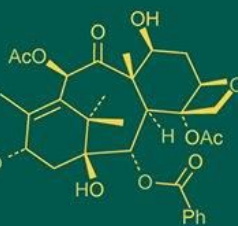
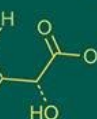
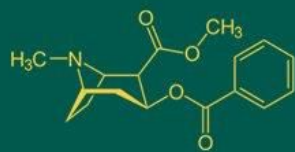


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Bacteriological profile and antimicrobial susceptibility pattern of canine pyometra in Central India

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Abstract

Background: Female dogs that are not spayed are at risk of developing a life-threatening canine pyometra, which is a severe uterine infection caused by bacterial overgrowth due to high levels of progesterone. This condition requires immediate veterinary care for a better prognosis and recovery. In Bhopal, Central India, the crucial information on the regional antimicrobial resistance patterns of the main bacterial causatives remains underreported.

Objectives: To determine the bacteriological profile and antimicrobial susceptibility pattern of canine pyometra cases presented in Bhopal district, Madhya Pradesh, India.

Methods: Thirty-one bitches clinically diagnosed with pyometra were sampled between July and September 2023. Vaginal swabs were subjected to culture and identification using standard microbiological techniques. Antimicrobial susceptibility testing (AST) was performed by the Kirby-Bauer disk diffusion method following CLSI (2021) guidelines.

Results: Of 31 samples, 29 (93.54%) were culture-positive, yielding 45 isolates. Gram-negative bacteria (57.77%) predominated over Gram-positive bacteria (42.22%). The most common isolates were *Escherichia coli* (28.88%), *Streptococcus pyogenes* (24.44%), *Staphylococcus aureus* (15.55%), *Pseudomonas aeruginosa* (13.33%), and *Klebsiella pneumoniae* (13.33%). Piperacillin/Tazobactam (87.61%), Ceftriaxone (84.55%), and Gentamicin (82.33%) showed highest overall sensitivity. Amoxicillin/Clavulanic acid exhibited the highest resistance (58.05%).

Conclusions: *E. coli* remains the predominant pathogen in canine pyometra in central India. Increasing resistance to commonly used beta-lactam antibiotics highlights the need for routine AST-guided therapy and antimicrobial stewardship in veterinary practice.

Keywords: Antibigram, antimicrobial resistance, Canine pyometra, CLSI, *E. coli*, veterinary microbiology

Introduction

Canine pyometra is a severe and potentially fatal uterine infection occurring predominantly during diestrus under progesterone influence (Hagman, 2022; Jitpean *et al.*, 2021) [3, 4]. Cystic endometrial hyperplasia (CEH), in combination with ascending bacterial infection, leads to the accumulation of purulent exudate within the uterine lumen (Kumar *et al.*, 2022; Schlafer and Gifford, 2020) [5, 9]. Reduced local uterine immunity during the luteal phase facilitates bacterial colonization and proliferation (Hagman, 2022; Santander *et al.*, 2021) [3, 8].

Globally, *E. coli* has been consistently reported as the most frequently isolated pathogen in canine pyometra, accounting for approximately 70-90% of cases (Oliveira *et al.*, 2021; Trautwein *et al.*, 2022; Hagman, 2022) [7, 10, 3]. Other commonly reported organisms include *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., and *Klebsiella* spp. (Trautwein *et al.*, 2022; Miranda *et al.*, 2023) [10, 6]. Endotoxin-producing strains of *E. coli* may induce systemic inflammatory response syndrome (SIRS) and septic shock through lipopolysaccharide-mediated inflammatory cascades (Hagman, 2022; Chen *et al.*, 2023) [3, 1]. The growing burden of antimicrobial resistance (AMR) in companion animals poses significant therapeutic challenges and zoonotic concerns (De Graef *et al.*, 2021; WHO, 2023) [2, 11]. Empirical antibiotic therapy without culture confirmation may contribute to treatment failure and selection of resistant strains (Miranda *et al.*, 2023) [6]. Therefore, region-specific data on bacterial prevalence and antimicrobial susceptibility patterns are critical for rational therapeutic decision-making and antimicrobial stewardship (De Graef *et al.*, 2021; WHO,

2023) [12, 11]. Hence, this study aimed to (i) identify bacterial pathogens associated with canine pyometra in central India and (ii) evaluate their antimicrobial susceptibility patterns.

2. Materials and Methods

2.1 Study area and sampling

The study was conducted between July and September 2023 in three tehsils (Huzur, Kolar, and Berasia) of Bhopal district, Madhya Pradesh, India. Laboratory investigations were performed at the State Animal Disease Investigation Laboratory (SADIL), Bhopal.

Thirty-one intact bitches clinically diagnosed with pyometra based on history, clinical signs, and ultrasonography were included. Sterile vaginal swabs were collected aseptically.

2.2 Bacterial isolation and identification

Samples were inoculated onto 5% sheep blood agar and incubated at 37 °C for 18-24 h. Gram staining and biochemical characterization (catalase, oxidase, IMViC, TSI, citrate, urease tests) were performed. Selective media including MacConkey agar, Eosin Methylene Blue (EMB) agar, and Mannitol Salt Agar were used for differentiation.

2.3 Antimicrobial susceptibility testing

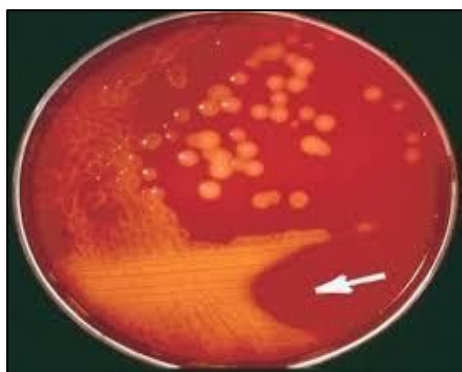
AST was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Eighteen antibiotics were tested. Zone diameters were interpreted according to CLSI (2021) guidelines. Intermediate isolates were considered resistant.

3. Results

3.1 Culture positivity and isolate distribution

Out of 31 samples, 29 (93.54%) were culture-positive, yielding 45 isolates. Gram-negative isolates accounted for 57.77% (n = 26), while Gram-positive isolates accounted for 42.22% (n = 19).

3.2 Bacterial profile



SL. No	Name of the pathogen	Number of isolates	Percentage (%)
1)	<i>Escherichia coli</i>	13	28.88
2)	<i>Streptococcus pyogenes</i>	11	24.44
3)	<i>Staphylococcus aureus</i>	7	15.55
4)	<i>Pseudomonas aeruginosa</i>	6	13.33
5)	<i>Klebsiella pneumoniae</i>	6	13.33
6)	<i>Enterococcus</i> spp.	1	2.22
7)	<i>Proteus</i> spp.	1	2.22

Mixed infections were observed in 48.27% of positive cases.

Antimicrobial susceptibility pattern



SL. No	Name of the antibiotic	Highest overall susceptibility
1	Piperacillin/Tazobactam	87.61%
2	Ceftriaxone	84.55%
3	Gentamicin	82.33%
4	Imipenem/Cilastin	79.78%

SL. No	Name of the antibiotic	Highest overall resistance
1	Amoxicillin/Clavulanic acid	58.05%
2	Ampicillin/Sulbactam	41.47%
3	Ciprofloxacin	38.85%

Gram-negative isolates demonstrated greater resistance to beta-lactams compared with Gram-positive isolates.

4. Discussion

The dominance of *E. coli* (28.88%) observed in the current study aligns with recent global research identifying *E. coli* as the primary etiological agent in canine pyometra (Oliveira *et al.*, 2021; Trautwein *et al.*, 2022; Hagman, 2022) [7, 10, 3]. The organism's capacity to adhere to endometrial epithelial cells through fimbrial adhesins (such as type 1 fimbriae), produce endotoxins, and evade host immune responses significantly contributes to its persistent presence in the uterus and systemic pathogenicity (Chen *et al.*, 2023) [1]. Recent molecular investigations have revealed that *E. coli* strains associated with pyometra frequently possess virulence-associated genes linked to uropathogenic lineages, indicating overlapping pathogenic mechanisms

between uterine and urinary tract infections in bitches (Miranda *et al.*, 2023) [6].

The analysis revealed a notable prevalence of Gram-negative isolates (57.77%) compared to Gram-positive isolates (42.22%), a difference that was statistically affirmed by chi-square analysis, indicating a non-random distribution favoring Gram-negative organisms. This finding is consistent with recent epidemiological reports highlighting the dominance of enteric Gram-negative bacteria in reproductive tract infections, largely attributed to ascending contamination from the perineal region (Trautwein *et al.*, 2022) [10]. Additionally, the relatively high incidence of mixed infections (48.27%) suggests polymicrobial synergy, which may contribute to enhanced biofilm formation and therapeutic resistance, as noted in recent veterinary microbiological studies (De Graef *et al.*, 2021) [2].

The antimicrobial susceptibility profile demonstrated a high *in vitro* efficacy for Piperacillin/Tazobactam (87.61%), Ceftriaxone (84.55%), and Gentamicin (82.33%). These results align with recent surveillance data showing maintained susceptibility of third-generation cephalosporins and β -lactam/ β -lactamase inhibitor combinations in companion animal isolates, although there are reports of emerging resistance trends (Oliveira *et al.*, 2021; WHO, 2023) [7, 11]. The significant resistance noted against Amoxicillin/Clavulanic acid (58.05%) is clinically concerning, particularly given its common use in small animal practice. Similar resistance patterns have been observed in recent studies conducted in Europe and Asia, indicating selective pressure resulting from frequent exposure to β -lactams (Miranda *et al.*, 2023; De Graef *et al.*, 2021) [6, 2].

The calculated mean resistance rate and Multiple Antibiotic Resistance (MAR) index emphasize the significant issue of antimicrobial resistance in the study region. A MAR index greater than 0.2 is typically regarded as indicative of high-risk contamination sources and considerable antimicrobial exposure (De Graef *et al.*, 2021) [2]. The MAR index observed in this study suggests a pattern of repeated or indiscriminate antibiotic use in settings involving companion animals, highlighting the necessity for antimicrobial stewardship programs.

From a clinical standpoint, strains of *E. coli* that produce endotoxins can lead to systemic inflammatory response syndrome (SIRS), septicemia, and multi-organ dysfunction. This occurs through the activation of pro-inflammatory cytokine cascades mediated by lipopolysaccharides (Hagman, 2022; Chen *et al.*, 2023) [3, 1]. Therefore, it is crucial to identify high-risk isolates early and to initiate effective antimicrobial therapy promptly to reduce morbidity and mortality.

The zoonotic aspect of antimicrobial resistance in companion animals is a significant concern that should not be overlooked. Close interactions between humans and animals enable the bidirectional transmission of resistant bacteria and resistance genes, especially those produced by extended-spectrum β -lactamase (ESBL) strains (De Graef *et al.*, 2021; WHO, 2023) [2, 11]. Region-specific surveillance studies, such as the one presented here, are crucial for establishing baseline data that can inform One Health-oriented strategies for mitigating antimicrobial resistance.

The findings collectively highlight three crucial implications:

- *E. coli* continues to be the predominant pathogen in canine pyometra;
- there is a concerning rise in resistance to commonly used β -lactams; and
- Routine culture and susceptibility testing should be prioritized over empirical therapy whenever possible.

To address the growing issue of resistance in veterinary clinical settings, it is essential to implement antimicrobial stewardship, regularly update regional antibiograms, and adopt rational prescription practices.

Conclusion

This study confirms that *E. coli* is the most common pathogen responsible for canine pyometra in central India. The high rates of antibiotic resistance observed highlight the importance of antibiotic susceptibility testing to guide therapy. Regular bacteriological examinations and antimicrobial stewardship are crucial for improving treatment outcomes and limiting the spread of AMR.

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