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## Evaluation of fertility response succeeding various therapeutic protocol in endometritic dairy cows under field condition

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### Abstract

Uterine infection is one of the major gynaecological problems, affecting reproductive efficiency and economy of milk production in dairy animals. The study is designed to isolate various bacterial population of uterus in endometritic cow and to compare different treatment measures for endometritis in cow. Out of the 130 dairy cows with various reproductive disorders, 18 endometritic cows were divided into 3 groups comprising 6 cows in each group. Group I were treated with Uraksha, group II were treated with *E. coli* LPS and group III were treated with levofloxacin (Lenovo AP) after culture and sensitivity test in cervicovaginal mucus of each cow. Gram negative bacteria were found to be the major cause of endometritis in dairy cows. The bacteria isolated were *E. coli*, *Proteus* spp., *Bacillus* spp. and *Staphylococcus* spp. The flouroquinolone groups of antibiotics, levofloxacin (88.89%), enrofloxacin (72.22%) and ciprofloxacin (72.22%) were most found to be most effective in sensitivity test while ampicillin (11.11%) and oxytetracycline (5.56%) were least effective. All the isolates in the present study were resistant to penicillin. The highest (83.33%) overall conception rate was found after treatment with *E. coli* LPS.

**Keywords:** antibiotic sensitivity, bacterial isolation, dairy cow, endometritis, fertility response, therapeutic protocol

### Introduction

Uterine infection is one of the major gynaecological problems, affecting reproductive efficiency and economy of milk production in dairy animals. Endometritis being the commonest uterine infection is prevalent in high-producing dairy cows and consequently results in economic losses [1]. Clinical endometritis and subclinical endometritis are mostly recorded after calving in dairy cows. Postpartum endometritis of dairy cows continues to be a major cause of poor fertility and delayed conceptions [2]. The greatest impact on the health and productivity is associated with microbial contamination [3]. The endometrial cell response to infection is initiated by the innate immunity and this has to do with cascade of events such as the role of lipopolysaccharides and bacterial lipopeptides [3]. Treatment of endometritis with antimicrobials has been reported with different degrees of success, inconsistent recovery rates, milk disposal due to withdrawal periods, increased rate of microbial resistance, and reduced phagocytic activity of polymorphonuclear leucocytes [4]. The indiscriminate and prolonged use of antimicrobials in the absence of in vitro sensitivity tests, have contributed to the emergence of resistant strains of bacteria [5]. Attention is gradually shifting from the conventional antibiotic treatment to alternatives such as herbal 'uraksha' and to methods that mimics the physiology of the uterus using immunomodulators like *E. coli* lipopolysaccharides (LPS). Hence the study is designed to isolate various bacterial population of uterine domain in endometritic cow and to compare different treatment measures for endometritis in cow.

### Materials and Methods

The study was carried out under formal approval of Institutional Ethics Committee. 18 cows suffering from endometritis were selected out of 130 cows with various reproductive disorders in an around Kamrup District of Assam based on history, gynaecological examination and nature of cervicovaginal discharge. The study was carried out during the year 2019-2020 under formal ethical approval of institutional ethics committee College of Veterinary Science, Assam Agricultural University, Khanapara. The 18 cows were divided into 3 groups each comprising

6 cows. Group I cows were treated with uraksha (Ayurved Limited) @30ml intrauterinely (I/U) for 3 consecutive days; Group II cows were treated with Single dose of *E. coli* LPS (Sigma life science available in lyophilized form as LB274-25MG Lipopolysaccharides from *Escherichia coli* 026:B6  $\geq 10000$  EU/mg) intrauterinely and Group III were treated with most sensitive antibiotic Levofloxacin (Lenovo AP; Intas Pharmaceuticals) @ 60 ml I/U ly for 3 consecutive days after culture and sensitivity test.

### Collection of cervico-vaginal discharge

Cervicovaginal mucus was collected from each cow with the help of glass catheter after proper restraining and cleaning of the perineal region of the cows. A part of the sample was subjected to White Side Test<sup>(6)</sup> which was carried out on the field and the rest samples were then transported to the laboratory for culture and identification of different microorganisms present and antibiotic sensitivity test.

### Bacterial isolation

Bacteria isolation from uterine discharge was done by directly streaking it on BHI agar plates to obtain discrete colonies. The plates were incubated at 37°C and examined for the presence of bacteriological growth after 24–72 hours of incubation. The smears were then prepared from single isolated colonies and gram staining was carried out as per method of Cruickshank and his coworkers<sup>(7)</sup>.

### Culture and sensitivity test

The isolates were tested for their sensitivities to different antibiotics by disc diffusion method<sup>(8)</sup>. The test was performed using Mueller Hinton agar (Hi-media) by employing 14 antibiotic discs (Hi-media) viz. Ciprofloxacin (CIP, 5 mcg/disc), Gentamicin (GEN, 10 mcg/disc), Amoxicillin (AMX, 10 mcg/disc), Cloxacillin (COX, 10 mcg/disc), Enrofloxacin (EX, 10 mcg/disc), Penicillin (P, 10 units/disc), Ofloxacin (OF, 5 mcg/disc), Oxytetracycline (O, 30 mcg/disc), Ampicillin (AMP, 10 mcg/disc) Metronidazole (MT, 5 mcg/disc), Levofloxacin (LE, 5 mcg/disc), Cephalexin (CN, 30 mcg/disc), Ceftriaxone (CTR, 30 mcg/disc) and Streptomycin (S, 10 mcg/disc). The zones of growth inhibition around each of the antibiotic discs were measured to the nearest millimeter. The diameter of the zone was related to the susceptibility of the drug.

### Conception rate

All treated cows were inseminated with good quality frozen semen at mid to late stage of subsequent oestrus. Pregnancy diagnosis was carried out by rectal examination after 60 days of insemination and first service conception rates were recorded in the different groups. The overall conception rates were also worked out taking two post-treatment A.I. into consideration.

### Statistical analysis

Analysis was done in MS Office Excel 2016.

### Results and Discussion

#### Isolation of bacteria from cervico-vaginal discharge in endometritic dairy cows

A total 18 bacterial isolates obtained from cervico-vaginal discharge of endometritic dairy cows were typed up to the last genus species level. Out of 18 samples tested, the bacteria isolated were presented in Table 1.

Out of 18 samples cultured from cervico-vaginal discharge from endometritic dairy cows, the majority of the bacteria were Gram negative (55.55%), followed by mixed infection (33.33%) and then Gram positive bacteria (11.11%). The present finding of Gram negative bacteria was in agreement with the finding of Sharma and his coworkers<sup>(9)</sup> who reported 55.06 per cent Gram negative bacteria, while the mixed infection in the present study was higher and Gram positive bacteria was lower than the report of Sharma *et al.* (2017)<sup>(9)</sup>. The result of the present study revealed that the most common bacteria present in uterine environment of endometritic dairy cows were Gram negative *E. coli* and *Proteus* which was in agreement with the findings of earlier workers<sup>(10),(11)</sup>. Williams and his coworkers<sup>(12)</sup> and Sheldon and his coworkers<sup>(13)</sup> also reported that Gram negative bacteria are most commonly associated with uterine infection. The Gram-positive bacteria, *Staphylococcus* and *Bacillus* spp. were isolated in the present study which was in agreement with the finding of Sheldon *et al.* (2006)<sup>(14)</sup> and Wakayo *et al.* (2015)<sup>(15)</sup> who reported a high percentage of *Staphylococcus* spp from uterine discharge of endometritic cows and buffaloes. However, *Streptococcus* spp<sup>(16)</sup>, *A. Pyogenes* and *F. necrophorum*<sup>(12)</sup> were reported by other workers, although these were not isolated in the present study which might be due to absence of these bacteria in the surrounding atmosphere.

#### Antibiogram of bacteria isolated from cervico-vaginal discharge in endometritic dairy cows

A total of 18 bacteria isolates obtained from cervico-vaginal discharge of endometritic dairy cows were subjected to antibiotic sensitivity study against 14 different antimicrobial agents and antibiogram was recorded and presented in the Table 2.

The result of the antibiotic sensitivity of different bacteria isolated from cervico-vaginal discharge in the present study was in agreement with the report of Sharma *et al.* (2017)<sup>(9)</sup>, who reported that the flouroquinolone family was the most effective, to both Gram negative and Gram-positive bacteria and penicillin was not effective. The result was in agreement with the report of Patel *et al.* (2009)<sup>(17)</sup>, who reported enrofloxacin and ciprofloxacin as the most effective antibiotics and the  $\beta$ -lactams as least effective. The present study was in agreement with Singh *et al.* (2001)<sup>(18)</sup>, who reported that levofloxacin had higher efficacy compared to other antibiotics. Sadig (2010)<sup>(19)</sup> reported high sensitivity of bacteria to penicillin and ampicillin as 100 per cent and 90 per cent respectively as against the present findings of 0.00 per cent and 9.88 per cent respectively. This difference could be due to overuse of penicillin and ampicillin in this locality thereby causing drug resistant to bacteria. Results obtained in the present study differed from the report of Udhayavel *et al.* (2013)<sup>(20)</sup> who reported, ceftriaxone as the most effective antibiotic for treating uterine infection followed by gentamicin and enrofloxacin, as against the present findings of flouroquinolones (levofloxacin, enrofloxacin and ciprofloxacin) followed by cephalexin, ceftriaxone and then gentamicin. The present study slightly differed from the report of Gani *et al.* (2008)<sup>(21)</sup>, who reported high sensitivity of bacteria to tetracycline, amoxicillin along with ciprofloxacin but the present study showed low sensitivity of bacteria to tetracycline and amoxicillin but high sensitivity to ciprofloxacin. The difference in the efficacy of antibiotics against the microorganisms isolated might be due to the

difference in the type of bacteria isolated and / or due to the development of drug resistance as a result of indiscriminate use of the drug used in the treatment or as feed additives.

### Conception rate of endometritic dairy cows following different treatment protocols

The conception rate after administering three different treatment protocols is mentioned in Table 3.

In this study, the result of *E. coli* LPS corresponds to the findings of Bhuyan *et al.*, (2015) [22] who reported 83.33 per cent conception in metritic cows, 50.00 per cent conception rate with antibiotics (ciprofloxacin). The present findings were similar to the report of Wakayo *et al.* (2015) [15] where

they got 93.30 per cent success rate in treatment of endometritis in cows and buffaloes. The present result corresponded to the report of Singh *et al.* (2000) [23] who reported 75.00 per cent conception rate and 100 per cent conception rate on subsequent oestrus. Such a better result obtained in the treatment of endometritis with *E. coli* LPS, could be because of the fact that it greatly reduced uterine microflora in endometritis cows [24]. The result of the present study on treatment of endometritis with uraksha was similar to that of Das (2018) [25] and Ahmed *et al.* (2014) [26] who reported conception rate of 60 per cent in cows suffering from endometritis.

**Table 1:** Isolation Of Bacteria From Cervico-Vaginal Discharge In Endometritic Dairy Cows

Type	Number of samples tested	Bacteria Isolated	Number of samples positive	Percentage (%)
Gram negative	18	<i>Escherichia coli</i> and <i>Proteus</i> spp.	10	55.55
Gram positive	18	<i>Staphylococcus</i> spp. And <i>Bacillus</i> spp.	2	11.11
Mixed	18	<i>Escherichia coli</i> , <i>Staphylococcus</i> spp. and <i>Bacillus</i> spp.	6	33.33

**Table 2:** Antibiogram Of Bacteria Isolated From Cervico-Vaginal Discharge In Endometritic Dairy Cows

Antimicrobial agents	No of sensitive samples (n=18)	Degree of sensitivity	
		Sensitive (%)	Resistant (%)
Enrofloxacin	13	72.22	27.78
Ciprofloxacin	13	72.22	27.78
Ofloxacin	10	55.56	44.44
Gentamicin	4	22.22	77.78
Ceftriaxone	8	44.44	55.56
Oxytetracycline	1	5.56	94.44
Streptomycin	4	22.22	77.78
Cephalexin	10	55.56	44.44
Cloxacillin	1	5.56	94.44
Levofloxacin	16	88.89	11.11
Amoxicillin	2	11.11	88.89
Ampicillin	2	11.11	88.89
Penicillin	0	0.00	100
Metronidazole	7	38.89	61.11

**Table 3:** Conception Rate of Endometritic Dairy Cows Following Different Treatment Protocols

Treatment	No. of animals treated	Overall conception rate (%)		
		First service AI (%)	Second service AI (%)	Overall
Uraksha	6	33.33 (2)	16.67 (1)	50.00 (3)
<i>E. coli</i> LPS	6	50.00 (3)	33.33 (2)	83.33 (5)
Antibiotics (Levofloxacin)	6	50.00 (3)	16.67 (1)	66.66 (4)

Figures in the parenthesis indicate number of cows conceived.

### Conclusion

Gram negative bacteria being the major cause of endometritis, Levofloxacin is the most effective antibiotic treatment in dairy cows. However, treatment with *E. coli* LPS is the best treatment of endometritis in dairy cows.

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