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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 by using E-test method and MIC values of the antimicrobials were investigated. The genes that play 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 MIC₉₀ values of 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 skin, urogenital and upper respiratory tract of the animals (Queen et al. 1994, Rzewuska et al. 2006, Silva et al. 2008).

T. pyogenes strains are isolated from pneumonia cases and abscess as well as metritis and endometritis. Also, the bacteria is identified as etiological agent of 'summer mastitis' in cattle (Zambrano et al. 2011, Rzewuska et al. 2016, Tamai et al. 2018). In addition, *T. pyogenes* can cause purulent infections like encephalitis, pyelonephritis, lenfadenitis, endocarditis, arthritis and omphalitis in cattle (Hijazin et al. 2011, Riseti 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, Riseti et al. 2017).

Aminoglycoside, beta-lactam, tetracycline, macrolide and fluoroquinolone group antimicrobial agents are frequently used in the treatment of *T. pyogenes* infections. But, resistant isolates cause problems in terms of effective antimicrobial therapy for *T. pyogenes* infections in recent years (Kwiecien et al. 2020). In addition to the unconscious use 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 (Riseti et al. 2017), *fimA*, *fimC*, *fimE*, and *fimG* genes encode fimbrial proteins (Zastempowska and Lassa 2012).

To date, a number of studies about determination of

antimicrobial susceptibility and virulence related genes of *T. pyogenes* have been conducted (Zastempowska and Lassa, 2012, Ribeiro et al. 2015, Alkasir et al. 2016, Dong et al. 2017, Moreno et al. 2017, Riseti et al. 2017, Tamai et al. 2018, Dong et al. 2019, Rezanejad et al. 2019, Galán-Relaño et al. 2020). In Turkey, there have been a limited number of studies on *T. pyogenes* infections. In these studies, antimicrobial susceptibility of *T. pyogenes* isolates have been generally determined using 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 Yapıcıer 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 MIC₅₀ and MIC₉₀ values of various antimicrobial agents in the isolates. Therefore, in this study, it was aimed i) to determine MIC₅₀ and MIC₉₀ 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.

Material and Method

Bacterial strains

In this study, 32 *T. pyogenes* strains isolated from cattle (abscess:9, mastitis:1), calf (abscess:4, omphalitis:7, arthritis:5), sheep (abscess:4, footrot:1) and goat (mastitis:1) were investigated. ATCC 19411 was used as a positive control for all examination in the study.

Isolation and identification of the 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 (Ulbeği-Mohyla et al. 2010). Genomic DNA was obtained through boiling method. Amplification protocol was slightly modified according to recommendations of the manufacturers that synthesized the primers and shown in Table 1. To prepare 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

by gel imaging system (Gen-Box ImagER, Ankara, Türkiye).

Antimicrobial susceptibility

MIC values of penicillin, cephalothin, sulfamethoxazole + trimethoprim, tetracycline, enrofloxacin, ciprofloxacin, streptomycin, gentamicin, erythromycin and tilmicosin were determined by using E-test strip (Himedia, India and Liofilchem, Italy). The criteria of European Committee on Antimicrobial Susceptibility Testing (2019) for *Corynebacterium* spp. were considered while applying the tests. For determination of MIC values using 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. 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. 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 control strains 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 and PCR conditions. PCR mix was prepared as described in the isolation and identification section. Amplification protocol was applied according to the recommendations of the manufacturers of the primers and mastermix.

Results

Isolation and identification

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

Antimicrobial susceptibility

While all of *T. pyogenes* isolates were susceptible to penicillin and cephalothin, the strains were resistant to the other antimicrobials at different rates (Table 2). Multidrug resistance was in 43.75% of the isolates (Table 3). Streptomycin resistance was most common in the strains isolated from abscess. 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.

Determination of antimicrobial resistance genes

Table 4 shows distribution of the antimicrobial resistance genes in resistant isolates. Two of tetracycline resistant isolates carried *tetW* gene and 8 macrolide resistant isolates harboured *ermX* gene. When aminoglycoside resistant isolates were examined by PCR, *aadA*, *aadA*₉, *strA-strB* and *aacC(6')-aph(2'')* genes were detected in 5, 14, 6 and 1 strains, respectively.

Determination of virulence genes

In the study, virulence related genes were determined at different rates in the isolates. While *nanP* and *fimA* genes were positive at the highest rate (93.75%) in the isolates, *fimG* gene was found in a limited number of the isolates. None of the isolates were positive for *cbpA* gene. Table 5 shows 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, Moreno et al. 2017, Riseti 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 through current data would allow the practitioners regulate effective initial antimicrobial therapy.

Data on clinical manifestations of *T. pyogenes* isolated cases mainly include abscesses, pneumonia, and lymphadenitis (Ribeiroa et al. 2015, Riseti 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.

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: AAGCAGGCCACGCGCAGG	122	Ulbegi-Mohyla et al. 2010
Antimicrobial resistance genes			
Tetracycline			
<i>tet(W)</i>	F: GACAACGAGAACGGACACTATG R: CGCAATAGCCAGCAATGAACGC	1843	Billington and Jost 2006
Macrolide			
<i>erm(X)</i>	F: GTTGCCTCTAACCGCTAAGGC R: CCATGGGGACCACTGAGCCGTC	571 / 657	Jost et al. 2003
<i>erm(B)</i>	F: GAAATTGGAACAGGTAAGG R: TTTACTTTGGTTTAGGATG	404	Jost et al. 2003
Aminoglycoside			
<i>aadA1</i>	F: CGGTGACCGTAAGCCTTGAT R: ATGTCATTGCGCTGCCATTC	193	Kwiecien et al. 2020
<i>aadA9</i>	F: ACGCCGACCTTGCAATTCT R: TAGCCAATGAACGCCGAAGT	373	Kwiecien et al. 2020
<i>aadA11</i>	F: CGTGCATTGTACGGCTCTG R: ACCTGCCAATGCAAGGCTAT	352	Kwiecien et al. 2020
<i>aacC</i>	F: TTGCTGCCTTCGACCAAGAA R: TCCCGTATGCCCAACTTTGT	256	Kwiecien et al. 2020
<i>strA-strB</i>	F: TATCTGCGATTGGACCCTCTG R: CATTGCTCATCATTGATCGGCT	538	Sunde et al. 2005
<i>aph(3')-IIIa</i>	F: GGCTAAAATGAGAATATCACCGG R: CTTTAAAAAATCATAACAGCTCGCG	523	Vakulenko et al. 2003
<i>aac(6')-aph(2'')</i>	F: CCAAGAGCAATAAGGGCATA R: CACTATCATAACCACTACCG	220	Ouoba et al. 2008
Virulence related genes			
<i>plo</i>	F: GGCCCGAATGTCACCGC R: AACTCCGCTCTAGCGC	270	Jost et al. 2002
<i>nanH</i>	F: CGCTAGTGCTGTAGCGTTGTTAAGT R: CCGAGGAGTTTTGACTGACTTTGT	781	Silva et al. 2008
<i>nanP</i>	F: TTGAGCGTACGCAGCTCTTC R: CCACGAAATCGGCCTTATTG	150	Silva et al. 2008
<i>cbpA</i>	F: GCAGGGTTGGTAAAAGAGTTTACT R: GCTTGATATAACCTTCAGAATTTGCA	124	Silva et al. 2008
<i>fimA</i>	F: CACTACGCTCACCATTACACAAG R: GCTGTAATCCGCTTTGTCTGTG	605	Silva et al. 2008
<i>fimC</i>	F: TGTCGAAGGTGACGTTCTTCG R: CAAGGTCACCGAGACTGCTGG	843	Silva et al. 2008
<i>fimE</i>	F: GCCCAGGACCGAGAGCGAGGGC R: GCCTTCACAAATAACAGCAACC	775	Silva et al. 2008
<i>fimG</i>	F: ACGCTTCAGAAGGTCACCAGG R: ATCTTGATCTGCCCCATGCG	929	Silva et al. 2008

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.

In this study, antimicrobial susceptibility of *T. pyogenes* isolates and MIC values of the antimicrobials were investigated. Also, it was found that antimicrobial resistance genes and virulence related genes

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)	<i>tetW</i> (Cattle:1, Calf:1)	2 (11.76)
Tilmicosin (n=1)	<i>ermX</i> (Calf:1)	1 (100)
Tilmicosin + Erythromycin (n=9)	<i>ermX</i> (Cattle:2, Calf:5)	7 (77.77)
	(-) (Cattle:1, Calf:1)	2 (22.22)
	<i>aadA9</i> (Cattle:2, Calf: 5, Sheep:1)	8 (61.54)
Streptomycin (n=13)	<i>aadA1+aadA9</i> (Cattle:1)	1 (7.70)
	(-) (Cattle:2, Calf:2)	4 (30.77)
Gentamicin (n=1)	<i>aadA1+aadA9+strA-strB</i> (Calf:1)	1 (100)
	<i>aadA1+aadA9+strA-strB</i> (Calf:1)	1 (16.66)
Streptomycin+Gentamicin (n=6)	<i>aadA9+strA-strB</i> (Cattle:1, Calf:2)	3 (50)
	<i>aadA1+strA-strB</i> (Calf:1)	1 (16.66)
	<i>aadA1+strA-strB+aacC(6')-aph(2'')</i> (Cattle:1)	1 (16.66)

explained pathogenesis of the infections caused by this microorganism.

The studies about 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 obtained from mastitis cases and uterus swab samples were resistant to penicillin. In their study investigating of MIC values, Zastempowska and Lassa (2012) reported that MIC value of penicillin was 0.06 µg/mL and all isolates were susceptible to penicillin. Galán-Relaño et al. (2020) found that the MIC₉₀ value of penicillin was 0.008 µg/mL and 6.3% of the isolates were resistant to penicillin. Likewise, in the present study MIC value of penicillin was low and all of the isolates were susceptible to penicillin.

In this study, MIC₉₀ 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 prevalence of sulfamethoxazole+trimethoprim resistant isolates was high (70%) (Alkasir 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 MIC₉₀ value of sulfamethoxazole+trimethoprim was 1.9 µg/mL. When compared to the present study, the other researchers found low MIC₉₀ value.

This study revealed that the MIC₉₀ value of tetracycline was 12 µg/mL and 53.12% of the isolates examined were resistant to tetracycline. In addition, the *tetW* gene responsible for tetracycline resistance was detected in only 2 of the tetracycline resistant isolates. The tetracycline resistance rate (9-30%) in *T. pyogenes*

isolates in previous studies (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 were resistant to tetracycline, respectively. In parallel with this study, Zastempowska and Lassa (2012) reported that the MIC₉₀ value of tetracycline was higher than 4 µg/mL. However, the *tetW* gene was found in the majority of isolates.

Fluoroquinolones group antimicrobial agents are commonly used in the treatment of soft tissue infections caused by Gram negative bacteria. In addition, they are known to have an effect on Gram positive bacterial agents (Akkaya 1997). The 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 isolated from cattle with mastitis and metritis 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 other hand, the present study revealed that MIC₉₀ value of ciprofloxacin and enrofloxacin was higher than 32 µg/mL, which was quietly higher than data revealed by Galan-Relano et al. (2020).

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

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

Previous studies indicated that 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 Kweicien et al. (2020). Conversely, it was observed that the rate of streptomycin and gentamicin resistance (>30%) in *T. pyogenes* isolates was remarkable (Alkasir 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 similar to other studies, more than half of the isolates were resistant to streptomycin. Also, MIC₉₀ value of streptomycin and gentamicin were higher than the value determined by Galan-Relano et al. (2020) and Kweicien 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 isolated from cattle with mastitis and metritis, 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 (Kweicien 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 gene positivity rate.

Previous studies reported that the prevalence of macrolide resistant *T. pyogenes* isolates varied (20-40%) (Alkasir et al. 2016, Ozturk et al. 2016, Tamai et al. 2018, Rezanejad et al. 2019). Likewise 28.12% of the isolates was resistant to erythromycin in this study. Nevertheless, MIC₉₀ 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, prevalence of *ermX* gene was higher than prevalence of *ermB* (Zastempowska and Lassa 2012, Tamai et al. 2018, Rezajenad et al. 2019). In this study, *ermX* gene was found to be positive in 80% of 10 macrolide resistant isolates.

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

The *cbpA* gene, which encodes proteins involved in adhesion to tissues that are rich for 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 *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, Riseti 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.

It is known that fimbrial proteins of *T. pyogenes* isolates are encoded by *fimA*, *fimC*, *fimE* and *fimG* genes. 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, Rezajenad 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, Riseti et al. 2017, Rezajenad et al. 2019, Tamai et al. 2018, Fujimato et al. 2020). Alkasir et al. (2016) found that *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. *fimC* and *fimE* positivity was found to be lower in the present study than other studies. 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, Alkasir et al. 2016, Riseti 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 Rezajenad 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, which result in economic losses by causing various disease cases in farm animals, but 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 *plc*, *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.

Declarations ethics approval

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

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