ORIGINAL RESEARCH ARTICLE

Comparison of antimicrobial resistance in bacterial isolates from dogs in a veterinary diagnostic laboratory in Colombia, between two consecutive 4-year periods

Comparación de resistencia antimicrobiana en aislamientos bacterianos de perros en un laboratorio de diagnóstico veterinario de Colombia, entre dos períodos consecutivos de 4 años

Comparação da resistência antimicrobiana em isolados bacterianos de cães em um laboratório de diagnóstico veterinário na Colômbia, entre dois períodos consecutivos de 4 anos

María Isabel García-Álvarez[1], David Villar[1], Sara López-Osorio[1], David A Gómez-Beltrán[2], Jenny J Chaparro-Gutiérrez[1]*.

[1]*Grupo de Investigación Centro de Investigaciones Básicas y Aplicadas en Veterinaria (CIBAV), Universidad de Antioquia.*
[2]*Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Colombia.*

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[1] **Corresponding author:** Jenny J Chaparro-Gutiérrez. Grupo de Investigación CIBAV, Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia, AA1226, Calle 70 No. 52-21, Medellín, Colombia. E-mail: jenny.chaparro@udea.edu.co; Tel.: +573138143231.

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Abstract

Background: Antimicrobial resistance (AMR) and the increase in multiresistant 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 dog isolates in two consecutive 4-year periods. **Methods:** Animal Microbiology Laboratory database at the University of Antioquia (Medellín, Colombia) was searched for routine dog submissions for which culture and antibiograms were performed. **Results:** A total of samples 1,146 were submitted between 2020 and August 2023 for culture and sensitivities from which 805 (70.2%) isolates could be recovered. Of those 805 isolates, sensitivities were performed in 799 samples. A significant decrease between 2016-2019 and 2020-August 2023 was noted in the susceptibility of dog isolates to 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 for *E. coli* to enrofloxacin (69.2-78.7%; $p<0.05$) and doxycycline (68.7-76.2%; $p=0.0745$) and *Enterobacteriaceae* to enrofloxacin (64.4-79.3%; $p<0.01$), and doxycycline (38.7-47.7%; $p=0.06$). For all types of bacteria there was an increased resistance pattern against 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 families of bacteria, the number of antimicrobials for which resistance was high (20-50%), or very high (50-70%) 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). This was attributed to a significant susceptibility reduction rather than susceptibility increases (28 *vs.* 20). **Conclusions:** High rates of resistance indicate continued surveillance and use of antibiograms is needed to guide clinical decisions.

Keywords: *antibiogram; antimicrobial resistance*; *dogs*; *E. coli*; *multidrug 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:** Se consultó la base de datos del Laboratorio de Microbiología Animal de la Universidad de Antioquia (Medellín, Colombia) en busca de envíos rutinarios de muestras clínicas de perros para los 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 senisibilidad en 799 muestras. Se observó una disminución significativa entre 2016-2019 y 2020-agosto 2023 en la susceptibilidad de las cepas 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 susceptibilidades significativamente mayores para *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 patrón de resistencia aumentado 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 cuya resistencia fue 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 una resistencia *in vitro* baja (<10%). El grado de multirresistencia aumentó ligeramente para el período 2020-agosto 2023 (19,7%; 150/761 aislamientos) en comparación con el período 2019-2020 (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). **Conclusiones**: Las altas tasas de resistencia indican que se necesita vigilancia continua y el uso de antibiogramas para guiar las decisiones clínicas.

Palabras clave: *antibiograma; E. coli; perros; resistencia a múltiples fármacos; resistencia antimicrobiana; 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 de la 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 2019-2020 (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: *antibiograma; cães; E. coli*; *resistência a múltiplas drogas; resistência antimicrobiana; Staphylococcus spp.; suscetibilidade.*

Introduction

In small animal practice, the choice of an antimicrobial treatment is often made empirically when a treatment needs to be initiated before the test results of cultures of susceptibility 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 possible resistance to antimicrobials is important.

Antimicrobial resistance among bacteria isolated from companion animals is an emerging problem as it narrows 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 up-to-date control strategies. A previous study in the city of 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 provided in our earlier study (Gómez-Beltrán *et al*., 2020) provided a baseline measurement for future surveillance, and so, the objective of this study was to compare changes in the antimicrobial resistance profile between two consecutive periods of 4 years (2016-2019 and 2020-August 2023) in the same area.

Materials and Methods

Clinical samples submitted for culture and susceptibility testing from dogs from 2020 to August 2023 were retrieved from the database of the Animal Microbiology Laboratory of the Faculty of Agrarian Sciences at the Universidad de Antioquia (Colombia). The total number of complete records in dogs was 1146. Blood agar plates were incubated with 5% CO_2 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. Antimicrobiological susceptibility was undertaken 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. Genera *Enterobacter, Klebsiella, Citrobacter, Proteus, Salmonella,* and *Serratia* were included within the *Enterobacteriaceae* group. *Escherichia coli* was considered separate from the *Enterobacteriaceae* group within the *Pseudomonas* group, *Pseudomonas, Flavimonas,* and *Acinetobacter* were included. The antimicrobials used to determine susceptibilities varied depending on specific requests by the veterinarian, 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), apud the joint guidelines of the European Centre for Disease Prevention and Control and the Center for Disease Control and Prevention of the USA. The clinical samples submitted, and antimicrobial susceptibilities were 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, 2015). Data were tabulated using a spreadsheet (Microsoft Excel® 2019) and are presented as percentages with respective 95% confidence intervals.

To evaluate changes in antimicrobial resistance overtime, the results of this study were compared with an earlier one (Gómez-Beltrán *et al*, 2020) that, using the same standard laboratory operating procedures, reported culture and sensitivities between 2016 and 2019. Overall multidrug sensitivities and individual antimicrobial resistance for the main type of bacteria isolated were used for comparison between both periods. Due to the limited number of samples received on a year basis, looking at year-to-year trends was not possible. Statistical analysis was performed by using the exact χ^2test (SPSS Statistics, version 21) with an alpha value of 0.05. A one-tailed test was used to determine if there was a difference between the two periods in the specific direction that we predicted. Significant susceptibility variations were classified according to the p-value.

Results

A total of samples 1,146 were submitted between 2020 and August 2023 for culture and sensitivities from which 805 (70.2%) isolates could be recovered. Of those 805 isolates, sensitivities were performed in 793 samples. Samples from ears (n = 335), skin/wounds (n = 127), and urine (n = 192) represented most of the samples over the study period (Table 1). The most frequent bacterium isolated from clinical samples were *Staphylococcus* coagulase-positive starting from ears (61.5%), skin (55.4.1%), eyes (50%), and abscesses (44.2%). The largest group of bacteria was represented by *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*, the species most common in urine samples (92/192; 47.9%). When ear and skin infections were combined, there was bacterial and/or fungal growth in 462 samples (Figure 1). The number of mixed infections with *Malassezia* spp. was 136 of pure *Malassezia* spp. was 77, and of pure *Staphylococcus* spp. was 247.

Antimicrobial susceptibilities for the 799 bacteria 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 *S. pseudointermedius* (n=279), 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 numbers of isolates were much lower to establish a reliable sensitivity profile.

232 **Table 1.** Bacterial isolates from clinical samples of dogs submitted to the Animal Microbiology Laboratory at the University of Antioquia
233 (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*

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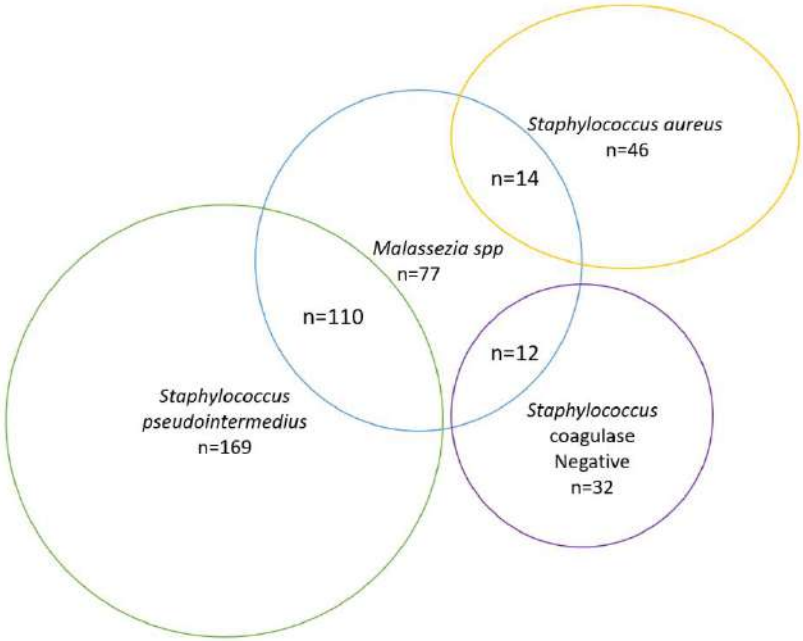

Figure 1. Number of infections by *Malassezia* spp. and *Staphylococcus* spp. isolated from skin and ear samples (n=460) in dogs between 2020 and August 2023.

252 **Table 2.** Antibacterial susceptibilities in bacteria isolated from clinical samples of 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

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For the *Enterobacteriaceae* group, there were low levels of resistance (<10%) to amikacin and gentamycin; 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 against which more antimicrobials were tested with the disk diffusion method. Resistance was also high (20–50%) for 10 antimicrobials (amoxicillin-clavulanate, ampicillin, cephalexin, enrofloxacin, trimethoprim-sulfadiazine, doxycycline, tetracycline, ceftiofur, cephalothin, and chloramphenicol), and low (<10%) against 2 antimicrobials (amikacin and gentamicin). For other groups of bacteria there were insufficient numbers to establish a susceptibility pattern.

Multidrug resistance was observed in 19.7% (150/761) of the dog isolates, ranging between 11.5% and 34.1% in different groups of bacteria. The degree of MDR by different bacteria in this study (2020-August 2023) was compared to a previous one (2016-2019; Gómez-Beltrán *et al*., 2020) using similar standard operating procedures (Table 3). A slight, but not significant, increase of MDR was observed for the main type of bacteria isolated in 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 between dates and type of bacteria (Table 4), significant susceptibility reductions rather than susceptibility increases were noted more frequently (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 gain in susceptibility from the first study to the present one (Table 4). For *S. pseudointermedius*, a reduced susceptibility resistance profile 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 increased resistance pattern by all types of bacteria against amoxicillin-clavulanate, ranging from 3.3% for *Enterobacteriaceae* to 20.5% for *Enterococcus* spp.

Table 3. Multidrug resistance (MDR) for groups of bacteria isolated at the Animal Microbiology Laboratory at the University of Antioquia between the periods 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 resistant. [a]Data for the period 2016-2019 was retrieved from Gómez-Beltrán *et al.* (2020).
[b]*Pseudomonas* was not included in the total number of bacteria because MDR was not determined.

Table 4. Antimicrobial sensitivity of selected bacteria isolated from dogs 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	183/183 100%	120/128 94.3%*	163/163 100%	149/154 97.0%*	393/406 96.80%	273/279 97.90%
Amoxicillin-clavulanate	140/183 76.70%	94/128 73.40%	109/163 76.70%	82/154 53.1%**	379/406 93.30%	249/279 89.2%*
Ampicillin	113/183 61.80%	58/128 45.70%*	110/163 67.70%	89/154 58.0%*	315/406 77.60%	279/279 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 38.70%	61/128 47.7%*	112/163 68.70%	117/154 76.20%	247/406 60.90%	121/279 43.4%**
Enrofloxacin	118/183 64.40%	101/128 79.3%**	113/163 69.20%	121/154 78.7*	334/406 82.30%	228/279 81.60%
Gentamicin	163/183 89%	116/128 91.00%	146/163 89.80%	142/154 92.2	259/406 63.90%	146/279 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 of the Universidad de Antioquia in the city of Medellin (Colombia). The first study was conducted for a similar period of 4-years between 2016 and 2019 (Gómez-Beltrán *et al.,* 2020) and showed that the level of resistance in all families of bacteria studied was high (20-50%) to at least six or more antimicrobials. Although the results are not entirely comparable to the present one because many antimicrobials used previously had been replaced, a similar pattern of high resistance was observed to 70-80% of the antimicrobials used *in vitro* by most bacteria. When changes in AMR between both periods were compared, there were more bacteria that gained resistance to antimicrobials than those for which there was no change or even increased in susceptibility. This translated in a slight increase of 1.4% in the level of MDR between studies, with levels that ranged from 11 to 34% in different families of bacteria.

Staphylococcus spp. were the dominant bacteria isolated from several sample sources, including skin, wounds, ears, abscesses, and eyes. This is not surprising as *Staphylococcus* spp. are normal flora of the integument and mucosae and are known to cause clinical diseases

311 such are pyoderma, and surgical and wound infections. Consistent with other studies,
312 *Staphylococcus* coagulase-positive (CoPS), and S. *pseudointermedius*, was the most common
313 organism isolated (Penna *et al*., 2009; Ludwig *et al*., 2016; Conner *et al*, 2018; Lee *et al*.,
314 2019). This was followed by *S. aureus* and coagulase-negative *Staphylococcus* (CoNS), the
315 latter of which have been identified mostly as *Staphylococcus schleiferi* (Lord *et al*., 2022).
316 In one of the largest recent retrospective studies of 4,972 *Staphylococcus* isolates in dogs in
317 a diagnostic laboratory, *S. pseudointermedius* was included within the *S. intermedius* group
318 and still accounted for the largest group (68%; 3388/4972) among the *Staphylococcus* spp.
319 infections (Conner *et al*., 2018). In their study, CoNS were the second largest group with
320 18.3% (907/4,972), and *S. aureus* was the third group with only 5.8% (290/4,972) of the
321 isolates. Studies that have characterized the staphylococcal population structure and
322 antimicrobial resistance profile in healthy dogs and cats have shown that it is the CoNS (with
323 up to 22 different *Staphylococcus* species), and not CoPS, that dominate the healthy skin and
324 mucosal surfaces of dogs and cats (Gandolfi *et al*., 2013; Schmidt *et al*., 2014). However,
325 CoPS tends to predominate over CoNS when there are infections. For example, a large
326 retrospective study in France that compiled 7,623 cases of dogs with otitis from 2012 to 2016
327 found a prevalence of *S. pseudointermedius* of 33% compared to a prevalence of 4.3% for all
328 other *Staphylococcus* spp. combined (Bourély *et al*., 2019). In our study, MDR was higher
329 for the CoPN isolates (34%) compared to the CoNS (18-20%). It was also the type of bacteria
330 that showed that highest increase in resistance to most antimicrobials, reaching 50% for
331 doxycycline. As it was observed in the previous study by our group, only amikacin and
332 amoxicillin-clavulanate met the criteria of low resistance (<10%) for empirical treatment of
333 *Staphylococcus* spp., despite increased resistance to both antimicrobials for the second
334 period. Unfortunately, neither ciprofloxacin nor cefoperazone, that attained a 100%
335 susceptibility against *S. pseudointermedius* in the first study period, were used in the present
336 one.

337 *Malassezia* spp. (formerly *Pityrosporum* spp.) was diagnosed by cytological examination
338 alone and in combination with *Staphylococcus* spp. Although it can be found as a commensal
339 organism in the skin and ear canals of normal dogs, it can also be involved with
340 dermatological disease in a suitably predisposed skin and ear canals. The two organisms
341 produce growth factors and micro-environmental alterations that benefit each other; thus,

there are increased numbers of *Staphylococcus* with concurrent *Malassezia* (Mauldin *et al*., 1997; Ben Sala *et al*., 1998). In fact, 40% of dogs with *Malassezia* overgrowth are diagnosed with *Staphylococcus* pyoderma due to the symbiotic relationship between the two organisms (Guaguere and Prelaud, 1996). Known factors that may predispose *Malassezia* spp. to become pathogenic, rather than remain commensal, may include increased humidity, skin folds, endocrine diseases, keratinization disorders, hypersensitivity diseases (i.e., atopy, flea allergies, cutaneous adverse food reactions) and increased numbers of symbiotic *Staphylococci* (Guillot and Bond, 2020).

Among the Gram-negative isolates, *Enterobacteriaceae* and *E. coli* were the most common urinary tract pathogens with 26.6 and 47.9% of the isolates, respectively. These dominant urinary bacterial isolates are consistent with other 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 the only amikacin and gentamicin retained enough efficacy (>90%) to be used as first line treatment for empirical therapy against both types of bacteria. Although there were changes in both directions for both type of bacteria, that is, gain or loss of susceptibility to many other antimicrobials, the level of resistance to all other antimicrobials (except for amikacin and gentamicin) tested in both periods was high or very high to recommend empirical use. Neither of the β-lactams tested (amoxicillin-clavulanate, ampicillin, cephalexin, cephalotin, ceftiofur), tetracyclines (doxycycline, tetracycline) or trimethropim-sulfadizine, could be recommended based on their high (20-50%) level of resistance. Again, on this occasion florfenicol, which attained a 100% susceptibility against both *Enterobacteriaceae* and *E. coli* in the first study, was not included in the present one. The International Society for Companion Animal Infectious Disease Committee (ISCAID) have formulated guidelines with first-line antimicrobials for uncomplicated urinary tract infections, which include amoxicillin and trimethoprim-sulfonamide (Weese *et al*., 2019). However, in our case, neither amoxicillin nor trimethoprim-sulfonamide can any longer be recommended as first-line drug options for urinary tract disease.

Of particular interest was the increased resistance trend by all types of bacteria against amoxicillin-clavulanate and ampicillin. Because these are probably the most prescribed

antimicrobials for companion animals, it is likely that selection pressure is driving the increased resistance (Lord et al., 2022). Similar loss of efficacy overtime has been reported for the same type of bacteria in other countries, warranting close monitoring (Awosile *et al*., 2018; Lord *et al*., 2022). The ISCAID considers a 10% increase in resistance within the population from baseline as a reasonable breakpoint to change the empirical drug choice (Weese *et al*., 2019). For amoxicillin-clavulanate and ampicillin this level was surpassed for 3 types of bacteria: *Enterococcus* spp., *E. coli* and CoNS.

Some of the limitations of this study were the reduced number of isolates for some bacterial species, and consequently the low precision for each one. Furthermore, we could not investigate the association between previous antimicrobial use and resistance patterns. It is likely that animals included in this study were mostly those that had not responded to empirical treatments and were examined for a second or third time, as opposed to first-time infections. Also, the lack of history data precluded knowing whether the isolates in this study part of the normal microflora or contamination were, and not necessarily the pathogenic organisms causing disease. Ideally, monitoring changes overtime should also be done year-to-year to properly detect trends of antimicrobial resistance. This was not possible in the present study due to the low number of samples tested.

Conclusion

This study provides information on susceptibility patterns that can assist clinicians, particularly in the city of Medellín, in making rational decisions on the use of antimicrobials in dogs. We propose that the samples submitted to our laboratory in this study reflect clinical cases of dogs within the city. It is obvious that knowledge of resistance patterns of bacteria to antimicrobial drugs requires constant vigilance. It is likely that antimicrobial overuse in veterinary practices previously reported from our surveys on prescribing practices (Gómez-Beltrán *et al*., 2021), are creating selection pressure. The antimicrobials that showed increasing resistance trends, such as amoxicillin-clavulanate, with a high proportion of previously susceptible isolates should be monitored closely in the future. Antimicrobial stewardship strategies and programs are needed in Colombian companion animal practices.

Declarations

Conflicts of interest

The authors declare no conflict 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 worked on 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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