SHORT COMMUNICATION

Isolation and Antibiogram of *Escherichia coli* from Canine Pyometra in Puducherry Region

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Abstract

Pyometra is a life-threatening and hormone induced cystic endometrial hyperplasia seen predominantly in older bitches, complicated with secondary bacterial infections. This study was performed with the objective of isolation and antibiogram of *Escherichia coli* from canine pyometra. Vaginal swabs were collected aseptically from 20 canine pyometra cases, presented to Veterinary Clinical Complex, Puducherry (India) for identification of *E. coli* isolates. Based on colony characters, microscopic observation, biochemical tests, 12 (60%) *E. coli* isolates were identified. All the 12 phenotypically positive *E. coli* isolates were confirmed further by PCR using the primer targeting *alr* gene. *E. coli* isolates were found sensitive to Gentamicin (100%), Ceftriaxone (83%), Enrofloxacin (75%) and Ciprofloxacin (66%), but were resistant to commonly used antibiotics like Amoxyclav (100%), Ceftazidime (58%) and Cefotaxime (42%). The results provided an authentic and reliable information to choose an effective antibiotic to treat *E. coli* infection causing canine pyometra.

Keywords: Antibiogram, Canine, *E. coli*, Hormonal imbalance, Pyometra,

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Introduction

Canine pyometra is a life-threatening condition seen predominantly in middle-aged to older aged bitches during diestrual phase. It is characterized by the accumulation of pus within the uterine lumen, because of progesterone dominance. This hormonal imbalance occurs during the luteal phase of estrous cycle when the uterine immunity is low and the contaminating microorganisms dominate over the protective mechanisms of the female reproductive tract (Roberts, 1986; Johnston *et al*., 2001; Niyas *et al*., 2020). Normally dog uterus does not accomodate any microbes but they migrate during the proestrous and estrous phases through vagina and cervix (Watts *et al*., 1996; Niyas *et al*., 2020). High or prolonged ovarian production of progesterone causes cystic endometrial hyperplasia and inhibition of myometrial contractility. This abnormal uterine environment allows bacterial colonization to cause pyometra. The most common bacterial infection of canine pyometra is reported to be *Escherichia coli.*

E. coli is a normal inhabitant of the intestinal tract of humans and animals, and considered to be one of the notorious multi-drug resistant bacterial pathogens causing variety of clinical condition in different animal species (Sangeetha *et al*., 2016; Tamilarasu *et al*., 2020). *E. coli* has high affinity to the epithelium and endometrium of the urinary tract especially when sensitized by a high level of progesterone and leads to canine pyometra (So *et al.,* 2000; Chen *et al.,* 2003; Niyas *et al.,* 2020). It is important to identify these *E.coli* causing pyometra cases, so as to proceed for the early identification, diagnosis and appropriate antibiotic intervention to avoid fatal consequences in animals. Therefore, this study was aimed to isolate and identify *E. coli* from canine pyometra cases in Puducherry and to determine their antibiogram profile. The long treated and recurrent cases were subjected to ABST and accordingly the therapy followed.

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Materials and Methods

Collection and Processing of Vaginal Swab Samples

A total of 20 clinical cases of canine pyometra, presented to Department of Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India were used for the study. The condition was diagnosed based on history, clinical signs and ultrasonography. Sterile cotton swabs individually packed in

polypropylene tube (M/s Hi-Media, Mumbai) were used for collection of vaginal swabs from dogs. The samples, after collection, were transported to Department of Veterinary Microbiology, of the Institute within 2 h and immediately processed for culture and isolation.

Isolation and Identification of *E. coli*

Each vaginal swab from the affected bitches was subjected to enrichment in 5 mL of Luria broth individually in sterile test tube, for 18 h at 37°C. A loopful of the enriched culture was streaked onto MacConkey's agar and incubated at 37°C for 24 h. The pink coloured colonies (Lactose fermenter, Fig. 1a) obtained were subjected to Gram's staining. The Gram negative bacilli were taken up for further study following standard method in practice.

The lactose fermenting colonies were sub-cultured onto Eosin Methylene Blue (EMB) agar, in which metallic green sheen colonies (Fig. 1b) were produced by *E. coli* (Kavitha *et al.,* 2013). The conventional biochemical tests such as Catalase, Oxidase, Indole, Methyl red, Voges-proskauer, Citrate, Urease and Triple sugar iron (TSI) agar tests were used for the phenotypic identification of *E. coli* as described by Krieg and Holt (1984). The phenotypically positive *E. coli* isolates were further confirmed by polymerase chain reaction (PCR).

Polymerase Chain Reaction for Detection of *E. coli*

The preparation of template DNA from *E. coli* strains was carried out as described by Zhang *et al.* (2015). The PCR was carried out with primers targeting *alr* genes specific for *E. coli* with the product size of 366 bp as per Yokoigawa *et al.* (1999). The PCR amplification was carried out in an automated thermal cycler (Eppendorf Mastercycler, Germany) according to the following protocol: Initial denaturation at 95°C for 6 min followed by 36 cycles of denaturation at 95°C for 20 s, annealing at 72°C for 45 s and extension at 72°C for 45 s and final extension at 72°C for 5 min. The PCR products were analyzed on 1.5% agarose gel electrophoresis.

Antibiotic Sensitivity Test (ABST) of *E. coli* Isolates

Isolated *E. coli* organisms were cultured on Mueller Hinton agar to obtain a smooth, homogeneous lawn culture and subjected to antibiotic sensitivity testing using 8 different antibiotics by the disc diffusion method (Bauer *et al.,* 1966). The antimicrobial agents used for the sensitivity testing were Gentamicin (GEN, 10 µg), Amoxyclav (AMC, 30 µg), Ciprofloxacin (CIP, 5 µg), Enrofloxacin (EX, 5 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Doxycycline (DO, 10 µg) and Ceftriaxone (CTR, 30 µg). The interpretation of zone diameter was carried out according to Clinical Laboratory Standard Institute (CLSI, 2019).

Results and Discussion

Based on colony characters, microscopic observation, biochemical tests, 12/20 (60%) *E. coli* isolates were identified. All the 12 (100%) phenotypically detected *E. coli* isolates in the study were further confirmed by PCR with *E. coli* species specific primers targeting *alr* gene (Fig. 2). Similarly Chen *et al.* (2003) and Xavier *et al.* (2022) by using both phenotypic and genotypic method detected 100% (24/24) and 56% (40/72) of *E. coli* isolates from pyometra cases in bitches.

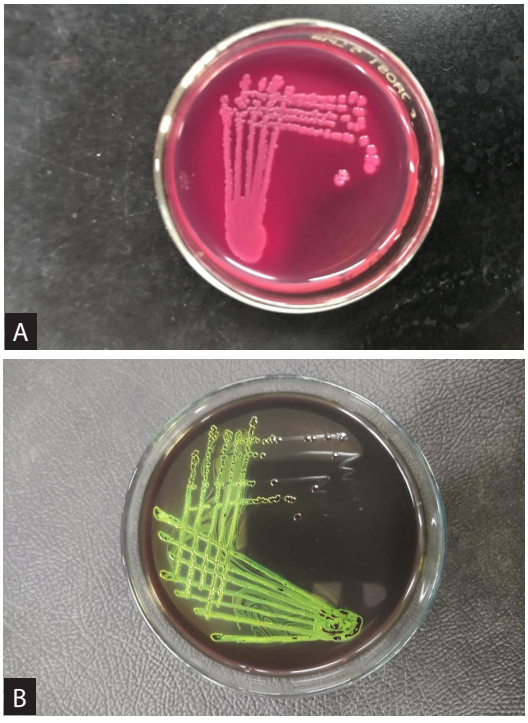

Fig. 1: (a) Lactose fermenting colonies in MacConkey's agar, (b) Metallic green sheen colonies in EMB agar.

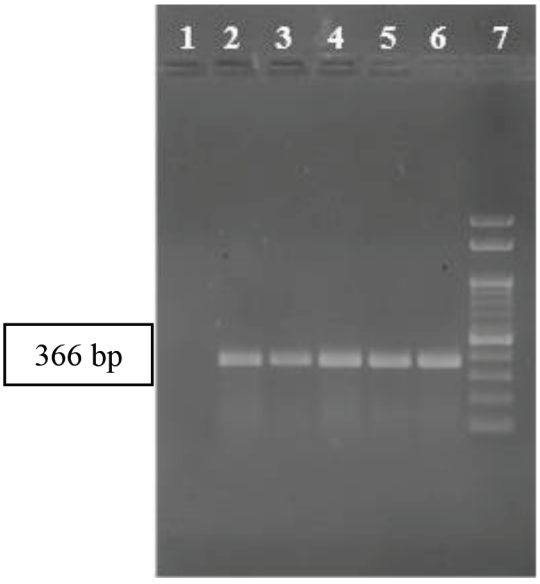

Fig. 2: Agarose gel electrophoresis showing the results of PCR amplified product of *alr* gene of *E. coli* with size 366 bp. Lane 1 & 2 Negative and Positive control, respectively; Lane 3, 4, 5 and 6 *E. coli* isolates; Lane 7 100 bp ladder.

Table 1: Antibiogram of the *E. coli* isolates (n=12)

Antibiotic	Sensitive	Intermediate	Resistant
Ceftriaxone	83% (10)	-	17% (2)
Ciprofloxacin	67% (8)	8% (1)	25% (3)
Enrofloxacin	75% (9)	-	25% (3)
Amoxyclav	-	-	100 % (12)
Doxycycline	8% (1)	67% (8)	25% (3)
Ceftazidime	25% (3)	17% (2)	58% (7)
Gentamicin	100% (12)	-	-
Cefotaxime	33% (4)	25% (3)	42% (5)

*(n)- no. of isolates

Antibiogram pattern of the *E. coli* isolates obtained from pyometra in this study showed resistant to most commonly used antibiotics (Table 1, Fig. 3). All the *E. coli* isolates were resistant against Amoxyclav (100%) followed by Ceftazidime (58%) and Cefotaxime (42%). Most sensitive antibiotics were found to be Gentamicin (100%), Ceftriaxone (83%), Enrofloxacin (75%) and Ciprofloxacin (66%). Gentamicin was detected as the most effective antibiotic which was in agreement with the previous reports of Bassessar *et al.* (2013) and Agostinho *et al.* (2014). But Gentamicin, being moderately nephrotoxic restricts its clinical usage in canine. Second most effective antibiotic was Ceftriaxone followed by Quinolones can be included for antibiotic therapy. Similar result was found by Rocha *et al.* (2021), who reported that the *E. coli* isolates were found sensitive to Ceftrioxone (83.33%) and Quinolones (66.67%), while Bassessar *et al.* (2013) reported about 55% to 65% sensitivity to Quinolone antibiotics.

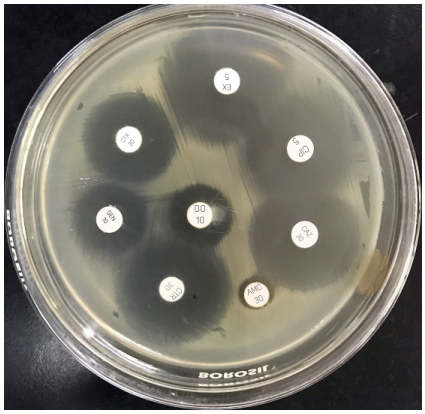

Fig 3: Antibiotic sensitivity Test (ABST) result of *E. coli* isolates

Conclusion

The present study showed that *E. coli* is the most common bacteria isolated from pyometra cases in different breeds of bitches in Puducherry region. Gentamicin and Ceftrioxone were detected as the most effective antibiotics and the isolates showed resistant to commonly used antibiotics such as Ceftazidime and Cefotaxime. Further studies are needed to determine the virulence genes of the bacteria isolated from pyometra cases in order to assess their pathogenic potential.

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