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Comparative Analysis of Microbial Prevalence in Normal and Endometritic cows

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Abstract

Endometritis, the inflammation of the endometrium, causes infertility in dairy herds. The aim of this study was to perform a comparative analysis of microbial prevalence in normal and endometritic cows. Therefore, cows were divided into four groups, comprising 10 cows in each. Group A: normal cows with no symptoms. Group B: Young stock (fit for breeding) cows suffering from endometritis. Group C: milking cows suffering from Endometritis. Group D: dry cows suffering from Endometritis. Three sterile swabs were collected from all cows of four groups, thus making a total of 120 samples. Bacterial isolation and identification were performed according to bergey's manual. Most common pathogens in the uterus of endometritic cows were *Escherichia coli* and then *Trueperella pyogenes* > *Salmonella enterica* > *Klebsiella pneumonia* > *Bacillus subtilis* > *Fusobacterium necrophorum* > *Proteus vulgaris* > *Staphylococcus aureus* > *Streptococcus pneumoniae.* In normal cows, mostly numbers of isolates were gram positive. *S. aureus* followed by *S. pneumoniae, B. subtilis, T. pyogenes, E. coli* and *K. pneumoniae.* In conclusion, higher numbers of pathogenic bacteria were found in endometritic cows as compared to normal cows. *E. coli*, being gram negative pathogen is the major cause of infection in female upper genital track and infertility. Appropriate combination of antibiotics and anti-inflammatory medicines along with herbal treatments should be adopted. Maintenance of proper hygiene of dairy herds is inevitable.

Key Words: *Trueperella pyogenes*; *Salmonella enterica*; *Klebsiella pneumonia*; *Fusobacterium necrophorum*; Endometritis.

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1. INTRODUCTION

One of the most prevalent reproductive disorders in dairy cows is endometritis [1,2]. The primary health issue in dairy cows is uterine inflammation [1,2,3]. It not only affects milk production but also reduces reproductive effectiveness, which results in a loss of income. The uterine lumen becomes contaminated with germs after parturition, according to [4,5]. Numerous organisms penetrate the birth canal and populate the uterus before, during, and after parturition. Purulent and mucopurulent discharge from the vagina can be used to detect clinical endometritis [6]. In mucopurulent discharge, 50% pus and 50% mucus are present, compared to 50% pus and 50% mucus in purulent discharge [7]. Subclinical endometritis is the inflammation of endometrial

cavity. When purulent material is not present in the vagina then we can determine clinical subendometritis by cytology. Cytobrush technique is mostly used for subclinical endometritis and it is easy and reliable technique [7,8,9].

Affected cows will show normal cycle but they will not conceive [10]. Affected cows have fewer chances to become pregnant at first service [11]. Loss of appetite, discomfort, weakness and depression are signs of endometritis [12]. Abnormal cows have fast pulse rate. Animals are dehydrated. Estrum mucous is not hazy while healthy estrum mucous is white [13].

Causes of endometritis might include difficulties in calving, abortion, twins or injury of birth canal during calving [10,13]. If fetal membrane does not eliminate from 8-48 hours after parturition then it can lead to endometritis. The causative agents of endometritis can be viruses, mycoplasma, Chlamydia, rickettsiae, bacteria and fungi but the most important cause of uterine infection are bacteria [14,15]. *E. coli* cause intestinal and urinary tract infection. It enters the uterus and cause endometritis. *T. pyogenes* cause uterine inflammation that leads to endometritis and metritis [16]. It is mostly found in the mucosal membrane of ruminants [17]. *F. necrophorum* cause uterine infection and mostly endometritis [16]. *S. aureus* live as a host in the genitalia and cause uterine diseases [18]. Various studies were conducted on Endometritis but the comparative analysis of microbial prevalence in normal and endometritic cows is unknown. Cows are mostly affected by *E. coli*, *T. pyogenes, S. enterica, K. pneumoniae, B. subtilis, F. necrophorum, P. vulgaris, S. aureus* and *S. pneumoniae*. In normal cows *S. aureus* and *S. pneumoniae* were in abundance [19].

2. MATERIALS AND METHODS

The study was conducted on the crossbred cows belonging to the military dairy farms located at Lahore to study the microbial regime of normal and endometritis cows. All cows were examined per month for diagnosing of endometritis. Normal animals without any abnormalities were also studied.

To determine the microbes' responible for causing endometritis, the cows were divided into four groups comprising 10 cows each:

Group A- Healthy cows with no signs and symptoms of endometritis.

Group B - Young stock (fit for breeding) cows suffering from endometritis.

Group C- In milk cows suffering from endometritis.

Group D- Dry cows suffering from endometritis.

Before collection of samples vulvar was cleaned with water and then swab was inserted into the vagina to sample the most front part. The sterile vaginal swabs were collected from all of groups, thus making a total of 120 samples. All samples were collected aseptically and then transmitted in ice packed flask to the Microbiology laboratory of Lahore Garrison University, Lahore. The samples were kept in refrigerator at 4°C.

Following diagnostic techniques were used for isolation of cows suffering from endometritis disease as described by the method of [3,5,20].

- Clinical signs
- Vaginal / uterine swabs
- Colour and odour of vaginal discharge
- Cervical diameter
- Endometrial biopsy

Clinical signs: - Less pregnancy rate / infertility, loss of appetite, depression, dehydration, vaginal discharge and dullness.

Vaginal/uterine swab: - After taking the sample, it was transferred to lab for isolation and identification of microbes. Different biochemical test and staining techniques were performed.

Colour and odour of vaginal discharge: - Clear vaginal discharge (mucous in nature) was found in normal cows while muddy and odorous discharge was present in cows suffering from endometritis.

Cervical diameter:-. Increased diameter of cervix was found cardinal finding.

Endometrial biopsy: - Tissues were taken from endometrial layer and examined histopatologically. Endometrial biopsy showed the inflammatory changes at cellular level as described by [21] also.

Isolation and screening of bacteria

The vaginal samples obtained was inoculated into the nutrient broth and incubated for 12 hours at 37°C. The nutrient broth was then checked for the turbidity that will indicate growth. A loopful of broth culture was streaked onto nutrient agar and selective media or non- selective media like MacConkey agar, Eosin Methylene Blue agar, blood agar and Mannitol salt agar incubated at 37°C for 24 hours. A tentative identification of bacteria was done based on colony morphology and Grams staining, capsule staing, spore staining. Biochemical tests such as, citrate, catalase, urease, coagulase, indole, motility, bile solubility, nitrate reduction, methyl red etc. test were also performed for the identification and characterization of organisms and find out different bacteria [22,23,24].

3. RESULTS AND DISCUSSIONS

Isolation and screening of bacterial isolates:

The results of the bacterial isolation and screening are as follows:

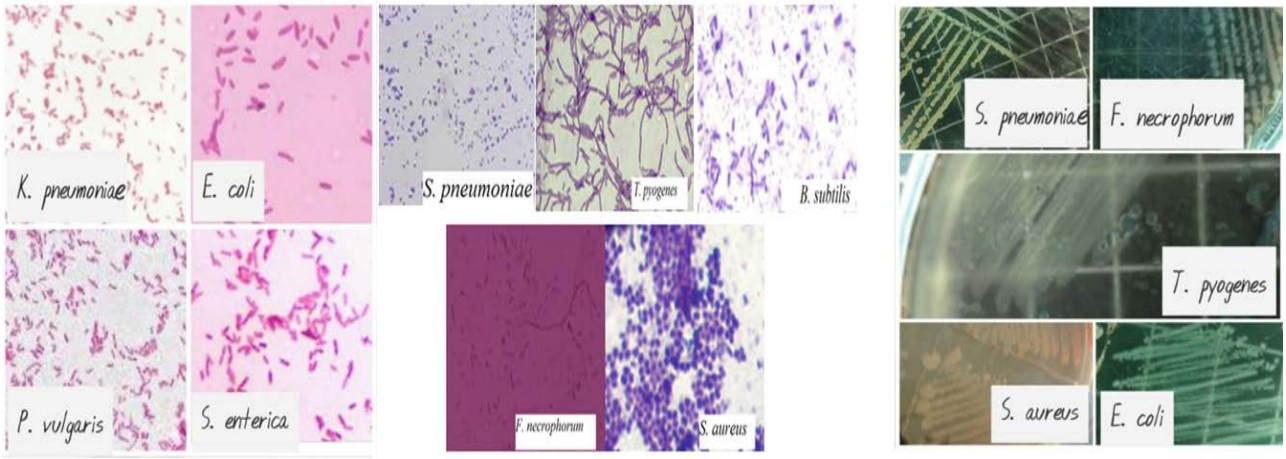

Fig. 1. Results of gram staining

Biochemical Characterization:

Different staining and biochemical techniques were performed for the identification and characterization of bacterial isolates following Bergey's manual and results have been compiled in table 1.

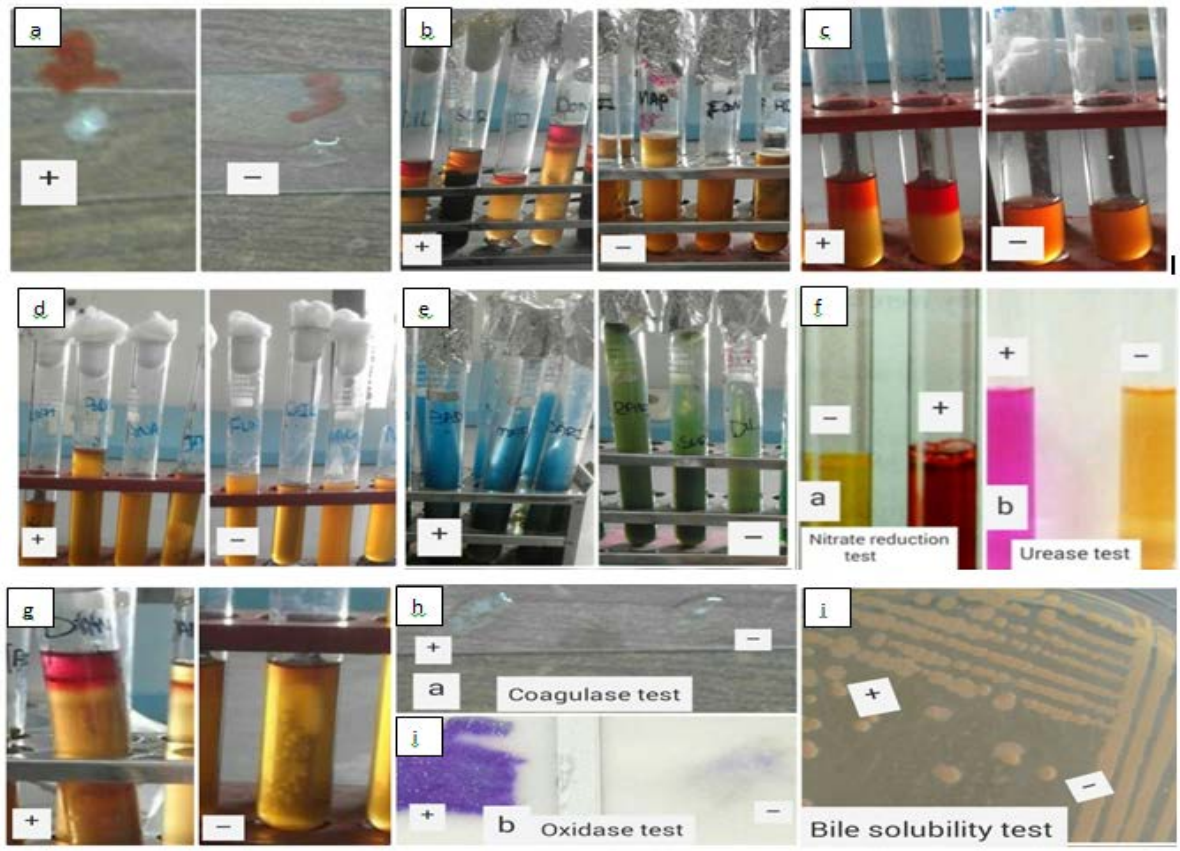

Fig. 2. (a) Catalase test; (b) Indole test; (c) Meythl red test (d) Voges proskauer test (e) Citrate utilization test (f) Nitrate reduction test & Urease test (g) Motility test (h) Coagulase test & Oxidase test (i) Bile solubility test

Table 1. Biochemical characterization of isolates

	E. coli	***S. aureus***	***S. pneumoniae***	***B. subtilis***	***K. pneumonia***	***P. vulgaris***	***F. necrophorum***	***T. pyogenes***	***S. enterica***
Media	EMB+ Mac	MSA + Blood agar	Blood agar	TSA	Blood Agar, Mac agar	Mac	Blood Agar	TSA	Salmonella shigella agar
Gram staining	-	+	+	+	-	-	-	+	-
Capsule	V	-	+	+	+	+	+	-	-
Spore	-	-	-	+	-	+	-	-	-
Catalase test	+	+	-	+	+	+	-	-	+
Indole test	+	-	-	-	-	+	+	+	-
Methyl red	+	+	NA	-	-	+	-	-	+

test									
Voges proskauer test	-	+	-	+	+	-	-	-	-
Citrate utilization test	-	+	NA	+	+	-	NA	-	-
Urease test	-	+	-	-	+	+	NA	-	-
Oxidase test	-	-	-	V	-	-	NA	-	-
Nitrate reduction test	+	+	-	+	+	+	-	+	+
Coagulase test	-	+	-	NA	-	NA	+	+	-
Bile solubility test	-	-	+	NA	-	NA	-	-	-
Motility test	+	-	-	+	-	+	-	+	+

EMB= Eosin-methylene-blue agar, Mac= MacConkey agar, MSA= Mannitol salt agar, TSA= Tryptic soy agar. V= Variable, NA= Not applicable.

Table 2. Microbial flora of normal and Endometritis cows

Sr. #	**Microbial flora of normal cows**	**Microbial flora of Endometritis cows**
1	*E. coli*	*E. coli*
2	*T. pyogenes*	*T. pyogenes*
3	*S. aureus*	*S. aureus*
4	*S. Pneumonia*	*S. Pneumonia*
5	*B. subtilis*	*B. subtilis*
6	*K. pneumonia*	*K. pneumonia*
7		*P. Vulgaris*
8		*F. necrophorum*

		S. enterica

Table 3. Percentage of bacteria in Normal and Endometritis cows

Sr. #	Normal cows flora	No. of samples containing respective bacteria	%	Endometritis cows flora	No. of samples containing respective bacteria	%
1	*E. coli*	5	11.11	*E. coli*	34	24.29
2	*T. pyogenes*	5	11.11	*T. pyogenes*	29	20.71
3	*S. aureus*	12	26.67	*S. aureus*	6	4.29
4	*S. pneumonia*	10	22.22	*S. pneumoniae*	4	2.86
5	*K.pneumoniae*	4	8.89	*K. pneumoniae*	14	10
6	*B. subtilis*	9	20	*B. subtilis*	12	8.57
7				*P. vulgaris*	10	7.14
8				*F. Necrophorum*	11	7.86
9				*S. enterica*	20	14.28
10	Total no. of isolates	45	100.00		140	100.00

Comparison of normal and endometritis microbial flora

Table 2 showing that in normal and abnormal cows *E. coli, T. pyogenes, S. aureus, S. pneumoniae, B. subtilis,* and *K. pneumonia* were common. These pathogens were opportunistic that's why these organisms' caused disease in cows. *P. vulgaris, F. necrophorum* and *S. enterica* were also present in diseased cows. Theses pathogens can enter from environmental agents, non-sterilized instruments and due to contamination.

Fig. 3. showing *E. coli, T. pyogenes, S. aureus. S. pneumoniae, B. subtilis, P. vulgaris, F. necrophorum* and *S. enterica* were isolated from normal and endometritic cows*. P. vulgaris, F. necrophorum* and *S. enterica* were absent in normal cows. In normal cows mostly numbers of isolates were gram positive. *S. aureus* was found to be most abundant in normal cows followed by *S. pneumoniae, B. subtilis, T. pyogenes, E. coli* and *K. pneumoniae.* Uterus of endometritic cows was mostly affected by gram negative bacteria and mostly bacteria were pathogenic while gram positive bacteria were in lesser qunatity. *E. coli* was found to be major pathogen in the uterus of endometritic cows followed by *T. pyogenes, S. enterica, K. pneumoniae, B. subtilis, F. necrophorum, P. vulgaris, S. aureus* and *S. pneumoniae.*

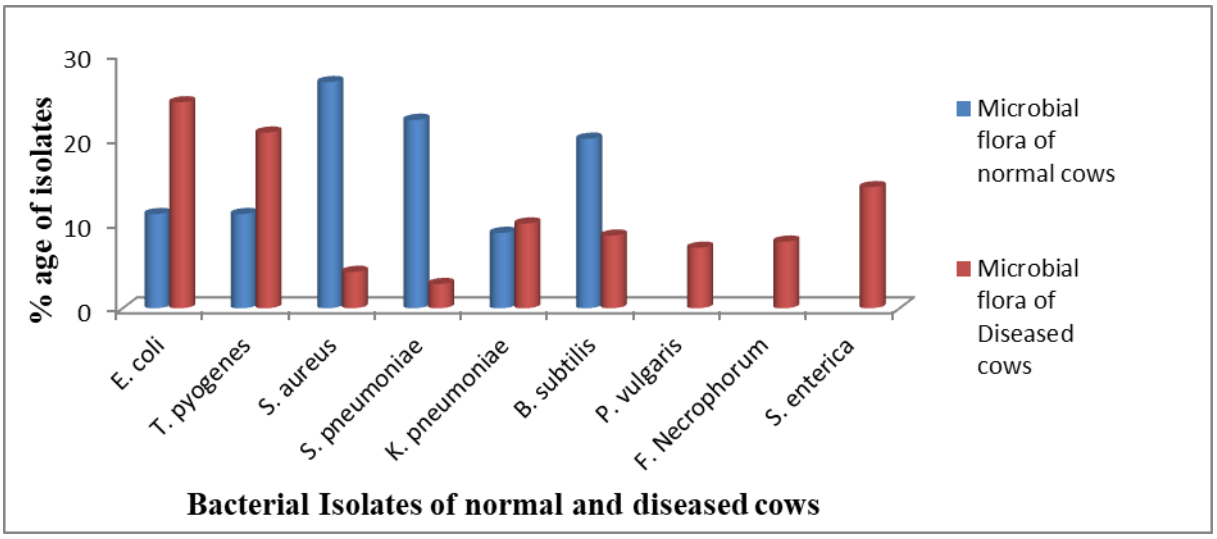

Fig. 3. Percentage of microbial flora of normal and Endometritis cows

Endometritis in cows is causing failure of pregnancy, increased calving interval and decreased milk production. This situation leads to an ultimate economy loss. In this study microbial flora of normal and endometritic cows were distinguished. The most common pathogens in the uterus of endometritic cows were *E. coli* and *T. pyogenes*. In normal cows *S. aureus* and *S. pneumoniae* were in abundance. Present study strengthens the results of [16,17,25]. According to them *E. coli* cause intestinal and urinary tract infection it enters the uterus and cause endometritis. *T. pyogenes* cause uterine inflammation that leads to endometritis and metritis. It mostly found in the mucosal membrane of ruminants. *F. necrophorum* cause uterine infection and mostly Endometritis. Present study agrees with [18] *S. aureus* that live as a host in the genitalia and cause uterine diseases. Current studies are in agree with [22] who also described that proteus species cause urinary tract infection and live in the intestinal tract of humans.

In Bangladesh, [15] collected vaginal discharge of local and crossbred normal cows and identified most common uterine bacterial species were *staphylococcus* (33%) and *streptococcus* (27.3%). Present study also showed that the most common pathogens isolated from normal cows are *staphylococcus aureus* and *streptococcus pneumoniae*. [19] also reported increased prevalence of *staphylococcus*, *Streptococcus, Eshcherichia, corynebacterium, haemophilis, Proteus* and *bacillus* species in normal flock.

In present study, the most abundant microorganisms includes *E. coli* and *T. pyogenes* followed by *S. enterica, K. pneumoniae, F. necrophorum, P. vulgaris, B. subtilis, S. aureus* and *S. pneumoniae.* [29] also isolated these organisms from uterine swab of cows, in Namakkal district of India. Different species of bacteria were isolated including *Escherichia coli* (36.66%), *Klebsiella spp.* (30%), *Proteus spp.* (13.33%), *Pseudomonas aeruginosa* (6.66%), *Clostridium spp.* (3.33%).

Current study showed that common isolates were *E. coli, A. pyogenes*. Our results confirm the findings of Takamtha *et al*., (2013) who also collected the samples of uterine swab from Holstein Frisian cows in Thailand. Similar results were reported by various research groups [26,27,28].

In present study, percentage of isolates were *E. coli* (24.29%) followed by *T. pyogenes* (20.71%), *S. enterica* (14.28%), *Klebsiella pneumoniae (10%), B. subtilis* (8.57%), *F. necrophorum* (7.86%), *P. vulgaris* (7.14%), *S. aureus* (4.29%) and *S. pneumoniae* (2.86%). There was no significant difference between the present study results and [28] results, who reported *E. coli* (28.97%), *Klebsiella spp* (16.28%), *salmonella spp* (14.95%), *proteus spp* (13.08%), *Staphlococcus aureus* (6.54%), *Staphylococcus epidermidis* (8.41%) and *Streptococcus spp* (6.54%).

4. CONCLUSIONS

Higher number of pathogenic bacteria was found in endometritic cows as compared to normal cows among which *E. coli* was the most abundant one. *E. coli* is associated with inflammation of endometrium walls in uterus of females and domestic animals. It also causes intestinal and urinary tract infection. Appropriate combination of antibiotics and anti-inflammatory medicines along with herbal treatments should be

adopted. Maintenance of proper hygiene of dairy herds is inevitable because random and frequent use of antibiotics causes exchange of resistant genes among microbes leading to chronic infection. Animal feed should be pure without antibiotic additives.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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