

## RESEARCH ARTICLE

# Determination of MIC Values of Various Antimicrobial Agents and Presence of Resistance Genes in *Pasteurella multocida* Strains Isolated from Bovine <sup>[1]</sup>

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## Abstract

*Pasteurella multocida* is an important bacterium that can cause respiratory infections in cattle. Due to the usage of antimicrobial agents in the treatment of the disease frequently, it is critical to follow the antimicrobial susceptibility of the isolates. In this study, minimal inhibitory concentrations (MIC) of various antimicrobial agents and presence of genes related to resistance were investigated in 59 *P. multocida* strains isolated from the respiratory tract of cattle. According to MIC values determined by E-test, all of the isolates were susceptible to enrofloxacin, chloramphenicol and gentamicin, but resistant to ceftiofur. In addition, high resistance to ampicillin (88.14%), tilmicosin (64.41%), clindamycin (83.05%) and streptomycin (59.32%) were observed in the isolates. When the resistance genes were examined by PCR, it was determined that *bla<sub>ROB-1</sub>*, *tet H*, *sul II*, *str A/aphA 1* and *erm 42* genes could play an important role in penicillin, tetracycline, sulfamethoxazole + trimethoprim, aminoglycoside and macrolide resistance, respectively. It was concluded that the usage of ampicillin, tetracycline, sulfamethoxazole + trimethoprim, macrolide and aminoglycosides should be considered for the treatment of respiratory tract infections caused by *P. multocida* in cattle. Also, it was determined that antimicrobial resistance genes could play an important role in the development of resistance in *P. multocida*.

**Keywords:** *Pasteurella multocida*, Antimicrobial susceptibility, MIC, Resistance gene

## Siğirlardan İzole Edilen *Pasteurella multocida* Suşlarında Çeşitli Antimikrobiyal Maddelerin MİK Değerlerinin ve Antimikrobiyal Direnç Genlerinin Belirlenmesi

### Öz

*Pasteurella multocida*, siğirlarda solunum yolu enfeksiyonlarına neden olan önemli bir bakteriyel etkindir. Hastalığın tedavisinde sıklıkla antimikrobiyal tedavi uygulanması nedeniyle etkene yönelik antimikrobiyal duyarlılık sonuçlarının takip edilmesi kritik öneme sahiptir. Bu çalışmada, siğirların solunum yolundan izole edilen 59 adet *P. multocida* izolatında çeşitli antimikrobiyal maddelerin minimal inhibitör konsantrasyonları (MİK) ve antimikrobiyal direnç ile ilişkili genlerin varlığı araştırıldı. E-test yöntemiyle belirlenen MİK değerlerine göre izolatların tamamı enrofloxacin, chloramphenicol ve gentamicine duyarlı, ceftiofur'a ise dirençli bulundu. Ayrıca ampicillin (%88.14), tilmicosin (%64.41), clindamycin (%83.05) ve streptomycin (%59.32) yüksek oranda direnç tespit edildi. PCR ile antimikrobiyal direnç genlerinin varlığı incelendiğinde ise penicillin, tetracycline, sulfamethoxazole + trimethoprim, aminoglikozid ve makrolid direncinde sırasıyla *bla<sub>ROB-1</sub>*, *tet H*, *sul II*, *str A/aphA 1* ve *erm 42* genlerinin önemli rol oynadığı belirlendi. Bu çalışmada, siğirlarda *P. multocida* suşlarının neden olduğu solunum yolu enfeksiyonlarının tedavisinde ampicillin, tetracycline, sulfamethoxazole + trimethoprim ile makrolid ve aminoglikozid antibiyotiklerin kullanımına dikkat edilmesi gerektiği sonucuna varıldı. Ayrıca, antimikrobiyal direnç ile ilişkili genlerin izolatlarda direnç gelişiminde önemli rol oynadığı belirlendi.

**Anahtar sözcükler:** *Pasteurella multocida*, Antimikrobiyal duyarlılık, MİK, Direnç genleri

## INTRODUCTION

Respiratory disease of cattle is one of the infections leading to significant economic losses in cattle breeding.

It is known that bacterial and viral factors, as well as stress factors caused by improper transport, weaning, and nutritional conditions are also involved in the etiology of this disease <sup>[1]</sup>. *Pasteurella multocida* is one of the

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bacterial agent that can cause respiratory disease in cattle [2].

There are a limited number of vaccine types that can achieve a specific immune response in the control of infections caused by *P. multocida*. Due to a wide host spectrum and having different capsular polysaccharides can affect the achievement of the vaccine negatively [3]. Therefore, antimicrobial therapy is often preferred for the treatment and control of pasteurellosis cases.

Prolonged and uncontrolled usage of antimicrobial agents can lead to development of resistance in isolates [4]. Because laboratory tests are time consuming, veterinarians have to use a broad spectrum antimicrobial agents, especially in the treatment of acute infections, which leads to the development of resistance in isolates. For this reason, it is critical that the antimicrobial susceptibility of *P. multocida* isolates should be monitored in national and international aspect, periodically [3].

It is known that the genes which can be located in chromosomal DNA or extra chromosomal structures in bacteria can also cause antimicrobial resistance. Aminoglycoside resistance genes (*str A*, *str B*, *aadA 14*, *aphA 1*, *aad B* and *aadA 25*) [5-7], macrolide resistance genes (*erm 42*, *msr E*, *mph E*, *erm A* and *erm C*) [6,8-10], tetracycline resistance genes (*tet H*, *tet B*, *tet M*, *tet C*, *tet L* and *tet O*) [3,5,8,11],  $\beta$ -lactam resistance gene (*bla<sub>ROB-1</sub>*) [12] and sulfonamide resistance gene (*sul II*) [3] have been reported to be associated with the antimicrobial resistance in *Pasteurellaceae* family.

In Turkey, there are various researches [13-15] that were conducted on identification of bacterial agents causing respiratory diseases in cattle and determination of their antimicrobial susceptibilities by disc diffusion method that can be obtained qualitative data about antimicrobial susceptibilities. However, investigation of MIC values of antimicrobial agents and the presence of the genes associated with the antimicrobial resistance can make to be clarified resistant mechanisms in bacterial agents and offers quantitative data.

In this study, MIC values of various antimicrobial agents and the presence of genes related to the antimicrobial resistance in *P. multocida* isolates isolated from the respiratory tract of cattle in Van, Turkey were investigated.

## MATERIAL AND METHODS

In this study, 59 *P. multocida* strains isolated from swab samples of upper and lower respiratory tract of the cattle between 2016 and 2019, were used. Nineteen of the isolates were obtained from nasal swab samples of cattle that had pneumonia symptom clinically. Also, 32 and 8 strains were isolated from nasal swabs and trachea-bronchial swabs of slaughtered cattle, respectively. This study was approved by Van Yuzuncu Yil University Animal Researches Local Ethic Committee with the number of 2019/01.

Preliminary identification of the isolates were performed according to hemolytic activity on blood agar, Gram staining, oxidase reaction and growth on MacConkey agar [16]. PCR method reported by Townsend et al. [17] was used for the identification of the isolates at the species level.

### Determination of MIC Values

MIC values of penicillin, ampicillin, tetracycline, sulfamethoxazole + trimethoprim, cephalotin, cefotaxime, ceftiofur, enrofloxacin, ciprofloxacin, erythromycin, tilmicosin, clindamycin, chloramphenicol, streptomycin and gentamicin were determined by using E-test strip (Himedia, India and Liofilchem, Italy). The criteria of European Committee on Antimicrobial Susceptibility Testing [18] and Clinical Laboratory Standards Institute [19,20] were considered in applying and evaluating the tests. For determination of MIC values using E-test method, overnight culture of the isolates on Columbia blood agar (Oxoid, CM 0331, England) supplemented with 5% defibrinated sheep blood were suspended into 2 mL sterile physiological saline (pH:7.0) and the suspension was adjusted to McFarland 0.5 turbidity. Then, 0.1 mL of suspension was inoculated Mueller Hinton agar (Oxoid, CM0337, England) supplemented with 5% defibrinated sheep blood. E-test strip was placed on the agar and incubated at 37°C for 18-24 h. After incubation period, the point where the inhibition ellipse intersected the strip was accepted as the MIC value. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, supplied from culture collection of Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Microbiology, were used as control strains.

### Determination of Antimicrobial Resistance Genes

The genes that were related to antimicrobial resistance were investigated by PCR using gene specific primer (Table 1). Genomic DNA was obtained by boiling method. For this purpose, *P. multocida* colonies were picked from Columbia blood agar and mixed into 200  $\mu$ L PCR water. Then, suspension was boiled at 100°C in a dry block for 10 min. After chilled on ice, suspension was centrifuged at 10,000 X g for 5 min and supernatant was used as genomic DNA. PCR mixture was consisted of 9.5  $\mu$ L of mastermix (Abm® 2X PCR Taq Plus Mastermix), 5  $\mu$ L of genomic DNA and 1  $\mu$ L of each primer (10  $\mu$ M) and the total volume was completed to 25  $\mu$ L with PCR water. Pre-denaturation was performed at 94°C for 5 min and the final extension was performed at 72°C for 10 min. The amplification process that was applied for each gene was shown in Table 1. Amplicons were electrophoresed in a 1.5% agarose gel at 80 V for 1.5 h and visualized in a gel imaging system (Spektrolin, GL-500).

## RESULTS

MIC values of penicillin, ampicillin, tetracycline, sulfamethoxazole + trimethoprim, cephalotin, ceftiofur,

Table 1. Primers used for the determination of antimicrobial resistance genes by PCR				
Gene	Oligonucleotid (5'-3')	bp	PCR Conditions (denaturation/annealing/elongation)	Reference
<b>β-lactamase</b>				
<i>bla<sub>ROB-1</sub></i>	F: CATTAAACGGCTTGTTCGC R: CTTGCTTTGCTGCATCTTC	852	94°C-30 sec/50°C-30 sec/72°C-30 sec 25 cycles	[21]
<b>Sulfonamide</b>				
<i>sul II</i>	F: ACAGTTTCTCCGATGGAGGCC R: CTCGTGTGTGCGGATGAAGTC	704	94°C-60 sec/56°C-60 sec/72°C-60 sec 30 cycles	[22]
<b>Tetracycline</b>				
<i>tet B</i>	F: CCTTATCATGCCAGTCTTGCC R: ACTGCCGTTTTTTTCGCC	774	94°C-30 sec/53°C-30 sec/72°C-90 sec 25 cycles	[23]
<i>tet H</i>	F: ATACTGCTGATCACCGT R: TCCCAATAAGCGACGCT	1076	94°C-60 sec/47°C-60 sec/72°C-60 sec 30 cycles	[11]
<i>tet M</i>	F: GTTAAATAGTGTCTTGGAG R: CTAAGATATGGCTCTAACAA	657	94°C-30 sec/48°C-30 sec/72°C-90 sec 30 cycles	[24]
<b>Macrolide</b>				
<i>erm 42</i>	F: TGCACCATCTTACAAGGAGT R: CATGCCGTCTTCAAGGTTT	173	94°C-30 sec/51°C-30 sec/72°C-45 sec 25 cycles	[10]
<i>msr E</i>	F: TATAGCGACTTTAGCGCAA R: GCCGTAGAATATGAGCTGAT	395	94°C-30 sec/52°C-30 sec/72°C-30 sec 25 cycles	[10]
<i>mph E</i>	F: ATGCCAGCATATAATCGC R: ATATGGACAAAGATAGCCCG	271	94°C-30 sec/52°C-30 sec/72°C-45 sec 25 cycles	[10]
<b>Aminoglycoside</b>				
<i>str A</i>	F: TGACTGGTTGCCTGTCAGAGG R: CAGTTGTCTTCGGCGTTAGCA	646	94°C-60 sec/57°C-60 sec/72°C-60 sec 30 cycles	[22]
<i>aph A1</i>	F: GCCGTTTCTGTAATGAAGGAG R: GGCAATCAGGTGCGACAATCT	642	94°C-30 sec/55°C-30 sec/72°C-30 sec 25 cycles	[25]

cefotaxime, enrofloxacin, ciprofloxacin, erythromycin, tilmicosin, clindamycine, chloramphenicol, streptomycin and gentamicin were determined as 0.125 - >256, 0.125 - >256, 0.25 - 32, 0.004 - 32, 0.016 - 32, 0.064 - >256, 0.002 - 0.094, 0.002 - 0.50, 0.002 - 3, 0.032 - >256, 2 - >32, 1.5 - >256, 0.25 - 8, 2 - >256, ve 0.19 - 2 µg/mL in *P. multocida* isolates, respectively (Table 2). According to these values, all of the isolates were found to be susceptible to enrofloxacin, chloramphenicol and gentamicine, but resistant to cefoxitin. In addition, 4 (6.77%), 52 (88.14%), 21 (35.59%), 23 (38.98%), 1 (1.69%), 2 (3.39%), 14 (23.73%), 18 (30.51%), 38 (64.41%), 49 (83.05%) and 35 (59.32%) of the isolates were resistant to penicilin, ampicillin, tetracycline, sulfamethoxazole + trimethoprim, cephalotin, cefotaxime, ciprofloxacin, erythromycin, tilmicosin, clindamycine and streptomycine, respectively (Table 3).

Distribution of antimicrobial resistance genes in the isolates were shown in Table 4.

*Bla<sub>ROB-1</sub>* gene was detected in 3 of 4 isolates that were resistant to both penicillin and ampicillin. However 48 isolates, found to be resistant to ampicillin only, did not harbour *bla<sub>ROB-1</sub>* gene.

*Tet H* gene were detected in 20 of the 21 tetracycline resistant isolates, but *tet B* gene was found only in 1 of

these isolates. *Tet M* could not be found in any of these resistant isolates.

*Sul II* gene was found in all 23 of the isolates which were determined to be resistant to sulfamethoxazole + trimethoprim.

Whereas eleven of 18 erythromycin resistant isolates harboured *erm 42* gene only, both *msr E* and *mph E* gene were detected only in 4 of the resistant isolates. Also, 12 and 4 of 38 isolates resistant to tilmicosine were observed to harbour *erm 42* and *msr E/mph E* genes, respectively. *Erm 42* and *msr E/mph E* genes were detected in 12 and 3 of the 49 isolates resistant to clindamycin, respectively but, macrolide resistance genes could not be found in the rest of the isolates. Additionally, 11 of the 17 *P. multocida* isolates resistant to both erythromycin, tilmicosine and clindamycin were determined to harbour *erm 42* gene, but *msr E* and *mph E* genes were detected only in 3 of macrolide resistant isolates. Any of macrolide resistance genes were not determined in other 3 of 17 macrolide resistant isolates (data not shown).

All of *P. multocida* isolates were susceptible to gentamicin, but 35 isolates were found to be resistant to streptomycin. The *str A* gene was determined in all streptomycin resistant isolates, while the *aph A1* gene was detected in 34 isolates.

**Table 2.** Distribution of MIC value of antimicrobial agents in *P. multocida* isolates

Antimicrobial	MIC (µg/ml)																																			
	0.002	0.003	0.004	0.006	0.008	0.012	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.50	0.75	1	1.5	2	3	4	6	8	12	16	24	32	>32	48	64	128	>256		
P	-	-	-	-	-	-	-	-	-	-	-	-	4	9	17 <sup>*</sup>	22	3	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
AMP	-	-	-	-	-	-	-	-	-	-	-	-	2	5	18 <sup>*</sup>	24	6	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
TE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	9	8	4	-	-	-	3	4	7	3 <sup>*</sup>	7	5	5	1	-	-	-	-	-	-	-
SXT	-	-	1	-	1	2	1	3	2	8	5	1	2	1	9 <sup>*</sup>	4 <sup>*</sup>	5	3	4	1	1	1	-	-	-	-	-	-	-	-	4	-	-	-	-	-
KF	-	-	-	-	-	1	1	1	-	2	5	7	13	9	8	3	5	3	1	-	-	-	-	-	-	-	-	-	-	1 <sup>*</sup>	-	-	-	-	-	-
FOX	-	-	-	-	-	-	-	-	-	-	1	-	2	2	14	15	17	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CTX	20	1	9	11	2	4	6	3	1 <sup>*</sup>	1 <sup>*</sup>	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ENR	4	4	10	12	8	1	4	6	6	3	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIP	2	-	-	2	6	9	10	5	4	4	3 <sup>*</sup>	5 <sup>*</sup>	4	-	3	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	7	15	15	3	-	-	-	-	-	1 <sup>*</sup>	2	1	2	-	3	3	-	-	6	
TIL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	5	7	2	4	-	-	-	-	-	38 <sup>*</sup>	-	-	-	-	-	
CLI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	6	7 <sup>*</sup>	6	3	2	1	-	-	-	-	-	-	-	-	-	30
CHL	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	13	27	10	2	-	2	-	1	1 <sup>*</sup>	-	-	-	-	-	-	-	-	-	-	-	-
S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	2	2	8	6	-	3 <sup>*</sup>	-	-	-	-	-	-	35	
GEN	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	6	17	18	13	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

P: Penicillin, AMP: Ampicillin, TE: Tetracycline, SXT: Sulfamethoxazole+trimethoprim, KF: Cephalothin, Fox: Cefoxitin, CTX: Cefotaxime, ENR: Enrofloxacin, CIP: Ciprofloxacin, E: Erythromycin, TIL: Tilimicosin, CLI: Clindamycin, CHL: Chloramfenikol, S: Streptomycin, GEN: Gentamicin  
 Underlined Value: MIC<sub>50</sub>  
 Bold Written Value: MIC<sub>90</sub>  
 \*: Interpretive criteria

**Table 3.** Antimicrobial susceptibilities of *P. multocida* determined by E-test

Antimicrobial Agent	S (%)	I (%)	R (%)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)
P <sup>†</sup>	30 (50.84)	25 (42.37)	4 (6.7)	0.25	0.50
AMP <sup>†</sup>	0	7 (11.86)	52 (88.14)	0.38	0.50
TE <sup>†</sup>	24 (40.68)	14 (23.73)	21 (35.59)	4	16
SXT <sup>††</sup>	36 (61.02)	0	23 (38.98)	0.25	1.5
KF <sup>†††</sup>	58 (98.31)	0	1 (1.69)	0.19	0.50
FOX <sup>*,††</sup>	0	0	59 (100)	0.38	0.75
CTX <sup>††</sup>	57 (96.61)	0	2 (3.39)	0.004	0.016
ENR <sup>†</sup>	58 (98.31)	1 (1.69)	0	0.006	0.032
CIP <sup>††</sup>	45 (76.27)	0	14 (23.73)	0.023	0.125
E <sup>†††</sup>	8 (13.56)	33 (55.93)	18 (30.51)	1	64
TIL <sup>†</sup>	21 (35.59)	0	38 (64.41)	>32	>32
CLI <sup>†††</sup>	0	10 (16.95)	49 (83.05)	>256	>256
CHL <sup>†</sup>	59 (100)	0	0	0.75	1
S <sup>††††</sup>	24 (40.68)	0	35 (59.32)	>256	>256
GEN <sup>†††</sup>	59 (100)	0	0	1	1.5

P: Penicilin, AMP: Ampicillin, TE: Tetracycline, SXT: Sulfamethoxazole + trimethoprim, KF: Cephalothin, Fox: Cefoxitin, CTX: Cefotaxime, ENR: Enrofloxacin, CIP: Ciprofloxacin, E: Erythromycin, TIL: Tilmicosin, CLI: Clindamycin, CHL: Chloramphenicol, S: Streptomycin, GEN: Gentamicin

\* Interpretive criteria for Cefotaxime was taken into consideration; <sup>†</sup> Interpretive criteria reported by CLSI, 2018<sup>[20]</sup> was taken into consideration; <sup>††</sup> Interpretive criteria reported by EUCAST, 2019<sup>[18]</sup> was taken into consideration; <sup>†††</sup> Interpretive criteria reported by CLSI, 2002<sup>[19]</sup> was taken into consideration; <sup>††††</sup> Interpretive criteria reported by Benedict et al.<sup>[26]</sup> was taken into consideration

**Table 4.** Presence of antimicrobial resistance genes in *P. multocida* isolates

Antimicrobial Agent	Resistance Genes	Number of Isolates	MIC (µg/mL)	
β-lactam	Penicillin	Phenotypic Resistance	4	12 - >256
		<i>bla<sub>ROB-1</sub></i>	3	16 - >256
	Ampicillin	Phenotypic Resistance	52	0.25 - >256
		<i>bla<sub>ROB-1</sub></i>	3	32 - >256
	Tetracycline	Phenotypic Resistance	21	8 - 32
		<i>tet B</i>	1	24
		<i>tet H</i>	20	3 - 32
		<i>tet M</i>	0	-
	Sulfonamide	Sulfamethoxazole + Trimethoprim	Phenotypic Resistance	23
<i>sul II</i>			23	0.38 - >32
Macrolide	Erythromycin	Phenotypic Resistance	18	8 - >256
		<i>erm 42</i>	11	32 - >256
		<i>msr E + mph E</i>	4	8 - 24
	Tilmicosin	Phenotypic Resistance	38	>32
		<i>erm 42</i>	12	>32
		<i>msr E + mph E</i>	4	>32
	Clindamycin	Phenotypic Resistance	49	4 - >256
		<i>erm 42</i>	12	>256
		<i>msr E + mph E</i>	3	2 - >256
Aminoglycoside	Streptomycin	Phenotypic Resistance	35	>256
		<i>str A</i>	35	>256
		<i>aphA 1</i>	34	>256
	Gentamicin	Phenotypic Resistance	0	0.19 - 2

## DISCUSSION

Because the identification and determination of antimicrobial susceptibility of the bacterial agents usually take a long time, the usage of broad-spectrum antimicrobial agents is preferred for the treatment of acute clinical disease and this can lead to the development of antimicrobial resistance in bacteria. Therefore, antimicrobial susceptibility of the bacterial agents and the determination of the MIC values of antimicrobial agents used for the treatment of bacterial infections, have a critical importance in national and international area. In this study, antimicrobial susceptibility of *P. multocida* strains isolated from bovine respiratory tract were evaluated by the determination of MIC values of various antimicrobial agents. Additionally, genes related to antimicrobial resistance were investigated to identify possible resistance mechanisms developing in the strains.

Yoshimura et al.<sup>[27]</sup> reported that MIC values of penicillin, dihydro-streptomycin, oxytetracycline and tilmicosin in *P. multocida* strains were 0.05-25 unit/mL, 0.39 - >100, 0.1-25 and 0.1-100 µg/mL, respectively. In another study, MIC values of tetracycline, tilmicosin and sulfamethoxazole + trimethoprim were determined as 0.06-256, 1-128 and 0.015-1 µg/mL, respectively<sup>[28]</sup>. Anholt et al.<sup>[29]</sup> found that MIC values of penicillin, ampicillin, tilmicosin, clindamycin and gentamicin were ≤0.12-8, 0.25-8, 4-64, 8-16 and 1-16 µg/mL, respectively. In another study and MIC values of oxytetracycline and ampicillin were 0.25 - >512 and 0.125-128 µg/mL, respectively. In the research, *tet* H gene was found in 89% of oxytetracycline resistant isolates, while *tet* B gene was reported to be detected in 4.76% of them. Also, 16 of 22 ampicillin resistant isolates were reported to be harboured *bla*<sub>ROB-1</sub> gene<sup>[30]</sup>.

In the presented study, phenotypic and genotypic findings about resistance to penicillin were similar to the findings reported by Dayao et al.<sup>[31]</sup>, whereas the MIC values of penicillin and ampicillin (0.125 - >256 µg/mL) was higher than the values reported by Anholt et al.<sup>[29]</sup> and Katsuda et al.<sup>[30]</sup>. Also, that the genes associated with resistance to β-lactam antibiotics are mostly encoded by plasmids, may cause that these genes are found in a low level in chromosomal DNA of ampicillin resistant isolates.

In this study MIC value of tetracycline was found to be lower than those of reported by Garch et al.<sup>[28]</sup> and Katsuda et al.<sup>[30]</sup>. However, this value was higher than that of reported for oxytetracycline by Yoshimura et al.<sup>[27]</sup>. *Tet* H gene was detected in 20 (95.2%) of 21 tetracycline resistant isolates, while *tet* B gene was only found in 1 (4.8%) isolate. Additionally, *tet* M gene could not be detected in the isolates. These findings were similar to the findings reported by Katsuda et al.<sup>[30]</sup>. In contrast to this study, Dayao et al.<sup>[31]</sup> reported that *tet* H gene was not detected whereas *tet* B gene was found in 57% of the examined isolates. As

in our study, Dayao et al.<sup>[31]</sup> reported that *tet* M gene could not be detected in tetracycline resistant isolates.

In the presented study, the MIC value of sulfamethoxazole + trimethoprim was observed to be similar to value reported by Garch et al.<sup>[28]</sup>. However, it was observed that value detected for sulfamethoxazole + trimethoprim was highly lower than MIC value of sulfamethoxazole (≥512 µg/mL) reported by Kehrenberg and Schwarz<sup>[22]</sup>. It was assumed that the use of sulfamethoxazole without trimethoprim could lead to this difference. However, as in our study, *sul* II gene was reported to be detected in all resistant isolates in both studies.

Generally, MIC value of enrofloxacin was found to be low<sup>[27-29]</sup> and resistance to this antimicrobial agent was not significant in *P. multocida* isolates<sup>[32-33]</sup>. As indicated previous studies, MIC value of enrofloxacin was determined as 0.002-0.5 µg/mL and no isolates were found to be resistant to enrofloxacin in this study.

MIC values of streptomycin in *P. multocida* isolates were reported as 0.39 - >100, ≥128 and 1-32 µg/mL by Yoshimura et al.<sup>[27]</sup>, Kehrenberg and Schwarz<sup>[22]</sup> and Wang et al.<sup>[25]</sup>, respectively. But, in this study, this value was determined to be higher (2 - >256 µg/mL). Also, in the presented research, *str* A ve *aph* A 1 (excepting 1 isolate) genes were found in all streptomycin resistant isolates same as in other studies<sup>[22,25]</sup>. MIC value (0.19-2 µg/mL) of gentamicin was found to be lower than reported by Wang et al.<sup>[28]</sup> and Anholt et al.<sup>[28]</sup>.

Kadlec et al.<sup>[34]</sup> reported that 8 to 32-fold increase were determined in MIC values of erythromycin, tilmicosin and clindamycin when *erm* 42 gene was cloned into *P. multocida* isolates via plasmid vector. It was also reported that the MIC values of erythromycin and tilmicosin increased to 32-128 times when *msr* E+*mph* E genes were cloned. In another study, it was reported that MIC value of tilmicosin ranged from 128 to >128 µg/mL in *erm* 42 positive isolates while that was 32 µg/mL in *msr* E+*mph* E positive isolates. Additionally, in isolates were positive for all three genes, MIC value of tilmicosin was reported to be >128 µg/mL<sup>[10]</sup>. Similarly, in another study it was revealed that MIC values of tilmicosin and clindamycin in *erm* 42 positive isolates were 128 - >128 and 1024 µg/mL, respectively. It was also reported that MIC values of tilmicosin and clindamycin were 32 and 16 µg/mL in *msr* E and *mph* E genes positive isolates, respectively. In addition, these values were determined as 128 and >1024 µg/mL in the isolates harbouring all those three genes<sup>[9]</sup>.

In this study, MIC value of erythromycin was detected as 32 - >256 µg/mL in *erm* 42 positive isolates. While this gene was determined in 12 of 38 tilmicosin resistant isolates and 11 of 49 clindamycin resistant isolates, MIC values for both antibiotics were found to be >32 µg/mL and >256 µg/mL, respectively. On the other hand, MIC value of erythromycin

varied from 8 to >24 µg/mL in erythromycin resistant isolates that were positive for *msr E*+*mph E*. Whereas both genes were determined in 4 tilmicosin resistant and in 3 clindamycin resistant isolates, MIC values of both antibiotics were detected as >32 µg/mL and 2 - >256 µg/mL, respectively. However, Dayao et al.<sup>[31]</sup> reported that *msr E* and *mph E* genes could not be detected in *P. multocida* isolates that were resistant to macrolides.

Although in this study macrolide resistance in *P. multocida* isolates were determined to be higher than that of reported by other researcher, the presence of resistance genes were observed in a limited number. It was assumed that other genes or different resistance mechanisms<sup>[6,8,9]</sup> could play a role in the development of resistance.

In this study, it was determined that *P. multocida* isolates that cause respiratory diseases in cattle was highly susceptible to penicillin, cephalothin, cefotaxime, chloramphenicol, gentamicin and enrofloxacin. Also, it was determined that it should be paid attention to the use of ampicillin, tetracycline, sulfamethoxazole + trimethoprim, macrolide and aminoglycoside antibiotics for the treatment of infections caused by this agent. Although the genes associated with tetracycline, sulfonamide and aminoglycoside resistance have an important role in the development of resistance in *P. multocida* isolates, the presence of resistance genes in extra chromosomal elements as well as other mechanisms that are responsible for macrolide and β-lactam antibiotics should be investigated in further studies.

## CONFLICT OF INTEREST

There is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All authors contributed to laboratory examinations and writing manuscript.

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