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Investigation of antimicrobial susceptibility and resistance genes in *mannheimia haemolytica* isolates obtained from respiratory tracts

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Abstract

The potential transfer of antimicrobial agent residues and resistance genes in foods between bacteria creates a significant risk for public health. This research was objectived to investigate the susceptibility of *Mannheimia haemolytica* isolates from the respiratory tract of animals to antibiotics and antibiotic resistance genes. In the study, 48 *Mannheimia haemolytica* isolates were cultured by conventional methods. The susceptibilities of the isolates to antimicrobial agents were investigated comparatively by disk diffusion and e-test methods. The presence of antibiotic resistance genes in isolates found to be resistant to various antimicrobial agents was investigated by Real-Time PCR method. In this research, 66.6% of the 9 isolates detected to be resistant to antibiotics were resistant to more than one antibiotic. The macrolide resistance genes (*erm42, mphE, msrE*) were detected in 6 (66.6%) isolates found resistant by E test. The aminoglycoside resistance gene (*strA*) was also found in 5 of these 6 isolates, while none of the *bla*$_{ROB}$*-1, tetH* and *sulII* genes were detected in these isolates. Isolates resistant to macrolide group antibiotics had at least one of the erm42, mphE and msrE genes, while all isolates resistant to aminoglycoside group antibiotics had the *strA* gene. Furthermore, MIC values were higher in isolates with resistance genes. In conclusion, this study showed that macrolide and aminoglycoside resistance genes are responsible for the phenotypic resistance of *Mannheimia haemolytica* to these antibiotic groups. Furthermore, *tetH, bla*$_{ROB}$*-1* and *sulII* genes are thought to be transported via extrachromosomal genetic elements. With this study, it was concluded that a broad perspective approach is required in the management of infections caused by *Mannheimia haemolytica*, in the protection of public health and in the fight against antibiotic resistance, and that it is important to conduct new studies to investigate alternative resistance mechanisms.

Keywords: *Mannheimia haemolytica*, antimicrobial resistance genes, one health, real time-PCR, e-test, MIC

Introduction

Animal derived proteins are among the essential nutrients indispensable for healthy human development. The necessity for adequate and balanced nutrition in a healthy society remains a priority for countries worldwide. In today's world, described as an era of technology and industrialization, the livestock sector still retains its strategic significance for many nations. According to global statistics, 30% of the world's meat production originates from cattle and 5% from small ruminants. In Turkey, red meat production differs from global averages, with 74.8% coming from cattle and 24.6% from small ruminants such as sheep and goats [1-3].

Food safety is defined as the presence of microorganisms, chemicals, biotoxins, and additives within specified safe limits to maintain health and prevent disease [4]. The production of safe food of animal origin is closely linked to the maintenance of animal well-being and health. In this regard, antibiotics administered for animal therapy serve as essential means for the prevention and management of infectious outbreaks [5,6].

Since the 1950s, antimicrobial agents have been used in the animal breeding sector not only for therapeutic and prophylactic intentions but also as feed additives to promote growth [7]. As a consequence, medicine residues accumulate in animal-source foods (meat, honey, eggs, milk, etc.) and may

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be transmitted to humans through consumption. Antimicrobial residues in these products may contribute to the emergence of bacterial resistance, allergic responses, tissue injury, digestive and nervous system disorders, and in severe cases, anaphylactic reactions, thereby constituting a risk to human health. Additionally, consumption of these products may result in congenital anomalies and carcinogenic effects in humans [8,9]. Antimicrobials including gentamicin, penicillin, oxytetracycline, sulfamethazine, and chloramphenicol may induce various adverse effects in humans, such as carcinogenicity, mutagenic potential, renal dysfunction, liver toxicity, recurrent conditions, bone marrow suppression, and hypersensitivity reactions [10]. These effects may result from both short- and long-term exposure. Beyond these effects, the most concerning issue is the increase in bacterial resistance to antimicrobial agents, which has become a critical problem globally. The Organisation for Economic Co-operation and Development has projected that antimicrobial resistance accounts for nearly 700.000 fatalities across the globe each year, and this figure could escalate to 9.5 million should resistance levels increase by 40% [11].

Global entities (like WHO and FAO) highlight the critical role of responsible antibiotis usege in animals raised for food production. Recommendations include observing withdrawal periods, raising awareness among animal owners, implementing scientific protocols for residue monitoring, conducting pre-slaughter residue testing through blood and urine samples, limiting antibiotic use to veterinary prescriptions, and preferring narrow-spectrum antibiotics following antibiogram testing [12].

Among the diseases in which antimicrobial agents are intensively used in domestic cattle and sheep, respiratory tract infections are of primary importance. *Mannheimia haemolytica (M. haemolytica)*, frequently isolated in these infections, can cause pneumonia, brain abscess, and urinary system infections in animals, and has also been associated with endocarditis and septicemia in humans [13-19].

M. haemolytica is a Gram-negative, encapsulated coccobacillus that does not form spores, and exhibits hemolytic activity on blood agar [14,20,21].

A broad spectrum of antibiotics is employed in the therapeutic management of respiratory tract infections in animals. These encompass sulfonamides and their synergistic formulations, β-lactams such as penicillin G, amoxicillin, ampicillin, ceftiofur, and cefquinome; tetracyclines, particularly oxytetracycline; macrolides including erythromycin, tylosin, tilmicosin, and tulathromycin; as well as phenicols like florfenicol. Additionally, aminoglycosides (e.g., gentamicin, spectinomycin, streptomycin, dihydrostreptomycin), lincosamides (lincomycin), and fluoroquinolones are frequently utilized to counteract bacterial pathogens associated with respiratory ailments [22].

This research aims to comparatively evaluate the antibiotic sensitivity patterns and MIC values of *M. haemolytica* isolates collected from the upper and lower respiratory systems of domestic cattle and sheep. Additionally, it examines antibiotic resistance genes present in resistant isolates, thereby aiding in the detection and control of antibiotic resistance associated with respiratory tract infections.

Material and Methods

Detection and Identification of *M. haemolytica* from Respiratory Samples

For the isolation and identification of the pathogen, 650 swab specimens obtained from cattle and sheep were cultured on 5-7% sheep blood agar using conventional microbiological methods. After aerobic incubation at 37°C for 24-72 hours, bacterial colonies exhibiting hemolytic activity were distinguished for further analysis. Preliminary identification included Gram staining as well as catalase, oxidase, and indole biochemical tests. Subsequently, 48 isolates presumptively identified as *M. haemolytica* were stored for further molecular verification. [14,23-27].

The identification of *M. haemolytica* strains and detection of antibiotic resistance genes were conducted using the Real-Time PCR method. Genomic bacterial DNA was isolated from the cultures and subsequently analyzed by Real-Time PCR utilizing gene-specific primers.

Research Ethics: This research was approved by the Local Ethics Committee for Animal Studies of Van Yüzüncü Yıl University (approval date: 01.03.2018, decision number: 02).

Reference Culture: The *M. haemolytica* reference strain employed in this study was generously supplied by Prof. Dr. Arzu Fındık from the Department of Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayıs University.

DNA Isolation: Genomic bacterial DNA from presumptive the isolates was obtained using a commercial bacterial DNA isolation kit (Vivantis, Malaysia), according to the manufacturer's protocol.

Real-Time PCR Protocol: Identification of *M. haemolytica* strains was applied using primers specific to the 325 bp fragment of the PHSSA gene, as formerly reported by Hawari et al. [28] (F: TTCACATCTTCATCCTC; R: TTTTCATCCTCTTCGTC).

The qPCR reaction mixture comprised 12 µl of 2X commercial master mix (Ampliqon, Denmark), 2 µl of template genomic DNA, and 1 µl of forward and reverse primers, with PCR-grade water added to reach a total reaction volume of 25 µl. The amplification process was conducted for 35 cycles (Table 2).

Table 1. Primer sequences used for the detection of antimicrobial resistance genes

Antibiotics	Gene		Primer Sequences (5’-3’)	References
Tetracycline	*tet*H	F	ATACTGCTGATCACCGT	35
		R	TCCCAATAAGCGACGCT	
β-lactam	*bla*ROB-1	F	AATAACCCTTGCCCCAATTC	35
		R	TCGCTTATCAGGTGTGCTTG	
Macrolide	*erm*42	F	TGCACCATCTTACAAGGAGT	36
		R	CATGCCTGTCTTCAAGGTTT	
	*mph*E	F	ATGCCCAGCATATAAATCGC	36
		R	ATATGGACAAAGATAGCCCG	
	*msr*E	F	TATAGCGACTTTAGCGCCAA	36
		R	GCCGTAGAATATGAGCTGAT	
Sulfonamide	*Sul*II	F	CAGTTTCTCCGATGGAGGCC	37
		R	CTCGTGTGTGCGGATGAAGTC	
Aminoglycoside	*Str*A	F	TGACTGGTTGCCTGTCAGAGG	37
		R	CCAGTTGTCTTCGGCGTTAGCA	

Table 2. PCR amplification protocols used in real-time PCR

Gene	First Denaturation (°C/dk.)	PCR Cycles (°C / sec)			Final Extention (°C/min)
		Denaturation	**Annealing**	**Extention**	
PHSSA	94/5	94/60	56/45	72/60	72/5
***tet*H**	95/5	94/30	53/60	72/60	72/8
bla$_{ROB}$**-1**	95/5	94/30	51/60	72/60	72/8
***erm*42**	94/5	94/30	50/30	72/45	72/5
***msr*E**	94/5	94/30	50/30	72/45	72/5
***mph*E**	94/5	94/30	50/30	72/45	72/5
***Str*A**	94/5	94/60	56/60	72/60	72/7
***Sul*II**	94/5	94/60	56/60	72/60	72/7

Determination of Antimicrobial Susceptibility

In this study, antimicrobials commonly used by veterinarians and known to have high resistance potential in both animal and human Gram-negative bacterial infections were selected. The antimicrobial susceptibility of confirmed *M. haemolytica* isolates to erythromycin, tilmicosin, streptomycin, gentamicin, tetracycline, penicillin-G, ampicillin, ceftazidime, sulfamethoxazole-trimethoprim, and enrofloxacin was determined using both disk diffusion [29] and E-test methods [30].

All susceptibility tests were applied following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [31,32] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [33].

The disk diffusion results were evaluated according to the interpretive standards outlined by the CLSI [31,32] and EUCAST [33].

In the E-test analyses, the MIC value for each antibiotic were assessed based on the interpretive breakpoints recommended by CLSI [31], EUCAST [33], and the National Antimicrobial Resistance Monitoring System (NARMS) [34].

Detection of Antibiotic Resistance Genes

Following MIC determination via E-test, resistance-associated genes were analyzed in nine phenotypically resistant isolates using Real-Time PCR. The screening focused on genes linked to resistance against macrolides (*erm*42, *msr*E, *mph*E), tetracyclines (*tet*H), β-lactams (bla$_{ROB}$-1), aminoglycosides (*str*A), and sulfonamides (sulII).

The PCR reaction mixture for the amplification of macrolide, tetracycline, and β-lactam resistance genes was prepared with 10 µl of qPCR Master Mix, 2 µl of genomic DNA, 1 µl of each primers (forward and reverse), and PCR-grade water adjusted to a final volume of 20 µl. In contrast, for the detection of aminoglycoside and sulfonamide resistance genes, the mixture consisted of 12 µl SYBR Green Master Mix, 2 µl of genomic DNA, 1 µl of each primer, and sufficient PCR-grade water to reach a total volume of 25 µl.

The Real-Time PCR protocols for each resistance gene were run for 35 cycles. The specific protocols are detailed in Table 1 and Table 2. Samples that yielded a characteristic sigmoidal amplification curve following real-time PCR were interpreted as positive for the presence of the target gene.

Results

Antimicrobial Susceptibility Test

Disk Diffusion Test: Among the bacterial isolates examined in this study, 10.4% showed resistance to erythromycin and tilmicosin, 8.3% to tetracycline, 4.2% to penicillin and ampicillin, and 2.1% to streptomycin. All tested isolates exhibited susceptibility to gentamicin, ceftazidime, enrofloxacin, and sulfamethoxazole-trimethoprim (Figure 1, Table 3).

Figure 1. Inhibition zone diameters observed in *M. haemolytica* isolates using the Kirby-Bauer disk diffusion method

Table 3. In vitro susceptibility of *M. haemolytica* isolates to antibiotics using the Kirby-Bauer disk diffusion method

Antibiotics	Evaluation Criteria (mm)	S (%)	I (%)	R (%)
Erythromycin	≤13–≥23[a]	22 (45.9)	21 (43.7)	5 (10.4)
Tilmicosin	≤10–≥14[b]	43 (89.6)	0	5 (10.4)
Streptomycin	≤12–≥15[c]	38 (79.2)	7 (14.5)	3 (6.3)
Gentamicin	≤12–≥15[a]	46 (95.8)	2 (4.2)	0
Tetracycline	<24–≥24[d]	44 (91.7)	0	4 (8.3)
Penicillin	<17–≥17[d]	46 (95.8)	0	2 (4.2)
Ampicillin	<17–≥17[e]	46 (95.8)	0	2 (4.2)
Ceftazidime	≤17–≥21[f]	47 (97.9)	1 (2.1)	0
Enrofloxacin	≤16–≥21[b]	48(100)	0	0
Sulfamethoxazole-Trimethoprim	<23–≥23[d]	48 (100)	0	0

a: Criteria from CLSI 2002 were used; b: Criteria from CLSI 2018 were used; c: Gentamicin was evaluated according to CLSI 2002 criteria; d: EUCAST 2022 criteria for *Pasteurella multocida* were used; e: Penicillin-G was evaluated according to EUCAST 2022 criteria for *Pasteurella multocida*; f: Ceftiofur was evaluated based on CLSI 2018 criteria; S: Susceptible; I: Intermediate; R: Resistant

Determination of MIC Values

The MIC values obtained via E-test for erythromycin, tilmicosin, streptomycin, gentamicin, tetracycline, penicillin, ampicillin, ceftazidime, enrofloxacin, and sulfamethoxazole-trimethoprim were recorded within the ranges of 0.19–32 µg/ml, 0.012–32 µg/ml, 0.75–64 µg/ml, 0.125–0.75 µg/ml, 0.064–32 µg/ml, 0.047–24 µg/ml, 0.016–32 µg/ml, 0.016–0.19 µg/ml, 0.002–0.032 µg/ml, and 0.002–0.064 µg/ml, respectively. Based on internationally accepted antimicrobial susceptibility guidelines, resistance was identified in 14.6% of isolates for penicillin, 12.5% for tilmicosin, 10.4% for erythromycin and streptomycin, 8.3% for tetracycline, and 6.3% for ampicillin. All isolates were susceptible to enrofloxacin, ceftazidime, gentamicin, and sulfamethoxazole-trimethoprim (Figure 2, Table 4).

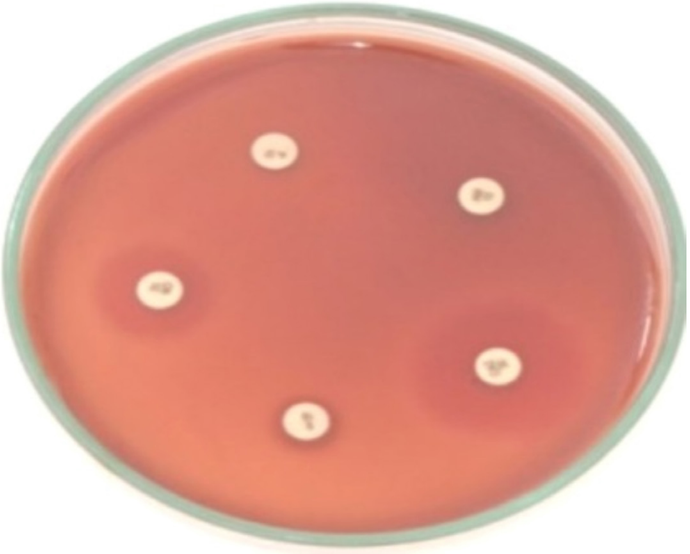

Figure 2. Determination of MIC values in *M. haemolytica* isolates using the E-test method

Differences were observed between disk diffusion and E-test results regarding resistance to tilmicosin, streptomycin, penicillin, and ampicillin.

Based on the analysis of MIC values, MIC_{50}–MIC_{90} values for the isolates were reported in Table 4.

Antimicrobial Resistance Genes

Among the nine resistant isolates identified by E-test, six contained macrolide resistance genes, while no tetracycline, β-lactam, or sulfonamide resistance genes were detected. Of the six macrolide-resistant isolates, one carried the *mph*E gene, three carried both *mph*E and *msr*E genes, and two carried the erm42 gene. Additionally, five of the six isolates harboring macrolide resistance genes also contained the aminoglycoside resistance gene strA (Table 6, Figure 3-5).

Comparing phenotypic resistance profiles and resistance gene profiles, the isolate resistant only to erythromycin by both methods carried the *mph*E gene. Two isolates resistant only to penicillin and one to both penicillin and tilmicosin did not harbor any resistance genes.

One isolate resistant to tilmicosin, streptomycin, and penicillin carried *erm*42 and *str*A genes. Another isolate with a similar profile was resistant to erythromycin, tilmicosin, streptomycin, and tetracycline.

Three isolates showing multidrug resistance to erythromycin, tilmicosin, streptomycin, tetracycline, penicillin, and ampicillin by E-test harbored *mph*E, *msr*E, and *str*A genes, but not erm42. Two of these isolates showed higher MIC values (32, 24, 32 µg/ml) compared to another isolate which was susceptible in the disk diffusion test (8, 4, 1.5 µg/ml) (Table 6).

Table 4. MIC levels of *M. haemolytica* isolates against antimicrobial agents determined by E-test

Antibiotics		E	TIL	STR	CN	T	P	A	CAZ	ENR	SXT
Evaluation Criteria (μg/ml)		≤0.5–≥8[b]	≤8–≥32[c]	≤32–≥64[d]	≤4–≥16[d]	≤2–≥8[c]	≤0.25–≥1[c]	≤1–>1[e]	≤2–≥8[f]	≤0.25–≥2[c]	≤0.25–>0.25[e]
MIC (μg/ml)[a]	0.002	0	0	0	0	0	0	0	0	7	1
	0.003	0	0	0	0	0	0	0	0	5	3
	0.004	0	0	0	0	0	0	0	0	**10**	3
	0.006	0	0	0	0	0	0	0	0	5	6
	0.008	0	0	0	0	0	0	0	0	5	**4**
	0.012	0	1	0	0	0	0	0	0	6	15
	0.016	0	0	0	0	0	0	2	7	5	9
	0.023	0	0	0	0	0	0	0	1	2	3
	0.032	0	0	0	0	0	0	1	4	3	2
	0.047	0	0	0	0	0	1	1	**5**	0	1
	0.064	0	0	0	0	1	1	0	12	0	1
	0.094	0	0	0	0	1	4	8	11	0	0
	0.125	0	0	0	1	1	8	**10**	6	0	0
	0.19	1	0	0	3	9	**9**	6	2	0	0
	0.25	0	1	0	2	**11**	3*	6	0	0*	0*
	0.38	0	0	0	**10**	15	6	6	0	0	0*
	0.50	2*	1	0	24	6	4	5	0	0	0
	0.75	**15**	2	2	8	0	5	0	0	0	0
	1.0	16	7	0	0	0	3*	0*	0	0	0
	1.5	5	**6**	3	0	0	1	1*	0	0	0
	2	3	9	5	0	0*	0	0	0*	0*	0
	3	1	1	**8**	0	0	0	0	0	0	0
	4	0	5	10	0*	0	1	0	0	0	0
	6	0	7	9	0	0	0	0	0	0	0
	8	0*	2*	4	0	1*	0	0	0*	0	0
	12	0	0	2	0	1	0	0	0	0	0
	16	2	0	0	0*	0	0	0	0	0	0
	24	1	0	0	0	0	2	0	0	0	0
	32	2	6*	0*	0	2	0	2	0	0	0
	64	0	0	5*	0	0	0	0	0	0	0
Number of resistant bacteria (%)		5 (10.4)	6(12.5)	5 (10.4)	0	4 (8.3)	7 (14.6)	3 (6.3)	0	0	0

a: Bold values indicate MIC_{50}; underlined values indicate MIC_{90}; b: Evaluation criteria reported by CLSI (2002) were used; c: Evaluation criteria reported by CLSI (2018) were used; d: Evaluation criteria reported by NARMS (2009) were used; e: Evaluation criteria for *Pasteurella multocida* from EUCAST 2022 were used; f: Evaluation criteria for Ceftiofur from CLSI (2018) were used; E: Erythromycin; TIL: Tilmicosin; STR: Streptomycin; CN: Gentamicin; TET: Tetracycline; P: Penicillin; A: Ampicillin; CAZ: Ceftazidime; ENR: Enrofloxacin; SXT: Sulfamethoxazole-Trimethoprim

Table 5. Phenotypic resistance profiles of *M. haemolytica* isolates resistant to antimicrobial agents as determined by E-test and disk diffusion

Disc diffusion		E-test		
Antibiotic resistance profile	n:6	Antibiotic resistance profile	n:9	MIC (μg/ml)
E	1	E	1	16
-	0	P	2[a]	1, 1.5
-	0	Til, P	1[b]	64<, 1
Til	1	Til, Str, P	1[c]	32, 64, 1
E, Til, T	1	E, Til, Str, T	1	24, 32, 64, 12
E, Til, Str, T, P, A	2	E, Til, Str, T, P, A	2	32, 32, 64, 32, 24, 32
E, Til, Str, T	1	E, Til, Str, T, P, A	1[d]	32, 32, 64, 8, 4, 1.5

a: Isolates found to be resistant by E-test but susceptible by disk diffusion; b: Isolates susceptible to Penicillin and Tilmicosin in the disk diffusion test; c: Isolate susceptible to Penicillin and Streptomycin in the disk diffusion test; d: Isolate susceptible to Penicillin and Ampicillin in the disk diffusion test; n: Number of isolates

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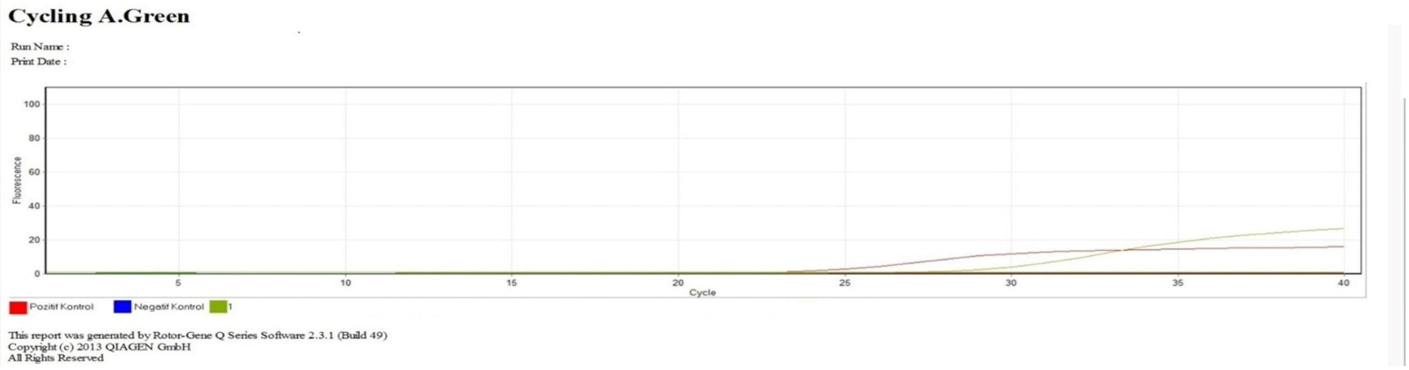
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Figure 3. Positive real-time PCR amplification curve of the *erm*-42 macrolide resistance gene in a *M. haemolytica* isolate

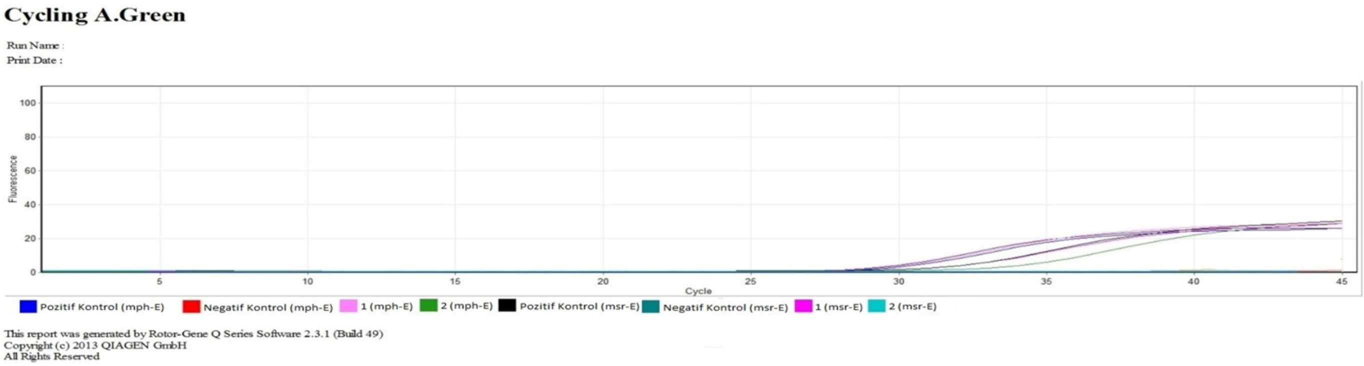
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Figure 4. Positive real-time PCR amplification curve of the *mph*-E ve *msr*-E macrolide resistance genes in a *M. haemolytica* isolate

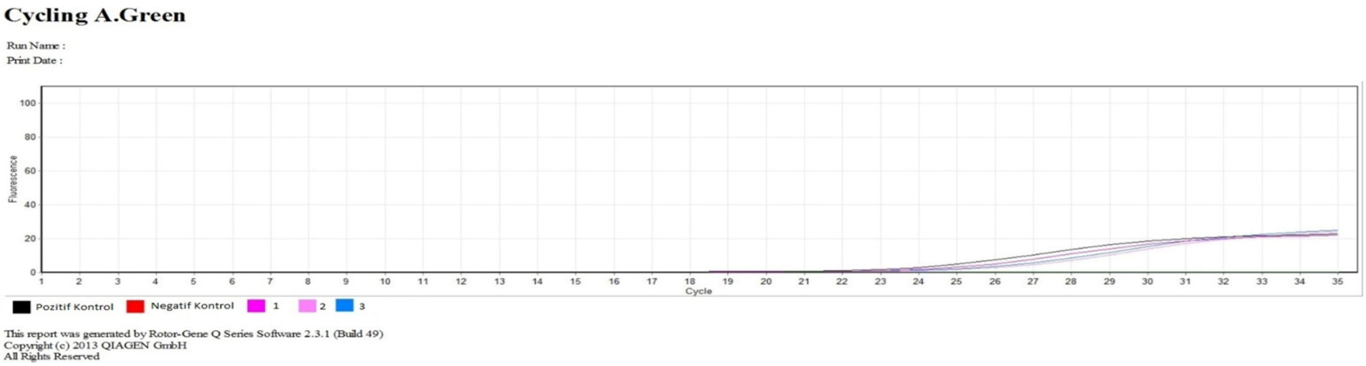
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Figure 5. Positive real-time PCR amplification curve of the *Str*-A aminoglycoside resistance gene in a *M. haemolytica* isolate

Table 6. Distribution of resistance gene profiles among *M. haemolytica* isolates showing phenotypic resistance by E-test

Antibiotic resistance profile	n:9	MIC (µg/ml)	Resistance gene profile
E	1	16	*mph*E
P	2	1, 1.5	-
Til, P	1	64<, 1	-
Til, **Str, P**	1	32, 64, 1	*erm*42, *str*A
E, Til, **Str, T**	1	24, 32, 64, 12	*erm*42, *str*A
E, Til, Str, T, P, A	2	32, 32, 64, 32, 24, 32	*mph*E, *msr*E, *str*A
E, Til, Str, T, **P, A**	1	32, 32, 64, 8, 4, 1.5	*mph*E, *msr*E, *str*A

*: The isolates found to be susceptible in the disk diffusion test are indicated in bold

Discussion

This study primarily aimed to investigate antibiotic resistance and resistance-associated genes in *Mannheimia haemolytica* within the One Health framework, emphasizing the critical link between animal and human health.

The findings reveal significant resistance to commonly used antibiotics, particularly macrolides and aminoglycosides, which may have profound implications for both veterinary and public health.

M. haemolytica, recognized as a major etiological agent of bovine respiratory disease complex (BRDC), may also present zoonotic potential, particularly in individuals with compromised immune systems [14,35]. It is therefore crucial to monitor its antimicrobial resistance patterns to prevent treatment failures in veterinary practice and limit the potential spread to human populations.

According to the E-test results, resistance was most frequently observed against penicillin, tilmicosin, erythromycin and streptomycin, and tetracycline. All isolates were susceptible to ceftazidime, enrofloxacin, gentamicin, and sulfamethoxazole-trimethoprim. Comparable results were observed in the studies conducted by Marshall et al. [36] and Girma et al. [37], which indicated that susceptibility to fluoroquinolones and cephalosporins remained consistently high.

The findings of this study, when evaluated in light of previous reports [38,39], reveal patterns of antibiotic resistance and resistance-associated genes in *Mannheimia haemolytica* that are largely consistent with existing literature, while also underscoring the necessity of interpreting such results within the broader One Health framework.

The findings underscored the emergence of multidrug resistance, reinforcing the concern from a One Health perspective. This is consistent with the increasing trend of MDR in veterinary pathogens, which may be attributed to the overuse or misuse of antibiotics in livestock. Studies by Melchner et al. [39] and Kostova et al. [40] have emphasized the role of irrational antimicrobial use in the development of MDR phenotypes in *M. haemolytica*.

In this study, some discrepancies were observed between the disk diffusion and E-test results, particularly for penicillin and tilmicosin, emphasizing the need for standardized testing protocols and careful interpretation of susceptibility data. Isolates resistant in E-test but susceptible in disk diffusion showed significantly higher MIC values, underlining the importance of MIC-based evaluation in resistance surveillance.

The outcomes of this research carry significant relevance for public health considerations. The identification of macrolide and aminoglycoside resistance genes in *M. haemolytica*, a pathogen with recognized zoonotic potential, raises concerns about the possible transmission of these resistance determinants to human-associated bacterial populations. This could lead to therapeutic challenges and limit the effectiveness of commonly used antimicrobials in both human and veterinary medicine.

In alignment with global strategies aimed at mitigating antibiotic resistance, there is an urgent necessity for comprehensive action in the veterinary field. This includes the strict regulation and oversight of antibiotic use in animal health practices, the establishment of robust surveillance systems to monitor resistance patterns, and the promotion of judicious antibiotic use supported by targeted education for veterinary professionals. Furthermore, investigating alternative therapeutic approaches such as bacteriophage therapy and the application of probiotics holds promise in reducing antibiotic dependency. Strengthening biosecurity and implementing effective infection control protocols at the farm level are also critical components in limiting the emergence and spread of resistant pathogens.

Conclusion

In conclusion, the emergence of antimicrobial resistance and the spread of resistance-associated genes in zoonotic pathogens such as *M. haemolytica* pose a significant threat to both animal and human health. The potential dissemination of these genes through horizontal gene transfer highlights the complexity of the problem and the risk of cross-species transmission. Addressing this challenge requires a comprehensive One Health approach that integrates veterinary, medical, and environmental disciplines to ensure effective prevention and control strategies.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
This research was approved by the Local Ethics Committee for Animal Studies of Van Yüzüncü Yıl University (approval date: 01.03.2018, decision number: 02).

Acknowledgments:
This study constitutes a part of the first author's PhD dissertation. A portion of this study was presented as an oral presentation at the 14th National Veterinary Microbiology Congress, held on October 13–16, 2020.

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