# ANTIBIOGRAM AND TOTAL VIABLE BACTERIA COUNT IN UTERINE LAVAGE OF NORMAL, SUB-CLINICAL AND CLINICAL ENDOMETRITIC POSTPARTUM BUFFALOES

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## ABSTRACT

The present study was conducted to study antibiogram of bacteria isolated from normal, sub-clinical and clinical endometritic postpartum Murrah buffaloes. A total of 250 postpartum (28 to 45 days) apparently healthy buffaloes with normal calving history and free from peripartum disorders were screened. After recording history all the animals were subjected to gynaeco-clinical examination, transrectal ultrasonography and endometrial cytology by cytobrush technique. On the basis of above tests performed, total 42 animals were selected and were divided into three groups: Normal (n=06), Clinical endometritis (n=06) and Sub-clinical endometritis (n=30). Uterine lavage was collected aseptically from all the animals and microbial assay like total viable bacteria count, culture and isolation and antibiotic sensitivity tests were performed. Total viable bacterial count in clinical and sub-clinical endometritic buffaloes were 0.425±0.13 and 0.185±0.47 x $10^6$ CFU/ml, while, uterine lavage samples from normal buffaloes were sterile. Bacterial isolates were obtained from 73.80% (31/42), out of these 93.54% (29/31) and 6.54% (2/31) were single and mixed bacterial isolates, respectively. Among the bacteria isolated in the study *Escherichia. coli* (30.03%) was highly prevalent followed by *Staphylococcus* spp. (27.27%), *Acenatobacter* spp. (9.09%), *Pseudomonas* spp. (9.09%) and others (24.52%). The antibiotic sensitivity of the isolates was found to be maximum for ceftriaxone + salbactum (93.54%) followed by levofloxacin (87.08%), ceftriaxone (80.64%), ciprofloxacin (74.19%), enrofloxacin (58.06%) and gentamycin (54.83%), respectively. It was concluded that *E.coli* was highly prevalent bacteria isolated from uterine lavage and a combination of ceftriaxone + salbactum was found to be highly sensitive.

**Keywords**: *Bubalus bubalis*, buffalo, antibiogram, total viable bacteria count, antibiotic sensitivity, uterine lavage, sub-clinical endometritis

## INTRODUCTION

Optimum fertility of buffaloes is the key to economically successful dairy farming.

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Postpartum uterine infections have negative impact on reproductive performance leading to drastic reduction in farm return. Among postpartum uterine infections, endometritis is most commonly encountered complication under field or farm conditions in buffaloes. Histologically, endometritis is defined as a disruption of the epithelium with the presence of inflammatory cells (Bodurant, 1999). Postpartum sub-clinical endometritis (SE) is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis (CE) is indicated by the presence of purulent/mucopurulent discharge (Sheldon *et al*., 2006). The presence of bacteria in the uterus causes inflammation, histological lesions of the endometrium and delays uterine involution (Sheldon *et al*., 2004). Uterine bacterial infections or bacterial products also suppress pituitary LH secretion and perturb postpartum ovarian follicle growth and function, which disrupts ovulation in cattle. Studies on clinical and sub-clinical endometritis reported the prevalence of these diseases ranging from 18 to 37% (Drillich *et al*., 2005) and 12 to 94% (Barlund *et al*., 2008), respectively. Dystocia, retention of fetal membranes, bacterial contamination of uterus and metabolic diseases like ketosis, hypocalcemia etc. are some of the conditions which predispose cows to endometritis (Arthur *et al*., 2009). *Escherichia coli* and *Arcanobacterium pyogenes* are the most prevalent bacteria isolated from the uterine lumen of cattle with uterine disease followed by a wide range of anaerobic bacteria (Sheldon *et al*., 2009). Bacteria are also isolated from the uterus of animals that do not develop clinical disease. Use of antibiotics have been also tried to combat uterine infections with variable success and efficacy of antibiotics have been evaluated from time to time due to continuous emergence of drug resistant bacterial strains (Barman *et al*., 2013). Keeping above facts in mind, the present study was conducted to study antibiogram of bacteria isolated from normal, sub-clinical endometritic and clinical endometritic postpartum Murrah buffaloes.

## MATERIALS AND METHODS

A total of 250 postpartum (28 to 45 days) apparently healthy buffaloes with normal calving history and free from peripartum disorders from college livestock farm and organized dairy farms in and around Jabalpur were screened. After recording history all the animals were subjected to gynaeco-clinical examination, Whiteside test, transrectal ultrasonography and endometrial cytology by cytobrush technique. On the basis of above tests performed a total of 42 animals were selected for the study. They were divided into three groups as follows: Normal (n=06), Clinical endometritis (n=06) and Sub-clinical endometritis (n=30).

All the animals were subjected to aseptic collection of uterine fluid by low volume lavage technique for microbial assay, total viable bacteria count and antibiogram. These samples were collected aseptically in autoclaved Brain Heart infusion (BHI) broth tubes and brought to laboratory in ice boxes. These tubes were incubated for 6 to 8 h using nichrome loops. They were gently steaked on BHI, MLA and EMB agar medium in petri dishes and incubated for 48 h at 37$^{o}$C (Quinn *et al*., 1999a). Isolation and identification of bacteria were based on the morphology, cultural characters and biochemical tests as described by Quinn *et al*. (1999b). All the isolates were characterized morphologically using Gram staining (Quinn *et al*., 1999b). Total viable bacteria count was calculated using spread plate technique as per the methods

described by Koshy and Padmanaban (1989) and Sarkar *et al*. (1996) with minor modifications. The pin head shaped colonies were counted with the help of digital colony counter and the results were interpreted as standard plate count/ml. The total number of colonies was determined as follows:

No. of colonies per plate x Ratio of dilution.

Broths (Samples) were aseptically processed for antibiotic sensitivity spectrum as per the method described by Barry (1976). The samples were 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/salbactum (30/15 mcg/disc). The diameter of zone of inhibition was taken into consideration for determining sensitivity of the organisms against the specific antibiotics.

## RESULTS AND DISCUSSION

The results of present study clearly show the difference in bacterial load between normal, clinical and sub-clinical endometritic buffaloes which hampers reproductive efficiency. Total viable bacteria count in normal group buffaloes differed significantly ($P<0.05$) with clinical endometritis group buffaloes. However, difference between sub-clinical endometritis group and normal group and also between sub-clinical and clinical endometritis buffaloes did not differ significantly ($P>0.05$) (Table 1).

**The means with the same superscript did not differ significantly (P>0.05)**

The findings of present study are in accordance with that reported by Singh, (1996); Singla *et al*. (2004); Biswal *et al*. (2014); Singh (2014) regarding total bacterial count in endometritic cow. Two out of 30 uterine fluid samples of sub-clinical endometritic buffaloes were found to be sterile. This may be due to spontaneous elimination of bacterial load by immune system or may be due to a reflection of cytologic endometritis.

A wide variety of bacteria have been isolated from bovine uterus postpartum. Uterine lavage obtained from normal buffaloes group were sterile. All the 06 uterine lavage samples screened for bacterial isolates were found to be positive in clinical endometritis group. Out of these 06 samples that were found to be positive for bacterial isolates, 05 (83.33%) samples yielded single bacterial isolates while 01 (16.67%) isolate was of mixed type.

Among the 07 isolates obtained, 02 (28.57%) *E. coli* and 02 (28.57%) *Streptococcus*

Table 1. Total viable bacterial count obtained from uterine lavage of normal, clinical and sub-clinical endometritic postpartum buffaloes.

| Groups | Total viable bacterial count ($10^6$ CFU/ml) |
|---|---|
| Normal group (n=06) | $0.00^{b}\pm0.00$ |
| Clinical endometritis group (n=06) | $0.425^{a}\pm0.134$ |
| Sub-clinical endometritis group (n=30) | $0.186b^{ab}\pm0.047$ |

species followed by 01 (14.28%) *Citrobacter* sp., 01 (14.28%) *Proteus* sp. and 01 (14.28%) *Enterococcus* sp.

Out of 30 samples from sub-clinical endometritic buffaloes, 25 (83.33%) samples were found positive while only 05 (16.67%) samples were negative for bacterial isolates. Among these 25 positive samples 24 (96.00%) samples yielded single isolates while in only 01 (04.00%) sample mixed isolates were obtained. Among the 26 bacterial isolates 08 (30.76%) *E. coli* was highly prevalent followed by 07 (26.92%) *Staphylococcus* spp., 03 (11.53%) *Streptococcus* spp., 03 (11.53%) *Proteus* sp., 03 (11.53%) *Acinetobacter* sp. and 01 (07.69%) *Bacillus* sp. Overall irrespective of groups, out of 42 samples analysed, 31 (73.80%) samples were found to be positive for bacterial isolates. From 31 positive samples, 29 (93.54%) samples yielded bacterial isolates consisting of single type of isolates whereas only 02 (6.45%) samples yielded mixed bacterial isolates.

Among the overall total of 33 isolates from uterine lavage samples, *E. coli*, *Staphylococcus* sp., *Streptococcus* sp., *Proteus* sp., *Acinetobacter* sp., *Bacillus* sp., *Citrobacter* sp., *Proteus* sp. and *Enterococcus* sp. were 10 (30.33%), 09 (27.27%), 03 (09.09%), 03 (09.09%), 03 (09.09%), 02 (06.06%), 01 (03.03%), 01 (03.03%) and 01 (03.03%), respectively (Table 2 and Figure 1).

The prevalence of *E. coli* as observed in the present study was in accordance with the findings of Kusum *et al*. (2003), Udhayavel *et al*. (2013) and Biswal *et al*. (2014). Similarly, Bhat and Bhattacharya (2012) isolated *Staphylococcus* spp., *E. coli*, *Bacillus* spp., *Corynebacterium* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp. and *Streptococcus* spp. from crossbred cows affected with metritis.

All the samples found positive for bacterial isolates were subjected to antibiotic sensitivity test. The per cent sensitivity of the bacterial isolates of uterine lavage samples having single isolates (n=29) was found to be maximum 27 (93.10%) for ceftriaxone and salbactum combination followed by 25 (86.20%) levofloxacin, 23 (79.31%) cefriaxone, 21 (72.41%) ciprofloxacin, 16 (55.17%) enrofloxacin and 16 (55.17%) gentamycin.

The percent sensitivity of the bacterial isolates of uterine lavage samples having mixed isolates (n=02) was found to be maximum 02 (100.00%) for ceftriaxone and salbactum combination, 02 (100.00%) levofloxacin, 02 (100.00%) cefriaxone, 02 (100.00%) ciprofloxacin, 02 (100.00%) enrofloxacin followed by 01 (50.00%) gentamycin (Table 3 and Figure 2).

The overall percent sensitivity of the uterine lavage samples irrespective of single or mixed bacterial isolates was found to be 29 (93.54%) for ceftriaxone and salbactum combination followed by levofloxacin 27 (87.09%), cefriaxone 25 (80.64%), ciprofloxacin 24 (74.19%%), enrofloxacin 18 (58.06%) and gentamycin 17 (54.83%), respectively. Udhayavel *et al.* (2013) reported cefriaxone to be most sensitive followed by gentamycin, enrofloxacin and chlorotetracycline while chloramphenicol was found to be least sensitive. Literature of antibiotic sensitivity using above antibiotics is scarce.

## CONCLUSION

It can be concluded from the study that *E. coli* is highly prevalent bacteria causing clinical and sub-clinical endometritis. A combination of ceftriaxone + salbactum is found to be highly sensitive against bacteria causing endometritis.

Table 2. Bacterial isolates in uterine lavage of normal, clinical and subclinical endometritic buffaloes.

| Groups | Bacterial isolation | | Positive bacterial isolates | | | Types of bacterial isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | -ve sample | +ve sample | Single type | Mixed type | Total | *Strepto-coccus* spp. | *Bacilli* spp. | *P. aeruginosa* | *E. coli* | *Staphylococcus* spp. | *Acinet-bacter* spp. | *Citro-bacter* spp. | *Proteus* spp. | *Enterococ-cus* spp. |
| Normal group (n=06) | 6 (100.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| Clinical endometritis group (n=06) | 0 (0.00) | 6 (100.00) | 5 (80.00) | 1 (20.00) | 7 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 2 (28.57) | 2 (28.57) | 0 (0.00) | 1 (14.28) | 1 (14.28) | 1 (14.28) |
| Sub-clinical endometritis group (n=30) | 5 (16.67) | 25 (83.33) | 24 (96.00) | 1 (4.00) | 26 | 3 (11.53) | 2 (7.69) | 3 (11.53) | 8 (30.76) | 7 (26.92) | 3 (11.53) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| Overall (Normal + Clinical endomeritis + Sub-clinical endometritis) (n= 42) | 11 (26.19) | 31 (73.80) | 29 (93.54) | 2 (6.45) | 33 | 3 (9.09) | 2 (6.06) | 3 (9.09) | 10 (30.03) | 9 (27.27) | 3 (9.09) | 1 (3.03) | 1 (3.03) | 1 (3.03) |

Figures in paranthesis indicate percentage.

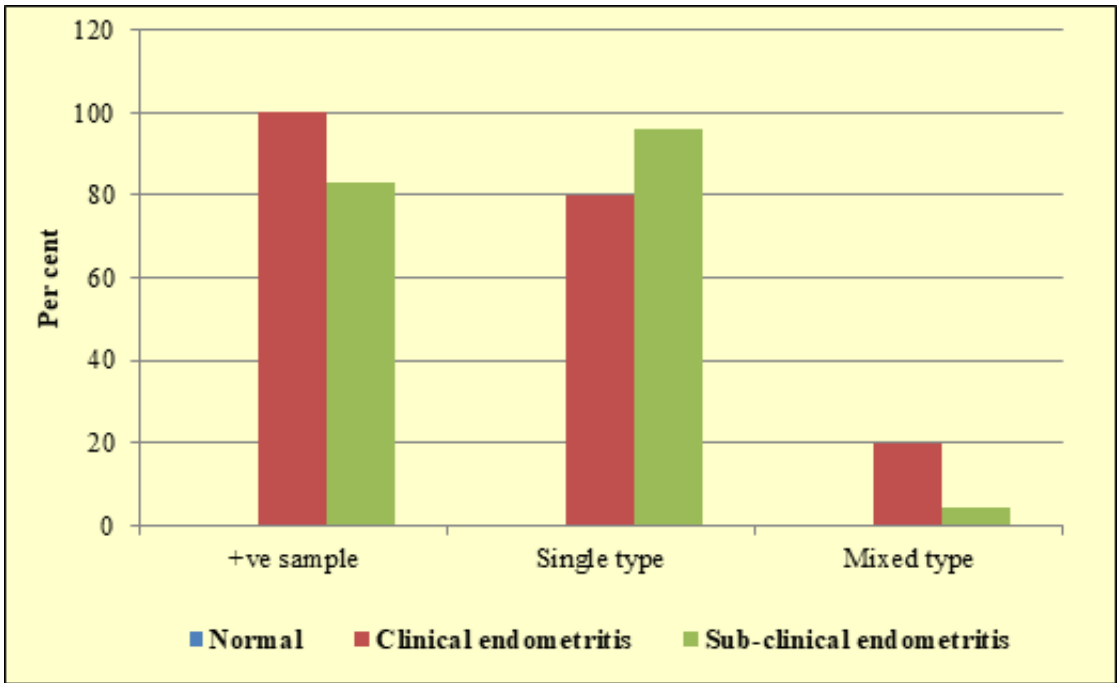


Figure 1. Bacterial isolates in normal, clinical and sub-clinical endometritic postpartum buffaloes.

Table 3. Antibiotic sensitivity of bacterial isolates from uterine lavage of clinical and sub-clinical endometritic buffaloes.

| Type of isolate | Percent sensitivity to antibiotics | | | | | |
|---|---|---|---|---|---|---|
| | Gentamycin | Levofloxacin | Ciprofloxacin | Enrofloxacin | Ceftriaxone | Cefriaxone + Salbactum |
| Single (n=29) | 16 (55.17) | 25 (86.20) | 21 (72.41) | 16 (55.17) | 23 (79.31) | 27 (93.10) |
| Mixed (n=02) | 1 (50.00) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) |
| Overall total (n=31) | 17 (54.83) | 27 (87.09 ) | 23 (74.19) | 18 (58.06) | 25 (80.64) | 29 (93.54) |

Figures in paranthesis indicate percentages.

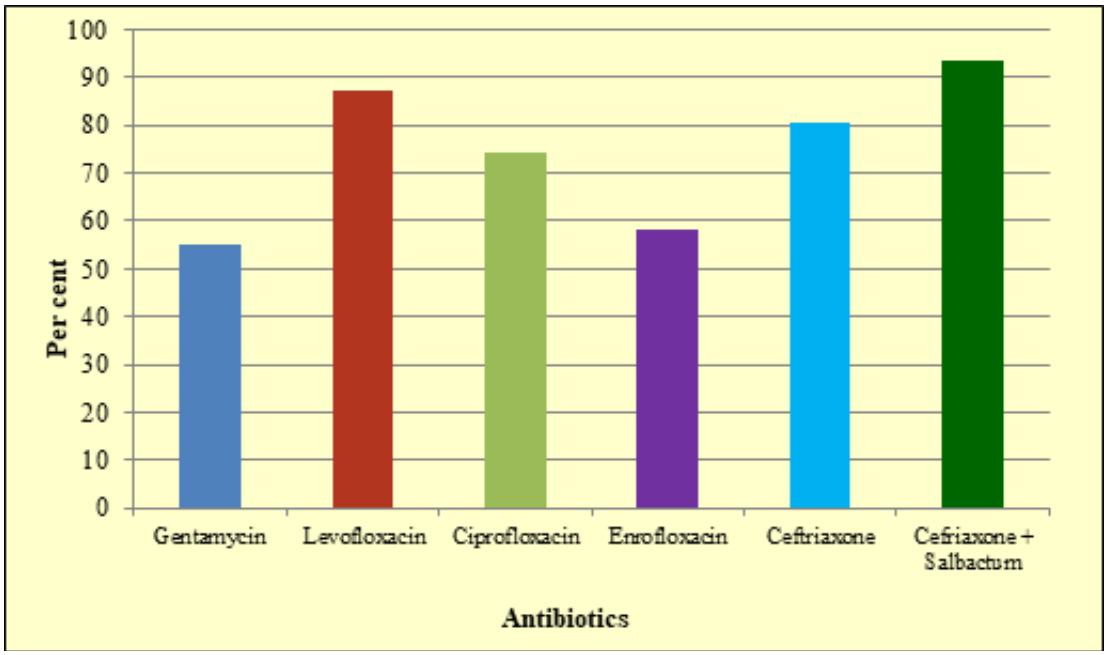


Figure 2. Antibiotic sensitivity of bacterial isolates obtained from clinical and sub-clinicalendometritic postpartum buffaloes.

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