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## Isolation of *Staphylococcus* from repeat breeder crossbred cattle and their antibiotic sensitivity pattern

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### Abstract

The uterine infectious problems play important role in the lower conception in dairy animals and one of the major hurdles for the profitable dairy industry. Repeat breeding due to the involvement of bacteria is an important aspect for the economic loss of the farmers in respect of increased inseminations, increased calving interval and treatment. There are number of bacteria involved in repeat breeding e.g., *Streptococcus* spp., *Staphylococcus* spp., *Clostridium* spp., *Archanobacterium* spp., *fusobacterium* and *Escherichia coli*. In the present study 22 Cervico-vaginal mucus samples from the repeat breeder crossbred cows were examined for the presence of *Staphylococcus* spp. The samples were also screened for the presence of other bacteria and fungi. Out of these 22 samples, 9 samples were found positive for the presence of bacteria out of which 7 samples were found positive for the *Staphylococcus*. All the samples were found negative for the fungi. *In vitro* sensitivity of these isolated *Staphylococcus* was also checked for the 9 different antibiotics. Out of these 9 antibiotics used, ciprofloxacin and ofloxacin were showing a maximum area of sensitivity than the other antibiotics used. The study revealed that *Staphylococcus* also play role in repeat breeding in cattle and found sensitive for the ciprofloxacin and ofloxacin, which can be used for the treatment.

**Keywords:** repeat breeding, cattle, antibiotic sensitivity, *Staphylococcus*

### Introduction

The uterine infectious problems play important role in lower conception in dairy animals and one of the major hurdles for the profitable dairy industry. The profit of dairy farming largely depends on pregnancy rate after insemination. Bovine genital infections, either specific or non-specific in nature, responsible for a large number of pregnancy failure in cows (Sirohi *et al.*, 1989) [9]. Repeat breeding (RB) is one of the major constraint in cattle breeding, causes heavy economic losses to the farmer in respect of more inseminations, increased calving interval and increased culling rates. Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced artificial inseminations (Zemjanis 1980) [12]. The causes of repeat breeder syndrome are many and may be categorized into three main reasons including ovulation delay, uterine environment and the luteal deficiency (Noakes *et al* 2009) [7]. But various other factors may lead to repeat breeder syndrome such as infectious agents, hormonal imbalance and luteal problems, immunologic problem, nutritional status of animal and environmental factor also responsible for the condition (Yusuf *et al* 2010, Sood *et al* 2017) [11, 10]. The bacterial infection is the most important among the various factors listed. (Dholakia *et al.*, 1987) [4]. These bacterial infectious conditions may cause cervicitis or endometritis of various degrees, which in turn may lead to embryonic death and repeat breeding problems. Persistent bacterial infection of the uterus may cause the metritis and endometritis in buffaloes and cows. Most common bacteria involved in endometritis are *Streptococcus* spp., *Staphylococcus* spp., *Clostridium* spp., *Archanobacterium* spp., *fusobacterium* and *Escherichia coli*. In any form clinical and subclinical endometritis plays a major role in repeat breeding in animals. Out of these pathogens different species of *Staphylococcus* spp. actively participate in the development of the endometritis. Other than that, the indiscriminate use of broad-spectrum antibiotics and corticosteroids for the treatment of reproductive disorders or the insemination of animals with contaminated semen may be responsible for various gynecological conditions and may be led to microbial infections of the uterine environment (Gani *et al* 2008) [5].

The involvement of various bacteria and the role of antibiotics kept in mind the objective of the present study was, to find out the presence of *Staphylococcus* in cervicovaginal mucus of repeat breeder cattle and to determine the *in-vitro* antibiotic sensitivity to various antibiotics.

### Material and Methods

**Experimental location:** The study was conducted in the department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, in collaboration with the department of Veterinary Gynecology and obstetrics, College of Veterinary Science and Animal Husbandry, Mhow.

**Experimental design:** Total 22 Cervico-vaginal mucus samples from the crossbred cows were collected in and around Mhow, during the gynecological examinations which are not conceived after three successive artificial inseminations. These cervicovaginal mucus will be transported immediately to the laboratory of Veterinary Microbiology for further analysis. Each sample is inoculated into the brain heart infusion broth and incubated at 37 °C overnight. After incubation these samples were inoculated by streaking on Nutrient agar, EMB and Baired Parker agar. The samples were also tested for the presence of fungus. The inoculated media were incubated at 37 °C for 24 hrs. The growth characteristics of the isolates were noted. Each isolate was characterized on the basis of staining, colony character and biochemical tests. The cultures were purified and stored in nutrient agar slants in refrigerator for further tests. All the isolates were tested for *in vitro* antibiotic sensitivity tests. All the colonies were inoculated separately in BHI broth and incubated for overnight at 37 °C. Then maintain the turbidity with 0.5 Mcfarland tube and by using the sterile cotton swab prepare the lawn of bacterial culture on the Muller-Hinton agar plates. The antibiotic sensitivity was checked for 9 different antibiotics named Ciprofloxacin, Ofloxacin, Ceftriaxone, Cefoparazone, Ampicillin plus sulbactam, Ceftazidime plus tazobactam, Cefotaxime plus clavulanic acid, Amoxicillin and Amoxiclav.

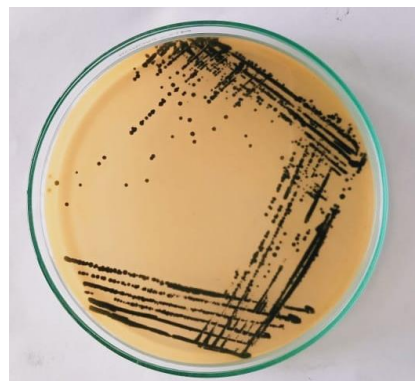
### Result and Discussion

There were 9 samples found positive for the bacteria and the remaining 13 showed no bacterial growth. The different types of bacteria isolated from the Cervico-vaginal mucus samples of the crossbred cows are presented in the table1. In 9 Cervico-vaginal mucus samples different types of gram positive and negative bacteria were present. Total 7 no. of *Staphylococcus* spp. were isolated from these 9 samples. In one sample gram negative *E. coli* was isolated. One sample was positive for the presence of gram-positive rods. All the samples were found negative for the fungal presence. A similar finding is found and supported by a previous report of Gani *et al.* 2008 [5] and Rautella *et al.* 2012.

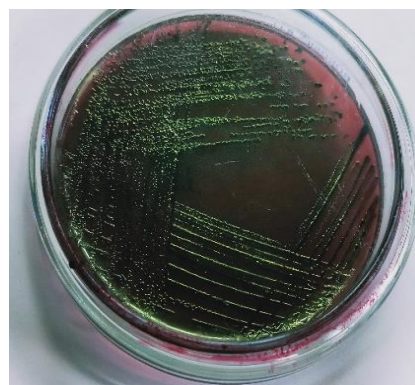
All the 7 isolates of *Staphylococcus* spp. were tested for the *in-vitro* sensitivity towards 9 antibacterial drugs against different antibiotics and found that ciprofloxacin and ofloxacin were showing the maximum area of sensitivity while ceftriaxone and cefoparazone were showing moderate area of sensitivity. All the other antibiotics were found resistant. The sensitivity of individual isolates to various drugs was interpreted according to the manufacturer's (Hi Media) instructions and is presented in table no. the result is summarized I table no.

**Table 1:** Different types of bacteria isolated from the sample

S.N.	Type of bacteria	Isolates	No. of isolate
1	Gram positive cocci	<i>Staphylococcus</i> spp.	7
2	Gram negative rods	<i>E. coli</i>	1
3	Gram positive rods	<i>Bacillus</i> spp.	1



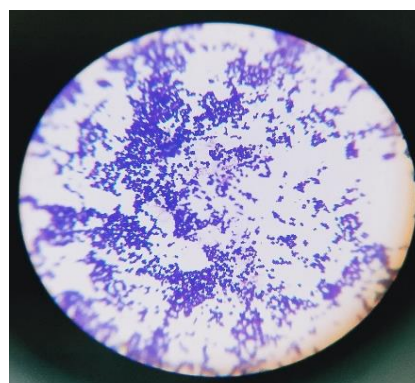
**Fig 1:** *Staphylococcus* spp. growth on the Baird Parker agar



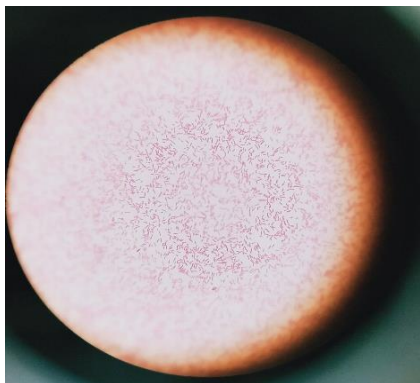
**Fig 2:** Growth of *E. coli* on the Eosine methylene blue agar



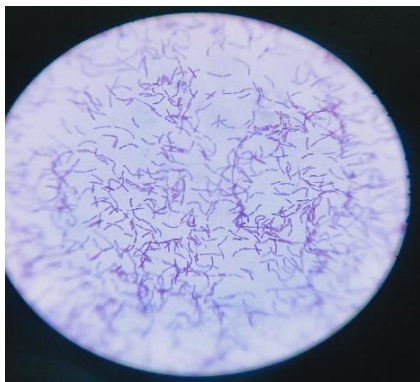
**Plate 1:** MHA plate showing zone of sensitivity against different antibiotics



**Fig 3:** Slide showing Gram's positive cocci (100x)



**Fig 4:** Slide showing Gram's negative rods (100x)



**Fig 5:** Slide showing Gram's positive rods (100x)

In the present study, 13 samples did not show any bacterial growth. Since these samples were taken from the repeat breeder animals, there may be the chance that the animal was not conceiving due to some other regions like hormonal and anatomical. One reason may be that the media and condition which are used in the study are not favorable for the bacterial entity involved.

Bhat and Bhattacharya, 2012 also isolate the *Staphylococcus* spp., *E. coli* and other bacteria from the case of metritis which supports our study that *Staphylococcus* spp. may be an important bacteria and play role in the cases of repeat breeder animals. Costa *et al.*, 2010<sup>[3]</sup>, Shweta, 2003<sup>[8]</sup> and Bonneett *et al.*, 1991<sup>[2]</sup> also found involvement of multiple gram positive and negative bacteria which supports our finding.

In our study we found ceftriaxone maximum sensitive. However, Muneer *et al.*, 1991<sup>[6]</sup> and Bhat and Bhattacharya, 2012<sup>[1]</sup> reported oxytetracycline to be the best antibiotic for the treatment of metritis in cows. From our findings it appears that Ciprofloxacin and Ofloxacin more effective than the other used antibiotics in treating endometritis in cows.

### Conclusions

After this study we can conclude that the *Staphylococcus* spp. play a significant role in repeat breeding cases along with other infectious bacteria. In our study most of the isolates of *Staphylococcus* were found resistant to many antibiotics. This is because of the abrupt use of antibiotics. The antibiotic ciprofloxacin and ofloxacin shows sensitivity for the staphylococcus must be used in the treatment. The detail study is required for the screening of bacteria involved in repeat breeding cases and also prepare data of the antibiotic resistance in that locality.

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