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Original article

Determination of antimicrobial susceptibility and virulence-related genes of *Trueperella pyogenes* strains isolated from various clinical specimens in animals

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Abstract

In this study, a total of 32 *Trueperella pyogenes* strains isolated from different disease specimens in cattle, sheep and goats were examined. Antimicrobial susceptibility of the isolates to 10 antimicrobials were determined using the E-test method and MIC values of the antimicrobials were investigated. The genes that play a role in the antimicrobial resistance or virulence of *T. pyogenes* were determined by PCR using gene specific primers. In the study, all the isolates were susceptible to penicillin and cephalosporin. The highest resistance rate in the isolates was determined against streptomycin (56.25%) and tetracycline (53.12%) and MIC_{90} values for these antimicrobials were found to be >256 µg/ml and 12 µg/ml, respectively. The *ermX* gene was found to be positive in 8 (80%) of 10 isolates that were resistant to macrolide group antimicrobials. Among 20 aminoglycoside resistant isolates, *aadA1*, *aadA9*, *strA-strB*, and *aac(6')-aph(2")* genes were determined in 5 (25%), 14 (70%), 7 (35%) and 1 (5%) of the isolates, respectively. When the presence of virulence-related genes in the isolates was examined, *nanP* (93.75%), *fimA* (93.75%) and *plo* (90.62%) genes were detected in the majority of the isolates. While the *cbpA* gene was negative in all isolates, the *fimG* gene was found in a limited number of the isolates (15.62%). It was concluded that streptomycin and tetracycline resistance should be considered in *T. pyogenes* isolates. Also, *nanP*, *fimA* and *plo* genes may have an important role in the pathogenesis of the infections.

Keywords: antimicrobial susceptibility *Trueperella pyogenes*, virulence-related genes

Introduction

Trueperella pyogenes (*T. pyogenes*), previously known as *Arcanobacterium pyogenes* (Ramos et al. 1997), *Actinomyces pyogenes* and *Corynebacterium pyogenes* (Rogosa et al. 1974) is Gram positive, coccobacilli, non-spore forming, non-motile, non-capsulated and facultative anaerobic microorganism which can be found as a commensal on the skin, and urogenital and upper respiratory tract of animals (Silva et al. 2008).

T. pyogenes strains are isolated from pneumonia cases and abscess as well as metritis and endometritis. Also, the bacterium is identified as an etiological agent of 'summer mastitis' in cattle (Zambrano et al. 2011, Rzewuska et al. 2016, Tamai et al. 2018). *T. pyogenes* can cause purulent infections such as encephalitis, pyelonephritis, lenfadenitis, endocarditis, arthritis and omphalitis in cattle (Hijazin et al. 2011, Risseti et al. 2017, Tamai et al. 2018). Similarly, it is also isolated from abscesses, genital tract infections, pneumonia, arthritis, mastitis and septicemia cases in small ruminants (Urumova et al. 2009, Ribeiro et al. 2015, Risseti et al. 2017).

Aminoglycoside, beta-lactam, tetracycline, macrolide and fluoroquinolone group antimicrobial agents are frequently used in the treatment of *T. pyogenes* infections. However, resistant isolates have caused problems in terms of effective antimicrobial therapy for *T. pyogenes* infections in recent years (Kwiecien et al. 2020). In addition to the overuse of antibiotics, it is known that various genes in genomic DNA and extra-chromosomal elements are also known to play a role in the formation of resistance in the isolates. The *tetW* gene in the transposon causes tetracycline resistance (Billington and Jost 2006), while the *ermB* and *ermX* genes in the plasmid or transposon cause macrolide resistance (Jost et al. 2003). However, integrons play an important role in resistance against trimethoprim, chloramphenicol, aminoglycoside and beta-lactam group antibiotics (Liu et al. 2009, Zhao et al. 2011, Dong et al. 2017).

Virulence factors that are responsible for pathogenesis of *T. pyogenes* infections have been identified as pyolysin, protease, neuraminidase, and fimbriae (Wickhorst et al. 2018). Pyolysin is encoded by the *plo* gene and is involved in the lysis of immune system cells. The protease involved in adhesion to epithelial cells is the surface protein encoded by the *cbpA* gene (Huang et al. 2018). While neuraminidase enzymes, encoded by *nanH* and *nanP* genes, function in colonization (Risseti et al. 2017), *fimA*, *fimC*, *fimE*, and *fimG* genes encode fimbrial proteins (Zastempowska and Lassa 2012).

To date, a number of studies on the determination of antimicrobial susceptibility and virulence related genes of *T. pyogenes* have been conducted (Zastempowska and Lassa, 2012, Ribeiro et al. 2015, Alkasır et al. 2016, Dong et al. 2017, Moreno et al. 2017, Risseti et al. 2017, Tamai et al. 2018, Dong et al. 2019, Rezanejad et al. 2019, Galán-Relaño et al. 2020). In Türkiye, there have been a limited number of studies on *T. pyogenes* infections. In these studies, the antimicrobial susceptibility of *T. pyogenes* isolates has been generally determined using the disc diffusion method and virulence related genes have been investigated by PCR (Hadimli et al. 2010, Hadimli and Kav 2011, Uluışık and Erbaş 2014, Ozturk et al. 2016, Sahan Yapicier et al. 2022).

Since there are no specific evaluation criteria for determination of antimicrobial susceptibility of *T. pyogenes* isolates, it is critical to determine the MIC_{50} and MIC_{90} values of various antimicrobial agents in the isolates. Therefore, in this study, it was aimed i) to determine MIC_{50} and MIC_{90} values of different antimicrobials in *T. pyogenes* isolates, ii) to investigate tetracycline, macrolide and aminoglycoside resistance genes and iii) to determine virulence related genes that are involved in the pathogenesis of infections caused by *T. pyogenes* isolates.

Materials and Methods

Material

In this study, 32 *T. pyogenes* strains isolated from various clinical samples that were brought to the microbiology laboratory for routine bacteriological examinations between the years 2021-2022 were used. ATCC 19411 was used as a positive control for all examination in the study.

This study was approved by Siirt University Animal Researches Local Ethic Committee with the number of 2021/01/04.

Method

Isolation and identification of isolates

The samples were inoculated onto blood agar base (1.10886, Merck, Darmstadt, Germany) with 5% defibrinated sheep blood and incubated at 37°C in microaerophilic conditions for 24-48 h. After the incubation period, the pin point colonies that were Gram positive, coccoid, and negative for catalase reaction were considered as suspicious for *T. pyogenes* (Quinn et al. 2011). Identification of the isolates was carried out by PCR (Ulbegi-Mohyla et al. 2010). Genomic DNA

was obtained using the boiling method. For this purpose, colonies were picked from blood agar and mixed into 200 µL PCR water. The suspension was then boiled at 100°C in a dry block for 10 min. and subsequently centrifuged at 10,000 X g for 5 min and the supernatant was used as genomic DNA. The amplification protocol was slightly modified according to the recommendations of the manufacturers that synthesized the primers and is shown in Table 1. To prepare the PCR mixture, 12.5 µl mastermix (2X PCR Mastermiks, ABT®, Ankara, Türkiye), 5 µl genomic DNA, 1.5 µl each primer (10 µM) and 4.5 µl PCR water were used. Amplicons were electrophoresed at 80 V for 1.5 h and visualized using a gel imaging system (Gen-Box ImagER, Ankara, Türkiye).

Antimicrobial susceptibility

MIC values for penicillin, cephalothin, sulfamethoxazole + trimethoprim, tetracycline, enrofloxacin, ciprofloxacin, streptomycin, gentamicin, erythromycin and tilmicosin were determined using E-test strips (Himedia, India and Liofilchem, Italy). The criteria of the European Committee on Antimicrobial Susceptibility Testing (2019) for *Corynebacterium* spp. were considered while applying the tests. For determination of MIC values using the E-test method, overnight culture of the isolates on blood agar was suspended into 2 mL sterile physiological saline (pH:7.0) and the suspension was adjusted to McFarland 0.5 turbidity. 0.1 mL of suspension was the inoculated on Mueller Hinton agar (Oxoid, CM0337, England) supplemented with 5% defibrinated sheep blood. The E-test strip was placed on the agar and incubated at 37°C for 18-24 h. After the incubation period, the point where the inhibition ellipse intersected the strip was accepted as the MIC value. The evaluation criteria reported by Liu et al. (2009) were used for penicillin (≥2 µg/mL), tetracycline (≥4 µg/mL), enrofloxacin (≥4 µg/mL), ciprofloxacin (≥16 µg/mL), gentamicin (≥2 µg/mL), erythromycin (>1 µg/mL) and tilmicosin (>2 µg/mL). In addition, the criteria reported by Zastempowska and Lassa (2012), Galan-Relano et al. (2020) and Kwiecien et al. (2020) were considered for cephalothin (>8 µg/mL), sulfamethoxazole + trimethoprim (>0.12 µg/mL) and streptomycin (>4 µg/mL), respectively. *Streptococcus pneumoniae* ATCC 49619 was used as a control strain in the test.

Determination of antimicrobial resistance genes and virulence genes

The genes were detected in the isolates through PCR using gene specific primers. Table 1 shows primers, amplicon size and the references that reported the primers. The PCR mix was prepared as described in the isolation and identification section. The amplification protocol was applied according to the recommendations of the manufacturers of the primers and mastermix. The protocols that consisted of 35 cycles were perfomed as preliminary denaturation at 94°C for 10 min, denaturation at 94°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. Annealing steps were adjusted for 1 min and at 63°C for *tet*(W), *cbpA* and *fimG*, 68°C for *erm*(X), 53°C for *erm*(B), 60°C for *aadA1*, *aadA9*, *aadA11* and *nanP*, 59°C for *aacC*, 62°C for *strA-strB*, *fimA* and *fimC*, 57°C for *aph(3')-IIIa* and *aac(6')-aph(2")*, 58°C for *plo*, 65°C for *nanH*, 72°C for *fimE*.

Results

Thirty-two suspected strains were isolated from the samples (Table 2) and 122 bp amplicons were obtained from the all the isolates by PCR.

While all the *T. pyogenes* isolates were susceptible to penicillin and cephalothin, the strains were resistant to the other antimicrobials at different rates (Table 3). Multidrug resistance was in 43.75% of the isolates (Table 4). Streptomycin resistance was most common in the strains isolated from abscesses. Sulfamethoxazole + + trimethoprim, tetracycline and streptomycin resistance was determined in 80% of the isolates identified from arthritis cases. Mastitis isolates were susceptible to all antibiotics, except for sulfamethoxazole + trimethoprim and streptomycin. Tetracycline resistance was observed in 71.42% of isolates obtained from omphalitis cases.

Table 5 shows the distribution of the antimicrobial resistance genes in resistant isolates. Two of the tetracycline resistant isolates carried the *tetW* gene and 8 macrolide resistant isolates harboured the *ermX* gene. When aminoglycoside resistant isolates were examined by PCR, $aadA_1$, $aadA_9$, *strA-strB* and *aacC(6')-aph(2")* genes were detected in 5, 14, 6 and 1 strains, respectively.

The $aadA_1$ gene was most common in the strains isolated from omphalitis cases (28.57%). The *aadA9* gene was detected in the majority (80%) of strains isolated from arthritis cases and was found positive in more than half of the calf isolates. While *ermX* and *strA-strB* genes were found in 60% and 40% of the strains isolated from arthritis cases, respectively, these genes were found to be higher in calf isolates than those obtained from other animals.

nanP and *fimA* genes were positive at the highest rate (93.75%) in the isolates, and the *fimG* gene was found in a limited number of the isolates. None of the

Table 1. Primer sequences used for identification of *Trueperella pyogenes* strains and determination of antimicrobial resistance genes and virulence-related genes by PCR.

Genes	Oligonucleotide (5'-3')	Amplicon Size(bp)	References
16S-23S rDNA	**F:** GTTTTGCTTGTGATCGTGGTGGTTATGA **R:** AAGCAGGCCCACGCGCAGG	122	Ulbegi- Mohyla et al. 2010
	Antimicrobial resistance genes		
Tetracycline			
tet(W)	**F:** GACAACGAGAACGGACACTATG **R:** CGCAATAGCCAGCAATGAACGC	1843	Billington and Jost 2006
Macrolide			
erm(X)	**F:** GTTGCGCTCTAACCGCTAAGGC **R:** CCATGGGGACCACTGAGCCGTC	571 / 657	Jost et al. 2003
erm(B)	**F:** GAAATTGGAACAGGTAAAGG **R:** TTTACTTTTGGTTTAGGATG	404	Jost et al. 2003
	Aminoglycoside		
aadA1	**F:** CGGTGACCGTAAGGCTTGAT **R:** ATGTCATTGCGCTGCCATTC	193	Kwiecien et al. 2020
aadA9	**F:** ACGCCGACCTTGCAATTCT **R:** TAGCCAATGAACGCCGAAGT	373	Kwiecien et al. 2020
aadA11	**F:** CGTGCATTTGTACGGCTCTG **R:** ACCTGCCAATGCAAGGCTAT	352	Kwiecien et al. 2020
aacC	**F:** TTGCTGCCTTCGACCAAGAA **R:** TCCCGTATGCCCAACTTTGT	256	Kwiecien et al. 2020
strA-strB	**F:** TATCTGCGATTGGACCCTCTG **R:** CATTGCTCATCATTTGATCGGCT	538	Sunde et al. 2005
aph(3')-IIIa	**F:** GGCTAAAATGAGAATATCACCGG **R:** CTTTAAAAAATCATACAGCTCGCG	523	Vakulenko et al. 2003
aac(6')-aph(2")	**F:** CCAAGAGCAATAAGGGCATA **R:** CACTATCATAACCACTACCG	220	Ouoba et al. 2008
	Virulence related genes		
plo	**F:** GGCCCGAATGTCACCGC **R:** AACTCCGCCTCTAGCGC	270	Jost et al. 2002
nanH	**F:** CGCTAGTGCTGTAGCGTTGTTAAGT **R:** CCGAGGAGTTTTGACTGACTTTGT	781	Silva et al. 2008
nanP	**F:** TTGAGCGTACGCAGCTCTTC **R:** CCACGAAATCGGCCTTATTG	150	Silva et al. 2008
cbpA	**F:** GCAGGGTTGGTGAAAGAGTTTACT **R:** GCTTGATATAACCTTCAGAATTTGCA	124	Silva et al. 2008
fimA	**F:** CACTACGCTCACCATTCACAAG **R:** GCTGTAATCCGCTTTGTCTGTG	605	Silva et al. 2008
fimC	**F:** TGTCGAAGGTGACGTTCTTCG **R:** CAAGGTCACCGAGACTGCTGG	843	Silva et al. 2008
fimE	**F:** GCCCAGGACCGAGAGCGAGGGC **R:** GCCTTCACAAATAACAGCAACC	775	Silva et al. 2008
fimG	**F:** ACGCTTCAGAAGGTCACCAGG **R:** ATCTTGATCTGCCCCCATGCG	929	Silva et al. 2008

isolates were positive for the *cbpA* gene. Table 6 shows the distribution of the virulence gene profile among sources of the isolates.

Discussion

T. pyogenes causes various purulent infections in ruminants and economic losses in herds (Urumova et al. 2009, Ozturk et al. 2016, Dong et al. 2017,

Table 2. The distribution of *Trueperella pyogenes* isolates according to the animal species and the clinical cases.

Cases	Cattle	Calf	Sheep	Goat
Abscess	9	4	4	-
Mastitis	1	-	-	1
Omphalitis	-	7	-	-
Arthritis	-	5	-	-
Footrot	-	-	1	-

Table 3. Frequencies of antimicrobial resistance in *T. pyogenes* isolates (n=32).

Antimicrobial Agent	S (%)	R (%)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Evaluation criteria (R)
Penicillin	100	0	0.016	0.016	≥2 µg/mL
Cephalothin	100	0	0.016	0.032	> 8 µg/mL
Sulfamethoxazole+trimethoprim	62.5	37.50	0.094	>32	> 0.12 µg/mL
Tetracycline	46.88	53.12	4	12	≥ 4 µg/mL
Enrofloxacin	78.13	21.87	0.50	>32	≥ 4 µg/mL
Ciprofloxacin	87.50	12.50	2	>32	≥ 16 µg/mL
Streptomycin	43.75	56.25	16	>256	> 4 µg/mL
Gentamicin	78.13	21.87	0.125	12	≥ 2 µg/mL
Erythromycin	71.88	28.12	0.016	>256	> 1 µg/mL
Tilmicosin	68.75	31.25	0.016	32	> 2 µg/mL

S – Susceptible, R – Resistant

Table 4. Multidrug resistance profile.

Samples	Resistance profile	Resistant isolates n (%)
Abscess (n=17)	TE-ENR-STR	1 (5.88)
	TE-STR-E-TIL	2 (11.76)
	SXT-STR-GEN-E-TIL	1 (5.88)
	SXT-TE-ENR-CIP-STR	1 (5.88)
	ENR-CIP-STR-GEN-E-TIL	1 (5.88)
Arthritis (n=5)	SXT-TE-ENR-CIP-STR-GEN-E-TIL	1 (20)
	SXT-TE-STR-GEN-E-TIL	1 (20)
	TE-ENR-STR	1 (20)
	SXT-TE-STR-GEN-E-TIL	1 (20)
	SXT-ENR-STR	1 (20)
Omphalitis (n=7)	SXT-TE-CIP-GEN-E-TIL	1 (14.28)
	SXT-STR-GEN-TIL	1 (14.28)
	SXT-TE-STR	1 (14.28)
	TOTAL	14 (43.75)

TE – Tetracycline, ENR – Enrofloxacin, STR – Streptomycin, E – Erythromycin, TIL – Tilmicosin, SXT – Sulfamethoxazole+trimethoprim, CIP – Ciprofloxacin, GEN – Gentamicin

Table 5. Distribution of antimicrobial resistance genes among resistant isolates.

Phenotypic Resistance	Genotypic Resistance	n (%)
Tetracycline (n=17) (Cattle: 2, Calf:5, Sheep: 1)	*tetW* (Cattle:1, Calf:1)	2 (11.76)
Tilmicosin (n=1)	*ermX* (Calf:1)	1 (100)
Tilmicosin + Erythromycin (n=9)	*ermX* (Cattle:2, Calf:5)	7 (77.77)
	(-) (Cattle:1, Calf:1)	2 (22.22)
Streptomycin (n=13)	*aadA9* (Cattle:2, Calf: 5, Sheep:1)	8 (61.54)
	aadA1+aadA9 (Cattle:1)	1 (7.70)
	(-) (Cattle:2, Calf:2)	4 (30.77)
Gentamicin (n=1)	*aadA1+aadA9+strA-strB* (Calf:1)	1 (100)
Streptomycin+Gentamicin (n=6)	*aadA1+aadA9+strA-strB* (Calf:1)	1 (16.66)
	aadA9+strA-strB (Cattle:1, Calf:2)	3 (50)
	aadA1+strA-strB (Calf:1)	1 (16.66)
	aadA1+strA-strB+aacC(6')-aph(2") (Cattle:1)	1 (16.66)

Table 6. Distribution of virulence related gene profile among sources of the isolates.

Samples	Virulence related gene profile	n (%)
Abscess (n=17)	*plo+nanP+fimA+fimE*	1 (5.88)
	plo+nanH+fimA	1 (5.88)
	plo+nanP+fimA	2 (11.76)
	plo+nanP+fimA+fimE	2 (11.76)
	plo+nanH+nanP+fimA+fimE	3 (17.64)
	plo+nanH+nanP+fimA+fimE+fimG	1 (5.88)
	plo+nanH+nanP+fimA+fimC+fimG	1 (5.88)
	plo+nanP+fimA+fimC+fimE	1 (5.88)
	Plo+nanH+nanP+fimA	1 (5.88)
	nanH+nanP+fimA+fimE	1 (5.88)
	plo+nanH+nanP+fimA+fimC+FimE	1 (5.88)
	plo+nanP+fimC	1 (5.88)
	none	1 (5.88)
Arthritis (n=5)	*plo+nanP+fimA+fimE*	1 (20)
	plo+nanH+nanP+fimA	1 (20)
	plo+nanH+nanP+fimA+fimG	1 (20)
	plo+nanP+fimA+fimC	2 (40)
Omphalitis (n=7)	*plo+nanP+fimA+fimC+fimE*	1 (14.28)
	plo+nanH+nanP+fimA	1 (14.28)
	plo+nanP+fimA	2 (28.57)
	plo+nanH+nanP+fimA+fimE	1 (14.28)
	plo+nanP+fimA+fimE	1 (14.28)
	plo+nanH+nanP+fimA+fimG	1 (14.28)
Mastitis (n=2)	*plo+nanP+fimA+fimE+fimG*	1 (50)
	nanH+nanP+fimA	1 (50)
Footrot (n=1)	*plo+nanH+nanP+fimA+fimE*	1 (100)

Moreno et al. 2017, Risseti et al. 2017, Dong et al. 2019). Because the lack of an effective vaccine against *T. pyogenes* isolates, antimicrobial therapy is the unique choice for preventing and treating the infections caused by this microorganism (Quinn et al. 2011). Following the distribution of antimicrobial resistance profile in isolates using current data would allow practitioners to regulate effective initial antimicrobial therapy.

Data on clinical manifestations of *T. pyogenes* isolated cases mainly include abscesses, pneumonia, and lymphadenitis (Ribeiroa et al. 2015, Risseti et al. 2017, Tamai et al. 2018). While abscesses also accounted for a significant rate in the present study, omphalitis and arthritis exhibited distinct rates, unlike other studies. These diseases affect mostly neonatal and young animals. High isolation rates of *T. pyogenes* in these diseases compared to the others may be due to the lack of the proper navel cord care by the local farmers.

Studies on the determination of the antimicrobial susceptibility of *T. pyogenes* isolates by disc diffusion method revealed that penicillin resistance was 2-8% in the isolates (Ribeiroa et al. 2015, Alkasir et al. 2016, Ozturk et al. 2016, Tamai et al. 2018). On the other hand, in another study (Rezanejad et al. 2019) it was reported that 98.63% of the isolates were resistant to penicillin. In their study investigating MIC values, Zastempowska and Lassa (2012) reported that the MIC value of penicillin was 0.06 µg/. Galán-Relaño et al. (2020) found that the MIC_{90} value of penicillin was 0.008 µg/mL. Likewise, in the present study, the MIC value of penicillin was low and all of the isolates were susceptible to penicillin.

In this study, the MIC_{90} value of sulfamethoxazole+ +trimethoprim was found to be >32 µg/mL and 37.50% of the isolates were resistant to this antimicrobial. Likewise, previous studies reported that the prevalence of sulfamethoxazole+trimethoprim resistant isolates was high (70%) (Alkasır et al. 2016, Tamai et al. 2018, Rezanejad et al. 2019, Galán-Relaño et al. 2020). In addition, Galán-Relaño et al. (2020) reported that the MIC_{90} value of sulfamethoxazole+trimethoprim was 1.9 µg/mL. When compared to the present study, the other researchers found low MIC_{90} value.

This study revealed that the MIC_{90} value of tetracycline was 12 µg/mL and 53.12% of the isolates examined were resistant to tetracycline. Tetracycline resistant isolates were obtained from 6 cattle with abscess (n=5), mastitis (n=1), 10 calves with omphalitis (n=5), arthritis (n=4) and abscess (n=1) and 1 sheep with footrot. In addition, the *tetW* gene responsible for tetracycline resistance was detected in only 2 of the tetracycline resistant isolates (cattle:1, calf:1). The tetracycline resistance rate (9-30%) in *T. pyogenes* isolates in previous studies conducted on farm animals with different clinical cases (Ribeiroa et al. 2015, Tamai et al. 2018) was determined to be lower than the rate detected in the present study. Zastempowska and Lassa (2012), Alkasir et al. (2016) and Rezanejad et al. (2019) found that 85.5%, 70% and 50.68% of *T. pyogenes* isolates obtained from bovine clinical mastitis cases were resistant to tetracycline, respectively. In parallel with this study, Zastempowska and Lassa (2012) reported that the MIC_{90} value of tetracycline was higher than 4 µg/mL. However, the *tetW* gene was found in the majority of isolates.

Studies aiming to determine the antimicrobial resistance profile of *T. pyogenes* isolates, reported that ciprofloxacin resistance was generally low (Ribeiroa et al. 2015, Alkasir et al. 2016, Ozturk et al. 2016, Tamai et al. 2018). Similarly, in the present study, 12.50% of the isolates were found to be resistant to ciprofloxacin. On the other hand, another study reported that 63.01% of *T. pyogenes* strains were resistant to ciprofloxacin (Rezanejad et al. 2019). In the present study, the enrofloxacin resistance rate was found to be 21.87%. When compared with other studies, it was observed that the rate of enrofloxacin resistance in this study was higher than the rate reported by Ribeiroa et al. (2015) and Ozturk et al. (2016) but lower than the rate reported by Rezanejad et al. (2019). On the other hand, Alkasir et al. (2016), Tamai et al. (2018) and Galan-Relano et al. (2020) detected the rates similar to this study. On the other hand, the present study revealed that the MIC_{90} value of ciprofloxacin and enrofloxacin was higher than 32 µg/mL, which was slightly higher than data revealed by Galan-Relano et al. (2020).

Previous studies indicated that the aminoglycoside resistance rate in *T. pyogenes* strains was high. However, the gentamicin resistance rate was reported in a limited number of strains by Ribeiroa et al. (2015) and Kwiecien et al. (2020). Conversely, it was observed that the rate of streptomycin and gentamicin resistance (>30%) in *T. pyogenes* isolates was remarkable (Alkasır et al. 2016, Ozturk et al. 2016, Tamai et al. 2018, Rezanejad et al. 2019). In this study, the rate of gentamicin resistance detected in isolates (21.87%) was lower than the value detected in other studies, but similarly to other studies, more than half of the isolates were resistant to streptomycin. Also, the MIC_{90} values for streptomycin and gentamicin were higher than the value determined by Galan-Relano et al. (2020) and Kwiecien et al. (2020).

Some studies reported that the genes related to aminoglycoside resistance in *T. pyogenes* isolates obtained from different disease cases in large and small ruminants were found at different rates. Rezanejad et al. (2019) reported that *aadA1* and *aacC* genes were found

in 63.01% and 68.49% of 73 *T. pyogenes* strains, respectively. In another similar study, it was reported that *aadA9*, *strA-strB*, *aph(3')-IIIa* genes were detected in 20.51%, 2.56% and 2.56% of the isolates, respectively (Kwiecien et al. 2020). In this study, *aadA1*, *aadA9*, *strA-strB* and *aacC(6')-aph(2'')* genes were positive in 25%, 70%, 35% and 5% of 20 aminoglycoside resistant isolates, respectively. Even though other studies have investigated the presence of antimicrobial resistance genes in all isolates, determination of resistance genes only in phenotypically resistant isolates may have caused a difference in the gene positivity rate.

Previous studies reported that the prevalence of macrolide resistant *T. pyogenes* isolates varied (20-40%) (Alkasır et al. 2016, Ozturk et al. 2016, Tamai et al. 2018, Rezajenad et al. 2019). Likewise, 28.12% of the isolates was resistant to erythromycin in this study. Nevertheless, the MIC_{90} value of erythromycin were determined as 0.125 µg/mL by Zastempowska and Lassa (2012) but it was higher than 256 µg/mL in this study. In *T. pyogenes* isolates, the prevalence of the *ermX* gene was higher than the prevalence of *ermB* (Zastempowska and Lassa 2012, Tamai et al. 2018, Rezajenad et al. 2019). In this study, the *ermX* gene was found to be positive in 80% of 10 macrolide resistant isolates.

The *nanH* and *nanP* genes associated with neuraminidase synthesis are involved in adhesion to the host cell (Jost and Billington 2005). Studies have reported that the positivity rate of *nanP* is generally higher than the positivity rate of *nanH* (Hadimli and Kav 2011, Ozturk et al. 2016, Risseti et al. 2017, Fujimato et al. 2020). In addition, the positivity of the *nanH* gene was higher in the strains isolated from cattle with metritis (Tamai et al. 2018). In the present study, the number of *nanP* positive isolates was higher than *nanH* positive isolates, which was compatible with other studies. The rate of *nanP* positivity in isolates was also compatible with other studies. In the present study, *nanH* positivity was detected at a low rate compared to other studies. This may have been caused by obtaining the isolates from different clinical cases.

The *cbpA* gene, which encodes proteins involved in adhesion to tissues that are rich in collagen, is mostly detected in *T. pyogenes* strains isolated from collagen-rich tissues. In this context, previous studies reported that 20-60% of the strains isolated from mastitis and metritis cases in cattle carried the *cbpA* gene (Zastempowska and Lassa 2012, Tamai et al. 2018, Rezajenad et al. 2019). Some others reported that the *cbpA* gene was detected at lower rates (6-11%) in strains isolated from different clinical cases in large and small ruminants (Ozturk et al. 2016, Risseti et al. 2017, Fujimato et al. 2020). However, the *cbpA* gene was not detected in the isolates in this study. This may be associated with sampling from abscess tissue rather than udder and uterus tissue.

Although almost all of the isolates were positive for the *fimA* gene in some studies (Hadimli and Kav 2011, Zastempowska and Lassa 2012, Ozturk et al. 2016, Tamai et al. 2018, Rezanejad et al. 2019, Fujimato et al. 2020), Alkasir et al. (2016) reported that the rate of *fimA* positive isolates was 26.28%. In the present study, *fimA* gene positivity was high (93.75%) in the isolates. In the literature review, it was reported that *fimC* and *fimE* genes were detected at high rates in *T. pyogenes* isolates (Hadimli and Kav 2011, Zastempowska and Lassa 2012, Ozturk et al. 2016, Risseti et al. 2017, Tamai et al. 2018, Rezanejad et al. 2019, Fujimato et al. 2020). Alkasir et al. (2016) found that the *fimC* gene was positive in 23.42% of the strains isolated from mastitis cases. In the present study, the *fimC* gene was positive in 21.88% of the isolates, which is compatible with the study by Alkasir et al. (2016). However, in this study, it was determined that the number of *fimE* gene positive isolates was higher (46.88%) when compared to *fimC* positivity. In studies in which the presence of virulence-related genes was determined in *T. pyogenes* isolates, it was reported that *fimG* was the gene with the lowest rate among fimbrial protein genes (Hadimli and Kav 2011, Zastempowska and Lassa 2012, Alkasır et al. 2016, Rissetti et al. 2017, Fujimato et al. 2020). Similarly, in the present study, the *fimG* gene was determined as the virulence gene with the lowest rate (15.63%) among the isolates. On the other hand, this rate was determined as 34.09% by Ozturk et al. (2016), 61.50% by Tamai et al. (2018), and 43.83% by Rezanejad et al. (2019).

In conclusion, this study revealed that penicillin and cephalosporin group antimicrobial agents can be used to effectively treat infections caused by *T. pyogenes* isolates. However, the resistance developed in isolates especially against streptomycin, tetracycline and sulfamethoxazole + trimethoprim should be considered. It is thought that determining the MIC values of antimicrobial agents in the isolates would contribute to studies on determining clinical breakpoints in particular. Identification of genetic resistance mechanisms in the isolates would contribute to the explanation of phenotypic resistance against macrolide and aminoglycoside antimicrobial agents. The *plo*, *nanP* and *fimA* genes could play an important role in the development of infections caused by *T. pyogenes* isolates and these antigenic structures should be taken into considerations in vaccine studies.

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