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Comparison of antimicrobial resistance in bacterial isolates from dogs in a veterinary diagnostic laboratory in Colombia between two consecutive four-year periods

Comparación de resistencia antimicrobiana en aislamientos bacterianos de perros en un laboratorio de diagnóstico veterinario de Colombia entre dos períodos consecutivos de cuatro años

Comparação da resistência antimicrobiana em isolados bacterianos de cães em um laboratório de diagnóstico veterinário na Colômbia entre dois períodos consecutivos de quatro anos

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Abstract

Background: Antimicrobial resistance (AMR) and the increase in multi-drug-resistant bacteria are among the most important threats to human and veterinary medicine, according to the World Health Organization. **Objective:** To compare the antimicrobial susceptibility patterns in bacterial isolates from dogs over two consecutive four-year periods. **Methods:** The Animal Microbiology Laboratory database of the Agrarian Sciences Faculty at the University of Antioquia (Medellín, Colombia) was searched for routine canine submissions in which culture and antibiograms were performed. **Results:** A total of 1,146 samples were submitted between 2020 and August 2023 for culture and susceptibility testing, from which 805 (70.2%) isolates were recovered. Of these 805 isolates, susceptibility testing was performed on 799 samples. A significant decrease in susceptibility between 2016-2019 and 2020-August 2023 was noted in dog isolates for some antimicrobials: *Escherichia coli* to amoxicillin-clavulanate (66.7-53.1%; p<0.01) and ampicillin (67.7-58%; p<0.05), *Enterobacteriaceae* to amikacin (100-94.3%; p<0.01), ampicillin (61.8-45.7%; p<0.01), and trimethoprim-sulfadiazine (83.9-75.6%; p<0.05), *Staphylococcus pseudointermedius* to gentamicin (63.9-52.5%; p<0.01), trimethoprim-sulfadiazine (57-50%; p<0.05), and doxycycline (60.9-43.4%; p<0.01). Significantly increased susceptibilities were also noted in *E. coli* to enrofloxacin (69.2-78.7%; p<0.05) and doxycycline (68.7-76.2%; p=0.0745) and in *Enterobacteriaceae* to enrofloxacin (64.4-79.3%; p<0.01), and doxycycline (38.7-47.7%; p=0.06). For all bacterial isolates, an increasing resistance trend was observed for amoxicillin-clavulanate. All *Staphylococcus* species showed low resistance to amikacin (<10%); moderate resistance (10-20%) to amoxicillin-clavulanate, cephalexin, cefovecin, and enrofloxacin; high resistance (20-50%) to ampicillin, gentamicin, trimethoprim-sulfadiazine, and clindamycin; and very high resistance (50-70%) to doxycycline. For other bacterial families, the number of antimicrobials with high (20-

50%) or very high (50-70%) resistance rates was: *Enterobacteriaceae* (7/9), *Enterococcus* spp. (4/7), *E. coli* (10/12), and *Streptococcus* spp. (4/6). For urinary tract infections caused by *E. coli* or *Enterobacteriaceae* (*Klebsiella* spp., *Proteus* spp.), amikacin and gentamicin were the only drugs that demonstrated low (<10%) *in vitro* resistance. Multidrug resistance slightly increased from 2016-2019 (18.7%; 247/1,316) to 2020-August 2023 (19.7%; 150/761). decrease in susceptibility rather than an increase in resistance (28 vs. 20). **Conclusion:** High resistance rates highlight the need for continued surveillance and reinforce the importance of antibiograms to guide clinical decisions.

Keywords: *antibiotics; antibiogram; antimicrobial resistance; antibiotic resistant bacteria; dogs; E. coli; multidrug resistance; multi-resistance; Staphylococcus spp.; susceptibility.*

Resumen

Antecedentes: La resistencia a los antimicrobianos (RAM) y el aumento de bacterias multirresistentes se encuentran entre las amenazas más importantes para la medicina humana y veterinaria según la Organización Mundial de la Salud. **Objetivo:** Comparar los patrones de susceptibilidad a los antimicrobianos en aislamientos de perros en dos períodos consecutivos de 4 años. **Métodos:** Pesquisamos no banco de dados do Laboratorio de Microbiología Animal de la Facultad de Ciencias Agrarias de la Universidad de Antioquia (Medellín, Colombia) en busca de envíos rutinarios de muestras clínicas de perros para las cuales se realizaron cultivos y antibiogramas. **Resultados:** Un total de 1.146 muestras fueron enviadas entre 2020 y agosto de 2023 para cultivo y determinación de sensibilidad, de las cuales se pudieron recuperar 805 (70,2%) aislamientos. De esos 805 aislamientos se determinó sensibilidad en 799 muestras. Se observó una disminución significativa entre 2016-2019 y 2020-agosto 2023 en la susceptibilidad de las cepas bacterianas aisladas de perros a algunos antimicrobianos: *Escherichia coli* a amoxicilina-clavulanato (66,7-53,1%; p<0,01) y ampicilina (67,7-58%; p<0,05), Enterobacterias a amikacina (100-94,3%; p<0,01), ampicilina (61,8-45,7%; p<0,01) y trimetoprim-sulfadiazina (83,9-75,6%; p<0,05), *Staphylococcus pseudointermedius* a gentamicina (63,9-52,5%; p<0,01), trimetoprim-sulfadiazina (57-50%; p<0,05) y doxiciclina (60,9-43,4%; p<0,01). También se observaron aumentos significativos en la susceptibilidad de *E. coli* a enrofloxacina (69,2-78,7%; p<0,05), doxiciclina (68,7-76,2%; p=0,0745), *Enterobacteriaceae* a enrofloxacina (64,4- 79,3%; p<0,01) y doxiciclina (38,7-47,7%; p=0,06). Para todos los tipos de bacterias hubo un incremento de la resistencia contra la amoxicilina-clavulanato. Todas las especies de *Staphylococcus* mostraron baja resistencia a la amikacina (<10%); resistencia moderada (10-20%) a amoxicilina-clavulanato, cefalexina, cefovecina y enrofloxacina; alta resistencia (20-50%) a ampicilina, gentamicina, trimetoprim-sulfadiazina y clindamicina; y resistencia muy alta (50-70%) a la doxiciclina. Para otras familias de bacterias, el número de antimicrobianos con resistencia alta (20-50%) o muy alta (50-70%) fue: *Enterobacteriaceae* (7/9), *Enterococcus* spp. (4/7), *E. coli* (10/12) y *Streptococcus* spp. (4/6). Para las infecciones del tracto urinario causadas por *E. coli* o *Enterobacteriaceae* (*Klebsiella* spp., *Proteus* spp.), la amikacina y la gentamicina fueron los únicos fármacos que demostraron baja resistencia *in vitro* (<10%). El grado de multirresistencia aumentó ligeramente para el período 2020-agosto 2023 (19,7%; 150/761 aislamientos) en comparación con el período 2016-2019 (18,7%; 247/1.316). Esto se atribuyó a una reducción significativa de la susceptibilidad en lugar de a un aumento de la susceptibilidad (28 vs. 20). **Conclusión:** Las altas tasas de resistencia indican que se necesita vigilancia continua y el uso de antibiogramas para guiar las decisiones clínicas.

Palabras clave: *antibiótico; antibiograma; E. coli; perros; multiresistencia; resistencia a múltiples fármacos; resistencia antimicrobiana; resistencia antibiótica; Staphylococcus spp.; susceptibilidad.*

Resumo

Antecedentes: A resistência antimicrobiana (RAM) e o aumento de bactérias multirresistentes estão entre as ameaças mais importantes à medicina humana e veterinária, de acordo com a Organização Mundial da Saúde. **Objetivo:** Comparar os padrões de suscetibilidade antimicrobiana em isolados de cães em 2 períodos consecutivos de 4 anos. **Métodos:** Pesquisamos no banco de dados do Laboratorio de Microbiología Animal da Faculdade de Ciências Agrárias da Universidad de Antioquia (Medellín, Colombia) os envios dos isolados de cães de rotina para os quais foram realizados cultura e antibiogramas. **Resultados:** Foram enviadas 1.146 amostras entre 2020 e agosto de 2023 para cultura e determinação de sensibilidade, das quais 805 (70,2%) isolados puderam ser recuperados. Destes 805 isolados, a sensibilidade foi determinada em 799 amostras. Foi observada uma diminuição significativa entre 2016-2019 e 2020-agosto 2023 na suscetibilidade de isolados de cães aos alguns antimicrobianos: *Escherichia coli* à amoxicilina-clavulanato (66,7-53,1%; p<0,01) e ampicilina (67,7-58%; p<0,05), *Enterobacteriaceae* para amicacina (100-94,3%; p<0,01), ampicilina (61,8-45,7%; p<0,01) e trimetoprim-sulfadiazina (83,9-75,6; p<0,05), *Staphylococcus pseudointermedius* à gentamicina (63,9-52,5%, p<0,01), trimetoprim-sulfadiazina (57-50%; p<0,05) e doxiciclina (60,9-43,4%; p<0,01). Suscetibilidades significativamente aumentadas também foram observadas como segue: *E. coli* à enrofloxacina (69,2-78,7%; p<0,05), doxiciclina (68,7-76,2%; p=0,0745), Enterobacteriaceae à enrofloxacina (64,4-79,3%; p<0,01) e doxiciclina (38,7-47,7%; p=0,06). Para todos os tipos de bactérias houve um aumento do padrão de resistência contra amoxicilina-clavulanato. Todas as espécies de *Staphylococcus* apresentaram baixa resistência à amicacina (<10%); resistência moderada (10-20%) à amoxicilina-clavulanato, cefalexina, cefovecina e enrofloxacina; alta resistência (20-50%) à ampicilina, gentamicina, trimetoprim-sulfadiazina e clindamicina; e resistência muito elevada (50-70%) à doxiciclina. Para outras famílias de bactérias, o número de antimicrobianos para os quais a resistência foi alta (20-50%) ou muito alta (50-70%) foi: *Enterobacteriaceae* (7/9), *Enterococcus* spp. (4/7), *E. coli* (10/12) e *Streptococcus* spp. (4/6). Para infecções do trato urinário causadas por *E. coli* ou *Enterobacteriaceae* (*Klebsiella* spp., *Proteus* spp.), a amicacina e a gentamicina foram os únicos medicamentos que demonstraram baixa (<10%) resistência *in vitro.* O grau de multirresistência aumentou ligeiramente no período de 2020-agosto 2023 (19,7%; 150/761 isolados) em comparação com o período de 2016-2019 (18,7%; 247/1.316). Isto foi atribuído a uma redução significativa da suscetibilidade, e não a aumentos de suscetibilidade (28 vs. 20). Conclusões: Altas taxas de resistência indicam vigilância contínua e o uso de antibiogramas é necessário para orientar as decisões clínicas.

Palavras-chave: *antibiótico; antibiograma; bactérias resistentes a antibióticos; cães; E. coli; multirresistência; resistência a múltiplas drogas; resistência a antibióticos; resistência antimicrobiana; Staphylococcus spp.;*

Introduction

In small animal practice, the choice of antimicrobial therapy is often made empirically when treatment must begin before the results of culture and susceptibility tests are known (Gómez-Beltrán et al., 2021). For the veterinarian, knowing the bacterial species possibly involved in the most frequently encountered infectious conditions and their potential resistance to antimicrobials is important.

Antimicrobial resistance among bacteria isolated from companion animals is an emerging problem as it limits the potential use of antimicrobials for the treatment of infections. Because antimicrobial resistance is constantly evolving, knowledge of antimicrobial resistance trends among bacteria is critical to guide therapeutic decisions and develop updated control strategies.

A previous study in Medellin (Colombia) showed that multidrug resistance is commonly present in bacteria isolated from animal infections in companion animals (Gómez-Beltrán et al., 2020). Most studies in different countries investigate trends and/or patterns in resistance by focusing on a specific pathogenic bacterium (i.e., *Escherichia coli*) or a specific organ/system (i.e., urinary tract infections). However, few studies from veterinary diagnostic laboratories have provided information on antimicrobial resistance patterns in bacteria isolated from clinical samples submitted over the course of 10-20 years (Authier et al., 2006; Awosile et al., 2018; Lord et al., 2022). The data from our earlier study (Gómez-Beltrán et al., 2020) served as a baseline measurement for future surveillance.

Therefore, the objective of this study was to compare changes in the antimicrobial resistance profile between two consecutive 4-year periods (2016-2019 and 2020-August 2023) in the same area.

Materials and Methods

Clinical samples submitted for culture and susceptibility testing from dogs between 2020 and August 2023 were retrieved from the Animal Microbiology Laboratory database at the

Faculty of Agrarian Sciences of the Universidad de Antioquia (Colombia). The total number of complete records for dogs was 1146.

Blood agar plates were incubated with 5% CO2 while MacConkey agar plates were incubated aerobically. All samples were incubated at 37°C for 18 to 24 h until adequate growth was present. Identification was based on colony type and morphology, Gram staining characteristics, and standard biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method (Biemer, 1973). Zones of growth inhibition were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2018). Intermediate isolates were infrequent and regarded as resistant. The Enterobacteriaceae group included the genera *Enterobacter*, *Klebsiella, Citrobacter, Proteus, Salmonella*, and *Serratia*. *Escherichia coli* was considered separately from the *Enterobacteriaceae*. Within the *Pseudomonas* group, *Pseudomonas, Flavimonas*, and *Acinetobacter* were included. The antimicrobials used to determine susceptibilities varied according to specific requests from veterinarians, but typically included amikacin, amoxicillin-clavulanate, ampicillin, cephalothin, cephalosporin, enrofloxacin, gentamicin, trimethoprim-sulfadiazine, doxycycline, tetracycline, ciprofloxacin, and florfenicol. Isolates showing resistance to three or more antimicrobial classes were classified as multidrug-resistant (MDR) as defined by Magiorakos et al., (2012), as per the joint guidelines of the European Centre for Disease Prevention and Control and the United States Centers for Disease Control and Prevention.

Clinical sample submissions and antimicrobial susceptibilities are presented as proportions with their respective confidence intervals. The frequency of antimicrobial resistance was considered as follows: rare: 1–10%; moderate: >10–20%; high: >20–50%; very high: >50–70%; extremely high: >70%; according to the European Food Safety Authority and the European Centre for Disease Prevention and Control (EFSA and ECDC, 2015). Data were tabulated using Microsoft Excel® 2019 and are presented as percentages with their respective 95% confidence intervals.

To evaluate changes in antimicrobial resistance over time, the results of this study were compared with those of an earlier study (Gómez-Beltrán et al, 2020) that reported culture and susceptibility results from 2016 to 2019 using the same standard laboratory operating procedures. Overall MDR prevalence and individual antimicrobial resistance rates for the major bacterial isolates were compared between both periods. Due to the limited number of samples received annually, analyzing year-to-year trends was not possible.

Statistical analysis was performed using the exact x^2 test (SPSS Statistics, version 21) with an alpha value of 0.05. A one-tailed test was used to determine whether there was a difference between the two periods in the specific direction predicted. Significant susceptibility variations were classified based on the p-value.

Results

A total of 1,146 samples were submitted between 2020 and August 2023 for culture and susceptibility testing, from which 805 (70.2%) isolates could be recovered. Of those 805 isolates, susceptibility tests were performed on 793 samples. Samples from ears (n = 335), skin/wounds (n = 127), and urine (n = 192) represented most of the samples collected during the study period (Table 1).

The most frequent bacterium isolated from clinical samples was *Staphylococcus* coagulase-positive found in ears (61.5%), skin (55.4.1%), eyes (50%), and abscesses (44.2%). The largest group of bacteria belonged to *Staphylococcus* spp. with 383 isolates distributed as follows: *Staphylococcus pseudintermedius* (n= 279), *Staphylococcus aureus* (n=60) and *Staphylococcus* coagulase-negative (n=40). The exception was *E. coli*, which was the most common species in urine samples (92/192; 47.9%). When ear and skin infections were combined, bacterial and/or fungal growth was detected in 462 samples (Figure 1). The number of mixed infections with *Malassezia* spp. was 136, while pure isolates of *Malassezia* spp. totaled 77, and pure isolates of *Staphylococcus* spp. totaled 247.

Antimicrobial susceptibilities for the 799 bacterial isolates are presented in Table 2. *Staphylococcus* spp. (n = 383) accounted for the most common tested group, followed by *E. coli* (n = 154), *Enterobacteriaceae* (n = 128), and *Enterococcus* spp. (n = 61). Within the *Staphylococcus* group, the most frequently isolated species was *S. pseudointermedius* (n = 279), which exhibited low resistance to amikacin (<10%); moderate resistance (10-20%) to amoxicillin-clavulanate, cephalexin, cefovecin, and enrofloxacin; high resistance (20-50%) to ampicillin, gentamicin, trimethoprim-sulfadiazine, and clindamycin; and very high resistance (50-70%) to doxycycline. A very similar pattern was observed for *S. aureus* and coagulase-negative *Staphylococcus* (CoNS), although the number of isolates was much lower, limiting the reliability of the sensitivity profile.

Table 1. Bacterial isolates from clinical samples of dogs analyzed at the Animal Microbiology Laboratory, University of Antioquia (2020–August 2023).

Matrix	N	*Enterobacteriaceae*[a] % (95%CI)	*Enterococcus* spp.[b] % (95%CI)	*Escherichia coli* % (95%CI)	*Pseudomonas*[c] % (95%CI)	*Staphylococcus* coagulase negative[d] % (95%CI)	*Staphylococcus* coagulase positive[e] % (95%CI)	*Streptococcus* spp. % (95%CI)	Others[f] % (95%CI)
Ear	335	8.4 (6.9-9.8)	8.7 (7.2-10.1)	5.1 (3.9-6.2)	6.9 (5.5-8.2)	7.8 (6.4-9.2)	61.3 (58.9-64)	0.9 (0.4-1.4)	0.9 (0.4-1.4)
Wound	15	20.0 (10.1-29.9)	13.3 (4.9-21.8)	26.7 (15.7-37.5)	0.0	6.7 (0.5-12.8)	20.0 (10.1-29.9)	0.0	13.3 (4.9-21.8)
Urine	192	26.6 (23.5-29.6)	6.3 (4.6-7.9)	47.9 (44.5-51.4)	1.6 (0.7-2.4)	0.5 (0.0-1.0)	15.0 (12.6-17.6)	1.6 (0.7-2.4)	0.5 (0.0-1.0)
Skin	112	15.2 (11.9-18.4)	6.3 (4.1-8.4)	7.1 (4.8-9.5)	5.4 (3.3-7.4)	8.9 (6.3-11.5)	55.4 (50.8-59.9)	0.0	1.8 (0.6-3.0)
Nasal cavity	18	44.4 (33.2-55.7)	11.1 (4.0-18.2)	11.0 (4.0-18.2)	5.6 (0.4-10.7)	3.3 (0.2-6.5)	36.7 (28.2-45.1)	0.0	27.8 (17.6-37.9)
Abscess	52	11.5 (7.3-15.8)	5.8 (2.7-8.9)	9.6 (5.7-13.5)	9.6 (5.7-13.5)	5.8 (2.7-8.9)	44.3 (37.6-50.8)	9.6 (5.7-13.5)	3.8 (1.3-6.4)
Eyes	8	37.5 (21.1-53.9)	0.0	12.5 (1.3-23.7)	0.0	0.0	50.0 (33,0-67,0)	0.0	0.0
Surgical	28	14.3 (7.9-20.6)	3.6 (0.2-6.9)	32.2 (23.7-40.6)	0.0	7.1 (2.5-11.8)	14,3 (7.9-20.6)	7.1 (2.5-11.8)	21.4 (14.0-28.6)
Fecal	17	23.5 (13.7-33.4)	0.0	76.5 (66.6-86.3)	0.0	0.0	0.0	0.0	0.0
Vaginal discharge	16	25.0 (14.6-35.4)	31.3 (20.1-42.4)	18.8 (9.4-28.1)	0.0	0.0	18.8 (9.4-28.1)	0.0	6.1 (0.4-12.1)

[a] *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Proteus* spp., *Serratia* spp., *Shigella* spp., *Yersinia* spp., *Salmonella* spp.
[b] *Enterococcus* spp., *Enterococcus* faecalis.
[c] *Pseudomonas* spp., *Flavimonas* spp., *Acinetobacter* spp.
[d] *Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus haemolyticus.*
[e] *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus pseudointermedius.*
[f] *Corynebacterium* spp., *Gardnerella vaginalis, Stenotrophomonas maltophilia, Morganella morganii, Gemella palaticanis, Chromobacterium violaceum, Sphingomonas paucimobilis, Pasteurella multocida.*

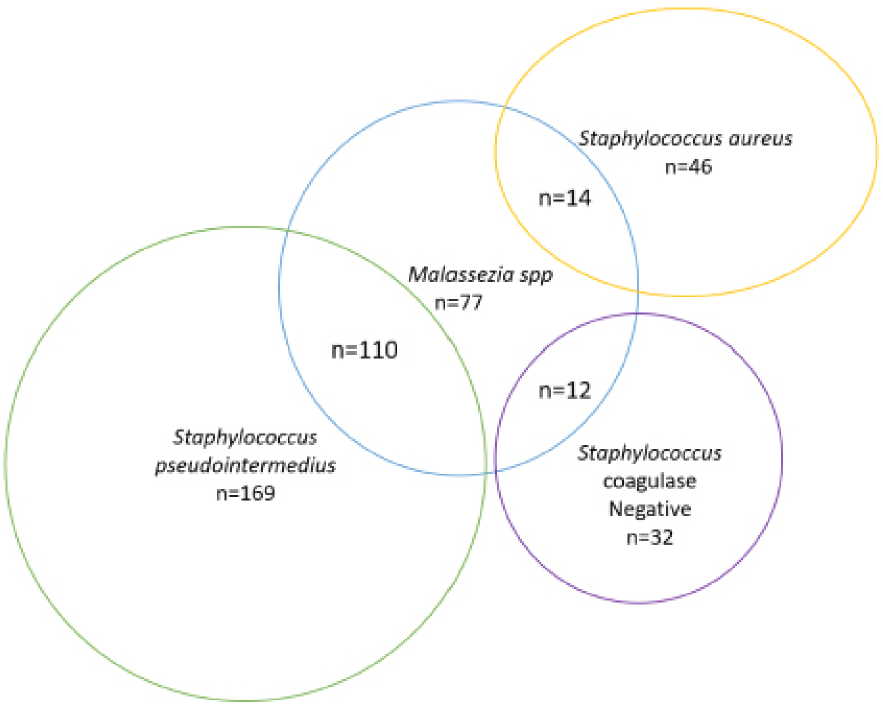

Figure 1. Number of infections caused by *Malassezia* spp. and *Staphylococcus* spp. identified in skin and ear samples (n=460) of dogs between 2020 and August 2023.

Table 2. Antibacterial susceptibilities profiles of bacteria isolated from clinical samples from dogs (2020–August 2023).

Bacteria	% of susceptibility																
	n	AMK	AMC	AMP	CEX	ENO	GEN	TMS	DOX	TET	PEN	CET	CEF	CHL	ERY	CEFO	CLY
Enterobacteriaceae	128	94.3	73.4	45.7	56	79.3	91	75.6	47.7	54.5	-	-	-	-	-	-	
Enterococcus spp.	61	-	69.2	76.6	-	-	-	-	88.9	47.8	88.1	-	-	88.9	43.4	-	
Escherichia coli	154	97	53.1	58	75.7	78.7	92.2	78.1	76.2	65	-	73.7	56.4	62.5		-	
Others*	22	83.3	73.3	85.7	-	68.8	100	-	-	100	-	-	-	-	-	-	
Pseudomona spp.	38	94.6	-	-	-	58.3	92.1	-	-	-	-	-	-	50	-	-	

Staphylococcus coagulase negative	44	88.9	86.8	60	65.7	79.1	76.7	63.6	50	58.3	-	-	-	-	-	46.2	50
Staphylococcus aureus	60	89.6	91.8	76.9	79.5	77.6	80.6	55	38.9	51.6	-	-	-	-	-	61.1	63.2
Staphylococcus pseudointermedius	279	97.9	89.2	75.2	88.6	81.6	52.5	50	43.4	-	-	-	-		-	84.1	60.9
Streptococcus spp.	13	54.5	100	77.8	-	50	-	-	-	30	-	-	-	-	-	87.5	-

AMK: Amikacin; AMC: Amoxicillin-clavulanate; AMP: Ampicillin; CEX: Cephalexin; ENO: Enrofloxacin; GEN: Gentamicin; TMS: Trimethoprim-sulfadiazine; DOX: doxycyclin; TET: Tetracycline; PEN: Penicillin; CET: ceftiofur; CEF: Cephalothin; CHL: Chloramphenicol; ERY: Erythromycin; CEFO: cefovecin; CLY: clindamycin; MDR: Multidrug resistant. (-) not determined. *Others: *Corynebacterium* spp., *Gardnerella vaginalis, Stenotrophomonas maltophilia, Morganella morganii, Gemella palaticanis, Chromobacterium violaceum, Sphingomonas paucimobilis, Pasteurella multocida*.
Interpretation of colors: DARK BLUE: 0.1–1% very low resistance, BLUE: >1–10% low resistance, PURPLE: >10–20% moderate resistance, RED: >20–50% high resistance, Light Green: >50–70% very high resistance, Dark Green: >70% extremely high resistance

For the *Enterobacteriaceae* group, there were low levels of resistance (<10%) to amikacin and gentamicin; high resistance (20-50%) to amoxicillin-clavulanate, cephalexin, enrofloxacin, trimethoprim-sulfadiazine, and tetracycline; and very high resistance (50-70%) to ampicillin and doxycycline (Table 2).

Escherichia coli was the organism for which more antimicrobials were tested using the disk diffusion method. Resistance was also high (20–50%) to 10 antimicrobials (amoxicillin-clavulanate, ampicillin, cephalexin, enrofloxacin, trimethoprim-sulfadiazine, doxycycline, tetracycline, ceftiofur, cephalothin, and chloramphenicol), and low (<10%) to two antimicrobials (amikacin and gentamicin). For other bacterial groups, the number of isolates was insufficient to establish a susceptibility pattern.

Multidrug resistance was observed in 19.7% (150/761) of isolates, ranging from 11.5% to 34.1% across different bacterial groups. The degree of MDR in different bacteria during this study (2020-August 2023) was compared with a previous one (2016-2019; Gómez-Beltrán et al., 2020) that followed similar standard procedures (Table 3). A slight, but not significant, increase in MDR was observed among the main type of bacteria isolated during this study period: *E. coli* (17.2 vs 18.8%), *S. aureus* (19.2 vs 20.0%), and *S. pseudointermedius* (16.7 vs 18.3%).

When individual antimicrobials were compared by date and bacterial species (Table 4), significant susceptibility declines were noted more frequently than susceptibility increases (28 vs. 20). For example, *E. coli* susceptibility decreased for amikacin (100-97%; p<0.01), amoxicillin-clavulanate (76.7-53.1%; p<0.01), and ampicillin (67.7-58.0%; p<0.05). However, for other antimicrobials, such as enrofloxacin and doxycycline, there was a slight increase in susceptibility from the first study to the present one (Table 4). For *S. pseudointermedius*, a reduction in susceptibility was observed for gentamicin (63.9-52.5%; p<0.01), trimethoprim-sulfadiazine (57-52.5%; p<0.05) and doxycycline (60.9-43.4%; p<0.01), but no major changes in susceptibility were observed for other antimicrobials (Table 4). Of particular interest was the overall increase in resistance to amoxicillin-clavulanate among all bacterial groups, ranging from 3.3% in *Enterobacteriaceae* to 20.5% in *Enterococcus* spp.

Table 3. Multidrug resistance (MDR) in bacterial groups isolated at the Animal Microbiology Laboratory, University of Antioquia, during 2016-2019 and 2020-August 2023.

Bacteria	MDR 2016-2019[a] % (n)	MDR 2020-2023 % (n)	p-value
Enterobacteriaceae	18.6 (183)	23,4 (128)	0.1485
Enterococcus spp.	20.0 (90)	11,5 (61)	0.0833
Escherichia coli	17.2 (163)	18,8 (154)	0.3508
Others	7.1 (14)	22,7 (22)	0.1106
Pseudomona spp.	49.4 (79)	Not determined (38)[b]	Not determined
Staphylococcus coagulase negative	10.9 (101)	34,1 (44)	0.0004
Staphylococcus aureus	19.2 (104)	20,0 (60)	0.4523
Staphylococcus pseudointermedius	16.7 (406)	18,3 (279)	0.3017
Streptococcus spp.	12.5 (16)	30,8 (13)	0.1136
Total	18.3 (1,156)	19.7 (761)	0.2264

MDR: Multidrug resistance. [a]Data from the period 2016-2019 were retrieved from Gómez-Beltrán et al. (2020). [b]*Pseudomonas* was not included in the total number of bacteria because MDR was not assessed.

Table 4. Antimicrobial susceptibility of selected bacteria isolated from dog samples between 2016-2019 and 2020-August 2023.

	Enterobacteriaceae		***Escherichia coli***		***Sthaphylococcus pseudointermedius***	
	2016-2019 n = 183	2020-2023 n = 128	2016-2019 n = 163	2020-2023 n = 154	2016-2019 n = 406	2020-2023 n =279
Amikacin	183/183	120/128	163/163	149/154	393/406	273/279
	100%	94.3%*	100%	97.0%*	96.80%	97.90%
Amoxicillin-clavulanate	140/183	94/128	109/163	82/154	379/406	249/279
	76.70%	73.40%	76.70%	53.1%**	93.30%	89.2%*
Ampicillin	113/183	58/128	110/163	89/154	315/406	279/279
	61.80%	45.70%*	67.70%	58.0%*	77.60%	75.20%
Cephalexin	111/183	72/128	118/163	117/154	331/406	247/279
	60.90%	56.00%	72.30%	75.70%	81.60%	88.6%**
Doxycycline	71/183	61/128	112/163	117/154	247/406	121/279
	38.70%	47.7%*	68.70%	76.20%	60.90%	43.4%**
Enrofloxacin	118/183	101/128	113/163	121/154	334/406	228/279
	64.40%	79.3%**	69.20%	78.7*	82.30%	81.60%
Gentamicin	163/183	116/128	146/163	142/154	259/406	146/279
	89%	91.00%	89.80%	92.2	63.90%	52.5%**

Tetracycline	96/183 52.20%	70/128 54.6%	105/163 64.50%	100/154 65.00%	255/406 62.80%	84/279 30.0%**
Trimethoprim-sulfadiazine	154/183 83.90%	97/128 75.6%*	126/163 77.60%	120/154 78.10%	231/406 57.00%	139/279 50.0%*

Statistically significant differences between 2016-2019 and 2020–August 2023: ($p<0.05$)*, ($p<0.01$)**.

Discussion

This is the second study to describe the prevalence of bacterial pathogens isolated from clinical samples submitted for culture and susceptibility testing from dogs in the area served by the veterinary diagnostic laboratory at the University of Antioquia in Medellin (Colombia). The first study covered a similar four-year period between 2016 and 2019 (Gómez-Beltrán et al., 2020) and showed that the level of resistance among all bacterial families studied was high (20-50%) to at least six or more antimicrobials. Although the results are not fully comparable with the present study because many antimicrobials previously used had been replaced, a similar pattern of high resistance was observed in 70-80% of the antimicrobials tested *in vitro* against most bacteria. When AMR changes between both periods were compared, more bacteria developed resistance to antimicrobials than those that showed no change or even an increase in susceptibility. This resulted in a slight increase of 1.4% in MDR levels between studies, with levels ranging from 11 to 34% among different bacterial families.

Staphylococcus spp. was the dominant bacterial group isolated from various sample sources, including skin, wounds, ears, abscesses, and eyes. This is not surprising as *Staphylococcus* spp. are part of the normal flora of the skin and mucosae and are known to cause clinical diseases such as pyoderma, as well as surgical and wound infections. Consistent with other studies, coagulase-positive *Staphylococcus* (CoPS), particularly *S. pseudointermedius,* was the most common organism isolated (Penna et al., 2009; Ludwig et al., 2016; Conner et al, 2018; Lee et al., 2019). This was followed by *S. aureus* and coagulase-negative *Staphylococcus* (CoNS), the latter mainly identified as *Staphylococcus schleiferi* (Lord et al., 2022). In one of the largest recent retrospective studies analyzing 4,972 *Staphylococcus* isolates from dogs in a diagnostic laboratory, *S. pseudointermedius* was included within the *S. intermedius* group and still accounted for the largest proportion (68%; 3388/4972) of *Staphylococcus* spp. infections (Conner et al., 2018). In their study, CoNS were the second largest group with 18.3% (907/4,972), while *S. aureus* accounted for only 5.8% (290/4,972) of the isolates. Studies characterizing staphylococcal population structure and antimicrobial resistance profiles in healthy dogs and cats have shown that CoNS (with up to 22 different *Staphylococcus* species), rather than CoPS, dominate the healthy skin and mucosal microbiota of dogs and cats (Gandolfi et al., 2013; Schmidt et al., 2014). However, CoPS tends to predominate over CoNS in infectious conditions. For example, a large retrospective study in France analyzing 7,623 dog otitis cases (2012 -2016) found that *S. pseudointermedius* was the predominant species (33%), while all other *Staphylococcus* spp. combined accounted for only 4.3% (Bourély et al., 2019). In our study, MDR rates were higher in CoPS isolates (34%) compared with CoNS (18-20%). CoPS also exhibited the highest increase in resistance to most antimicrobials, reaching 50% resistance for doxycycline. As reported in our previous study, only amikacin and amoxicillin-clavulanate met the low-resistance criteria (<10%) for empirical treatment of *Staphylococcus* spp., despite increasing resistance to both antimicrobials during the second period. Unfortunately, ciprofloxacin and cefoperazone, which had previously shown 100% susceptibility against *S. pseudointermedius* in the first study period, were not tested in the present study.

Malassezia spp. (formerly *Pityrosporum* spp.) was diagnosed via cytological examination alone and in combination with *Staphylococcus* spp. Although it is a commensal organism on the skin and in the ear canals of healthy dogs, it can contribute to dermatological diseases in predisposed individuals. The two organisms mutually benefit from environmental factors, leading to increased numbers of *Staphylococcus* in conjunction with *Malassezia* (Mauldin et al., 1997; Ben Sala et al., 2010). In fact, 40% of dogs with *Malassezia* overgrowth are also diagnosed with *Staphylococcus* pyoderma due to this synergistic relationship (Guaguère and Prelaud, 1996). Known factors that may predispose *Malassezia* spp. to become pathogenic, rather than remain commensal, include increased humidity, skin folds, endocrine diseases, keratinization disorders, hypersensitivity diseases (i.e., atopy, flea allergies, cutaneous food reactions), and an increased number of symbiotic *Staphylococci* (Guillot and Bond, 2020).

Among the Gram-negative isolates, *Enterobacteriaceae* and *E. coli* were the most common urinary tract pathogens, representing 26.6 and 47.9% of the isolates, respectively. These dominant urinary bacterial isolates align with reports from Canada (Authier et al., 2006; Awosile et al., 2018), the United States (Thungrat et al., 2013), and our previous study (Gómez-Beltrán et al., 2020). Our results showed that only amikacin and gentamicin retained sufficient efficacy (>90%) for empirical therapy against both bacterial groups. Although susceptibility trends fluctuated—with both gains and losses for various antimicrobials—the resistance level to all other antimicrobials (except amikacin and gentamicin) remained high or very high, making them unsuitable for empirical use. None of the β-lactams tested (amoxicillin-clavulanate, ampicillin, cephalexin, cephalothin, ceftiofur), tetracyclines (doxycycline, tetracycline), or trimethoprim-sulfadiazine could be recommended due to their high (20–50%) resistance rates. Once again, florfenicol, which had shown 100% susceptibility against both *Enterobacteriaceae* and *E. coli* in the first study, was not included in the present analysis. The International Society for Companion Animal Infectious Disease (ISCAID) has formulated guidelines recommending first-line antimicrobials for uncomplicated urinary tract infections, including amoxicillin and trimethoprim-sulfonamide (Weese et al., 2019). However, in our study, neither amoxicillin nor trimethoprim-sulfonamide can be recommended as first-line treatment options for urinary tract disease.

Of particular interest was the increasing trend of resistance to amoxicillin-clavulanate and ampicillin among all bacterial groups. As these are among the most prescribed antimicrobials for companion animals, it is likely that selection pressure has driven this increase in resistance (Lord et al., 2022). Similar declines in efficacy over time have been reported for these bacteria in other countries, warranting continuous monitoring (Awosile et al., 2018; Lord et al., 2022). The ISCAID suggests that a 10% increase in resistance from baseline within a population is a reasonable threshold for modifying empirical antimicrobial selection (Weese et al., 2019). For amoxicillin-clavulanate and ampicillin, this threshold was exceeded in three bacterial groups: Enterococcus spp., E. coli, and CoNS.

Study Limitations

Some of the limitations of this study included the relatively small number of isolates for certain bacterial species, resulting in lower precision for each analysis. Additionally, we were unable to investigate associations between prior antimicrobial use and resistance patterns. It is likely that most of the animals included in this study had already failed empirical treatments and were undergoing second or third evaluations, rather than being first-time infection cases. Furthermore, the lack of historical data prevented us from determining whether the isolates represented part of the normal microbiota or were contaminants, rather than the true pathogenic agents responsible for the disease. Ideally, monitoring antimicrobial resistance over time should be conducted on an annual basis to properly track resistance trends. However, this was not feasible in the present study due to the limited number of samples tested per year.

Conclusion

This study provides information on antimicrobial susceptibility patterns that can assist clinicians, particularly in Medellín, in making rational decisions regarding the use of antimicrobials in dogs. We propose that the samples submitted to our laboratory represent clinical cases of dogs within the city. Monitoring bacterial resistance patterns to antimicrobial drugs requires constant vigilance. It is likely that antimicrobial overuse in veterinary practice, previously reported in our surveys on prescribing practices (Gómez-Beltrán et al., 2021) has contributed to selection pressure. Antimicrobials showing increasing resistance trends, such as amoxicillin-clavulanate, which previously had a high proportion of susceptible isolates, should be monitored closely in the future. Antimicrobial stewardship strategies and programs are urgently needed in Colombian companion animal practices.

Declarations

Conflicts of interest

The authors declare no conflicts of interest

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Author Contributions

M.I.G.A., D.V., and D.A.G.-B. conducted the interviews. D.V., D.A.G.-B., S.L.O., and J.J.C.G were responsible for methodology and statistical analysis. D.V. wrote and prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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