FULL ARTICLE

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Detection of resistance genes in pyometra isolated bacteria in bitches

Detecção de genes de resistência em bactérias isoladas de piometra em cadelas

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ABSTRACT

Pyometra has several immunological and molecular changes that are responsible for uterine inflammation and the disease may or may not have infections. This study aimed to isolate and identify bacteria in the uterine content of bitches with pyometra, to analyze the susceptibility profile to antibiotics, detect β-lactamase enzyme production by phenotypic tests, and resistance genes to β-lactams. Eighteen samples of uterine content were collected by aspiration puncture. The samples were inoculated in bacteriological media and identified by biochemical tests. Subsequently, antibiogram tests, screening for detection of β-lactamases, and Real-Time PCR for detection of resistance genes was performed. *Escherichia coli*, *Klebsiella* spp., *Enterobacter aerogenes*, *Citrobacter* spp., *Staphylococcus* spp., and *Streptococcus* spp. were identified in the analyzed samples of uterine content. In the antibiogram test, 90.5% of the isolates showed resistance to at least one antibiotic, and of these, 36.8% were considered MDR, with three *Staphylococcus* spp., three *E. coli,* and one *Klebsiella* spp. Concerning bacterial resistance to the groups of antibiotics tested, 38.1% of the isolates were resistant to at least one type of β-lactam, 33.3% to tetracycline, 19.0% to aminoglycosides, and 14.3% to fluoroquinolones, macrolides, and trimethoprim-sulfamethoxazole. In the phenotypic test to detect β-lactamase production, *E. coli* samples were negative and *Klebsiella* spp. was positive for the production of AmpC, which presented the *bla*CMY, *bla*SPM, and *bla*SIM genes. Bacteria that are resistant to antibiotics represent a great challenge and laboratory support is therefore essential, without which therapeutic success decreases and death may be inevitable.

Keywords: Antibiotics. *bla*CMY. *bla*SPM. *bla*SIM. Resistance.

RESUMO

A piometra apresenta diversas alterações imunológicas e moleculares que são responsáveis pela inflamação uterina, e a doença pode ser infecciosa ou não. O objetivo deste estudo foi isolar e identificar bactérias no conteúdo uterino de cadelas com piometra, analisar o perfil de suscetibilidade aos antibióticos, detectar a produção de enzimas β-lactamase por testes fenotípicos e genes de resistência aos β-lactâmicos. Dezoito amostras de conteúdo uterino foram coletadas por punção aspirativa. As amostras foram inoculadas em meio bacteriológico e identificadas por testes bioquímicos. Posteriormente, foram realizados testes de antibiograma, triagem para detecção de β-lactamases e PCR em tempo real para detecção de genes de resistência. *Escherichia coli*, *Klebsiella* spp., *Enterobacter aerogenes*, *Citrobacter* spp., *Staphylococcus* spp. e *Streptococcus* spp. foram identificados nas amostras de conteúdo uterino analisadas. No teste de antibiograma, 90,5% dos isolados apresentaram resistência a pelo menos um antibiótico, e destes, 36,8% foram considerados MR, sendo três *Staphylococcus* spp., três *E. coli* e uma *Klebsiella* spp. Sobre a resistência bacteriana aos grupos de antibióticos testados, 38,1% dos isolados foram resistentes a pelo menos um tipo de β-lactâmico, 33,3% à tetraciclina, 19,0% aos aminoglicosídeos e 14,3% às fluorquinolonas, macrolídeos e trimetoprim-sulfametoxazol. No teste fenotípico para detecção da produção de β-lactamase, as amostras de *E. coli* foram negativas, e *Klebsiella* spp. foi positiva para a produção de AmpC, que apresentou os genes *bla*CMY, *bla*SPM e *bla*SIM. As bactérias resistentes aos antibióticos representam um grande desafio e, portanto, o suporte laboratorial é essencial, sem o qual o sucesso terapêutico diminui e a morte pode ser inevitável.

Palavras-chave: Antibióticos. *bla*CMY. *bla*SPM. *bla*SIM. Resistência.

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Introduction

Pyometra is the most common gynecological disease in female dogs and results from hormonal disorders in the endometrium combined with bacterial superinfection (Porowska et al., 2018). The disease is mediated by hormones, most frequently seen in diestrus when there is an increase in progesterone and a decrease in estrogen. The predominance of progesterone results in endometrial proliferation, increased uterine glandular secretion, decreased contraction of the uterus, and induced closure of the cervix (Rautela & Katiyar, 2019). Also, immunological suppression is observed due to the inhibition of dendritic cell maturation, which indirectly induces an increase in the incidence of the disease (Wijewardana et al. 2015) when associated with bacteria such as *Escherichia coli*, *Enterobacter* spp., *Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa, Streptococcus* spp., and *Staphylococcus* spp. (Oliveira et al., 2016).

Antibiotics are used in the moderate and severe stages of pyometra to prevent or treat sepsis. Broad-spectrum antibiotics should be administered concomitantly with the specific treatment protocol, and it is necessary to determine anti-microbial activity against the bacteria isolated from vaginal discharge before the initiation of antibiotic therapy (Rautela & Katiyar, 2019). However, this practice is not always performed, which can result in treatment failures and patient death.

One reason for pyometra treatment failure is the bacterial resistance to antibiotics, which has become a worldwide problem. Resistance can occur by mutation or acquisition of resistance genes by horizontal transfer (Bello-López et al., 2019).

In medical practice, one of the most used antibiotics in animals and humans is β-lactams. The main resistance mechanisms developed by bacteria are the production of an altered PBP with a lower affinity or inactivation of the drug due to the production of enzymes called β-lactamases, which is one of the most important resistance mechanisms found in bacteria, mainly gram-negative (Eiamphungporn et al., 2018).

β-Lactamase enzymes can be encoded chromosomally or on extrachromosomal elements (Alby & Miller, 2018) and are responsible for the hydrolysis of the β-lactam ring, which results in the inactivity of antibiotics (Zango et al., 2019). There are several types of β-lactamase produced by gram-negative bacteria, especially enterobacteria isolated from animals, such as extended-spectrum β-lactamase (ESβL), AmpC (Kaur et al., 2016), metallo-β-lactamase (Pruthvishree et al., 2018), and carbapenemase (Meletis, 2016).

Pyometra is considered a clinical emergency with a high rate of complications and must be diagnosed early, using the appropriate choice of effective antibiotics for a good prognosis. Thus, the identification of pathogenic microorganisms, the performance of the antibiogram, and resistance detection are essential tools to guide the clinician in choosing the appropriate antibiotic therapy. Therefore, this study aimed to analyze the phenotypic and genetic profile of bacteria isolated from the uterine content of bitches with pyometra, which showed resistance to the tested antibiotics.

Material and Methods

This study was submitted to and approved by the Ethics Committee on the Use of Animals at UFG, protocol 043/14.

This is a descriptive, prospective study conducted at the Veterinary Hospital of the School of Veterinary and Zootechnics of the Federal University of Goiás, Goiânia, Goiás, Brazil, from August 2016 to July 2017.

The study sample was non-probabilistic. The dogs with pyometra included were those with a closed or open uterine cervix, regardless of race or age. The diagnosis was confirmed by clinical and complementary exams, such as a leukogram and ultrasound. Of the 18 bitches, 14 had leukocytosis (20.200μL to 124.000 μL) with neutrophilia (14.592μL to 81.840μL), but only eight had monocytosis (1.368 μL to 6.200 μL). Of these, eight had neutrophilia with regenerative left-shift and six had neutrophilia without left shift. Four bitches showed no changes in the leukogram. The abdominal ultrasound revealed a uterus with both regular and irregular contour, with increased volume, which ranged from 1.77 to 5.4 cm in longitudinal section, thickened wall, irregular mucosa, and hypoechogenic content with hyperechogenic foci in suspension.

Sample collection

After ovariohysterectomy, 2 mL of uterine content from each animal was collected by aspiration puncture using a 5mL syringe and a 25x8 or 40x12 hypodermic needle. Each sample was placed in a tube containing brain heart infusion broth, transport medium, and then immediately sent for processing in a Styrofoam box with ice to the Anaerobic, Phenotyping and Molecular Biology Laboratory of the Institute of Tropical Pathology and Public Health at the Federal University of Goiás.

Bacterial isolations and identification

The samples were grown on three types of agar, Columbia supplemented with 5% defibrinated horse blood, MacConkey, and salted mannitol. The plates were incubated in a bacteriological incubator at 37ºC for 48 h in aerobiosis. Afterward, the morphocolonial description, bacterial isolation in nutrient agar, morphotintorial characterization by the gram staining method, and biochemical tests were performed (Brasil, 2013).

Gram-negative bacteria were tested for oxidase, acid and gas production from glucose, motility, pigment production, arginine dihydrolase, lysine decarboxylase, indole production, hydrogen sulfide, citrate utilization, gelatin hydrolysis, urea hydrolysis, phenylalanine deaminase, acid production from lactose.

Gram-positive bacteria, positive to catalase, were tested for motility, oxidase, growth in 5%, 10%, and 15% NaCl medium, while for Gram-positive bacteria, negative to catalase, the type of hemolysis was verified, as well as the bacitracin, optoquine and trimethoprim-sulfamethoxazole sensitivity, CAMP test, esculin hydrolysis, growth in the presence of bile and growth in 6.5% NaCl medium.

Antibiogram and screening tests for the detection of β-lactamases

Subsequently, an antibiogram test was performed, according to the methodology described by the Clinical and Laboratory Standards Institute (2017).

The antibiotic disks used for enterobacteria were amoxicillin-clavulanate, ampicillin, piperacillin-tazobactam, ceftriaxone, ceftazidime, cefoxitin, cefazolin, cefepime, aztreonam, imipnem, tetracycline, trimethoprim-sulfamethoxazole, gentamicin, tobramycin, and ciprofloxacin; for *Staphylococcus*, penicillin, cefoxitin, gentamicin, erythromycin, tetracycline, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, rifampin, linezolid were tested; and for *Streptococcus,* penicillin, ceftriaxone, vancomycin, erythromycin, tetracycline, clindamycin, and linezolid.

Gram-negative bacteria that showed resistance to β-lactams were subjected to screening tests to detect the production of β-lactamases EsβL, AmpC, metallo-β-lactamase, and carbapenemase, according to methodologies contained in Clinical and Laboratory Standards Institute (2017) and Martínez Roja (2009). The results were evaluated descriptively.

Detection of resistance genes for β-lactams

Bacteria with positive results in the phenotypic detection of β-lactamases had their plasmid DNA extracted, according to the Pharmacia® FLEXIPREP extraction kit manual.

After executing the plasmid DNA extraction protocol, the samples were kept frozen for quantification of the genetic material and other analyses. The quantification step was performed on the Nanodrop 2000 Thermo Fisher Scientific device and software Nanodrop 2000/2000c (version 1.6). The quantification was performed individually and sterile Milli-Q water was used as a blank, for an adequate calibration between the quantification of each sample.

Specific primers were used, based on the sequences deposited in GenBank for the Real-Time PCR Methods on the BioRad IQ5 platform. The oligonucleotides used for amplification comprised gene sequences from 5 'to 3', with a forward and reverse for each gene related to resistance to β-lactams. The researched genes were *bla*CMY, *bla*CTX-M, *bla*DHA, *bla*GIM, *bla*IMP, *bla*KPC, *bla*NDM, *bla*OXA, *bla*SHV, *bla*SIM, *bla*SME, *bla*SPM, *bla*TEM, and *bla*VIM.

The reactions were prepared using the methodology of the kit for Real-Time PCR of the SYBR Green (SYBR Green qPCR Master Mix LOW ROX-100 reactions x 25 µL), from LGC Biotecnologia®, with the addition of specific primers to amplify each gene. Initially, the reaction mix containing 2X SYBR Green Master Mix Low ROX and primers for amplification of the target region was prepared. The reaction mix was agitated in a vortex and rapidly centrifuged to collect the entire reaction volume at the back of the tube. To maintain viable components, the tubes were kept on ice until amplification, which had the following amplification parameters: Initial denaturation (hold) at 95ºC for 2 min, followed by 40 cycles with denaturation at 95ºC for 15 sec, ringing at 50º-60ºC for 15 sec and extension at 68ºC-72ºC for 45 sec. For the endogenous control of the reaction, the RNaseP gene was amplified and, for the negative control, water was added in place of the DNA.

Results

From 18 bitches with pyometra, 21 bacteria were isolated and identified, and 55.5% (10/18) showed the presence of enterobacteria. The isolated enterobacteria were *E. coli* (6/18),

Klebsiella spp. (2/18), *Enterobacter aerogenes* (1/18), and in a pyometra mixed infection of *E. coli* with *Citrobacter* spp. (1/18). *Staphylococcus* spp. (6/18) were also isolated, and two other pyometra with mixed *Staphylococcus* spp. with *Streptococcus* spp. (2/18).

In the antibiogram test, 90.5% (19/21) of the isolates showed resistance to at least one antibiotic (Figures 1, 2, 3, and 4) and of these, 36.8% (7/19) were considered multidrug-resistant, with three *Staphylococcus* spp., three *E. coli,* and one *Klebsiella* spp. Two isolates, *Enterobacter aerogenes,* and *Citrobacter* spp., were sensitive to all tested antibiotics.

Concerning bacterial resistance to the groups of antibiotics tested, 38.1% (8/21) of the isolates were resistant to at least one type of β-lactam, 33.3% (7/21) to tetracycline, 19.0% (4/21) to aminoglycosides, and 14.3% (3/21) to fluoroquinolones, macrolides, and trimethoprim-sulfamethoxazole.

In the phenotypic test to detect β-lactamase production in gram-negative bacteria MDR, *E. coli* samples were negative and *Klebsiella* spp. was negative for the production of ESβL, carbapenemase, and metallo-β-lactamase, but positive for the production of AmpC. The result of the antibiogram test for this bacterium is described in Table 1.

In the molecular tests performed on the *Klebsiella* spp., from 14 genes surveyed, three were identified, *bla*CMY, *bla*SPM, and *bla*SIM (Figure 5).

Discussion

According to the data presented, 55.5% of the cases had enterobacteria as the etiological agent, with *E. coli* the most isolated. This is justified because *E. coli* is part of the vaginal and vulvar microbiota of bitches, which favors migration to the uterus (Baithalu et al., 2010), depending on the stage of the estrous cycle. The dog has four to five phases of the estrous cycle, proestrus, estrus, metestrus/diestrus, and anestrus (Silva & Lima, 2018).

In the metestrus phase, progesterone sensitizes the endometrium and myometer, besides developing endometrial receptors for *E. c*oli (Baithalu et al., 2010). In addition to the presence of receptors at this stage, *E. coli* pathogenic strains have type 1 fimbrial adhesin, called FimH, which facilitates binding to the endometrium, and can be an important factor in the pathogenesis of canine pyometra (Krekeler et al., 2012). Other bacterial agents besides *E. coli* were also isolated, such as *Klebsiella* spp., *Enterobacter aerogenes*, *Citrobacter* spp., *Staphylococcus* spp., *Streptococcus* spp. and mixed infections, which was also found by Coggan (2005).

Santos (2006) observed that it was possible to isolate all bacterial genera from the vaginal mucosa of healthy bitches. Carneiro et al. (2005) also isolated bacteria from the genera *Staphylococcus* spp., *E. coli,* and *Streptococcus* spp. of the vaginal mucosa of healthy bitches. These data indicate that the bacteria isolated in this study are normally present in the vaginal mucosa of healthy bitches, and at certain stages of the estrous cycle they can reach the endometrial mucosa and multiply.

A factor that can contribute to the maintenance of the intrauterine bacterial presence is the immunosuppression that occurred during the diestrus period since the increase in progesterone levels and the decrease in estrogen inhibit the maturation of dendritic cells (Wijewardana et al., 2015), which hinders a response adequate immune system. Another factor would be endometrial hyperplasia with increased glandular secretion, in which secretion becomes an excellent site for bacterial growth (Baithalu et al., 2010).

Although two bacteria were sensitive to the tested antibiotics, it was possible to detect resistance in the other

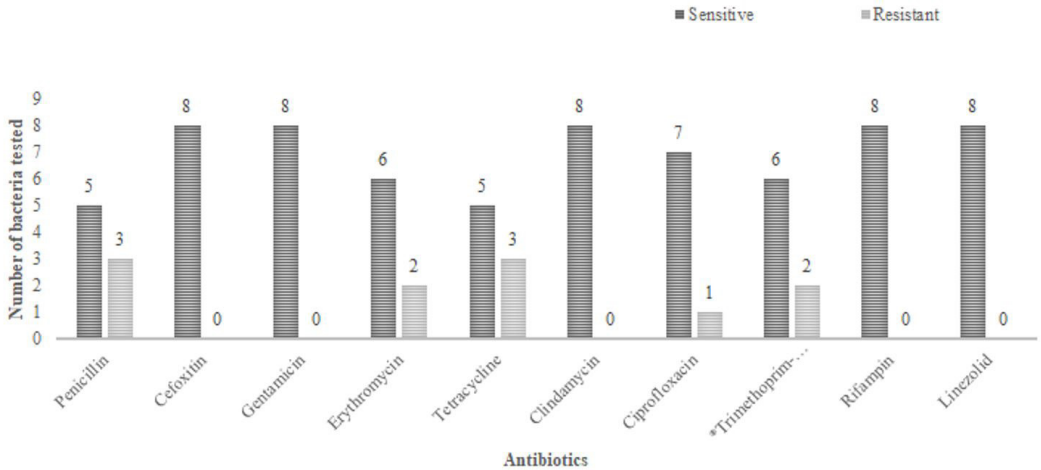

Figure 1 – Result of the antibiogram test of eight *Staphylococcus* spp. isolated from the uterine content of bitches with pyometra (*Trimethoprim-Sulfamethoxazole).

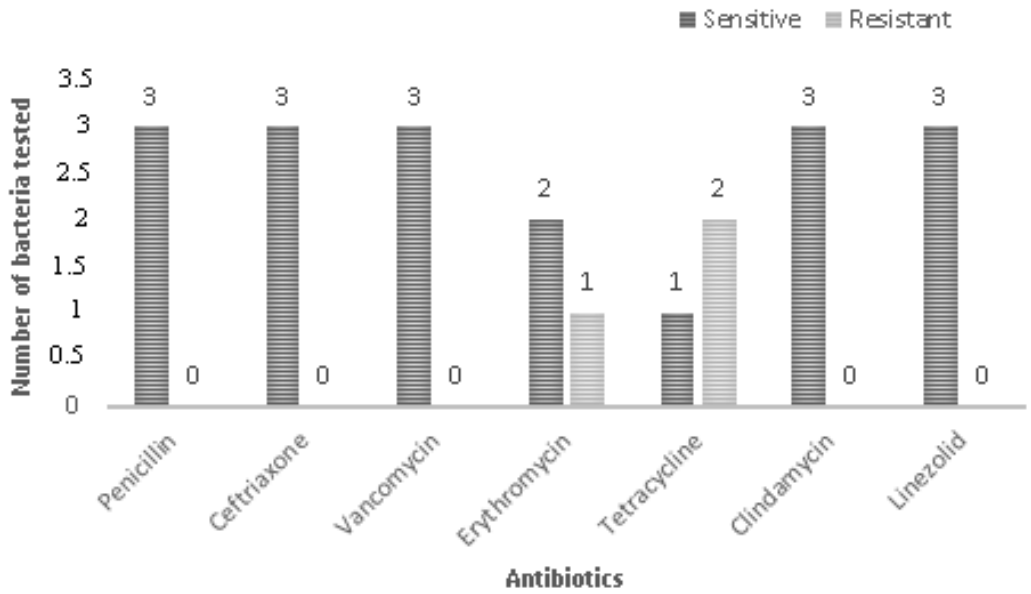

Figure 2 – Result of the antibiogram test of three *Streptococcus* spp. isolated from the uterine content of bitches with pyometra.

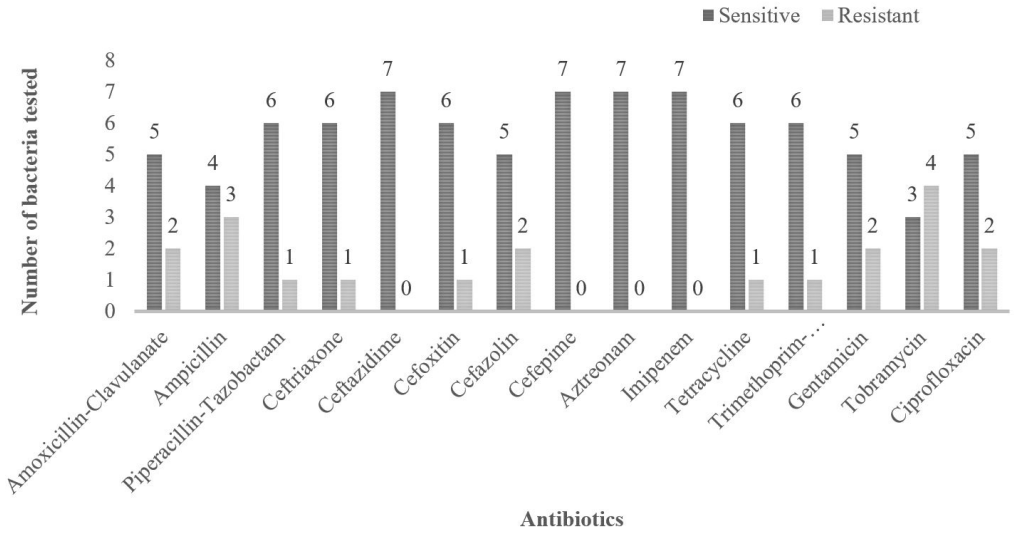

Figure 3 – Result of the antibiogram test of seven *Escherichia coli* isolated from the uterine content of bitches with pyometra.

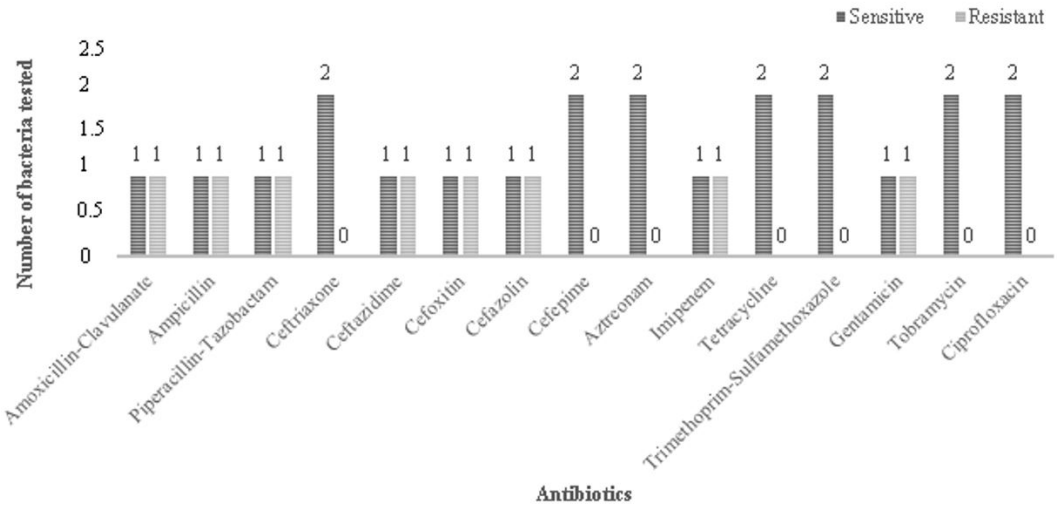

Figure 4 – Result of the antibiogram test of two *Klebsiella* spp. isolated from the uterine content of bitches with pyometra.

Table 1 – Result of *Klebsiella* spp. antibiogram test isolated from uterine content of bitches with pyometra, positive for the AmpC test. (09:14, Goiânia-GO)

Bacteria	AMC[1]	AMP[2]	PPT[3]	CRO[4]	CAZ[5]	CFO[6]	CFZ[7]	CPM[8]	ATM[9]	IPM[10]	TET[11]	SUT[12]	GEN[13]	TOB[14]	CIP[15]
Klebsiella spp.	R[16]	R	R	S[17]	R	R	R	S	S	R	S	S	R	S	S

AMC[1]: Amoxicillin-Clavulanate; AMP[2]: Ampicillin; PPT[3]: Piperacillin-Tazobactam; CRO[4]: Ceftriaxone; CAZ[5]: Ceftazidime CFO[6]: Cefoxitin; CFZ[7]: Cefazolin; CPM[8]: Cefepime; ATM[9]: Aztreonam; IPM[10]: Imipenem; TET[11]: Tetracycline; SUT[12]: Trimethoprim-Sulfamethoxazole; GEN[13]: Gentamicin; TOB[14]: Tobramycin; CIP[15]: Ciprofloxacin, R[16]: Resistant; S[17]:Sentitive.

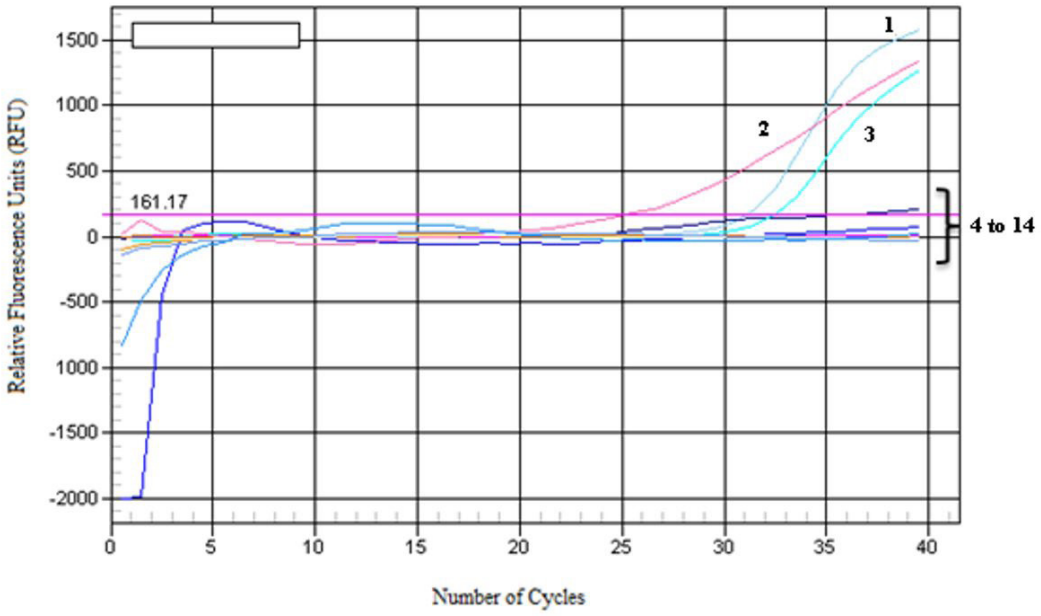

Figure 5 – Real-time PCR test result of *Klebsiella* spp. isolated from bitch uterine content with pyometra. The *bla*CMY (1), *bla*SPM (2), and *bla*SIM (3) genes were detected. 1.*bla*CMY: capri blue. 2.*bla*SPM: rose; 3.*bla*SIM: light blue; 4.*bla*CTX-M: red; 5.*bla*DHA: lilac; 6.*bla*GIM: medium blue; 7.*bla*IMP: france blue; 8.*bla*KPC: sky blue; 9.*bla*NDM: marsala; 10.*bla*OXA: white; 11.*bla*SHV: navy blue; 12.*bla*SME: orange; 13. *bla*TEM: blue; 14.*bla*VIM: light orange.

19 isolates as the result of the antibiogram test. Among the most prescribed antibiotics for pyometra cases are amoxicillin, amoxicillin-clavulanate, cephalosporins, trimethoprim-sulfamethoxazole, doxycycline, metronidazole, and enrofloxacin (Baithalu et al., 2010; Fieni et al., 2014; Yoon et al., 2017). The isolated bacteria showed resistance to all of the most prescribed antibiotics in pyometra cases, except metronidazole, which is an antibiotic indicated for the treatment of infections caused by anaerobic bacteria (Vicente & Pérez-Trallero, 2010), and not included in the present study.

Magiorakos et al. (2012) proposed definitions for multidrug-resistant (MDR), extreme drug-resistant (XDR) and pandrug-resistant (PDR) strains of pathogenic bacteria that are frequently found in healthcare settings. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and PDR as non-susceptibility to all agents in all antimicrobial categories. The list of antimicrobial categories was created using the results of the antimicrobial susceptibility tests contained in the documents Clinical Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the United States Food and Drug Administration (FDA).

According to the proposed document, seven bacteria were MDR in this study, with emphasis on the resistance of *Staphylococcus* spp. to ciprofloxacin, *E. coli* to ampicillin, amoxicillin, ceftriaxone and ciprofloxacin, and *Klebsiella* spp. to ampicillin. The emphasis is necessary because the protocol for the treatment of pyometra used in the veterinary hospital where the animals were treated considers ceftriaxone, enrofloxacin, and ampicillin as the antibiotics of choice in these cases. Thus, it is evident that the microbiological diagnosis, together with the antibiogram test, is essential for the prescription of a correct and effective antibiotic therapy.

The genes for β-lactamases production detected in the present study can encode resistance to various β-lactam antibiotics. The AmpC enzyme is produced in numerous microorganisms, mainly in the group of enterobacteria, such as *E. coli*, *Enterobacter* spp. and *Citrobacter* spp., which have chromosomal induction by antimicrobials such as ampicillin, cefepime, or clavulanate. *Klebsiella* spp. also presents AmpC

as identified in this study, but from plasmid origin, which increases the prevalence of resistance due to the constant exchange of genetic material between bacteria (Santiago et al., 2016). The AmpC genes derived from chromosomal genes are incorporated and mobilized by plasmids, which facilitates their propagation (Campana et al., 2013).

Santos et al. (2020) found *bla*CMY codes resistance for ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, ceftazidime, cefoxitin, cefazolin, cefepime, ceftriaxone, imipenem, and aztreonam; *bla*SIM for ampicillin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem and aztreonam; and *bla*SPM for ampicillin, piperacillin-tazobactam, ceftazidime, cefoxitin, cefepime, imipenem, and aztreonam. According to the results obtained, *Klebsiella* spp. was resistant to β-lactams amoxicillin-clavulanate, ampicillin, piperacillin-tazobactam, ceftazidime, cefoxitin, cefazolin, and imipenem, which is consistent with the molecular findings as mentioned by Santos et al. (2020).

The reason for *Klebsiella* spp. having been sensitive to the antibiotics cefepime, ceftriaxone and aztreonam can be explained by recent findings that suggest that the environment and/or the genetic context may modify the phenotypic expression of specific resistance genes/mutations, which implies that a particular genotype does not always result in the expected phenotype even with resistance genes (Hughes & Andersson, 2017).

Thus, bacteria that show resistance to antibiotics represent a great challenge, both for their laboratory detection and the appropriate infection treatment. The absence of laboratory tests to identify pathogenic bacteria and the susceptibility profile to antibiotics in the veterinary clinic can worsen the condition of patients with infectious bacterial diseases, especially in cases of pyometra, an emergency condition susceptible to septicemia. Laboratory support is therefore essential, without it therapeutic success will decrease and death may be inevitable.

Conclusion

Escherichia coli was the bacteria most isolated from the uterine content of bitches with pyometra. Mixed infections were found, *E. coli* and *Citrobacter* spp., *Staphylococcus* spp. and *Streptococcus* spp. Bacterial resistance to frequently used antibiotics in the veterinary clinical routine was found, with one strain of *Klebsiella* spp., producer of AmpC, from which the *bla*CMY, *bla*SPM, and *bla*SIM genes were detected.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics Statement

The research was conducted under the approval of the Ethical Committee for Animal Research of UFG (protocol 043/14).

References

Alby K, Miller MB. Mechanisms and detection of antimicrobial resistance. In: Long S, Prober C, Fischer M. Principles and pactice of pediatric infectious diseases. 5. ed. Philadelphia: Elsevier; 2018. p. 1467-78.

Baithalu RK, Maharana BR, Mishra C, Sarangi L, Samal L. Canine pyometra. Vet World [Internet]. 2010 [cited 2020 Aug 26];3(7):340-2. Available from: http://www.veterinaryworld.org/Vol.3/July/Canine%20Pyometra.pdf

Bello-López JM, Cabrero-Martínez OA, Ibáñez-Cervantes G, Hernández-Cortez C, Pelcastre-Rodríguez LI, Gonzalez-Avila LU, Castro-Escarpulli G. Horizontal gene transfer and its association with antibiotic resistence in the genus Aeromonas spp. Microorganisms. 2019;7(9):363. http://dx.doi.org/10.3390/microorganisms7090363. PMid:31540466.

Brasil. Agência Nacional de Vigilância Sanitária. Manual de microbiologia clínica para o controle de infecção relaciona à assistência à saúde. Módulo 6: Detecção e identificação de bacérias de importância médica [Internet]. Brasília, DF: ANVISA; 2013 [cited 2020 Aug 26]. Available from: https://www20.anvisa.gov.br/segurancadopaciente/index.php/publicacoes/item/deteccao-e-identificacao-de-bacterias-de-importancia-medica.

Campana EH, Barbosa PP, Fehlberg LCC, Gales AC. Frequency of plasmid-mediated AmpC in Enterobacteriaceae isolated in a Brazilian Teaching Hospital. Braz J Microbiol. 2013;44(2):477-80. http://dx.doi.org/10.1590/S1517-83822013000200023. PMid:24294242.

Carneiro AP, Toniollo GH, Schocken-Iturrino RP. Avaliação microbiológica da flora vaginal e do corpo uterino de cadelas (*Canis familiaris*) submetidas a ovariossalpingohisterectomia. Ars Vet. 2005;21(3):361-7. http://dx.doi.org/10.15361/2175-0106.2005v21n3p361-367.

Clinical and Laboratory Standards Institute – CLSI. Performance standards for antimicrobial susceptibility

testing; twenty-seven informational supplement. M100. USA: CLSI; 2017.

Coggan JA. Estudo microbiológico de conteúdo intrauterino e histopatológico de útero de cadelas com piometra e pesquisa de fatores de virulência em cepas de *Escherichia* coli e o potencial risco à saúde humana [dissertação]. São Paulo: Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo; 2005.

Eiamphungporn W, Schaduangrat N, Malik AA, Nantasenamat C. Tackling the antibiotic resintance caused by class A β-lactamase inhibitory protein. Int J Mol Sci. 2018;19:1-24. http://dx.doi.org/10.3390/ijms19082222.

Fieni F, Topie E, Gogny A. Medical treatment for pyometra in dog. Reprod Domest Anim. 2014;49(Suppl 2):28-32. http://dx.doi.org/10.1111/rda.12302. PMid:24947858.

Hughes D, Andersson DI. Environmental and genetic modulation of the phenotypic expression of antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):374-91. http://dx.doi.org/10.1093/femsre/fux004. PMid:28333270.

Kaur J, Mahajan G, Chand K, Chopra SS. Enhancing phenotytic detection of ESBL in AmpC co-producers by using Cefepime and Tazobactam. J Clin Diagn Res. 2016;10(1):5-8. http://dx.doi.org/10.7860/JCDR/2016/15264.7041. PMID: 26894064.

Krekeler N, Marenda MS, Browning GF, Holden KM, Charles LA, Wright PJ. Uropathogenic virulence factor FimH facilities binding of uteropathogenic Escherichia coli to canine endometrium. Comp Immunol Microbiol Infect Dis. 2012;35(5):461-7. http://dx.doi.org/10.1016/j.cimid.2012.04.001. PMid:22554919.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standar definitions for acquidred resitance. Clin Microbiol Infect. 2012;18(3):268-81. http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x. PMid:21793988.

Martinez Rojas DDV. Betalactamasas tipo AmpC: generalidades y métodos para detección fenotípica. Rev Soc Venez Microbiol [Internet]. 2009 [cited 2020 Aug 26];29:78-83. Available from: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S1315-25562009000200003&lng=es.

Meletis G. Carbapenem resistance; overview of the problem and future perspectives, Ther Adv Infect Dis. 2016;3(1):15-21. http://dx.doi.org/10.1177/2049936115621709. PMID: 26862399.

Oliveira FS, Paz LN, Mota TM, Oriá AP, Silva MCA, Pinna MH. Perfil de resitência de isolados de *Escherichia coli* a partir de piometra canina. Cienc Anim Bras. 2016;17(4):615-21. http://dx.doi.org/10.1590/1089-6891v17i438817.

Porowska E, Kulus M, Jankowski M, Kocherova I, Jeseta M, Chamier-Gliszczyńska A, Stefańska K, Borowiec B, Bukowska D, Brüssow KP, Kempisty B, Antosik P. Selected aspects of endometritis – pyometra complex in dogs – current troubles and treatment perspectives. Med J Cell Biol. 2018;6(3):108-13. http://dx.doi.org/10.2478/acb-2018-0017.

Pruthvishree BS, Vinodh Kumar OR, Sivakumar M, Tamta S, Sunitha R, Sinha DK, Singh BR. Molecular characterization of extensively drug resistant (XDR), extended spectrum beta-lactamases (ESBL) and New Delhi Metallo beta-lactamase-1 (blaNDM1) producing Escherichia coli isolated from a male dog - a case report. Vet Arh. 2018;88(1):139-48. http://dx.doi.org/10.24099/vet.arhiv.160924.

Rautela R, Katiyar R. Review on canine pyometra, oxidative stress and current trends in diagnostics. Asian Pac J Reprod. 2019;8(2):45-55. http://dx.doi.org/10.4103/2305-0500.254645.

Santiago GS, Motta CC, Bronzato GF, Gonçalves D, Souza MMS, Coelho IS, Ferreira HN, Coelho SMO. Revisão: produção de β-lactamases do tipo AmpC em enterobacteriaceae. Rev Bras Med Vet [Internet]. 2016 [cited 2020 Aug 26];38(3):17-30. Available from: https://repositorio-aberto.up.pt/bitstream/10216/111009/2/255803.pdf

Santos AG. Avaliação da microbiota vaginal de cadelas usando como diagnóstico o isolamento microbiológico e a colpocitologia [dissertação]. Seropédica: Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro; 2006 [cited 2020 Aug 26]. Available from: http://www.dominiopublico.gov.br/pesquisa/DetalheObraForm.do?select_action=&co_obra=65770.

Santos AL, Dos Santos AP, Ito CRM, Queiroz PHP, de Almeida JA, de Carvalho MAB Jr, de Oliveira CZ, Avelino MAG, Wastowski IJ, Gomes GPLA, Souza ACSE, Vasconcelos LSNOL, Santos MO, da Silva CA, Carneiro LC. Profile of enterobacteria resistant to beta-lactams. Antibiotics (Basel). 2020;9(7):410. http://dx.doi.org/10.3390/antibiotics9070410. PMid:32679663.

Silva LDM, Lima DBC. Aspectos da fisiologia reprodutiva da cadela. Rev Bras Reprod Anim [Internet]. 2018 [cited 2020 Aug 26];42(3-4):135-40. Available from: http://www.cbra.org.br/portal/downloads/publicacoes/rbra/v42/n3-4/p135-140%20(RB750).pdf.

Vicente D, Pérez-Trallero E. Tetracyclines, sulfonamides, and metronidazole. Enferm Infecc Microbiol Clin. 2010;28(2):122-30. http://dx.doi.org/10.1016/j.eimc.2009.10.002. PMid:20097452.

Wijewardana V, Sugiura K, Wijesekera DPH, Hatoya S, Nishimura T, Kanegi R, Ushigusa T, Inaba T. Effect of ovarian hormones on maturation of dendritic cells from peripheral blood monocytes in dogs. J Vet Med Sci. 2015;77(7):771-5. http://dx.doi.org/10.1292/jvms.14-0558. PMid:25715707.

Yoon HY, Byun JY, Park KH, Min BS, Kim JH. Sterile pyometra in two dogs. Immune Netw. 2017;17(2):128-31. http://dx.doi.org/10.4110/in.2017.17.2.128. PMid:28458625.

Zango UU, Ibrahim M, Shawai SAA, Shamsuddin IM. A review on β-lactam antibiotic drug resistance. MOJ Drug Develop Ther. 2019;3(2):52-8. http://dx.doi.org/10.15406/mojddt.2019.03.00080.

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