%)	5 (12.5%)
9.	Cloxacillin	COX	25 (62.5%)	15 (37.5%)

**Methicillin Resistance *S. aureus* isolates**

In this study 5 isolates (12.5%) were methicillin resistant (5 out of 40) by disc diffusion method which was further confirmed by MRSA Detection EZY MIC strip. These 5 isolates were further tested by Oxacillin Ezy MIC Strip (0.016-256 mcg/ml) of Hi-Media that further confirmed the resistance to Methicillin in 5 isolates.

**Multiple drug resistance (MDR)**

Multiple drug resistance has been observed in this study. Among these 40 isolates, 15 isolates showed resistance against 2 antibiotics (37.5%), 5 isolates were found resistant to three, four and eight antibiotics (12.5% each) and 10 isolates have showed resistance against five antibiotics (25%). Out of 40 isolates, 5 isolates were found to be resistant against Methicilline (12.5%).

In the current study antibiotic resistance pattern of *S. aureus* isolates was tested against 9 antibiotics viz. Ampicillin, Cloxacillin, Tetracycline, Oxacilline, Cefotaxime, Ofloxacin, Gentamicin, Ceftazidime-Tazobactam and Methicillin. The resistant pattern observed was cefotaxim > ampicillin > ceftazidime and tazobactam > oxacillin > cloxacillin > methicillin, gentamicin and ofloxacin. Multi Drug Resistance exists in cows of Eastern Plain Zone. The most effective antibiotic against *S. aureus* in this region against subclinical mastitis is Tetracycline followed by Ofloxacin, Gentamicin and Methicillin. Previous studies from North west India and Chennai reported methicillin resistant *Staphylococcus aureus* positive percentages as 13% and 10.44% respectively (Kumar, *et. al.* and Chandrasekaran, 2014).

**CONCLUSION**

It can be concluded from the study that Methicillin-resistant *Staphylococcus aureus* (MRSA) is the predominant cause of bovine mastitis and holds importance among the growers of eastern plain zone of Uttar Pradesh and it has emerged as one of the most challenging task for veterinarians. The most effective antibiotic against *S. aureus* in this region against subclinical mastitis is Tetracycline followed by Ofloxacin, Gentamicin and Methicillin.

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