

PREVALENCE OF BIOFILM FORMING *STAPHYLOCOCCUS* SPP. IN CANINE PYOMETRA: AN AREA OF CONCERN

Surya Sankar¹, Amrutha M.S.², Arya Krishnan^{1,2}, Binu K. Mani³ and M. Mini⁴

¹Assistant Professor, ²M.V.Sc. Students, ³Associate Professor, ⁴Professor and Head, Department of Veterinary Microbiology, College of Veterinary and Animal Science, Mannuthy, Thrissur (Kerala).

[Received: 16.01.2023; Accepted: 23.05.2023]

{DOI 10.29005/IJCP.2023.15.1.94-96}

The current study was conducted to evaluate the predominant bacterial species associated with canine pyometra and its biofilm forming potential and antibiotic resistance profile. A total of 55 samples from canine pyometra was included and put for microbial investigation. 28 isolates were to be *Staphylococcus* spp. and 19 of *Escherichia coli*. Among the *Staphylococcus* spp., 12 and among *E. coli*, 6 were found to be producers. The biofilm forming species exhibited resistance to a wide repertoire of antibiotics

Keywords: Pyometra; *Staphylococcus*; Biofilm.

Pyometra is one of the most common reproductive emergencies and a serious life threatening condition affecting intact bitches. The disease is characterized by accumulation of pus in the uterus and mostly occurs secondary to the physiological changes taking place in the reproductive tract during the normal hormonal cycle, which make the tract prone to develop infection. The most common infectious cause associated with pyometra is of bacterial origin, and the predominant bacterium implicated is *Escherichia coli*, (Hagman, 2018). The other organisms implicated include *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp. and *Proteus* spp. (Rautela and Katiyar, 2019). Though the treatment of choice is ovariohysterectomy, medical management of the condition is resorted in case of valuable breeding bitches or when the patient is not a suitable candidate for an immediate surgery. Recurrences were noticed in some cases, even after successful medical management (Fieni *et al.*, 2014). One of the major reasons behind the recurrence is the problem of antibiotic resistance, and among the various factors associated with antibiotic resistance, the ability of microorganisms to form biofilm *in vivo* is a significant one (Katongole *et al.*, 2020).

Biofilm is a thick layer consisting of a heterogenous population of microbial communities embedded in extra cellular

polymeric substances, which protects the microbes from harsh external conditions and also helps to evade host immune system and various treatments directed against the infection, including antibiotic therapy. Very few literatures are available regarding the biofilm forming potential of the predominant bacterial species associated with pyometra. The present study was envisaged in that direction.

Materials and Methods

The experimental material involved guarded anterior vaginal swabs and uterine discharges collected from 55 pyometra affected bitches presented to Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai, during the period from 2021-2022. Brain Heart Infusion Agar (BHIA), MacConkey Agar (MAC), Eosin Methylene Blue Agar (EMB) and Mannitol Salt Agar (MSA) were used for isolation of the bacteria, and identification was done based on cultural, morphological and biochemical characteristics. Muller-Hinton agar (MHA) was employed to observe the antibiogram of the isolates employing Kirby-Bauer disc diffusion method. Biofilm forming potential of the *Staphylococcus* spp. and *E. coli* isolates were determined using tissue culture plate method.

Results and Discussion

Among the 55 samples, 28 isolates of *Staphylococcus* spp. and 19 of *E. coli* were recorded. The isolates were subjected to antibiogram using the common antibiotics employed for pyometra, which included amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftriaxone-tazobactam, co-

trimoxazole, ciprofloxacin, enrofloxacin, gentamicin, metronidazole and tetracycline. The results are depicted in table 1. All isolates were found to be multi-drug resistant, showing resistance to at least two classes of antibiotics.

Table 1. Antibiogram of *Staphylococcus* spp and *Escherichia coli*

Isolates	Sensitivity	Resistance (100 per cent)
<i>Staphylococcus</i> spp	50 per cent sensitivity towards Tetracycline followed by Enrofloxacin, Co-trimoxazole	Amoxicillin-clavulanic acid, Ceftriaxone, Ceftriaxone-tazobactam, Ciprofloxacin, Gentamicin, Metronidazole
<i>Escherichia coli</i>	70 per cent sensitivity towards Tetracycline followed by Enrofloxacin, Co-trimoxazole	Amoxicillin-clavulanic acid, Cefotaxime, Ceftriaxone, Ciprofloxacin, Metronidazole

In tissue culture plate method, among the *Staphylococcus* spp. 12 Out of 19 *E.coli* isolates, 6 were biofilm producers. Other bacterial isolates obtained in the study included *Streptococcus* spp. *Klebsiella* spp. and *Pseudomonas* spp. In the present study, *Staphylococcus* spp. were predominant followed by *E.coli*. Similar observations were made by Khan *et al.*, 2007 and Singathia *et al.*, 2013, where, *Staphylococcus* spp. were the predominant organism from cases of pyometra followed by *E. coli* and *Pseudomonas* spp. *Staphylococcus* was the predominant bacterial spp identified in canine pyometra as also reported by Niyas *et al.*, 2020,.

Most isolates of *Staphylococcus* spp. were sensitive to tetracycline followed by enrofloxacin and co-trimoxazole. Isolated *E. coli* and *S. aureus* from cases of pyometra and these isolates were more susceptible to enrofloxacin, followed by norfloxacin, nalidixic acid, chloramphenicol, trimethoprim-sulfamethazole, tetracycline and gentamicin. The biofilm forming isolates of *Staphylococcus* spp. and *E. coli* showed high degree of resistance to a wide repertoire of antibiotics compared with non-biofilm producers. Similar observations were reported

by Singh *et al.*, 2017, where biofilm forming *Staphylococcus* spp. exhibited a high rate of antibiotic resistance. In contrast to various studies on pyometra which documented *E. coli* as the predominant bacterial spp. associated, the current study documented *Staphylococcus* spp. as the most prevalent organisms in comparison with *E.coli* and other bacterial spp. This is an area of concern because *Staphylococcus* spp. normally resides as commensals on the skin of animals and human and are considered as opportunistic pathogens. The multi-drug resistant biofilm forming strains of these organisms, in addition to causing recurrence and life threatening conditions in canine pyometra, could act as potential source of transfer of antibiotic resistance to humans who live in close proximity with companion animals. The biofilm forming *Staphylococcus* spp, are responsible for many life threatening conditions in humans, since they associate with indwelling medical devices like catheters, stents and valves. Also, the resistance could be transferred to normal commensal spp. also making the therapy of many clinical conditions in humans and animals worse.

Reference

- Fieni, F., Topie, E. and Gogny, A. (2014). Medical treatment for pyometra in dogs. *Reprod. Domest. Anim.*, **49**: 28-32.
- Hagman, R. (2018). Pyometra in small animals. *Vet. Clin. Small Anim.*, **48**: 639–661.
- Katongole, P., Nalubega, F., Florence, N.C., Asiimwe, B. and Andia, I. (2020). Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic *Escherichia coli* isolated from clinical isolates in Uganda. *BMC Infect. Dis.*, **20**: 453.
- Khan, L.A., Raghuwanshi, D.S., Vhora, S.C., Utage, S.G. and Khan, W.A. (2007). Cultural and sensitivity pattern of bacterial flora of canine pyometra. *R. Vet. J. India.*, **3**: 89-91.
- Niyas, E., Reshma, S., Shrivya, G.S., Jayakumar, C., Kumar, R.H.N., Sarika, N. and Easaw, A.M. (2020). AntibioGram of isolated organisms in canine pyometra. *Int. J. Current Microbiol. Appl. Sci.*, **9**: 263-268.
- Rautela, R. and Katiyar, R. (2019). Review on canine pyometra, oxidative stress and current trends in diagnostics. *Asian Pac. J. Reprod.*, **3**: 45-55.
- Singathia, R., Gupta, S.R., Yadav, R. and Lakhotia, R.L. (2013). AntibioGram profile of bacteria isolated from canine pyometra. *Vet. Prac.*, **14**: 242-243.
- Singh, A.K., Prakash, P., Achra, A., Singh, G.P., Das, A. and Singh, R.K. (2017). Standardization and classification of in vitro biofilm formation by clinical isolates of *Staphylococcus aureus*. *J. Glob. Infect. Dis.*, **9**: 93-101.