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Hematobiochemical alterations and antimicrobial resistance profiles of uterine pathogens in postpartum metritic crossbred cows

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Abstract

A Post-partum metritis (PPM) is one of the major reproductive disorders causes a significant economic loss to the dairy farmers due to reduced fertility, impaired milk production, increased culling rates, and higher therapeutic expenses. The present study was designed to analyse the changes in hematological and biochemical profiles, identify and isolate the uterine bacterial pathogens, and detect antimicrobial resistance patterns of the isolates in the post-partum crossbred cows suffered with metritis. A total of 24 crossbred cows were divided into two groups as Group 1 (healthy post-partum cows, n = 12) and Group 2 (cows with clinically diagnosed metritis, n = 12). Blood samples were collected and analyzed for the hematological and biochemical parameters. Uterine samples were collected for bacterial study. Isolation and identification of bacteria were done with the standard cultural, morphological, and biochemical techniques. Antibiotic sensitivity test was conducted with use of the disc diffusion method following CLSI guidelines. Cows infected with metritis had shown a significant reduction of hemoglobin concentration ($p < 0.01$) and quantitatively reduced glucose and albumin concentrations indicated that the animals suffered with systemic inflammation and metabolic stress. *Escherichia coli*, *Enterobacter spp.*, and *Staphylococcus spp.* were the predominant pathogens isolated from metritic cows, especially in animals with mixed infections. Antibiogram profiles revealed that complete resistance to amoxicillin-cloxacillin and high resistance to ampicillin, ceftriaxone, oxytetracycline, and ciprofloxacin were observed. Gentamicin and amikacin had the highest sensitivity rates. PPM was associated with hematometabolic alterations and polymicrobial uterine infections indicated by extensive antibiotic resistance. Regular bacterial culture test and selected antimicrobial therapy, and suitable metabolic support are important for effective management of PPM and improving the reproductive outcomes in dairy herds. Therefore, an integrated approach is required with use of strategic antibiotics, metabolic profiling, and improved postpartum management which in turn can significantly reduce the incidence and impact of post-partum metritis in dairy herds, thereby improving animal welfare and farm productivity.

Key words: *Post-partum metritis, dairy cow, hematology, uterine pathogens, antibiotic resistance, E. coli, gentamicin, amikacin*

Introduction

Post-partum metritis is one of the significant reproductive disorders affecting the dairy cows, leads to considerable economic losses due to reduced fertility, impaired milk production, higher culling rates, and higher therapeutic costs (Adnane and Chapwanya, 2024). PPM is characterized by inflammation of the endometrium and is often associated with systemic illness like fever, anorexia, and reduced milk yield within the first 21 days of post-partum (Bazzano et al., 2022). The etiology of PPM is multifactorial with involvement of bacterial infections, metabolic stress, and immune suppression during the periparturient period. PPM is primarily

associated with bacterial contamination of the uterine lumen during and after calving, specifically in animals with dystocia, retained fetal membranes, or unhygienic calving conditions (Adnane and Chapwanya, 2024).

PPM is caused by invasion and colonization of the uterus by opportunistic and pathogenic bacteria which in turn impaired the uterine involution and delayed resumption of ovarian cyclicity (Shi *et al*., 2025). Bacterial species like *E. coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and anaerobes like *Prevotella* spp. are commonly isolated from the metritic uterus (Sayed *et al.,* 2024). Similarly, bacterial pathogens such as *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.*, and *Staphylococcus spp.* were isolated from PPM affected cows (Poit *et al.,* 2024). These pathogens exploit the immunosuppressed state of postpartum cows leads to persistent uterine infections and compromised reproductive performances. Antimicrobial resistance prevalance among these uterine pathogens has further complicated with treatment protocols, therefore, periodic evaluation of antibiotic sensitivity patterns is required to design the effective therapeutic protocols (Paiano and Baruselli, 2022).

Diagnosis of PPM is by use of clinical examination, hematological and biochemical profiling, and microbiological culture of uterine contents (Fukushima *et al.,* 2020). Haematological and biochemical parameters serve as the valuable markers to assess the systemic as well as severity of the infection. Hematological parameters such as hemoglobin, red blood cell counts, and white blood cell counts are often altered in the PPM infected cows, indicating the systemic inflammatory response (Premi *et al.,* 2021). Biochemical parameters such as glucose, total protein, albumin, and liver functional enzymes provide about the metabolic disturbances related with PPM (Barcarolo *et al.,* 2024). Similarly, alterations in calcium (hypoglycaemia), albumin (hypoalbuminemia), urea (hyper uraemia) and creatinine (hyper creatinine) have been associated with immune dysfunction and hepatic stress during the peri and post parturient period in dairy cows (Fukushima *et al.,* 2020; Cheng *et al*., 2022). Further, antibiotic sensitivity test (ABST) is a critical for assess the effective treatment protocols as antibiotic resistance is a growing concern among the uterine pathogens (Gajic *et al.,* 2022).

Even after thorough understanding about PPM, treatment approach and it management are challenging due to the development of multidrug-resistant bacterial strains in the uterus (Pereira *et al.,* 2025). The inappropriate use of antibiotics has created severe antibiotic resistance; therefore, evidence-based treatment protocols formulation is required (Liu *et al*., 2025). Furthermore, the interaction between the metabolic imbalances such as hypoglycaemia and hypocalcemia, and immune dysfunction need to be understand for comprehensive therapeutic approaches to treat PPM (Cheng *et al*., 2022). Therefore, understand these metabolic, biochemical and uterine pathogenic profiles is required for proper diagnosis, prognosis, and treatment, and supportive care of cows with PPM.

This study was designed to estimate the hematological, biochemical, and microbiological profiles of crossbred cows affected with PPM, with a focus on isolation and identification of prevalent aerobic uterine bacterial pathogens and their antibiotic resistance patterns in post-partum crossbred cows suffered with metritis, and evaluate their antibiogram profiles, and correlate them with haematological and serum biochemical parameters. Comparison of these findings with healthy postpartum cows, the study will provide significant diagnostic markers and recommend rational antibiotic usage to manage PPM more effectively under field conditions in tropical regions. These research findings will support to develop the targeted treatment strategies which in turn improve the reproductive performances and herd productivity.

Materials and Methods

Study location and animals

This study was conducted at the Veterinary Clinical Complex, Obstetrics Unit, TANUVAS-Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India. A total of 24 crossbred cows in the early postpartum period were selected. These animals were presented to the clinic with the different reproductive histories. Based on the clinical examination and diagnostic criteria, animals were divided into two groups:

Group 1 (Control group): Consisted of 12 clinically healthy post-partum crossbred cows with no signs of uterine infection.

Group 2 (PPM group): Consisted of 12 crossbred cows diagnosed with post-partum metritis (PPM) based on clinical signs and diagnostic evaluation.

Diagnosis of PPM was confirmed by thorough **general physical examination, rectal palpation**, and **vaginal inspection**. Affected cows presented with fetid uterine discharge, uterine enlargement, and systemic signs such as elevated body temperature. The study protocol was approved by the institutional ethics committee.

Sample collection

Blood sampling

Blood samples (10 mL) were collected aseptically from the jugular vein with use of a sterile 18-gauge needle and vacutainer tubes. Blood samples were divided into: EDTA tubes for **haematological analysis**, and clot activator tubes for **serum biochemical analysis**. The samples were immediately transported on ice to the laboratory and processed within 2 h of collection. Serum was separated by centrifugation at 3000 rpm for 15 min at 4°C and stored at −20°C until analysis (Radostits *et al.,* 2000).

Uterine swab collection

Sterile vaginal specula were used to expose the cervix, and double-guarded sterile swabs were introduced through trans-cervically into the uterus to collect the uterine discharge. **Uterine swabs** were collected aseptically from both the groups with use of sterile swabs, transported in Amies transport medium, and processed within 2 h for microbiological analysis (Quinn *et al.,* 2011). This was done to avoid the contamination. Swabs were placed in a sterile nutrient broth for transport and cultured within 1 h at the Microbiology Laboratory, TANUVAS-VCRI, Orathanadu.

Bacterial isolation and identification

Primary enrichment and culture

The uterine swab samples were initially inoculated into the **nutrient broth** and **buffered peptone water and** followed by incubation at 37°C for 16–24 h under the aerobic conditions (Louise *et al.,* 2008). These cultures were then sub-cultured onto the solid selective and differential media including: **MacConkey agar (**Enterobacteriaceae like *E. coli*, *Klebsiella*)**, Eosin Methylene Blue agar (EMB;** differential identification of lactose fermenters**), Mannitol Salt Agar (MSA;** *Staphylococcus* spp.**), Nutrient Agar, and Xylose Lysine Deoxycholate (XLD) agar** (*Proteus* spp.; Cruickshank *et al.,* 1975). All the media and reagents were procured from HiMedia Laboratories, Mumbai, India.

Identification of bacterial species

Morphological characteristics such as colony appearance, pigmentation, and hemolysis patterns were observed. Each isolate was subjected to **Gram's staining**, size, and arrangement (Cruickshank *et al.,* 1975), **motility test** in the semisolid agar media, **biochemical characterization** with the use of the standard **IMViC tests**, **oxidase**, **catalase**, **TSI**, and **urease** tests as per the methods of Collee *et al.* (1996) and Louise *et al.* (2008). Additionally, the **API 20E biochemical test kit** (HiMedia KB001, Mumbai, India) was used for confirmatory identification of *Enterobacteriaceae* members (Nazarzadeh *et al.,* 2014).

Hematological and biochemical analysis

Haematological parameters such as **hemoglobin (Hb)**, **total RBC**, and **WBC counts** were measured with use of an automated hematology analyzer (Mindray BC-2800 Vet; Kaneko *et al.,* 1997). Biochemical parameters such as **serum glucose**, **total protein**, **albumin**, **urea (BUN)**, **creatinine**, **ALT**, **AST**, **ALP**, **calcium**, **phosphorus**, and **magnesium** were estimated with use of a semi-automated biochemistry analyzer (Erba Chem-5 Plus V2) using standard diagnostic kits (Aspen Diagnostics, India). The reference ranges used for the interpretation were based on

published sources (Kaneko *et al.,* 1997; Radostits *et al.,* 2000; López *et al.,* 2023).

Antibiotic sensitivity test (ABST)

The antibiotic susceptibility of isolated uterine bacterial pathogens was tested by use of the **Kirby-Bauer disc diffusion method** on Mueller-Hinton agar as per CLSI guidelines (CLSI, 2021). The antibiotic discs were selected based on their common therapeutic use in veterinary practice, representation of major antibiotic classes (β-lactams, tetracyclines, fluoroquinolones, aminoglycosides, and sulphonamides), clinical relevance, and recommendations by CLSI for antimicrobial susceptibility testing. The selection also reflected regional usage patterns and aimed to assess broad- and narrow-spectrum activity against both Gram-positive and Gram-negative bacteria. The following antibiotic discs used included: **Amoxicillin + Cloxacillin (β-lactams), Ampicillin, Ceftriaxone, Oxytetracycline, Ciprofloxacin (Fluoroquinolones), Gentamicin (Aminoglycosides), Amikacin (Aminoglycosides), Enrofloxacin (Fluoroquinolones), and Cotrimoxazole.** Zones of inhibition were measured and categorized as *sensitive*, *intermediate*, or *resistant* based on established interpretive criteria as per CLSI guidelines.

Statistical analysis

Data were analyzed using SAS, Version 9.3.1 (SAS Institute, Inc., Cary, NC, 2011). Continuous variables (hematological/biochemical parameters) were compared via independent t-tests or Mann-Whitney U tests (non-normal data). Categorical data (ABST results) were expressed as percentages and analyzed using Chi-square tests. $p < 0.05$ was considered significant (Snedecor and Cochran, 1989).

Results

The study was designed to analyse the hematological, biochemical, and microbiological parameters in postpartum crossbred cows suffered with metritis as compared to healthy controls. Additionally, antimicrobial susceptibility patterns of uterine pathogens were analyzed to determine the therapeutic strategies.

Hematological parameters

The comparative hematological parameters between the normal postpartum cows (control group) and post-partum metritis affected cows revealed the significant differences (Table 1). A **highly significant lower hemoglobin concentration** was observed in PPM cows (7.10 ± 1.30 g/dL) as compared to control animals (9.29 ± 2.62 g/dL; $F = 6.70$; $p < 0.01$) indicated that there was possible anemia due to chronic infection (Radostits *et al.* 2000). This result indicated that PPM affected cows had a systemic inflammatory stress associated with chronic infection and compromised erythropoiesis. Although the **mean RBCs** and WBCs were quantitatively lower in the PPM affected cows than those in controls, these differences were not statistically significant ($p > 0.05$). However, the decreasing trend in RBCs and WBCs indicated mild anemia and immunosuppression secondary to the systemic infection and metabolic imbalance as reported by Lv *et al*. (2024).

Table 1: Hematological parameters of normal postpartum cows and postpartum metritis cows

Sl. No.	Parameters	Values		F-Value	Significance
		Control	PPM		
1.	Hb (Hb) (g/dl)	9.29± 2.62	7.10± 1.30	6.70	** (p<0.01)
2.	RBC ($\times 10^6/\mu L$)	5.30± 1.79	4.71± 0.73	1.12	NS
3.	WBC ($\times 10^3/\mu L$)	8.90± 3.94	7.36± 1.74	1.54	NS

NS = Not significant; ** = Highly significant

Serum biochemical profile

Non-significant differences were observed in the serum biochemical parameters between the PPM affected and unaffected cows; but numerical trends were observed (Table 2). Biochemical profiles revealed that serum glucose concentrations were lower in PPM -affected cows (44.80 ± 21.81 mg/dL) than in the unaffected control cows (63.17 ± 9.79 mg/dL); however, non-significant difference was observed (F = 2.62). The hypoglycemic tendency in PPM affected cows was due to higher glucose utilization for milk synthesis and immune function during the early lactation period in association with decreased gluconeogenic activity due to hepatic stress (Barcarolo *et al.,* 2024; Cheng *et al*., 2022).

The serum albumin was also lower in the PPM group (2.90 ± 0.48 g/dL) as compared to unaffected control animals (3.22 ± 0.54 g/dL) indicated that there was a potential hepatic dysfunction and a negative acute-phase response in PPM cows. In contrast, total serum protein levels were relatively stable in both the groups. Urea (BUN) and serum creatinine levels were remained within physiological reference ranges (Andjelić *et al.,* 2022), although creatinine was slightly elevated in the PPM cows indicated that altered renal function or muscle catabolism. Calcium and phosphorus concentrations were slightly higher in the PPM affected cows due to hormonal fluctuations after calving (Mun *et al.,* 2019; Mota *et al.,* 2023).

Serum ALT, AST, and ALP activities are indicators of hepatic function and had showed no significant differences between the affected and unaffected groups; however, ALT was numerically lower in PPM cows indicated that subclinical liver dysfunction due to the systemic inflammatory burden (Kaneko *et al.,* 1997).

Table 2: Biochemical parameters of normal postpartum cows and postpartum metritis cows

Sl. No.	Parameters	Experimental groups		F-Value	Significance
		Control	PPM		
1.	Glucose(mg/dl)	63.17±9.79	44.80± 21.81	2.62	NS
2.	Total Protein (g/dl)	5.96±1.38	6.09± 0.97	0.74	NS
3.	Albumin (g/dl)	3.22±0.54	2.90± 0.48	2.16	NS
4.	BUN (mg/dl)	65.59±26.62	55.43±39.35	0.550	NS
5.	Creatinine (mg/dl)	23.16±5.14	28.19±4.10	0.060	NS
6.	AST (U/L)	70.36±33.27	72.91±43.69	0.026	NS
7.	ALP (U/L)	90.18±17.72	18.18±11.28	1.98	NS
8.	ALT (U/L)	49.42±24.30	33.67±16.14	3.50	NS
9.	Calcium (mg/dl)	9.85±1.79	11.10±1.20	0.375	NS
10.	Phosphorus (mg/dl)	5.68±2.27	6.59±2.42	0.908	NS
11.	Magnesium (mg/dl)	3.33±1.99	2.60±0.87	0.55	NS

BUN: Blood Urea Nitrogen, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase ALT: Alanine aminotransferase

Bacterial isolates from uterine swabs

The bacterial culture and identification from the uterine swabs revealed polymicrobial infections. In PPM affected cows, *E. coli* was the most frequently isolated organism and identified in 58% of samples as compared to control animals **(33%)**. Other pathogens were also prevalent in PPM cows such as *Enterobacter* spp. (42%), *Staphylococcus* spp. (33%), *Pseudomonas* spp. (17%), *Klebsiella* spp. (8%), and *Bacillus* spp. (8%). Interestingly, *Proteus* spp. was not isolated from any PPM cases, but present in 17% of controls. In contrast, uterine swabs from the normal postpartum cows showed *E. coli* and *Enterobacter* spp. in 33% of samples, along with isolates of *Bacillus* spp. (25%), *Proteus* spp. (17%), and *Staphylococcus* spp. (8%) (Figure 1; Table 3).

Mixed bacterial infections were also reported particularly in the PPM group where the multiple combinations such as *E. coli* + *Enterobacter spp.*, and *E. coli* + *Staphylococcus spp.* were frequently observed. This polymicrobial nature of uterine infection complicates the treatment; therefore, precise microbial identification and targeted therapy are important in the treatment of PPM (Cruickshank *et al.,* 1975; Collee *et al.,* 1996).

Colony morphology **Biochemical characters**

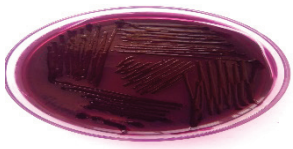


Plate 1. *Klebsiella*

Plate 1a. *Klebsiella*

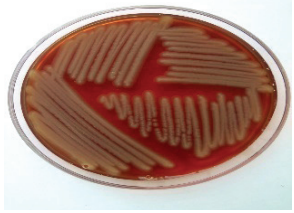


Plate 2. *Bacillus*

Plate 2a. *Bacillus*

Plate 3. Mucoid colony Enterobactor aerogenes

Plate 3a. *Enterobactor*

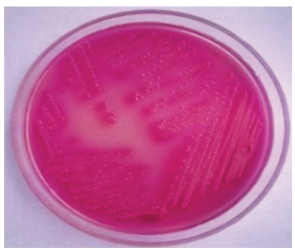


Plate 4. *E. coli*– MacConkey agar

Plate 4a. *E. coli*

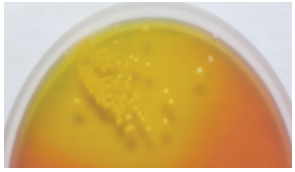


Plate 5. *Staphylococcus aureus*- Manitaol salt agar

Plate 5. *Staphylococcus aureus*

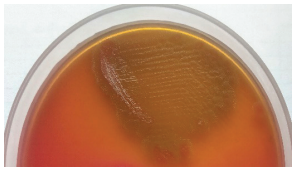


Plate 6. Pseudomonas spp. – blood agar

Plate 6. Pseudomonas spp.

Plate 7. Proteus spp.

Plate 7a. Proteus spp.

Figure 1. Morphological and biochemical characteristics of bacterial isolates

Table 3: Bacterial agents isolated individually and in combination with other bacterial spp.

Sl. No.	Bacterial isolates	Normal postpartum cows (n=12)		Postpartum metritis cows (n=12)	
		No.	%	No.	%
1	*E. coli*	4	33	7	58
2	*Enterobacter spp*	4	33	5	42
3	*Klebsiella spp.*	2	17	1	8
4	*Proteus spp.*	2	17	-	-
5	*Pseudomonas spp.*	2	17	2	17
6	*Bacillus spp.*	3	25	1	8
7	*Staphylococcus spp.*	1	8	4	33

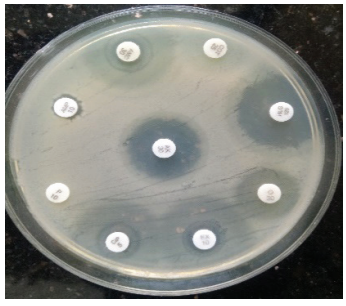
Antibiotic sensitivity profile

The antibiotic sensitivity test (ABST) revealed that a high level of multidrug resistance among the bacterial isolates from both the control and PPM affected groups. All isolates (100%) from the PPM affected group were resistant to the Amoxicillin + Cloxacillin and 92% to Ampicillin. Higher resistance was also observed for Ceftriaxone (75%), Oxytetracycline (75%) and Ciprofloxacin (75%) indicated that there was reduced efficacy of these commonly used antibiotics. On the other hand, Amikacin and Gentamicin had showed the highest sensitivity rates (42% each) and making them potential therapeutic choices for treatment of PPM. However, caution must be exercised with Amikacin because its nephrotoxic potential requires concurrent monitoring of renal parameters such as BUN and creatinine (Tsige, 2017). Unaffected control cows also showed higher resistance to Amoxicillin/Cloxacillin (84%) suggested that there was potential misuse or environmental antibiotic selection pressure (Tsige, 2017). In unaffected control cows, a similar trend was observed with higher resistance to the beta-lactam antibiotics and moderate sensitivity to the Enrofloxacin, Gentamicin, and Cotrimoxazole (Figure 2; Table 4 and Table 5).

The present study revealed that significant reduction in hemoglobin concentration in PPM affected cows was due to systemic inflammatory impact and anemia.

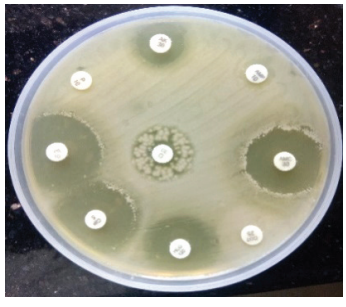
Quantitatively, decreased glucose and albumin levels in PPM affected cows indicated that there was energy deficit, hepatic response and metabolic strain. Multidrug resistance was observed among the uterine isolates; Amikacin and Gentamicin had showed the relative efficacy. *E. coli* was the predominant pathogen in PPM affected cows; often in mixed infections with Enterobacter and Staphylococcus Spp. Amoxicillin/Cloxacillin needs to avoided in the therapeutic due to 100% resistance. In Amikacin therapy, renal function need to be monitored. These research findings highlighted that there is need for targeted antibiotic therapy and nutritional management in postpartum cows to mitigate metritis-associated complications in the crossbred cows.

Zone of inhibition around Amikacin (size=18 mm) and zone of resistance around Ampicillin (size=6 mm)



Zone of inhibition around Enrofloxacin (size = 30 mm) and zone of resistance around Cotrimaxazole (size = 6 mm)

Zone of partially sensitive to Amikacin (size = 18 mm) and complete sensitive to Ciprofloxacin (size = 22.5 mm)



Zone of partially sensitive to Enrofloxacin (size = 15 mm)and complete resistance to Oxytetracycline (size = 6 mm)

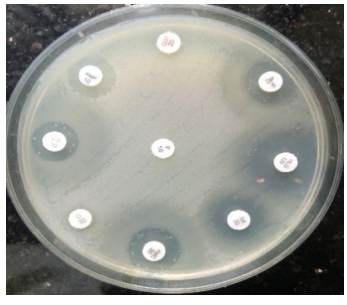


Figure 2. ABST plates with different antibiotics and their zones of sensitivity, resistance and intermediate

Table 4: ABST pattern in postpartum metritis samples

Antibiotics	Resistant	Sensitive	Intermediate
Amoxicillin and Cloxacillin	12(100.0%)	NIL (0%)	- NIL(0%)
Ceftriaxone	9(75.0%)	2(17.0%)	1(8.0%)
Gentamicin	3(25.0%)	5(42.0%)	4(33.0%)
Enrofloxacin	8(66%)	2(17%)	2(17.0%)
Amikacin	3(25.0%)	5(42.0%)	4(33.0%)
Oxytetracycline	9(75.0%)	1(8.0%)	2(17.0%)
Ciprofloxacin	9(75.0%)	1(8.0%)	2(17.0%)
Ampicillin	11(92.0%)	NIL (0%)	1(8.0%)
Cotrimaxazole	7(58.0%)	2(17.0%)	3(25.0%)

Table 5: ABST pattern in postpartum control samples

Antibiotics	Resistant	Sensitive	Intermediate
Amoxicillin and Cloxacillin	10(84%)	1(8.0%)	1(8.0%)
Ceftriaxone	9(75.0%)	2(17.0%)	1(8.0%)
Gentamicin	2(17.0%)	5(42.0%)	5(42.0%)
Enrofloxacin	6(50.0%)	3(25.0%)	3(25.0%)
Amikacin	3(25.0%)	4(33.0%)	5(42.0%)
Oxytetracycline	9(75.0%)	2(17.0%)	1(8.0%)
Ciprofloxacin	5(42.0%)	5(42.0%)	2(17.0%)
Ampicillin	10(84%)	1(8.0%)	1(8.0%)
Cotrimaxazole	7(58.0%)	5 (42.0%)	Nil (0%)

Discussion

The result of the present study revealed that the hematological, biochemical, and microbiological parameters were altered in the crossbred cows suffered with PPM as compared to healthy normal cows. PPM remains one of the most significant reproductive disorders in the dairy cows particularly during the early puerperal period due to its detrimental effects on uterine health, milk production, and overall fertility (Várhidi *et al*., 2024). The present study designed to analyse the haematological, biochemical, and bacteriological profiles between healthy postpartum cows and those affected with PPM, alongside the assessment of antimicrobial resistance patterns in isolated uterine pathogens.

The significantly lower hemoglobin concentration in PPM-affected cows due to an ongoing inflammatory or infectious process, and oxidative stress associated with uterine infection leads to anemia of inflammation (Radostits *et al.,* 2000). The reduction of hemoglobin might be due to chronic bacterial infections that cause iron sequestration, reduced erythropoiesis, or even blood loss through lochial discharges as similar to the previous findings (Lv *et al.,* 2024). Anemia in metritis impair the oxygen delivery to tissues which in turn exacerbating the immune dysfunction during the critical postpartum period (Cheng *et al*., 2022). Although RBC and WBC counts were not statistically different between the groups, the quantitatively lower values were observed in the PPM group suggested that immunosuppression or systemic metabolic strain aligns with the previous reports of immunosuppression in metritic cattle (Barcarolo *et al.,* 2024) and observed in periparturient cows (Cheng *et al*., 2022).

Glucose metabolism plays an important role in the postpartum period as an energy supplement. In this study, cows suffered with PPM exhibited lower serum glucose concentrations as reported earlier by Barcarolo *et al.* (2024) and López *et al.* (2023) who observed that hypoglycaemia in cows with uterine infections. The hypoglycemic tendency due to higher glucose demand for milk synthesis combined with insufficient hepatic gluconeogenesis and immune responses. This predisposed the cows to secondary infections due to impaired leukocyte function (Cheng *et al*., 2022), which was exacerbated by the systemic infections. Although serum total protein levels were stable in both the groups, albumin concentrations were reduced in the PPM cows. As a negative acute-phase protein, decreased albumin levels signify the hepatic response to inflammation (Kaneko *et al.,* 1997). Albumin and BUN were quantitatively lower in PPM affected cows indicated the protein catabolism and energy diversion toward the inflammation resolution (Andjelić *et al.,* 2022). Likewise, the non-significant but elevated creatinine levels due to muscle catabolism or mild renal stress as reported by Andjelić *et al.* (2022). Concentrations of calcium and phosphorus were within physiological limits; however, they were quantitatively higher in PPM affected cows. This could be due to compensatory hormonal regulation in response to early lactation demands and due to subclinical mineral imbalances contributing to uterine inertia and susceptibility to metritis (Mun *et al.,* 2019;

Tsige *et al.,* 2017) or mineral mobilization from bones (Mun *et al.,* 2019). Serum liver enzymes (ALT, AST, ALP) were remained within the broad reference ranges defined for the cattle; however, ALT and ALP levels were slightly lower in PPM affected cows due to impaired hepatic function or reduced enzyme synthesis under stress conditions as reported by Kaneko *et al.* (1997).

The uterine bacteriology of cows with PPM revealed that a high incidence of *E. coli***,** Enterobacter spp., and Staphylococcus spp., with *E. coli* being the most frequently isolated organism (58%) in PPM as compared to control cows (33%), corroborating its role as a key pathogen in bovine metritis (Adnane and Chapwanya*,* 2024). These findings were aligned with earlier works by Sayed *et al.* (2024) and Paiano and Baruselli (2022), who reported that *E. coli* serves as a primary uterine contaminant and a facilitator of secondary bacterial infections by other pathogens through endometrial damage. Enterobacter spp. (42%) and Staphylococcus spp. (33%) were also prominent suggested that the polymicrobial infections complicated the uterine recovery (Poit *et al.,* 2024). Interestingly, Proteus spp., often implicated in the mixed infections and were absent in the PPM group but found in healthy controls, suggested that their mere presence was not sufficient to cause metritis, and host factors likely modulate the disease expression and also due to the geographic or herd-specific microbial ecology (Tsige *et al.,* 2017).

The antimicrobial susceptibility profiles indicated that a disturbing trend of multidrug resistance was observed. Total resistance to Amoxicillin + Cloxacillin (100%) and high resistance to Ampicillin (92%), Ceftriaxone, and Oxytetracycline among the isolates from PPM cows was alarming and indicative of indiscriminate use of antibiotics in the field conditions (Tsige *et al.,* 2017), underscores the futility of β-lactams for empirical therapy. Similar resistance trends were reported in Indian dairy herds (Nazarzadeh *et al.,* 2014). Amikacin and Gentamicin revealed the highest sensitivity rates (42%) indicated their potential utility in the targeted therapy and supporting their use as the first-line of treatments. However, caution is warranted due to the nephrotoxic potential of aminoglycosides (elevated BUN), particularly in dehydrated or metabolically compromised animals; therefore, required concurrent renal function monitoring (Radostits *et al.,* 2000). Resistance to Ceftriaxone (75%) and Ciprofloxacin (75%) indicated emerging multidrug resistance due to indiscriminate use of antibiotics in the livestock (WHO, 2017).

This resistance pattern showed the importance of rational antibiotic usage and the integration of regular antibiogram surveillance into the herd health programs. Irregular use of antibiotics leads to treatment failures and further antibiotic resistance. The results of this study revealed that the PPM was due to multifactorial etiologies. The interaction between metabolic status, uterine microbial colonization, and immune function appeared a central key to the disease process. Nutritional and mineral imbalances (hypocalcemia and hypoglycaemia), exacerbated the condition, impaired the uterine contractility, and delayed the involution (Radostits *et al.,* 2000; Mota *et al.,* 2023). Moreover, identification of polymicrobial infections in PPM affected cows with combinations of *E. coli***,** Klebsiella, Staphylococcus, and Pseudomonas, supports the need for multi-pronged treatment strategies including uterine lavage, systemic antibiotics based on culture sensitivity, and supportive metabolic therapy.

In summary, cows affected by PPM exhibited signs of systemic inflammatory response, metabolic stress, and hepatic strain, indicated in their hematobiochemical profiles. The uterine microflora was diverse with *E. coli* was predominance and high levels of antimicrobial resistance. The findings indicated that there is need for proactive monitoring, evidence-based treatment, and improved periparturient management to enhance the uterine health and reproductive performance in dairy herds.

Targeted antimicrobial therapy needs to be focussed. Given the resistance profiles, Amikacin/Gentamicin should be prioritized for PPM, while avoiding β-lactams. Adjunctive therapies (NSAIDs, uterine lavage) may reduce reliance on antibiotics (Barcarolo *et al.,* 2024). Nutritional and metabolic support needs to be improved. Glucose supplementation could mitigate immunosuppression. Calcium and phosphorus homeostasis must be monitored to prevent postpartum hypocalcemia (Liu *et al.,* 2022).

Herd-level interventions are required. Biosecurity measures like clean calving environments may reduce bacterial load. Antibiotic stewardship programs are critical to curb resistance (WHO, 2017).

Conclusion

The present study concluded that PPM in crossbred dairy cows was associated with hematological alterations, metabolic imbalances, and a high prevalence of multidrug-resistant uterine bacterial pathogens. A significant reduction in hemoglobin levels, along with quantitatively lower glucose and albumin concentrations, suggested that the presence of systemic inflammation and energy deficits, which compromised the immune function and delayed uterine involution. Bacteriological examination revealed that *E. coli*, *Enterobacter spp.*, and *Staphylococcus spp.* were the predominant pathogens in the PPM cows and often associated in mixed infections. The antimicrobial susceptibility patterns highlighted a concerning level of resistance to commonly used antibiotics such as ampicillin, amoxicillin, oxytetracycline, and ceftriaxone. In contrast, gentamicin and amikacin exhibited the highest efficacy, making them suitable candidates for therapeutic intervention, albeit with caution due to potential nephrotoxicity. These findings suggested the importance of routine uterine culture and sensitivity testing before initiating the antibiotic therapy. They also pointed to the need for better nutritional and metabolic support during the periparturient period to enhance the disease resistance and reproductive performances. An integrated approach involving the strategic antibiotic use, metabolic profiling, and improved postpartum management can significantly reduce the incidence and impact of PPM in the dairy herds, thereby improving the animal welfare and the farm productivity. However, this study had the following limitations: small sample size (n=12/group) limited the statistical power; larger cohorts are needed, long-term fertility outcomes were not assessed; future studies should evaluate conception rates post-treatment and molecular characterization (PCR for virulence genes) could enhance the pathogen profiling.

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