Molecular characterization of antimicrobial resistance genes in bacterial isolates from canine cutaneous infections.

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Abstract

Introduction: Antimicrobial resistance is one of the most significant challenges in contemporary medicine, affecting human and animal health, agriculture, and the environment. This issue has become increasingly critical due to the rising prevalence of antimicrobial-resistant cases, which has led to adverse consequences, including prolonged hospital stays, increased treatment costs, and higher morbidity and mortality rates within the population.

Material and Methods: A total of 30 bacterial isolates were analyzed. The isolates were biochemically characterized, and their phenotypic antimicrobial resistance was determined, showing a predominance of resistance to β-lactam antibiotics. After total DNA extraction, the presence of resistance genes to different antimicrobial classes was evaluated using specific primers and end-point PCR.
Results: The analyzed isolates showed the presence of resistance genes, with the highest frequencies observed for β-lactams (*blaCMY-2* and *blaTEM*), trimethoprim (*dfrA1*), sulfonamides (*sul1*), quinolones (*qnrS*), and tetracyclines (*tetB*). Significant associations ($p < 0.05$) were observed between most resistance genes and their corresponding phenotypic profiles, indicating a possible genotype–phenotype concordance.
Conclusions: The rational use of antimicrobials, combined with antimicrobial susceptibility testing and molecular methods, may help limit the spread of bacterial resistance. Finally, the association between genotypic and phenotypic resistance profiles provided a better understanding of the resistance mechanisms present in the studied isolates.
Keywords: Antimicrobial resistance; canine skin infections; resistance bacteria; molecular biology.

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1. Introduction

In Colombia, studies have evaluated antibiotic use and resistance in small animal practice. In methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates, the *mecA* gene has been detected, showing a possible correlation between phenotypic and genotypic resistance [1]. Additionally, Duque [2] and Morales et al [3] reported a high prevalence of *Staphylococcus pseudintermedius, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Proteus mirabilis* in dogs from Medellín, with notable resistance to gentamicin, doxycycline, neomycin, and azithromycin. Studies in Medellín and Valle del Cauca have also evaluated antibiotic use and risk factors associated with antimicrobial resistance. These studies identify clinical settings, animal production systems, and animal–human interactions as key drivers of multidrug resistance [4].

In the department of Tolima, some studies have focused on production animals, such as the evaluation of phenotypic and genotypic resistance in *Salmonella* spp. isolates from chicken carcasses sold in Ibagué [5,6] Other studies have assessed antimicrobial resistance in bacterial isolates from veterinary clinics, which are considered potential causes of nosocomial infections [7]. Despite available data on antimicrobial resistance in other regions, information from small animal clinical practice in Tolima remains limited. This highlights the need to characterize local bacterial epidemiology and resistance genes, contributing to a better understanding of antimicrobial resistance in companion animals and supporting global surveillance efforts.

2. Materials and methods

2.1 Study population

In this study, 30 bacterial isolates from skin infections of different patients were used, which come from patients with a clinical course longer than 3 days, characterized by suppurative reaction, reddening and increase of temperature at the infection zone. Patients were attended in the Veterinary Hospital Bernardino Rodríguez Urrea from University of Tolima and Dermatol Veterinary Clinic, following the standard guidelines of each medical center. The wounds were classified according to the type of infection as localized or generalized, and according to the type of injury as caused by pyoderma or by avulsion. Informed consent for sample collection was obtained from the owners or legal guardians of all patients.

Samples were taken by swabbing of the skin by using ProbactTM 5-18 (M40, TSC, UK) and kept refrigerated until their transport to the Clinical Laboratory Analicemos (Ibagué, Tolima); Bacterial identification began with a phenotypic characterization of the isolates, using Gram staining and catalase and coagulase tests to guide identification and determine the bacterial group. Isolates compatible with *Staphylococcus* were subsequently processed using the automated system (Vitek 2 Compact, Biomereaux, Spain) with the VITEK 2 GP card for the identification of Gram-positive bacteria. The system determined the bacterial species based on the biochemical profile of the isolate and its comparison with the

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equipment's database. The same general identification procedure was applied to the other bacterial isolates, using the corresponding card according to their microbiological characteristics.

Antimicrobial susceptibility was evaluated against benzylpenicillin, oxacillin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, linezolid, daptomycin, vancomycin, tetracycline, nitrofurantoin, rifampicin, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, ceftazidime, imipenem, meropenem, amikacin, ampicillin/sulbactam, ceftazidime/avibactam, ceftolozane/tazobactam, cefepime, aztreonam, ertapenem, and tigecycline. To determine susceptibility, AST-N438 and AST-GP67 cards were used. The results were interpreted according to the criteria established by CLSI M100, using the corresponding breakpoints for each antimicrobial. As part of quality control, reference strains established by the laboratory were used. Finally, the isolates were transported to the Laboratory of Immunology and Molecular Biology at the University of Tolima, for their posterior analysis.

2.2 Genomic DNA (gDNA) extraction

Fresh cultured strains of isolated bacterial species were used for DNA extraction by PCI (Phenol-Chloroform-isoamyl acid). Extracted DNA was suspended in TE buffer and stored at -20 °C until its use.

2.3 Identification of resistance genes by conventional PCR

Polymerase chain reaction (PCR) amplification was performed using a ProFlex (Applied Biosystem, USA) thermocycler in a volume of 25 µl, using five µl of Colorless 5x GoTaq® Flexi Buffer, one µl of dNTPs, one µl of each primer (Forward and Reverse; Table 1), one µl of MgCl2, 0.5 U GoTaq® Flexi DNA polymerase (Promega, Madison, USA) and one µl of DNA template. PCR settings were: one cycle of denaturation (95 °C, three min), followed by 35 cycles of denaturation (95 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 30 s), then one cycle of final extension (72 °C, five min). Amplicons were documented in a UV transilluminator (Enduro™ GDS, Labnet Intl, USA) after agarose gel (2%, Ultrapure ™ Agarose, ThermoFisher Scientific, USA) electrophoresis run in TBE buffer with Hydragreen™ as DNA dye (ACTGene, Piscataway, NJ). 100 bp DNA Ladder (Canvax BrightMAX™ 100 – 1 000 bp DNA Ladder, Spain) was used as molecular weight marker. The amplicons were confirmed by Sanger sequencing from previous laboratory projects. These sequence-verified amplicons were used as positive controls in the present study and served as references to confirm the expected amplification of the target resistance genes in the bacterial isolates.

Table 1. Primers for antibiotic resistance genes.

	Gen		Sequence (5´-3´)	Amplicon (pb)	Reference
B-lactamics	*blaCMY2*	F	AAATCGTTATGCTGCGCTCT	244	This study
		R	CCGATCCTAGCTCAAACAGC		
	blaCTX-M	F	TTCGCTAAATACCGCCATTC	236	
		R	TATCGTTGGTTGTGCCGTAA		

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	blaCTX-M-9	F	ATGGTGACAAAGAGAGTGCA	876
		R	TTACAGCCCTTCGGCGATGA	
	blaCTX-M-15	F	CAATGTGCAGCACCAGTAA	542
		R	CGCAATATCATTGGTGGTG	
	blaTEM	F	ATCAGTTGGGTGCACGAGTG	608
		R	ACGCTCACCGGCTCCAGA	
	blaPSE-1	F	GCAAGTAGGGCAGGCAATCA	422
		R	GAGCTAGATAGATGCTCACAA	
	blaOXA-1	F	TATCAACTTCGCTATTTTTTTA	807
		R	TTTAGTGTGTTTAGAATGGTGA	
	blaOXA-48	F	AAATCACAGGGCGTAGTTGTG	555
		R	GACCCACCAGCCAATCTTAG	
	blaSHV	F	ATGCGTTATATTCGCCTGTG	753
		R	TGCTTTGTTATTCGGGCCAA	
	blaSHV-2	F	CGATCGTCCACCATCCACTG	201
		R	CACTTCCTTTTTGCGCCAGA	
	blaSHV-5	F	ACGCTTTCCCATGATGAGCA	522
		R	CTAGCTCCGGTCTTATCGGC	
	blaCIT	F	TGGCCAGAACTGACAGGCAAA	462
		R	TTTCTCCTGAACGTGGCTGGC	
	bla-NDM1	F	GGGCAGTCGCTTCCAACGGT	476
		R	GTAGTGCTCAGTGTCGGCAT	
	blaIMP	F	GGAATAGAGTGGCTTAATTCTC	233
		R	GGTTTAATAAAACAACCACC	
	blaVIM	F	GATGGTGTTTGGTCGCATA	390
		R	CGAATGCGCAGCACCAG	
	blaKPC	F	CGTCTAGTTCTGCTGTCTTG	798
		R	CTTGTCATCCTTGTTAGGCG	
	bla1[1]	F	TCGCCTGTGTATTATCTCCC	768
		R	CGCAGATAAATCACCACAATG	
	bla2[1]	F	TGGCCAGAACTGACAGGCAAA	462
		R	TTTCTCCTGAACGTGGCTGGC	
	ampC	F	CCCCGCCTTATAGAGCAACAA	635
		R	TCAATGGTCGACTTCACACC	
	blaZ	F	CAAAGATGATATAGTTGCTTATTCTCC	421
		R	TGCTTGACCACTTTTATCAGC	
	mecA	F	AAAATCGATGGTAAAGGTTGGC	533
		R	AGTTCTGCAGTACCGGATTTG	
Chloramphenicols	*catA*	F	AAATCGTTATGCTGCGCTCT	454
		R	CCGATCCTAGCTCAAACAGC	
	cmlA	F	TGGACCGCTATCGGACCG	642
		R	CGCAAGACACTTGGGCTGC	
	floR	F	CACGTTGAGCCTCTATATGG	888
		R	ATGCAGAAGTAGAACGCGAC	
Aminoglycosides	*aac(6´)-Ib*	F	TTGCGATGCTCTATGAGTGGCTA	482
		R	CTCGAATGCCTGGCGTGTTT	
	aadA1	F	CTCCGCAGTGGATGGCGG	629
		R	GATCTGCGCGCGAGGCCA	
	aadA2	F	CATTGAGCGCCATCTGGAAT	500
		R	ACATTTCGCTCATCGCCGGC	
	strA	F	CTTGGTGATAACGGCAATTC	548
		R	CCAATCGCAGATAGAAGGC	
	strB	F	ATCGTCAAGGGATTGAAACC	509
		R	GGATCGTAGAACATATTGGC	
	dfrA1	F	CAATGGCTGTTGGTTGGAC	254

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Trimethoprim		R	CCGGCTCGATGTCTATTGT	
	dfrA10	F	TCAAGGCAAATTACCTTGGC	432
		R	ATCTATTGGATCACCTACCC	
	dfrA12	F	TTCGCAGACTCACTGAGGG	330
		R	CGGTTGAGACAAGCTCGAAT	
Sulfonamides	*sul1*	F	CGGACGCGAGGCCTGTATC	591
		R	GGGTGCGGACGTAGTCAGC	
	sul2	F	GCGCAGGCGCGTAAGCTGAT	514
		R	CGAAGCGCAGCCGCAATTC	
	sul3	F	GGGAGCCGCTTCCAGTAAT	500
		R	TCCGTGACACTGCAATCATTA	
Quinolones	*oqxA*	F	GGTGAAGTCGATCAGTCAGT	154
		R	ATCTATCGTGAACAGCACCT	
	qnrA	F	TCAGCAAGAGGATTTCTCA	625
		R	GGCAGCACTATTACTCCCA	
	qnrB	F	GTACGTGAAAGCCAGAAAGG	469
		R	ACGATGCCTGGTAGTTGTCC	
	qnrC	F	GGGTTGTACATTTATTGAATCG	308
		R	CACCTACCCATTTATTTTCA	
	qnrD	F	CGAGATCAATTTACGGGGAATA	582
		R	AACAAGCTGAAGCGCCTG	
	qnrS	F	ACGACATTCGTCAACTGCAA	417
		R	TAAATTGGCACCCTGTAGGC	
	qepA	F	GCAGGTCCAGCAGCGGGTAG	218
		R	CTTCCTGCCCGAGTATCGTG	
Tetracyclines	*tetA*	F	GCTGTCGGATCGTTTCGG	658
		R	CATTCCGAGCATGAGTGCC	
	tetB	F	CTGTCGCGGCATCGGTCAT	615
		R	CAGGTAAAGCGATCCCACC	
	tetM	F	ACAGAAAGCTTATTATATAAC	171
		R	TGGCGTGTCTATGATGTTCAC	
	tetD	F	GGATATCTCACCGCATCTGC	436
		R	CATCCATCCGGAAGTGATAGC	
	tetG	F	CAGCTTTCGGATTCTTACGG	844
		R	GATTGGTGAGGCTCGTTAGC	
Macrolides	*ereA*	F	GCCGGTGCTCATGAACTTGAG	420
		R	CGACTCTATTCGATCAGAGGC	
	ermB	F	CATTTAACGACGAAACTGGC	425
		R	GGAACATCTGTGGTATGGCG	
Colistin	*mcr-1*	F	AGTCCGTTTGTTCTTGTGGC	320
		R	AGATCCTTGGTCTCGGCTTG	
Mobile genetic elements	Intl-I	F	GGCATCCAAGCAGCAAG	1664
		R	AAGCAGACTTGACCTGA	
	Intl-II	F	CACGGATATGCGACAAAAAGGT	789
		R	GTAGCAAACGAGTGACGAAATG	
	Intl-III	F	CCGGTTCAGTCTTTCCTCAA	155
		R	GAGGCGTGTACTTGCCTCAT	
	Tn5	F	GGTGGAAAATGGCCGCTTTT	598
		R	TGCCTGCAAGCAATTCGTTC	

[1]Beta-lactamasa genes

2.4 Statistical analysis

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To evaluate the potential statistical association between the presence of resistance genes and the phenotypic expression of antibiotic resistance, statistical test was Irwin–Fisher exact test. For each pair of variables, 2×2 contingency tables were constructed. Odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) were calculated as measures of association. Statistical significance was evaluated at p = 0. 05. Statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA). the data were reanalyzed using the Benjamini–Hochberg procedure to control the false discovery rate (FDR), and the wording was revised to avoid overinterpretation of the results and to accurately describe the observed associations.

3. Results

The findings related to genotypic antimicrobial resistance were summarized and visualized using a Sankey diagram (figure 1). In this diagram, the thickness of the flows on the left side represents the distribution of the bacterial species analyzed, whereas the flows on the right side indicate the frequency of detection of resistance genes associated with different antimicrobial families in the bacterial isolates. Phenotypic analysis revealed considerable variability in antimicrobial resistance profiles among the bacterial isolates (Table 2).

Table 2. Bacterial strains and phenotypic resistance patterns.

ID	*Strain*	*Phenotypic resistance*
NP1	*Staphylococcus pseudintermedius*	--
NP2	*Pseudomonas fluorescens*	--
NP3	*Escherichia coli*	CIP, AZT
NP4	*Streptococcus pneumoniae*	P, E
NP5	*Enterobacter cloacae* complex	--
NP6	*Staphylococcus warneri*	P, CIP, LEV, E, DA, TE, SXT
NP07	*Staphylococcus haemolyticus*	--
NP08	*Enterococcus faecium*	TE
NP09	*Klebsiella pneumoniae* ssp *pneumoniae*	--
NP10	*Escherichia coli*	AZT
NP11	*Staphylococcus pseudintermedius*	P, OX, E, TE, SXT
NP12	*Escherichia coli*	AZT
NP13	*Staphylococcus pseudintermedius*	P, TE
NP 14	*Staphylococcus aureus*	P, OX, CIP, LEV, E, DA, TE, SXT
NP15	*Staphylococcus hyicus*	TE

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NP16	*Staphylococcus haemolyticus*	--
NP17	*Staphylococcus pseudintermedius*	TE, SXT
NP18	*Staphylococcus pseudintermedius*	E, DA, TE, SXT
NP19	*Staphylococcus pseudintermedius*	E, DA, SXT
NP20	*Enterobacter cloacae* complex	CIP, CAZ, CEFTO/TAZO, FEP, AZT
NP21	*Escherichia coli*	--
NP22	*Enterococcus faecium*	TE
NP23	*Pseudomonas aeruginosa*	--
NP24	*Staphylococcus pseudintermedius*	TE
NP25	*Staphylococcus pseudintermedius*	CIP, LEV, E, DA, TE, RD, SXT
NP26	*Staphylococcus pseudintermedius*	SXT
NP27	*Staphylococcus pseudintermedius*	E, DA, TE, SXT
NP28	*Staphylococcus pseudintermedius*	CIP, LEV, E, DA, TE, SXT
NP29	*Staphylococcus pseudintermedius*	--
NP30	*Escherichia coli*	CIP, AZT

CIP (Ciprofloxacin), AZT (Aztreonam), P (Benzylpenicillin), E (Erythromycin), LEV (Levofloxacin), DA (Clindamycin), TE (Tetracycline), SXT (Trimethoprim/Sulfamethoxazole), OX (Oxacillin), CAZ (Ceftazidime), CEFTO/TAZO (Ceftolozane/Tazobactam), FEP (Cefepime), RD (Rifampin).

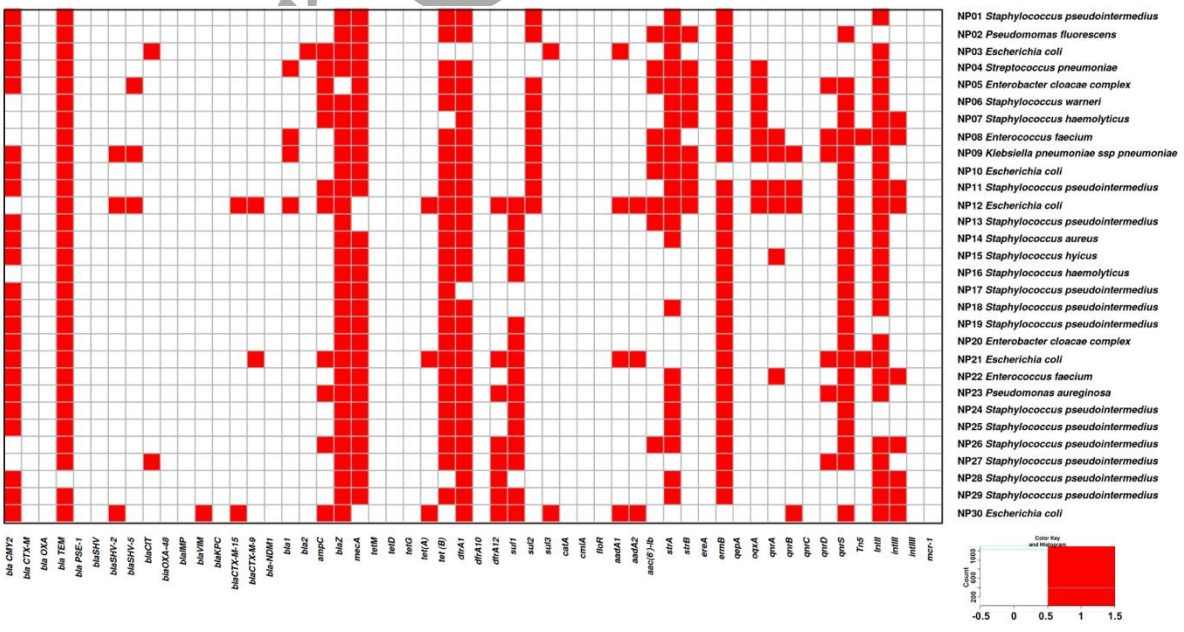


Figure 1. The heatmap showing the consolidated distribution of genotypic antimicrobial resistance determinants among the different bacterial species. β-lactams (*mecA* = 27, *blaZ* = 29, *blaTEM* = 29, *blaCMY-2* = 23, *ampC* = 11, *blaSHV* = 2, *blaSHV-2* = 3, *blaSHV-5* = 3, *blaCTX-M-9* = 2, *blaCTX-M-15* = 2, *bla1* = 4, *bla2* = 1, *blaVIM* = 1, *blaCIT* = 2); trimethoprim (*dfrA1* = 28, *dfrA12* = 8); tetracyclines (*tetA* = 3, *tetB* = 26); aminoglycosides (*aac(6')-Ib* = 9, *strA* = 22, *strB* = 9, *aadA1* = 4, *aadA2* = 3); macrolides (*ermB* = 28); quinolones (*oqxA* = 8, *qnrA* = 6, *qnrB* = 4, *qnrD* = 6, *qnrS* = 25); sulfonamides (*sul1* = 16, *sul2* = 10, *sul3* = 2); and mobile genetic elements (*IntI1* = 26, *IntI2* = 9, *Tn5* = 2). Heat map generated using Morpheus. Red squares indicate positive results, whereas white squares indicate negative results.

Statistical analyses performed using Irwin–Fisher exact test revealed statistically significant associations ($p < 0.05$) between most of the resistance genes evaluated and their corresponding phenotypic resistance profiles against the antibiotics analyzed. *blaCMY2*, *blaTEM, blaZ, mecA, tetB, qnrS, ermB, dfrA1, and intI1* genes showed a consistent association with the observed resistance phenotypes. In contrast, no significant association was found between the sul1 gene and resistance to SXT. (Table 3).

Table 3. Association between antimicrobial resistance genes and phenotypic resistance patterns in the analyzed bacterial isolates.

Gen/antibiótic	OR (IC95%)	P	qValue
***blaCMY2*/ESBL**	95.29 (12.6-1008)	<0.001	0.0011
***blaTEM*/ESBL**	841.0 (52.31-8526.0)	<0.001	0.0011
***blaTEM*/AZT**	188.5 (20.51-1973)	<0.001	0.0011
***blaZ*/OX**	841 (52.31-8526)	<0.001	0.0011
***mecA*/OX**	261 (24.91-2715)	<0.001	0.0011
***tetB*/TE**	8.5 (2.430-26.01)	<0.001	0.0011
***qnrS*/CIP**	16.43 (4.597-58.19)	<0.001	0.0011
***qnrS*/LEV**	32.5 (7.396-107.3)	<0.001	0.0011
***ermB*/E**	32.67 (6.154-150.7)	<0.001	0.0011
***dfrA1*/SXT**	28.0 (5.384-129.2)	<0.001	0.0011
***sul1*/SXT**	2.286 (0.76-6.5)	0.1923	0.1923
Int-1/aadA1	58.5 (12.53-222.5)	<0.001	0.0011

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Odds ratios (OR), 95% confidence intervals (95% CI), and p-values obtained from association analyses are shown. qValue for method of Benjamini and Hochberg

4. Discussion

Antimicrobial resistance is a major challenge in human and veterinary medicine, especially in *Enterobacterales*, where plasmid-mediated β-lactamase genes are a key resistance mechanism [8]. In this context, extended-spectrum β-lactamases (ESBLs) significantly limit the use of third-generation cephalosporins and complicate the treatment of infections caused by Gram-negative bacilli. In the present study, isolates of *Escherichia coli* and *Klebsiella pneumoniae* carrying the *blaTEM, blaSHV-2, blaSHV-5, blaCTX-M-9, and blaCTX-M-15* genes were identified, which are commonly associated with the ESBL phenotype in both humans and animals [8]. These isolates are of One Health relevance due to their potential role as reservoirs of antimicrobial resistance determinants that may circulate between animal and human populations [9]. In our study, the *blaCMY-2* gene was detected in 76% of the isolates (Figure 1). AmpC β-lactamases are typically encoded by the chromosomal ampC gene; however, in species such as *Klebsiella* and *Salmonella*, AmpC enzymes are often plasmid-mediated (pAmpC) [10]. The *blaCMY-2* gene is one of the most frequently reported AmpC variants in enterobacteria isolated from animals [11], and in the present study it was identified as the most prevalent resistance determinant among the bacterial strains analyzed.

Methicillin-resistant staphylococci (MRS) represent a relevant problem in both human and veterinary medicine due to their resistance to β-lactam antibiotics, which considerably limits the available therapeutic options [12]. In this study, strain NP11 was identified as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and strain NP14 as methicillin-resistant Staphylococcus aureus (MRSA), based on their phenotypic resistance to oxacillin. The concomitant detection of the *mecA* gene, responsible for encoding the penicillin-binding protein 2a (PBP2a), confirmed the classification of both strains as MRS, in accordance with previously established criteria for this resistance phenotype [13]. *Staphylococcus spp.* has been reported as the most frequently isolated bacterium across different skin sites [14] and exhibits two major mechanisms of resistance to β-lactam antibiotics; drug inactivation

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through the production of a β-lactamase encoded by the *blaZ* gene, and modification of the target site via penicillin-binding protein 2a (PBP2a) [15]. In the present study, the *blaZ* gene was detected in 96% of the isolates, consistent with previous reports describing a high prevalence of this gene in clinical *Staphylococcus spp*. isolates of animal origin [16,17]. Additionally, 16 of the 17 strains analyzed concomitantly harbored the *blaZ* and *mecA* genes; however, only two were classified as methicillin-resistant, suggesting that although these strains may carry both resistance determinants, phenotypic expression of the MRS profile primarily depends on the functional production of PBP2a [18].

The *aac(6')-Ib-cr* gene is a plasmid-mediated resistance determinant derived from a*ac(6')-Ib.* In addition to acetylating aminoglycosides, the *aac(6')-Ib-cr* variant can acetylate fluoroquinolones, particularly ciprofloxacin and norfloxacin, due to specific amino acid substitutions that distinguish it from the parental *aac(6')-Ib* variant. Machuca et al. [19] reported that aac(6′)-Ib-cr increased ciprofloxacin and norfloxacin MICs by 4- to 8-fold in E. coli isolates; however, clinically relevant resistance generally requires additional chromosomal mutations, particularly in *gyrA* and *parC*, and alterations in marR. In strain NP3, ciprofloxacin resistance was observed alongside the presence of *aac(6')-I*b. However, since the gene was not sequenced to confirm the aac(6′)-Ib-cr variant and *gyrA/parC* mutations were not assessed, other uninvestigated mechanisms may contribute to the observed resistance.

Bacterial resistance to quinolones is mainly associated with mutations in the quinolone resistance–determining regions (QRDRs), which alter DNA gyrase and topoisomerase IV, as well as plasmid-mediated mechanisms involving *qnr* genes, and efflux pumps such as *qepA* and *oqxA* that reduce intracellular drug concentrations [20]. In this study, a high frequency of the *qnrS* gene was observed, suggesting its relevant role in the dissemination of plasmid-mediated quinolone resistance; additionally, the concomitant detection of *oqxA* indicates a possible complementary action between plasmid-mediated resistance and active efflux systems, promoting a more efficient reduction of intracellular antimicrobial accumulation [21].

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Erythromycin resistance is mainly caused by erm-mediated methylation of 23S rRNA, leading to MLSB cross-resistance [22]. In this study, 30% of the isolates exhibited phenotypic resistance to erythromycin, and in all cases the *ermB* gene was detected. Overall, this determinant was present in 93.3% (28/30) (figure 1) of the strains analyzed, and concomitant resistance to clindamycin was observed in some isolates, in agreement with previous reports describing this pattern in canine *Staphylococcus pseudintermedius* isolates [23].

In our study, statistically significant correlations were identified between specific resistance genes and their corresponding antibiotics, as well as between integrons and associated genes. The most relevant associations included *blaCMY-2*/ESBL, *blaTEM*/ESBL, *blaTEM*/AZT, *blaZ*/OX, *mecA*/OX, *tetB*/TE, *qnrS*/CIP, *qnrS*/LEV, *ermB*/E, *dfrA1*/SXT, and *intI1*/aadA1. These findings are consistent with reports by Chaisaeng [24] and Sánchez-Osuna [25], who demonstrated that, in cases where phenotypic resistance to specific antibiotics was detected, the corresponding resistance genes were also present. This suggests a potential circumvention of antimicrobial regulatory mechanisms, thereby promoting the expression and persistence of resistance.

The detection of antimicrobial resistance determinants in companion animals is relevant from a One Health perspective because dogs and cats have close and frequent contact with humans and may contribute to the circulation of antimicrobial-resistant bacteria and resistance genes. Evidence indicates that resistant organisms can be shared bidirectionally between humans and companion animals, highlighting the potential role of pets as reservoirs or sentinels of antimicrobial resistance [26]. Previous antimicrobial exposure is also an important risk factor for antimicrobial-resistant Enterobacterales in dogs [27]. These findings highlight the importance of including companion animals in antimicrobial resistance surveillance and integrated One Health strategies, although the detection of resistance determinants alone does not demonstrate zoonotic transmission.

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5. Conclusions

The findings of this study highlight the usefulness of molecular tools such as PCR for monitoring antimicrobial resistance determinants in canine bacterial isolates. Molecular detection should be considered complementary to phenotypic antimicrobial susceptibility testing, which remains essential for guiding antimicrobial treatment. The correlation between resistance genes and phenotypic resistance profiles contributes to a better understanding of resistance mechanisms and supports the surveillance of antimicrobial resistance. Continued monitoring of resistance determinants, together with phenotypic testing, may contribute to improved antimicrobial stewardship and control of resistance within a One Health.

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Authors' contributions

Christian Camilo Patiño Valencia, Maria Alejandra Bautista Paez, and Iang Schroniltgen Rondón Barragán contributed to the study concept and design, analysis and interpretation of data, drafting of the manuscript, and statistical analysis. Iang Schroniltgen Rondón Barragán was responsible for data acquisition and critical revision of the manuscript for important intellectual content. Administrative, technical, and material support, as well as study supervision, were provided by Christian Camilo Patiño Valencia, Maria Alejandra Bautista Paez, Valentina Rueda Garcia, and Iang Schroniltgen Rondón Barragán.

Conflict of interest

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The authors declared no conflicts of interest.

Compliance with ethical guidelines

All experimental procedures followed the Guidelines from the Local Committee of Bioethics of the Office of Research and Scientific Development from University of Tolima based on the Law 84/1989, Resolution 8430/1993 and Law 576/2000, following the current guidelines of the Colombian Network CICUALES and ARRIVE for use and care of laboratory animals in research and teaching.

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Data availability

All data analyzed during this study are included in this article.

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