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Isolation and antimicrobial profiling of bacteria from domesticated animals with Metritis

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Abstract
Metritis is the inflammation of endometrium, underlying glandular tissue and muscular layer. The objective of this study was to determine the susceptibility pattern of bacteria isolated from metritis of domesticated animals. In the present study, a total 53 uterine discharge swabs were collected from domesticated animals (n=16), buffalo (n=14), and goats (n=23). From metritis cases 26 isolates identified on the basis of morphological and cultural characteristics were of *E. coli*, 8 were of *Enterococcus* spp and 6 isolates of were *Acenetobacter lwoffii.* Evaluation of antibiotic sensitivity pattern of bacterial isolates indicated that *E. coli* was resistant to oxytetracycline (88.46). The *Enterococcus faecalis* showed resistant to oxytetracycline (75%) and ampicillin (75%). The isolates of *Acinetobacter lwoffii* showed resistance to oxytetracycline (83.33%) and ampicillin (66.66). This study revealed that *E. coli* is the most commonly isolated bacteria in metritis. Chloramphenicol was the most effective antibiotic against isolated bacteria.

Keywords: Metritis, bacteria, domesticated animals and antimicrobial sensitivity

1. Introduction
India ranks first in the bovine population, which has brought us on top of the world as the largest milk producing country. Unfortunately, reproductive tract infections have always led to a diminished reproductive capacity, which is noticed mainly in cattle and buffalo with high milk production. Reproductive efficiency is the main determining factor for female livestock that get distressed due to reproductive disorders. The most crucial reproductive health problems are metritis and endometritis occurred due to inflammation of the uterus. Metritis influenced the endometrium, the underlying glandular tissue and the muscular layer (Bartlett *et al*., 1986 and Lewis 1997) [1-2], while in endometritis only the endometrium infected with underlying glandular tissue (Bonnett *et al*., 1993) [3] without systemic signs (Bondurant 1997) [4].

Bacteriological contamination of the uterus after birth and metabolic variations in the transition phase are supreme etiological factors for the inception of metritis and endometritis (Kaufmann *et al*., 2009) [5]. Uterine bacterial contamination always had an impact on a huge number of dairy cows after parturition and was one of the important causes of infertility thereby interrupting uterine and ovarian function (Sheldon *et al*., 2008) [6]. After parturition, there is a period when animals are simply affected by bacteria, followed by bacterial clearance from uterine immune defence and or recontamination. If animals could not resolve the contamination, pathogenic bacteria in the uterus caused clinical endometritis distinguished by purulent secretions from the vagina of the infected animals (LeBlanc 2014) [7].

Metritis is often marked by an abnormal uterine secretion with local or sometimes involving systemic signs. Systemic or toxic metritis was identified by a watery and foul-smelling uterine outflow, usually accompanied by a sharp drop in milk production and fever. This could be life-threatening. Some predisposing factors for metritis include RFM (Retained Fetal Membrane), calving difficulties, fat calf cows, twins or dead parts and metabolic disorders. Metritis (MET) clinical endometritis (CE) and subclinical endometritis (SE) were common uterine diseases in dairy cows, associated with decreased reproductive performance and higher abatement rates (LeBlanc 2008; Sheldon *et al*., 2008 & Madoz *et al*., 2013) [8, 6, 9].

Risk factors associated with uterine infectious diseases mainly related to problems around parturition and subsequent negative energy balance (Giulidori *et al*., 2013 & de Boer, 2015) [10-11]. Metritis was originated by the number of etiological factors and bacterial pathogens were the most important among them. The most abundant uterine organisms isolated from cows with MET, CE, and in some cases SE, were *Escherichia coli* and *Truperella pyogens* (Prunner *et al*., 2014) [12]. Other commonly isolated

organisms were *Fusobacterium nucleatum*, *Fusobacterium necrophorum* and Prevotella (Foldi *et al*., 2006 & Sheldon *et al*., 2009) [13-14]. Wagener *et al*., (2015) [15] revealed that the postpartum uterine bacterial community is encompassed over 200 bacterial species.

Bacterial infections of the uterus were cured with antibiotics. In order to select an antimicrobial agent suitable for the treatment of a postpartum uterine infection, it is vital to know if the agent is sensitive to antibiotics. Antimicrobial resistance has unfolded as one of the most critical issues in human and veterinary medicine, the most important factor in the disclosure, selection and distribution of antimicrobial resistant bacteria (Takamtha *et al*., 2013) [16].

2. Materials and method

2.1 Sample collection

Total 53 samples comprising of uterine discharge and intra-vaginal swabs were collected from cattle (n=16), buffalo (n=14), and goats (n=23) located at various animal farms and government clinics in and around Parbhani city. The samples were collected from December 2018 to April 2019.

2.2 Intravaginal swab collection and bacterial isolation

The intra vaginal swab/discharges were collected from clinical cases of metritis of affected domesticated animals in to sterile vials and then were transferred to the laboratory on ice. The samples were collected on the basis of clinical signs and by observation of abnormal uterine discharge. The metritis cases were observed with red brown discharge with fetid smell while in some cases purulent vaginal discharge observed with only inflammation of endometrium. All the samples were then screened for isolation and identification of bacteria. Immediately after the samples were collected, the inoculums from swab were prepared by adding sterile nutrient broth to each sample and incubated at 37˚C for overnight. The test tubes expressing turbidity indicated the bacterial growth in the nutrient broth (Ejiofor *et al*., 2018) [17].

Each sample was inoculated for cultural examination on eosin methylene blue agar, nutrient agar, brain heart infusion agar and anaerobic agar. The inoculated plates and tubes were incubated at 37˚C by maintaining anaerobic and aerobic conditions for 24 to 48 hrs (Udhayavel *et al*., 2013) [18]. The anaerobic condition is maintained by placing the petri plates in an anaerobic jar of 3.5 L volume which contained catalyst chamber that facilitated reaction by eliminating oxygen and provided completely anaerobic atmosphere for the bacteria. The methylene blue strip used as an indicator that placed in a jar which automatically turned colourless under anaerobic conditions and it was proved that the jar was in anaerobic state. Then anaerobic gas pack was used which allowed the reaction that occurred in catalyst chamber. The sealed anaerobic jar then incubated at 37˚C for 24 hrs. The very next day plates were observed for the growth of bacteria on the basis of colony characteristics, morphology and biochemical test.

2.3 Antimicrobial susceptibility test

Sensitivity of bacterial isolates to various antibiotics was studied by agar diffusion method, using single disc of antibiotic by Moges *et al*., (2013) [19]. The Muller Hinton agar plates were prepared and for sterility checking kept for incubation at 37˚C overnight. The nutrient broth was inoculated with single isolated colony and tubes were incubated at 37˚C for 5 hours until a visible turbidity appeared. The turbidity was measured with 0.5 Mcfarland standards. Broth cultures were uniformly spread over Muller Hinton Agar plates. After 15 minutes of plate inoculation, antibiotic impregnated discs were applied to the surface of the inoculated plates with sterile forceps.

All discs were gently pressed down onto the agar plates with forceps to ensure complete contact with the agar surface; the plates were inverted and then incubated for 24 hours at 37˚C. The diameter of zone of inhibition was measured to the nearest millimetre using zone inhibition scale. The antibiotic sensitivity test for 26 *E. coli*, 8 *Enterococcus faecalis* and 6 *Acinetobacter lwoffii* isolates was carried out.

3. Results

From the 53 uterine discharge samples of metritis 26 isolates identified on the basis of morphological and cultural characteristics (Table 1) were of *E. coli*, 8 were of *Enterococcus* spp and 6 isolates of were *Acenetobacter lwoffii* and 6 samples showed no bacterial growth. The bacteria which were identified are presented in Table 2.

Table 1: Morphological and cultural characteristics of bacterial isolates

Sr.No.	Pathogen	Media used	Colony characteristics	Gram stain and morphology
1.	*Escherichia coli*	EMB agar	Metallic sheen coloured	Negative (Rod)
2.	*Enterococcus faecalis*	Nutrient agar	Smooth, cream/white	Positive (Cocci)
3	*Acinetobacter lwoffii*	Nutrient agar	Mucoid, pale yellow	Negative (Cocobacillary)

Table 2: Bacterial isolates from metritis of domesticated animals

Sr. No.	Species	Total no. of isolates	Percentage (%)
1.	*Escherichia coli*	26	49.05
2.	*Enterococcus faecalis*	08	15.09
3.	*Acinetbacter lwoffii*	06	11.32
4.	Untyped	07	13.20
5.	Bacterial sterile	06	11.32
Total No. of samples		53	99.98

The most commonly isolated bacteria was *E. coli* (49.05%) followed by *Enterococcus faecalis* (15.09%) and *Acinetobacter lwoffii* (11.32%).

The result of antimicrobial sensitivity test indicated that the all isolates of E. coli were resistant to oxytetracycline (88.46%), followed by Ampicillin (69.23%), ciprofloxacin (65.38%), ceftriaxone (61.53%), and norfloxacin (57.69%). The *Enterococcus faecalis* showed resistant to oxytetracycline (75%), ampicillin (75%) norfloxacin (50%). The isolates of *Acinetobacter lwoffii* showed resistance to oxytetracycline (83.33%), ampicillin (66.66%), and ceftriaxone (50%). The isolates of *E. coli* and *Acinetobacter lwoffii* were sensitive to chloramphenicol and enrofloxacin wehereas *Enterococcus faecalis* was susceptible to chloramphenicol (Figure 1, Figure 2 and Figure 3).

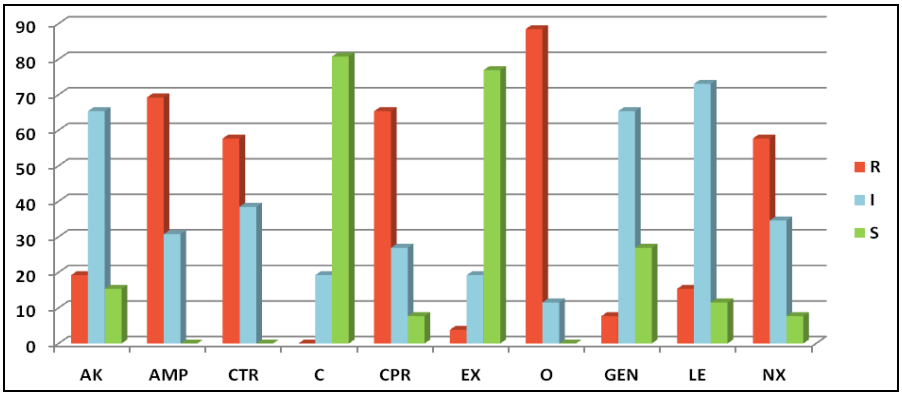

Fig 1: *In-vitro* antibiotic sensitivity pattern of *E. coli* isolated from metritis cases: AK= amikacin, AMP= ampicillin, CTR= ceftriaxone, C= chloramphenicol, CPR= ciprofloxacin, EX= enrofloxacin, O= oxytetracycline, GEN= gentamycin, levofloxacin and NX= norfloxacin.

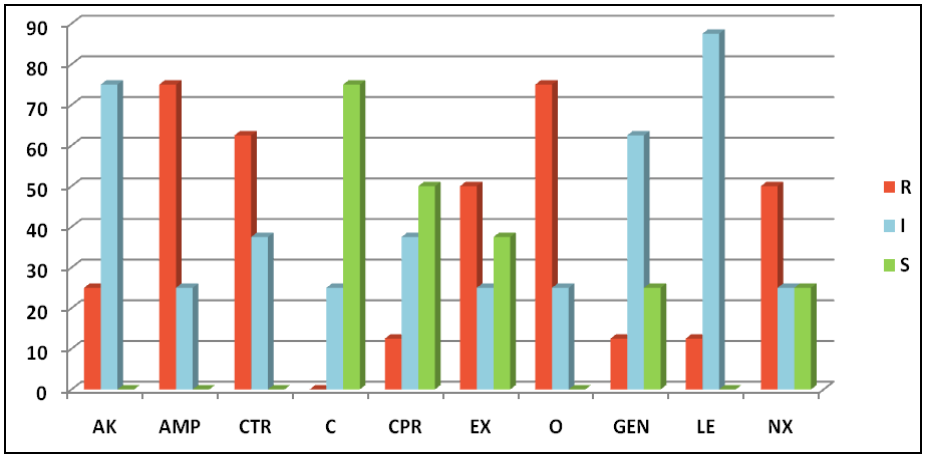

Fig 2: In-vitro antibiotic sensitivity pattern of Enterococcus faecalis isolated from metritis cases: AK= amikacin, AMP= ampicillin, CTR= ceftriaxone, C= chloramphenicol, CPR= ciprofloxacin, EX= enrofloxacin, O= oxytetracycline, GEN= gentamycin, LE= levofloxacin and NX= norfloxacin.

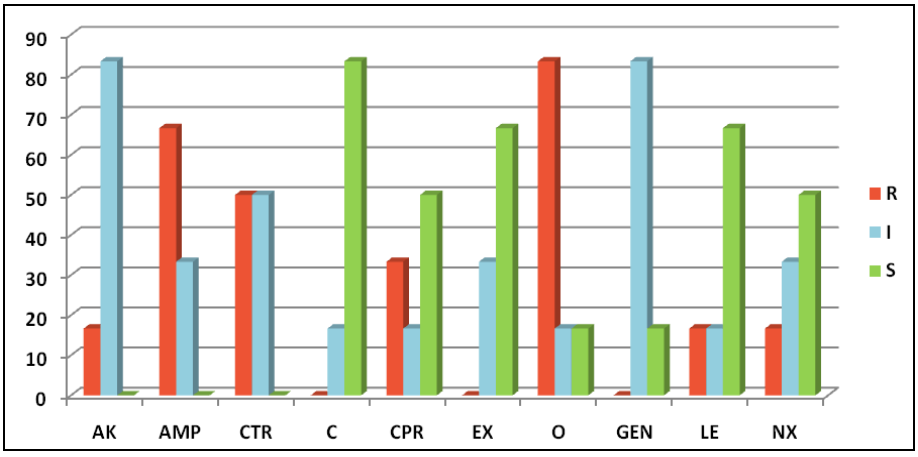

Fig 3: *In-vitro* antibiotic sensitivity pattern of *Acinetobacter lwoffii* isolated from metritis cases: AK= amikacin, AMP= ampicillin, CTR= ceftriaxone, C= chloramphenicol, CPR= ciprofloxacin, EX= enrofloxacin, O= oxytetracycline, GEN= gentamycin, LE= levofloxacin and NX= norfloxacin.

4. Discussion

In the present study at the time of sample collection animals were noted with different clinical signs that included anorexia, foul smelling purulent vaginal discharge, lethargy, depression, reduced milk yield and acute collapse. Physical examination revealed dehydration, distended uterus and rectal temperature was found to be more than 102.74°F. In few animals metritis cases were noted with frequent necrotic and malodorous discharges and secretions that contained remnants of fetal membranes. The clinical signs observed by Umamageswari *et al*., (2015) [20] in the cases of bovine endometritis were revealed that no palpable abnormalities found with yellowish, watery vaginal discharge which was included pus flakes. The clinical signs similar with present study observed by Sheldon *et al*.,(2008) [6] that in the uterine diseases purulent discharges were detected, while in metritis cases red vaginal discharge associated with rectal temperature >39.5°C and reduced milk yield was reported.

In this study the most frequently isolated bacteria were *E. coli* (49.05%) followed by *Enterococcus faecalis* (15.09%)

and *Acinetobacter lwoffii* (11.32%). According to many researchers (Moges *et al*., 2013; Takamatha *et al*., 2013; Azizunnesa 2011) [19,16,21] *E. coli* was the most commonly isolated bacteria in uterine disorders while some researchers have mentioned about the presence of Enterococcus and Acinetobacter spp in the endometritis and reproductive diseases (Wagener *et al*., 2015 and Wang *et al*., 2018) [15,22]. Earlier in a similar study on prevalence of bacteria isolated from clinical endometritis in dairy cows conducted by Takamatha *et al*., (2013) [16] observed that *Staphylococcus aureus* 28.2%, *Corynebacterium pyogens* 23.1%, *Acinetobacter calcoaceticus* 10.3% and *E. coli* 17.9% and they concluded that the most frequently isolated bacteria were *E. coli*, *Corynebacterium* spp and *Arcanobacterium pyogens*. In our study *Corynebacterium* spp and *Arcanobacterium pyogens* were not detected.

In the presnt study, from metritis cases of goats 12 *E. coli*, 5 *Enterococcus faecalis* and *1 Acinetobacter lwoffii* was isolated. From cow uterine discharge 7 *E. coli*, 3 *Enterococcus faecalis* and 1 *Acinetobacter lwoffii* was isolated while from Buffalo uterine swab 7 *E coli*, 4 *Acinetobacter lwoffii* were isolated but no *Enterococcus spp* could be isolated from buffalo.

Acinetobacter spp. and Enterococccus spp. were not observed commonly to cause metritis, but in this study, 6 *Acinetobacter lwoffii* were detected and 8 of *Enterococcus faecalis* isolates were obtained. The etiology of metritis and uterine diseases reported by researchers were not in agreement with the influence of Acinetobacter. *Acinetobacter lwoffii* are commensal of the body of animals and are associated with environment (Müller *et al*., 2014) [23]. Previously Nam *et al*., (2009) [24] reported *Acinetobacter lwoffii* involvement in bovine mastitis milk samples. The presence of *Acinetobacter lwoffii* in the metritis of the uterine samples might be due to breach of the uterine mucosa or otherwise environmental contamination. *Enterobacter faecalis* has been reported as the common vaginal microbiota in cow earlier by Wang *et al*., (2013) [22] which indicated that the bacteria might be involved in metritis cases due to overgrowth of pathogenic bacteria like *E. coli* and other bacteria like *Acinetobacter lwoffii* and *Enterobacter faecalis* which are opportunistic pathogens and may have invaded the uterus and caused metritis.

In one of the study on etiology of metritis was reported by Ordell *et al*., (2016) [25] where they got *Fusobacterium necrophorum*, Clostridium spp. and *Truperella pyogens* most influential bacteria which could not be detected in the present study.

The antibiogram of *E. coli* indicated that maximum resistance was observed in case of oxytetracycline (88.46%), followed by Ampicillin (69.23%), ciprofloxacin (65.38%), ceftriaxone (61.53%), and norfloxacin (57.69%) whereas high sensitivity observed in chloramphenicol (80.76%) and enrofloxacin (73.07%). In earlier study by Moges *et al*., (2013) [19], *E. coli* showed resistance to tetracycline (100%), gentamycin (40%), polymixin (100%), oxacillin (40%) and cefoxitin (100%). The variation in resistance reported might be due to uncontrolled use of that particular antibiotic in studied animal population.

Enterococcus faecalis showed resistant to oxytetracycline (75%), ampicillin (75%) norfloxacin (50%). Highl sensitivity to chloramphenicol (75%) and moderate sensitivity was observed to levofloxacin (87.5%), amikacin (75%), ceftriaxone (62.5%) and gentamycin (62%). Similar kind of results were observed by Sharma *et al*., (2017) [26], they reported that gram positive aerobic bacteria were sensitive to levofloxacin and enrofloxacin (94.08 and 93.88%), respectively.

Antibiogram of *Acinetobacter lwoffii* indicated that high sensitivity observed to chloramphenicol (83.33%), enrofloxacin (66.66%) and levofloxacin (66.66%). Maximum resistance was observed to oxytetracycline (83.33%), ampicillin (66.66%), and ceftriaxone (50%). Contraindicatory results were reported by Constantiniu *et al*., (2004) [27] who have found slightly different results in which 16.6 % strains of Acinetobacter were sensitive to ampicillin and 25% to ceftriaxone and gentamycin while Sharma *et al*., (2017) [26] observed the similar degree of antimicrobial resistance in gram negative bacteria isolated from uterine discharge after parturition. They reported maximum resistance in ampicillin (60.72%), amoxicillin (57.14%) and oxytetracycline (46.23%).

While in 2013, antimicrobial susceptibility in postpartum dairy cows studied by Takamatha *et al*., (2013) [16] found that gentamicin (91%), cephalexin (77%) and amoxicillin (74%) most susceptible antibiotics while oxytetracycline (43%), streptomycin (25%) and sulfamethoxazole-trimethoprim (27%) were the most resistant antibiotics. In gram negative bacteria oxytetracycline (41%), amoxicillin (26%), ampicillin (24%) and streptomycin (24%) were the most resistant.

Different factors have emerged for the development of antimicrobial resistance, continuous and indiscriminate uses of antibiotics have been resulted into the development of reduced efficacy of antibiotics against pathogens. The enzymes engaged in biosynthesis of the cell wall, as well as the synthesis of nucleic acids and metabolites, served as an antibiotic immediate goal. Structural changes in these enzymes were associated with the resistance mechanism. The enzymatic modifications of the structural elements affected by antibiotics were associated with another mechanism. The main reasons for Gram-positive bacteria developed resistance to β-lactam antibiotics included mutations in native PBPs, their hyperproduction and the synthesis of new PBPs that were not sensitive to β-lactam inhibition (Nikolaidis *et al*., 2014) [28].

The main mechanism behind bacterial resistance to chloramphenicol was the production of chloramphenicol acetyltransferases (CATs) which prevented antibiotic molecules from binding to the ribosome (Egorov *et al*., 2018) [29].

5. Conclusion

This study revealed updated information of pathogens causing metritis and it is found that *E. coli* was the commonly isolated bacteria in metritis of domesticated animals. Chloramphenicol was the most effective antibiotic and oxytetracycline was the most resistant for *E. coli*, *Enterococcus faecalis* and *Arcanoacterium lwoffii*. Metritis is one of the frequently occurred reproductive diseases in domesticated animals and responsible for the huge economic losses in the livestock industry so it is crucial to plan the preventive measures and control the disease.

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