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Prevalence of drug-resistant uropathogenic bacteria in canines in the city of Merida, Yucatan

Prevalencia de bacterias uropatógenas drogorresistentes en caninos de la ciudad de Mérida, Yucatán

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Abstract

Antibiotic treatment is key to the improvement of canine patients with urinary tract infections; however, the irrational use of antimicrobials has led to the emergence of resistance mechanisms in uropathogenic bacteria. The objective of the study was to identify the bacteria present in urine cultures from canines in the state of Yucatán, determine their sensitivity to antibiotics, the prevalence of resistance to methicillin (MR) and production of extended-spectrum beta-lactamase (ESBL). Identification and sensitivity to antibiotics were performed using the MicroScan commercial kit and the Kirby-Bauer technique. MR was determined by sensitivity to oxacillin/cefoxitin and ESBL production through synergy techniques with beta-lactamase inhibitors. The most frequently isolated bacteria was *Proteus mirabilis* and the antibiotic with the highest percentage of resistant strains was norfloxacin; 30.2% of the Enterobacterales strains manifested ESBL production and 39.4% of the *Staphylococcus* spp. showed MR. The development of drug resistance is an important problem, only the knowledge about the prevalence of these uropathogenic bacteria and their drug-resistant strains in our state will allow us to propose effective treatment protocols.

ESBL, UTI, Canine

Resumen

El tratamiento antibiótico es clave para la mejoría de los pacientes caninos con infecciones del tracto urinario, sin embargo, el uso irracional de los antimicrobianos ha provocado el surgimiento de mecanismos de resistencia en las bacterias uropatógenas. El objetivo del estudio consistió en identificar las bacterias presentes en urocultivos provenientes de caninos del estado de Yucatán, determinar su sensibilidad a antibióticos, la prevalencia de resistencia a meticilina (MR) y producción de betalactamasa de espectro extendido (BLEE). Se realizó la identificación y sensibilidad a antibióticos mediante el kit comercial MicroScan y la técnica de Kirby-Bauer. Se determinó la MR mediante la sensibilidad a oxacilina/cefoxitina y la producción de BLEE a través de técnicas de sinergia con inhibidores de betalactamasas. La bacteria aislada con mayor frecuencia fue *Proteus mirabilis* y el antibiótico con mayor porcentaje de cepas resistentes fue norfloxacina; 30.2% de las cepas de Enterobacterales manifestaron producción de BLEE y 39.4% de las cepas de *Staphylococcus* spp. mostraron MR. El desarrollo de farmacorresistencia es un problema importante, sólo el conocimiento sobre la prevalencia de estas bacterias uropatógenas y sus cepas farmacorresistentes en nuestro estado nos permitirá proponer protocolos de tratamiento eficaces

BLEE, ITU, Canino

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Introduction

In the small animal clinic, one of the most frequent causes of consultation is urinary tract infection (UTI), caused by the adhesion, multiplication and persistence of an infectious agent in the urogenital tract (Wong *et al.*, 2015; Yu *et al.*, 2020).

In México, lower urinary tract infections occur regularly in clinical practice; however, scientific reports are limited. Mendoza, *et al.* (2017), conducted a retrospective study in which bacterial lower urinary tract infection was identified as the most frequent cause of UTI (34.02%). The main bacterial agent causing acute UTI in canines is *Escherichia coli*, and in recurrent or persistent infections, *Enterococcus spp.* and *Pseudomonas aeruginosa.* (Thompson *et al.*, 2011). In another research conducted by Brložnik *et al*., (2016) reported that 39% of isolates in canines with UTI correspond to *Escherichia coli*; 27.3% of cases to *Staphylococcus spp*., 13.5% to *Proteus spp.* and only 8.5% to *Enterococcus spp*. Many of the frequently isolated bacteria are considered potentially zoonotic; an example is *Escherichia coli*, which can produce infections in the human urogenital tract.

On the other hand, the high frequency of resistance to antibiotics used empirically for their treatment has made UTIs relevant to public health (Johnson *et al.*, 2003). Initial empiric antimicrobial therapy is indicated to alleviate patient discomfort in most cases while awaiting the results of urine culture and antibiotic sensitivity testing. However, antimicrobial treatments have an impact on the resistance patterns of the dog's resident microbiota, as well as opportunistic pathogens (especially after the use of broad-spectrum antibiotics) (Galarce *et al.*, 2019; Garza *et al.*, 2018; J. Weese *et al.*, 2011).

The irrational use of antimicrobials in infections where they are not required (viral diseases), inadequate therapeutics and lack of commitment on the part of the owners, contribute to the generation of selective pressure on bacteria and with it the selection of resistance genes that allow the survival of microorganisms. This is the case of the emergence of methicillin resistance (MR) in *Staphylococcus*, a mechanism that confers resistance to all β-lactam antibiotics. Another resistance mechanism of special importance is the production of extended-spectrum beta-lactamases (ESBL) in Enterobacteriaceae, which confers resistance to penicillins, cephalosporins and monobactams (Acosta & Vargas, 2018; Angles, 2018; Garza *et al.*, 2018; J. Gómez & Sánchez, 2018; Villegas *et al.*, 2016).

There are classifications that catalog antibiotic resistance in bacteria: multidrug resistance (MDR), resistance to 3 or more groups of antibiotics; extended drug resistance (XDR), resistance to all categories except 1 or 2 groups of antibiotics; and pandrug-resistance (PDR), when there is resistance to all antibiotics available for treatment (Angles, 2018; Jiménez *et al.*, 2019).

Multidrug-resistant pathogens in animals and humans produce diseases with greater lethal potential due to their virulence and frequent treatment failures (Galarce, *et al*., 2019). In this context, taking measures to improve the use of antibiotics to reduce bacterial resistance becomes more important. The World Health Organization (WHO), during 2016, raised the need to contain and delay the emergence of bacterial resistance, for this purpose it promoted epidemiological surveillance as the first step of multiple strategies against this serious public health problem (Angles, 2018; J. Gómez & Sánchez, 2018; Madero & Justo, 2021; OMS, 2016). Despite the evidence of increased percentages of antibiotic resistance in bacteria from UTI in canines, there are no studies in the state of Yucatán that evaluate the presence of drug resistance, so the use of empirical therapies may not be in accordance with our local epidemiology.

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The objective of this study was to identify the bacteria isolated from urine cultures from canine patients, by means of biochemical tests and to determine their sensitivity profile to frequently used antibiotics, to determine the prevalence of bacterial species, drug-resistant strains, and antibiotics with a higher percentage of resistance. Likewise, to identify the prevalence of MR strains or extended spectrum beta-lactamase (ESBL) producers to determine if there is a relationship between these and the presence of resistance to other important antibiotics.

This study is a milestone for future research focused on the identification of circulating resistance genes in bacteria isolated in canine urinary tract infections in the state. In addition, it will allow knowing the best treatment options based on the frequency of resistance in the local environment.

Methodology

Sampling

Dog urine samples for aerobic urine culture were received from different veterinary centers in Yucatán, between June 2020 - August 2022; they were also reviewed and catalogued according to the collection procedure: spontaneous urination, catheter collection and cystocentesis. The sample submitter was responsible for the choice of the appropriate collection method for each case, handling, transport, and preservation of the sample.

Microbiological culture

Inoculation of the sample was performed using a previously sterilized round calibrated bacteriological loop, on CHROMagar™ Orientation agar (Becton Dickinson, Mexico) for the identification and differentiation of pathogens in the urinary tract, and incubated for 24-48 hours in a bacteriological oven at 37°C. The amounts of CFU/mL isolated for each case were interpreted as proposed by Feijoó & Gómez, (2012).

Bacterial identification

After the incubation period, presumptive identification was performed by interpretation of CHROMagar™ Orientation chromogens (guide provided by the manufacturer), complementary tests (oxidase, catalase, bound coagulase, growth on Mac Conkey agar) and Gram staining; Based on the results of this staining, the commercial panel "MicroScan Neg/Urine Combo Panel Type 84" or "MicroScan Combo Panel Type 33" (Beckman Coulter, México) was used for biochemical identification and antibiogram. The analysis of the results was performed based on the biochemical reaction tables proposed by Procop *et al*. (2017), Cowman *et al*. (2016) and the ABIS online platform. (Online Advanced Bacterial Identification Software, 2022).

Based on the identified bacteria and the results of the antibiotics contained in the MicroScan panel (broth microdilution technique), an antibiogram was performed by the Kirby-Bauer method with antibiotic discs: ciprofloxacin 5 µg, enrofloxacin 5 µg, levofloxacin 5 µg, cefotaxime 30 µg, ceftazidime 30µg, cefoxitin 30 µg, penicillin 10 U, ceftazidime 30µg, amoxicillin/clavulanic acid 20/10 µg and fosfomycin 200 µg (Biorad, EU).

For phenotypic identification of ESBL production, the disc approach technique described by Calvo *et al*. (2011), combined double-disc synergy described in document M100 (CLSI, 2022) and microdilution in broth contained in the MicroScan panel were used. On the other hand, for the phenotypic detection of MR strains, the cefoxitin disk induction test and broth microdilution with oxacillin described in M100 (MicroScan panel) were used.

Statistical analysis

The numbers of isolates were compared using Kruskal-Wallis, ANOVA and Pearson's correlation statistics. The existence of association between ESBL/MR production and the presence of antibiotic resistance was performed through the χ^2 test and Fisher's exact F test; an $\alpha = 0.05$ was used for all tests. Statistical analyses were performed using Past 4.05 and IBM SPSS statistics 25 statistical software.

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Results

Urine culture was performed on 260 urine samples from canines from the city of Mérida, Yucatán, collected in the period June 2020 - August 2022, of which 108 were from males, 141 were from females and in 11 cases the sex was not specified. According to the way the sample was collected; 180 were obtained by cystocentesis, 29 by catheter, 9 by spontaneous urination and in 42 cases it was not stated.

Once identification was performed, 143 positive urine cultures were obtained (55%, 143/260), of which 139 had only one isolation (97.2%) and 4 had two (2.8%). *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus aureus* were isolated in greater proportion (Table 1).

The data were analyzed to determine whether there was any correlation between the total number of cases with respect to time (months), using the r test (Pearson) with a significance of α=0.05. However, no significant correlation was found between the variables (r = 0.0859, p=0.6699). The same statistic was used to determine whether there is a correlation between positive cases and time (months), and no significant correlation was found (r = -0.1367, p=0.4962).

Microorganism	Percentage
Proteus mirabilis	21.8 %
Escherichia coli	15.0 %
Staphylococcus aureus	14.3 %
Klebsiella pneumoniae	12.2 %
Enterococcus faecalis	10.9 %
Citrobacter freundii	6.1 %
Pseudomonas aeruginosa	5.4 %
Staphylococcus saprophyticus	4.1 %
Enterococcus spp.	2.0 %
Staphylococcus epidermidis	2.0 %
Acinetobacter baumannii/calcoaceticus Complex	1.4 %
Proteus vulgaris	1.4 %
Staphylococcus pseudintermedius	1.4 %
Edwardsiella tarda	0.7 %
Kluyvera ascorbata	0.7 %
Staphylococcus spp.	0.7 %

Table 1 Bacterial isolates in canine urine cultures

The mean, median and variance of monthly cases were calculated for each year: 2020, $\bar{X}$=10.29, $\tilde{X}$=11, S=11.9; 2021: $\bar{X}$=8.66, $\tilde{X}$=6.5, S=15.51; 2022, $\bar{X}$=10.5, $\tilde{X}$=10, S=17.14. The Kruskal-Wallis's test with a significance of α=0.05 was used to determine whether there are differences between the medians of monthly cases according to the year. No significant differences were found (p=0.4845).

On the other hand, to determine if there are any variations in the number of monthly positive cases according to the year (2020: $\bar{X}$= 6.43, $\tilde{X}$=7, S= 3.1; 2021: $\bar{X}$= 4.75, $\tilde{X}$=3.5, S= 10.35; 2022: $\bar{X}$= 5.13, $\tilde{X}$=4, S= 5.61), variances were compared by ANOVA test with a significance of α=0.05. It was found that there is no significant difference (p= 0.4591) between the groups (year variable).

The sensitivity profiles of each isolate to different antibiotics were evaluated (Table 2). The average percentage of resistance per antibiotic was 36.4%.

Antibiotic	Sensitive	Medium	Resistant
Penicillin	21.6% (11/51)	-	78.4% (40/51)
Oxacillin	59.1% (13/22)	-	40.9% (9/22)
Ampicillin	34.3% (23/67)	-	65.7% (44/67)
Ampicillin/Sulbactam	51.3% (45/78)	14.1% (11/78)	34.6% (27/78)
Amoxicillin/Clavulanic Acid	52% (39/75)	8% (6/75)	40% (30/75)
Piperacillin/Tazobactam	97.7% (86/88)	-	2.3% (2/88)
Cefazolin	58.8% (30/51)	-	41.2% (21/51)
Cefuroxime	66.7% (2/3)	-	33.3% (1/3)
Cefoxitin	57.6% (57/99)	-	42.4% (42/99)
Ceftriaxone	51.1% (45/86)	5.7% (5/88)	43.2% (38/88)
Cefotaxime	52.9% (46/87)	5.7% (5/87)	41.4% (36/87)
Ceftazidime	64.6% (62/96)	4.2% (4/96)	31.3% (30/96)
Cefepime	68.3% (56/82)	1.2% (1/82)	30.5% (25/82)
Imipenem	74.5% (70/94)	14.9% (14/94)	10.6% (10/94)
Meropenem	93.8% (90/96)	5.2% (5/96)	1% (1/96)
Gentamicin	54.4% (68/125)	1.6% (2/125)	44% (55/125)
Amikacin	78.9% (71/90)	5.6% (5/90)	15.6% (14/90)
Norfloxacin	20% (1/5)	-	80% (4/5)
Ciprofloxacin	49.3% (71/144)	9% (13/144)	41.7% (60/144)
Levofloxacin	51.8% (29/56)	5.4% (3/56)	42.9% (24/56)
Enrofloxacin	41.4% (48/116)	12.1% (14/116)	46.6% (54/116)
Trimethoprim/Sulfamethoxazole	47.7% (53/111)	-	52.3% (58/111)
Nitrofurantoin	69.8% (60/86)	16.3% (14/86)	14% (12/86)
Vancomycin	75% (15/20)	10% (2/20)	15% (3/20)
Fosfomycin	83.3% (5/6)	-	16.7% (1/6)

Table 2 Overall results of susceptibility profiles by antibiotic

To determine the antibiotics with the highest and lowest percentages of resistant strains, the data were grouped for analysis with respect to the overall mean, 36.4% (graph 1).

It was found that the 3 antibiotics with the highest percentage of resistant strains were: norfloxacin, penicillin and ampicillin; while the drugs with the lowest percentage were: piperacillin/tazobactam, meropenem and imipenem. It was also observed that of the group of third generation cephalosporins, only ceftazidime had a lower-than-average percentage of resistance. In addition, it was found that the 4 quinolones studied (norfloxacin, ciprofloxacin, levofloxacin and enrofloxacin) showed above-average resistance.

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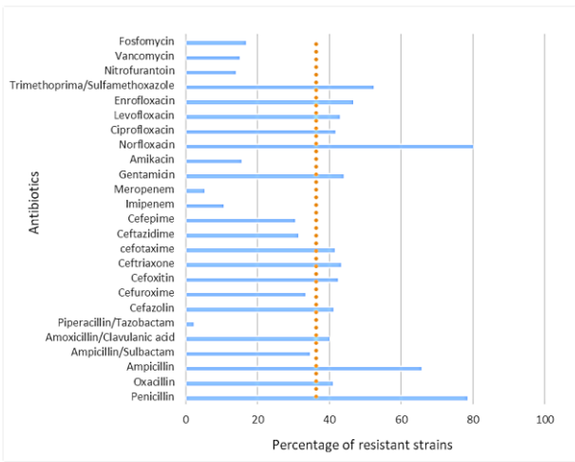
On the other hand, the results of the sensitivity tests for strains of each species identified were classified by level of resistance: multidrug resistance (MDR), extended drug resistance (XDR) and pan-drugresistance (PDR). The above was performed based on the classification proposed by the Pan American Health Organization (PAHO) and other works (Jiménez *et al*., 2019; Magiorakos *et al*., 2012; Ríos, 2016); however, no strains classified as XDR or PDR were observed. (Table 3).



Graphic 1 Comparison of percentages of resistant strains among antibiotics. The dotted orange line represents the overall mean (36.4%)

Microorganism	MDR	XDR	PDR	Non MDR
Proteus mirabilis	59.4% (19/32)	-	-	40.6% (13/32)
Escherichia coli	59.0% (13/22)	-	-	41.0% (9/22)
Klebsiella pneumoniae	66.7% (12/18)	-	-	33.3% (6/18)
Citrobacter freundii	66.7% (6/9)	-	-	33.3% (3/9)
Proteus vulgaris	50% (1/2)	-	-	50% (1/2)
Edwardsiella tarda	100% (1/1)	-	-	-
Kluyvera ascorbata	100% (1/1)	-	-	-
Pseudomonas aeruginosa	50% (4/8)	-	-	50% (4/8)
COMPLEJO *Acinetobacter baumannii/calcoaceticus*	50% (1/2)	-	-	50% (1/2)
Staphylococcus aureus	42.9% (9/21)	-	-	57.1% (12/21)
Staphylococcus saprophyticus	16.7% (1/6}	-	-	83.3% (5/6)
Staphylococcus epidermidis	33.3% (1/3)	-	-	66.7% (2/3)
Staphylococcus pseudintermedius	50% (1/2)	-	-	50% (1/2)
Staphylococcus spp.	-	-	-	100% (1/1)
Enterococcus faecalis	31.2% (5/16}	-	-	68.8% (11/16)
Enterococcus spp.	33.3% (1/3)	-	-	66.7% (2/3)

Table 3 Comparison of degree of resistance between species. MDR: resistant ≥ 1 antibiotic in ≥ 3 antimicrobial categories; XDR: resistant ≥ 1 antibiotic in all antimicrobial categories except to 1 or 2 groups; PDR: resistant to all antibiotics; non-MDR: not resistant or resistant ≥ 1 antibiotic in < 3 antimicrobial categories

On the other hand, phenotypic detection of ESBL production in isolates of Enterobacteriaceae with low- or high-level resistance (intermediate or resistant strains) to third generation cephalosporins was performed.

Of a total of 85 Enterobacteriaceae isolates, 26 were identified as ESBL producers (30.2%), of these, 8 were identified in *Klebsiella pneumoniae* strains (8/18, 44.4%) ¸ 7 in *Escherichia coli* (7/22, 31.8%), 6 *in Proteus mirabilis* (6/32, 18.7%), 4 in *Citrobacter freundii* (4/9,44.4%) and 1 in *Proteus vulgaris* (1/2, 50%).

The production of ESBL in Enterobacteriaceae leads to the development of resistance to cephalosporins, particularly to third and fourth generation cephalosporins. In this study, resistance to ceftriaxone and cefotaxime was observed in all 26 ESBL -producing strains.

We analyzed whether there is an association between the presence of ESBL and the level of resistance to ceftazidime and cefepime, using the $\chi 2$ and Fisher's exact tests. Likewise, because ESBL-producing strains have been related to the development of corresistances to quinolones and aminoglycosides, the existence of association between the presence of ESBL and the level of resistance to ciprofloxacin, enrofloxacin, gentamicin and amikacin was determined through χ^2 and Fisher's exact tests (Table 4).

Dependent variable	Test	P value	Other Coefficients
Ceftazidime resistance level	Exact F	0.000*	Cramer´s V = 0.590 λ= 0.419
Presence of resistance to Cefepime	χ^2=35.55	0.000*	Cramer´s V = 0.708 λ= 0.609
Resistance level to Ciprofloxacin	Exact F	0.000*	Cramer´s V = 0.564 λ= 0.455
Resistance level to Enrofloxacin	χ^2=19.82	0.000*	Cramer´s V = 0.480 λ= 0.273
Resistance level to Gentamicin	Exact F	0.027*	Cramer´s V = 0.268 λ= 0.034
Resistance level to Amikacin	Exact F	0.25	-

Table 4 Analysis of variables associated with the existence of ESBL (independent variable) (*) Statistically significant P-value

Phenotypic detection of MR in Staphylococcus isolates was also performed. Of a total of 33 *Staphylococcus* isolates, 13 were identified as MR (39.4%), of these, 7 were in *Staphylococcus aureus* (7/21, 33.3%) ¸ 2 in *Staphylococcus pseudintermedius* (2/2, 100%), 2 in *Staphylococcus saprophyticus* (2/6, 33.3%), 1 in *Staphylococcus epidermidis* (1/3, 33.3) and 1 in *Staphylococcus spp.* (1/1, 100).

MR strains have been related to the development of quinolone and aminoglycoside resistance, so we determined the existence of an association between the presence of MR and the level of resistance to ciprofloxacin, levofloxacin, enrofloxacin and gentamicin by χ^2 and Fisher's exact tests (Table 5).

ISSN-On line: 2414-8849

Dependent variable	Test	P Value
Ciprofloxacin Resistance	χ^2=0.075	0.784
Levofloxacin Resistance	Exact F	0.411
Enrofloxacin resistance	Exact F	1
Gentamicin resistance	Exact F	0.119

Table 5 Analysis of variables associated with the existence of RM (independent variable) (*) Statistically significant P-value

Discussions

Urinary tract infections (UTI) are considered an important reason for consultation with the veterinarian. Studies have estimated that about 14% of dogs present some UTI event during their lifetime, so it is also considered as the second cause of antibiotic use in canines. On the other hand, reports indicate that UTIs usually involve the presence of uropathogenic bacteria frequently from gastrointestinal origin (Hernando *et al.*, 2021; Yamanaka *et al.*, 2019).

With the intention of observing whether there is any trend in the results of total cases or positive cases, an analysis of the results was performed using Pearson's r statistic; however, no correlation was found. Likewise, no differences were detected between the monthly mean of total or positive cases according to the year. This made it clear that there has been no significant increase or decrease in total or positive cases.

In this study, the most frequently isolated bacteria was *Proteus mirabilis* (21.8%); this microorganism is strongly related to the generation of UTI, the precipitation of phosphate-ammonium-magnesium crystals (struvite) in urine and the formation of uroliths. *Proteus mirabilis* utilizes urea and generates ammonium and carbon dioxide, this raises the urine pH and increases the availability of phosphates and ammonium to finally, by increasing their concentration together with magnesium, lead to the formation of crystals (Mendóza *et al.*, 2017; Rinkardt & Houston, 2004).

The prevalence of *Proteus mirabilis*, as the most frequently isolated bacteria in this study, contrasts with reports from other investigations. Marques *et al*., (2018), reported that 45.1% of canine UTI cases in Portugal were caused by *Escherichia coli*. With similar results, a study conducted in Spain by Hernando *et al*., (2021), found the presence of *Escherichia coli* in 45.3% of UTI cases in dogs. In our study, *Escherichia coli* was the second most frequent isolate, with 15% of the cases. The search for virulence factors in circulating clones of *Proteus mirabilis* isolated from UTI in canines from our state may provide information on the high prevalence of this microorganism.

On the other hand, the choice of the appropriate antibiotic is crucial for the improvement of patient's health. The International Society for Companion Animal Infectious Diseases (ISCAID) proposes the use of antibiotics empirically for the treatment of cystitis; specifically, it proposes the use of amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole (J. Weese *et al*., 2011; J. S. Weese *et al*., 2019). In our study, the percentages of resistance for the two antibiotics were 40% and 52.3%, respectively. These results contrast with those described by other authors. McGovern *et al*., (2019); in a retrospective study conducted in Louisiana, United States, it was reported the presence of resistance to amoxicillin/clavulanic acid in 24.6% of isolates and to trimethoprim/sulfamethoxazole in 14.1%. For their part, Hariharan *et al*. (2016) found that 35.7% and 57.1% of isolates were resistant to amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole, respectively.

In the human medical field, it has been established that the use of empirical treatments for UTI should contemplate drugs whose resistance percentages do not exceed 20% in the local environment; however, this has not been properly determined in the veterinary field. (Batalla *et al.*, 2007; Gupta *et al.*, 2011).

Veterinary guidelines only suggest that the choice of antibiotic should be based on local epidemiology (J. Weese *et al*., 2011; J. S. Weese *et al*., 2019). In our case the observed percentages were higher than the global mean (36.4%), which discourages its use empirically.

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The use of appropriate alternatives for their use as empirical treatments is under discussion. ISCAID suggests the use of nitrofurantoin or fosfomycin as an alternative in the treatment of multidrug-resistant infections; in contrast, these drugs are considered excellent options for empirical treatment of cystitis in the human setting due to their high concentration in urine, short treatment regimens, low resistance rates, etc. (Batalla *et al.*, 2007; J. S. Weese *et al.*, 2019).

In our study, we found a percentage of resistant strains of 14% and 16.7% for nitrofurantoin and fosfomycin, respectively. It is worth mentioning that the results reported for fosfomycin are limited only to *Escherichia coli* and *Enterococcus spp*. due to the lack of reference values for other species.

It is necessary to consider that some frequent bacteria, according to the local epidemiology observed, may be intrinsically resistant to certain antibiotics recommended for UTI, for example: *Proteus spp.* against nitrofurantoin (CLSI, 2022). Likewise, the use of broad-spectrum drugs with distribution in multiple tissues (fluoroquinolones, third generation cephalosporins, etc.) may generate the selection of multiresistant strains during or after treatment. (Yudhanto *et al.*, 2022).

In the present study, resistance percentages higher than the overall average (36.4%) were observed for all fluoroquinolones and 2 of the 3 third generation cephalosporins studied. This is a concerning problem, because it is an indication of selection for resistance genes, such as genes coding for ESBL (Bush, 2018).

The results found in studies on resistance to members of fluoroquinolones and third generation cephalosporins are contrasting with each other. Chan *et al.*, (2022), conducted a retrospective study with data obtained from canine urine cultures between 2018 and 2020 in China. The team found resistant strain percentages of 20% for ceftriaxone, ciprofloxacin, and enrofloxacin. In contrast, Amphaiphan *et al*., (2021), noted percentages above 50% of *Staphylococcus spp*. strains, *Escherichia coli, Proteus spp. strains, Klebsiella spp.* resistant to enrofloxacin.

Gómez *et al*. (2020) reported that more than 30% of the strains of the Enterobacteriaceae family were resistant to enrofloxacin. In the case of ciprofloxacin in *Escherichia coli* it was 35% and in other Enterobacteriaceae it was 15.6%. For their part, García *et al*. (2019), described that of the total number of strains isolated from *Proteus spp*. and *Escherichia coli* (period 2012 - 2017) were resistant to ceftriaxone 27.3% and 38.7%; for ciprofloxacin, 27.6% and 35.9% and for enrofloxacin, 51.9% and 75%, respectively.

The percentages of resistant strains vary according to the region in which they are found, although percentages higher than 25% prevail. It is possible that the abuse of these broad-spectrum drugs in unnecessary treatments is an important factor in the development of resistance. (Bush, 2018; WHO, 2016; Valdés, 2017; Yudhanto *et al.*, 2022).

Resistance to fluoroquinolones has been mainly related to the presence of mutations in the *gyrA* and *gyrB* genes (coding for DNA gyrase subunits, as well as mutations in *parC* (coding for topoisomerase IV); These mutations have been described in Enterobacterales and *Pseudomonas aeruginosa* isolated in dogs and are even implicated in the existence of resistance to last generation quinolones such as enrofloxacin, marbofloxacin and pradofloxacin (Liu *et al.*, 2012; Vingopoulou *et al.*, 2018). Likewise, the above-mentioned mutations are related to cross-resistance between multiple quinolones (EUCAST, 2021). In the same context, Vingopoulou *et al*., (2018) reported the presence of plasmid-mediated quinolone resistance genes in *Escherichia coli* and *Pseudomonas aeruginosa* strains in canine samples; the identified genes (*qnrA1*, *qnrB1*, *qnrS1* and *qnrS2*) encode for proteins that protect DNA gyrase from the action of fluoroquinolones by preventing their binding.

On the other hand, they determined the percentage of MDR, XDR and PDR strains for each of the bacterial species identified. He emphasized that in all cases of Gram-negative bacteria, a percentage of MDR higher than 50% was observed; this correlates with findings reported by Amphaiphan *et al*. (2021).

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The group reported MDR percentages higher than 50% for *Escherichia coli* (69.7%), *Proteus spp.* (81.5%), *Klebsiella spp.* (92.9%), *Enterobacter spp.* (100%), *Pseudomonas aeruginosa* (92.3%) and *Acinetobacter spp.* (100%). As for Gram-positive bacteria (*Staphylococcus* and *Enterococcus*), the results were lower than those reported in the previous investigation. The high percentages of MDR in Gram-negative bacteria observed in the present study are concerning in view of the continuous development of resistance; these raise the need for the correct management of antibiotics in the veterinary clinical environment.

The phenotypic manifestation of ESBL in Enterobacterales and *Staphylococcus* MR strains was determined. In the present study it was found that 30.2% of the Enterobacterales strains presented ESBL production; the most frequent strains were *Klebsiella pneumoniae* and *Escherichia coli*. These results contrast with the data observed in the study conducted in China by Li *et al*., (2017), where the group found 3 ESBL-producing *Escherichia coli* strains (3/118, 2.54%); additionally, the team identified ESBL type CTX-M-15 and TEM-1, these enzymes have been found in strains isolated from humans and animals. On the other hand, a study conducted in Europe by Bogaerts *et al*., (2015), noted the identification of genes coding for ESBL type CTX-M-15 and CTX-M-14 from *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from canine samples; these proteins have been described in strains from human and animal samples.

ESBLs are hydrolytic enzymes that degrade certain β-lactams such as penicillins, cephalosporins and monobactams, and are therefore related to resistance to these antibiotics. Treatment alternatives have been proposed (carbapenemics, fluoroquinolones, etc.) which should be confirmed by antibiogram. The use of antibiotics with inhibitors such as amoxicillin/clavulanic acid for the treatment of ESBL-producing bacteria lacks conclusive studies, so they should be used with caution and in particular situations (Esparza *et al.*, 2015; Mederos *et al.*, 2018; Villegas *et al.*, 2016; J. S. Weese *et al.*, 2019).

It is evident that the observed percentages of ESBL-producing strains are a particularly important problem for public health; the genes involved are found in plasmids that allow their transfer between strains, in conjunction with other resistance genes. Likewise, the isolation of these bacteria from companion animals, such as dogs, poses a potential risk of zoonosis (Bogaerts *et al.*, 2015).

The association between ESBL production in Enterobacterales and the level of resistance to ceftazidime (third generation cephalosporin), the presence of resistance to cefepime (fourth generation cephalosporin) and the level of resistance to aminoglycosides and fluoroquinolones (co-resistance) was evaluated. An association was observed between the presence of ESBL (independent variable) and the level of resistance to ceftazidime (dependent variable), as well as the presence of resistance to cefepime (dependent variable). For ceftazidime, the intensity of association is moderate and the ability to reduce the error to predict the dependent variable is moderate. In the second case, the association is strong and the ability to reduce the error to predict the dependent variable is high. This result was expected, because cephalosporins are substrates of these enzymes; more than 300 classes have been described with greater or lesser affinity for some third generation cephalosporins. The emerging problem of ESBL-producing strains has led to the proposal of alternative antibiotics that allow the correct management of infections caused by these bacteria, avoiding the abuse of carbapenemics (Calvo *et al.*, 2011; Dueñas *et al.*, 2021; Mederos *et al.*, 2018; J. S. Weese *et al.*, 2019).

The presence of corresistance to other antibiotics is frequent in ESBL-producing strains, since the plasmids where the genes are transported also have coding sequences for other resistance mechanisms (Torres & Zarazaga, 2007). Bogaerts *et al*., (2015), reported the presence of corresistances to trimethoprim/sulfamethoxazole and gentamicin; in another study, Li *et al*., (2017), described isolates of ESBL-producing Escherichia coli strains with simultaneous resistance to enrofloxacin, marbofloxacin, trimethoprim/sulfamethoxazole and tetracycline.

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The present study analyzed whether there was an association between the presence of ESBL in the strains studied and the presence of corresistance (level of resistance) to ciprofloxacin, enrofloxacin, gentamicin and amikacin. In general, associations with moderate intensity were observed for the two fluoroquinolones, as well as a moderate capacity to reduce the error to predict the dependent variable. In the case of aminoglycosides, we only found an association with the level of resistance to gentamicin with a moderate intensity and a low capacity to reduce the error. The above findings could be explained if we consider that the presence of corresistance genes to other families of antibiotics in plasmids is variable; therefore, it is proposed to perform molecular tests to know which genes cause the development of resistance in this group of bacteria, as well as the frequency in which they occur. This information will allow us to know the genes and plasmids of resistance circulating in the veterinary environment of our state.

On the other hand, the phenotypic manifestation of MR in *Staphylococcus* strains was determined; it was observed that about 40% of *Staphylococcus* isolates presented the MR phenotype. The data obtained contrast with reports from several studies: Marques *et al*. (2018) reported that 9.2% of the isolates of *Staphylococcus* species during the study period presented MR; they also pointed out that the main gene identified was the *mecA* gene. Chan *et al*., (2022) conducted another contrasting study; the team reported that 19% of *Staphylococcus spp*. isolates in the period 2018-2020 presented the MR phenotype (oxacillin resistance). On the other hand, McGovern *et al*., (2019); reported that approximately one third of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* cases presented MR. It is evident that the prevalence of MR *Staphylococcus* cases is a function of the geographical area in which the study was conducted, however, the data observed in this study are alarming, because they surpass the percentages reported in other investigations.

MR comes from the acquisition of *mecA* or mecC genes coding for a PBP2a (penicillin binding protein 2a) that has a lower affinity for beta-lactams; therefore, it preserves the integrity of the peptide-glycan layer (cell wall) by maintaining transpeptidase activity (Aguayo *et al.*, 2018).

According to several studies, *Staphylococcus* strains with MR should be considered resistant to all beta-lactams (CLSI, 2022). Moreover, *mecA* or *mecC* genes, regulatory genes (*mecI* and *mecR*) and other resistance genes are located within a mobile chromosomal element called staphylococcal chromosomal cassette (*SCCmec*), so sometimes MR strains can also develop co-resistance to other antibiotic families. (Aguayo *et al.*, 2018).

The search for therapeutic options for the treatment of Staphylococcus MR is under debate; in general, it is proposed that the management of cases should be individualized due to the presence of corresistances; thus, antibiotics such as vancomycin should be reserved exclusively for cases indicated by the specialist in charge due to their importance in human medicine. ISCAID proposes the use of various antibiotics in whose spectrum of action encompasses *Staphylococcus* MR (trimethoprim/sulfamethoxazole, nitrofurantoin, quinolones, etc.) (J. S. Weese *et al*., 2019). In recent years, the possible use of doxycycline has been explored as an "off-label" alternative in the use of urinary tract infections by multidrug-resistant strains such as *Staphylococcus* MR, although this has not been proven. (Jodlowski *et al.*, 2021; Rubi & Gaunt, 2011).

Finally, this study analyzed the association between the presence of MR in the *Staphylococcus strains* studied and the presence of corresistance (presence of resistance) to ciprofloxacin, levofloxacin, enrofloxacin and gentamicin. In none of the cases was an association found, which could be explained if we consider that the presence of resistance genes to other antibiotic families in the plasmids is variable. Therefore, it is proposed to perform molecular tests to know the type of *SCCmec* circulating in the MR strains isolated in the state of Yucatán; likewise, it is proposed to identify resistance genes to other families in *Staphylococcus* (Aguayo *et al.*, 2018).

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Conclusions

The development of antibiotic resistance in the veterinary field is a major problem affecting our state; it compromises the health status of patients by reducing effective therapeutic options. The indiscriminate use of broad-spectrum antibiotics for the treatment of UTIs has led to the selection of bacteria with resistance genes such as ESBL-producing Enterobacterales and *Staphylococcus* MR. Only the knowledge about the prevalence of bacterial species causing UTI in our state and antibiotic resistance will allow us to propose better treatment protocols based on the reality of our environment.

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