Full Length Article

BACTERIAL ISOLATION AND ANTIBIOGRAM STUDIES IN DOGS WITH OPEN AND CLOSED-CERVIX PYOMETRA

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ABSTRACT

The emergence of antimicrobial resistance is raising public health concerns globally and random use of ineffective antibiotics in dogs creates great trouble during therapeutic interventions. Antibiogram determines the antibiotics to which a particular microorganism is susceptible, allowing clinicians to select the most effective treatment. This approach ensures use of antibiotics judiciously and in proper doses, helping to curb the development of resistant strains. Antibiogram studies were performed in 12 dogs affected with open and closed-cervix pyometra. Group I contained six dogs with open-cervix pyometra and Group II contained six dogs with closed-cervix pyometra. All the dogs were treated with mifepristone @ 2.5 mg/kg body weight twice daily for five days orally and from third day onwards, after ensuring the cervical patency, cloprostenol was administered @ 5 µg/kg body weight subcutaneously as once daily injection, on every third day, till complete emptying of uterus, as evaluated by ultrasonography. Bacterial isolation and antibiogram studies were carried out using sterile anterior vaginal swabs collected from the affected dogs on the day of presentation in Group I and once the discharge was started in Group II. Gram's reaction revealed mixed isolates in eight out of 12 samples collected. Gram negative bacilli were the predominant isolate obtained from both the groups. On antibiogram studies, maximum sensitivity in Group I isolates was noticed to ceftriaxone-sulbactam (66.67%), followed by gentamicin (50%), amoxicillin clavulanic acid (50%), amoxicillin (33.33%), ciprofloxacin (33.33%), cefotaxime (33.33%), co- trimoxazole (33.33%), metronidazole (33.33%) and enrofloxacin (16.67%). Maximum sensitivity in Group II was noticed to amoxicillin-clavulanic acid and gentamicin (83.33%) followed by ciprofloxacin and metronidazole (50%), cefotaxime (33.33%), amoxicillin (16.67%), ceftriaxone-sulbactam (16.67%), co-trimoxazole (16.67%), enrofloxacin (16.67%).

Key words: Open-cervix pyometra, Closed-cervix pyometra , Antibiogram, Bacterial isolation, Dogs

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INTRODUCTION

Chronic oestrogenic or progestinic activity in uterus causes endometrial alterations in dogs (De Cock et al., 2002). It is an amplified response of the uterus to the persistent progesterone stimulation during the dioestrous phase of the oestrous cycle causing increased production of fluid within the endometrial glands which gets complicated with bacteria later on and results in pyometra (De Bosschere *et al.,* 2001). Pyometra can be either open or closed cervix pyometra, wherein closed-cervix pyometra cases are more severe due to the lack of drainage of accumulated pus (Devarajan *et al.,* 2021).

Hagman and Greko (2005) suggested Escherichia coli as the most common infective agent in pyometra. Additionally, bacteria from the Streptococcus, Staphylococcus and Enterococcus genera are significant contributors to the condition (Xavier *et al.,* 2023). The emergence of antimicrobial resistance is raising several public health concerns. Avoiding the use of unnecessary antibiotics and focusing treatment on patients who truly need it are key strategies for preventing antimicrobial resistance (Ylhainen *et al.,* 2023). Early recognition, diagnosis and suitable intervention were of utmost importance to avoid fatal consequences. For that, timely identification of causative agents before administration of therapy was considered more effective while treating pyometra in dogs. The present study deals with the isolation of bacteria and evaluating antibiogram in closed and open-cervix pyometra.

MATERIALS AND METHODS

Female dogs (n=12) presented to University Veterinary Hospitals with history and clinical signs suggestive of pyometra were utilised for the study. These dogs were subjected to detailed clinical, gynaecological, laboratory and ultrasonographic evaluation for confirmation of the condition. Six dogs exhibiting sanguineous to mucopurulent vaginal discharge of varying consistency, colour, odour and with evidence of definite uterine sacculations on ultrasonography, were selected as animals with open-cervix pyometra (Group I). Six dogs which were not exhibiting any vaginal discharge, presented with other symptoms of pyometra and confirmed with evidence of definite uterine sacculations on ultrasonography, was selected as animals with closed-cervix pyometra (Group II).

All the dogs were treated with mifepristone @ 2.5 mg/kg body weight twice daily for five days orally and from third day onwards, after ensuring the cervical patency, cloprostenol was administered @ 5 µg/kg body weight subcutaneously as once daily injection, on every third day, till complete emptying of uterus, as evaluated by ultrasonography. Supportive therapy with intravenous fluid and antibiotics was initiated in all the cases according to the clinical condition of each case, and necessary modifications were made as per the progress of the condition and results of culture and sensitivity studies of anterior vaginal swab collected.

Collection of vaginal swabs

Dogs were adequately restrained on an examination table and the perivaginal area was scrubbed with 70 per cent isopropyl alcohol. Sterile cotton swab sticks were passed into the vagina, after parting the vaginal labia with the help of vaginoscope. Initially the stick was directed cranio-dorsally, avoiding the clitoral fossa and the urethral orifices and then longitudinally, as described by Kustritz (2010). The swab was then gently rolled around the vaginal wall, withdrawn carefully, transferred into a labelled, sterile container and transported to the laboratory immediately after collection.

Bacterial isolation and antibiogram

The collected swab was streaked on Brain heart infusion agar (BHIA) and incubated at 37 ºC for 24-72 hrs to obtain bacterial colonies. A minute amount of colony was transferred to a glass slide contained a drop of water and made a very thin layer of smear by spreading with inoculation loop. Smear was dried completely and heat fixed. Gram's staining was performed and identified Gram positive or Gram negative cocci and bacilli.

Kirby-Bauer disc diffusion method was performed on Muller-Hinton agar (MHA) plates to determine susceptibilities of different beta-lactam and non-beta-lactam sensitive bacteria. Sterile MHA plates were prepared with a medium thickness of about 4 mm for rapidly growing aerobic microorganisms. Pure culture was used as inoculum. Three to four similar colonies were selected and transferred into three mL of brain heart infusion broth (BHIB) and incubated at 37ºC for 2-8 hrs till light to moderate turbidity was developed. A sterile non - toxic cotton swab on a wooden applicator was dipped into the standardized inoculum and was rotated. The soaked swab was pressed firmly against the upper inside wall of the tube to remove excess fluid in the swab. The entire agar surface of the plate was streaked with the swab three times, turning the plate at 60º between the streaking and the inoculum was allowed to dry for 5-10 min with the lid in place.

The antibiotic discs with known concentration of antibiotics in microgram (µg) per disc, as mentioned in Table I were applied under aseptic conditions. The discs were deposited with a distance of at least 24 mm between each other. The plates were incubated immediately at 37ºC and examined after14-18 hrs or later. The clear zones of inhibition were measured and the diameter of the zones to the nearest millimeter was recorded. The zones were compared with the standard inhibition zone chart to find out sensitive and resistant antibiotics. The obtained results were demonstrated as sensitive (S) and resistant (R).

RESULTS AND DISCUSSION

Bacterial isolation and antibiogram

The results of bacterial isolates obtained with Gram's reaction are shown in Table - 2 and Figure I.

Gram's reaction showed that, out of 12 cases, mixed isolates were obtained

for eight samples. Gram's reaction showed that in Group I, Gram positive cocci, Gram negative cocci, Gram positive bacilli and Gram negative bacilli were 30.77, 7.69, 15.38 and 46.15 per cent, respectively. Gram's reaction showed that, in Group II, Gram positive cocci, Gram negative cocci, Gram positive bacilli and Gram negative bacilli were 20, 10, 20 and 50 per cent, respectively (Figure I). The predominant bacterial isolate obtained from both the groups of animals were Gram negative bacilli.

These findings are in accordance with Ros (2014) who observed 72.72 per cent of isolates were Gram negative and the remaining were Gram positive bacteria. Gram's reaction revealed that out of 53 bacterial isolates, 23 isolates were Gram positive cocci (43.39%) and 30 isolates (56.60%) were Gram negative bacilli (Unnikrishnan, 2018). Krekeler *et al.* (2012) observed that E. coli was the most commonly isolated organism in canine pyometra, which bound to the receptors present in uterine endometrium, which was previously stimulated by progesterone and contributed to the initiation of infection. Rainey *et al.* (2018) also suggested that E. coli is one of the most prevalent microorganisms, responsible for up to 90% of canine pyometra cases. This Gram negative, facultative anaerobic bacterium is also a leading cause of uterine infections in various species, including humans. Given that E. coli is part of the gut microbiota, it is believed that the bacterium can migrate from the rectum to the uterus, thereby causing the infection.

The sensitivity profile of bacterial isolates of Group I and Group II are depicted in Table - 3. Maximum sensitivity in Group I isolates was noticed to ceftriaxone-sulbactam (66.67%), followed by gentamicin (50%), amoxicillin clavulanic acid (50%), amoxicillin (33.33%), ciprofloxacin (33.33%), cefotaxime (33.33%), co-trimoxazole (33.33%), metronidazole (33.33%) and enrofloxacin (16.67%). Maximum sensitivity in Group II was noticed to amoxicillin-clavulanic acid and gentamicin (83.33%) followed by ciprofloxacin and metronidazole (50%), cefotaxime (33.33%), amoxicillin (16.67%), ceftriaxone-sulbactam (16.67%), co-trimoxazole (16.67%), enrofloxacin (16.67%). Chinnu (2016) reported that antibiotics like ciprofloxacin, gentamicin, netilmicin, amikacin, ceftriaxone, cefaperazone are sensitive in pyometra cases. Unnikrishnan (2018) obtained maximum sensitivity of organism to amikacin, followed by gentamicin. Vidya (2019) reported maximum sensitivity of organisms to gentamicin followed by amikacin.

In the present study, ceftriaxone-sulbactam and amoxicillin-clavulanic acid demonstrated high sensitivity across various cases, likely attributed to their broad-spectrum activity and strong efficacy against Gram negative bacteria. Similarly, gentamicin exhibited notable sensitivity, consistent with findings in comparable studies, which its classification can explain as an antimicrobial agent particularly effective against Gram negative organisms.

The resistance profile of bacterial isolates of Group I and II are depicted in Table - 4. Maximum resistance in Group I isolates was noticed to enrofloxacin (83.33%), followed by amoxicillin, ciprofloxacin, cefotaxime, cotrimoxazole and metronidazole (66.67%), gentamicin (50%) and ceftriaxone-sulbactam (33.33%). Maximum resistance in Group II isolates was noticed to amoxicillin, ceftriaxone-sulbactam, cefotaxime, co-trimoxazole and enrofloxacin (83.33%) followed by metronidazole and ciprofloxacin (50%), gentamicin and amoxicillin-clavulanic acid (16.67%). Unnikrishnan (2018) reported maximum resistance of isolated organisms to amoxicillin, followed by amoxicillin-clavulanic acid, norfloxacin, tetracycline and metronidazole. Renukaradhya (2011) observed maximum resistance of pyometra organisms to gentamicin. Vidya (2019) reported maximum resistance of bacteria to cefotaxim, followed by enrofloxacin, norfloxacin and ceftriaxone. According to Prescott (2013), sulfadoxine-trimethoprim was selected as the treatment medication based on Finnish national guidelines, which recommend it for both surgical prophylaxis and postoperative management of uncomplicated pyometra. Sulfonamide-diamine pyrimidine combinations exhibit bactericidal activity against a broad spectrum of bacteria, including E. coli, the most frequently isolated pathogen in canine pyometra.

Enrofloxacin, a fluoroquinolone antibiotic commonly used in veterinary medicine, exhibited the highest resistance in Group I. The frequent administration of enrofloxacin in livestock and companion animals without appropriate diagnostic testing contributes significantly to the development of resistance. The significant resistance observed against amoxicillin could be attributed to the presence of beta-lactamase-producing bacteria. Beta-lactamase enzyme effectively inhibits activity of penicillin-class antibiotics, rendering them less effective. The significant resistance to ceftriaxone-sulbactam observed in Group II may be attributed to advanced resistance mechanisms beyond beta-lactamase production.

Different antibiogram studies provided different results by different scientists and this may be due to the variation in sensitivity and resistant pattern exhibited by field isolates of pathogens (Mane et al., 2012). The results vary with the geographical location of the work and the type of the bacteria isolated. The main reason behind the antibiotic resistance exhibited by organisms is the antibacterial residues in the female dogs and the indiscriminate use of antibiotics without performing any antibiotic sensitivity test (Bassessar et al., 2013). Research-based evidence is necessary to create treatment guidelines to ensure the efficacy of antimicrobials and to slow or even prevent the development of antimicrobial resistance.

Table-1 Antibiotic concentration present in the discs used for antibiogram

S. No.	Antibiotics	Microgram (µg) per disc
1	Gentamicin	10 µg
2	Amoxycillin	30 µg
3	Amoxycillin-clavulanic acid	30 µg
4	Cefotaxime	30 µg
5	Ceftriaxone-sulbactam	30 µg
6	Co-trimoxazole	25 µg
7	Enrofloxacin	10 µg
8	Ciprofloxacin	30 µg
9	Metronidazole	5 µg

Table- 2 Types of bacterial isolates obtained in Group I and Group II (n=6), per cent

Group	N	Per cent of bacterial colonies			
		Gram +ve cocci	Gram –ve cocci	Gram +ve bacilli	Gram –ve bacilli
I	13	30.77	7.69	15.38	46.15
II	10	20.00	10.00	20.00	50.00

Table- 3 Antibiotic sensitivity profile of bacterial isolates (n=12), per cent

S. No.	Antibiotic	Group I		Group II	
		No of cases with sensitive isolates	Sensitivity (%)	No of cases with sensitive isolates	Sensitivity (%)
1	Amoxicillin	2	33.33	1	16.67
2	Amoxicillin-clavulanic acid	3	50.00	5	83.33
3	Ceftriaxone-sulbactam	4	66.67	1	16.67
4	Ciprofloxacin	2	33.33	3	50.00
5	Cefotaxime	2	33.33	1	33.33
6	Co – trimoxazole	2	33.33	1	16.67
7	Enrofloxacin	1	16.67	1	16.67
8	Gentamicin	3	50.00	5	83.33
9	Metronidazole	2	33.33	3	50.00

Table - 4 Antibiotic resistance profile of bacterial isolates (n=12), per cent

Sl. No.	Antibiotic	Group I		Group II	
		No of cases with sensitive isolates	Sensitivity (%)	No of cases with sensitive isolates	Sensitivity (%)
1	Amoxicillin	4	66.67	5	83.33
2	Amoxicillin-clavulanic acid	3	50.00	1	16.67
3	Ceftriaxone -sulbactam	2	33.33	5	83.33
4	Ciprofloxacin	4	66.67	3	50.00
5	Cefotaxime	4	66.67	5	83.33
6	Co - trimoxazole	4	66.67	5	83.33
7	Enrofloxacin	5	83.33	5	83.33
8	Gentamicin	3	50.00	1	16.67
9	Metronidazole	4	66.67	3	50.00

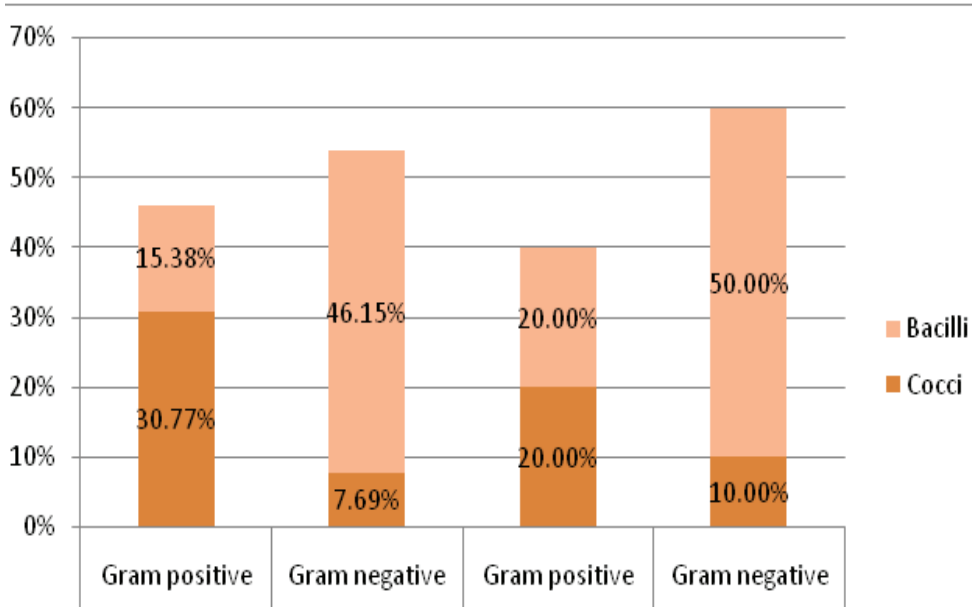

Fig.1. Types of bacterial isolates obtained from Group I and Group II (n=6), per cent

CONCLUSION

Antibiotic sensitivity testing is pivotal in effectively managing bacterial infections by identifying the most appropriate antibiotics for treatment. This approach not only enhances clinical outcomes but also minimizes the misuse of antibiotics, thereby curbing the emergence of antimicrobial resistance. The present study resulted in the identification of Gram negative bacilli as the primary pathogen responsible for canine pyometra. From antibiogram, ceftriaxone-sulbactam, gentamicin and amoxicillin-clavulanic acid showed the highest sensitivity across the samples, whereas enrofloxacin and amoxicillin demonstrated significant resistance. The findings of this study emphasize the importance of antibiotic sensitivity testing, particularly in conditions like canine pyometra, where tailored therapy is essential for successful outcomes. Continued research and broader sample sizes are necessary to refine treatment protocols and ensure their reliability in diverse clinical scenarios.

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