



## Bacterial Isolation and Antibiogram of Uterine Lavage from Repeat Breeder Cows Suffering from Subclinical Endometritis

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### ABSTRACT

A total 500 cows were randomly selected from college livestock farm, Kuthuliya and different villages in and around Rewa (M.P.). After recording history all the animals were subjected to gynaeco-clinical examination, Whiteside test and endometrial cytology by cytobrush technique. On the basis of above tests performed all the animals were selected for the study. They were divided into three groups as follows: Normal (n=280), Clinical endometritis (n=80) and Subclinical endometritis (n=140). All the animals were subjected to aseptic collection of uterine fluid by low volume lavage technique. Uterine fluid samples obtained were used for microbial assay and antibiotic sensitivity tests. Among the bacterial isolates *Staphylococcus* species (36.31%) was highly prevalent. The antibiotic sensitivity of isolates was found to be maximum for ceftriaxone and sulbactam combination 91.67 per cent followed by levofloxacin 89.07, ciprofloxacin 79.69, ceftriaxone 73.43, enrofloxacin 61.45 and gentamicin 56.78 per cent, respectively. It was concluded that *Staphylococcus* species was highly prevalent bacteria isolated and a combination of ceftriaxone and sulbactam was found to be highly sensitive.

**Keywords:** Subclinical endometritis, repeat breeder, uterine lavage microbial assay, antibiotic sensitivity

Good fertility in dairy cows is considered as key to economically successful dairy farming. It is widely accepted that uterine disorders in the postpartum period have a negative impact on reproductive performance. Postpartum uterine infection is the major cause of economic loss in dairy cows. A cow that has normal or nearly normal oestrus cycle and bred to 3 or more time with a fertile bull or AI by fertile semen yet fail to conceive is known as repeat breeder. Repeat breeding syndrome results in lowered dairy profit via wastage of semen and increased insemination cost, and reducing fertility. After the postpartum period, repeat breeding is considered one of the most important reproductive disorders in cattle (Yusuf *et al.*, 2010) which affect reproductive efficiency. Generally, non-specific infection of the genitalia is considered to be the main cause of repeated conception failure.

Postpartum uterine infections can delay the endometrial regeneration and disrupt the resumption of ovarian cyclicity which leads to the postponement of first insemination (AI), increase in number of inseminations per conception and thus calving interval is prolonged. Histologically, endometritis is defined as a disruption of the epithelium with presence of inflammatory cells. Subclinical endometritis is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis is indicated by the presence of purulent/ mucopurulent discharge.

Fertility of cow is affected by many nonspecific and specific pathogens of the genital tract. Uterine bacterial infections or bacterial products also suppress pituitary LH secretion and perturb postpartum ovarian follicle growth and function, which disrupts ovulation in cattle.

A higher percentage of cows (80 to 100%) are found to have bacterial contamination of the uterus in the first 2 weeks postpartum. Many of these bacteria are eliminated during the first 5 weeks after parturition, but the remaining bacteria cause uterine disease in some cows. Common bacteria isolated from cows with uterine infections are *Escherichia coli*, *Arcanobacterium pyogenes* (Sheldon *et al.*, 2009).

Major advantages of intrauterine treatment with antibiotics are reduced interval of treatment, rapid recovery rate and improved conception rate but their unselective use has led to development of resistant bacterial strains making further use of such therapy useless. The antibiotics have been used to uterine infections with variable success and the efficacy of antibiotics evaluated from time to time due to continuous emergence of drug resistant bacterial strains (Barman *et al.*, 2013).

Keeping this in view, the present study was planned with an objective to study antibiotic sensitivity of bacterial isolates obtained from normal, clinical and subclinical endometritis of repeat breeder cows.

## MATERIALS AND METHODS

A total 500 cows were randomly selected from college livestock farm, Kuthuliya and different villages in and around Rewa (M.P.). After recording history all the animals were subjected to gynaeco-clinical examination, Whiteside test and endometrial cytology by cytobrush technique. On the basis of above tests performed all the animals were selected for the study. They were divided into three groups as follows: Normal (n=280), Clinical endometritis (n=80) and Subclinical endometritis (n=140).

All the animals were subjected to aseptic collection of uterine fluid and endometrial tissue by low volume lavage technique. These samples were collected aseptically in autoclaved Brain Heart infusion (BHI) broth tubes and brought to laboratory in ice boxes. The aseptically collected fluid from all the animals were subjected to culture isolation and identification of bacterial microorganisms (Cruickshank, 1965) as well as for antibiogram as per method described by Barry (1976). Antibiotic sensitivity discs supplied by HI-MEDIA Laboratories Limited, Mumbai (India) were used and tested for their sensitivity with 6 antibiotics using antibiotic sensitivity

discs *viz.*, ceftriaxone (30 mcg/disc), levofloxacin (05 mcg/disc), gentamicin (10 mcg/disc), ciprofloxacin (5 mcg/disc), enrofloxacin (10 mcg/disc) and ceftriaxone/sulbactam (30/15 mcg/disc). The results were interpreted as per the chart furnished by the company.

## RESULTS AND DISCUSSION

Different types of bacteria were isolated from uterine fluid samples obtained by low volume uterine lavage technique. All the uterine fluid samples obtained from normal group animals were sterile. All the 80 uterine lavage samples obtained from clinical endometritic cows screened for bacterial isolates were found to be positive. Out of these, 80 samples that were found to be positive for bacterial isolates, 04 (5.00%) samples given mixed bacterial isolates while 76 (95.00%) isolates were of single type. Out of 84 isolates, 35 isolates (41.67%) were of *E. coli*, 6 (7.14%) of *Streptococcus* species, 34 (40.48%) of *Staphylococcus* species and 9 (10.71%) of *Bacillus* species.

Out of 140 uterine lavage samples obtained from cows suffering from subclinical endometritis, 112 (80.00%) samples were found to be positive for bacterial isolates while only 28 (20.00%) samples were recorded negative for bacterial isolates. Among these 112 positive samples, 107 (95.53%) yielded single type bacterial isolates while in only 5 (4.47%) sample mixed bacterial isolates were obtained. Among the 117 bacterial isolates, 39 isolates (33.33%) of *Staphylococcus* species, was most prevalent followed by 32 isolates (27.35%) of *E. coli*, 18 isolates (15.39%) of *Streptococcus* species, 18 isolates (15.39%) of *Bacillus* species and 10 isolates (8.54%) were of *Pseudomonas* spp.. Overall irrespective of groups, 192 (38.40%) samples out of 500 samples were found to be positive for bacterial isolates. Among these 192 positive samples, 9 (4.69%) samples yielded mixed type bacterial isolates whereas 183 (95.31%) samples yielded single type isolate.

Among the overall total of 201 isolates obtained from uterine lavage samples, *Staphylococcus* species, *E. coli*, *Streptococcus* species, *Bacillus* species and *Pseudomonas* species were 73 (36.31%), 67 (33.33%), 24 (11.94%), 27 (13.43%) and 10 (4.98%), respectively (Table 1).

The prevalence of *Staphylococcus* species isolates as observed in the present study was in accordance with the

findings of Behera *et al.* (2015) and Ahuja *et al.* (2017). They observed that *E. coli* and *Staphylococcus* species was highly prevalent in endometritic buffaloes. Aforesaid findings of present study are in agreement with Samatha *et al.* (2013) who reported the bacterial isolates in the order of *Staphylococcus* species (28.12%), *E. coli* (21.87%) and *Pseudomonas* (15.62%) in endometritic buffaloes. Above findings are in close accordance with Patel *et al.* (2009) who reported that *Staphylococcus* species was most prevalent bacterial isolated in buffaloes.

All the uterine lavage samples found positive (192) for bacterial isolates were subjected to antibiotic sensitivity tests for 6 different antibiotic agents (ceftriaxone, levofloxacin, gentamicin, ciprofloxacin, enrofloxacin and ceftriaxone + salbactam). The results of antibiotic sensitivity of the cultures of uterine lavage of cows suffering from clinical and subclinical endometritis. (Table 2).

The sensitivity of the bacterial isolates obtained from uterine lavage samples having single bacterial isolates (n=183) was found to be maximum for ceftriaxone and sulbactam combination 167 (91.25%) followed by levofloxacin 162 (88.52%), ciprofloxacin 145 (79.23%), ceftriaxone 132 (72.13%), enrofloxacin 109 (59.57%) and gentamicin 105 (57.38%), respectively. The sensitivity of uterine lavage samples having mixed bacterial isolates (n=9) was found to be 100.00% for ceftriaxone and sulbactam combination and levofloxacin, enrofloxacin and ceftriaxone followed by ciprofloxacin 8 (88.29%) and gentamicin 4 (44.44%).

The overall per cent sensitivity of samples irrespective of single or mixed bacterial isolates was found to be maximum for ceftriaxone and sulbactam combination 176 (91.67%) followed by levofloxacin 171 (89.07%), ciprofloxacin 153 (79.69%), ceftriaxone 141 (73.43%), enrofloxacin 118 (61.45%) and gentamicin 109 (56.78%).

Ahuja *et al.* (2017) reported that maximum number of isolates was highly sensitive to ciprofloxacin (100%) and less sensitive to cefotaxim (26%), cefuroxime (25%) and ampicillin (15%). However, Udhayavel *et al.* (2013) reported ceftriaxone to be the most sensitive followed by gentamicin, enrofloxacin and chlorotetracycline while chloramphenicol was found to be the least sensitive. Bajaj *et al.* (2018) in their study on antibiogram on uterine lavage of normal, sub-clinical and clinical endometritic

postpartum buffaloes found maximum sensitivity for ceftriaxone+salbactam (93.54%) followed by levofloxacin (87.08%), ceftriaxone (80.64%), ciprofloxacin (74.19%), enrofloxacin (58.06%) and gentamycin (54.83%), respectively.

## CONCLUSION

It can be concluded that *Staphylococcus* species is highly prevalent bacteria causing clinical and sub-clinical endometritis. A combination of ceftriaxone + salbactam was found to be highly sensitive against bacteria causing subclinical and clinical endometritis in postpartum repeat breeder cows.

## REFERENCES

- Ahuja, A.K., Cheema, R.S., Narang, D. and Dhindsa, S.S. 2017. Bacterial pathogens and antibiotic susceptibility patterns of cervico-vaginal discharges in cross bred repeat breeding heifer cows. *Int. J. Curr. Microbiol. Appl. Sci.*, **6**:1769-1775.
- Bajaj, N.K., Agrawal, S., Jain, S.K., Sharma, V., Mourya, A. and Shrivastava, O.P. 2018. Antibiogram and total viable bacteria count in uterine lavage of normal, sub-clinical and clinical endometritis postpartum buffaloes. *Buffalo Bull.*, **37**: 17-24.
- Barman, P., Yadav, M.C., Bangthai, A. and Kumar, H. 2013. Antibiogram of bacteria isolated from bovine endometritis. *Vet. Res. Int.*, **1**: 20-24.
- Barry, A.L. 1976. The antimicrobial susceptibility test: Principles and practices. Lea and Febiger, Philadelphia, pp. 72-80.
- Behera, S., Chandrashekarmurthy, V., Krishnaswamy, A., Kumar, G., Rao, S. and Nagaraj, N. 2015. Studies on bacterial profile of repeat breeding cows with subclinical endometritis. *Int. J. Sci. Res.*, **6**: 2319- 7064.
- Cruickshank, R. 1965. Medical Microbiology, 12<sup>th</sup> Edn., Vol. II. Churchill Livingstone, Edinburg, London and New York, pp. 170-172.
- Patel, P.P., Panchal, M.T., Kalyani, I.H. and Kavani, F.S. 2009. Antibiotic sensitivity spectrum of bacteria isolated from cervico-vaginal mucus of postpartum rural buffaloes. *Intas Polivet*, **10**: 29-31.
- Samatha, V. and Babu, K.R. 2013. Bacteriological and cytological studies of endometritis in buffaloes. *Int. J. Food, Agric. and Vet. Sci.*, **3**: 6-9.
- Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G. and Schuberth, H.J. 2009. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biol. Reprod.*, **81**: 1025-1032.



Udhayavel, S., Malmarugan, S., Palanisamy, K. and Rajeswar, J. 2013. Antibioqram pattern of bacteria causing endometritis in cows. *Vet. World*, **6**(2): 100-102.

Yusuf, M., Nakao, T., Ranasinghe, R.B., Gautam, G., Long, S.T., Yoshida, C., Koike, K. and Hayashi, A. 2010. Reproductive performance of repeat breeders in dairy herds. *Theriogenology*, **73**: 1220–1229.