

Antibiogram of bacterial species isolated from canine pyometra

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Abstract

Aim: The aim of the present work was to ascertain the bacterial flora causing pyometra in female dogs and their antibiotic sensitivity.

Materials and Methods: A study was conducted to determine the antibiogram of bacterial species isolated from 20 female dogs diagnosed with pyometra. The vaginal discharge was collected by sterile swab and streaked smoothly over Mueller Hinton medium and sensitivity towards antibiotics was determined by measuring the zone of inhibition using a Hi-media scale.

Results: The antibiogram showed that Gentamicin was the most sensitive (85%) antibiotic followed by Enrofloxacin, Ciprofloxacin and Amoxicillin (65%, 65% and 55%), respectively. The isolates were most resistant to Oxytetracycline (85%) followed by Tetracycline, Ampicillin, Chloramphenicol, Cloxacillin and Erythromycin (80%, 80%, 75%, 70% and 70%), respectively.

Conclusion: Gentamicin was found to be most effective antibiotic against the bacterial species isolated from canine pyometra.

Keywords: antibiotic, antibiogram, canine, pyometra, sensitivity test

Introduction

Canine pyometra, also known as cystic endometrial hyperplasia complex, is a disease of the adult dog with inflammation of the uterus and accumulation of pus, and normally occurs in the luteal phase of the oestrous cycle. Pyometra refers to uterine infection that occurs when contaminating bacteria overcome the normal uterine protective mechanisms. The bacterial infection is a secondary condition. Bacteria ascend through the cervix and into the uterus during oestrous. Bitches with cystic endometrial hyperplasia, seem to be incapable of eliminating bacteria that can survive in the cystic fluid. The toxic state affects the function of several organs like bone marrow, kidney, liver etc. [1].

It is of particular importance to the veterinary practitioner, since early recognition, diagnosis and appropriate intervention is required to avoid fatal consequences in animals [2]. Therefore, identification of the causative agent before institution of appropriate therapy is considered to be more rationale while treating this problem in female dogs.

Materials and Methods

Location of work and study period: The work was conducted in Department of Veterinary Pathology and Teaching Veterinary Clinical Service Complex

(TVCC), College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh. The study period was of 8 months extending from January –August, 2009.

Collection of samples: Twenty female dogs with a presumptive diagnosis of pyometra, irrespective of their age or breed, were included in the study. The diagnosis was based on history, clinical examination, laboratory parameters and ultrasonography. Discharge material from the vagina of the pyometric animals was collected with the help of sterilized cotton swabs under aseptic conditions.

Processing of samples: The swabs were then processed for isolation of bacteria and antibiotic sensitivity test. The vaginal discharge was spread on simple and enriched media, and left for incubation at 37°C for a period of 24 to 48 hrs.

Materials used: Commercially available various ready to use dehydrated medias (Hi-Media Laboratories Limited, Mumbai, India) were used for the preparation of culture media. The following media were utilized during the present study: Nutrient broth, Nutrient agar, MacConkey agar, Brilliant green agar, Eosinmethylene blue agar, Mannitol salt agar, Muller-Hinton agar, Sabouraud's agar

Antibiotic sensitivity test: Growth from isolated colonies was used for identification studies based on morphology, staining, cultural and biochemical charac-

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teristics [3]. The discharge material was streaked over Mueller Hinton medium in such a way that a smooth, homogenous lawn culture was obtained. Plates were allowed to dry for 5 to 10 minutes at room temperature after inoculation. Standard disc of 10 antibiotics (Hi-media) were placed with the help of forceps and pressed gently to ensure full contact with the media. Plates were incubated at 37°C for 18 to 24 hrs. The inhibition zones of different antibiotics were recorded in mm and results were expressed in terms of sensitivity (S) and resistant (R). The sensitivity towards antibiotics was determined using the single disc diffusion method [3]. Zone of inhibition was measured using a Hi-media scale. Interpretation was made according to guidelines of clinical laboratory standards institute (CLSI, 2003) proposed for animal pathogens. Antibiotic discs used in the present study are as shown in Table-1.

Table-1. Antibiotics used for determination of the antibiogram of bacterial spp. isolated from canine pyometra.

Sr. No.	Name and concentration of antibiotic	Symbol
1	Tetracycline (30µ/disc)	T
2	Erythromycin (15mcg/disc)	E
3	Ciprofloxacin (10mcg/disc)	Cf
4	Enrofloxacin (10mcg/disc)	E
5	Cloxacillin (5mcg/disc)	Cx
6	Oxytetracycline (30mcg/disc)	O
7	Gentamicin (10mcg/disc)	G
8	Chloramphenicol (30mcg/disc)	C
9	Amoxicillin (10mcg/disc)	Am
10	Ampicillin (10mcg/disc)	A

Results

The microbiological examination of the vaginal discharge from the 20 pyometric dogs revealed 9 samples of *Escherichia coli*, 4 samples of *Salmonella* spp., 2 samples of *Pseudomonas* spp., 3 samples of *Staphylococcus* spp. and 2 samples of mixed infection with *E. coli* and *Staphylococcus* spp. The antibiogram of 20 vaginal discharge showed that Gentamicin (85%) was the most sensitive antibiotic followed by Enrofloxacin (65%). However, Ciprofloxacin and Amoxicillin showed 55% sensitivity. The isolates were most resistant to Oxytetracycline (85%), Tetracycline (80%), Ampicillin (80%), Chloramphenicol (75%) Cloxacillin (70%) and Erythromycin (70%).

In *in-vitro* study, the isolates of *E. coli* were found to be 100% sensitive to the Gentamicin followed by Enrofloxacin (75%), Ciprofloxacin (65%), Amoxicillin (55%). The organisms were most resistant to Tetracycline and oxytetracycline (100%), respectively.

All the *Staphylococcus* spp. Were most effective to Enrofloxacin (100%) followed by Gentamicin, Erythromycin, Amoxicillin and Chloramphenicol (65%), respectively, while the other antibiotics were ineffective against the obtained isolates.

Salmonella spp. isolates were effective to Gentamicin (75%) and Amoxicillin (75%), Ciprofloxacin (50%) and Enrofloxacin (50%), whereas Chloramphenicol (0%) was least sensitive followed by Cloxacillin and Oxytetracycline (25%), respectively.

Isolates of *Pseudomonas* spp. showed 50% effectiveness in case of Gentamicin and Tetracycline, while complete resistance was seen in case of other antibiotics used.

Discussion

Several studies have been conducted to determine the antibiogram pattern of bacterial isolates from various infections in canines. The results vary with the geographical location of the work and the type of the bacteria isolated. In the present study *E. coli* were found to be most frequently associated with canine pyometra. Aminoglycosides like Gentamicin and Fluoroquinolone like Enrofloxacin were found to be most effective. However, since mixed infections were prevalent 100% efficacy of a single antibiotic was not observed.

Silva *et al.* [4] during their etiological and therapeutic study of pyometra in female dogs found that Gentamicin was the most effective *in-vitro* antibiotic (97.83%) followed by Chloramphenicol (75.51%) and Streptomycin (65.79%), whereas Cephalosporin (14.81%) was the least effective drug. Ball *et al.* [5] studied the antimicrobial resistance and prevalence of canine uropathogens. In the study they found that the bacterial isolates were most likely to be susceptible to Gentamicin, Fluoroquinolones, Amoxicillin-Clavulanic acid, Cephalosporins (third generation). However, Murugavel *et al.* [6] reported that the bacterial isolates from pyometra were sensitive to Ciprofloxacin only while they were resistant to Cephalexin, Gentamicin, Cloxacillin and tetracycline. Roy *et al.* [7] collected 234 genital swabs from canine genital infections including pyometra cases which yield 98 (41.8%) *Escherichia coli* strains. Among these positive samples highest percentage (46.2) were observed from uterine infections (pyometra) cases. The antibiotic drugs like Gentamicin, Chloramphenicol, Cefixime (each 100%), Ciprofloxacin (90%) were found to be sensitive against these isolates when tested for antibiogram *in vitro*. But Amikacin (95%), Tetracycline, Kanamycin (approx. 80-82%), Vancomycin, Erythromycin and Novobiocin (78-80%) showed their infectivity against these isolates. Our study correlates with the reports of Ghose *et al.* [8], Bhattacharya [9] and Chhabra and Arora [10].

Mane *et al.* [11] observed that the antibiotic sensitivity pattern to field isolates of *E. coli* showed considerable variation in sensitivity and resistance pattern. The workers found highest rate of resistance was against Tetracycline (80%), followed by Doxycycline (70%), Trimethoprim (55%), Ampicillin (50%), Amoxicillin (40%) and Ciprofloxacin (40%). Ghanbarpour and Akhtardanesh [12] reported that eighty four isolates of *E. coli* from canine pyometra exhibited the maximum resistance against Sulfadiazine and Tetracycline. Similarly, resistance to Amoxicillin, Cloxacillin and Tetracycline by *E. coli* in colibacillosis has been observed [13] in Japanese Quails. Hariharan *et al.* [14] employing 1819 samples from canine otitis reported all 178 *E. coli* isolates to be susceptible to

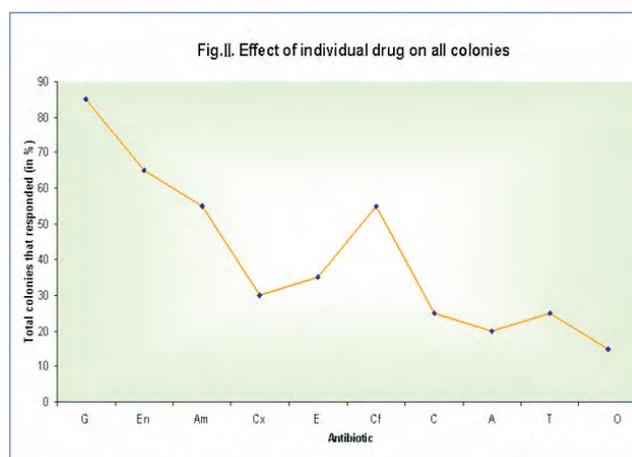
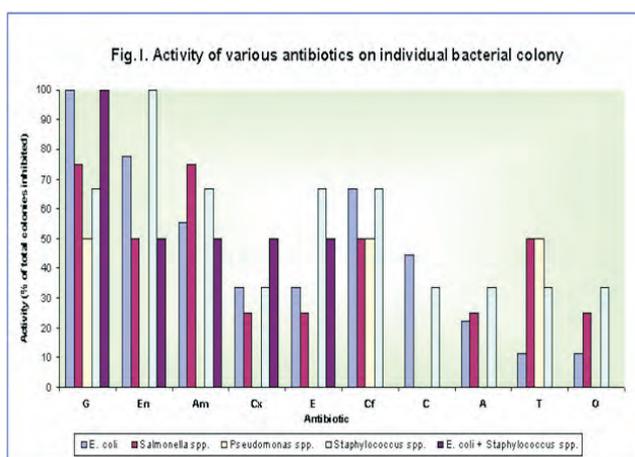
Table-2. Overall sensitivity and resistance profiles of bacterial spp. isolated from canine pyometra

Sr. No.	Antibiotics	No. of sensitive isolates	Sensitivity %	No. of resistant isolates	Resistance %
1	Tetracycline (30 µg/disc)	5	25%	15	75%
2	Erythromycin (15 µg/disc)	6	30%	14	70%
3	Ciprofloxacin (10 µg/disc)	11	55%	9	45%
4	Enrofloxacin (10 µg/disc)	13	65%	7	35%
5	Cloxacillin (5 µg/disc)	6	30%	14	70%
6	Oxytetracycline (30 µg/disc)	3	15%	17	85%
7	Gentamicin (10 µg/disc)	17	85%	3	15%
8	Chloramphenicol (30 µg/disc)	5	25%	15	75%
9	Amoxicillin (10 µg/disc)	11	55%	9	45%
10	Ampicillin (10 µg/disc)	4	20%	16	80%

Table-3. Antibiotic sensitivity and resistance profiles of bacterial spp. isolated from canine pyometra

Name of bacteria	No. of isolates	Number of bacterial isolates found sensitive to the antibiotic discs									
		G	En	Am	Cx	E	Cf	C	A	T	O
<i>E. coli</i>	9	9	7	5	3	3	6	4	2	1	1
<i>Salmonella</i> Spp.	4	3	2	3	1	1	2	0	1	2	1
<i>Pseudomonas</i> Spp.	2	1	0	0	0	0	1	0	0	1	0
<i>Staphylococcus</i> Spp.	3	2	3	2	1	2	2	1	1	1	1
<i>E. coli</i> + <i>Staphylococcus</i> Spp.	2	2	1	1	1	1	0	0	0	0	0

G – Gentamicin, En - Enrofloxacin, Am – Amoxicillin, Cx - Cloxacillin, E - Erythromycin, Cf - Ciprofloxacin, C - Chloramphenicol, A – Ampicillin, T - Tetracycline, O – Oxytetracycline



Amikacin, Cefotaxime, Chloramphenicol and Ceftriaxone. Rajeev *et al.* [15] isolated *E. coli* and *S. aureus* and found that the antibiogram profile of them is highly variable. Chloramphenicol and Enrofloxacin were found to be promising antibiotics as most of the isolates were sensitive to them. These results correlated with the findings of Wani and Bhat [16], Balakrishnan *et al.* [17], Palinivel *et al.* [18] and Anderson *et al.* [19] who observed high sensitivity of *E. coli* serotypes to Chloramphenicol, Amikacin and Enrofloxacin. Ramani Pushpa *et al.* [20] reported that out of 10 *E. coli* isolates about 87.5% were sensitive to Cephalexin, 79.6% to Chloramphenicol, 58.6% to Ciprofloxacin, 52.6% to Enrofloxacin and 52.6% to Gentamicin. Shambulingappa and Anand Manegar [21] conducted studies on clinical metritis in female dogs and found Gram negative bacteria in 54.05 % cases and major isolates were *E. coli* (18.92%) and *Proteus spp.* (16.22%). Major gram positive isolates were *S. aureus* (24.32%) and *S. pyogenes* (10.18%). Majority of the above isolates were highly sensitive to Ciprofloxacin and Gentamicin. Lowered sensitivity was noticed towards Ampicillin, Streptomycin and Nitrofurantoin. In agreement to the findings of several workers, in the

present study also, maximum sensitivity of *E. coli* was found to Gentamicin (85%) followed by Enrofloxacin and the organisms were most resistant to Tetracycline and Oxytetracycline.

Maity, *et al.* [22] found that *Staphylococcus* spp. was the predominant one (44.19%) from 43 canine pyometra cases screened in veterinary clinics. All the isolates were sensitive to Enrofloxacin, Ciprofloxacin, Gentamicin and Ceftriaxone, but were generally resistant to Lincomycin and Oxytetracycline. Thus, our findings are in agreement with those of earlier workers, with 100% efficacy of Enrofloxacin observed against *Staphylococcus* spp.

In a study carried out to screen and analyze the characteristics of antibiotic resistance in *Salmonella* strains isolated from foods of animal origin the *Salmonella* isolates were found to be resistant against Trimetoprim, Kanamycin, and Co-trimoxazole [23]. However in agreement to our findings the workers reported efficacy of Gentamicin against *Salmonella* isolates. The bacterial profile of some isolates exhibited antibacterial resistance due to the antibacterial residue in the female dogs and indiscriminate use of antibiotics in the previous cases of illness without performing the

antibiotic sensitivity test in female dogs.

Conclusion

Thus, it can be concluded that gentamicin was found to be the most effective antibiotic against bacteria like *E. coli*, *Staphylococcus* spp. and *Salmonella* spp. causing canine pyometra in this region whereas tetracycline and oxytetracycline were the least effective.

Authors' contributions

This study was a part of postgraduate thesis of VB, submitted to Department of Veterinary Pathology, Veterinary College, Jabalpur. All the authors contributed equally in design of the research, analysis of the data, drafting and revision of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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