





Isolation of *Escherichia coli* and *Klebsiella pneumoniae* strains producing extended-spectrum β -lactamases from dog urine of the Metropolitan Area of the Aburrá Valley (Antioquia, Colombia)

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ABSTRACT

Escherichia coli and *Klebsiella pneumoniae* are the most common pathogens causing urinary tract infections in humans and animals. Close contact between humans and companion animals can facilitate the spread of multidrug resistant pathogens between both species. The objective of the research was to characterize extended-spectrum β -lactamases (ESBL)-producing *E. coli* and *K. pneumoniae* isolated from dogs with urinary tract infections in the metropolitan area of Valle del Aburrá (Antioquia, Colombia). Three-hundred seventy-one urine samples collected from March 2018 to March 2019 in a veterinary clinical laboratory were analyzed. *E. coli* and *K. pneumoniae* isolates were detected in chromogenic agar and identified by biochemical tests. Susceptibility testing was performed by disc diffusion and ESBL production was evaluated by the double disk test in all isolates. MIC determination of ESBL-positive isolates were performed on the automated VITEK®2 system. Multiple PCR was used for the detection of CTX-M beta-lactamases (group 1, 2, 9 and 8/25), SHV, TEM, and AmpC of plasmid origin in ESBL-positive isolates. In total 22 out 371 isolates were positive for ESBL production by double disc test, 11 *E. coli* (ESBL-*Ec*) and 11 *K. pneumoniae* (ESBL-*Kp*). The multiple PCR detected CTX-M group 1 in the 22 ESBL-positive isolates. Multi-drug resistance was observed in all ESBL-producing isolates. In conclusion, a high frequency of antibiotic multi-resistance was found in ESBL-*Ec* and ESBL-*Kp*. The main ESBL detected was CTX-M group 1, which also prevails in human isolates.

Keywords: beta-lactam, resistance, dogs, enterobacteriaceae, public health, urinary tract infection.

Aislamiento de *Escherichia coli* y *Klebsiella pneumoniae* productoras de betalactamasas de espectro extendido a partir de orina de perros del Área metropolitana del Valle de Aburrá (Antioquia, Colombia)

RESUMEN

Escherichia coli y *Klebsiella pneumoniae* son los patógenos más comunes causantes de infecciones en tracto urinario en humanos y animales. El contacto estrecho con los animales

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de compañía puede favorecer la diseminación de patógenos multiresistentes entre ambas especies. El objetivo de la investigación fue caracterizar *E. coli* (Ec-BLEE) y *K. pneumoniae* (Kp-BLEE) productores de betalactamasas de espectro extendido provenientes de aislados de caninos con infecciones del tracto urinario del Área Metropolitana del Valle de Aburrá. 371 muestras de orina de caninos colectadas entre marzo de 2018 y marzo 2019 en un laboratorio clínico veterinario fueron analizadas. *E. coli* y *K. pneumoniae* se detectaron en agar cromogénico y se identificaron mediante pruebas bioquímicas. La prueba de susceptibilidad se realizó por difusión en disco y la producción de BLEE se evaluó por test de doble disco en todos los aislados. La determinación de la CIM en aislados positivos a BLEE se realizó en el sistema automatizado VITEK®2. Se utilizó PCR múltiple para la detección de betalactamasas tipo CTX-M (grupo 1, 2, 9 y 8/25), SHV, TEM y AmpC de origen plasmídico en aislados positivos a BLEE. Un total de 22 de 371 aislados fueron positivos a BLEE por test de doble disco, 11 *E. coli* (Ec-BLEE) y 11 *K. pneumoniae* (Kp-BLEE). La PCR detectó CTX-M grupo 1 en los 22 aislados positivos a BLEE. Se observó multiresistencia en todos los aislamientos productores de BLEE. En conclusión, se encontró una alta frecuencia de multiresistencia en Ec-BLEE y Kp-BLEE. La principal BLEE detectada fue CTX-M grupo 1, que también predomina en aislados humanos.

Palabras clave: resistencia a betalactámicos, perros, enterobacteriales, infección del tracto urinario, salud pública.

INTRODUCTION

Bacterial urinary tract infections (UTIs) are a common cause of disease in dogs. The most frequently isolated bacteria in both humans and dogs are *Escherichia coli*, followed by *Staphylococcus*, *Proteus*, and *Klebsiella* species (Byron 2019; Gómez Beltrán *et al.* 2020; Sierra González and Arango Uribe 2017). UTIs and other bacterial infections are frequently treated with β -lactam antibiotics, however resistance to this antibiotic group has increased in the last decade. The main mechanism of resistance to β -lactam in Enterobacteriaceae is the production of extended-spectrum β -lactamases (ESBL). These enzymes confer resistance to penicillins, cephalosporins (including oximinocephalosporins), and aztreonam. On the contrary, ESBL are unable to hydrolyze cefamycins (cefoxitin and cefotetan) and carbapenems (Paterson and Bonomo 2005).

Most ESBLs can be classified into three groups: (i) TEM and (ii) SHV—derived from gene mutations in the classic narrow-spectrum β -lactamases TEM-1, TEM-2, and SHV-1-, and (iii) CTX-M group which predominates over the other ESBL-types globally. CTX-M enzymes possess preferential hydrolytic activity over cefotaxime and have been classified into five groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25), based on amino acid identities (Bonnet 2004). ESBL-type enzymes are usually encoded in plasmids, facilitating their rapid dissemination between different species through horizontal transfer by conjugation (Morejón García 2013). In addition, the same plasmid can carry genes that encode resistance to quinolones, aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole, which contribute to antibiotic multi-resistance

(Tafur *et al.* 2011). Due to their impact on morbidity, mortality, and frequent resistance and limited therapeutic options, carbapenem-resistant and ESBL-producing Enterobacteriaceae are included in the priority pathogens list on antibiotic resistance of the World Health Organization (Tacconelli *et al.* 2017).

Close contact between humans (owners, vets, animal caretakers, etc.) and pets, as well as direct contact with animal secretions during the performance of routine actions related to their care (feeding, grooming, cleaning living spaces, petting), implies a potential transmission risk of pathogens between both species, as well as the transfer of resistance genes. Moreover, since it is very common that dogs urinate in public areas, the contamination of the soil and water sources could represent a problem for the community and the environmental health.

In Colombia, in the clinical practice in companion animals the use of antibiotics that are also used in human medicine is frequent (Cabrera García 2010), mainly β -lactams (Astaiza Martínez *et al.* 2016; Gutiérrez *et al.* 2002). In addition, it is common in daily practice the recognition of pathologies based on presumptive diagnoses and the scarce use of diagnostic aids, such as culture with antibiogram, resulting in the establishment of empirical antibiotic therapies is common in the daily practice (Gómez Beltrán *et al.* 2021; Sánchez *et al.* 2015).

Estimating the use of antimicrobials in companion animal species in Colombia is also difficult. Usually, its magnitude is assessed in reference to the sales reported by the manufacturing laboratories, without considering the dispensing of authorized antimicrobials for human use, as well as injectable antimicrobials designed for

food-producing animals that are also used in companion ones (Cabrera García 2010).

The widespread and excessive use of antibiotics, mainly third-generation cephalosporins have usually been attributed as a selection pressure that drives ESBL evolution (Paterson and Bonomo 2005). Thus, inappropriate use of antibiotics in animals can contribute to the spread of resistant bacteria that cause complicated infections in both animals and humans, and that are associated with higher morbidity, mortality, and treatment costs in humane medicine (Alós 2015). Therefore, understanding the phenomenon of antimicrobial resistance from a veterinary medicine perspective is important to determine its impact on public health.

Although urinary infections in dogs are very common, as well as the use of antibiotics in companion animals is high and contaminated urine can be discharged into the environment, information about the phenomenon of antimicrobial resistance in companion animals in Colombia is limited. Therefore, this study aimed to characterize isolates of ESBLs producing *E. coli* and *K. pneumoniae*, obtained from urine samples of dogs from the metropolitan area of Valle del Aburrá (Antioquia, Colombia).

MATERIALS AND METHODS

Location

In this study urine samples from canines collected at TESTLAB© Veterinary Clinical Laboratory were included. This laboratory performs ESBL-detection test routinely in *E. coli* and *K. pneumoniae* urine isolates. In addition, the laboratory is certified under the Colombian law (ICA ISO 17025 standard). The laboratory

receives samples from veterinary clinics located in the metropolitan area of Valle del Aburrá (Province of Antioquia, Colombia), where Medellín is the main city surrounded by the municipalities of Barbosa, Girardota, Copacabana, Bello, Itagüí, Sabaneta, Envigado, La Estrella, and Caldas. It is estimated that the study region had a population of 480,659 dogs in 2018 (Área Metropolitana del Valle de Aburrá 2018).

Data collection

Information on dogs' characteristics and clinical practices related to the presence of ESBL producing *E. coli* (ESBL-*Ec*) and ESBL producing *K. pneumoniae* (ESBL-*Kp*) in urine was retrieved from clinical records. Information on the use of antibiotics, infectious diseases, hospitalizations, and surgeries during the last 12 months, as well as the method used to obtain the urine sample and cohabits with other animals were obtained through the medical history and the attending veterinarian.

Ethics committee

The study was approved by the Ethics Committee for Animal Experimentation (CEEAA) of the Universidad de Antioquia (act number 120, October 9th, 2018).

Samples

All urine samples from dogs (n=371, one sample from each dog) analyzed at the laboratory for microbiological culture and antibiogram between March 2018 and March 2019 were included in the study. The urine sample was considered as the sampling unit, and the dogs and the isolates as analysis units. A urine sample was considered positive when the growth of at least one (1) isolate of ESBL-*Ec* or ESBL-*Kp* was confirmed.

Bacterial isolation and identification

The BBL™ CHROMagar™ Orientation (Becton, Dickinson and Company, Sparks, USA) was used for the isolation, characterization, differentiation, and enumeration of the bacteria present in the urine samples. In the cultures with significant growth of uropathogens, one colony compatible with *E. coli* and *K. pneumoniae* in each positive culture was selected for genus and species confirmation by biochemical oxidase tests, Simmons citrate, TSI agar, lysine decarboxylation, urease, indole production, and mobility of the bacteria.

Antimicrobial susceptibility testing and ESBL evaluation

Antimicrobial susceptibility testing by disk diffusion and ESBL evaluation by double disc test was performed simultaneously in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI M100-S25, 2015 y CLSI VET08, 2018). Briefly, the bacterial colonies were suspended in saline solution, adjusted to 0.5 McFarland turbidity standard and plated on Mueller-Hinton agar. According to the request of the attending veterinarian, different antibiotics were evaluated for each isolate, including amoxicillin 25 µg (AML), ampicillin (AMP), amoxicillin/clavulanic acid 30 µg (AMC), ampicillin/sulbactam 20 µg (SAM), cefotaxime 30 µg (CTX), ceftazidime 30 µg (CAZ), ceftriaxone 30 µg (CRO), imipenem 10 µg (IPM), ertapenem 10 µg (ETP), nitrofurantoin 300 µg (F), trimethoprim/sulfamethoxazole 25 µg (SXT), enrofloxacin 5 µg (ENR), ciprofloxacin 5 µg (CIP), chloramphenicol 30 µg (C), norfloxacin 10 µg (NOR), amikacin 30 µg (AK), gentamicin 10 µg (CN), doxycycline

30 µg (DO), erythromycin 15 µg (E), tetracyclines 30 µg (TE), oxytetracycline 30 µg (OT), minocycline 30 µg (MH), oxacillin (OX), azithromycin 15 µg (AZM), and neomycin 30 µg (N).

ESBL production was evaluated by double-disk test as recommended by CLSI (CLSI M100-S25, 2015; Papich 2018). Briefly, a bacterial suspension with a turbidity pattern of 0.5 McFarland was plated on Mueller-Hinton agar. Ceftazidime/clavulanic acid (30/10µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg), and cefotaxime (30 µg) disks were used. Antibiotic disks were placed at a 25 mm center-to-center distance and incubated at 37° C for 18-24 h. An increase of ≥5 mm in the halo zone diameter for cephalosporin disks combined with clavulanic acid compared to the antibiotic alone was considered positive for ESBL-production. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls, respectively. Positive isolates for the ESBL-production were stored in cryobeads (CRYOBANK™) for preservation and subsequent determination of the minimum inhibitory concentration (MIC) and molecular analysis.

Determination of the minimum inhibitory concentration (MIC)

The MIC for ESBL-*Ec* and ESBL-*Kp* isolates was determined using the VITEK®2 automated system (bioMérieux, Marcy l'Étoile, France). A total of 14 antibiotics were evaluated and interpreted according to the standards (CLSI M100-S25, 2015 y CLSI VET08, 2018), including ampicillin/sulbactam (SAM), ceftoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), doripenem (DOR), ertapenem (ETP), imipenem (IPM), meropenem (MEM), amikacin (AK),

gentamycin (CN), ciprofloxacin (CIP), tigecycline (TGC), and colistin (CT).

Molecular detection of ESBL genes by multiple polymerase chain reaction (PCR)

Bacterial DNA extraction from positive ESBL-producing isolates was performed using the Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, USA) and according to the manufacturer's instructions. Subsequently, *bla* genes of the TEM, SHV, and CTX-M subtypes (groups 1, 2, 8, 9, and 25) were amplified using primers and conditions previously described (Dallenne et al. 2010). A non-ESBL- *Ec* strain (ATCC 25922) was used as negative control. The PCR products were visualized by ethidium bromide in 2% agarose gel electrophoresis (AMRESCO Inc, Solon Ohio, USA ISO 9002). The size of the bands was evaluated by comparison with a 100 bp molecular weight DNA ladder (Gene Ruler- Qiagen, Germantown, USA).

Statistical analysis

The information collected was stored in Excel 2010 (Microsoft, Redmond, USA). The data were analyzed using descriptive statistics. Absolute and relative frequencies were calculated for the qualitative variables, and summary and central tendency measures for the quantitative ones, according to the normality assumption of the data. STATA 15.1 (Statacorp, Texas, USA) software was used for the statistical analysis.

RESULTS

A total of 371 urine samples were sent to the laboratory for urine culture and antibiogram during March 2018 and March 2019. In 190 samples *E. coli* and *K. pneumoniae* were identified. *E. coli* was

isolated from 165 samples, of which 6.6% (11/165) were ESBL positive by double disc test. *K. pneumoniae* was isolated from 25 samples, of which 44% (11/25) were ESBL positive by double disc test.

The mean age of the 22 dogs with ESBL-positive isolates was 9.4 ± 3.1 years and most of the dogs 59.09% (13/22) were males. 81.8% (18/22) were purebred dogs (i.e., Jack Russell Terrier, Yorkshire Terrier, Labrador Retriever, Pitbull, Cocker Spaniel, Pug, Schnauzer, Beagle, Shih-Tzu, German Shepherd, Dachshund, Golden Retriever, French Bulldog, and English Bulldog), and 18.2% (4/22) were mixed-breed. Fifty percent (11/22) of dogs had undergone a previous sterilization process more than 6 months ago and 50% (11/22) lived with other dogs. A high frequency of hospitalization (77.3%, 17/22) and the use of antibiotic-based treatments (77.3%, 17/22) were found in the last year.

Regarding the ESBL-*Ec* isolates, resistance to enrofloxacin and ciprofloxacin was found in 87.5% (7/8) and 70% (7/10), respectively. The highest susceptibility was found to nitrofurantoin (70%, 7/10). Two of the isolates were resistant to all the antibiotics evaluated.

In addition, 82% (9/11) of the *K. pneumoniae* isolates were resistant to all the antibiotics evaluated, being nitrofurantoin 100% (9/9), trimethoprim/sulfamethoxazole 100% (6/6), enrofloxacin 88.8% (8/9), and ciprofloxacin 88% (7/8) the antibiotics with the highest proportion of resistance. One of the other two isolates was susceptible to enrofloxacin and ciprofloxacin and the other one to amikacin, imipenem, ertapenem, and meropenem.

In general, all isolates showed resistance to three or more antibiotics groups by the Kirby Bauer (disk diffusion) method. In

addition to the resistance to cephalosporins, a high resistance to ciprofloxacin (78%, 14/18), enrofloxacin (88%, 15/17), and trimethoprim-sulfamethoxazole (85%, 11/13) was found.

The MICs of the 14 antibiotics, obtained through the broth microdilution method using the VITEK[®]2 system for the 22 isolates of *E. coli* and *K. pneumoniae*, are shown in tables 1 and 2, respectively. These results showed all isolates were susceptible to carbapenems (ertapenem, imipenem, meropenem and doripenem), and most of them were susceptible to amikacin, and tigecycline. In addition, resistance to gentamicin was more frequent in *K. pneumoniae* than *E. coli*.

The 22 isolates harbored group 1 CTX-M genes, based on the multiple PCR analysis. The CTX-M group (2, 8, 9, and 25 genes) were not detected in any isolate. Two of the 22 isolates were also positive to TEM and SHV genes, carrying all three genes simultaneously.

DISCUSSION

In this study, a general frequency of 11.6% (22/190) of *E. coli* and *K. pneumoniae* producing ESBL was found. When compared to similar studies where both bacteria were analyzed in dog urine, this frequency was slightly higher than the one found in Brazil of 9.6% (2/62) (Melo *et al.* 2018) but lower than that reported in Switzerland of 25% (39/156) (Zogg *et al.* 2018). However, if we consider the difference in the length of the study of those researches (two and four years respectively) compared with this one-year study, our findings may represent a major concern by showing a greater number of diseased dogs carrying *E. coli* and

TABLE 1. Minimum inhibitory concentration (MIC) values for *Escherichia coli* isolates obtained from urine samples of dogs from the metropolitan area of Valle del Aburrá (Province of Antioquia, Colombia), 2018-2019

ISOLATE	SAM	TZP	FOX	CAZ	CRO	FEP	DOR	ETP	IPM	MEM	AMK	CN	CIP	TGC
2-241447-KP	>=32	8	<=4	4	>=64	4	<=0,12	<=0,5	1	<=0,25	<=2	<=1	2	<=0,5
3-237353-KP	>=32	<=4	<=4	4	>=64	2	<=0,12	<=0,5	<=0,25	<=0,25	<=2	>=16	<=0,25	<=0,5
4-242407-KP	>=32	16	<=4	16	>=64	>=64	<=0,12	<=0,5	<=0,25	<=0,25	4	>=16	>=4	<=0,5
7-243889-KP	>=32	16	<=4	8	>=64	4	<=0,12	<=0,5	<=0,25	<=0,25	<=2	>=16	2	1
8-247697-KP	>=32	16	<=4	16	>=64	32	<=0,12	<=0,5	<=0,25	<=0,25	4	>=16	>=4	<=0,5
14-255779-KP	>=32	16	<=4	8	>=64	4	<=0,12	<=0,5	<=0,25	<=0,25	<=2	>=16	>=4	1
15-259003-KP	>=32	16	<=4	4	>=64	4	<=0,12	<=0,5	<=0,25	<=0,25	<=2	<=1	2	<=0,5
16-259320-KP	>=32	32	>=64	>=64	>=64	32	<=0,12	<=0,5	0,5	<=0,25	>=64	>=16	2	2
20-271234-KP	>=32	16	<=4	8	>=64	2	<=0,12	<=0,5	<=0,25	<=0,25	<=2	>=16	1	1
21-274461-KP	>=32	>=128	8	16	>=64	32	<=0,12	<=0,5	0,5	<=0,25	<=2	>=16	>=4	2
27-295328-KP	>=32	64	>=64	>=64	>=64	2	<=0,12	<=0,5	<=0,25	<=0,25	>=64	>=16	2	2

SAM (ampicillin/sulbactam), TZP (piperacillin/tazobactam), FOX (cefoxitin), CAZ (ceftazidime), CRO (ceftriaxone), FEP (cefepime), DOR (doripenem), ETP (ertapenem), IPM (imipenem), MEM (meropenem), AK (amikacin), CN (gentamicin), CIP (ciprofloxacin), TGC (tigecycline). Gray shade indicates intermediate or resistance to the antibiotic. Source: self-made.

TABLE 2. Minimum inhibitory concentration (MIC) values for *Klebsiella pneumoniae* isolates obtained from urine samples of dogs from the metropolitan area of Valle del Aburrá (Province of Antioquia, Colombia), 2018-2019

ISOLATE	SAM	TZP	FOX	CAZ	CRO	FEP	DOR	ETP	IPM	MEM	AK	CN	CIP	TGC
5-242857-EC	16	4	4	16	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	>=16	>=0,4	<=0,5
6-243280-EC	4	4	4	8	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	<=0,25	<=0,5
9-248019-EC	4	4	4	4	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	<=0,25	<=0,5
10-253735-EC	8	4	8	16	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	1	<=0,5
11-253824-EC	4	4	4	4	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	1	<=0,5
18-267995-EC	16	4	4	16	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	>=16	>=4	<=0,5
19-268858-EC	8	4	8	16	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	1	<=0,5
22-276305-EC	4	4	4	4	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	<=0,25	<=0,5
24-278733-EC	4	4	4	4	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	<=0,25	<=0,5
25-281253-EC	4	4	4	4	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	<=0,25	<=0,5
26-294656-EC	4	5	5	4	>=64	4	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	<=0,25	<=0,5
28-300603-EC	4	4	4	4	>=64	2	<=0,12	<=0,5	<=0,2	<=0,25	<=2	<=1	>=4	<=0,5

SAM (ampicillin/sulbactam), TZP (piperacillin/tazobactam), FOX (cefoxitin), CAZ (ceftazidime), CRO (ceftriaxone), FEP (cefepime), DOR (doripenem), ETP (ertapenem), IPM (imipenem), MEM (meropenem), AK (amikacin), CN (gentamicin), CIP (ciprofloxacin), TGC (tigecycline), MIC (minimum inhibitory concentration). Gray shade indicates intermediate or resistance to the antibiotic.
Source: self-made.

K. pneumoniae producing ESBL in a shorter period of time.

In the same way, the frequency of isolates ESBL producing *E. coli* and *K. pneumoniae* observed is also remarkably higher than those obtained from diseased companion animals between 2008 and 2010 in a European surveillance program (Bogaerts *et al.* 2015) where only five *E. coli* and one *K. pneumoniae* were isolated. Such a difference can probably be explained by the fact that all chronically diseased animals, as well as those who had been recently treated with antibiotics, were excluded in that study, while the majority of the animals in our study had a history of hospitalization and/or use of antibiotics in the previous year.

Other studies have also evaluated the presence of ESBL-*Ec* in dogs suffering from UTI worldwide. Among the isolates of *E. coli* in this study, the observed frequency of 6,6% (11/22) of ESBL producers is higher but not so far than that found previously in the United States (4%), Switzerland (3,4%) and China (3,8%) (Huber *et al.* 2013; Li *et al.* 2017; O'Keefe *et al.* 2010), although data from those studies were single-institution based and may not be entirely comparable.

As it has been mentioned, research — particularly in animals — has been more focused on studying ESBLs produced by *E. coli*, yet the predominance of ESBL production in this study was higher in *K. pneumoniae* isolates than in *E. coli*. Some studies in Japan, Italy, Portugal, Germany, and other European countries have also reported a high number of multidrug resistance and third-generation cephalosporin resistant *K. pneumoniae* strains among companion animals (Donati *et al.* 2014; Ewers *et al.* 2012; Harada *et al.* 2012;

Marques *et al.* 2018). The latest suggests that ESBL-*Kp* is frequent in clinical samples from companion animals and that it is necessary to continue monitoring resistance mechanisms in this species.

The predominant gene class detected in our study was CTX-M. The CTX-M family of enzymes is grouped based on the similarities in amino acid sequences; for example, within the group CTX-M-1 are the enzymes CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12, CTX-M-15, and CTX-M-32 (Galvis and Moreno 2019). Several studies suggest that CTX-M-1 is the most frequent genotype, not only in companion animals, but also in food-producing animals, food, wild animals, and veterinarians that work with dogs and cats (Abbas *et al.* 2019; Baede *et al.* 2015; Cormier *et al.* 2019; Darwich *et al.* 2019; Ewers *et al.*, 2012; Irrgang *et al.* 2018).

In Colombia, a few studies had analyzed the presence of *bla*_{CTX-M} genes. From the poultry chain, Castellanos *et al.* (2017) identified *Escherichia coli* carrying *bla*_{CTX-M-2}, *bla*_{CTX-M-8} and *bla*_{CTX-M-15} and then, in 2018, *bla*_{CTX-M-2} group was found to be the most prevalent ESBL gene among *Salmonella enterica* isolates (Castellanos *et al.* 2018). In addition, CTX-M group 1 was detected in *enterobacteriaceae* isolated from bulk tank milk samples (Vásquez Jaramillo *et al.* 2017).

In addition, the predominant circulation of the CTX-M group 1 in dogs described here is consistent with the epidemiology of CTX-M in *E. coli* and *K. pneumoniae* causing UTI in humans in Colombia, both at hospital and community settings (Blanco *et al.* 2016; Galvis and Moreno 2019; Leal *et al.* 2013; Martínez *et al.* 2012; Rada *et al.* 2019). They all reported the presence of the CTX-M group 1, with

$bla_{CTX-M-1}$, $bla_{CTX-M-3}$, $bla_{CTX-M-15}$ and $bla_{CTX-M-32}$ being the mainly found enzymes. We also detected TEM and SHV-like genes in some isolates, but it is important to note that these genes could also encode narrow-spectrum beta-lactamases and confirmation of ESBL-type should be performed by gene sequencing.

The detection of CTX-M type enzymes in dog's uropathogens increases the risk of clinical failure because of the use of inappropriate empirical treatments and therefore results in complicated urinary infections. In our study, the highest frequency of resistance was found to enrofloxacin and ciprofloxacin, while the lowest resistance was observed against nitrofurantoin among the *E. coli* isolates, in addition to resistance to penicillins and cephalosporins.

Regarding *K. pneumoniae* isolates, it was observed that the vast majority were multidrug-resistant, highlighting the resistance to nitrofurantoin, trimethoprim/sulfamethoxazole, enrofloxacin, and ciprofloxacin. These findings agreed with a previous retrospective study carried out in a veterinary diagnostic laboratory in Medellín, Colombia, which evaluated the susceptibility to antimicrobials of bacterial isolates from dogs and reported that in general, the level of resistance was high (20-50%) to at least 6 or more antimicrobials. Specifically for urinary tract infections caused by *E. coli* or *enterobacteriaceae* (*Klebsiella* spp., *Proteus* spp.), amikacin and florfenicol were the only drugs that demonstrated 100% in vitro efficacy (Gómez Beltrán *et al.* 2020).

The resistance of ESBL-producing strains to other classes of antimicrobial agents can be explained by the fact that plasmids harboring $bla_{CTX-M-like}$ genes frequently carry genes that confer resistance

to fluoroquinolones, aminoglycosides, and SXT among other antibiotics (Coque *et al.* 2018; Wieler *et al.* 2011), as already reported in *E. coli* and *K. pneumoniae* isolates from urine of dogs with UTIs (Huber *et al.* 2013; Zogg *et al.* 2018). This suggests that veterinarians are encouraged to improve antibiotic prescriptions, based on susceptibility testing in order to reduce the selection pressure on the pathogens.

Surveillance studies implementing a One Health approach are required to assess the level of genetic relatedness among resistant bacteria from animal and human origins as well as to help elucidate the complex epidemiology of transmission and the role of companion animals in the spread of multi-resistant bacteria. Additionally, these findings contribute to the design of policies related to the use of antibiotics in both human and veterinary medicine, as well as other factors such as hygiene procedures, that may be associated with the emergence of the antimicrobial resistance phenomenon in the country.

This study has some limitations. First, it was difficult to obtain accurate data from the patients' clinical record, such as the type of UTI and details on previous antimicrobial treatment. Because veterinarians request urine culture and antibiogram more frequently from complicated than uncomplicated cases our results likely correspond to those of complicated UTI patients. Second, the selection of antibiotics to be evaluated in each patient was defined by the treating veterinarian rather than being standardized, which makes the comparison of susceptibility results in all the isolates difficult. Finally, it will be important to include in future studies additional molecular techniques to investigate the predominant ESBL-positive clones causing UTI in dogs.

CONCLUSIONS

An overall frequency of 11,6% (22/190) ESBL-producing *E. coli* and *K. pneumoniae* isolates in urine samples from dogs with UTI. CTX-M group 1 was the main ESBL detected, and a multidrug resistance profile was identified in *K. pneumoniae*. The timely identification of ESBL-producing strains of enterobacteriaceae is essential for evaluating the development of antimicrobial resistance and for establishing successful antibiotic therapies in the small-species daily practice, as well as to strengthen the implementation of infection control measures and the rational administration of antibiotics for the protection of public health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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