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Antibiogram of staphylococcal 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, their antibiotic sensitivity and biochemical profile.
Materials and Methods: A study was conducted to determine the antibiogram of bacterial species isolated from 2 female dogs diagnosed with pyometra. One sample was uterine fluid collected from one of the uterine horns using sterile needle and syringe in a sterile tube and other sample was a vaginal discharge collected by using sterile swab. The bacteria from both the samples were isolated, biochemical profiling was done and sensitivity towards antibiotics was determined by measuring the zone of inhibition on Muller Hinton agar.
Results: Staphylococcal species was isolated from both the samples. The antibiotic sensitivity of uterine fluid showed that lincomycin was the most sensitive antibiotic followed by enrofloxacin and gentamicin. Methicillin was most resistant antibiotic followed by kanamycin and ampicillin. While the vaginal discharge showed that cloxacillin was the most sensitive antibiotic followed by ceftriaxone and lincomycin, tetracycline was most resistant antibiotic followed by Ampicillin.
Conclusion: Lincomycin was found to be the most effective antibiotic against the bacterial species isolated from canine pyometra uterine fluid. While Cloxacillin was the most effective antibiotic against bacterial species isolated from vaginal discharge.

Keywords: Antibiotic, antibiogram, canine pyometra, sensitivity test, biochemical profile

Introduction
Canine pyometra is a disease of adult dog with inflammation of the uterus and accumulation of pus and normally occurs in the luteal phase of the oestrus cycle. Pyometra is a hormonally mediated diestrual disorder characterized by cystic endometrial hyperplasia with secondary bacterial infection which occurs due to repeated and prolonged response to oestrogen followed by long intervals of progesterone dominance in the intact bitches [1]. Pyometra is reported primarily in older bitches (>5 year old), 4-6 weeks after oestrus [2]. Bacteria ascend through the cervix into the uterus during oestrus. Bitches with pyometra, seem to be incapable of eliminating bacteria that can survive in the cystic fluid [1]. Bitches with pyometra may present either with a vaginal discharge present (open cervix pyometra) or without a vaginal discharge (closed cervix pyometra) [3]. Closed cervix pyometra is particularly dangerous because it may lead to septicaemia and toxaemia, hence early diagnosis is much important [4].

Materials and Methods
Collection of samples
Samples from 2 female dogs with a presumptive diagnosis of pyometra, presented to TVCC, Rajendranagar, Hyderabad were collected. The diagnosis was based on history, clinical examination, laboratory parameters and ultrasonography. Discharge material from vagina of one of the pyometric dogs was collected with the help of a sterile cotton swab under aseptic conditions. Other sample was uterine fluid collected from one of the uterine horns by using sterile syringe and needle and placed in a sterile tube. They are further processed at the department of veterinary microbiology, college of veterinary science, Hyderabad.

Sample Processing
The swabs were then processed for isolation of bacteria, antibiotic sensitivity test and biochemical profile. The vaginal discharge and uterine fluid were streaked on nutrient agar media and left for incubation at 37 °C for 24 to 48 hrs. The single colonies formed on nutrient agar was again spread on mannitol salt agar media, Eosin methylene blue agar, MacConkey agar media and incubated at 37 °C for 24 to 48 hrs.

Materials used

Commercially available various ready to use dehydrated media and reagents (Hi-media laboratories limited, Mumbai, India) were used for preparation of culture media and reagents. The following media were used during the present study: Nutrient broth, Nutrient agar, Mannitol salt agar, Eosin methylene blue agar, MacConkey agar, Simmons citrate agar, Triple sugar iron agar, 40% Urea, Muller Hinton agar. The reagents used are: Kovac's reagent, Methyl red indicator, Alpha naphthol, 40% KOH, Hydrogen peroxide and Oxidase discs.

Antibiotic Sensitivity Test (ABST)

Growth from isolated colonies were used for identification studies based on morphology, staining, cultural and biochemical characteristics [5]. The vaginal discharge and uterine fluid were streaked over Muller Hinton agar medium in such a way that a smooth, homogenous lawn culture was obtained. Plates were allowed to dry for 5-10 minutes at room temperature after inoculation and standard discs of 10 antibiotics (Hi-media) were placed with the help of forceps and pressed gently to ensure full contact with the media and then 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 and resistant. Zone of inhibition was measured using a Hi-media scale. Antibiotic discs used in the present study and the results of their zone of inhibition are shown in table-1.

Table 1: Antibiotics used for determination of the antibiotic sensitivity of bacteria isolated from canine pyometra and the results of their zone of inhibition in mm.

S. No.	Name of the Antibiotic	Concentration	Symbol	Zone of inhibition in mm (uterine fluid)	Zone of inhibition in mm(vaginal sample)
1	Gentamicin	10mcg	GEN	19	27
2	Tetracycline	10mcg	TE	13	27
3	Ceftriaxone	30mcg	CTR	17	36
4	Ampicillin	10mcg	AMP	13	18
5	Amoxycillin Sulbactum	30/15mcg	AMS	26	32
6	Methicillin	5mcg	MET	0	21
7	Kanamycin	30mcg	K	10	34
8	Lincomycin	15mcg	L	29	28
9	Enrofloxacin	10mcg	EX	26	25
10	Cloxacillin	30mcg	COX	17	>40

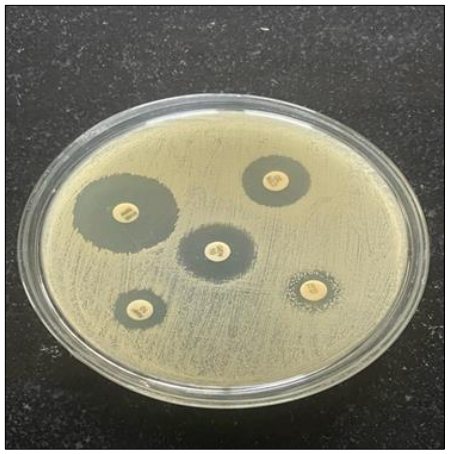

Fig 1a: Antibiotic sensitivity test of uterine fluid:GEN-Gentamicin, CTR- Ceftriaxone, TE- Tetracycline, AMS-Amoxycillin sulbactum, AMP- Ampicillin

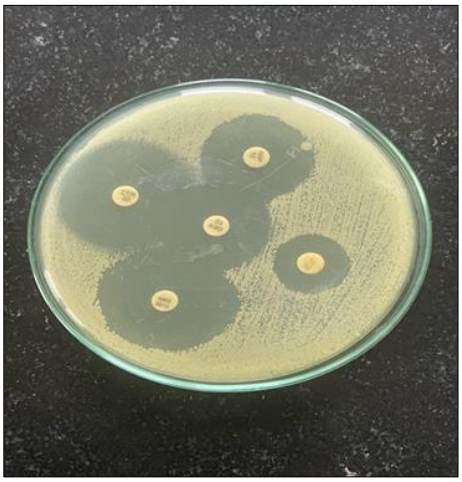

Fig 2a: Antibiotic sensitivity test of vaginal discharge:GEN-Gentamicin, CTR- Ceftriaxone, TE-Tetracycline, AMS-Amoxycillin sulbactum, AMP- Ampicillin

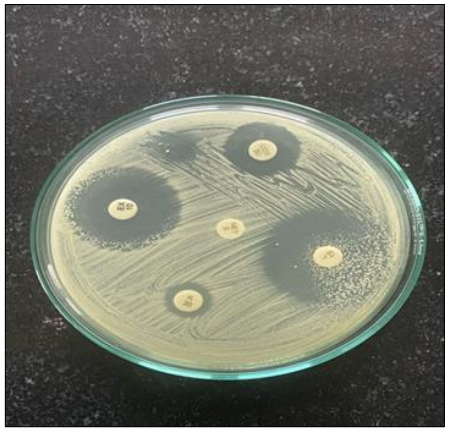

Fig 1b: Antibiotic sensitivity test of Uterine fluid: COX- Cloxacillin, MET-Methicillin, K-Kanamycin, L-Lincomycin, EX- Enrofloxacin

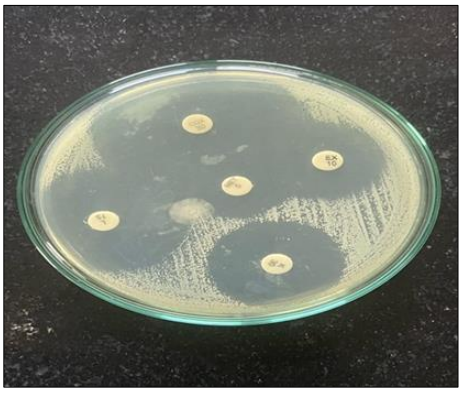

Fig 2b: Antibiotic sensitivity test of vaginal discharge: COX-Cloxacillin, MET-Methicillin, K-Kanamycin, L-Lincomycin, EX-Enrofloxacin

Results and Discussion

The microbial examination of the vaginal discharge and uterine fluid revealed that both the samples were Staphylococcus species and they are gram positive cocci (Fig 3 and Fig 4), appearing like cluster of grapes. The antibiotic sensitivity of uterine fluid had shown that lincomycin was the most sensitive antibiotic followed by Enrofloxacin and Gentamicin. Methicillin was the most resistant antibiotic followed by Kanamycin, Ampicillin, Tetracycline, Cloxacillin, Amoxycillin Sulbactum and Ceftriaxone was intermediate resistant (Fig 1a and Fig 1b). Methicillin was found to be most resistant antibiotic (As per CLSI guidelines). Hence it was identified as methicillin resistant staphylococcus aureus[MRSA]. Tajima *et al*. (2013) [6] reported a case of methicillin resistant staphylococcal aureus [MRSA] in a dog with keratitis and they revealed MRSA was sensitive to Lincomycin. Maity *et al*. (2009) [7] and Bassessar *et al*. (2013) [8] have found that Staphylococcus species was the predominant one (44.19%) from 43 canine pyometra cases screened by them. All the isolates were sensitive to enrofloxacin, ciprofloxacin, gentamicin and ceftriaxone. Thus, our findings are in agreement with those of earlier workers, with 100% efficacy of enrofloxacin observed against Staphylococcus species.

While the vaginal discharge showed that cloxacillin was most sensitive antibiotic followed by ceftriaxone, lincomycin, kanamycin, gentamicin, enrofloxacin, methicillin and tetracycline was most resistant antibiotic followed by ampicillin and amoxycillin sulbactum (Fig 2a and Fig 2b). Dimitrova *et al*. (2002) [9] analysed the therapeutic effect of cloxacillin in 6 dogs with experimental staphylococcal infection and observed that intramuscular applied cloxacillin led to normalization of changes in the clinical condition and the laboratory parameters. Riviere and papich, (2009) [10]; Papich (2018) [11] mentioned in their studies that cloxacillin was effectively used to treat staphylococcal infections such as pyoderma in dogs.Mark and Sykes, (2013)[12] have found that majority of the Staphylococci are sensitive to lincosamides like lincomycin. Our results are in agreement with the above studies.

The biochemical profiles of 2 samples are shown in table 2.

Table 2: The biochemical characters of 2 samples isolated from Canine Pyometra

S. No.	Test	Uterine fluid	Vaginal discharge
1	Urease	+	+
2	Citrate	+	+
3	Triple Sugar Iron	-	-
4	Indole	-	-
5	Methyl Red	+	+
6	Voges Proskauer	+	+
7	Catalase	+	+
8	Oxidase	-	-

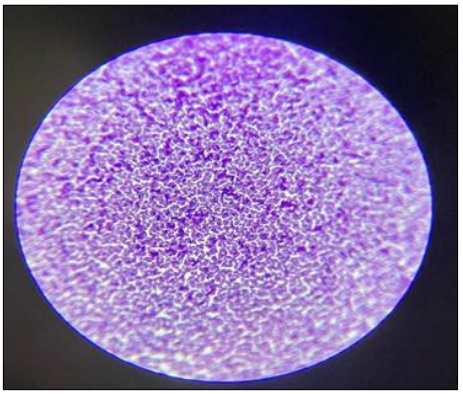

Fig 3: Grams staining of Uterine fluid showing staphylococcus

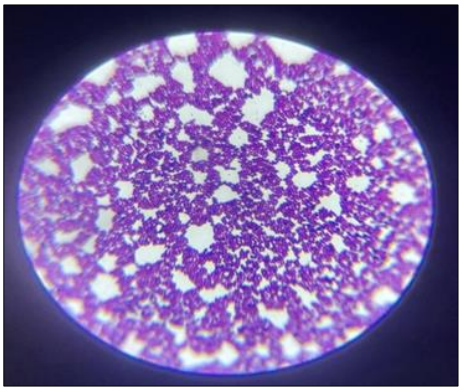

Fig 4: Grams staining of vaginal discharge showing staphylococcus

Conclusion

Our study concluded that lincomycin was found to be most effective towards staphylococcal species isolated from uterine fluid followed by enrofloxacin and gentamicin in this study. Tetracycline and ampicillin was found to be least effective. While the vaginal discharge showed that cloxacillin was most sensitive antibiotic followed by ceftriaxone and lincomycin. Tetracycline was most resistant antibiotic.

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References

1. Kustritz MVR. Cysticendometrial hyperplasiaand pyometra. In: S.G. Ettinger and E.C. Feldman (ed.) Textbook of Veterinary Internal Medicine. 6thedn., Pubu., Elsevier Saunders, St. Louis, 2005, 1676-1680.
2. Pyometra-The Merck Veterinary Manual, 2006. Retrieved on 2006-12-14.
3. Smith FO. Canine pyometra. Theriogenology. 2006;66:610-612.

4. Baithalu Rubina Kumari, Maharana BR, Mishra C, Sarangi L, Samal L, Canine pyometra, Vet. World 2010;3(7):340-342.
5. Cruickshank R, Dugie JP, Marimon BP, Swain RHA. Medical Microbiology, 12thedn, Publ. Churchill Livingstone, New York, 1975, 202-203.
6. Tajima K, Sinjyo A, Ito T, Noda Y, Goto H, Ito N. Methicillin-resistant Staphylococcus aureus keratitis in a dog. Veterinary Ophthalmology. 2013;16:240-243.
7. Maity S, Sarkar S, Saha T. Bacteriology and antibiogram of canine pyometra. Indian Vet. J. 2009;86:896-897.
8. Bassessar V, Verma Y, Swamy M. Antibiogram of bacterial species isolated from canine pyometra, Vet World. 2013;6(8):546-549.
9. Dimitrova D, Borissov I, Andonova M, Pashov D, Dimitrov D, Sotirova P, *et al*. Studies on the therapeutic effect of cloxacillin in dogs with experimental staphylococcal infection. Bulg. J Agric. Sci. 2002;8:103-110
10. Riviere JE, Papich MG. Veterinary Pharmacology and Therapeutics, 9th Ed., Wiley Blackwell, 2009.
11. Papich MG. β-Lactam Antibiotics: Penicillins, Cephalosporins, and Related Drugs. In J. E. Riviere & M. G. Papich (Eds.), Veterinary Pharmacology & Therapeutics. Hoboken, New Jersey, USA: Wiley Blackwell, John Wiley & Sons. 2018;10:826-857.
12. Sykes, Jane, Papich, Mark. Antibacterial Drugs. Canine and Feline Infectious Diseases, 2013, 66-86.
13. Niyas E, Reshma S, Shravya GS, Jayakumar C, Naveen Kumar RH, Sarika N, *et al*. Antibiogram of Isolated Organisms in Canine Pyometra. Int. J Curr. Microbiol. App. Sci. 2020;9**(**06):263-26.