BACTERIAL RESISTANCE AND ANTIBIOTIC SENSITIVITY PATTERN IN RELATION TO THERAPEUTIC MANAGEMENT OF CANINE PYOMETRA

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By

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CERTIFICATE – I

This is to certify that the thesis entitled "**BACTERIAL RESISTANCE AND ANTIBIOTIC SENSITIVITY PATTERN IN RELATION TO THERAPEUTIC MANAGEMENT OF CANINE PYOMETRA**" submitted in partial fulfilment of the requirement for the award of the degree of Master Veterinary Science in the discipline of **Veterinary Gynaecology & Obstetrics** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is a bonafide research work carried out by **Dr. DEEPSHIKHA RAJ, Registration No-VM0018/2019-2020**, daughter of **Shri. RAJKUMAR MAHTO** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledged.

Dr. Dipyaman Sengupta
Major Advisor

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CERTIFICATE – II

This is to certify that the thesis entitled, "**BACTERIAL RESISTANCE AND ANTIBIOTIC SENSITIVITY PATTERN IN RELATION TO THERAPEUTIC MANAGEMENT OF CANINE PYOMETRA**" submitted by **Dr. DEEPSHIKHA RAJ, Registration No-VM0018/2019-2020**, daughter of **Shri. RAJKUMAR MAHTO** to the Bihar Animal Sciences University, Patna in partial fulfilment of the requirements for the degree of Master Veterinary Science.in the discipline of **Veterinary Gynaecology & Obstetrics** has been approved by the Student's Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Place _____

Date _____ *(Deepshikha Raj)*

CONTENTS

ABBREVIATIONS

ºC	:	Degree Centigrade
kg	:	Kilo-gram
g	:	Gram
mg	:	Milli-gram
µg	:	Micro-gram
ng	:	Nano-gram
pg	:	Pico-gram
µl	:	Micro-liter
ml	:	Milli-liter
dl	:	Deci-liter
mm	:	Milli-meter
cm	:	Centi-meter
L	:	Liter
IU	:	International Unit
U	:	Unit
I/M	:	Intra-muscular
S/C	:	Sub-cutaneous

CEH	:	Cystic Endometrial Hyperplasia
BUN	:	Blood Urea Nitrogen
GH	:	Growth Hormone
$PGF_{2\alpha}$	:	Prostaglandin 2Alpha
ALT	:	Alanine Aminotransferase
AST	:	Aspartate Aminotransferase
EUCAST	:	The European Committee on Antimicrobial Susceptibility Testing
CLSI	:	The Clinical and Laboratory Standards Institute
MIC	:	Minimum Inhibitory Concentration
BHI	:	Brain Heart Infusion
UTI	:	Urinary Tract Infections
MH	:	Mueller Hinton
MDR	:	Multiple Drug Resistant
TNF-α	:	Tumour Necrosis Factor- Alpha
IFN-γ	:	Interferon- Gamma
IL	:	Interleukin
LPS	:	Lipopolysaccharide

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Introduction

Background

Pyometra is the accumulation of pus in the uterine lumen and is one of the most commonly reported reproductive disorders of bitches in Veterinary Clinical Complex. The incidence of pyometra is more common in un-spayed bitches of all ages but more common in older age. Due to prolonged pro-estrus and estrus, the reproductive tract is under estrogen dominance that result in cervix to remain partially open (Egenvall *et al.*, 2001). This will allow the entry of bacteria from the anterior vagina to the uterus (ascending infection). The high Progesterone level during met-estrus promotes glandular secretion that acts as an ideal media for bacterial proliferation.

Rationale and Research Gap

The most common bacteria isolated in cases of canine pyometra are *Escherichia coli* (90%) and anaerobic bacteria such as *Bacteroids sp. and Fusobacterium sp.,* (Green and Prescott 2006; Bartoskova *et al.*, 2007 and Verstegen *et al.*, 2008). The most effective antibiotics reported against these pathogens are Ceftiofur Sodium and Levofloxacin - Ornidazole. These antibiotics have been reported to deposit in the uterus at concentration above the MIC level of these pathogens (Fieni *et al.,* 2014). Ceftiofur Sodium has been reported to be sensitive against anaerobic organism (Dow, 1957). Infact many cases presented in the clinics are treated successfully by these antibiotics. However, recently there is increase in the number of cases of canine pyometra that are refractory to these antibiotics which need further study. These cases are referred to surgery for ovario-hysterectomy (Coggan *et al.*, 2008 and Otto, 2007).

There is paucity of information available regarding the organisms present in canine pyometra, resistant to the aforementioned antibiotics and their pattern of antibiotics sensitivity. Moreover, a detailed study is warranted to test their clinical efficacy in cases of canine pyometra. Therefore, this work seeks to isolate the resistant bacteria from aseptically taken pus samples from the excised uterus in cases of canine pyometra following treatment with levofloxacin- ornidazole and Ceftiofur sodium and test their antibiotics sensitivity as well as clinical efficacy. The finding of this work will assist the clinicians to select the correct combination of antibiotics in these refractory cases of canine pyometra.

Objectives

The main objectives of this work are:

1. To assess the percentage of clinical efficacy in case of canine pyometra treated with Ceftiofur Sodium & Levofloxacin Ornidazole.
2. Isolation of resistant pathogens from cases of canine pyometra refractory to the treatment with Ceftiofur Sodium and Levofloxacin Ornidazole.
3. To test the antibiotic sensitivity of the resistant pathogens.

REVIEW OF LITERATURE

1. Incidence of canine pyometra in India & Abroad

Bosschere *et al.* (2001) studied that pyometra occur mostly during estrus cycle of bitch. There are number of morphologic changes that occur in canine uterus due to the influence of progesterone and estrogen level. Cystic endometrial hyperplasia (CEH) develop in the uterus due to abnormal response of ovarian hormone, mainly due to chronic or repeated progestational stimulation during the luteal phase of the estrous cycle which cause the accumulation of fluid within the endometial glands and the uterine lumen. Canine pyometra occur due to interaction of bacteria and hormonal which is predisposing factor in the development of the disease. Therefore it is refers to as the cystic endometrial hyperplasia-pyometra complex.

Cock *et al.* (2002) studied that certain growth factors are responsible for canine pyometrawhich cause inflammation of the canine uterus. Several growth factors, their receptor and regulatory protein all are found in the endometrium of canine uterus. Insuline-like growth Factor-I (IGF-I) is most important growth factor and it has a potential mitogenic effect on the uterus. IGF-I is a single- chain polypeptide with structural homology to pro-insulin. It regulates proliferation and differentiation of a multitude of cell types and capable of exerting insulin- like metabolic effect. IGF-I is mainly regulated by growth hormone. Endogeneously or exogenously injecting GH or a Progesterone induced increase in GH is associated with rising serum IGF-I levels. IGF-I causes excessive endometrial proliferation found in dogs with pyometra/ CEH (Cystic endometrial hyperplasia).

Bigliardi and Pamigiani (2004) reported that canine pyometra develops only during the diestrus period of estrus cycle, more specifically between 20 -70 days after heat. During the estrus period estrogen level is high and promotes over proliferation of endometrium and lengthens the period in which the uterine cervix remains open. This period is followed by prolonged interval of progesterone dominance during the diestrus period. Progesterone causes endometrial proliferation with increased uterine glandular secretions and decreased myometrial contraction and closing of cervix.

Pretzer (2008) studied that pyometra mostly occur in mature bitches which have undergone repeated estrous cycling and they were found at mean age of 7.25 years.

Nulliparous bitches and their age greater than 4 years were more prone to pyometra. Hormonal therapies such as progestins for estrus suppression or estrogen for estrus induction or pregnancy termination was the main cause of development of pyometra in young bitches and anatomic abnormalities of the vagina and vestibule, such as strictures and septum, was also predisposed factor for development of these conditions in young bitches.

Baithalu *et al.* (2010) reported that pyometra affects bitch irrespective of age and mostly occurs after first estrus cycle. Pyometra occurs in bitches as young as four months to as old as 16 years of age. Incidence of pyometra was more in nulliparous bitches as well as in bitches of >4years of age, with an increased frequency in 7-8years old bitches.

Simon *et al.* (2011) reported that genetic factors are responsible for pyometra. Higher prevalence was observed in the Collie and Belgian shepherd breed and low prevalence in the Dachshund and Poodle breed. Some bitch breeds such as Golden Retriever, Irish Terrier, Saint Bernard and Rottweiler were observed to be at higher risk. In Indian conditions, breeds such as Spitz, Labrador, Alsatian, Doberman Pinscher, Boxer, Daschund and Rottweiler were more affected than Golden Retriever, Spaniel, Irish Terrier.

Jitpean *et al.* (2014) studied that pyometra is the most common disease in intact bitches which affects approximately 25% of dogs before 10 years of age. They reported that it's incidence rates is greater in older bitches and also depended on breeds of bitch. The age range for pyometra was one to 15 years. Most of the bitches were > 10 years old. Pyometra most probably occured in estrous cycle.

Kumar *et al.* (2019) studied that canine pyometra or chronic purulent endometritis is the most frequent disease of the reproductive tract and is occurring in middle-aged to old bitches during diestrus period.It mostly affects nearly one fourth of all female dogs before they reach ten years of age. Incidence of canine pyometra in nulliparous (58.33%) as compared to parous (41.67%) dogs.

Rautela and Katiyar (2019) studied that the incidence is very high in bitches of <10 year of age and specifically between 6-8 years. It is the most common and serious reproductive disorders that occur during diestrus period in adult intact bitches. Among the breeds, Rottweiler, Saint Bernard, Chow Chow, Golden Retriever,

Miniature Schnauzer, Irish Terrier, Spaniel and Collie are most susceptible, while German Shepherd, Daschund are less susceptible to pyometra. In Indian conditions, breeds such as Spitz, Labrador, Alsatian, Doberman Pinscher, Boxer, Daschund, Rottweiler are more affected than Golden Retriever, Spaniel, Irish Terrier. The nulliparous bitches found to be 75.00% to 77.78% of all pyometra cases. The condition develops only during diestrus period of estrous cycle, more specifically between 20-70 days after heat.

Singh *et al.* (2020) studied that one of the most common uterine pathologies of intact bitch at middle to advanced age is pyometra. Middle to aged nulliparous bitches or those frequently exposed to hormonal therapy are at high risk of developing cystic endometrial hyperplasia, pyometra and neoplasia of the uterus, ovaries and mammary glands mature bitches develop pyometra at an average 7.25 years of age due to recurrent oestrous cycles. Pyometra can be occurs 15-20 days after oestrus or may also appear at proestrus, post-mating or even at anestrus stage. 75% cases of canine pyometra found higher in nulliparous bitches. Breed and genetic factors strongly predispose to the development of pyometra. In India, the breeds at high risk of development of pyometra include: Labrador, Spitz, German Shepherd, and Dalmatian; however, Doberman, Dachshund, Great Dane, Pug, Boxer, Lhasa Apso, Cocker Spaniel, Saint Bernard, English Bulldog and Neapolitan Mastiff show lower risk of pyometra. It is highest in the Labrador (28.89%), followed by Spitz (22.22%), non-descript (20.00%) and German Shepherd (8.89%). It is relatively lower in the Doberman, Pug, Saint Bernard, Rottweiler and Dalmatian breeds.

Chandrakar *et al.* (2021) studied that factor responsible for pyometra was the age, breed and parity. The incidence of pyometra was higher (61%) in bitches above 5 years of age than bitches upto 5 years (39%) of age. Pyometra is a disease of middle aged and older bitches due to the repetitive exposure to the normal long luteal phase of the estrous cycle. Younger bitches were more prone to pyometra in absence of hormonal treatment. Non-descript breed and Pomeranian (28%) followed by Labrador (22%), German Shepherd (17%) and Pug (5%) were more prone to pyometra. The majority of the pyometra cases seen in Spitz, followed by German Shepherd, Pomeranian, Cocker Spaniel, Doberman and Lhasa Apso. Smaller breeds were affected more than the large breeds of dogs. Nulliparous bitches were having highest incidence of pyometra (61%) cases followed by primiparous (28%) and pluriparous (11%) bitches.

2. *Progesterone favouring the growth of uterine pathogen*

Nelson and Feldman (1986) studied that the plasma progesterone concentration during anestrus is relatively low (less than 0.5ng per ml) in the bitch and it's level increased after 9 to 12 weeks following ovulation in each estrous cycle, often exceeding 40 ng per ml. During this period, progesterone promotes or supports endometrial growth and glandular secretion while suppressing myometrial activity and result in allowing accumulation of uterine glandular secretions. These secretions provide an excellent environment for bacterial growth. Bacterial growth is further enhanced by inhibition of the leukocyte response to infection in the progesterone-primed uterus.

Bosschere *et al.* (2001) studied that pyometra caused due to exposure of uterus to chronic or repeated progestational secretion during the luteal phase of the estrous cycle, with accumulation of fluid within the endometrial glands and the uterine lumen. The endometrium is thickened due to an increase in the size and number of endometrial glands. The hyperplastic and hypertrophic endometrial glands have an increased secretory activity, and sterile fluid may accumulate in the glands and in the lumen of the uterus. These secretion provide ideal media for bacteria proliferation and causes pyometra.

Noakes *et al.* (2001) studied the use of hormones progestagens for estrus suppression and found that uterine biopsies, scarification and uterine irritants such as suture material are also the other contributing agents of endometritis-pyometra complex. It is believed that inflammation of canine uterus occurred due to late administration of progestagensin pro-oestrus period in order to break the heat.

Fransson and Ragle (2003) studied that Progesterone stimulate endometrial glandular secretion and suppress contractions of the uterus which creating an intrauterine environment predisposed to bacterial growth. During progesterone influence in diestrus condition cause the bacteria proliferate in the uterus and cause infection in uterus.

Leitner *et al.* (2003) studied that normally progesterone favours the expression of glycocalyx on apical cell surface of uterine epithelium which is required for embryo recognition and implantation. In CEH, bacteria attach to these "glycocalyx" sugar residues which are found in the uterine glandular regions together with epithelial surface. The adherence of bacteria to endometrial glandular and epithelial cells is mediated by the fimbriae present on bacteria and cause canine pyometra.

Pretzer (2008) studied that Cystic endometrial hyperplasia develops due to repeatedly stimulation of progestational hormone during the luteal phase of the estrous

cycle in bitches. During diestrus period, progesterone hormone gets dominant which increases endometrial gland secretory activity, increases endometrial proliferation, decreases myometrial contractility, and causes closure of the cervix. Due to high progesterone concentration in the early luteal phase cause the suppression of cellular immunity which reduced local immunity and provide ideal uterine conditions for bacterial colonization.

Verstegen *et al.* (2008) studied that progesterone play very important role in the pathogenesis of pyometra. It suppress the immune responses, stimulate the endometrial gland secretions which provide a suitable environment for bacterial growth, functional closure of the cervix which inhibits drainage of uterine exudates, and mediation of cystic endometrial hyperplasia. Pyometra was caused by excessive or prolonged exposure to progesterone, if exogenous progesterone administer.

Baithalu *et al.* (2010) reported that progesterone plays very important role in initiating the pathogenesis of Cystic Endometrial Hyperplasia (CEH)-Pyometra complex. During follicular phase, the estrogen level remains high and cause the cervix to remain open and allow the entrance of bacteria into the uterus followed by high progesterone level which suppress the myometrial contraction, proliferates the endometrium and provide good environment for bacterial growth. Progesterone also inhibits the neutrophil migration favouring canine pyometra.

Dennis and Hamm (2012) studied that canine pyometra caused by chronic recurrent exposure of the endometrial lining to progesterone produced by the corpus luteum during diestrus. Progesterone binds to uterine receptors and induces endometrial gland proliferation, stimulates endometrial gland secretions, decreases myometrial contractility, and induces closure of the cervix. Progesterone has also interfere with immune function within the uterus and increase its susceptibility to bacterial infection. Progesterone's effect on the endometrium is cumulative from reproductive cycle to reproductive cycle. Due to accumulating uterine secretions, prominent endometrial gland crypts, and immune suppression caused by progesterone stimulation during diestrus make the uterus an ideal environment for bacterial proliferation leading to pyometra. Also term cystic endometrial hyperplasia-pyometra complex.

Kempisty *et al.* (2013) reported that the progesterone concentration (>40ng/ml) increases after the ovulation and promotes endometrial growth (hyperplasia) and glandular secretion; accumulation of this uterine glandular secretion provides excellent media for

bacterial growth. In the presence of progesterone, the uterine migration of leucocytesis also inhibited which results in the growth of infection. Progesterone also suppresses the myometrial contractions and facilitates the closing of cervix, which enhances the bacterial proliferation in uterine lumen.

Hayati *et al*. (2016) studied that the corpus luteum starts to produce progesterone 24–48 h after ovulation. Progesterone causes hyperplasia of the endometrium, especially the epithelium and endometrial glands, cervix closure, increase in endometrial gland secretion and a decrease in myometrial contractibility. Estrogens production increase progesterone receptors in the endometrium, dilate the uterine cervix, allow bacterial ascent and can influence endometrial changes Changes in the uterine microenvironment and decreased contractility would favour ascendant bacterial infection; *E. coli* are the most frequently isolated microorganisms.

3. *Common bacterial pathogen presents in canine pyometra*

Nelson and Feldman (1986) studied that source of the bacteria that cause a uterine infection is the resident bacteria of the vaginal vault and these bacteria have the potential of ascending through the relatively dilated cervix and into the uterus during proestrus and estrus period. A predominance bacteria found was *Escherichia coli* in uterine infections along with *Staphylococci, Streptococci, Pseudomonas* and *Proteus.*

Watts *et al.* (1996) isolated different types of bacteria from the selected cases of canine pyometra on the basis of cultural, morphological and biochemical characterization and they were identified as *Escherichia coli, Staphylococcus sp., Streptococcus sp., Pseudomonas sp.* and *Bacillus sp.* Major isolates were *E. coli, Staphylococcus sp*. followed by *Streptococcus sp.* and *Pseudomonas sp.* and the isolated microbes in the order of frequency were *Escherichia coli, Haemophilus* species, *α-haemolytic streptococci, Corynebacterium* species, *Streptococcus canis, Alcaligenesfaecalis, Bacteroides sp., Pasteurella sp.* and *Proteus mirabilis.*

Fransson *et al.* (1997) reported that the bacteria causing pyometraisolated from pus filled uterus were *Escherichia coli (E. coli), Staphylococcus aureus, Steptococcus sp., Pseudomonas sp.,* and *Proteus sp*. Bacteria causing sub-clinical urinary tract infection were also responsible for pyometra cases. *E. coli* bacteria was isolated from 90% of bitches having pyometra. The lipopolysaccharide released from Gram-negative bacteria results in

dysfunction of leucocytes. The endotoxins released from Gram-negative bacteria causes uncontrolled production of inflammatory mediators. These mediators cause irreversible damage to internal organs, sepsis and death of animal

Johnston *et al.* (2001) reported that the key feature of canine pyometra is the hormonal imbalance which 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. The most commonly isolated organism included *Escherichia coli* a member of the normal vaginal flora and additional bacteria isolates included *Staphylococcus, Streptococcus, Pseudomonas, Proteus* and *Pasteurella.*

Chen *et al.* (2003) reported that the bacteria present in canine pyometra are same as found in the normal microflora of the vagina of healthy bitches. The main bacteria "*Escherichia coli"* was isolated from pus filled uterus which was naturally found in the vaginal flora that entered into the uterus during pro-estrus and estrus period. It was reported that the urinary tract served as a bacterial reservoir and bacteria ascend into the uterus during a susceptible stage in the estrus cycle.

Smith (2006) reported that the hormonally compromised uterus becomes better environment for infection by the opportunistic bacteria that are found in normal vaginal microflora. Bacteria proliferate in the lumen containing excessive secretory fluid. The presence of adhesive factors and cysts along with local tissue and leukocyte inhibition reducing local immunity favour bacterial growth. With each estrous cycle the effect gets cumulative resulting in the uterine pathology.

Susi *et al.* (2006) reported that in about 90% of cases of canine pyometra, bacteria *Escherichia coli* is the main causal agent together with other perennial faecal bacteria like *Streptococcus sp., Staphylococcus sp., Klebsiella sp., Proteus sp., and Pseudomonas sp.* These bacteria might often damage the uterus and produce clinical symptoms, especially if these bacteria have not been cleaned out of the uterus at the beginning of the luteal phase.

Pretzer (2008) studied that the most common bacteria "*Escherichia coli*" is isolated in cases of canine pyometra which is usually found in the faeces of affected bitches. Usually uterus gets contaminant by bacteria just prior to diestrus period when the cervix is open, and in cases of CEH, the bacteria cannot be cleared prior to the luteal phase, leaving opportunistic organisms in an ideal environment for colonization and proliferation. *E. coli* is a particularly dangerous organism due to endotoxin release which may result in septic shock.

Verstegen *et al.* (2008) studied that primarily *Escherichia coli* present in the uterus and it is opportunistic pathogens which invade from the vagina. It will proliferate and establish infection within the uterus due to excessive amounts of secretory fluids accumulated within the lumen, the presence of numerous crypts and cysts where bacteria can proliferate, and reduced local immunity, either associated with or resulting from local tissue degeneration. The bacteria most frequently isolated from the uterus in case of pyometra include *Staphylococcus aureus, Streptococcus sp., Pseudomonas sp.,* and *Proteus sp.*

Cramer (2010) studied that *Escherischia coli,* beta-haemolytic *streptococci*, *Pasteurella multocida*, coagulase positive *Staphylococcus sp.* and *Klebsiella sp*. are amongst the organisms mostly isolated from pyometra cases.

Krekeler *et al.* (2012) reported that the most common bacteria found in almost 70% of all pyometra cases was *Escherichia coli*. Prevalence of this high proportion of *E. coli* might be due to their natural presence in the vaginal passage and theymove to the uterus during pro-estrus and estrus phase. After gaining entrance into theuterine tissue, *E. coli* colonize and proliferate in the epithelial lining of the uterus. Certain pathogenic strains of *E. coli* are having virulence factors that have the ability to bind specific receptors in the endometrium of the canines.

Kitshoff *et al.* (2015) studied that bacteria *Citrobacter diversus* (3.0%*), Morganella morgani* (1.0%) *and Corynebacterium jeikeium* (1.0%) were found in canine pyometra. *Enterobacter* (5.7%), *Actinomycetaceae sp.* (2.4%), unidentified gram negative (0.8%) and unidentified gram positive (0.8%) bacteria were also associated with canine pyometra.

4. *Role of Prostaglandin in the treatment of canine pyometra*

Pharriss *et al.* (1970) studied that the prostaglandins induce regression of the corpora lutea (luteolysis) by constricting the flow of blood in the utero-ovarian vein responsible for oxygen delivery, and thus, decreasing the flow of blood to the ovaries. As the corpora lutea regress, progesterone production drops and cause the relaxation (opening) of the cervix, allowing the uterine contents to escape. Prostaglandins also directly stimulate myometrium contraction, there by promoting expulsion of the infected uterine contents.

Nelson *et al.* (1982) studied that $PGF_{2\alpha}$ is effective in the treatment of metritis or open pyometra in healthy young bitches with normal kidney and liver function, and in the

absence of uterine hypertrophy. The use of prostaglandin is not indicated in cases of closed pyometra due to the risk of peritonitis, following the forced passage of purulent fluid up to the uterine tubes into the ovarian bursae and out into the peritoneal cavity, or through rupture of the uterine wall.

Nelson and Feldman (1986) studied that prostaglandin$F_{2\alpha}$ has several physiologic effects on the female reproductive system. It causes contraction of the myometrium and relaxation of the cervix which result in expulsion of the exudate from the uterus. Lysis of the corpora lutea or transitory inhibition of luteal steroidogenesis is done by $PGF_{2\alpha}$ which results in decreased plasma progesterone concentration and reduces the stimulus for endometrial growth and glandular secretion. They reported that $PGF_{2\alpha}$ also has side effect, such as restless, pacing, hypersalivation and occasional panting followed by some or all of the following: abdominal pain, tachycardia, fever, vomiting, and defecation. Therefore they opined that age, body condition, any diseases, etc. must be considered before administration of $PGF_{2\alpha}$.

Arnold *et al.* (1988) studied that the $PGF_{2\alpha}$ is effective in treatment of pyometric bitches without obvious hormonal imbalance, if given at low doses @ 20µg/kg body weight three times daily on consecutive days

Fransson and Ragle (2003) studied that $PGF_{2\alpha}$ causes contraction of the myometrium and relaxation of the cervical canal which leads to expulsion of exudate from the uterine lumen. The side effects shown by $PGF_{2\alpha}$ include abdominal pain, emesis, defecation, tachycardia, hypersalivation, dyspnea, panting and fever. A low-dosage treatment (0.025 mg/kg injected S/C q 12h for 5 days or to effect) of a natural $PGF_{2\alpha}$, such as dinoprost tromethamine, has been shown to be effective with fewer side effects. Intravaginal administration of $PGF_{2\alpha}$ has been used with no side effect. . A natural $PGF_{2\alpha}$ (such as dinoprost tromethamine) at a dose of 0.15 mg/kg (0.3 ml/10 kg) was infused vaginally using a sterile plastic catheter. Immediately after infusion, the hindquarters of the animal were raised for 3 to 5 minutes to prevent loss of the infused substance. Recurrence of pyometra was not noted within the 12 months following treatment.

Feldman and Nelson (2004) studied several physiologic effects of prostaglandin ($PGF_{2\alpha}$) on the female reproductive system such as contraction of the myometrium and reduction of circulating progesterone concentration level. It also causes relaxation of the cervix. Myometrial contraction results in expulsion of exudates from the uterus. The

primary function of the corpora lutea is to synthesis and secrete the progesterone hormone which is responsible for canine pyometra. Prostaglandin cause the lysis of corpora lutea and transitory inhibition of luteal steroidogenesis. Thus, $PGF_{2\alpha}$ has both luteolytic and uterotonic properties.The adverse effect of injecting prostaglandins such as abdominal discomfort, vomiting, defecation, urination, tachycardia, restlessness, anxiety, fever, hyper-salivation, dyspnea, or panting which usually occur within minutes of administration and can persist for up to an hour or more afterward. To minimizing the severity of these adverse effect, walk with the patient for 20 to 60 minutes. If the adverse effects are persistent or severe, pre-treatment with anti-cholinergics and anti-emetics has been suggested.

Hagman *et al.* (2006) reported that prostaglandins originate from arachidonic acid and it play very important roles in reproduction and inflammation. Uterine tissue is synthesis and release prostaglandins during inflammation and mainly prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$). The metabolite 15-keto-13, 14-dihydro-$PGF_{2\alpha}$ (PG-metabolite) is more stable in circulating blood than $PGF_{2\alpha}$. The concentrations of PG-metabolite are highly elevated in bitches with pyometra.

Verstegen *et al.* (2008) studied that the repeated administration of $PGF_{2\alpha}$, causes luteolysis which reduces plasma progesterone concentrations. Reduction in progesterone concentrations induces cervical relaxation, a decrease in uterine secretions and since prostaglandins also have a uterine spasmogenic action, the expulsion of uterine fluid. Higher doses of prostaglandins are associated with substantial adverse effects, including salivation, vomiting, straining, diarrhea, pyrexia, some occasional respiratory distress, as well as cases of shock and death. $PGF_{2\alpha}$ apart from its luteolytic effects mediates functional opening of the cervix, which permits drainage of exudate, and promotes myometrial contractions, facilitating uterine drainage.

Cramer (2010) studied that Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) facilitates smooth muscle contraction in the uterine wall leading to expulsion of uterine contents in cases of pyometra. The flushing of the uteri may also have had the effect of releasing endogenous prostaglandins which in turn could have induced luteolysis, uterine motility and expulsion of septic debris. Prostaglandin F_{2alpha} ($PGF_{2\alpha}$) involves induction of luteolysis, stimulation of uterine contraction and cervical dilation.

Hamm and Dennis (2012) studied as the corpora lutea regress, progesterone production drops. The reduction in progesterone concentrations promotes relaxation (opening) of the cervix and allowing the uterine contents to escape. Prostaglandins also directly stimulate myometrium contractions thus promoting expulsion of the infected uterine contents. They reported some adverse effects of prostaglandin such as abdominal discomfort, vomiting, defecation, urination, tachycardia, restlessness, anxiety, fever, hypersalivation, dyspnea and panting.

Jena *et al.* (2013) studied that in order to conserve breeding capability of valuable females, repeated administration of prostaglandins can be used as medical treatment which causes lysis of the corpora lutea and a reduction in plasma progesterone concentration. Low progesterone level causes relaxation (opening) of the cervix and allows expulsion of the uterine content which cause reduction in uterine secretions and increased uterine contractions. Prostaglandins have also a uterine spasmogenic action which cause the expulsion of uterine fluid. As corpora lutea of bitches are insensitive to the effects of administered prostaglandins but repeated doses cause luteal regression. Prostaglandins also cause adverse effects such as salivation, vomiting, diarrhoea, pyrexia and occasionally respiratory distress.

Kumar *et al.* (2019) studied that Prostaglandin $F_{2\alpha}$ and systemic antibiotics are the most effective medical therapy in pyometric bitches to evacuate the uterine fluid and increase uterine defense mechanism. They administered Prostaglandin $F_{2\alpha}$ @ 0.10–0.20 mg/kg S/C. Prostaglandin $F_{2\alpha}$ administered parenterally can cause side effects such as salivation, vomiting, diarrhoea, hyperpnoea, ataxia, urination, anxiety and pupillary dilatation followed by contraction. These side effects may last for up to 120 min after Prostaglandin $F_{2\alpha}$ administration.

5. Haemato Biochemical parameters in cases of pyometra in bitch

Rechfeld (1954) studied that the average leucocyte count in clinical cases of canine pyometrawas 44,800 to 1,66,400 and found significant increase in the number of immature neutrophil.

Renton *et al.* (1971) studied that the total white cell count was a useful diagnostic feature in differentiating pyometra and early pregnancy. In closed or open type pyometra,

the white cell count was increased from normal to 20,000 per cm. The mean of total white cell count in closed type was higher than that of open type.

Hardy and Osborne (1974) studied that total white cell count was higher in closed cervix pyometra than that in open cervix pyometra and toxic state produced non-regenerative normocytic normochromic anaemia and a degenerative left shift. Anaemic in dogs with pyometra is due to either toxic depression of bone marrow and /or loss of red cells into the uterine lumen.

Greene (1984) studied that a non- regenerative microcytic hypochromic anaemia with higher WBC count was more which indicated more blood loss. When number of immature neutrophil exceeds the number of segmented neutrophil, indicates the presence of intense suppurative diseases with more chronic character.

Jain (1986) studied about the total leucocyte count (TLC) and differential leucocytes counts (DLC) as per routine clinical test. The leukocytes were moderate (16,999-29,999cells/mm^3) in one, marked (29,999-50,000 cells/mm^3) in two and extreme (>75,000cells/mm^3) in two. Neutrophil was 80% in two, marked (>80-99%) in four bitches and extreme in (>90%) in two bitches. In dogs, the normal range of TLC is 6,000-17,000 cells /mm^3 while that of neutophil is 60-77%. So moderate to extreme leukocytes and neutrophil in these cases may help to diagnosis these cases as pyometra

Sokolowski (1992) studied that white blood cell count in excess of 30,000per cm was a more common finding in bitches suffering from pyometra. They assessed ESR and blood urea concentration and found that urea concentration had no diagnostic value for the cystic endometrial hyperplasia/pyometra syndrome. ESR could be used for confirmation of diagnosis and to confirm negative cases. The cut-off values for ESR associated with the highest sensitivity and specificity was 12mm/hour.

Feldman and Nelson (1996) studied that during anestrus phase, the plasma progesterone concentration is low (<0.5 ng/ml). Progesterone concentrations remain below 1.0 ng/mL during proestrus and then begin to rise at the onset of estrus, typically being greater than 2.0 ng/mL. Progesterone concentrations continue to increase throughout estrus and the first several weeks of metestrus, then a slow return toward basal concentrations. The return to concentrations less than 1.0 ng/mL marks the end of metestrus

Bartoskova *et al.* (2007) studied that whether a combination of hysterectomy and antibiotics treatment leads to an improvement of altered haematological and immunological parameters in bitches affected by pyometra. Leucocytesis parameters was

most affected due to it's inhibition. Seven days after hysterectomy, all affected parameters returned to normal levels comparable to clinically healthy dogs.

Pretzer (2008) studied that a common pathologic clinical finding in canine pyometra is a peripheral leukocytosis, which is more pronounced in closed-cervix pyometra. A left shift is found when a differential cell count is performed and having a normocytic, normochromic anemia with packed cell volumes ranging from 21 to 48%. Abnormalities in serum chemistry include azotemia, hypergammaglobulinemia and hypoalbuminemia. Metabolic acidosis and proteinuria is also a common finding.

Verstegen *et al.* (2008) studied that marked leukocytosis characterized by neutrophilia with a left shift and toxic degeneration of neutrophils, as well as a monocytosis. Many affected bitches have a mild to moderate normocytic, normochromic anemia (PCV 30–35%). serum alkaline phosphatase elevated in approximately 50–75% of cases. These changes reflect hepato-cellular damage in response to toxemia, or diminished hepatic circulation due to dehydration. Hyperproteinemia may develop in response to dehydration, and hyperglobulinemia reflects the chronic antigenic stimulation present with this disease. Serum blood urea nitrogen and creatinine concentrations are not usually elevated, unless pre-renal azotemia develops as a consequence of dehydration. Severe proteinuria progress to renal failure.

Cramer (2010) studied that Pyometra cases show marked haematological and biochemical changes. These changes include elevated total white blood cell counts (WBC), marked left shift, toxic degeneration of neutrophils, elevated serum ALP levels and detection of plasma endotoxin in some cases.

Kumar *et al.* (2019) studied that Marked neutrophilic leukocytosis with shift to left occurs because pyometra being a severe bacterial infection stimulates bone marrow to release more number of immature neutrophils into the peripheral circulation in an attempt to combat the infection. Hyperproteinemia (Hypoalbuminemia Hyperglobulinemia) occurs in acute phage of pyometra due to renal loss of albumin. Blood urea nitrogen (BUN) and plasma creatinine indicate about kidney damage. Hypoglycemia causes sepsis in these cases.

Singh *et al.* (2020) studied that there was marked alteration in the haematology and serum biochemistry found in canine pyometra. Leukocytosis with increased band

neutrophils, monocytosis, toxic degeneration of neutrophils, anaemia are observed in bitches with pyometra. A normocytic, normochromic anaemia is an indicator of chronic pyometra. In pyometra, increased serum concentrations of creatinine, blood urea nitrogen, hypoalbuminemia, hyper gamma-globulinaemia and proteinuria. In pyometra, increased serum concentrations of creatinine, blood urea nitrogen, hypoalbuminemia, hyper gamma-globulinaemia and proteinuria. Salivary adenosine deaminase activity (ADA) is more in cases of pyometra.

6. Efficacy of Ceftiofur Sodium in the treatment of canine pyometra

Jaglan *et al.* (1992) studied that Ceftiofur has a broad antibacterial spectrum and is effective against both Gram positive and Gram negative bacteria as well as some anaerobic bacteria. It has good efficacy against *Escherichia coli, Pasteurella multocida, Actinobacillus pleuropneumoniae, Haemophilus and Salmonella sp.*

Brown *et al.* (1995) studied that the minimum concentration required to inhibit the growth of 90% of the isolates (MIC_{90}) for targeted urinary tract pathogens, is 4.0 pg/mL for *Escherichfn coli* and 1.0 pg/mL for *Proteus mirabilis*. It's eficacy is optimized by maintenance of urine concentrations above the MIC, for targeted urinary tract bacterial pathogens, then concentrations should remain above 4.0 pg/mL for *E.coli* and above 1.0 pg/mL for *P. mirabili*.

Sunkara *et al.* (1999) studied the antibiotics activity of Ceftiofur that it is quickly metabolized to its active metabolite desfuroylceftiofur and it's activity is higher in an infected environment. This increase in activity was due to the infected tissue chambers collected higher concentrations of ceftiofur as well as desfuroylceftiofur which was attributed to binding of ceftiofur and desfuroylceftiofur to macromolecules including proteins which then served a depot effect as the reversible binding was undone over time.

Bosch *et al.* (2006) studied that ceftiofur's action is a time-dependent for antimicrobial action and its concentration should be above the minimum inhibitory concentration (MIC) of the pathogen for an extended period of time. Ceftiofur sodium is a third-generation cephalosporin with broad-spectrum bactericidal activity. It consist typically an aminothiazole group which are active against Gram-negative bacteria, retain good activity against Gram-positive bacteria and are resistant to most b-lactamase enzymes. Ceftiofur is rapidly metabolized to desfuroylceftiofur after injection. The bacteria

"*Staphylococcus aureus"* is two- to eightfold less sensitive to desfuroylceftiofur than to ceftiofur.

Rang *et al.* (2007) studied that Ceftiofur is a third-generation cephalosporin and a β-lactam antibiotic that interferes with cell-wall synthesis by inactivating transpeptidase. Ceftiofur's antimicrobial properties are time-dependent, meaning the degree of bacterial killing is determined by the duration of exposure to the drug. Because of their pharmacokinetic–pharmacodynamic properties, the therapeutic goal is to maintain the plasma concentration above the minimal inhibitory concentration (MIC) of target pathogens for the duration of the treatment period.

Meyer *et al.* (2009) studied that ceftiofur sodium is a broad-spectrum third generation cephalosporin. Ceftiofur exerts good in vitro activity against many Gram-negative and Gram-positive bacterial pathogens of veterinary importance, including *E. coli.* The MIC of ceftiofur required to inhibit growth of 90% of isolates of *Escherichia coli, Pasteurella sp., Klebsiella sp*., and β-hemolytic *streptococci* was <0.5 μg/mL.

Prescott (2013) studied that Ceftiofur is a third generation cephalosporin, fairly resistant to β-lactamases, bactericidal and time-dependent antibiotic. Time-dependent antibiotics are those which are active against susceptible bacteria for the duration of time the drug concentrations in the patient exceed the minimum inhibitory concentration (MIC) of the bacterium. Ceftiofur is distributed evenly in the extracellular fluid, but does not pass adequately across the plasma membranes of cells. However, inflammation of these membranes can allow therapeutic penetration. It is considered to distribute throughout the body, as well as have effective penetration into tissue spaces and fluids.

Hassan *et al.* (2016) studied the broad antibacterial spectrum of ceftiofur and found that it is effective against both Gram positive and Gram negative bacteria along with some anaerobic bacteria. It showed good efficacy against *Escherichia coli, Pasteurella multocida, Actinobacillus pleuropneumoniae, Haemophilus* and *Salmonella sp.* Ceftiofur is being resistant to inactivation by enzymes β- lactamase produced by some bacteria and it is due to presence of bulky imino- methoxy side chain which cause the bactericidal action by killing the bacteria by disrupting the cell wall synthesis.

Hooper *et al.* (2016) studied that Ceftiofur is a third-generation cephalosporin and is a β-lactam antibiotic that interferes with cell-wall synthesis by inactivating

transpeptidase. Ceftiofur is having antimicrobial properties which are time-dependent. It maintain the plasma concentration above the minimal inhibitory concentration (MIC) of targets pathogens such as *Actinobacillus pleuropneumniae, Pasteurella multocida, Haemophilus parasuis, Streptoccus suis, Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*.

Wang *et al.* (2019) studied that Ceftiofur sodium (CEF-Na) is a third-generation broad-spectrum cephalosporin (β-lactam antibiotic) which is effective against Gram-positive, Gram-negative, anaerobic, and β-lactamase producing bacteria. Ceftiofur is metabolised by rapid cleavage of the thioester bond to the active metabolite desfuroylceftiofur (DFC) and furoic acid after parenteral administration. Free DFC (which contains an intact β-lactam ring) is the primary microbiologically active metabolite of ceftiofur. It is further metabolized to disulfides and also bound to macromolecules in plasma and tissues which are DFC-glutathione disulfide, DFC-cysteine disulfide, 3, 3-DFC-disulfide (DFC-dimer), and DFC-protein.

7. Efficacy of levofloxacin and Ornidazole combination in the treatment of canine pyometra

Fish and Chow (1997) studied that levofloxacin is a drug of fluoroquinolone group and having antibacterial properties. It possesses a wide spectrum of bactericidal activity against both Gram-positive and Gram negative pathogens, as well as *Mycoplasma, Legionella, Chlamydia* and *Mycobacteria sp.* and *mycobacteral* species. Levofloxacin is active against both intracellular and extracellular pathogens due to its wide distribution throughout the body and extensive intracellular penetration. It is rapidly absorbed from the gastrointestinal tract with the time to maximum plasma concentrations (t_{max}) ranging from 0.8 to 2.4 hours after the administration of levofloxacin 50 to 1000mg with or without food. It's killing activity against *Staphylococcus aureus* is more rapid than other drug due to the greater ratio of its C_{max} to the MIC (peak: MIC ratio) value.

Croom and Goa (2003) studied that levofloxacin is a fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria. It is active against both penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae*. Levofloxacin is highly active against *Haemophilus influenzae* (MIC_{90} 0.008–0.12 mg/L), *Moraxella catarrhalis* (MIC_{90} ≤0.03–0.06 mg/L) and also shows activity against the *Enterobacteriaceae* (MIC_{90} ≤2.0 mg/L for most isolates). The susceptibility

rates of *Pseudomonas aeruginosa* to levofloxacin were 62–74%. Levofloxacin is rapidly absorbed after oral administration, reaching Cmax after 1–2 hours. Levofloxacin is highly active against *Haemophilus influenzae, Moraxella catarrhalis* and the *Enterobacteriaceae* (including *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, cloacae* and *Citrobacter* species), with MIC_{90} values generally between 0.008 and 2.0 mg/L. Levofloxacin is well tolerated, and is associated with few of the phototoxic, cardiac or hepatic adverse events seen with some other quinolones. It's a pharmacokinetic profile that is compatible with once-daily administration and allows for sequential intravenous to oral therapy.

Hizarcioglu *et al.* (2004) studied that ornidazole is a 5-nitroimidazole derivative drug and is used in the treatment of susceptible protozoal infections and also in anaerobic bacterial infections. It kills parasites and anaerobic bacteria that cause infections by damaging their DNA. It's absorption is maximum from the small intestine when given orally, with bioavailability of >90% and t_{max} ranging between 2 and 4 hours. It is widely distributed in body tissues and fluids, including cerebrospinal fluid. Antibacterial concentrations are maximum in vaginal secretions, appendix and intestinal tissues. The mean half-life of elimination from plasma is 11 to 14 hours

Zhou *et al.* (2014) studied that levofloxacin(LVX) is a broad-spectrum antibiotic of the fluoroquinolone drug class. It is rapidly and completely absorbed after oral administration. Peak plasma concentration (C_{max}) is usually attained 1–2 hours after oral dosing. It is excreted largely (87%) as unchanged drug in the urine. The mean terminal plasma elimination half-life ($t^{1/2}$) of LVX ranges from approximately 6 to 8 hours.

Landoni and Albarellos (2019) studied that levofloxacin show bactericidal effects that caused the inhibition of both bacterial DNA gyrase (a type-II topoisomerase) and topoisomerase IV. It is broad antibacterial spectrum that includes many Gram negative (most *Enterobacteriaceae*) and Gram-positive bacteria (methicillinsusceptible strains of *Staphylococcus sp.* and *Streptococcus sp.*), atypical and intracellular bacteria (*Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae,* and *Chlamydia pneumonia*). Levofloxacin works by preventing the bacterial cells from dividing and repairing, there by killing the bacteria.

Madsen *et al.* (2019) studied the effect of levofloxacin in six healthy adult beagles that the levofloxacin was highly absorbed when administered orally and it's elimination half-life was moderately longer than other fluoroquinolones. Levofloxacin was water solubility of approximately 200 mg/ml and it meets the criteria of a highly soluble drug according to biopharmaceutical classification system criteria. Levofloxacin was orally given due to more reliable absorption and good antimicrobial activity against a wide range of pathogens. Antimicrobial susceptibility breakpoint for *Enterobacteriaceae* is ≤ 0.5 µg/ml and for *Pseudomonas aeruginosa* is ≤ 1 µg/ml. Therefore levofloxacin administered orally at dose 25 mg/kg, once daily which was sufficient to reach pharmacokinetic-pharmacodynamic therapeutic targets for canine bacteria.

Urzúa *et al.* (2020) studied that levofloxacin is the optically pure levorotatory isomer of ofloxacin, is a 3rd generation fluoroquinolone, broad-spectrum antibiotic, effect against gram-negative, some gram-positive microorganisms, *mycoplasmas* and limited activity on anaerobic bacteria by inhibition of enzymes DNA gyrase II and topoisomerase IV. It's action is concentration-dependent bactericidal effect. The breakpoint susceptibility of levofloxacin in the presence of *Enterobacteriaceae* and *P. aeruginosa* was at ≤0.5 and ≤1 µg/ml, respectively. Oral administration with fasting was reported as C_{max} and AUC of 3.2 ± 0.69 mg/ml and 32.91 ± 9.81 µg-h/ml, respectively, and oral bioavailability of 60.9 ± 14.8% and 104 ± 30% and the absorption half-life ($^{t1/2}$abs)≤0.73 hours and $t^{1/2}\beta$≤11.07 hours.

Sitovs *et al.* (2021) studied that levofloxacin is a third-generation fluoroquinolone drug and it is effective against Gram-positive bacteria and atypical intracellular pathogens. Its spectrum of activity includes Gram-positive aerobic bacteria, Gram-negative aerobic bacteria, some anaerobic bacteria, and other microorganisms including *Chlamydia sp*., *Mycoplasma sp*., and *Mycobacterium sp*. Ornidazole is a nitroimidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria.

Materials and Methods

The work was done on bitches presented with pyometra in Veterinary Clinical Complex, Bihar Veterinary College, Patna. Bitches were diagnosed for pyometra based on:

1. **Haemato-biochemistry**- Hemoglobin(%), RBC, WBC, neutrophil, lymphocytes, BUN, creatinine, Albumin, ALT, AST.
2. **Clinical parameters**- anorexia, lethargy, vomition, polydipsia, polyuria, pus in vaginal discharge.
3. **Ultrasonograph**y (Figure 1).
4. **Neutrophil in Vaginal Cytology** (Figure 2).

Treatments

Group	No. of Bitches	Treatment
CEF	10	Ceftiofur Sodium @ 2.2mg/kg body weight I/M + Cloprostenol Sodium @ 5µg/kg body weight S/C twice daily for 5 days
LO	10	Levofloxacin (@ 5mg/kg bd. wt.) + Ornidazole (@ 10mg/kg body weight) Orally twice +Cloprostenol Sodium @ 5µg/kg body weight S/C twice daily for 5 days

The bitches were evaluated for recovery based on haemato-biochemistry and allocation to one of the grades of clinical parameter.

Grading system of clinical parameters in cases of pyometra in bitches

Clinical Parameters	Grade A	Grade B	Grade C
Anorexia	Nil	Partial	Complete
Lethargy	Nil	Partial	Complete
Vomition	Nil	Partial	Complete
Pus in Vaginal Discharge	Nil	Partial	Complete
Neutrophil in Vaginal Cytology	Nil	Partial	Complete
Ultrasonography	Nil	Partial	Complete

Figure 1: Ultrasonographic image of anechoic sacs (denoted by triangles) in the uterus of a bitch with pyometra

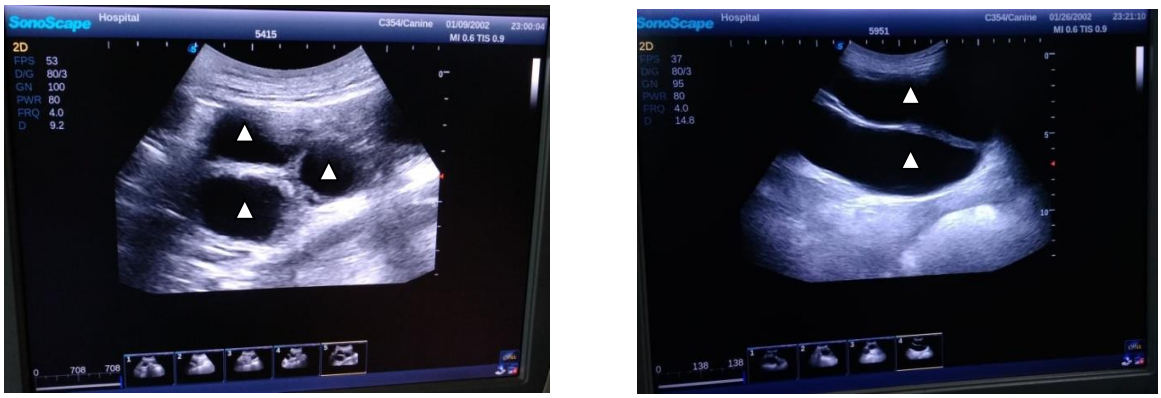
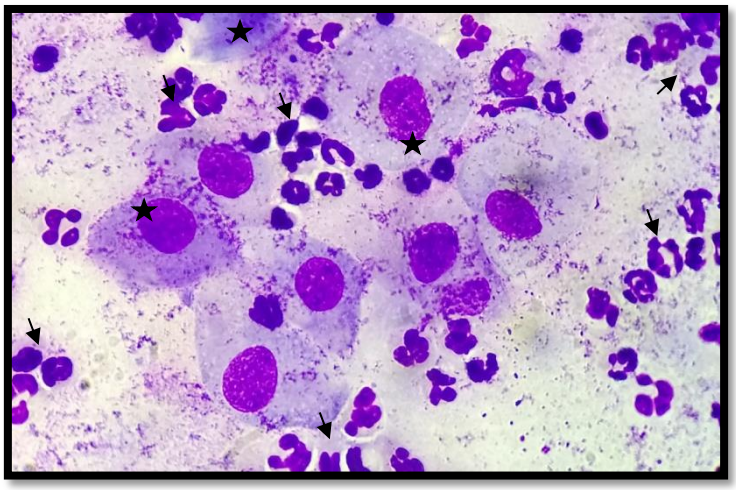


Figure 2: Exfoliative vaginal cytology of a bitch with pyometra (arrow in the picture denotes neutrophils and star denotes small intermediates)

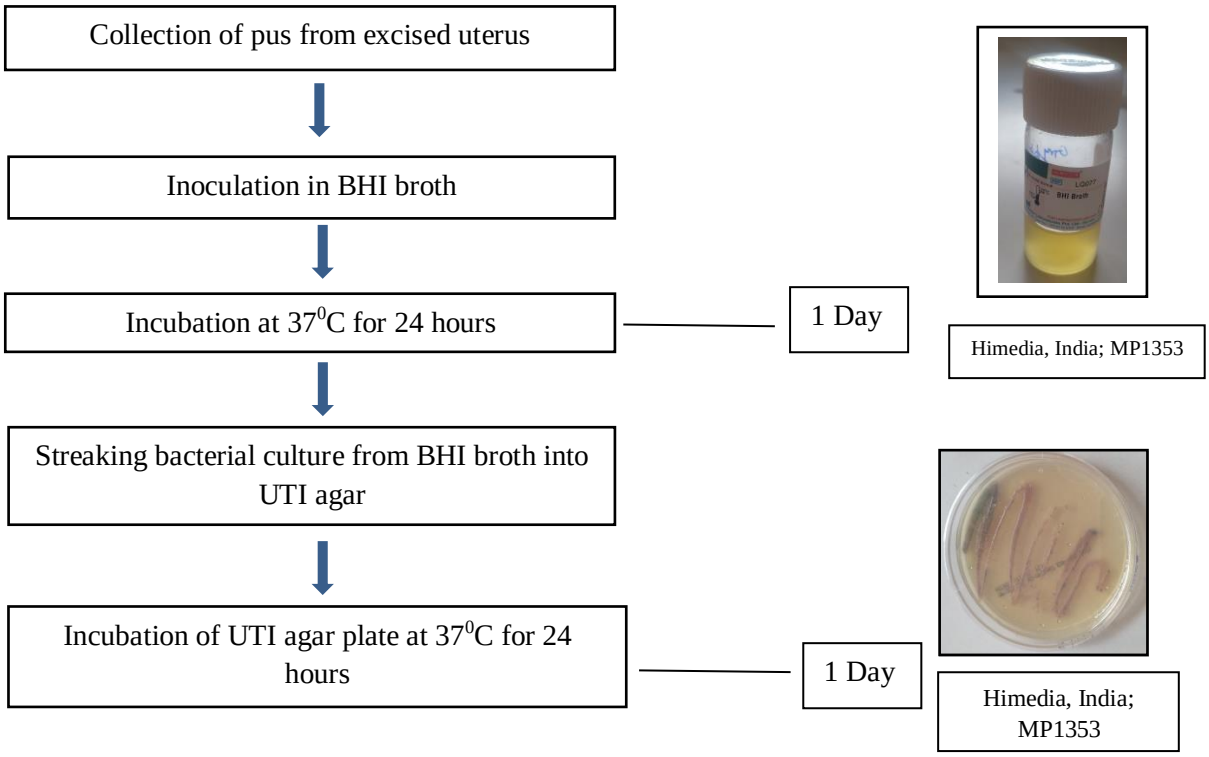


Methods for bacterial isolation

Ovario-hysterectomy (OH) was performed 7 days after the start of treatment with Ceftiofur (n=5) & Levofloxacin (n-=5) bitches with in-complete clinical recovery. Four bitches underwent OH without prior antibiotics treatment.

The pus was withdrawn from the uterus by puncturing it with sterile hypodermic needle and drawing the pus in a sterile syringe. The pus was collected from the anterior vagina by means of a sterile cotton swab sticks. The pus after collection was immediately transferred to BHI broth and cultured for 24 hours.

Flow diagram of activities for identification, isolation and antibiotics sensitivity test of bacteria from the excised uterus of bitches with pyometra



Himedia, India; MP1301

Identification of bacteria based on colour of colonies in differential agar

↓

Re-confirmation of identified bacteria by gram staining, inoculation in selective agar e.g. cetrimide agar, sheep blood agar with neomycin & catalase test (Table 1)

↓

Inoculation of BHI broth with isolated bacterial colonies from UTI agar to obtain pure culture

↓

Inoculation of BHI broth at 37^0C for 24 hours — 1 Day

↓

Streaking pure culture in BHI broth into UTI agar or selective agar — 1 Day

↓

Picking up colonies from UTI agar & diluting in Normal Saline and comparing the turbidity with 0.5 Mcfarland standard

↓

Taking 50µl of diluted bacterial suspension & spreading over 90mm Mueller Hinton (MH) agar plates & antibiotics disc is placed on it

↓

Incubation of MH agar plates at 37^0C for 24 hours — 1 Day

Himedia, India; MP173

↓

Measurement of the diameter of zone of inhibition & comparison with the EUCAST/CLSI standard

Total Number of Days Required to complete the test — 5 Day

Figure 3: Collection of pus from excised uterus

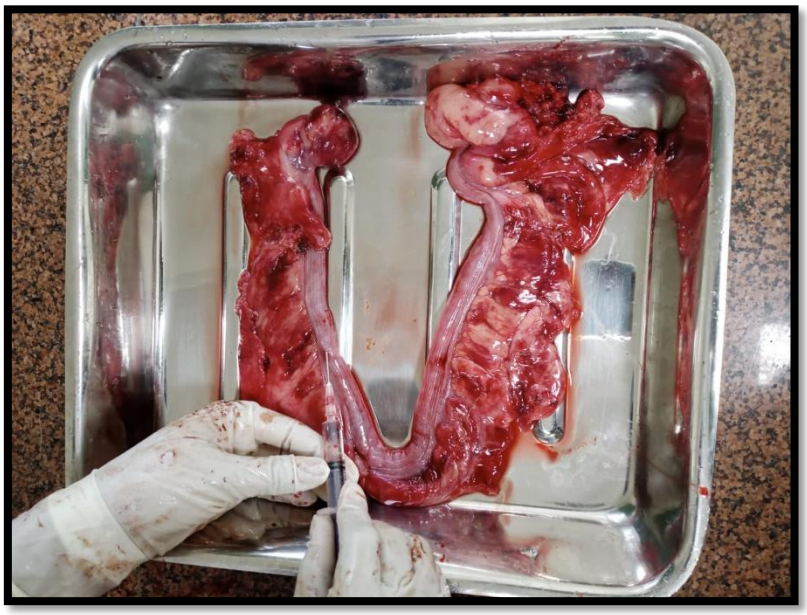


Table 1: Protocol for identification of bacteria in pus collected from the excised uterus of bitch with pyometra

Bacterial sp.	Colony Character			Gram Stain	Catalase Test
	UTI agar (Himedia, India; MP1353)	**Anaerobic sheep blood agar with neomycin (Himedia, India; MP1301)**	**Cetrimide agar (Himedia, India; MP024)**		
Enterococcus sp.	Green	Luxurient Non haemolytic white colonies	-	Gram positive cocci/ cocco-bacilli arranged in short chain	Negative
Staphylococcus sp.	Yellow	-	-	Gram positive cocci arranged in bunches	Positive
Bacillus sp.	Greenish Yellow	-	-	Gram positive rods	Positive
Pseudomonas sp.	White	-	Luxurient White colonies	Gram negative rods	Positive
Proteus sp.	Brown	-	-	Gram negative rods	Positive
E. coli	Purple	-	-	Gram negative rods	Positive
Gram negative ObligateAnaerobe	Yellow	-	-	Gram negative rods	Negative

Following antibiotics disc were used to perform antibiotics sensitive test

Antibiotics Disc*	Concentration	Disc Code
Amikacin (AK)	30µg	SD035
Amoxyclav(Amoxycillin/Clavulanic acid)	30 µg (20/10 µg)	SD063
Ampicillin/ Salbactam (A/S)	10/10 µg,	SD112
Ciprofloxacin(CIP)	5 µg	SD060
Gentamicin(GEN)	10 µg	SD016
Levofloxacin (LE)	5 µg	SD216
Moxifloxacin (MO)	5 µg	SD217
Penicillin-G (P)	10U	SD028
Tetracycline (TE)	30 µg	SD037
Ampicillin/ Cloxacillin (AX)	10 µg	SD113
Cefoperazone/ Salbactam (CFS)	75/30 µg	SD203
Ceftriaxone/ Tazobactam (CIT)	30/10 µg	SD256
Chloramphenicol (C)	30 µg	SD006

***-** Himedia® India

Statistical Analysis

All the collected data was statistically analyzed using SPSS software version 23. Independent sampleT-test and Paired T-test was used to compare haemato-biochemical parameters between treatment groups as well as before and after treatments. Bitches falling under different grades of clinical parameters was compared by Chi-Sq. test.

Results & Discussion

Three out of ten bitches with pyometra showed complete recovery after treatment with levofloxacin ornidazole and two out of ten bitches showed complete recovery after treatment with ceftiofur sodium (Table 2), however chi-square test revealed non-significant difference with respect to clinical recovery. In ceftiofur group, three bitches died during the treatment. Further there was non- significant difference in clinical recovery amongst the two antibiotics treated groups (Figure 4).

Amongst the bitches that showed complete clinical recovery with respect to haemato-biochemical parameters after antibiotics treatment, there was significant difference in haemato-biochemical parameters (Table 3, Figure 5A) in LO & CEF groups w.r.t. haemoglobin (12.92 g/dl ± 0.22 VS 12.02 ± 0.01 g/dl in LO & CEF groups respectively; $P<0.05$), RBC (6.4 ± 0.05 $x10^6$/µl VS 5.77 ± 0.06 $x10^6$/µl in LO & CEF groups respectively; $P<0.01$), BUN (12.3 ± 1.09 mg/dl VS 19.04 ± 0 mg/dl in LO & CEF groups respectively; $P<0.05$), creatinine (1 ± 0.07 mg/dl VS 1.34 ± 0.04 mg/dl in LO & CEF groups respectively; $P<0.05$), ALT (33.9 ± 0.25 IU/L VS 41.58 ± 0.06 IU/L in LO & CEF groups respectively; $P<0.01$), AST (37.54 ± 0.04 IU/L VS 43.97 ± 0.1 IU/L in LO & CEF groups respectively; $P<0.01$); wherein levofloxacin ornidazole treatment showed better results compared to CEF group. Comparison of haemato-biochemical parameters before and after antibiotics treatment showed significant improvement in all parameters except RBC (5.97 ± 0.33 $x10^6$/µl VS 6.4 ± 0.05 $x10^6$/µl; $P>0.05$) in LO group, WBC (20.13 ± 0.01 $x10^3$/µl VS 19.08 ± 0.16 $x10^3$/µl; $P>0.05$) and creatinine (1.85 ± 0.09 mg/dl VS 1.34 ± 0.04 mg/dl; $P>0.01$) in CEF group (Table 3).

Amongst the bitches that showed incomplete clinical recovery with respect to haemato-biochemical parameters after antibiotics treatment, there was significant difference in all haemato-biochemical parameters (Table 4, Figure 5B) between LO & CEF groups, wherein levofloxacin ornidazole treatment showed better results compared to CEF group. There was significant improvement before and after treatment in LO & CEF groups w.r.t all haemato-biochemical parameters except creatinine (1.82 ± 0.0 5mg/dl VS 1.7 ± 0 mg/dl; $P>0.05$) in LO group (Table 4).

The bacteria isolated from the excised uterus of bitches with pyometra without prior antibiotics treatment were *E.coli, gram negative anaerobes, Enterococcus sp., Proteus sp., Pseudomonas sp.* and *Staphylococcus sp*. (Table 5).

Table 2: Allocation of bitches with pyometra into each of the three gradations (A, B and C) of clinical parameters before and after treatment with levofloxacin ornidazole and ceftiofur sodium

Clinical Parameters	LO Group (n=10)						Sig. (Chi-Sqaure)	CEF Group (n=7)*						Sig. (Chi-Sqaure)
	Before Treatment			After Treatment				Before Treatment			After Treatment			
	A	B	C	A	B	C		A	B	C	A	B	C	
Anorexia		4	6	3	5	2	NS		2	5	2	2	3	NS
Lethargy		5	5	2	5	3	NS		1	6	1	3	3	NS
Vomition		6	4	3	5	2	NS		3	4	1	4	2	NS
Pus in Vaginal Discharge		6	4	3	5	2	NS		2	5	2	3	2	NS
Neutrophil in Vaginal Cytology		7	3	3	5	2	NS		3	4	2	3	2	NS
Ultrasonography		4	6	3	5	2	NS		1	6	2	3	2	NS

*: Three bitches died during treatment

Anorexia: Grade A- Nil, Grade B- Partial, Grade C- Complete

Lethargy: Grade A- Nil, Grade B- Partial, Grade C- Complete

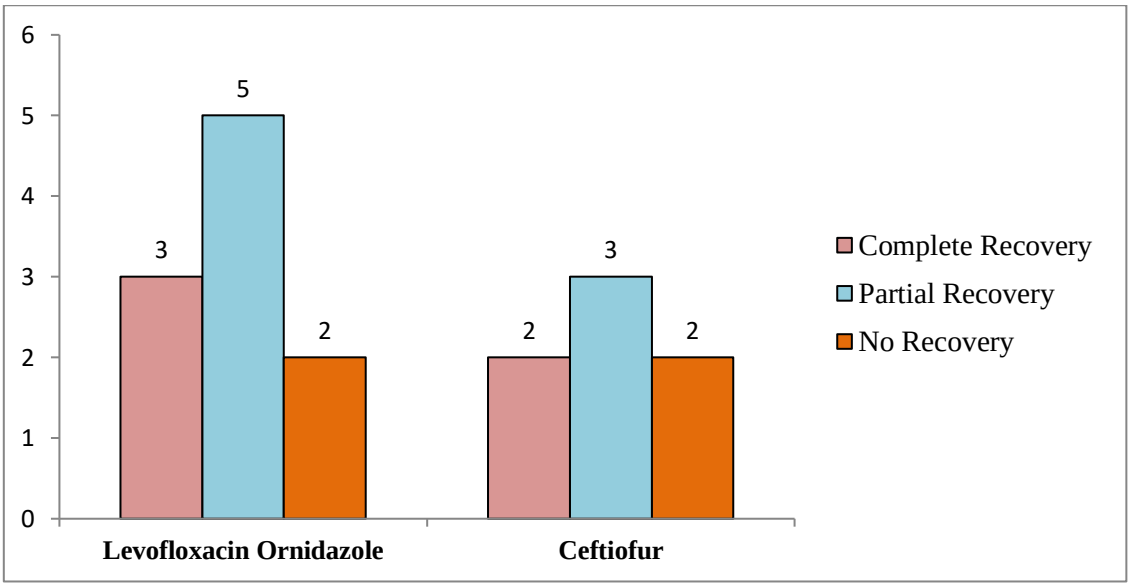
Vomition: Grade A- Nil, Grade B- Partial, Grade C- Complete

Pus in Vaginal Discharge: Grade A- No pus, Grade B- Scanty, Grade C- Copious foul smelling

Neutrophil in Vaginal Cytology: Grade A- Nil, Grade B- Few, Grade C- Large number

Ultrasonography: Grade A- Normal, Grade B- Anechoic sacs less than 1 cm, Grade C- Anechoic sacs greater than 1.5 cm

Figure 4: Allocation of bitches into three different categories of clinical recovery following treatment with Levofloxacin Ornidazole and Ceftiofur



Chi-Sqaure test reveals non-significant difference

Table 3: Comparison of haemato-biochemical parameters of bitches showing complete clinical recovery with levofloxacin Ornidazole and Ceftiofur treatment

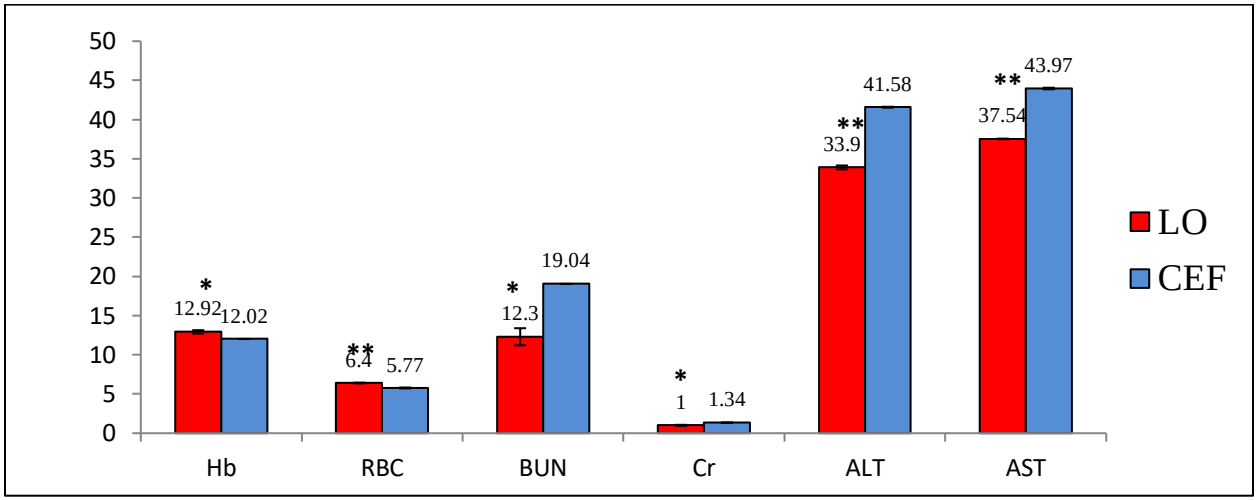
Parameters	Unit	Normal Range	Significance (between LO & CEF Groups post treatment; independant -t)	LO Group (n=3)		Significance (before & after treatment in LO Group; paired-t)	CEF Group (n=2)		Significance (before & after treatment in CEF Group; paired-t)
				Before Treatment	After Treatment		Before Treatment	After Treatment	
Hemoglobin	g/dl	12-16	P<0.05	8.86 ± 0.13	12.92 ± 0.22	P<0.01	9.95 ± 0.09	12.02 ± 0.01	P<0.05
RBC	$x10^6$ /µl	5-7	P<0.01	5.97 ± 0.33	6.4 ± 0.05	NS	4.57 ± 0.11	5.77 ± 0.06	P<0.05
WBC	$x10^3$ /µl	6-17	NS	21.03 ± 0.29	18.61 ± 0.15	P<0.05	20.13 ± 0.01	19.08 ± 0.16	NS
Neutrophils	$x10^3$ /µl	3.6-10.2	NS	13.95 ± 0.1	7.37 ± 0.75	P<0.05	11.95 ± 0.09	9.75 ± 0.03	P<0.05
Lymphocytes	$x10^3$ /µl	1.8-6.8	NS	5.47 ± 0.1	4.56 ± 0.75	P<0.01	6.31 ± 0.09	5.76 ± 0.11	P<0.05
BUN	mg/dl	10-20	P<0.05	26.45 ± 0.05	12.3 ± 1.09	P<0.01	29.03 ± 0.24	19.04 ± 0	P<0.05
Creatinine	mg/dl	<1.4	P<0.05	2.11 ± 0.04	1 ± 0.07	P<0.01	1.85 ± 0.09	1.34 ± 0.04	NS
Albumin	g/dl	2-4	NS	1.33 ± 0.09	3.11 ± 0.34	P<0.01	1.65 ± 0.15	3.17 ± 0.05	P<0.01
ALT	IU/L	<90	P<0.01	104.82 ± 0.22	33.9 ± 0.25	P<0.01	120.39 ± 0.15	41.58 ± 0.06	P<0.01
AST	IU/L	23-40	P<0.01	80.92 ± 0.19	37.54 ± 0.04	P<0.01	70.45 ± 0.02	43.97 ± 0.1	P<0.01

Table 4: Comparison of haemato-biochemical parameters of bitches showing incomplete clinical recovery with levofloxacin Ornidazole and Ceftiofur treatment

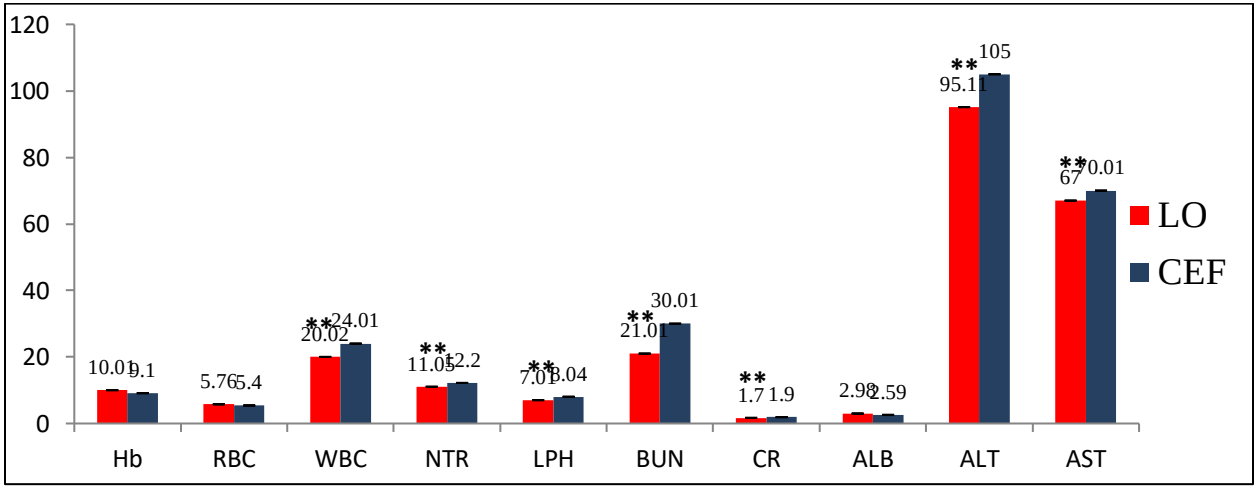
Parameters	Unit	Normal Range	Significance (between LO & CEF Groups post treatment; independant-t)	LO Group (n=7)		Significance (before & after treatment in LO Group; paired-t)	CEF Group (n=5)		Significance (before & after treatment in CEF Group; paired-t)
				Before Treatment	After Treatment		Before Treatment	After Treatment	
Hemoglobin	g/dl	12-16	P<0.01	7.99 ± 0.05	10.01 ± 0.03	P<0.01	8.7 ± 0.13	9.1 ± 0	P<0.05
RBC	$x10^6/\mu l$	5-7	P<0.05	5.03 ± 0.09	5.76 ± 0.09	P<0.01	4.39 ± 0.07	5.4 ± 0.07	P<0.01
WBC	$x10^3/\mu l$	6-17	P<0.01	21.96 ± 0.12	20.02 ± 0.03	P<0.01	25.1 ± 0.09	24.01 ± 0.01	P<0.01
Neutrophils	$x10^3/\mu l$	3.6-10.2	P<0.01	12.14 ± 0.04	11.05 ± 0.03	P<0.01	13 ± 0	12.2 ± 0	P<0.01
Lymphocytes	$x10^3/\mu l$	1.8-6.8	P<0.01	6.42 ± 0.08	7.01 ± 0	P<0.01	7.32 ± 0.06	8.04 ± 0.01	P<0.01
BUN	mg/dl	10-20	P<0.01	30.69 ± 0.11	21.01 ± 0	P<0.01	38.97 ± 0.05	30.01 ± 0	P<0.01
Creatinine	mg/dl	<1.4	P<0.01	1.82 ± 0.05	1.7 ± 0	NS	2.09 ± 0.05	1.9 ± 0.01	P<0.05
Albumin	g/dl	2-4	P<0.05	1.75 ± 0.07	2.98 ± 0.12	P<0.01	1.47 ± 0.12	2.59 ± 0.06	P<0.01
ALT	IU/L	<90	P<0.01	101.03 ± 0.03	95.11 ± 0	P<0.01	121.03 ± 0.02	105 ± 0	P<0.01
AST	IU/L	23-40	P<0.01	70 ± 0	67 ± 0	P<0.01	75.01 ± 0.01	70.01 ± 0	P<0.01

Figure 5: Comparison of haemato-biochemical parameters post treatment with Levofloxacin Ornidazole (LO) and Ceftiofur (CEF) in cases of canine pyometra

A. Complete Clinical Recovery



B. Incomplete Clinical Recovery



The Bacteria isolated from excised uterus after treatment were *Enterococcus sp., Pseudomonas sp., Staphylococcus sp.* and *Bacillus sp.* with levofloxacin ornidazole and *Pseudomonas sp., Staphylococcus sp., Bacillus sp. and Enterococcus sp.* and gram negative anaerobes with ceftiofur.

E.coli and *Proteus sp.* were absent in the antibiotics treated groups compared to the non-treated groups, thus ceftiofur and levofloxacin ornidazole was effective against both of these pathogens. Gram negative catalase negative (Figure 6B) bacteria were isoloated from the excised uretus of bitches with levofloxacin ornidazole but not ceftiofur. Since all gram negative bacteria are catalase positive the bacteria isolated are obligate anaerobes, ceftiofur is ineffective against all gram negative anaerobic bacteria. Some bacteria were neither effective against levofloxacin ornidazole nor ceftiofur and found common in both groups such as *Enterococcus sp., Pseudomonas sp., Staphylococcus sp.* and *Bacillus sp.* (Table 6).

The bacteria isolated from bitches with incomplete clinical recovery following antibiotics treatment from the vagina were *Enterococcus sp., Pseudomonas sp., Staphylococcus sp.* and *Bacillus sp.* The bacterial population from pus sample taken from vagina did not always represent the bacterial population in pus sample from the excised uterus (Table 6), e.g. the bacteria isolated from vagina in bitch no 3 was *Pseudomonas sp.* compared to *Bacillus sp.* and *Enterococcus sp.* in uterus in LO group. Thus it can be inferred that the bacteria isolated from vaginal pus sample do not always represent the pathogen present in the uterus.

Enterococcus sp. was found to be highly sensitive to tetracycline and ciprofloxacin; sensitive to chloramphenicol, gentamicin, pencillin- G, amikacin and moxifloxacin and resistant to amoxicillin/clavunanic acid, ampicillin/cloxacillin, ampicillin/sulbactam and levofloxacin (Table 7A i & ii). *Pseudomonas sp.* was found to be highly sensitive to amikacin, tetracycline, cefoperazone/sulbactam; sensitive to chloramphenicol, gentamicin and resistant to amoxicillin/clavunanic acid, ciprofloxacin moxifloxacin, levofloxacin, ceftriaxone/tazobactam (Table 7B i & ii). *Staphylococcus sp.* was found to be highly sensitive to amikacin and moxifloxacin; sensitive to chloramphenicol, gentamicin, tetracycline, cefoperazone/sulbactam and ceftriaxone/tazobactam and resistant to ampicillin/cloxacillin, pencillin- G, ciprofloxacin, ampicillin/sulbactam, amoxicillin/clavunanic acid and levofloxacin (Table 7C i & ii). *Bacillus sp.* was found to be highly sensitive to amikacin; sensitive to chloramphenicol, gentamicin, tetracycline and

Table 5: Bacteria isolated from the excised uterus of bitches with pyometra without prior antibiotics treatment

Dog No.	Bacteria Isolated
1	*E. coli* Gram Negative Anaerobes^{+} *Enterococcus sp.*
2	*Proteus sp.* *E. coli* Gram Negative Anaerobes^{+}
3	Gram Negative Anaerobes^{+} *E. coli* *Enterococcus sp.*
4	*Pseudomonas sp.* *E. coli* *Staphylococcus sp.*

+- based on negative reaction to catalase test

Table 6: Bacteria isolated from the vagina and excised uterus of bitches with pyometra following treatment with levofloxacin ornidazole and ceftiofur

Dog No.	Levofloxacin Ornidazole Treated		Ceftiofur Treated	
	Uterus	**Vagina**	**Uterus**	**Vagina**
1	*Enterococcus sp.*	*Bacillus sp.*	*Pseudomonas sp.* Gram Negative Anaerobes^{+}	*Pseudomonas sp.* *Bacillus sp.*
2	*Enterococcus sp.* *Pseudomonas sp.* *Staphylococcus sp.*	*Enterococcus sp.* *Bacillus sp.*	*Staphylococcus sp.* *Pseudomonas sp.* *Bacillus sp.*	*Enterococcus sp.* *Staphylococcus sp.*
3	*Bacillus sp.* *Enterococcus sp.*	*Pseudomonas sp.*	*Pseudomonas sp.* Gram Negative Anaerobes^{+}	*Enterococcus sp.*
4	*Pseudomonas sp.* *Staphylococcus sp.*	*Enterococcus sp.* *Staphylococcus sp.*	*Enterococcus sp.* *Staphylococcus sp.*	*Bacillus sp.* *Pseudomonas sp.*
5	*Bacillus sp.* *Staphylococcus sp.*	*Pseudomonas sp.*	*Staphylococcus sp.* Gram Negative Anaerobes^{+}	*Staphylococcus sp.*

NB: Uterus was excised by ovario-hysterectomy (OH); vaginal pus samples were collected just before performing OH

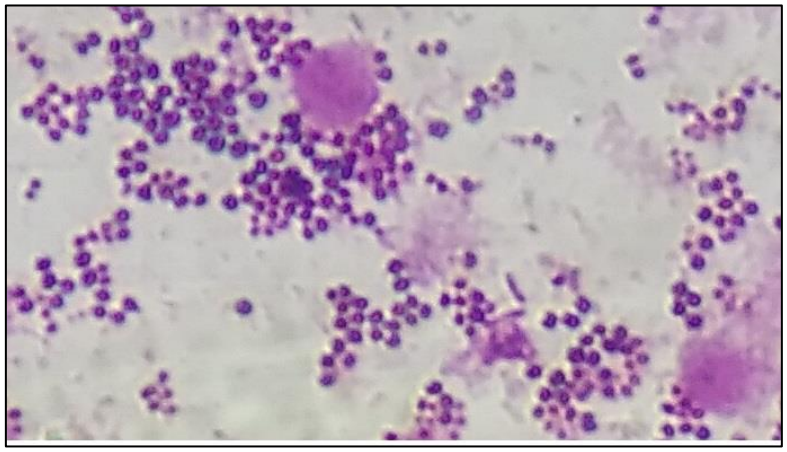
+- based on negative reaction to catalase test

Figure 6: Identification of resistant bacteria isolated from the excised uterus of bitches with pyometra based on culture characteristic, gram staining and catalase test

A. *Staphylococcus sp.* colonies in UTI Agar (Himedia, India; MP1353)

Yellow colonies in UTI agar

Gram Stained Smear of Pure *Staphylococcus sp.* culture



X1000 (digitally zoomed)

Gram positive cocci arranged in bunches

Positive in Catalase Test

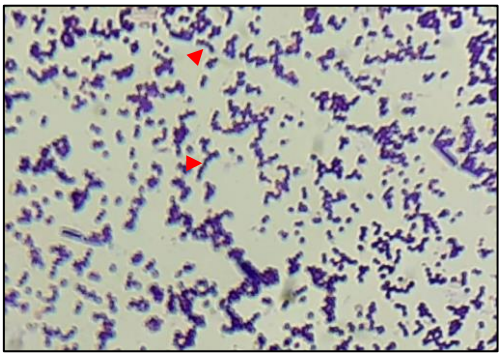
Effervescence of *Staphylococcus sp*. culture upon addition of hydrogen peroxide

B. *Enterococcus sp*. colonies

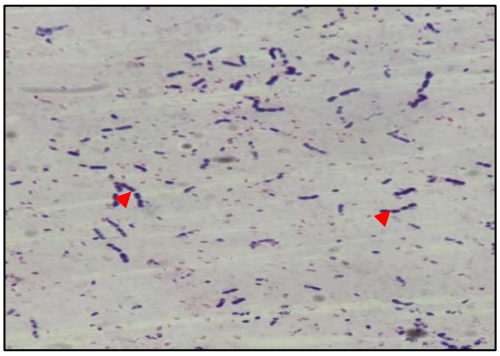
Green colonies in UTI agar
(Himedia, India; MP1353)

Non haemolytic colonies in sheep blood agar with Neomycin
(Himedia, India; MP1301)

Gram Stained Smear of Pure *Enterococcus sp*. culture



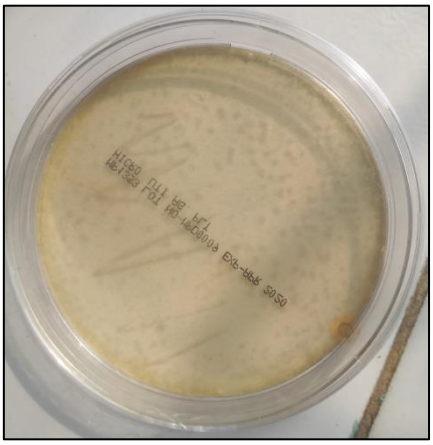
Triangle denotes gram positive cocci arranged in chains



Triangle denotes gram positive coccio-bacilli arranged in shorts chains

NO Effervescence in Catalase Test

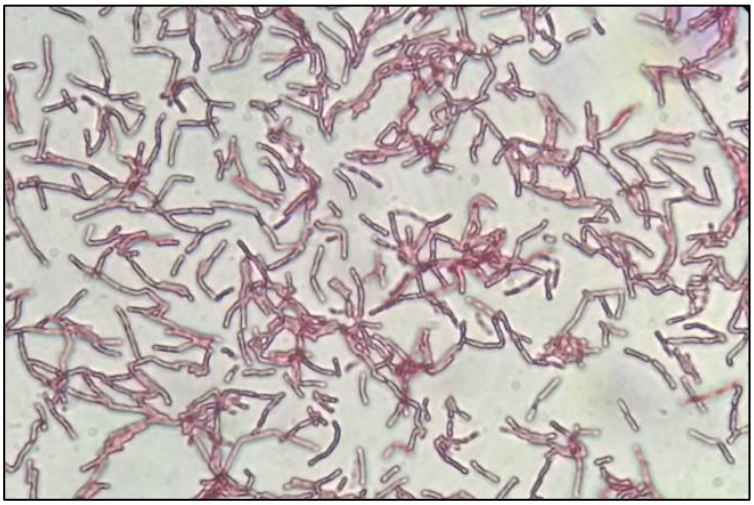
C. *Pseudomonas sp*. colonies



White colonies in UTI agar (Himedia, India; MP1353)

Rich Growth in Cetrimide agar (Himedia, India; MP024)

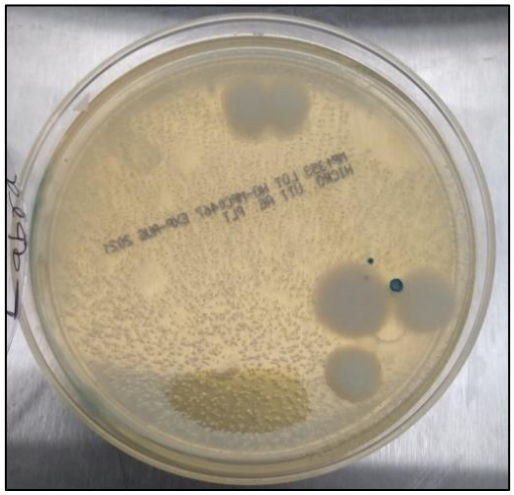
Gram Stained Smear of Pure *Pseudomonas sp*. culture



Gram negative rods

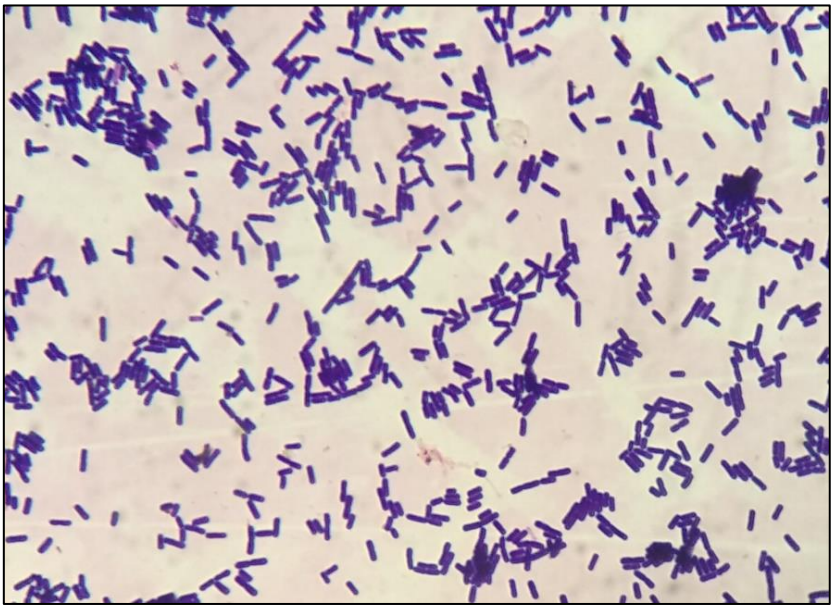
X 1000 (Digitally Zoomed)

D. *Bacillus sp*. colonies



Greenish yellow colonies in UTI agar (Himedia, India; MP1353)

Gram Stained Smear of Pure *Bacillus sp*. culture

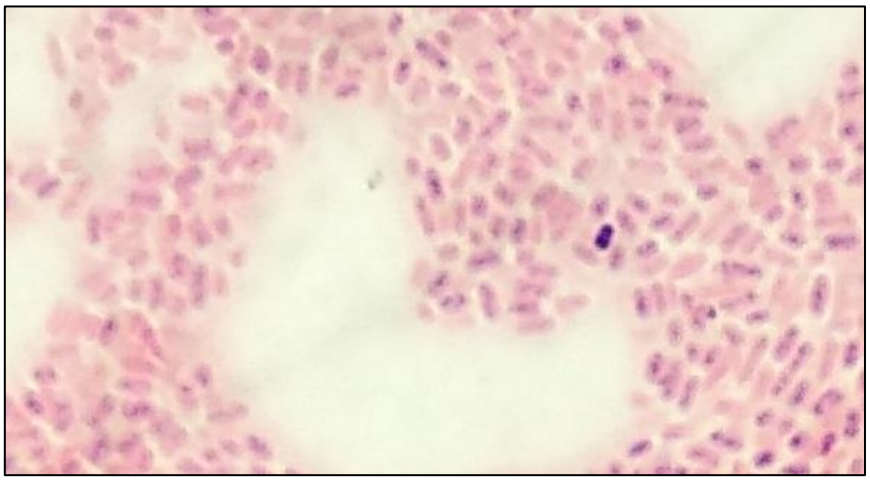


X1000 (Digitally Zoomed) Gram positive rods

E. *Proteus sp*. culture in UTI agar

Brown colonies in UTI agar
(Himedia, India; MP1353)

Gram Stained Smear of Pure *Proteus sp*. culture



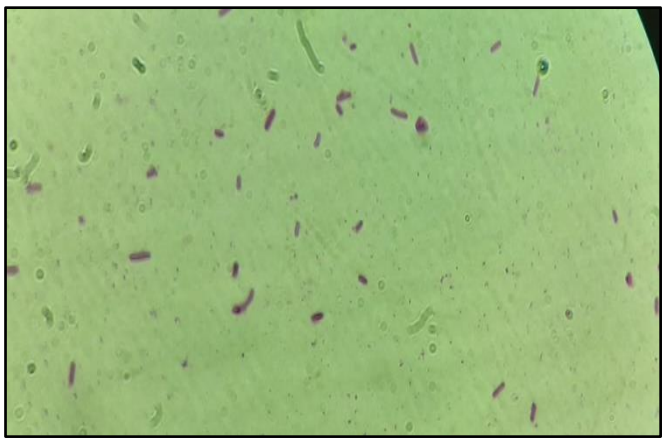
Gram negative rods

X 1000 (Digitally Zoomed)

F. *Escherichia coli* culture in UTI agar

Pink colonies in UTI agar
(Himedia, India; MP1353)

Gram Stained Smear of Pure *Escherichia coli* culture



Gram negative rods	X 1000 (Digitally Zoomed)

resistant to amoxicillin/clavunanic acid, ampicillin/sulbactam, ciprofloxacin, levofloxacin, ampicillin/cloxacillin, pencillin- G, cefoperazone/sulbactam, ceftriaxone/tazobactam (Table 7D i & ii).

Thus antibiotics sensitive test of pathogens recovered from the excised uterus following treatment with LO & CEF reveal multiple antibiotics resistant bacteria. The antibiotics effective against these multiple drug resistant (MDR) pathogens were tetracycline, amikacin, gentamicin and chloramphenicol against *Enterococcus sp., Pseudomonas sp., Staphylococcus sp.* and *Bacillus sp.* (Table 8).

Although the haemato-biochemical parameters improved following treatment with levofloxacin Ornidazole and Ceftiofur, clinical recovery was incomplete. The bacteria isolated from the excised uterus were *Enterococcus sp., Pseudomonas sp., Staphylococcus sp., Bacillus sp.* and gram negative anaerobics. The bitches that had ovario-hysterectomy without prior antibiotics treatment had *E. coli* and *Proteus sp.* in addition to the pathogen recovered after antibiotics treatment. Thus it is clear that Levofloxacin Ornidazole and Ceftiofur were effective in the elimination of *E. coli* and *Proteus sp.* that corroborates with the findings of Fish and Chow, 1997; Croom and Goa, 2003; Landoni and Albarellos, 2019; Sitovs *et al.*, 2021 .

Sachan *et al.* (2019) reported that the bacteria isolated from pus culture of canine pyometra were *Escherischia coli, Staphylococcus sp., beta-haemolytic Streptococci, Pasteurella multocida* and *Klebsiella sp.* Kumar *et al.* (2016) reported that the main pathological agent found in most of canine pyometra was *Escherischia coli*. Biswas *et al.* (2012) found that *Streptococcus sp.* along with *Escherischia coli* are normal flora of vagina which ascend into the uterus through dilated cervix during estrous. Kempisty *et al.* (2013) reported that *E. coli* was isolated along with *Staphylococci, Streptococci* and *Pseudomonas aeruginosa*. In our case major bacteria isolated from uterus of bitches with pyometra were *E. coli* and *Staphylococcus sp.* that corroborated with the results of the aforementioned authors but our study could not isolate *Pasteurella multocida, Klebsiella sp.* and *beta-haemolytic Streptococci.*

Ceftiofur, a third generation cephalosporin is the drug of choices for the treatment of uterine infection in pet and farm animals due to the fact it reaches a high enough

Table 7: Antibiotics Sensitivity of resistant bacteria isolated from the excised uterus of bitches with pyometra following treatment with levofloxacin ornidazole and ceftiofur sodium

A(i): EUCAST/CLSI Result

Name of bacteria: *Enterococccus sp.*

Antibiotics	Sensitivity (mm)	Resistant (mm)	Intermediate Zone (mm)	Result (mm)	Remarks
Amikacin (AK)- **30µg**	12-18			19	Sensitive
Amoxyclav(Amoxicillin/Clavulanic acid)- **30 µg (20/10 µg)**	10	8		<8	Resistant
Ampicillin/ Sulbactam (A/S)*- **10/10 µg**	15-21	8		<8	Resistant
Ciprofloxacin(CIP)- **5 µg**	21	15	16-20	27	Highly Sensitive
Gentamicin(GEN)- **10 µg**	12-18			21	Sensitive
Levofloxacin (LE)- **5 µg**	19-25			20	Resistant
Moxifloxacin (MO)- **5 µg**		12		25	Sensitive
Penicillin-G (P)- **10U**	15	14		20	Sensitive
Tetracycline (TE)- **30 µg**	19	14	15-18	30	Highly Sensitive
Ampicillin/Cloxacillin (AX)*- **10 µg**	15-21			18	Resistant
Chloramphenicol (C)- **30 µg**	18	12	13-17	24	Sensitive

***** Ampicillin resistance is uncommon in *E. faecalis* but common in *E. faecium* as per EUCAST/CLSI

A(ii): *Enterococcus sp.*[1]

Highly Sensitive	Sensitive	Resistant
Tetracycline	Chloramphenicol	Amoxicillin/Clavulanic acid
Ciprofloxacin	Gentamicin	Ampicillin/Cloxacillin
	Pencillin-**G**	Ampicillin/Sulbactam
	Amikacin	Levofloxacin
	Moxifloxacin	

B(i): EUCAST/CLSI Result

Name of bacteria: *Pseudomonas sp.*

Antibiotics	Sensitivity (mm)	Resistant (mm)	Intermediate Zone (mm)	Result (mm)	Remarks
Amikacin (AK)- **30µg**	17	14	15-16	30	Highly Sensitive
Amoxyclav(Amoxicillin/Clavulanic acid)- **30 µg (20/10 µg)**	18	13	14-17	10	Resistant
Ciprofloxacin(CIP)- **5 µg**	50	26	23-24	36	Resistant
Gentamicin(GEN)- **10 µg**	15	15		20	Sensitive
Levofloxacin (LE)- **5 µg**	50	18	18-19	18	Resistant
Moxifloxacin (MO)- **5 µg**	17-25			20	Resistant
Tetracycline (TE)- **30 µg**	≥19	≤14	15-18	36	Highly Sensitive
Cefoperazone/ Sulbactam (CFS)- **75/30 µg**	22-28			34	Highly Sensitive
Ceftriaxone/ Tazobactam (CIT)- **30/10 µg**	23	23		20	Resistant
Chloramphenicol (C)- **30 µg**	18	12	13-17	20	Sensitive

B(ii): *Pseudomonas sp.*[1]

Highly Sensitive	Sensitive	Resistant
Amikacin Tetracycline Cefoperazone/Sulbactam	Chloramphenicol Gentamicin	Amoxicillin/Clavulanic acid Moxifloxacin Levofloxacin Ceftriaxone/Tazobactam Ciprofloxacin

C(i): EUCAST/CLSI Result

Name of bacteria: *Staphylococcus sp*.

Antibiotics	Sensitivity (mm)	Resistant (mm)	Intermediate Zone (mm)	Result (mm)	Remarks
Amikacin (AK)- **30µg**	17	14	15-16	33	Highly Sensitive
Amoxyclav(Amoxicillin/Clavulanic acid)- **30 µg (20/10 µg)**		29		28	Resistant
Ampicillin/Sulbactam (A/S)- **10/10 µg**		29		24	Resistant
Ciprofloxacin(CIP)- **5 µg**	50	21	16-20	41	Resistant
Gentamicin(GEN)- **10 µg**	18	12	13-14	29	Sensitive
Levofloxacin (LE)- **5 µg**	50	24	16-18	32	Resistant
Moxifloxacin (MO)- **5 µg**	24	20	21-23	38	Highly Sensitive
Penicillin-G (P)- **10U**	29	28		25	Resistant
Tetracycline (TE)- **30 µg**	22	19	12-14	34	Sensitive
Ampicillin/ Cloxacillin (AX)- **10 µg**	35-37			21	Resistant
Cefoperazone/ Sulbactam (CFS)- **75/30 µg**	23-30			24	Sensitive
Ceftriaxone/ Tazobactam (CIT)- **30/10 µg**	24-32			25	Sensitive
Chloramphenicol (C)- **30 µg**	18	12	13-17	18	Sensitive

C(ii): *Staphylococcus sp.*[1]

Highly Sensitive	Sensitive	Resistant
Amikacin Moxifloxacin	Tetracycline Gentamicin Chloramphenicol Cefoperazone/Sulbactam Ceftriaxone/Tazobactam	Ampicillin/Cloxacillin Penicillin-G Ciprofloxacin Levofloxacin Ampicillin/Sulbactam Amoxicillin/Clavulanic acid

D(i): EUCAST/CLSI Result

Name of bacteria: *Bacillus sp.*

Antibiotics	Sensitivity (mm)	Resistant (mm)	Intermediate Zone (mm)	Result (mm)	Remarks
Amikacin (AK)- **30µg**	17			24	Highly Sensitive
Amoxyclav(Amoxicillin/Clavulanic acid)- **30 µg (20/10 µg)**		29		15	Resistant
Ampicillin/Sulbactam (A/S)- **10/10 µg**		29		14	Resistant
Ciprofloxacin(CIP)- **5 µg**	50	23	23-49	30	Resistant
Gentamicin(GEN)- **10 µg**	18			23	Sensitive
Levofloxacin (LE)- **5 µg**	50	24	23-49	25	Resistant
Penicillin-G (P)- **10U**	29			19	Resistant
Tetracycline (TE)- **30 µg**	22	19	21-22	23	Sensitive
Ampicillin/ Cloxacillin (AX)- **10 µg**	37			15	Resistant
Cefoperazone/ Sulbactam (CFS)- **75/30 µg**	30			27	Resistant
Ceftriaxone/ Tazobactam (CIT)- **30/10 µg**	32			24	Resistant
Chloramphenicol (C)- **30 µg**	18			25	Sensitive

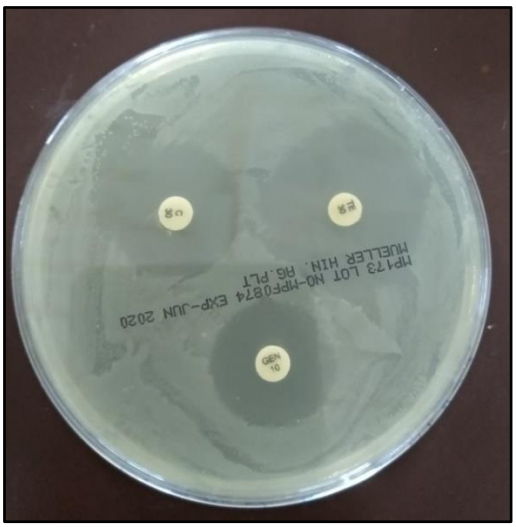
D(ii): *Bacillus sp.*[2]

Highly Sensitive	Sensitive	Resistant
Amikacin	Gentamicin Chloramphenicol Tetracycline	Amoxicillin/Clavulanic acid Ampicillin/Sulbactam Ciprofloxacin Levofloxacin Penicillin-G Ampicillin/Cloxacillin Cefoperazone/Sulbactam Ceftriaxone/Tazobactam

1: Antibiotics Sensitivity Test performed according to the EUCAST/CLSI guidelines and zone of inhibition compared according to the set standards

2: The breakpoints used for the above antibiotics were for *Staphylococcus sp.* according to the zone diameters of antimicrobial agents in accordance with CLSI guidelines 2011. It has been reported that breakpoints of antibiotics for *Staphylococcus sp.* can be used to test *Bacillus sp.* (*non-Bacillus anthracis*; Meena *et al.* 2000)

Figure 7: Zone of inhibition following incubation of evenly distributed bacterial suspension with antibiotics discs in Muller Hinton agar plate for 24 hours at 37º C



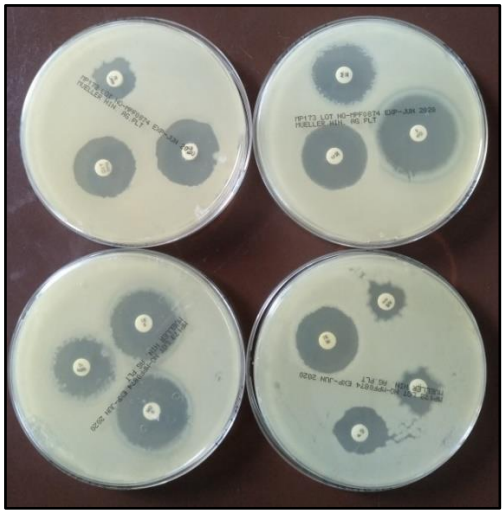


Table 8: Antibiotics sensitive to multiple pathogenic bacteria recovered from uterus in cases of canine pyometra

Antibiotics	Pathogenic bacteria
Tetracycline Amikacin Gentamicin Chloramphenicol	*Bacillus sp.* *Pseudomonas sp.* *Enterococcus sp.* *Staphylococcus sp.*

concentration in the uterus to kill most common pathogenic bacteria (Bosch *et al.,* 2006) and its broad spectrum activity attributed in part to its resistance to attack by bacterial beta lactamases rendered by methoxy side chain of imino group (Neu, 1982). Though excellent activity of ceftiofur was reported against *E. coli* (Yancey *et al.,* 1987; Brown *et al.,* 1991; Jaglan *et al.,* 1992), *Staphylococcus aureus* (Yancey *et al.,* 1987 and Brown *et al.,* 1991), gram negative anaerobic bacteria (Brown *et al.,* 1991), ceftiofur was resistant to *Pseudomonas aeruginosa* (Brown *et al.,* 1991). *Pseudomonas sp*. isolated from the pus samples of bitches treated with ceftiofur in our work corroborates with the findings of Brown *et al.* 1991 which indicates intrinsic resistance of *Pseudomonas sp*.to ceftiofur that did not change with time.

However we could recover gram negative anaerobic bacteria from uterine pus samples treated with ceftiofur that is inconsistent with the findings of Brown *et al.* 1991. More recent studies revealed that amongst the gram negative anaerobes ceftiofur had good activity against *Fusobacterium sp*.(Jeon *et al.*, 2018) but *Bacteroides sp.* was able to degrade ceftiofur by the release of beta lactamases (Wagner *et al.*, 2011). Thus from the above discussion it can be inferred that gram negative anaerobes resistant to ceftiofur in our samples could be *Bacteroides sp.*

As opposed to the findings of Yancy *et al.,* 1987 and Brown *et al.*, 1991 wherein they reported that ceftiofur was sensitive to *Staphylococcus aureus,* we recovered *Staphylococcus sp.* from excised uterus following ceftiofur treatment implying that *Staphylococcus sp.* has acquired resistance to ceftiofur with time. This finding is corroborated with the work of Prescott, 2013 who reported *Staphylococci aureus* was resistant to ceftiofur. *Enterococcus sp*. recovered from samples following ceftiofur treatment is well supported by the findings of Prescott, 2013 and Hooper *et al.*, 2016 who reported the intrinsic resistance of ceftiofur and cephalosporins as a class being resistant to *Enterococcus sp.* We also recovered *Bacillus sp.* following ceftiofur treatment which proves its resistant due to presence of ceftiofur degrading beta lactamase Wagner *et al.*, 2011.

Levofloxacin is the ideal antibiotics of choice for the treatment of uro-genital infection in human as well as in animals due to fact that it is excellently distributed in the uro-genital tract and sensitive to most common pathogenic bacteria (Gao *et al.,* 2014; Casas *et al*., 2019; Landoni & Albarellos, 2019 and Madsen *et al.,* 2019). In our case we

administered levofloxacin ornidazole for the treatment of pyometra in bitches. Pus samples collected from excised uterus following treatment with levofloxacin ornidazole recovered *Bacillus sp. Enterococcus sp., Pseudomonas sp.,* and *Staphylococcus sp.* but unlike in the ceftiofur treated bitches gram negative anaerobes were not recovered. Further *E. coli* and *Proteus sp.* were not recovered from pus samples of excised uterus of bitches that had pre-treatment with levofloxacin compare to bitches that had an ovario-hysterectomy without antibiotics pre-treatment. From the above discussion it can be inferred that levofloxacin ornidazole was effective in elimination of anaerobic bacteria, *E. coli* and *Proteus sp*.

The resistance of *Pseudomonas aeruginosa* has been reported by Morissey and Smith, 1994 and Davis and Bryson, 1994 and has also been found in our study which point to the fact that this bacteria has intrinsic resistance to levofloxacin ornidazole. Earlier reports have pointed out sensitivity of levofloxacin to *Staphylococcus aureus* (Davis and Bryson, 1994; Tarshish *et al.,* 2001), *Enterococcus faecalis* (Davis and Bryson, 1994, Croom and Goa., 2003) and *Bacillus sp*. (Deziel *et al.,* 2005, Yee *et al.*, 2010) which contradicts with our results wherein we found these bacteria resistant to levofloxacin. More recent studies have reported the resistance of levofloxacin to *Staphylococcus aureus* (Prescott, 2013) and moderate susceptibility to *Enterococcus faecalis* which indicates the emergence of resistance strains of *Staphylococcus sp.* and *Enterococcus sp*. against levofloxacin. There is Paucity of recent literature regarding resistant of *Bacillus sp*. to levofloxacin but in our study we had recovered *Bacillus sp*. from uterine pus samples of bitches treated with levofloxacin ornidazole. Thus it can be inferred from our work that there is recently developed resistance of *Bacillus sp*. to levofloxacin ornidazole.

The bitches with pyometra show clinical symptoms such as lethargy, vomition, anorexia and vulvar discharge (Hardy and Osborne, 1974) is due to the release of endotoxin (lipopolysaccharide) presence in cell wall of *E. coli* and gram negative bacteria either result of bacterial death or vigorous growth of the bacteria (Borresen, 1975; Hagman & Greko, 2005). Higher release of endotoxin give rise to cardiovascular and gastrointestinal effects such as mucoid, bloody diarrhea and vomiting. Timely therapeutic interventions of the bitches with pyometra helps to bring the haemoto-biochemical parameters to normalcy if diagnosed earlier, otherwise it leads to progressive hypotension (Hardie, 1995, Panciera *et al*., 2003) leading to endotoxic shock and myocardial failure and high risk of mortality (Hardie, 1995; Brady & Otto, 2001; Hagman *et al.,* 2006).

High values of aspartate aminotransferase and alanine aminotransferase seen in cases of canine pyometra is due to hepatocellular damage as a result of endotoxemia (Nishida *et al.* 1990 and Holst *et al.,* 1993). Endotoxemia caused by bacterial lipopolysaccharide, a component of the outer cell wall of Gram-negative bacteria, that enters the liver via circulating portal blood (Borresen and Skrede, 1980 and Panciera *et al.,* 2003) and inhibits bile flow and biliary excretion of organic anions causing bile acid induced hepatocyte apoptosis (Woolbright and Jaeschk, 2012). Furthermore endotoxemia results in dehydration and decreased hepatic blood flow leading to hypoxia and hepatic damage (Wheaton *et al*., 1989). The increase in BUN and creatinine level in cases of canine pyometra as found in our study and also by other workers (Kuplulu *et al.*, 2009 and Shah *et al*., 2017) is due to glomerulonephritis due to immune complex deposition in the glomerular basement membrane (Pretzer, 2008) as well as hypoxia caused by renal perfusion rate.

The peptidoglycan and lipoteichoic acids (LTA) is the component of the cell wall of gram positive bacteria (Millar and Thiemermann, 1997; Thiemermann, 1997 and Ceppi *et al.,* 1997) and released the enterotoxins and exotoxins which caused toxic shock syndrome toxin 1 which are involved in the pathogenesis of sepsis (Bone, 1994 and Kimpe *et al.*, 1995). It leads to the release of endogenous cytokines which are responsible for the development of septic shock which increased the levels of Nitric oxide, TNF-α, IL-1β, IFN-γ and IL-6 in their serum (Kilbourn, 1997). The development of shock results in a progressive failure of the circulation to provide blood and oxygen to vital organs of the body resulting in impaired tissue perfusion and oxygen extraction (Thiemermann, 1997). The key symptoms include a severe fall in blood pressure (hypotension) with hyporeactivity to vasoconstrictor agents (vasoplegia) which may lead to the dysfunction or failure of major organs including lungs, liver, kidneys and brain (multiple organ dysfunction, MODS) and ultimately death (Rackow and Astiz, 1991; Thiemermann, 1997 and Kilbourn, 1997).

Our results corroborates with aforementioned discussion wherein there was incomplete recovery of bitches with pyometra following treatment with either levofloxacin ornidazole or ceftiofur sodium. We observed that the liver function and kidney function did not return to normal level following antibiotics therapy and both gram negative as well as gram positive bacteria isolated from the excised uterus. However the liver and kidney

function were better in levofloxacin ornidazole treated bitches compare to ceftiofur which could be explained by the fact levofloxacin ornidazole able to complete eliminate gram negative anaerobes which ceftiofur could not. Thus it can be inferred that LPS from gram negative anaerobes is highly toxic and antibiotics therapy should target these pathogens for complete cure of canine pyometra.

Although some bacteria were common in vagina and uterus but most of them were totally different in uterus and vagina of the bitches with pyometra. This was consistent with the findings of Hadley, 1975; Schlafer and Gifford, 2008 and Lyman *et al.*, 2019 who reported that the bacterial population of uterus was more diverse than vagina bacterial population and that the endometrium and vagina has its own resident microbiome thus contradicting the hypothesis that pyometra developed as an ascending infection from the vagina through the open cervix.

Enterococcus sp. was found to be resistance against Amoxicillin/Clavulanic acid, Ampicillin/Sulbactam and Levofloxacin in our study. Miskeen and Deodhar (2002), Rodrigues *et al.* (2002) and Kapoor *et al.* (2005) found *Enterococcus* to be resistant to Amoxicillin/Clavulanic acid, however sensitivity of *Enterococcus* to amoxicillin/Clavulanic acid was reported by Orrett and Connors, (2001); Li *et al.* (2014) and Maasjost *et al.* (2015). Miskeen and Deodhar (2002), Rodrigues *et al.* (2002), Mendiratta *et al.* (2008) and Parameswarappa *et al.* (2013) found *Enterococcus* to be resistant to Ampicillin/Sulbactam, however sensitivity of *Enterococcus* to Ampicillin/Sulbactam was reported by Lee *et al.* (2011), Yumi and Gilho, (2013) and Gilho, (2013). Amipicillin resistance is uncommon in *E. faecalis* but common in *E. faecium* as per EUCAST/CLSI, therefore *Enterococcus* isolated in our study could have been *E. faecium* since it was resistant to amipicillin. Hayden *et al.* (1995), Hoogkamp-Korstanje (2000) and Li *et al.* (2014) found *Enterococcus* to be resistant to Levofloxacin, however sensitivity of *Enterococcus* to Levofloxacin was reported by Yasufuku *et al.* (2011), Yum and Gilho (2013) and Gilho, (2013).

In our study *Enterococcus sp.* found to be sensitive to Tetracycline, Ciprofloxacin, Chloramphenicol, Gentamicin, Pencillin-**G,** Amikacin and Moxifloxacin. In our support of our findings *Enterococcus* was also found to be sensitive to tetracycline (Rudy *et al.*, 2004; Pinheiro *et al.*, 2004 and García-Solache and Rice, 2019), Ciprofloxacin (Hoogkamp-Korstanje, 2000, Rudy *et al.*, 2004; Pinheiro *et al.*, 2004 and Yumi and Gilho,

2013), Chloramphenicol (Lautenbach *et al.*, 1998; Ricaurte *et al.*, 2001 and Pinheiro *et al.*, 2004), Gentamicin (Traub *et al.*1986; Lefort *et al.*, 2000 and Yumi and Gilho, 2013), Pencillin-**G (**Sibel *et al.*, 2012; Yumi and Gilho, 2013 and Maasjost *et al.*, 2015), Moxifloxacin (Hoogkamp-Korstanje, 2000; Pinheiro *et al.*, 2004 and Schubert and Dalhoff, 2012). However reports of resistant of *Enterococcus* to Tetracycline (Chatterjee *et al.*, 2007, Yumi and Gilho, 2013 and Gilho, 2013), Ciprofloxacin (Chatterjee *et al.*, 2007, Mendiratta *et al.*, 2008, Parameswarappa *et al.*, 2013 and Gilho, 2013), Chloramphenicol (George and Uttley, 1980; Peters *et al.*, 2003 and Chatterjee *et al.*, 2007), Gentamicin (Miskeen and Deodhar, 2002; Aslangul *et al.*, 2006; Mendiratta *et al.*, 2008 and Parameswarappa *et al.*, 2013), Pencillin-**G (**Orrett and Connors, 2001, Feizabadi *et al.* 2004 and Chatterjee *et al.*, 2007), Amikacin (Neu, 1992; Gonzalez and Spencer, 1998 and Mir *et al.*, 2011), Moxifloxacin. (Tankovic *et al.*, 1999) have also been reported.

In our study *Pseudomonas sp*. was found to be resistant to Amoxicillin/Clavulanic acid, Ciprofloxacin, Moxifloxacin, Ceftriaxone/Tazobactam and Levofloxacin. In support of our findings, *Pseudomonas* was also found to be resistant to Amoxicillin/Clavulanic acid (Brown and Izundu, 2004; Goel *et al.*, 2009 and Walelign *et al.*, 2016), Ciprofloxacin (Zanel *et al.*, 2008; Javiya *et al.*, 2008 and Sivanmaliappan and Sevanan, 2011), Moxifloxacin (Bauernfeind, 1997; Bebear *et al.*, 1998 and Culley *et al.*, 2001), Ceftriaxone/Tazobactam (Javiya *et al.*, 2008; Zanel *et al.*, 2008 and Walelign *et al.*, 2016), Levofloxacin (Javiya *et al.*, 2008; Noreddin and Elkhatib, 2010 and Yayan *et al.*, 2015). However reports of sensitivity of *Pseudomonas* to Ciprofloxacin (Decimo *et al.*, 2016; Walelign *et al.*, 2016 and Meng *et al.* 2020), Moxifloxacin (Zhanel *et al.*, 1999 and Soussy *et al.*, 2003**),** Ceftriaxone/Tazobactam (Greenwood and Eley, 1982; Von Graevenitz and Bucher, 1982 and Richards *et al.*, 1984), Levofloxacin (Trivedi *et al.*, 2015; Decimo *et al.*, 2016 and Meng *et al.*, 2020) have also been reported.

In our study *Pseudomonas sp.* found to be sensitive to Tetracycline, Chloramphenicol, Gentamicin and Cefoperazone/Sulbactam acid. In support of our findings, *Pseudomonas* was also found to be sensitive to Tetracycline (Morgan, 2014; Decimo *et al.*, 2016 and Meng *et al.* 2020), Chloramphenicol (Morgan, 2014 and Decimo *et al.*, 2016), Gentamicin (Trivedi *et al.*, 2015; Walelign *et al.*, 2016 and Meng *et al.*, 2020), Cefoperazone/Sulbactam (Hinkle *et al.*, 1980, Jones *et al.*, 1981 and Jones and Barry, 1983). However reports of resistant of *Pseudomonas* to Tetracycline (Brown and

Izundu, 2004 and Sivanmaliappan and Sevanan, 2011 and Decimo *et al*., 2016), Chloramphenicol (Javiya *et al.,* 2008; Trivedi *et al.,* 2015 and Meng *et al.,* 2020), Gentamicin (Zanel *et al.,* 2008, Javiya *et al.,* 2008 and Sivanmaliappan and Sevanan, 2011), Cefoperazone/Salbactam (Neu *et al.,* 1979 Javiya *et al.,* 2008 and Sivanmaliappan and Sevanan, 2011).

In our study *Staphylococcus sp*. was found to be resistant to Ampicillin/Sulbactam, Penicillin-G, Ciprofloxacin, Levofloxacin and Amoxicillin/Clavulanic acid. In support of our findings, *Staphylococcus* was also found to be resistant to Ampicillin/Sulbactam (Rajaduraipandi *et al.,* 2006, Akanbi *et al.,* 2017 and Okonkwo *et al.,* 2018), Penicillin-G (Rajaduraipandi *et al.,* 2006; Onwubiko and Sadiq, 2011 and Akanbi *et al.,* 2017) Ciprofloxacin (Sharma and Mall, 2011; Singh *et al.,* 2016 and Pramodhini *et al.,* 2017), Levofloxacin (Zhanel *et al.,* 2006; Fritsche *et al.,* 2007 and Okonkwo *et al.,* 2018), Amoxycillin/Clavulanic acid (Hogi *et al.,* 1998; Shibabaw *et al.,* 2014 and Okonkwo *et al.,* 2018). However reports of sensitivity of *Staphylococcus* to Penicillin-G (Afsari and Rezaiian, 1977; Hoogkamp-Korstanje, 2000 and Aldman and Pahlman, 2020), Ciprofloxacin (Rajaduraipandi *et al.,* 2006; Onwubiko and Sadiq, 2011 and Akanbi *et al.,* 2017), Levofloxacin (Moran *et al.,* 2006; Onwubiko and Sadiq, 2011 and Akanbi *et al.,* 2017), Amoxicillin/Clavulanic acid (Rajaduraipandi *et al.,* 2006; Onwubiko and Sadiq, 2011 and Naimi *et al.,* 2017) have also been reported.

In our study *Staphylococcus sp.* found to be sensitive to Amikacin, Moxifloxacin, Gentamicin, Chloramphenicol, Cefoperazone/Sulbactam, Ceftriaxone/Tazobactam and Tetracycline. In our support of our findings, *Staphylococcus* was also found to be sensitive to Amikacin (Khanal and Jha, 2010; Mahmood *et al.,* 2010 and Bhatt *et al.,* 2014), Moxifloxacin (Zhanel *et al.,* 1999; Hoogkamp-Korstanje, 2000 and Soussy *et al.,* 2003), Gentamicin (Onwubiko and Sadiq, 2011; Akanbi *et al.,* 2017 and Okonkwo *et al.,* 2018), Chloramphenicol (Onwubiko and Sadiq, 2011; Akanbi *et al.,* 2017 and Naimi *et al.,* 2017), Cefoperazone/Sulbactam (Ragamy *et al.,* 1975; Goldman & Petersdorf, 1980 and Chambers and Fournier, 1993), Ceftriaxone/Tazobactam (Tan *et al.,* 2010; Bushra *et al.,* 2016 and Naimi *et al.,* 2017), Tetracycline (Moran *et al.,* 2006; Onwubiko and Sadiq, 2011 and Akanbi *et al.,* 2017). However reports of resistant of *Staphylococcus* to Amikacin (Gilbert, 1995) Moxifloxacin (Thomson *et al.,* 1991; Yoshida *et al.,* 1991 and Thomson and Sanders, 1994), Gentamicin (Rajaduraipandi *et al.,* 2006; Sharma and Mall, 2011 and

Naimi *et al.,* 2017), Chloramphenicol (Hogi *et al.,* 1998; Sharma and Mall, 2011 and Okonkwo *et al.,* 2018), Cefoperazone/Sulbactam (Hall *et al.,* 1980; Pulliam *et al.,* 1981 and Jones and Barry, 1983), Ceftriaxone/Tazobactam (Shoaib *et al.,* 2001; Masood and Aslam, 2010 and Gashe *et al.,* 2018), Tetracycline (Hogi *et al.,* 1998; Sharma and Mall, 2011 and Shibabaw *et al.,* 2014) have also been reported.

In our study *Bacillus sp*. was found to be resistant to Amoxicillin/Clavulanic acid, Ampicillin/Sulbactam, Ciprofloxacin, Levofloxacin, Penicillin-G, Cefoperazone/Sulbactam and Ceftriaxone/Tazobactam. In support of our findings, *Bacillus* was also found to be resistant to Amoxicillin/Clavulanic acid (Logan and Turnbull., 2003; Luna *et al*., 2007 and Adewumi *et al.,* 2009), Ampicillin/Sulbactam (Jensen *et al*., 2001; Logan and Turnbull, 2003 and Luna *et al*., 2007), Ciprofloxacin (Hooper *et al*., 1987; Qin *et al.,* 2006 and Magiorakos *et al*., 2012), Levofloxacin (Horii *et al.,* 2011; Milan *et al.,* 2001 and Gururaju *et al*., 2015), Penicillin-G (Ciffo, 1984; Al-Khatib *et al.,* 2007 and Oladipo and Adejumobi, 2010), Cefoperazone/Sulbactam (Kucutkates and Kocazeybek, 2002; Levin, 2002 and Gupta *et al.,* 2006), Ceftriaxone/Tazobactam (Logan and Turnbull, 2003; Al-Khatib *et al.,* 2007 and Oladipo and Adejumobi, 2010). However reports of sensitivity of *Bacillus* to Ampicillin/Sulbactam (Odendaal *et al.,* 1991; Ikeda *et al.,* 2015 and Gao *et al.,* 2018), Ciprofloxacin (Andrews and Wise, 2002; Adewumi *et al.,* 2009; and Oladipo and Adejumobi, 2010), Levofloxacin (Siegrist *et al.,* 1999; Ikeda *et al*., 2015 and Sharma *et al.,* 2019), Penicillin-G (Odendaal *et al.,* 1991) have also been reported.

In our study *Bacillus sp.* found to be sensitive to Amikacin, Gentamicin, Chloramphenicol and Tetracycline. In support of our findings, *Bacillus* was also found to be sensitive to Amikacin (Betts *et al*., 1984; Chambers and Sande, 1996 and Ikeda *et al.,* 2015), Gentamicin (Hoa *et al.,* 2000; Luna *et al*., 2007 and Oladipo and Adejumobi, 2010), Chloramphenicol (Jensen *et al*., 2001; Luna *et al*., 2007 and Oladipo and Adejumobi, 2010), Tetracycline (Jensen *et al.,* 2001; Logan and Turnbull, 2003 and Luna *et al*., 2007). However reports of resistant of *Bacillus* to Amikacin (Priceet *et al.,* 1981; Moody *et al.,* 1982 and Levine *et al.,* 1985), Gentamicin (Kassimi, 1988; Moaz *et al.,* 1989 and Adewumi *et al.,* 2009), Chloramphenicol (Ciffo *et al*., 1984; Hoa *et al.,* 2000 and Huang *et al.,* 2008), Tetracycline (Ciffo, 1984; Al-Khatib *et al.,* 2007 and Oladipo and Adejumobi, 2010) have also been reported.

It can be inferred from our work that there is emergence of multidrug resistant pathogens that throw new challenges in the treatment of canine pyometra. In fact none of the resistant pathogens in our study was sensitive to amoxicillin/Clavulanic acid which proves the wide spread resistance to beta-lactam antibiotics. This can be attributed to overuse and misuse of these antibiotics. Antibiotics are often prescribed incorrectly that leads to sub-inhibitory and sub-therapeutic concentration promoting the development of resistance through mutagenesis and plasmid mediated a horizontal gene transfer (HGT) (Von Wintersdorff *et al.*, 2016). Further bacteria was found to have narrow spectrum antibiotics sensitivity and considering the fact that most cases of canine pyometra have mixed bacterial infection therefore treatment of pyometra with single antibiotics is not enough.

Summary and Conclusion

Pyometra is the most common reproductive disorder of diestrum in middle-aged to old bitches. It occurs due to prolonged proestrous and estrous phase in bitches leading to prolonged estrogen dominance and open cervix that facilitates the entry of bacteria from the vagina to the uterus. After ovulation, the corpus luteum (C. L.) secretes progestrone which provides the ideal environment for the growth of pathogens by causing the endometrium to release histotrophs or uterine milk that serves as an excellent media for the growth of bacteria as well as suppressing the local immunity.

The most common pathogenic bacteria responsible for causing pyometra are *Escherichia coli* (90%), *Bacteroids sp.* and *Fusobacterium sp.* The most common antibiotics used for the treatment of canine pyometra is Levofloxacin Ornidazole and Ceftiofur Sodium, however there are recent reports of incomplete recovery of bitches treated with these antibiotics. Recent data of resistant organisms is lacking and antibiotics sensitivity of these resistant pathogens needed to be determined. Thus this work was taken up with the following objectives- (i) to assess the clinical efficacy of Ceftiofur Sodium & Levofloxacin Ornidazole in the treatment of canine pyometra, (ii) to isolates the resistant pathogens after the treatment and (iii) to test the antibiotics sensitivity of the resistant pathogens.

The bitches with pyometra were taken from Veterinary Clinical Complex, Bihar Veterinary College, Patna. The bitches were diagnosed on the basis of haemato-biochemistry and clinical parameters includes anorexia, foul smell pus discharge from vulva, polydipsia, polyuria, lethargy, ultrasonography and evaluated for recovery based on pre-defined grading system following treatment with Ceftiofur Sodium & Levofloxacin Ornidazole along with Cloprostenol Sodium for 7 days. Cases which did not get cured after treatment were subjected to ovario-hysterectomy after seven days of antibiotics. Pus was collected from excised uterus and identified on the basis of growth in selective agar, biochemical test and grams stain.

There was non-significant difference in improvement of clinical parameters between Levofloxacin Ornidazole treated and Ceftiofur treated groups as well as before and after treatment with these antibiotics. Haemato-biochemistry parameters were significantly better with Levofloxacin Ornidazole treated compared to Ceftiofur treated

bitches. The most common bacteria isolated from the excised uterus following treatment with ceftiofur and levofloxacin ornidazole combination were *Enterococcus sp., Staphylococcus sp., Pseudomonas sp.* and *Bacillus sp.* However, *E. coli, Proteus sp.,* Gram negative anaerobes, were isolated in addition to aforementioned bacteria from excised uterus of bitches without any antibiotics pre-treatment thus, these two antibiotics were effective against these pathogens. Improvement in liver function and kidney function may be attributed to efficacy of these antibiotics against *E. coli, Proteus*, and gram negative anaerobes as lipopolysaccharides released from the cell wall of these bacteria have been found to cause the release of cytokines that directly damage these organs and indirectly result in decrease in organ perfusion and hypoxia. Moreover, gram negative anaerobes were isolated in ceftiofur treated bitches implying its resistance to ceftiofur. This explained incomplete recovery of haemato- biochemistry parameters in ceftiofur treated bitches compare to levofloxacin ornidazole treated bitches.

Tetracycline, amikacin, gentamicin and chloramphenicol were found to be effective against *Bacillus sp., Pseudomonas sp., Enterococuus sp.* and *Staphylococcus sp*. There was wide spread resistance of these bacteria to fluoroquinolones, amino-penicillins and cephalosporin. Further narrow spectrum of sensitivity of these bacteria to antibiotics warrants a combination therapy for effective treatment of canine pyometra.

Conclusions

We found the emmergence of gram positive and gram negative bacterial resistant to ceftiofur & levofloxacin ornidazole. *Staphylococcus sp., Bacillus sp. and Enterococcus sp*. were the most common gram positive, multi- drug resistant (MDR) pathogens in cases of canine pyometra. *Pseudomonas sp.* was the major MDR gram negative pathogen in cases of canine pyometra. Wide spread beta– lactam antibiotics resistance was found amongst MDR pathogen. Narrow spectrum antibiotics sensitivity was observed in MDR pathogens. Therefore treatment of pyometra with mixed MDR pathogens, single antibiotics therapy is not enough. Since antibiotics resistance of bacteria changes with time and it’s usage, this work must be repeated over time and in different localities.

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ABSTRACT

This work was to find out the organism resistant to ceftiofur and levofloxacin ornidazole in cases of canine pyometra. Bitches were treated with ceftiofur and levofloxacin ornidazole and clinical parameters were evaluated based on vomition, lethargy, anorexia, polydipsia, polyuria and pus discharge from vagina. Haemato- biochemistry was also formed and following parameters evaluated. Pus was collected from excised uterus after ovario-hysterectomy after 7 days of antibiotics treatment and cultured in BHI broth and identified by the growth in differential/selective, biochemical test and gram stain. None of the antibiotics showed complete recovery, however levofloxacin ornidazole treatment resulted in better recovery as compared to ceftiofur sodium as ceftiofur was unable to clear gram negative anaerobic infection which was confirmed by better results in the haemato-biochemistry with levofloxacin ornidazole treated bitches. Resistant organisms isolated with either antibiotics treatment were *Bacillus sp., Pseudomonas sp., Staphylococcus sp., and Enterococcus sp.* Antibiotics highly effective against these organisms were tetracycline, amikacin, gentamicin and chloramphenicol. Wide spread resistance was found against fluoroquinolones, amino-penicillins and cephalosporin. Narrow spectrum antibiotics sensitivity in these multi-drug resistant pathogens warrants combination antibiotics therapy.

Keyword: Canine Pyometra, Ceftiofur Sodium, Levofloxacin Ornidazole, Multi-Drug Resistant Pathogens

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Abstract

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