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Multidrug-resistant Escherichia coli isolated from a dog with a history of urolithiasis: case report

[*Escherichia coli multirresistente isolada de cão com histórico de urolitíase: relato de caso*]

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ABSTRACT

Bacterial resistance is a reality in both human and veterinary health, it limits the therapeutic arsenal and raises the costs of the patient's treatment. A dog with signs of cystitis received treatment with 5mg/kg enrofloxacin at three consecutive times, with low effectiveness. The presence of urethral uroliths was identified and urohydropulsion was done. The animal presented a new obstruction, for which a cystotomy was performed, but continued with signs of infection. Uroculture and antimicrobial susceptibility test were then performed. *Escherichia coli* was identified, which was resistant to 13 antibiotics, being sensitive only to piperacillin-tazobactam and amikacin. In the screening test for β-lactamase, the production of ESβL was detected. The qPCR indicated the presence of the *bla*$_{CTXm}$, *bla*$_{DHA}$, *bla*$_{OXA}$, *bla*$_{IMP}$, *bla*$_{TEM}$, *bla*$_{GIM}$, *bla*$_{SIM}$, *bla*$_{SPM}$ and *bla*$_{SME}$ genes, which may lead to a phenotypic resistance profile for ampicillin, amoxicillin-clavulanate, aztreonam, cefepime cefoxitin, cefuroxime, ceftazidime, ceftriaxone, imipenem, and piperacillin-tazobactam. This case reaffirms the value that laboratory analysis adds to the diagnosis and treatment of cystitis and urolithiasis, which can define the direction of evolution of the prognosis and the speed at which the patient's health will be restored.

Keywords: veterinary microbiology, clinical analysis, antibiotics, cystitis

RESUMO

A resistência bacteriana aos antibióticos é uma realidade, tanto na saúde humana quanto veterinária, limita o arsenal terapêutico e eleva os custos relacionados ao tratamento do paciente. Um cão, com sinais de cistite, recebeu tratamento com enrofloxacina, na dose de 5mg/kg, em três momentos seguidos, com baixa efetividade. Identificou-se presença de urólitos uretrais e foi feita uro-hidropropulsão. O animal apresentou nova obstrução, para a qual foi realizada uma cistotomia, mas continuou com sinais de infecção. Realizou-se, então, urocultura e teste de antibiograma. Foi identificada Escherichia coli, *que se mostrou resistente a 13 antibióticos, sendo sensível somente à piperacilina-tazobactam e amicacina. No teste de triagem para β-lactamase, detectou-se a produção de ESβL. A qPCR indicou presença dos genes* bla$_{CTXm}$, bla$_{DHA}$, bla$_{OXA}$, bla$_{IMP}$, bla$_{TEM}$, bla$_{GIM}$, bla$_{SIM}$, bla$_{SPM}$ e bla$_{SME}$, *que podem conduzir um perfil fenotípico de resistência para ampicilina, amoxicilina-ácido clavulânico, aztreonam, cefepima, cefoxitina, cefuroxima, ceftazidima, ceftriaxona, imipenem, piperacilina-tazobactam. Este caso reafirma o valor que a análise laboratorial agrega ao diagnóstico e tratamento da cistite e da urolitíase, podendo definir o sentido de evolução do prognóstico e a velocidade em que a saúde do paciente será restabelecia.*

Palavras-chave: microbiologia veterinária, análises clínicas, antibióticos, cistite

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INTRODUCTION

Bacterial resistance to antibiotics is a reality in both human and veterinary health and has been reported frequently in dogs (Shaheen *et al*., 2010). *Escherichia coli* is a bacterium capable of developing resistance phenotypes from gene expression (Gandolfi-Decristophoris *et al*., 2013), and the production of enzymes called betalactamases is common.

One of the enzymes produced is extended spectrum beta-lactamase (ESβL), which can inactivate penicillin and cephalosporins, as well as antibiotics from other classes such fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole and tetracyclines. This is due to the co-selection process in which plasmids carrying ESβL genes can often carry a wide range of resistance genes to other non-β-lactam antimicrobials (Winokur *et al*. 2001). Thus, bacteria such as *E. coli*, carriers of ESβL genes, limit the therapeutic arsenal, which makes patient treatment more difficult and expensive (Hordijk *et al*., 2013), especially in cases of urinary tract infections (UTI).

As an opportunistic microorganism, *E. coli* can be found in the urinary tract of animals such as dogs and cats (Ewers *et al*., 2012) and is constantly related to UTI, which may arise as a result of urolithiasis. Urolithiasis results from the formation of uroliths, which are mineral crystals formed mainly due to supersaturation of the urine, which can result in urinary flow obstruction (Oyafuso *et al*., 2010) and, consequently, lead to UTI.

Due to the clinical status of patients coming to veterinary care with signs of UTI and because of the socioeconomic profile of their tutors, the treatment protocol is not always standardized. Thus, the use of fluoroquinolones is common due to its broad spectrum against gram positive and negative bacteria. However, even being common, treatment of UTI can become challenging (Weese *et al*., 2011) due to bacterial resistance to routine antibiotics.

Laboratory techniques have been used to integrate diagnosis to improve treatment. An example is the phenotypic detection of antibiotic resistance by screening tests, as well as molecular detection of resistance genes by the real time polymerase chain reaction technique (qPCR).

CASUISTRY

An undefined breed male dog was seen at a private veterinary hospital, with difficulty urinating, and when he did, the urine was dark and fetid. The clinical suspicion was cystitis. Initially, the proposed treatment was enrofloxacin antibiotic therapy at a dose of 5mg / kg for seven days. After one month, the dog had the same symptoms and was prescribed the same therapy. As there was no improvement, the animal was sent to the Veterinary Hospital of the School of Veterinary and Animal Science of the Federal University of Goiás (HV / EVZ / UFG).

After clinical evaluation, the same treatment was prescribed. However, the condition remained unchanged and the dog was taken back to HV / EVZ / UFG, and the presence of urethral uroliths was diagnosed by means of x-ray. An urohydropropulsion was performed to remove the crystals, and new antibiotic therapy with enrofloxacin was performed.

After a few days, the dog presented new obstruction and, when taken again to the HV / EVZ / UFG, a cystotomy was performed. After surgery, a bladder catheter was introduced into the dog, which remained for 15 days. However, the animal continued with signs of infection.

A sample of the urine was collected by cystocentesis for microbiological evaluation with a 5mL syringe and 0.80x30 needle. The urine was seeded on blood agar, MacConkey and Mannitol, incubated in aerobiosis, at 37ºC, for 24-48h. Afterwards, bacterial identification was performed by conventional biochemical tests and antibiogram test. The antibiotics tested were ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, ceftazidime, cefazolin, cefoxitin, cefepime, ceftriaxone, aztreonam, imipenem, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, gentamicin, and amikacin.

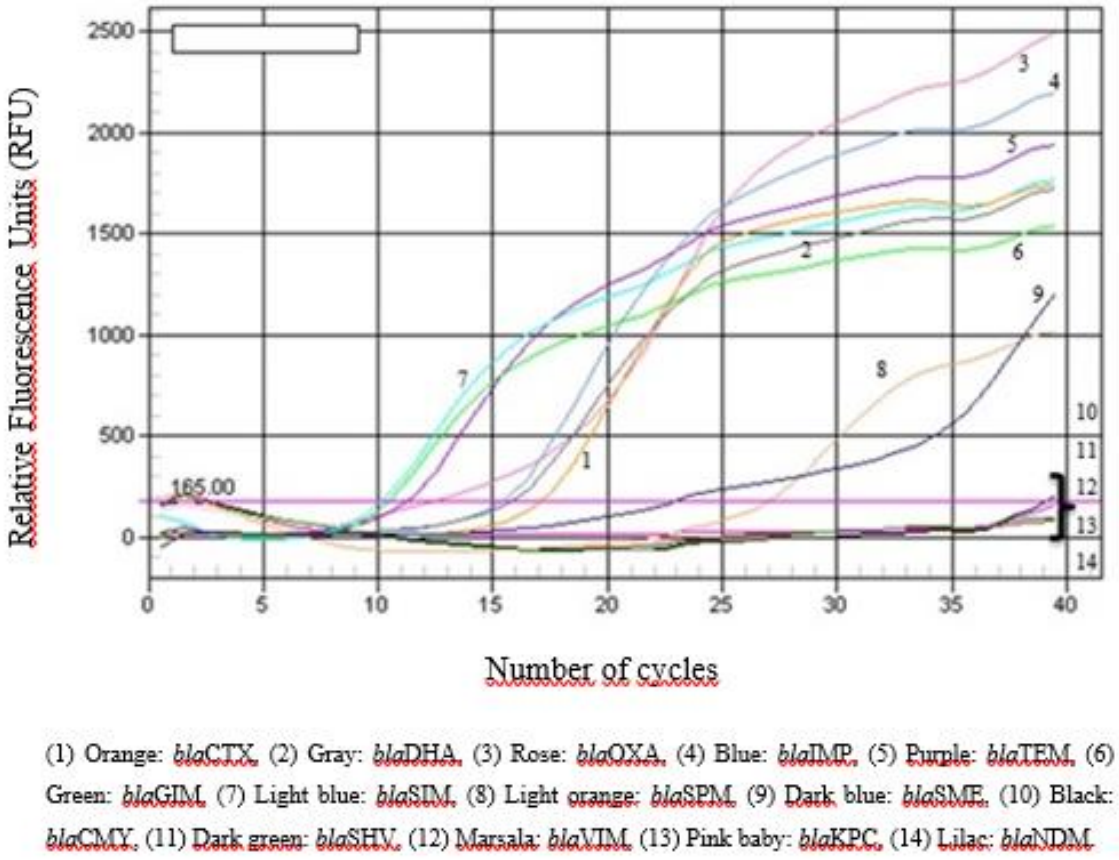
The identified bacterium was *E. coli*, which was resistant to 13 antibiotics, being sensitive only to piperacillin-tazobactam and amikacin (Table 1). Screening tests were performed for detection of the β-lactamase of the ESβL-type, carbapenemase, AmpC and metallo-β-lactamase, having been positive for ESβL.

Table 1. *Escherichia coli* antibiogram test result isolated from dog with cystitis and urolithiasis.

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

[1]*E. coli*: *Escherichia coli*. [2]AMP: Ampicillin, [3]AMC: Amoxicillin-clavulanate, [4]PPT: Piperacillin-tazobactam, [5]CAZ: Ceftazidime, [6]CFZ: Cefazolin, [7]CFO: Cefoxitin, [8]CPM: Cefepime, [9]CRO: Ceftriaxone, [10]ATM: Aztreonam, [11]IPM: Imipenem, [12]SUT: Trimethoprim-sulfamethoxazole, [13]TET: Tetracycline, [14]CIP: Ciprofloxacin [15]GEM: Gentamicin, [16]AMI: Amikacin.

Subsequently, resistance genes were identified by qPCR. Results indicated the presence of *bla*CTX-M, *bla*DHA, *bla*OXA, *bla*IMP, *bla*TEM, *bla*GIM, *bla*SIM, *bla*SPM and *bla*SME genes (Figure 1), which may lead to a phenotypic resistance profile for ampicillin, amoxicillin-clavulanate, aztreonam, cefepime cefoxitin, cefuroxime, ceftazidime, ceftriaxone, imipenem, piperacillin-tazobactam.



(1) Orange: *bla*CTX. (2) Gray: *bla*DHA. (3) Rose: *bla*OXA. (4) Blue: *bla*IMP. (5) Purple: *bla*TEM. (6) Green: *bla*GIM. (7) Light blue: *bla*SIM. (8) Light orange: *bla*SPM. (9) Dark blue: *bla*SME. (10) Black: *bla*CMY. (11) Dark green: *bla*SHV. (12) Marsala: *bla*VIM. (13) Pink baby: *bla*KPC. (14) Lilac: *bla*NDM.

Figure 1. Amplification of resistance genes in a sample of *Escherichia coli* isolated from a dog with cystitis and urolithiasis by real-time PCR technique.

DISCUSSION

In the past decade, urolithiasis was the third most common canine urinary tract health problem (Sosnar *et al*., 2005), which may be associated with UTI cases, which was detected in the present study. Among the most commonly isolated bacteria in cases of UTI is *E. coli*, considered the

largest reservoir of resistance genes today and may be responsible for treatment failures in both human and veterinary medicine. A growing number of resistance genes have been identified in *E. coli* isolates in recent decades and many of these genes have been acquired by horizontal transfer, being able to both donate and receive them from other bacteria. Thus, resistance in *E. coli* has been one of the biggest challenges worldwide in the treatment of human and animal infections and should be considered a real public health concern (Poirel *et al*., 2018).

The results of the antibiogram and qPCR reinforced, in this case, the low effectiveness of the strategy adopted for the treatment, which calls attention to the prescription of the same antibiotic, although without obtaining a desirable response, with gradual worsening of the animal. This highlights the importance of microbiological examination and antibiogram testing that, once applied at the beginning of treatment, could have contributed to a good prognosis and positive patient outcome.

These analyzes showed that although the gene encoding piperacillin-tazobactam resistance was identified in qPCR, the antibiogram indicated bacterial sensitivity to this combination and there was no phenotypic expression of the gene. In addition, E. coli was resistant to cefazolin, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin and gentamicin. The genetic profile of resistance to such antibiotics (Perreten *et al*., 2010) was probably also present. However, only testing for β-lactamase coding genes was performed.

In 2017, researchers in China (Li *et al*., 2017) isolated three strains of *E. coli* ESβL from the urinary tract of three dogs in a group of 80 dogs with acute or chronic cystitis. PCR was performed to analyze 3 genes (*bla*CTXm, *bla*TEM and *bla*SHV), which were also analyzed in this case. The phenotype found by the authors - resistance to ampicillin, tetracycline, trimethoprim-sulfamethoxazole and sensitivity to amikacin - was also detected in the present work.

It is understood that the aggravating factors of veterinary treatments vary, ranging from the difficulty of a quick and accurate diagnosis to the high costs that come with the therapy. However, it is essential to reinforce the importance of performing laboratory tests in the early stages of clinical follow-up, as well as the reformulation or implementation of care protocols that encourage prescription based on clinical evidence and laboratory results.

CONCLUSION

Bacterial resistance is a reality in the veterinary field, and treating infectious diseases today is a challenge. The present case reaffirms the value that laboratory analysis adds to the diagnosis and treatment of cystitis and urolithiasis and may define the direction in which the prognosis will evolve and the speed at which the patient's health will be restored.

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