

# Involvement of fungal species in bovine mastitis in and around Mathura, India

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## Abstract

**Aim:** To study the prevalence of mycotic mastitis in bovines of Mathura region.

**Materials and Methods:** A total of 100 milk samples collected from seven different regions of Mathura were examined by cultural, morphological and biochemical methods.

**Results:** Out of 100 milk samples processed 64 samples were found positive for fungal isolates. The most common isolates were *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus niger*.

**Conclusion:** The results of the present study suggest that *Candida spp* and *Aspergillus spp* are the main fungi involved in bovine mastitis in this region. Good hygiene and sanitation practices of animal farm and judicious use of antibiotics will lower incidence of bovine mycotic mastitis.

**Keywords:** *Aspergillus*, bovine, candida, fungus, mastitis, Mathura

## Introduction

Mastitis is the inflammation of the mammary gland. It is widespread in dairy herds and associated with a significant reduction in milk yield resulting in increased costs of production and deteriorated quality of milk and milk products [1]. Mastitis is characterized by partial or complete damage to udder tissues which is usually a consequence of microbial infection. A wide variety of microorganisms have been implicated as causative agent of bovine mastitis including bacteria and fungi [2].

Seriousness of mycotic infection of mammary glands depends upon the species of the fungus involved as well as the percentage of infectivity. Treatment of mycotic mastitis is a challenge as many of these fungi do not respond to the antibiotics therapy rather they use some of the antibiotics like tetracycline as their source of energy [3].

In routine mastitis therapy antibiotics are used more often and much emphasis is not given on the antifungal therapy. Due to this reason most of the mastitis cases not only become incurable but also act as a source of infection for other lactating animals in the herd. Therefore it has become necessary to know about the fungal spp. playing role in mastitis, which could be differentially diagnosed and pinpoint therapy should be given to the animals.

Though there are many reports of fungal involvement in bovine mastitis around the globe, still

very little work is done in India. Present study was undertaken to know the prevalence of fungal infections and the species involved in clinical and sub-clinical mastitis.

## Materials and Methods

A total of 100 milk samples were collected for fungal isolation from March to May 2010 (Table-1). Out of these 20 milk samples were collected from clinical mastitis cases and 80 milk samples were collected from apparently healthy cows and buffaloes. 15ml of milk was collected from each animal and transported to laboratory under ice. Milk samples were examined for colour, consistency and pH. Samples were streaked on Saboraud's dextrose agar plates. The plates were incubated at 37°C and examined for growth at 24, 48 and 72 hours and at weekly intervals for 4 weeks after which the plates showing no growth were considered negative [3]. The colonies were picked up and re-streaked on another SDA plate to get the pure cultures. These fungal isolates were studied for their cultural and morphological characteristics. The morphological characteristics were noted after staining with Gram's and Lacto phenol cotton blue stain. Isolates were identified by different sugar fermentation test. Briefly, 1% solution of different sugar solutions were dispensed into separate tubes and fungal isolates were inoculated in these tubes. The tubes were then incubated at 37°C for 24-48 hours. The results were indicated by the change of colour [4].

## Results

Out of 100 samples, 64 samples (14 from clinical

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Table-1. Samples collected from different places

Sr. No.	Milk Samples	Total	Areas	No. of samples
1.	Clinical Samples	20	Kothari Hospital Veterinary University, Mathura	20
2.	Subclinical samples	80	Haridayal Goshala, Vrindavan	10
			Shriji baba Goshala, Mathura	10
			Panchayanti Goshala, Raman Reti	20
			DDD Farm-Mathura	20
			Krishna farm-Mathura	10
			Daudayal farm-Baldeo Mathura	10
	Total	100		100

Table-2. Species wise isolation from different samples

Sr. No.	Type of sample	Total no of Sample	No. positive for fungus isolation	Species isolated	No. of isolates	Percentage infectivity
1	Clinical	20	14	<i>Candida albicans</i>	6	30
				<i>Aspergillus fumigatus</i>	3	15
				<i>Aspergillus niger</i>	5	25
2	Subclinical	80	50	<i>Candida albicans</i>	20	25
				<i>Aspergillus fumigatus</i>	14	17.5
				<i>Aspergillus niger</i>	16	20

cases and 50 from subclinical cases), were found positive for fungal isolation. Of the 64 isolates, 26 isolates yielded smooth white or yellowish, cottony colonies having resemblance with *Candida spp.* Upon microscopic examination these isolates showed oval-shaped budding cells. Sugar fermentation test revealed that the isolates can ferment glucose, maltose and trehalose but cannot ferment lactose and sucrose. These 26 isolates were tentatively identified as *Candida albicans* [5].

Remaining 38 isolates yielded rapidly growing colonies with green or black pigmentation, showing resemblance with *Aspergillus spp.* Out of 38 isolates 17, revealed granular to cottony colony, septate mycelium with their conidiophores long and have club-shaped vesicles. Vesicles were uniseriate and only the distal half was covered by conidia. These 17 isolates were identified as *Aspergillus fumigatus*. Rest 21 of *Aspergillus spp.* revealed long non-septate conidiophore with black spore heads with rapidly growing colonies. Vesicle was obscured due to profuse conidiation. These 21 isolates were tentatively identified as *Aspergillus niger* (Table-2) [6].

#### Discussion

Fungi as an etiological agent of mastitis have also been reported by various workers [7-12]. Prevalence of *Candida albicans* in clinical and subclinical cases was found to be 30% and 25% (Table-2). The overall infectivity of *Candida albicans* was 26%. Other researchers have also reported *Candida spp.* as a causative agent of bovine mastitis [13-18]. This higher rate of *Candida* infectivity may be due to the summer.

Prevalence of *Aspergillus spp.* in clinical and subclinical cases was found to be 40% and 37.5% (Table 2). The overall infectivity of *Aspergillus spp.* was 38%. Other researchers have also reported *Aspergillus spp.* as a causative agent of bovine mastitis [19-21]. As *Aspergillus spp.* is a common pathogen of human skin this high incidence of may be attributed to possible transmission during milking.

A higher percentage of isolation of fungi from clinical cases and subclinical cases reveal that the incidence of fungal mastitis is increasing which may be due to unhygienic conditions of the animal sheds supporting the growth of fungal spores and hyphae in the vicinity of lactating animals. Thereby, increasing the probability of fungal spores and yeast cells to enter into the udder parenchyma which provides the best environment for the growth of these fungi [22,23]. It may be due to the development of antibiotic resistance in the bacteria which prolongs the course of treatment favoring chances for the fungal species such as *Candida* and *Aspergillus* to infect as secondary invader, due to the physiological changes in udder and milk. There may be the seasonal influences like high humidity along with favorable ambient temperature. Besides management practice like bathing of animals in polluted Yamuna river may be contributory. Other practices like discarding first few streaks of milk on ground while milking may be contributing factor in the increased incidences of fungal mastitis. Further detailed investigation on the mycotic and bacterial mastitis is required in relation to the hygienic and management practices as well as pattern of antibiotic therapy adopted for the treatment of these cases.

#### Authors' contribution

All the authors contributed equally for this study. All authors read and approved the final manuscript.

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#### Competing interests

Authors declare that they have no competing interest.

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