
CANINE PYOMETRA: MICROBIOLOGICAL DIAGNOSIS AND THERAPY

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Abstract

Pyometra is a common illness in adult intact female dogs and cats and a less frequent diagnosis in other small animal species. The aim of this study was to evaluate the microbial flora isolated from uterine exudates of bitches suffering from pyometra and test their susceptibility to antibiotics in dogs presented to a clinic in North Rhine-Westphalia region, Germany. Between February and September 2018, a total of 40 dogs suffering from pyometra were included in this study. Cotton swabs were used to collect pus from the uterus. The identification of bacteria was possible using MALDI-TOF MS device, in a private clinic from Germany. The susceptibility to antibiotics was evaluated using Kirby-Bauer disk diffusion test. The results demonstrated that Escherichia coli was the most frequently isolated bacteria, present in 28 from 40 samples (70%), followed by Streptococcus sp. in 5 samples (12.5%), Staphylococcus pseudintermedius in 3 samples (7.5%), Enterococcus sp. and Pasteurella multocida, each in 2 samples (5%) and Staphylococcus haemolyticus, Pseudomonas aeruginosa, Pseudomonas putida, Klebsiella pneumoniae, Haemophilus hemoglobinophilus, Acinetobacter pittii, Pseudomonas koreensis and Enterobacter cloacae, each in 1 sample (2.5%). Regarding antibiotic susceptibility, the most efficient antibiotics were represented by Marbofloxacin, Enrofloxacin Doxycycline and Amoxicillin and clavulanic acid, while the least efficient were Penicillin G and Clindamycin.

Keywords: pyometra, bitches, isolation, susceptibility testing.

Introduction

Pyometra is a common illness in adult intact female dogs and cats and a less frequent diagnosis in other small animal species. (Hagman, 2018). The pathogenesis of pyometra is only partly understood, but it's generally acknowledged that primary hormonal imbalance or abnormal response to normal concentrations of estrogens, and progesterone affects the epithelial cells of the uterus and facilitates bacterial adherence, colonization and growth (Fieni, 2014).

The disease is defined by accumulation of purulent material within the uterus, which manifests in both local and systemic symptoms, and demands costly surgical or medical intervention in order to resolve (Gibson A., 2013). Progesterone induces changes in the uterus which prepare a suitable environment for early embryo development, including endometrial proliferation, increased uterine glandular secretions and decreased myometrial contractions, as well as a relaxation in normal uterine cellular immune defenses (Noakes D., 2009).

The clinical signs are usually expressed in the diestrus phase, 4-8 weeks after the estrus, bitches usually present anorexia, lethargy, polydipsia and polyuria. Fever is not common, but when the cervix is open, a purulent hemorrhagic discharge is observed. The most common bacterium isolated is *Escherichia coli*, normal approach is to grow the pathogens from the uterine exudates and test their susceptibility to antimicrobial agents.

Recent papers published in the field demonstrated that Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) offers the possibility of accurate, rapid, inexpensive identification of bacteria, fungi, and mycobacteria isolated in clinical microbiology laboratories (Murray P.R., 2012).

The aim of this study was to evaluate the microbial flora isolated from uterine exudates of bitches suffering from pyometra and test their susceptibility to antibiotics in dogs presented to a clinic in North Rhine-Westphalia region, Germany.

Materials and methods

Between February and September 2018, a total of 40 dogs suffering from pyometra were included in this study. The clinical cases presented for consultation had in most situations closed cervix (n=29; 72.5%), the rest were open cervix. Clinical findings included polyuria, polydipsia, lethargy, anemia, leukocytosis and, in chronic cases increased renal parameters. The diagnosis was established after anamnesis, physical examination and abdominal radiography. Ovariohysterectomy was the treatment of choice for all cases, associated with antimicrobial therapy according to the in vitro susceptibility test.

Cotton swabs were used to collect pus from the uterus. The swabs were initially used to prepare slides and examine them after Gram staining technique was performed. The examination aimed to evaluate the shape, size and arrangement of bacteria, in most cases Gram negative cocobacilli, typical for genus *Escherichia*. The inoculation of blood agar plated was then performed for the characterization of isolates.

The identification of bacteria was possible using MALDI-TOF MS device, in a private laboratory from Germany. Isolated 24 h colonies were selected and suspended in 70% ethanol with the purpose of inactivating bacteria, then centrifuged. The supernatant was removed and the cells resuspended in 70% formic acid for disruption of the cell wall. Acetonitrile is added for protein extraction and the sample is again centrifuged for concentration. The target plate is covered with 1 μ l of supernatant, allowed to dry and then covered with the matrix consisting of a saturated solution of α -cyano-4-hydroxy-cinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid.

The susceptibility to antibiotics was evaluated using Kirby-Bauer disk diffusion test. The isolated colonies grown on blood agar were used to prepare a suspension in broth of 0.5 density on McFarland scale. This suspension was then used to flood Mueller-Hinton agar plates. The excess fluid was removed and the surface allowed drying for 20 minutes. The following antibiotics as filter paper disks were used: Amoxicillin and clavulanic acid, Cefovecin, Clindamycin, Doxycycline, Enrofloxacin, Marbofloxacin, and Penicillin G.

Results and discussions

The results demonstrated that *Escherichia coli* was the most frequently isolated bacteria, present in 28 from 40 samples (70%), followed by *Streptococcus* sp. in 5 samples (12.5%), *Staphylococcus pseudintermedius* in 3 samples (7.5%), *Enterococcus* sp. and *Pasteurella multocida*, each in 2 samples (5%) and *Staphylococcus haemolyticus*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Klebsiella pneumoniae*, *Haemophilus hemoglobinophilus*, *Acinetobacter pittii*, *Pseudomonas koreensis* and *Enterobacter cloacae*, each in 1 sample (2.5%). Bacterial associations were identified in 9 (22.5%) cases, most common between *Escherichia coli* and other species, while 3 samples were negative for microbial growth.

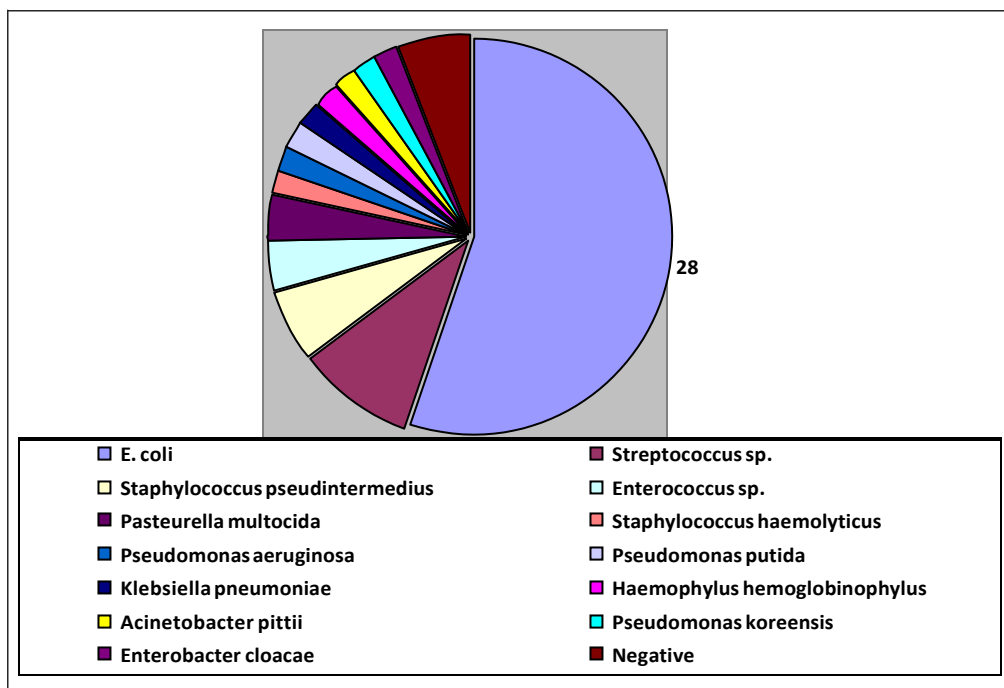


Figure 1. The type and number of bacterial species isolated from pyometra samples in dogs

Regarding antibiotic susceptibility, the most efficient antibiotics were represented by Marbofloxacin, with the average of inhibition area diameter of 23.17 mm, Enrofloxacin with 22.46 mm, Doxycycline with 22.09 mm, Amoxicillin and clavulanic acid with 20.94 mm and Cefovecin with 20.02 mm, while the least efficient were Penicillin G with 14.41 and Clindamycin with 16.19 mm.

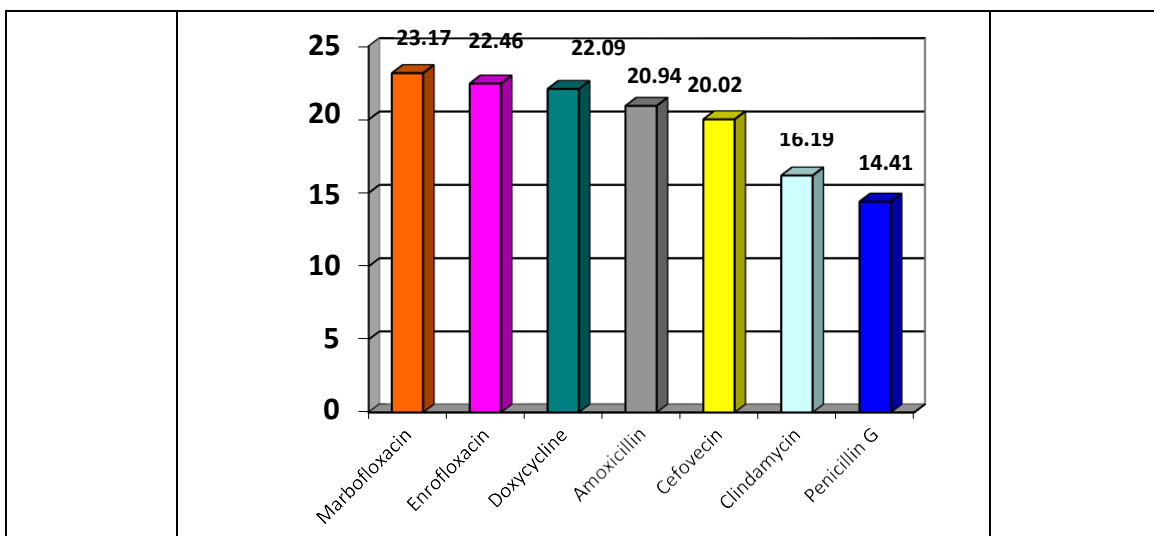


Figure 2. Average inhibition area diameter of the susceptibility testing

Conclusions

The study of canine pyometra concerning etiologic agents and susceptibility profiles concluded that:

- MALDI-TOF MS offers the possibility of accurate and rapid identification of bacteria isolated in clinical microbiology laboratories;
- *E. coli* was the most frequently isolated bacterium;
- Regarding the antimicrobial susceptibility, the most recommended antibiotics were represented by quinolones (Marbofloxacin and Enrofloxacin), Doxycycline and Amoxicillin and clavulanic acid;
- The least efficient antibiotics were Penicillin G and Clindamycin.

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