

# Studies on Bacterial Profile of Repeat Breeding Cows with Subclinical Endometritis

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**Abstract:** The study was conducted on 72 uterine cytology samples collected from repeat breeding cows with subclinical endometritis for bacteriological examinations to find out the prevalence of aerobic bacterial flora in subclinical endometritis. Presence of aerobic bacteria was detected in 61 samples (84.72%) of repeat breeding cows with subclinical endometritis. Of the 72 cows, 61 samples (84.72%) yielded bacterial isolates; among which *Staphylococcus aureus* isolates were recorded the highest percentage of total isolates (38.88 %) followed by *E. coli* spp. (36.11 %), *Streptococcus* spp. (33.33 %), *Enterobacter* spp. (22.22 %), *Proteus* spp. (18.05 %) and *Pseudomonas* spp. (16.67 %). It is concluded from the study that large proportion of cows will have subclinical endometritis due to aerobic bacteria which results in repeat breeding syndrome.

**Keywords:** Aerobic Bacteria, repeat breeding, subclinical endometritis

## 1. Introduction

Repeat breeding defined as cows with failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis, 1980). Repeat breeder syndrome is a major source of economic loss and poor reproductive performance in dairy cows (Bartlett *et al.*, 1986; Yusuf *et al.*, 2010). 12.5% subclinical endometritis is a major to the repeat breeder syndrome of bovines (Noakes *et al.*, 2002). Subclinical endometritis is defined as the inflammation of the uterus, resulting in a reduction of the reproductive performance, in the absence of uterine discharge (Sheldon *et al.*, 2006). Kasimanickam *et al.*, (2004) defined the assessment of inflammation at 40–60 days post partum corresponded approximately to >5% Neutrophils. Generally, non-specific infection of the genitalia is considered to be the main cause of repeated conception failure. A wide variety of micro flora infect female genitalia and play a significant role in causing inflammation of endometrium and lead to conception failure (Singh *et al.*, 1996).

Hence the present study was designed to isolate the aerobic bacteria present in uterine discharge of repeat breeder animals with subclinical endometritis.

## 2. Material and Methods

The study was conducted between August 2015 to February 2017 on Crossbred Dairy Cows of five Organized Dairy Farms (n = 387) in and around Bengaluru. The animals used for the study were crossbred dairy cows with a history of

repeat breeder syndrome. Only the cows aged not more than eight years and that had calved at least once, failed to conceive for three or more consecutive insemination with apparently normal genitalia and estrous cycle length without any signs of clinical endometritis. The cows with BCS of less than 3.0 were utilized for the study. Clinical endometritis was ruled out using Vaginoscopy. All repeat breeder cows were screened for the subclinical endometritis (SCE) based on endometrial cytobrush cytology (Baranski *et al.*, 2012) with a cut off percentage of PMN 5%.

The cytobrush samples obtained from endometrium (Baranski *et al.*, 2012) of repeat breeder cows with subclinical endometritis inoculated into the Nutrient broth (Hi-Media, India) and incubated for 12 hours at 37° C. The nutrient broth was then checked for the turbidity that indicated growth. A loopful of broth culture was then streaked onto Nutrient agar and selective media like Mac Conkey agar, Mannitol salt agar (Hi-Media, India) and incubated at 37°C for 24 hours. Plates were examined for bacterial growth after 24 hours of incubation. The colonies obtained after primary inoculation, were further inoculated to selective media like Eosin Methylene Blue agar. A tentative identification of bacteria was done based on colony morphology, Grams staining and various biochemical tests (Cruickshank *et al.*, 1980).

## 3. Results

A total of 72 (38.29%) cows of 188 repeat breeding cows were diagnosed as having subclinical endometritis based Endometrial cytology with a cut off percentage of PMN >5% (cytobrush technique). Among the 72 repeat breeders

with subclinical endometritis the 61 (84.72 %) cows were having uterine swab positive for aerobic bacterial isolates and 11 (15.27 %) swabs were free from bacterial isolates (Table 1). Among the aerobic bacteria isolates 19 (31.15 %) were single isolates and 42 (68.85 %) were mixed isolates (Table 1).

Six different genres of aerobic bacteria were identified in the uterine bacterial culture. It was observed that *Staphylococcus aureus* isolates were recorded at the highest percentage of total isolates (38.88 %) followed by *E. coli* sp (36.11 %), *Streptococcus* sp (33.33 %), *Enterobacter* sp (22.22 %), *Proteus* sp (18.05 %) and *Pseudomonas* (16.67 %) in cases of repeat breeding cows with subclinical Endometritis (Table 2).

#### 4. Discussion

Association of bacteria isolation in repeat breeding subclinical endometritic cows revealed that 84.72 % (61/ 72) samples yielded bacterial isolates while as 15.27% (11/72) samples were free of bacteria. The results of the present study were more or less in agreement with the findings of Chandrakar *et al.* (2002), Ahmed and Bhattacharyya (2005), Mane *et al.* (2009) who isolated bacteria from 80 to 100% cervical mucus samples of repeat breeding

In a Previous study, Gani *et al.* (2008) recorded only 62.2% bacterial infection in cervical mucus of repeat breeding animals. Bhat *et al.* (2014) reported 71.25% of repeat breeding animals showed bacterial infection whereas, 28.75% animals were free of infection. Difference in the rate of infection recorded in different studies might be due diversity in the severity of infection and variations in agro-climatic conditions of the areas where those studies were undertaken.

Single organism was isolated from 42 (68.85 %), whereas mixed infections consisting of more than one type of organisms were observed in 19 (31.15 %) of repeat breeding cows in the current study. This finding was more or less in accordance with the findings of Chandrakar *et al.* (2002), Mane *et al.* (2009), Bhat *et al.* (2014) who reported in 70, 80.00% and 76.67% samples with single isolate and 30, 20.00 and 23.33% with multiple isolates in repeat breeding cows. Several other authors also reported single isolate cases dominated multiple isolates in samples obtained from repeat breeding animals (Bhattacharya, 2004, Ahmed and Bhattacharyya, 2005, Mane *et al.*, 2009). However, Javed and Khan (1991) recorded single isolate in 45.45 % and mixed isolates in remaining samples.

In the present study *Staphylococcus aureus* isolates were recorded the highest % of total isolates (38.88 %) followed by *E. coli* spp. (36.11 %), *Streptococcus* spp. (33.33 %), *Enterobacter* spp. (22.22 %), *Proteus* spp. (18.05 %) and *Pseudomonas* spp. (16.67 %). Similar species of bacterial were also isolated by Javed and Khan (1991) in repeat breeding cows; Arora *et al.* (2000) in endometritic cows, Dohmen *et al.* (1995) ; Gani *et al.* (2008) in repeat breeder cows; Azawi *et al.* (2008) in repeat breeding buffaloes; Tek *et al.* (2010) in subclinical endometritis cows, Kavyashree *et al.* (2013) in repeat breeder cows, Palanisamy *et al.*

(2015) in endometritic cows. Further several authors reported *Staphylococcus* spp. was the most frequently isolated bacteria from the female genital tract with mild uterine infection or repeat breeding (Javed and Khan, 1991; Gani *et al.*, 2008; Palanisamy *et al.*, 2015) where as *E. coli* was reported to be in maximum frequency by Azawi *et al.* (2008); Kavyashree *et al.* (2013) and Bhat *et al.* (2014).

Currently, subclinical endometritis is being discussed as an important cause of reduced conception rates in dairy cows. Inflammation of the endometrium alters the uterine environment and disrupts conception or embryo survival. Uterine contents culture from the infertile and sub fertile cows demonstrated that subclinical endometritis is one of the most common cause of repeat breeding.

In tropical countries like India, the husbandry and sanitation practices commonly employed in the management of dairy cows are inadequate and the genital organs are more exposed to microbial invasion either at parturition or during estrus. These organisms are likely to multiply and invade the mucosal surface causing unhealthy uterine environment as a result of compromised uterine defence mechanism.

As observed from present study it was concluded a large proportion of cows will have subclinical endometritis which results in repeat breeding. The study also finds out that the hygiene level both at farm, individual cows owned by farmers needs to be strengthen in order to avoid any complication of cows leading to sub fertility.

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**Table 1:** Percentage of bacterial isolates from repeat breeder cows with subclinical endometritis (n = 72)

	Repeat breeder with SCE(n= 72)	
	Number (n)	Percentage (%)
Number of isolates positive for bacterial isolates	61	84.72
Number of isolates free from bacterial isolates	11	15.27
Number of isolates positive for single bacterial isolates	19	31.15
Number of isolates positive for mixed bacterial isolates	42	68.85

**Table 2:** Frequency of bacterial isolates (species wise) recovered from repeat breeder cows with subclinical endometritis (n = 72)

Bacteria Species	Before Treatment (n, %)
<i>E. coli</i>	26 (36.11 %)
<i>Staphylococcus</i>	28 (38.88 %)
<i>Streptococcus</i>	24 (33.33 %)
<i>Pseudomonas</i>	12 (16.67 %)
<i>Proteus</i>	13 (18.05 %)
<i>Enterobacter</i>	16 (22.22 %)