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# Antibiogram and Bacterial Profiling of Reproductive Tract Infections in Female Cattle and Buffaloes

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## ABSTRACT

Reproductive tract disorders are considered a major cause of low conception rate and poor productivity of livestock, leading to infertility or sterility of animals, and thus, results in high economic losses. The current study was designed to investigate the role of bacteria in reproductive tract disorders of cattle and buffalo, and the efficacy of different antibiotics against different bacterial isolates. A total of 100 reproductive tracts of slaughtered cattle and buffaloes were randomly collected from different abattoirs of District Lahore and examined for the presence of any gross abnormality. The swab samples were processed for bacteriology on suitable culture media. The bacterial isolates were identified based on colony characteristics and biochemical tests and subjected to *in vitro* antibiotic sensitivity assay. For microscopic examination, tissue samples of reproductive tracts were processed for histopathology. Out of 100 collected samples, 54 samples showed the presence of gross lesions including endometritis (21%), pyometra (17%), Para-ovarian cysts (8%), hydrosalpinx (3%), luteal cysts (3%), and follicular cysts (2%). *E. coli* was the major contaminant isolated from all the samples (100%) followed by *Staphylococcus aureus*, *Micrococcus* species, *Staphylococcus epidermidis*, *Lactobacillus* species, *Corynebacterium* species, and *Aeromonas* species. Histopathological examination showed infiltration of polymorph nuclear (PMN) cells in the endometrium, degeneration of endometrial glands, congestion, hemorrhage, and a proliferation of connective tissue in a few cases. The isolates showed higher sensitivity to norfloxacin, ciprofloxacin, and chlortetracycline while resistant to bacitracin, sulphaphenazole, and chloramphenicol. A significant association was found between the antibiotics and the pattern of their sensitivity. In conclusion, endometritis and pyometra are the leading pathological disorders of the reproductive tract of cattle and buffalo. *E. coli* has been found as the most frequently occurring bacteria in the reproductive tract of female buffaloes and cattle. Though Ciprofloxacin, Norfloxacin, and Chlortetracycline have been investigated as the most effective drugs in the treatment of bacterial infections in female bovine reproductive tracts, further explorations on antimicrobial sensitivity and continuous monitoring of antimicrobial resistance are needed as future considerations.

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**IA, AA, and AAA designed the research project, supervised the research work, collected and analysed data. AJ, AG, II, HQA and QN performed sample collection and laboratory procedures. MKR and AUR provided research material and validated laboratory procedures. MTM and MZA performed data analysis and drafted the manuscript. AS, SR, and YI contributed to Lab work and revised the manuscript.**

**Key words**
**Antibiotic, Bovine, Microbial profiling, Pathology, Reproduction, Resistance**

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## INTRODUCTION

Pakistan has 29.6 million cattle and 27.3 million buffaloes which are playing a vital role in the national economy by producing a significant proportion of milk and meat (Rehman *et al*., 2017). Reproductive health is very important for a profitable dairy enterprise. Animals may suffer from acquired and congenital anomalies of the

reproductive tract. Disorders of the reproductive tract cause economic losses to dairy farmers due to increased risk of stillbirth, retained placenta, prolonged calving intervals, extra service charges, treatment expenditure, and culling of animals (Azawi, 2010). The reproductive tract may get infected with specific pathogens via contaminated semen or opportunistic microbes may proliferate under favorable circumstances (Azawi, 2008; Sheldon *et al*., 2009). Dystocia, uterine prolapse, and retained placenta also predispose animals to get the infection. The most common problems of all commercial dairy farms are endometritis and pyometra (Hossein-Zadeh and Ardalan, 2011).

Normal microflora in the reproductive tracts of cattle and buffaloes include a variety of beneficial microorganisms. These microbes include, for instance, *E. coli*, *S. epidermidis, Y. enterocolitica, Lactobacillus* species, and *Micrococcus* species (Azawi, 2010). Some bacteria like *A. pyogenes*, *F. necrophorum*, *C. pyogenes*, *S. aureus* and *S. pyogenes* may lead to severe inflammation of the uterus resulting in endometritis, metritis and pyometra (Bajaj *et al*., 2018; Al-Dori *et al*., 2020). Bacterial profiling of the uterine tract in repeat breeders have revealed the presence of *Bacillus* species and *Staphylococcus* species while *E. coli*, *S. aureus*, *A. pyogenes* and *Klebsiella* species have been reported as fatal pathogens during ovarian inactivity (Ahuja *et al*., 2017).

During the uterine disease process, microorganisms, or pathogen-associated molecules, as well as inflammatory response, cause disruption of uterine tissue and endocrine dysfunction in the reproductive tracts of cattle (Price *et al*., 2013). Several antibiotics are being used by clinicians to treat post-partum infections of the reproductive tract in bovines (Dubuc *et al*., 2011). A large variety of antimicrobials are available in the market, being used in routine but excessive and prolonged usage resulted in multidrug resistance (Ma *et al*., 2018). Antimicrobial susceptibility testing has been conducted worldwide, and different kinds of tetracyclines, cephalosporins, and penicillins are widely used drugs for reproductive tract diseases (Naz *et al*., 2012; Verma *et al*., 2018). However, the efficacy of such antimicrobial agents needs to be evaluated continuously due to the emergence of antibiotic resistance of bacterial strains (Frieri *et al*., 2017).

As there could be several microbes involved in impairing the reproductive efficiency of animals and due to uncontrolled use of antimicrobials in animals in Pakistan which could lead to the emergence of multiple drug-resistant bacteria, continuous monitoring of the reproductive tract pathogens and their susceptibility to various antibiotics are essential. The current investigation has been undertaken to study the gross and histopathological lesions of the female bovine reproductive tract, the bacterial profile of reproductive tract infections, and the efficacy of different antibiotics against bacteria isolated from reproductive tract infections.

## MATERIALS AND METHODS

A total of 100 reproductive tracts were collected randomly from buffaloes (n = 60) and cattle (n = 40) slaughtered at Shera Kot and Shah Pur Kaanjran abattoirs, Lahore, Pakistan. Abattoirs were visited twice a week and reproductive tracts were collected in sterilized polythene bags. Samples were brought to the Department of Pathology, University of Veterinary and Animal Sciences, Lahore, for further processing.

The gross lesions in any part of reproductive tracts were visually inspected by incising them completely with sterilized scissors, scalpel, and blade, and any type of pus, hemorrhages, edema, abscess, etc. was recorded. Both ovaries of each sample were examined for any lesions like para-ovarian cysts, follicular cysts (FC), and luteal cysts (LC) and results were noted.

Different culture media (MacConkey's agar, Nutrient agar, and EMB agar) were prepared by following the procedures given in Bergey's Manual of Determinative Bacteriology (Bergey *et al*., 1994) to isolate and identify the bacterial organisms. The primary culture of micro-organisms was obtained by using the streak plate method (Servais and Passerat, 2009) and from this primary culture; the purification of bacteria was done by using the three-dimensional streaking technique (Krishna *et al*., 2019).

To study the morphology of bacteria, isolated bacteria were stained by the Gram staining method (Beveridge, 2001) and subjected to different biochemical tests to differentiate bacteria at genus and species level. Lactose fermentation test, Indole production test, Methyl red test, Voges Proskauer test, Citrate utilization test (Bergey *et al*., 1994), Catalase test (Gruner *et al*., 2007), Mannitol fermentation test (Makwana *et al*., 2012), Glucose fermentation test (Abbott *et al*., 2003), Starch hydrolysis test (Iverson and Millis, 1974), and test for the requirement of sodium for bacterial growth was used for differentiation among different bacterial isolates. Moreover, in addition to Gram's staining, spore staining and acid-fast staining were used for the identification of certain spore-forming and acid-fast bacteria respectively (Reynolds *et al*., 2009).

Antibiotic sensitivity of *E. coli*, *S. aureus*, *S. epidermidis*, *Corynebacterium* species, *Aeromonas* species, *Micrococcus* species, and *Lactobacillus* species to different antibiotics was determined by the disk diffusion method in Mueller Hinton agar. The following antimicrobial drugs were used: penicillin (P; 10 µg), streptomycin (S; 10 µg), ticarcillin/clavulanic acid (TC;

75/10 µg), norfloxacin (NOR; 10 µg), sulpaphenazole (Sp; 200 µg), chloramphenicol (C; 20 µg), bacitracin (B; 10 U), trimethoprim (Tr; 5 µg), ciprofloxacin (CIP; 5 µg), gentamicin (CN; 10 µg), chlortetracycline (CT; 30 µg). The results were declared as sensitive (S), intermediate (I), and resistant (R), based on the zone of inhibition given by the Clinical and Laboratory Standards Institute (CLSI).

For histopathology, samples of uterine tissue were preserved in 10% buffered formalin and processed through routine paraffin embedding technique as described by Bancroft and Gamble (2008). Tissue sections were cut at 5 µm and stained with H and E stains for examination of changes in the tissues.

The obtained data (2012 to 2013) was analyzed using the Chi-Square test through IBM SPSS statistics Ver. 22.0. Armonk, NY.

## RESULTS

A total of 100 randomly collected reproductive tracts of female buffaloes (*n*=60) and cattle (*n*=40) were inspected for gross lesions, of which 54% showed lesions. Out of the total of 60 reproductive tracts of buffaloes investigated, 34 reproductive tracts had gross pathological lesions including endometritis (13/60), pyometra and pyosalpinx (11/60), para-ovarian cyst (5/60), hydrosalpinx (2/60), luteal cyst (2/60), follicular cyst (1/34). In cattle (n = 40), gross lesions were found in 20 samples including endometritis (8/40), pyometra and pyosalpinx (6/40), para-ovarian cyst (3/40), hydrosalpinx (1/40), luteal cyst (1/40), and follicular cyst (1/40). Statistical analysis showed that the prevalence of different gross lesions in the reproductive tract of female bovines is not significantly associated ($p > 0.05$) with the species of the animal. The percentage prevalence of the different lesions found in the reproductive tract of female bovines is presented in Table I.

In endometritis, uterine tissues showed lesions such as hemorrhages in the stratum compactum, congestion, necrosis, and degeneration of endometrial glands, glandular tissue filled with mononuclear cells, destroyed secretary epithelial cells while in metritis, intact endometrial mucosa, the proliferation of connective tissue, infiltration of inflammatory cells and mononuclear cells in the myometrium were observed.

In pyometra samples, a thick layer of exudate on the surface of the endometrium, severe infiltration of mononuclear cells, sloughing of endometrial mucosa, necrosis of endometrial glands and glands filled with a heavy population of mononuclear cells, the proliferation of fibrous connective tissues in between endometrial glands, many macrophages are also present, and infiltration of polymorphs mononuclear cells was observed in stratum functional.

**Table I. Prevalence percentage (%) of gross lesions observed in the reproductive tract of the buffaloes and cattle.**

| Gross lesions | Buffaloes (n= 60) | Cattle (n = 40) |
|---|---|---|
| | Number[1] (%) | Number[2] (%) |
| Endometritis | 13 (21.26 %) | 8 (20 %) |
| Pyometra and pyosalpinx | 11 (18.33 %) | 6 (15 %) |
| Para-ovarian cysts | 5 (8.33 %) | 3 (7.50 %) |
| Hydrosalpinx | 2 (3.33 %) | 1 (2.5 %) |
| Luteal cysts | 2 (3.33 %) | 1 (2.50 %) |
| Follicular cysts | 1 (1.67 %) | 1 (2.50 %) |
| Total | 34 (56.67 %) | 20 (50 %) |
| p-value | 0.996 | |

[1]Buffalo showing gross lesions in the reproductive tract. [2]Cattle showing of gross lesions in the reproductive tract.

The collected samples were processed for bacterial isolation and identification. All the samples were found positive for the presence of bacteria. A total of 301 bacterial isolates from 100 reproductive tracts of female cattle and buffaloes were identified. These isolates were recognized as *E. coli, Staphylococcus* sp., *Micrococcus* sp., *Lactobacillus* sp., *Corynebacterium* sp. (*C. xerosis* and *C. kutsceri*), and *Aeromonas* sp.

Out of 60 reproductive tracts of buffaloes examined for bacteriological examination, *E. coli* (60/60) had the highest prevalence i.e., 100% followed by *S. aureus* (31/60), *Micrococcus* sp. (29/60), *S. epidermidis* (23/60), *Lactobacillus* sp. (19/60), *Corynebacterium* sp. (*C. xerosis* and *C. kutsceri*) (15/60) and *Aeromonas* sp. (3/60) as 51.6%, 48.3%, 38.3%, 31.6%, 20% and 5%, respectively (Fig. 1). Out of 40 reproductive tracts of cattle examined for bacteriological examination, *E. coli* (40/40) had the highest prevalence i.e., 100% followed by *S. aureus* (20/40), *Micrococcus* sp. (18/20), *S. epidermidis* (15/40), *Lactobacillus* sp. (15/40), *Corynebacterium* sp. (15/40), *Aeromonas* sp. (2/40) as 50%, 45%, 42.5%, 37.5%, 22.5%, and 5%, respectively. Statistically, the association between the host animal species (buffalo and cattle) and the presence of certain bacteria in the female bovine's reproductive tract was not found ($p > 0.05$). The percentage prevalence of different bacteria isolated from the reproductive tract of female buffaloes and cattle is shown in Table II.

**Table II. Prevalence (%) of different bacteria isolated from the female buffalo and cattle reproductive tracts.**

| Bacterial isolates | Buffalo (n = 60) | Cattle (n = 40) |
|---|---|---|
| | Number (%) | Number (%) |
| *E. coli* | 60 (100.0%) | 40 (100.0 %) |
| *S. aureus* | 31 (51.6 %) | 20 (50.0 %) |
| *Micrococcus* spp. | 29 (48.3 %) | 18 (45.0 %) |
| *S. epidermidis* | 23 (38.3 %) | 17 ( 42.5 %) |
| *Lactobacillus* spp. | 19 (31.6 %) | 15 (37.5 %) |
| *Corynebacterium* spp. | 15 (20.0 %) | 9 (22.5 %) |
| *Aeromonas* spp. | 3 (5.0 %) | 2 (5.0 %) |
| p-value | 0.998 | |

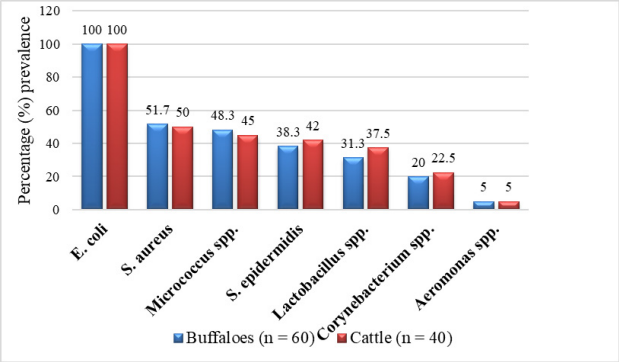


Fig. 1. Percentage prevalence (%) of different bacteria isolated from the female buffalo and cattle reproductive tracts.

All species of isolated bacteria including *C. xerosis, C. kutsceri, S. aureus*, *S. epidermidis, Lactobacillus* species, *E. coli, Aeromonas* sp., and *Micrococcus* sp. were sensitive to norfloxacin (NOR; 10µg), ciprofloxacin (CIP; 5 µg), and chlortetracycline (CT; 30µg). All species were sensitive to gentamycin (CN; 10µg) except only *Aeromonas* species that were resistant to gentamycin. *C. xerosis, C. kutsceri, S. aureus*, *S. epidermidis*, and *Lactobacillus* species were found sensitive to penicillin (P; 10µg) whereas *E. coli* and *Aeromonas* species were found resistant to the penicillin (P; 10µg). However, *Micrococcus* species were found intermediately sensitive to penicillin (P; 10 µg). *C. kutsceri, S. aureus, Lactobacillus* sp., and *Micrococcus* sp. were found sensitive to the streptomycin (S; 10 µg) whereas *C. xerosis, E. coli,* and *Aeromonas* sp., were found resistant to the streptomycin (S; 10 µg). However, *S. epidermidis* was found intermediate sensitive against streptomycin (S; 10 µg). *E. coli*, *S. epidermidis*, *Lactobacillus* sp., *Aeromonas* sp. and *Micrococcus* sp. were found sensitive to ticarcillin/clavulanic acid (TC; 75/10 µg), whereas *S. aureus* was found intermediately sensitive to the same drug. Only *S. epidermidis* and *Micrococcus* sp. were sensitive against suphaphenazole (Sp; 200 µg) while all other bacteria were found resistant to the same drug. Chloramphenicol (C; 20µg) was effective against all isolates except *E. coli and S. aureus* which were found intermediate sensitive to the same drugs. *S. epidermidis* was found unaffected by chloramphenicol. *Lactobacillus* sp., *Micrococcus* sp. and *S. aureus* were found sensitive to trimethoprim (Tr; 5 µg) whereas the rest of the bacterial isolates were found resistant. All bacterial isolates were resistant to bacitracin (B; 10 U).

Table III presents the efficacy of antibiotics against various strains of gram-negative and gram-positive bacteria. The results indicate the effectiveness, intermediate effectiveness, or ineffectiveness of antibiotics. The analysis reveals a significant correlation ($p < 0.05$) between the bacterial Gram staining characteristics and sensitivity against antibiotics, specifically highlighting that Gram-positive bacteria exhibit significantly higher sensitivity to antibiotics compared to Gram-negative counterparts.

**Table III. The sensitivity of different gram bacteria against different anti-biotic discs applied on MHA medium.**

| Bacteria | Effective antibiotics (n) | Intermediately effective antibiotics (n) | Non-effective antibiotics (n) | Sig. |
|---|---|---|---|---|
| **Gram-negative** | | | | |
| *E. coli* | 4 | 1 | 6 | 0.025* |
| *Aeromonas* spp. | 4 | 0 | 7 | |
| **Gram-positive** | | | | |
| *Lactobacillus* spp. | 8 | 0 | 3 | |
| *Micrococcus* spp. | 8 | 1 | 2 | |
| *C. kutsceri* | 8 | 0 | 3 | |
| *C. xerosis* | 7 | 0 | 4 | |
| *S. aureus* | 7 | 2 | 2 | |
| *S. epidermidis* | 6 | 1 | 4 | |

*A significant difference ($p < 0.05$) exists between sensitivity of gram negative and positive bacteria i.e., gram positive bacteria are more sensitive to the antibiotics applied than gram negative bacteria

## DISCUSSION

Reproductive tract lesions may affect animal breeding either by causing infertility or sterility resulting in high economic losses (Fareed *et al*., 2017). Gross lesions observed in the current study (54%) agree with the findings of Lazim *et al*. (2008) who reported that 53.3% of animals were affected with genital abnormalities in Iraq. Current

results differ in terms of prevalence from the study of Mohammad (2013) who reported 13.13% of cattle suffering from macroscopic pathologies in Yemen. Two different studies from Ethiopia reported that 36.8% and 35.9% of cattle had gross lesions (Abalti *et al*., 2006; Mekibib *et al*., 2013). However, in another study, the percentage of gross lesions observed in the reproductive tract of buffaloes was slightly higher i.e. 67.3% than that of the present study (Al-Fahad *et al*., 2004). These variations in the occurrence of gross lesions may be attributable to differences in breed, dam's age, parity, nutrition, management, geographic location/environment, and number of examined animals (Sharma *et al*., 2017).

Gross lesions in the case of endometritis were almost identical to those previously reported research findings (Lazim *et al*., 2008). The results of the current study agree with Ghanem *et al*. (2002) who found 22.4% of cows suffer from endometritis. However, in contrast, a higher prevalence of endometritis in Iranian and Indian buffaloes were found i.e. 44.73% and 72%, respectively (Vala *et al*., 2019). Similarly, a clinical study on buffalo reported a higher percentage (48.79%) of animals suffering from endometritis/metritis while only 0.22% with pyometra were detected (Modi *et al*., 2011). Moreover, a much lower prevalence rate of endometritis was noted in Egyptian buffaloes i.e. 8.53% (Hamouda *et al*., 2020). A low prevalence (3.9% and 4.93%) of endometritis was found and reported in previous studies (Abalti *et al*., 2006; Mekibib *et al*., 2013). Azawi (2008) found 12.3% and 0.5% incidence of endometritis and pyometra. Endometritis can be relatable to many predisposing factors including some extrinsic factors such as untrained artificial inseminators, unhygienic conditions during parturition, nutrition, and certain intrinsic factors: dystocia, negative energy balance, inflammation of the udder, retention of placenta, and decrease in body calcium, etc. (Onyango *et al*., 2014; Adnane *et al*., 2017). In comparison to the current study, Azawi and Ali (2015) observed 2.2% and 4.9% of pyosalpinx and hydrosalpinx, respectively. A relatively low incidence of hydrosalpinx (1.25%) was observed and reported previously (Mohammad, 2013). Similarly, Mekibib *et al*. (2013) reported the incidence of Hydrosalpinx and pyosalpinx as 2.32% and 0.87%, respectively. The prevalence of hydrosalpinx and pyosalpinx in buffalo varies between 0.7%-14.2% and 0.6%-11.9%, respectively (Purohit, 2014).

In contrast to the present study, observed a low incidence of para-ovarian and luteal cysts (1.25% and 0.63%), and a high incidence of follicular cysts (7.5%), respectively was reported in a previous study (Mohammad, 2013). Abalti *et al*. (2006) reported incidence of para-ovarian and ovarian cysts as 1.5% and 3.5%, respectively. In Iraqi buffaloes, the prevalence of para-ovarian cysts, luteal cysts, and follicular cysts was found at 4.4%, 0.2%, and 1.5%, respectively (Azawi and Ali, 2015). In another study, conducted on Pakistani non-descript cows, Ali *et al*. (2006) found the incidence of para-ovarian, luteal, and follicular cysts as 1.81%, 0.9%, and 2.72%, respectively. In comparison to the current study, a lower incidence of para-ovarian cysts (3.40% and 1.74%) luteal cyst incidence (1.5%, 0.87%), and a higher incidence of follicular cysts (8% and 4.35%) was also reported (Saxena *et al*., 2006; Mekibib *et al*., 2013). A review of ovarian and oviduct pathologies by Purohit, 2014 reported that 0.54% of buffalo have persistent corpus luteum while ovarian cysts range between 0.5-1.4%. A deficient luteinizing hormone is supposed to cause cystic ovaries (Vanholder *et al*., 2002). Moreover, this variation may be due to differences in sample size, breed, age and feeding practices. Moreover, several factors like breed, age, level of milk production, feeding, management and exercise are suggested as influencing the prevalence of cystic ovaries in cattle (Nelson *et al*., 2010).

In the current study, histological findings such as infiltration of the polymorphs and mononuclear cells in the endometrium, desquamation of epithelial cells, glandular degeneration, and peri-glandular fibrosis were in line with the work of other researchers (Hatipoglu *et al*., 2002; El-Sakkar *et al*., 2008; Yilmaz *et al*., 2012). Inflammation is a common process in the endometrium of postpartum dairy cattle. The presence of PMN cells in the endometrium was thought to be because of endometritis (Healy *et al*., 2014). Almost all cows have some inflammatory changes, especially in the epithelium and stratum compactum (Adnane *et al*., 2017). During the disease process of the uterus, inflammatory cells come into the endometrium and the superficial epithelium may become necrotic and the number of lymphocytes and neutrophils present in the endometrium increases (Carneiro *et al*., 2016). Thus, uterine disease causes a lower conception rate and increases calving intervals. These may result in the culling of the animals (Sheldon *et al*., 2009). In the current investigation, the prevalence of the bacterial isolates in normal cyclic animals was as follows: *E. coli* was most abundant followed by *Micrococcus* sp., *Staphylococcus* sp. and *Lactobacillus* sp. Previous studies told that normal microbial flora included *E. coli*, *Staphylococcus* sp., and *Bacillus* sp. (Gani *et al*., 2008). While others reported that normal microflora included *Y. enterocolitica*, *Citrobacter diversus*, *Enterococcus faecalis*, *Bacillus* sp., *Micrococcus* spp. and *Pasteurella multocida* (El-Jakee *et al*., 2008).

Different species of bacteria i.e. *Enterobacteriaceae* spp., *Escherichia coli*, *Corynebacterium* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Pseudomonas*

spp., and *Bacillus* spp. have been isolated from the reproductive tract of cattle suffering from endometritis (Barman *et al*., 2013). All the endometriotic or pyometric samples included in this study were positive for *E. coli*. Previous studies reported that endometrial biopsies in cows were positive for *E. coli* and *Streptococcus* sp. (Ata *et al*., 2010). In this study, the most abundant organism was *E. coli*, it was present in all animals whether they were pregnant, non-pregnant, or aborted. Likewise, there was a 100% prevalence of *E. coli* in the uterus from normal prepubertal cattle and buffaloes (El-Jakee *et al*., 2008). *E. coli* is known to be present in both normal and endometriotic animals (Hanafi *et al*., 2008). The *E. coli* invading the endometrium is different from intestinal or extra-intestinal pathogenic *E. coli*, and its more adherent and invasive to the endometrium in comparison to other strains of *E. coli* (Sheldon *et al*., 2010). *S. aureus* (51.6 %) and *S. epidermidis* (38.3 %) were also recovered in a higher percentage. *E. coli*, *S. aureus*, and *S. epidermidis* were isolated from healthy as well as inflamed uterus (Yilmaz *et al*., 2012; Raza *et al*., 2013). *S. aureus* is categorized as a potential uterine pathogen (Dolezel *et al*., 2010). Liu *et al*. (2013) found *S. aureus* (21.8%) as the major pathogen in cows with endometritis. *Micrococcus*, *S. epidermidis*, and *Lactobacillus* observed in the current study, are more prevalent in healthy uterine tissues and considered opportunistic contaminants (Williams *et al*., 2007; Raza *et al*., 2013). *Aeromonas* spp. was reported along with other uterine bacteria and BHV-4 in cases of mild endometritis (Fabian *et al*., 2008). The postpartum uterus is highly susceptible to the growth of a variety of aerobic and anaerobic microbes though many of them are removed by uterine defense. Some studies using conventional and molecular techniques have detected *E. coli* in very small numbers with a dominance of Fusobacteriaceae and Bacteriodetes from animals suffering from pyometra, endometritis, and repeat breeding (Knudsen *et al*., 2015; Pothmann *et al*., 2015). The current study has the limitation of covering only the aerobic microflora.

In the current study, all bacterial isolates were sensitive to chlortetracycline, ciprofloxacin, and norfloxacin and resistant to bacitracin. The results of this study partially agree with the findings of Malinowski *et al*. (2010) who found that *E. coli* was sensitive to norfloxacin and gentamicin while it was resistant to oxytetracycline. Low sensitivity of *T. pyogenes* to tetracycline and intermediate sensitivity to gentamicin has been reported. *E. coli* is highly resistant to tetracycline in a study conducted by Barman *et al*. (2013). Our study supports the findings of Gani *et al*. (2008) who found ciprofloxacin and oxytetracycline effective against uterine bacteria and a resistance of *E. coli* against penicillin. Udhayavel *et al*. (2013) reported that ceftriaxone, gentamicin, Norfloxacin, and chlortetracycline were effective against uterine pathogens. Oxytetracycline was the best antimicrobial against endometritis and metritis in cows (Frieri *et al*., 2017). *In vitro*, antibiotic sensitivity showed that the most effective antibiotic was ceftriaxone (64%). The sensitivity of gentamicin, norfloxacin, and chlortetracycline was found as 32% (Udhayavel *et al*., 2013). In a study conducted in India, it was revealed that the sensitivity of bacteria isolated from the uterine lavage of clinically endometriotic buffaloes was 74.19% and 54.83% against ciprofloxacin and gentamycin, respectively (Bajaj *et al*., 2018). In contrast to our results, bacteria isolated from the reproductive tract of endometriotic buffaloes have shown higher sensitivity against chloramphenicol (83.9%) and a lower sensitivity against gentamycin (80.06%) (Bhadaniya *et al*., 2019). Similar to our findings, the sensitivity of *E.coli* isolated from endometriotic buffaloes was found as 90% against gentamycin (Ingale *et al*., 2016). The variation in different antibiogram studies might be due to the improper large-scale administration and prescription of a variety of antimicrobial drugs and intrinsic factors of bacteria (Marston *et al*., 2016).



## CONCLUSION

The study has revealed that endometritis and pyometra are the most commonly occurring problems of female bovines in Pakistan. *E. coli* has been found as the most commonly occurring bacteria in the reproductive tract of female buffaloes and cattle. Though ciprofloxacin, norfloxacin, and chlortetracycline have been investigated as the most effective drug in the treatment of bacterial infections in female bovine reproductive tracts, further investigations on antimicrobial sensitivity and continuous monitoring of antimicrobial resistance are needed as future considerations.

## DECLARATIONS

*Ethics approval consent to participate*

The ethical approval was taken from the University's Advanced Studies and Research Board of UVAS, Lahore to conduct the proposed research in Lahore.

*Data analysis*

Data was analyzed by MS Office 2016, GraphPad Prism 5, and SPSS 2016.

*Availability of data and materials*

Data on this article is available for any sort of publicity after publication.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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