

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(6): 1273-1281
www.biochemjournal.com
 Received: 16-03-2026
 Accepted: 18-04-2026

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Profiling of *E. coli* and *Klebsiella* and their antibiotic resistance pattern in bovine endometritis

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6p.8885>

Abstract

The present investigation was undertaken in the Department of Animal Reproduction, Gynaecology and Obstetrics, C.V.Sc. & A.H., OUAT, Bhubaneswar with an objective to study the microbial profiling of oestral cervico-vaginal mucus and antibiotics resistance pattern of micro-organisms in bovine endometritis. Fifty endometritic cows were selected from the cases presented at outdoor of T.V.C.C, at owner's door step at Bhubaneswar, Nimapara and Niali under mobile health coverage scheme were studied after dividing them in 2 groups namely clinical endometritis (group I, n = 25) and subclinical endometritis (group II, n = 25). Clinical endometritis was diagnosed basing on presence of mucopurulent to purulent mucus discharge during estrus, thick uterine wall and alkaline pH of the cervicovaginal mucus. Sub-clinical endometritis was diagnosed basing on presence of positive reaction to white side test. pH of estrual cervical mucus shifted towards alkaline side during endometritis when compared with normal cyclic cows. Bacterial load increased significantly in clinical endometritic cows than that of sub clinical endometritic and normal cyclic cows. *Escherichia coli* were the most common bacterial isolate in estrual cervical mucus of clinical and sub clinical endometritic cows. Isolated species of *E. coli* bacteria were resistant to Erythromycin, Ceftazidime and Aztreonam. *Klebsiella* spp. bacteria showed resistant towards Erythromycin and Ampicillin antibiotics.

Keywords: Clinical endometritis, sub-clinical endometritis, *E. coli*, *Klebsiella*, antibiotic sensitivity

1. Introduction

The post-partum fertility greatly affects the economic viability of dairy farmers which in turn depends on involution of uterus coupled with re-establishment of first fertile estrus. The inflammatory as well as immune response to bacterial infection of the uterus compromises animal welfare and leads to subfertility as well as infertility (Sheldon *et al.*, 2004) [25]. Endometritis can take place after artificial insemination or after normal/abnormal parturition and is defined as the inflammation of endometrium and its underlying glandular tissue in absence of any systemic signs that occurs at least 21 days after calving. The bacterial attack of the uterus after the 3rd week post-partum may lead to uterine infection which cause delay in involution of uterus, prolongs the interval of time until the first estrus and the time interval to calving and in severe cases causes infertility among bovine population (Zerbe *et al.*, 2002) [35]. Development of uterine infection takes place upon the establishment of pathogenic microorganisms onto the uterine mucosa, leading to colonization which later penetrates the epithelial layer and produces harmful toxins (Sheldon *et al.*, 2006) [27]. Once these are absorbed from the uterine lumen, endotoxins by gram negative bacteria get access into the peripheral blood circulation, and hinder the secretion of the Luteinizing Hormone (LH) from the hypophysis as well as the Gonadotropin Releasing Hormone (GnRH) from the hypothalamus. These toxins also decrease the susceptibility of the hypophysis to the secretion of endocrine as well as exocrine GnRH (Sheldon *et al.*, 2004 and Mateus *et al.*, 2002a) [25, 14]. The morbidity of bovine endometritis ranges between 30-40%, but if it exceeds 90%, infertility cases of bovine were induced by it (Gilbert *et al.*, 1998) [7].

Clinical as well as subclinical endometritis due to postpartum infection of the uterus leads to infertility and sub-fertility in high producing dairy cattle by delaying the process of onset of ovarian cyclic activity after parturition, prolonging the luteal phases, extending the parturition-first insemination as well as parturition-conception intervals and decreasing the

rates of conception (Sheldon, 2009a and 2009b) [26]. Many previous studies have reported *Escherichia coli* in addition to many other bacterial organisms like *Proteus* spp, *Klebsiella* spp., *Clostridium* spp. and *Pseudomonas aeruginosa*, isolated from endometritic cows.

Antibiotics resistance has become a serious threat now-a-days because of emergence of resistant strain of bacteria. Incomplete or suboptimal uses of antimicrobial agents for treatment of microbial infections facilitate the emergence of resistant strain of micro-organism. A wide variety of resistance genes mediate resistance which is a global threat now-a-days. One of the most important example of antibiotic resistance is ESBL-producing *Escherichia coli* and multidrug-resistant (MDR) (Pormohammad *et al.*, 2019) [19, 20], which can cause life-threatening infections not only in animals but also in humans through direct or indirect contact (Pormohammad *et al.*, 2019) [19, 20]. *E. coli* is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer. The most problematic mechanisms in *E. coli* correspond to the acquisition of genes coding for extended-spectrum carbapenemases which confers resistance to carbapenems, β -lactamases which confers resistance to broad-spectrum cephalosporins, plasmid-mediated quinolone resistance (PMQR) genes which confers resistance to fluoroquinolones, 16S rRNA methylases which confers pan-resistance to aminoglycosides, and *mcr* genes which confers resistance to polymyxins (Poirel *et al.*, 2018) [18]. Similar genes responsible for multidrug resistance in staphylococci of animal origin have been reported by Schwarz *et al.*, (2018) [24]. Looking into the above consideration, the present study is taken for profiling of *E. coli* and *Klebsiella* and their antibiotic resistance pattern in bovine endometritis. Hence it can be concluded that development of antibiotics resistance in the microorganisms isolated from estrual cervical mucus discharge of endometritic cows can be considered as a serious threat towards reproductive and productive capacity of farm animals.

2. Materials and Methods

The current study was conducted in the Department of ARGO, C.V.Sc. & A.H., OUAT, Bhubaneswar. 50 endometritic cows were selected from the cases presented at outdoor of T.V.C.C, at owner's door step at Bhubaneswar, Nimapara and Niali under mobile health coverage scheme. Cases were studied after dividing them in 2 groups namely clinical endometritis (group I, n = 25) and subclinical endometritis (group II, n = 25) along with 6 normal cyclic animals. Clinical endometritis was diagnosed based on the presence of mucopurulent to purulent mucus discharge during estrus, thick uterine wall and alkaline pH of the cervicovaginal mucus. Sub-clinical endometritis was diagnosed on the basis of presence of positive reaction to white side test. The pH of estrual cervical mucus shifted towards alkaline side during endometritis in comparison with normal cyclic cows.

2.1 Collection of estrual cervical mucus

After restraining the animal properly, the tail, perineum, as well as external genitalia were cleaned with water

thoroughly and wiped out neatly with a clean and dry piece of cotton. Uterine discharge was collected from all the experimental and control cows. Mucus samples of the uterus were collected in sterilized vials from the cows in oestrous by using AI gun covered with sheath and was immediately transferred to the Microbiology lab. Samples so collected were inoculated on BHI by using streak plate technique.

A sterile glass uterine catheter which is attached with a 20 ml of glass syringe with a rubber adapter was utilized for aspirating uterine fluid following aseptic protocol. A sterile hollow aluminium cylindrical tube was introduced to the os cervi. It served as a protecting device in order to avoid vaginal contamination. The uterine content which was aspirated aseptically was transferred into a sterile glass vial. Immediately after collection, one half of the discharge was used for white side test. Other half of the uterine sample was used for bacteriological studies. Prior to bacteriological examination the uterine discharge was mucolysed by mixing with equal volume of sterile distilled water for 10-15 minutes using magnetic stirrer under sterile condition. The mucolysed samples were used for identification of the organisms.

2.2 Hydrogen ion concentration (pH) of estrual cervical mucus

The pH of uterine discharge was recorded by using digital pH meter as described by Palanisamy *et al.* (2014) [15]. For each sample, pH was determined in duplicate.

2.3 White side test

The estrual mucus discharge of each experimental cow with history of conception failure for more than three inseminations and having discharge of mucopurulent in nature was subjected to White side test as described by Pateria and Rawal (1990) [17].

Table 1: White side test

Sl. No.	Colour of the sample	Degree of endometritis
1	Clear	Normal
2	Light yellow colour	Mild infection
3	Yellow colour	Moderate infection
4	Dark yellow colour	Severe infection

2.4 Determination of bacterial load

The load of viable bacteria or the no of viable bacterial Colony Forming Unit (CFU) in uterine sample was counted by using "total plate count technique" as described by Malik (1967) [13].

2.5 Isolation of Enterobacteriaceae (*Escherichia coli* and *Klebsiella*)

The sample from swab/vial was serially diluted (10 fold dilution) in sterile peptone water. Loopful of appropriate dilution was streaked on MacConkey agar plate. The plates were incubated under aerobic conditions overnight at 37 °C and then bacterial growth was examined. Apparent colonies were sub cultured on nutrient agar plates/slants and were incubated at 35 °C for 18-48 hours. To confirm the isolates as *E. coli* or *Klebsiella* spp, standard biochemical tests were performed. Presence of large non-mucoid colonies with even pink colour: *E. coli*. Presence of large mucoid colonies with pale pink centres and colorless peripheral, or even pink color: *Klebsiella*

2.6 Identification of bacteria

Identification of bacteria was based on colonial morphology, Gram's stained smear, hemolysis and biochemical reactions as per standard methods described by Cruickshank *et al.* (1975) [3].

2.7 Morphological and staining characteristics

Gram-ve colonies were subcultured into MacConkey's Agar (MLA) by streak plate method as described by Collins and Lyne (1970) [2]. The plates were then incubated for 18-24 hrs aerobically at 37 °C and searched for pale non lactose fermenting colonies. The colonies were further subcultured into Eosin Methylene Blue Agar (EMBA) with overnight incubation at 37 °C. Colonies with metallic sheen suggestive of *Escherichia coli* (Fig. 3C) were further confirmed by biochemical characterization.

2.8 Biochemical characteristics

In order to identify certain bacterial isolates recovered in the present investigation, biochemical tests were carried out according to the methods described by Cruickshank *et al.* (1975) [3] and Edwards and Ewing (1972) [5]

2.9 Antibiotics sensitivity Test

Prior to performing antimicrobial sensitivity test, all isolates were revived by subculturing on Mueller-Hinton agar plates and incubating at 37 °C for 24 hours under aerobic conditions. In order to perform antimicrobial susceptibility test, isolates were streaked onto Mueller-Hinton agar plates and incubated at 37 °C for 24 hours aerobically. Isolates were suspended in 0.1 M sodium phosphate solution/normal saline in order to have turbidity equivalent to 0.5 McFarland standards. It was again streaked onto 150-mm Mueller-Hinton agar plates. Antimicrobial sensitivity was determined by using Kirby-Bauer disk diffusion assay following the guidelines published by the Clinical and Laboratory Standards Institute (CLSI; CLSI, 2008).

18 different antibiotic discs were placed on the surface of the Mueller-Hinton agar plate. Antimicrobial susceptibility to 18 antibiotics was assessed by utilizing discrete antibiotic discs (Himedia) as per the following panel: Ampicillin 30 µg, Cefepodoxime 30 µg, Ciprofloxacin 30 µg, Ceftriaxone 30 µg, Enrofloxacin 30 µg, Gentamicin 30 µg, Amoxicillin-clavulanic acid 20/10 µg, Nalidixic acid 30 µg, Amikacin 30 µg, Tetracycline 30 µg, Aztreonam 30 µg, and Erythromycin 30 µg, Trimethoprim-sulfamethoxazole 23.75/1.25 µg, Ceftazidime 30 µg, Colistin 30 µg, Cefoxitin 30 µg, Chloramphenicol 30 µg, and Cefotaxime 30 µg. The disks were applied to the plates and incubated at 37 °C for 12 to 18 h/24 h under aerobic conditions, following which the diameters of the inhibition zones were measured with the help of Kirby-Bauer scale and recorded to know the sensitivity or resistance to antibiotics (Fig. 10).

2.10 Statistical Analysis

Data generated from the present experiment were analyzed statistically as per the method suggested by Snedecor and Cochran (1994) [30].

3. Results

3.1 Appearance of estrual cervical mucus

The appearance of estrual cervical mucus in clinical endometritis was categorized as purulent, muco-purulent and clear. Out of these 25 cows, only 44 percent (n = 11) of

cows exhibited purulent mucus discharge. Fifty six percent of cows (n = 14) showed muco-purulent discharge during estrus. None of the cows with clinical endometritis voided clear mucus discharge. All the normal cyclic cows as well as cows with sub-clinical endometritis (100%) showed clear mucus discharge.

3.2 White side test

This test was used to confirm the positive cases of sub-clinical endometritis. Only the cows which have shown positive reaction to white side test were selected for the study in Gr. II. The cows which had shown negative reaction for white side test were considered as normal cyclic and placed under control group for further studies.

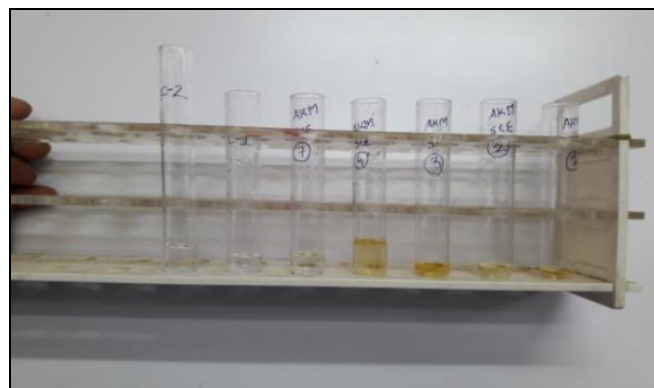


Fig 1: White Side Test.

3.3 Bacterial load

The mean total bacterial count ($\times 10^4$ CFU/ml) was found to be 0.37 ± 0.10 , 286.41 ± 11.05 and 131.23 ± 9.95 in the normal cyclic, clinical endometritis and subclinical endometritic cows respectively. Bacterial load in cows with clinical endometritis was significantly higher ($p < 0.01$) as compared to subclinical endometritic and normal cyclic cows. Bacterial load in cows with subclinical endometritis was significantly higher ($p < 0.01$) than normal cyclic cows. (Table 1)

Table 1: The pH and bacterial load ($\times 10^4$ CFU/ml) in estrual cervical mucus in normal cyclic and endometritic cows

Parameter	Normal cows (n = 16)	Gr I (n = 25)	Gr II (n = 25)	'F' value
Bacterial load	0.37 ± 0.10	286.41 ± 11.05	131.23 ± 9.95	106.526**

NS-Not significant, *-Significant ($p < 0.05$), **-Significant ($p < 0.01$)

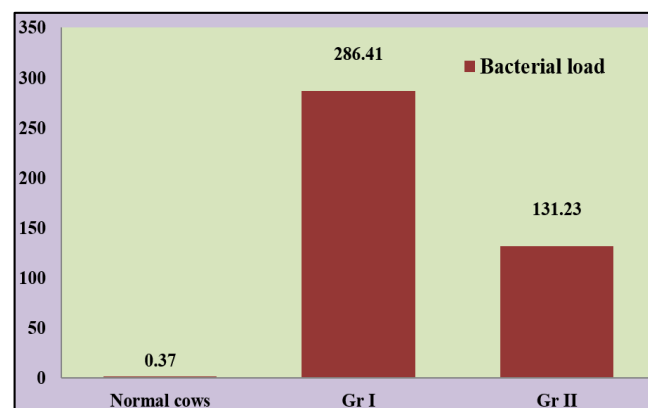


Fig 2: The bacterial load ($\times 10^4$ CFU/ml) in estrual cervical mucus in normal cyclic and endometritic cows

3.4 Bacterial isolation and identification

Fifty six uterine samples (25 clinical endometritis, 25 sub-clinical endometritis and 6 normal cyclic) were collected and subjected for bacteriological analysis using routine standard bacteriological technique. Result of bacteriological investigation of estrual cervical mucus of normal and endometritic cows have been presented in table 2.

Out of 6 normal cyclic cows, 1 cow (16%) was positive for bacterial infection of which solitary cases of single (100%) infection were observed. *Escherichia coli* were the predominant bacteria isolated in normal cyclic cows. All the endometritic cows (100%) were found positive for bacterial

infection.

In the cows suffering from clinical endometritis mixed infections were found in 19 (76%) cases, whereas single isolates were detected only in 6 (24%) cases. In the present study *Escherichia coli* (100%) was the most common isolate in clinical endometritic cows followed by *Klebsiella* spp. (52%),

In subclinical endometritis, mixed infections were found in 20 (80%) cases, whereas single isolates were detected only in 5 (20%) cases. In the present study *Escherichia coli* (100%) was the most common isolate in sub-clinical endometritic cows followed by *Klebsiella* spp. (32%),

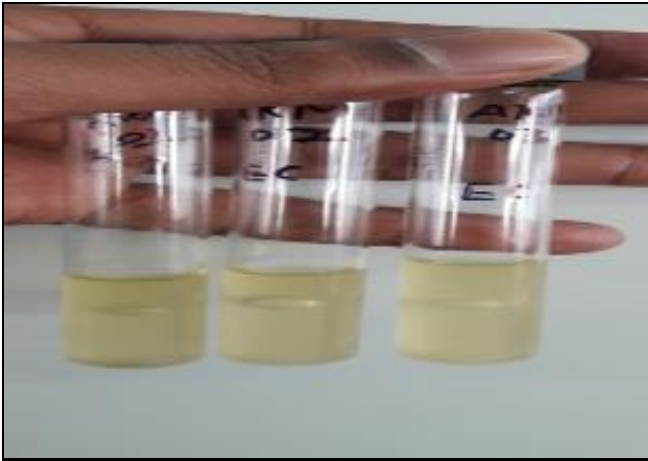
Table 2: Proportionate population of microorganisms isolated from cervico-vaginal mucus of endometritic cows (n = 50, including both clinical and sub-clinical endometritis)

Name of the bacterial organism	No. of Isolates	Percentage of total population
<i>Escherichia coli</i>	25 + 25 = 50	100
<i>Klebseilla</i> spp.	13 + 8 = 21	42

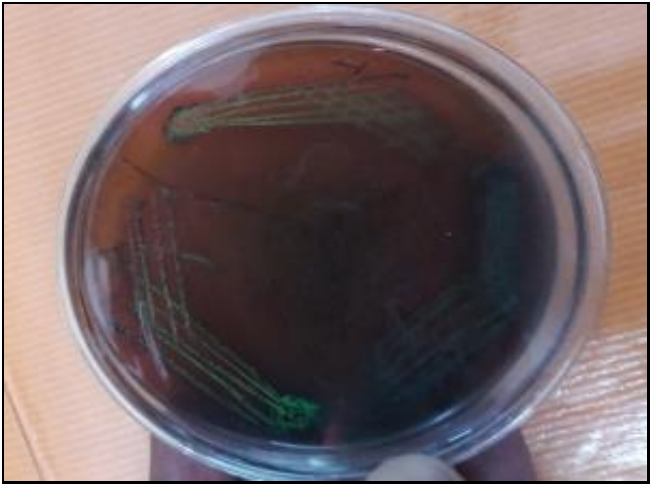
Table 3: Number of clinical and subclinical endometritic animals found positive for specific Bacterial population

Bacterial species isolated	No. of clinical endometritic cows found positive	No. of sub-clinical endometritic cows found positive
<i>E. coli</i>	25 (100%)	25 (100%)
<i>Klebsiella</i> spp.	13(52%)	8 (32%)

Figures in parenthesis indicates percentage



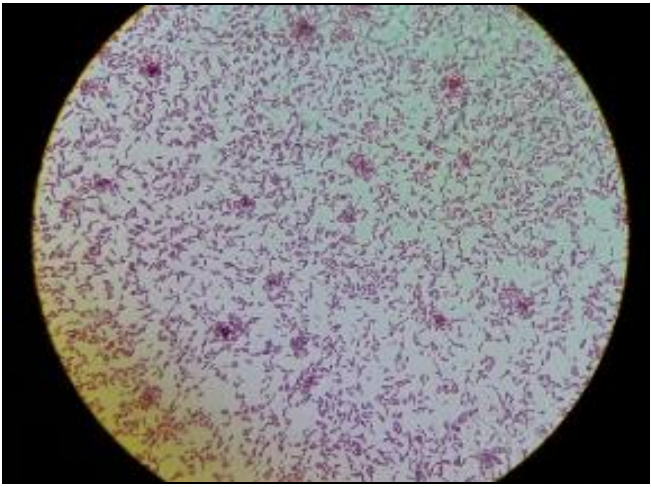
A. *E. coli* Broth media showing growth andgas production



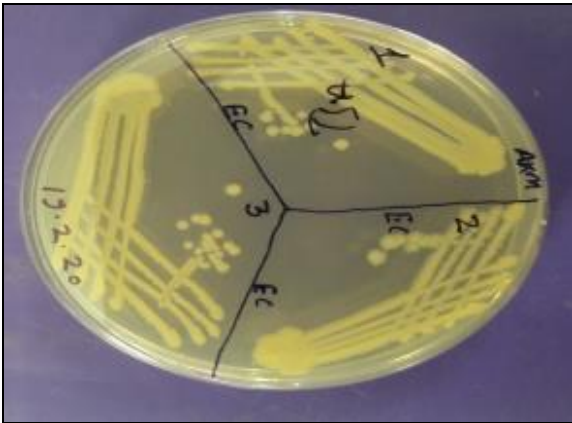
C. Metallic sheen in EMB Agar media D. Gram-ve rods in Gram staining



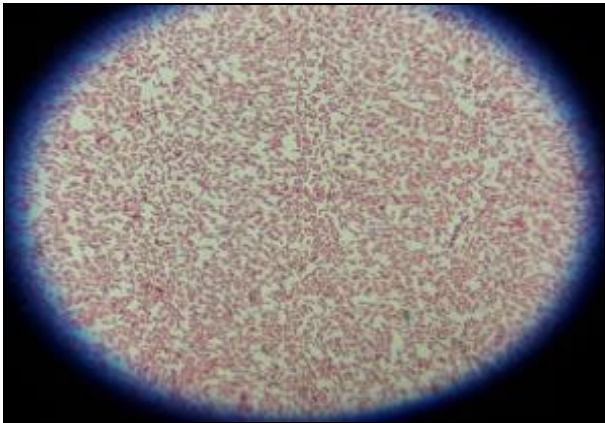
B. Bright Pink-Red colonies inMacConkey Agar



D.Gram-ve rods in Gram staining

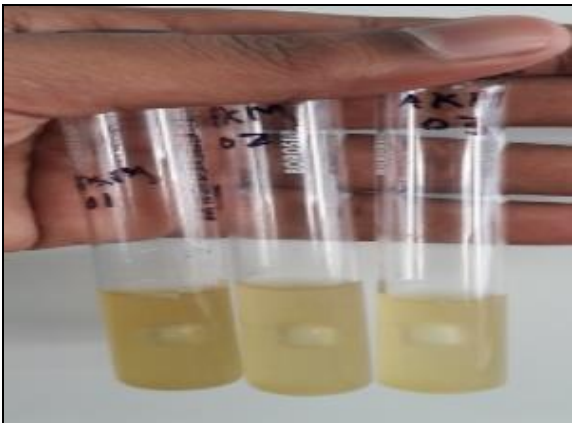


E. Growth in TSA media plate



D. Gram-ve rods in Gram staining

Fig 3: Identification and Isolation of *E. coli* bacteria



A. Growth in MacConckey broth

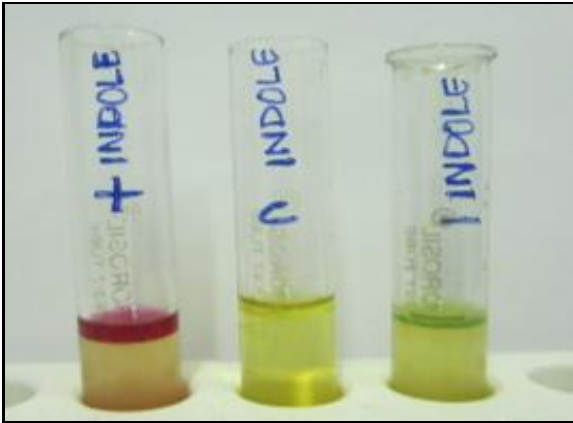


E. Growth in TSA media plate

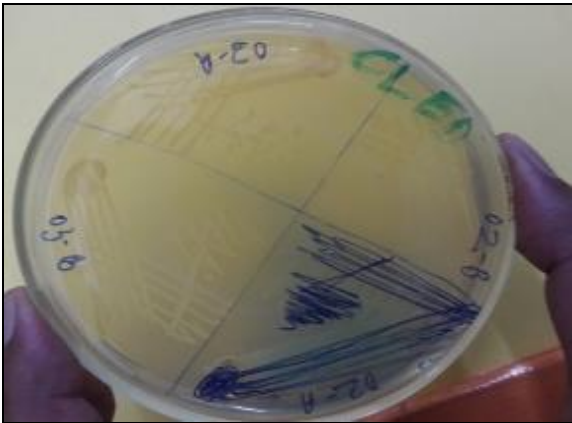
Fig 4: Identification and Isolation of *Klebsiella*



B. Growth in KSA media plate



A. Indole Test



C. Growth in CLED media plate



B. Methyl Red



C. Voges Proskauer (VP)



D. Citrate utilization test

Fig 5: Biochemical Test**Table 4:** Proportionate population of microorganisms isolated from cervico-vaginal mucus of endometritic cows (n = 50, including both clinical and sub-clinical endometritis).

Name of the bacterial organism	No. of Isolates	Percentage of total population
<i>Escherichia coli</i>	25 + 25 = 50	100
<i>Klebsiella</i> spp.	13 + 8 = 21	42

Table 5: Bacteria isolated from Clinical endometritic animals

S. No.	Sample No.	Name of isolated bacteria from samples
1.	1	<i>E. coli</i>
2.	2	<i>E. coli, Klebsiella</i> spp.,
3.	3	<i>E. coli, Klebsiella</i> spp.,
4.	4	<i>E. coli, Klebsiella</i> spp.,
5.	5	<i>E. coli, Klebsiella</i> spp.,
6.	6	<i>E. coli, Klebsiella</i> spp.
7.	7	<i>E. coli, Klebsiella</i> spp.
8.	8	<i>E. coli,</i>
9.	9	<i>E. coli,</i>
10.	10	<i>E. coli,</i>
11.	11	<i>E. coli, Klebsiella</i> spp
12.	12	<i>E. coli, Klebsiella</i> spp.,
13.	13	<i>E. coli</i>
14.	14	<i>E. coli, Klebsiella</i> spp.,
15.	15	<i>E. coli, Klebsiella</i> spp.,
16.	16	<i>E. coli</i>
17.	17	<i>E. coli</i>
18.	18	<i>E. coli, Klebsiella</i> spp.,
19.	19	<i>E. coli, Klebsiella</i> spp.,
20.	20	<i>E. coli,</i>
21.	21	<i>E. coli</i>
22.	22	<i>E. coli,</i>
23.	23	<i>E. coli,</i>
24.	24	<i>E. coli, Klebsiella</i> spp.

25.	25	<i>E. coli</i>
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Table 6: Bacteria isolated from Sub-Clinical endometritic animals

S. No.	Sample No.	Isolated species of bacteria from samples
1.	1	<i>E. coli, Klebsiella</i> spp.
2.	2	<i>E. coli, Klebsiella</i> spp.,
3.	3	<i>E. coli</i>
4.	4	<i>E. coli,</i>
5.	5	<i>E. coli,</i>
6.	6	<i>E. coli,</i>
7.	7	<i>E. coli, Klebsiella</i> spp.
8.	8	<i>E. coli, Klebsiella</i> spp.,
9.	9	<i>E. coli, Klebsiella</i> spp.,
10.	10	<i>E. coli,</i>
11.	11	<i>E. coli,</i>
12.	12	<i>E. coli, Klebsiella</i> spp.
13.	13	<i>E. coli</i>
14.	14	<i>E. coli,</i>
15.	15	<i>E. coli, Klebsiella</i> spp.
16.	16	<i>E. coli, Klebsiella</i> spp.
17.	17	<i>E. coli,</i>
18.	18	<i>E. coli,</i>
19.	19	<i>E. coli,</i>
20.	20	<i>E. coli,</i>
21.	21	<i>E. coli,</i>
22.	22	<i>E. coli</i>
23.	23	<i>E. coli,</i>
24.	24	<i>E. coli</i>
25.	25	<i>E. coli</i>

3.5 Antibiotics sensitivity test

The isolated bacterial species were subjected to antibiotics sensitivity test to ascertain their susceptibility towards the routinely used antibiotics by using the disc diffusion method. The diameter of zone of inhibition was measured for the specific antibiotics and represented in Table 7.

Out of the several species of bacteria isolated, *E. coli* bacteria were highly sensitive to Gentamicin, Ciprofloxacin, Enrofloxacin, Cefpodoxime, Amoxicillin-clavulanic acid, Tetracycline and Cefoxitin with a zone of inhibition of > 20 mm in diameter. They showed moderate sensitivity towards Colistine, Ceftriaxone, Ampicillin, Nalidixic acid, Chloramphenicol, Trimethoprim-sulfamethoxazole, Cefotaxime and Amikacin with a zone of inhibition of < 20 mm in diameter. However, the isolated species of bacteria were resistant to Erythromycin, Ceftazidime and Aztreonam antibiotics.

Klebsiella spp. bacteria were highly sensitive to Amoxicillin-clavulanic acid, Cefoxitin and Amikacin. They showed moderate sensitivity towards several antibiotics like Gentamicin, Ciprofloxacin, Enrofloxacin, Colistine, Ceftriaxone, Ceftazidime, Cefpodoxime, Aztreonam, Tetracycline, Nalidixic acid, Trimethoprim-sulfamethoxazole and Cefotaxime with a zone of inhibition of < 20 mm in diameter. However, the isolated species of

bacteria were resistant to Erythromycin and Ampicillin antibiotics

Table 7: Antibiotics sensitivity pattern of different micro-organisms isolated from endometritic cows against commonly used antibiotics with their relative zone of inhibition (ZI in mm)

S. No.	Antibiotics	Abbreviations	<i>E. coli.</i>	<i>Klebsiella spp.</i>
1	Gentamicin	GEN	23	16
2	Ciprofloxacin	CIP	27	13
3	Enrofloxacin	EX	28	13
4	Erythromycin	E	R	R
5	Colistine	CL	15	10
6	Ceftriaxone	CTR	14	11
7	Ceftazidime	CAZ	R	14
8	Cefpodoxime	CPD	26	19
9	Aztreonam	AT	R	10
10	Ampicillin	AMP	17	R
11	Amoxicillin-clavulanic acid	AMC	28	20
12	Tetracycline	TE	31	19
13	Nalidixic acid	NA	10	14
14	Chloramphenicol	C	10	-
15	Trimethoprim-sulfamethoxazole	COT	12	13
16	Cefoxitin	CX	28	20
17	Cefotaxime	CTX	12	12
18	Amikacin	AK	12	22
19	Oxacillin	OX	-	-
20	Metronidazole	LZ	-	-
21	Penicillin G	P	-	-

Table 11: Antibiotics sensitivity pattern of different micro-organisms isolated from endometritic cows

Bacterial spp.	Highly sensitive (ZI > 20 mm)	Moderately sensitive (ZI < 20 mm)	Resistant (ZI = 0)
<i>E. coli.</i>	Gentamicin, Ciprofloxacin Enrofloxacin, Cefpodoxime Amoxicillin-clavulanic acid Tetracycline, Cefoxitin	Colistine, Ceftriaxone Ampicillin, Nalidixic acid, Chloramphenicol, Trimethoprim-sulfamethoxazole, Cefotaxime, Amikacin	Erythromycin, Ceftazidime, Aztreonam
<i>Klebsiella spp</i>	Amoxicillin-clavulanic acid Cefoxitin Amikacin	Gentamicin, Ciprofloxacin, Enrofloxacin, Colistine Ceftriaxone, Ceftazidime Cefpodoxime, Aztreonam, Tetracycline Nalidixic acid, Trimethoprim-sulfamethoxazole Cefotaxime	Erythromycin Ampicillin



A. E. coli

Fig 6: Antibiotics sensitivity Test

4. Discussion

Endometritis is termed as the inflammation of endometrium and underlying glandular tissue without any systemic signs, occurring at least 21 days after calving. Uterine infections develop upon the establishment of pathogenic microorganisms onto the mucosa, their colonisation and penetration of the epithelial layer and the production of

toxins by these microorganisms. These microbial populations produce ample amount of toxin that interfere with sperm transport process, may destroy the spermatozoa or may result in death of the conceptus by altering the uterine environment that hamper their development and maintenance. Infertility on bovines has remained as one of the major contributing factor towards economic loss to dairy farmers under field condition due to reduction in conception rate, extending the calving interval and reduced milk production in farm animals (Sheldon *et al.*, 2006) [27]. In the current study the subclinical and clinical endometritic cows were investigated to ascertain the involvement of *E. coli* and *Klebsiella* as causal factor with simultaneous study of antibiotics sensitivity pattern of the isolated bacterial species.

In our study bacterial load in cows with clinical endometritis was significantly higher ($p < 0.01$) as compared to subclinical endometritic and normal cyclic cows. Bacterial load in cows with subclinical endometritis was significantly higher ($p < 0.01$) than normal cyclic cows. Our findings corroborates with the findings of Deori (2002) [4]. Several studies reported a higher bacterial load in endometritis as well as in healthy cows. In repeat breeder cows Singla *et al.* (1993) [29] reported a bacterial count of 189.39×10^6 per ml of uterine fluid. Rao and Seshagiri (1997) [22] reported that in their study bacterial load in uterine discharge was found to be $0.0013 \pm 0.0008 \times 10^6$ per ml in healthy cows and

47.0626±1.1589 × 10⁶ per ml of discharge in endometritic cows. A quite high bacterial load of 4.14±1.41 × 10⁴ CFU/ml was observed by Agrawal *et al.* (2002) [1] during their study and they opined that healthy uterine environment also can harbour the facultative micro-organisms without showing any external variation or any infertility conditions. A great variation has been observed among the previous reports and the result of our study which might be due to the different level of infection resulting in clinical and subclinical endometritis. El-Azab *et al.* (1988) [6] described that increased oxygen consumption by the polymorphonuclear inflammatory cells or for micro-organism metabolism can result in creation of anaerobic environment in uterus that can be indicated by reduced intrauterine oxygen reeducates potential (Eh) with simultaneous increase in pH.

Out of 6 normal cyclic cows, 1 cow (16%) was positive for bacterial infection of which solitary cases of single (100%) infection were observed. *Escherichia coli* were the predominant bacteria isolated in normal cyclic cows. All the endometritic cows (100%) were found positive for bacterial infection.

In the cows suffering from clinical endometritis mixed infections were found in 19 (76%) cases, whereas single isolates were detected only in 6 (24%) cases. In the present study *Escherichia coli* (100%) was the most common isolate in clinical endometritic cows followed by *Klebsiella* spp. (52%),

In subclinical endometritis, mixed infections were found in 20 (80%) cases, whereas single isolates were detected only in 5 (20%) cases. In the present study *Escherichia coli* (100%) was the most common isolate in sub-clinical endometritic cows followed by *Klebsiella* spp. (32%),

There is normal bacterial population which resides in vaginal vault of both normal cyclic and repeat breeder cattle which are voided through cervico-vaginal mucus during estrus which may or may not interfere with fertility (Panangala *et al.*, 1978 and Kumar *et al.*, 2004) [16, 12]. But clinical infection like endometritis will result when uterine immunological barrier is either compromised due to any stressful condition or might be breached during or after parturition (Kaufmann, 2009) [11]. Most bacteriological contaminations of uterus take place during parturition and in the phase of metabolic transition period. Sheldon (2006) [27] and Janeway *et al.* (2001) [10] observed that the pathogenic microorganisms invade on the uterine mucosal layer, colonize and penetrate up to the epithelial layer as well as release toxins which help in the establishment of uterine infection.

The isolated bacteria were subjected to antibiotics sensitivity test to observe the effectiveness of commonly used antibiotics. In our study *E. coli* were resistant to Erythromycin, Ceftazidime and Aztreonam antibiotics while isolated *Klebsiella* spp. were resistant to Erythromycin and Ampicillin antibiotics. The common antibiotics used in case of treatment of endometritis are enrofloxacin (Rane *et al.*, 2003) [21], its metabolites ciprofloxacin (Souza *et al.*, 2002) [31] which are effective against microorganisms which are resistant to other antimicrobial agents used for the treatment of endometritis, such as aminoglycosides, tetracyclines, macrolides and β-lactams (Shim *et al.*, 2003) [28]. Antibiotics are the drug of choice for treating uterine infectious conditions like metritis, endometritis or pyometra. But emergence of antibiotics resistant strain results in therapeutic failure (Srinivasan *et al.*, 2005) [32] which is a

serious global threat in this current era. In our study we have observed that almost all the bacteria showed resistance towards Erythromycin. This resistance in the microorganism might be induced by genetic modification of the bacteria as an adaptive consequence against antibiotics.

Goldstone *et al.* (2014) [8] isolated an extra-intestinal pathogenic *E. coli* (ExPEC) from uterus of metritic cow and named it as MS499 strain which showed resistance against several antibiotics like β-lactams, tetracyclines and macrolides. An important example of antibiotic resistance is multidrug-resistant (MDR) and ESBL-producing *Escherichia coli* (Pormohammad *et al.*, 2019) [19, 20], which can cause life-threatening infections not only in animals but also humans through direct or indirect contact (Pormohammad *et al.*, 2019) [19, 20]. Similarly Yang *et al.* (2016) [34] also isolated *E. coli* strains highly resistant towards tetracyclines, ampicillin, carbenicillin, and amoxicillin antibiotics. Santos *et al.* (2010) [23] opined that continuous use of antibiotics in dairy farm can be attributed towards emergence of antimicrobial resistance against commonly used antibiotics ending in ineffectiveness of antibiotics in treating the disease condition. Von Baum and Marre (2005) [33] reported that in both human and veterinary medicine, isolating *E. coli* showing resistance towards at least 2 classes of antimicrobial agent is a common finding. Hence, the availability of therapeutic options to treat the resultant infectious conditions like urinary tract infections, neonatal meningitis and nosocomial bacteraemia in human (Hammerum and Heuer, 2009) [9] and uterine infections in animals are limited

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