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Original Article
Özgün Araştırma

Isolation and antimicrobial susceptibility of *Streptococcus canis* from dogs

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Abstract: *Streptococcus canis* is an important opportunistic pathogen that infects animals and humans. It causes various infections in dogs and cats such as arthritis, skin infections, otitis externa, septicemia, facial necrosis, and streptococcal toxic shock syndrome. It can also lead to human infections. There are still very few specific studies on the identification and antimicrobial resistance of this pathogen. In this study, the phenotypic and biochemical properties of 12 *S. canis* isolates obtained from the vaginal discharge of 11 dogs and the open wound of one dog with facial necrosis, and their susceptibility to some antibiotics was investigated. The phenotypic and biochemical properties of 12 isolates, such as esculin hydrolysis, hippurate hydrolysis, synergistic CAMP-like hemolytic activity, growth on Brain Heart-Infusion Agar (BHIA) containing 6.5% NaCl, and carbohydrate fermentation were investigated by conventional bacteriological methods. It was determined that all isolates were in the G group according to Lancefield serogrouping. The presence of the species-specific 16S-23S rRNA gene intergenic spacer region (ISR) and $sodA_{int}$ gene was also detected in all the isolates using the polymerase chain reaction method. All the isolates were detected to be susceptible to penicillin G, cefoperazone, ampicillin, and amoxicillin-clavulanic acid, and resistant to gentamicin and neomycin.

Keywords: Antimicrobial susceptibility, intergenic spacer region (ISR), Lancefield group G, $sodA_{int}$ gene, *Streptococcus canis*.

Köpeklerden *Streptococcus canis*'in izolasyonu ve antimikrobiyal duyarlılığı

Özet: *Streptococcus canis*, hayvanları ve insanları enfekte eden önemli bir opportunistik patojendir. Köpeklerde ve kedilerde artritis, deri enfeksiyonları, otitis eksterna, septisemi, fasiyal nekroz ve streptokokkal toksik şok sendromu gibi çeşitli enfeksiyonlara neden olmaktadır. Ayrıca insan enfeksiyonlarına da yol açabilmektedir. Bu patojenin identifikasyonu ve antimikrobiyal direncine yönelik çalışmalar hala az sayıdadır. Bu çalışmada, hasta 11 köpeğin vajinal akıntısından ve bir fasiyal nekrozlu köpeğin açık yarasından izole edilen 12 *S. canis* izolatının fenotipik ve biyokimyasal özellikleri ile bazı antibiyotiklere duyarlılıkları incelendi. Oniki izolatın eskülin hidrolizi, hippurat hidrolizi, sinerjistik CAMP benzeri hemolitik aktivitesi, %6,5 NaCl içeren Brain Heart Infusion Agar (BHIA)'da üremesi ve karbonhidrat fermentasyonu gibi fenotipik ve biyokimyasal özellikleri konvansiyonel bakteriyolojik metodlar ile incelendi. Tüm izolatların Lancefield serogruplandırmasına göre G grubunda yer aldığı belirlendi. Polimeraz zincir reaksiyonu methodu ile tüm izolatlarda türe özgü 16S-23S rRNA geni intergenik ara bölge (ISR) ve $sodA_{int}$ geninin varlığı da tespit edildi. İzolatların tamamının penisilin G, sefaperazon, ampisilin, amoksisilin-klavulanik asit'e duyarlı, gentamisin ve neomisine dirençli olduğu belirlendi.

Anahtar kelimeler: Antimikrobiyal duyarlılık, intergenik ara bölge (ISR), Lancefield grup G, $sodA_{int}$ geni, *Streptococcus canis*.

Introduction

Streptococcus canis is a well-known pathogen of dogs and has been shown to cause mainly skin, respiratory, genital, and urinary tract infections (Lamm et al. 2010). *S. canis*, which has been reported to cause many serious diseases, such as otitis externa, polyarthritis, endocarditis, abortion, neonatal septicemia, streptococcal toxic shock syndrome, and facial necrosis in cats and dogs (DeWinter and Prescott 1999; Lysková et al. 2007; Pesavento et al. 2007; Avki et al. 2008; Lamm et al. 2010; Sharma et al. 2012; Abma et al. 2013; Mališová et al. 2019; Glassman et al. 2020), has also been reported in mastitis cases in cows (Chaffer et al. 2005; Hassan et al. 2005; Tikofsky and Zadoks 2005). *S. canis* infections are also seen in humans, with zoonotic infections having been documented by some authors (Bert and Lambert-Zechovsky 1997; Whatmore et al. 2001; Galpérine et al. 2007; Ohtaki et al. 2013; Pinho et al. 2013; Lacave et al. 2016).

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S. canis is a beta-hemolytic streptococcus included in Group G in Lancefield serogrouping (Devriese et al. 1986; DeWinter and Prescott 1999; Sharma et al. 2012). Group G hemolytic streptococcus species causing bacteremia is less known, and thus commercial kits are inadequate in determining the types of these streptococci, which are among the factors affecting the outcome of patients (Woo et al. 2001). Although API (bioMerieux Vitek), Vitek (bioMerieux Vitek, Hazelwood, MO), and ATB Expression (bioMerieux Vitek) systems are widely used for the identification of β-hemolytic streptococci, Group G β-hemolytic streptococci cannot be detected by these systems (Woo et al. 2001). It has been reported that the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method identifies β-hemolytic streptococcus species faster and more accurately than the VITEK2 GP ID system (Besli et al. 2018) but distinguishing between *S. canis*, *S. dysgalactiae*, and *S. pyogenes* can be difficult with the current version of Bruker's MALDI-TOF database (Schabauer et al. 2014; Nybakkenet et al. 2021). It has been suggested that the 16S rRNA gene is highly conserved within or between the species of the same genus and therefore can be used as the 'gold standard' for identification at the species level of bacteria (Woo et al. 2001). Hassan et al. (2003, 2005) reported that the detection of species-specific 16S-23S rRNA gene intergenic spacer region (ISR) and the *sodA*$_{int}$ gene was a fast and reliable method for the identification of *S. canis*. The authors also noted that there might be differences in the antibiotic susceptibilities of *S. canis* isolates obtained from various clinical cases of animals (Hassan et al. 2005; Lysková et al. 2007; Diren Sığırcı et al. 2012).

This study aimed to determine the biochemical properties of *S. canis* isolated from the vaginal discharge and open wound samples of dogs, to verify *S. canis* isolates by polymerase chain reaction (PCR) in terms of species-specific genes, and to determine their susceptibility to some antimicrobial agents.

Materials and Methods

***Bacteriological culture*:** In this study, 12 β-hemolytic streptococcal isolates obtained from a total of 12 dogs with vaginal discharge and facial necrosis were used. These isolates were obtained from swab samples taken from the vaginal mucosa (n = 11) of dogs with vaginal discharge and the open wound of one dog with facial necrosis and transferred to Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology Laboratory for analyses. The swab samples were cultured on blood agar (Oxoid, UK) containing 1% esculin (Merck, Germany) and 5% defibrinated sheep blood. The plates were incubated in aerobic atmosphere at 37°C for 24 hours.

***Phenotypical and biochemical properties*:** Gram staining was performed in the growing colonies, and the streptococci-suspected colonies were purified. The biochemical properties of each isolate were detected based on catalase, hippurate hydrolysis, esculin hydrolysis, and growing features in Brain Heart Infusion Agar (BHIA) containing 6.5% NaCl, and the isolates were identified at the genus level (Hassan et al. 2005; Lysková et al. 2007; Whiley and Hardie 2009). Phenol red broth (Merck) containing 1% salicin, mannitol, lactose, sucrose, trehalose, raffinose, and sorbitol were used for carbohydrate fermentation tests. All streptococci-suspected colonies were β-hemolytic, and the synergistic CAMP-like hemolytic activity was tested using β-hemolytic *Staphylococcus aureus* (Hassan et al. 2005; Lysková et al. 2007; Whiley and Hardie 2009). For the Lancefield serogrouping of the 12 *Streptococcus* sp. isolates, a commercial latex agglutination test kit (Strep Test Kit, Plasmatec, UK) was used according to the manufacturer's instructions.

***Polymerase chain reaction (PCR)*:** The isolates suspected as *S. canis* according to the biochemical tests and Lancefield serogrouping were verified using species-specific genes (ISR and *sodA*$_{int}$) by PCR. For this purpose, genomic DNA isolation from the isolates was performed according to the protocol of a commercial DNA extraction kit (Genejet, Thermo Scientific, Vilnius, Lithuania). The pure DNAs obtained were kept at -20 °C until use. Table 1 presents the primers, target gene, and PCR product size used in the study.

The following protocols were used to detect the ISR and *sodA*$_{int}$ genes for *S. canis* identification:

ISR: The assay was performed in a final volume of a 30 µl reaction mixture consisting of 5 µl template DNA, 13 µl PCR master mix (2X) (Thermo Scientific, Lithuania), 1 µl primer c-1 (100 pmol), 1 µl primer c-2 (100 pmol), and 10 µl ddH_2O (Steril ultra-pure water). Amplification was carried out in a thermal cycler (Apollo, ATC-401, Ramsey, Minnesota, USA) under the following conditions: An initial denaturation (94 °C, 4 minutes), followed by 30 cycles comprising 10 seconds at 94 °C, 30 seconds at 64 °C, 10 seconds at 72 °C and 5 minutes at 72 °C (Hassan et al. 2003, 2005).

Table 1. Species-specific oligonucleotide primers and genome sizes used for the amplification of the ISR and *sodA*$_{int}$ genes of *S. canis* (Hassan et al., 2003, 2005).

Target gene	Oligonucleotide primer	Sequence (5'-3')	PCR product size (bp)
ISR	c-I	TAAACCGAAAACGCTGTAAGTATTA	215
	c-II	ACCATTAGTTAGTGGGTTCCCCC	
sodA$_{int}$	canis-sod-I	AGAATTATTGGCAGATGTCACTA	263
	canis-sod-II	TTTCAAGTTGTCCTTCCTTATTG	

sodA$_{int}$: The assay was performed in a final volume of a 30 µl reaction mixture consisting of 5 µl template DNA, 13 µl PCR master mix (2X), 1 µl canis-sod-I (100 pmol), 1 µl primer canis-sod-II (100 pmol), and 10 µl ddH_2O (Steril ultra-pure water). Amplification was carried out in a thermal cycler under the following conditions: An initial denaturation (94 °C, 4 minutes), followed by 30 cycles comprising 25 seconds at 94 °C, 20 seconds at 60 °C, 25 seconds at 72 °C and 5 minutes at 72 °C (Hassan et al. 2005).

Using the universal 27F-1492R primers supplied by BMLabosis (Ankara), the 16S rRNA gene of an isolate suspected of *S. canis* was amplified and the bidirectional sequence analysis (ABI 3730XL, Applied Biosystems, Foster City, CA) was performed. The 16S rRNA nucleotide sequences obtained were compared with the NCBI GenBank for the identification of species. For this purpose, the Nucleotide-Nucleotide BLAST program available on the website of the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov) was used. According to the results, the isolate had 99.72% similarity to the *S. canis* strains NCTC12191 (LR134293.1) and ATCC 43496 (KP851852.1), and therefore this isolate was used as a positive control in all PCR tests. The DNA-free PCR mix was used as a negative control.

After the amplification process, the PCR products were electrophoresed on 1.5% agarose gel stained with EZ-Vision® In-Gel Solution (Code: N391, Amresco, USA) at 100 V for 45 min. The bands were photographed using the gel imaging system (EDAS-290, Eastman, Kodak Company, Rochester, NY, USA) under ultraviolet light.

Antimicrobial susceptibility test: The antibiotic susceptibility tests of bacteria were performed in Mueller Hinton agar (Merck) using the Kirby-Bauer disc diffusion test according to the method of the Clinical and Laboratory Standards Institute (CLSI 2018). Accordingly, bacterial suspension equivalent to a 0.5 McFarland standard was prepared with 0.85% physiological saline and spread onto the Muller Hinton agar (Oxoid, UK) containing 5% sheep blood using a swab. Amoxicillin-clavulanic acid (Oxoid, 30 µg), ampicillin (Oxoid, 25 µg), enrofloxacin (Bioanalyse, 5µg), erythromycin (Bioanalyse, 15 µg), gentamicin (Oxoid, 10 µg), chloramphenicol (Oxoid, 30 µg), lincomycin (Bioanalyse, 2 µg), neomycin (Bioanalyse, 30 µg), oxytetracycline (Oxoid, 30 µg), penicillin G (Oxoid, 10 IU), rifamycin (Bioanalyse, 30 µg), cefoperazone (Bioanalyse, 75 µg) and trimethoprim-sulfamethoxazole (Oxoid, 25 µg) discs were used for the test. The plates were incubated at 37 °C for 24 h aerobically, and the diameters of the inhibition zones formed around the antibiotic discs were measured. Their susceptibility to antibiotics was evaluated as susceptible, moderately susceptible, and resistant considering the limits determined by CLSI for Gram-positive cocci (CLSI 2018) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2019) for Group A, B, C, and G streptococci.

Results

In this study, it was determined that the *S. canis* isolates were Gram-positive and catalase-negative cocci, and all formed β-hemolysis. The obtained isolates showed synergistic CAMP-like hemolytic activity with *S. aureus* in sheep blood agar, and the carbohydrate fermentation tests revealed that the isolates hydrolyzed lactose, sucrose, and salicin but not all samples hydrolyzed raffinose and sorbitol. Four isolates obtained from the vaginal swabs of the dogs showed a mannitol-positive reaction. The isolates did not grow at 37 °C in BHIA containing 6.5% NaCl and did not hydrolyze hippurate and esculin. The biochemical properties of the isolates are given in Table 2. In addition, the 12 isolates identified as *S. canis* according to their biochemical properties were found to be in Group G according to Lancefield serogrouping.

Table 2. Biochemical test results of the *S. canis* isolates.

Biochemical tests	*S. canis* isolates (n=12)	
	n	%
Catalase	0	0
Beta-hemolysis	12	100
Hydrolysis of sodium hippurate	0	0
Hydrolysis of esculin	0	0
CAMP-like reaction	12	100
Carbohydrate fermentation		
Mannitol	4	33,33
Trehalose	9	75
Lactose	12	100
Sucrose	12	100
Raffinose	0	0
Sorbitol	0	0
Salicin	12	100
Growth on 6.5% NaCl	0	0

n: number of test-positive *S. canis* isolates

All the isolates suspected as *S. canis* by conventional bacteriological methods were confirmed to be *S. canis* using the species-specific ISR and $sodA_{int}$ genes by PCR (Figures 1 and 2).

The antibiotic susceptibility test revealed that all the *S. canis* isolates were susceptible to amoxicillin-clavulanic acid, ampicillin, penicillin G, and cefoperazone, and resistant to gentamicin and neomycin. Only two (16.66%) of the isolates were resistant to oxytetracycline. The susceptibility of the isolates to enrofloxacin, erythromycin, chloramphenicol, lincomycin, rifamycin, and trimethoprim-sulfamethoxazole was determined as 25%, 50%, 83.33%, 58.33%, 91.66%, and 75%, respectively. The susceptibility rates of the *S. canis* isolates to antibiotics used in the study are given in Table 3.

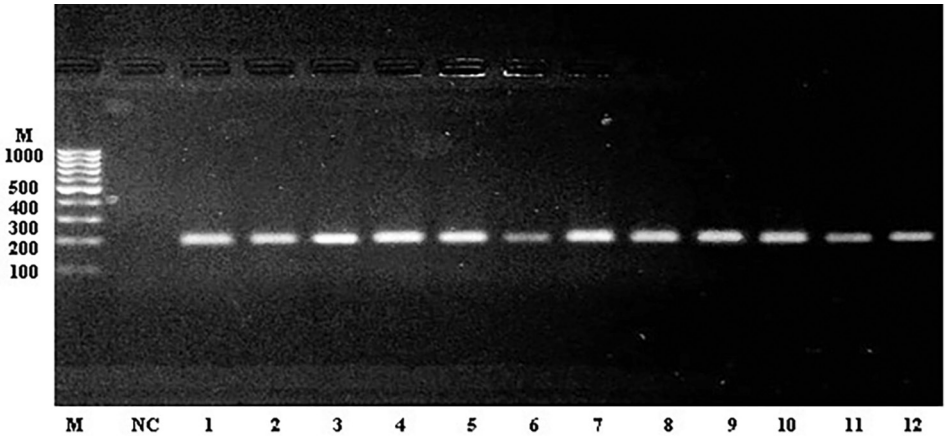

Figure 1. Lane M: 100-bp ladder DNA marker (Thermo Scientific, SM0241), Lane NC: negative control, Lane 1: positive control, Lanes 2-12: A 215-bp amplicon of the ISR gene of *S. canis* obtained by PCR with species-specific oligonucleotide primers c-I and c-II.

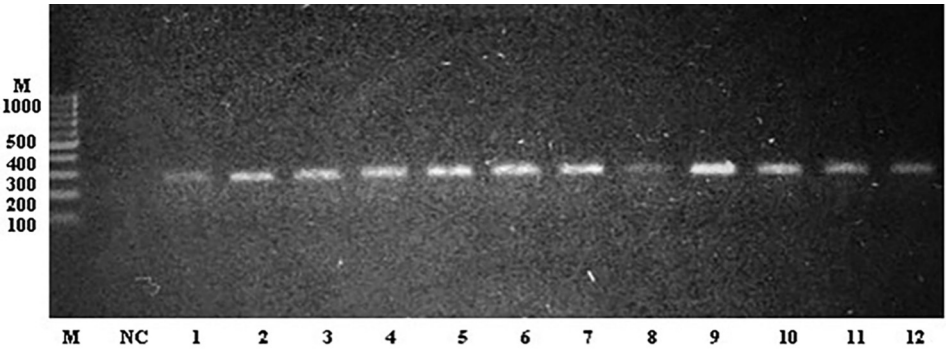

Figure 2. Lane M: 100-bp ladder DNA marker (Thermo Scientific, SM0241), Lane NC: negative control, Lane 1: positive control, Lanes 2- 12: A 263-bp amplicon of the $sodA_{int}$ gene of *S. canis* by PCR with species-specific oligonucleotide primers canis-sod-I and canis-sod-II.

Table 3. Antimicrobial susceptibility results of *S. canis* isolates obtained from dogs.

Antimicrobial agent	Disk content	*S. canis* (n=12)		
		Susceptible % (n)	Intermediate % (n)	Resistant % (n)
Amoxycillin-clavulanic acid	30µg	100 (12)	0	0
Ampicillin	25µg	100 (12)	0	0
Enrofloxacin	5µg	25 (3)	41.66 (5)	33.33 (4)
Erythromycin	15µg	50 (6)	50 (6)	0
Gentamicin	10µg	0	0	100 (12)
Chloramphenicol	30µg	83.33 (10)	16.66 (2)	0
Lincomycin	2µg	58.33 (7)	25 (3)	16.66 (2)
Neomycin	30µg	0	0	100 (12)
Oxytetracycline	30µg	83.33 (10)	0	16.66 (2)
Penicillin G	10 IU	100 (12)	0	0
Rifamycin	30µg	91,66 (11)	8.33 (1)	0
Cefoperazone	75µg	100 (12)	0	0
Trimethoprim-Sulfamethoxazole	25µg	75 (9)	25 (3)	0

n: number of *S. canis* isolates

Discussion

Pets and people often live in close contact, and in recent years, the 'one health' concept has become increasingly important due to zoonotic infections. It has been confirmed that the bacteria of the genus *Streptococcus* can cause serious or fatal diseases in pets and humans, some confirmed to be zoonotic (Lam et al. 2007; Fulde et al. 2013; Pinho et al. 2013; Lacave et al. 2016; Mališová et al. 2019). In particular, it has been reported that *S. canis* causes serious or fatal zoonotic infections in dog owners, and their constant contact with dogs' vaginal discharge can be a potential source for the risk of streptococcal disease, which is a concern for public health (Olufemi et al. 2017). However, due to the phenotypic, biochemical, and genetic similarities between this bacterial agent and other *Streptococcus* species, there are still uncertainties in its identification. Therefore, in this study, a total of 12 β-hemolytic *Streptococcus* sp. isolates obtained from the vaginal discharge of infected dogs (n=11) and the wound lesion of one dog were grouped according to Lancefield serogrouping, and some phenotypic and biochemical properties were evaluated. In addition, the identified isolates were confirmed by PCR testing in terms of the presence of species-specific genes, and their susceptibility to some antimicrobial agents was determined.

All isolates were determined to be in Group G according to Lancefield serogrouping and evaluated as *S. canis* according to the description of Devriese et al. (1986). Some researchers (Devriese et al. 1986; Hassan et al. 2005; Lysková et al. 2007) reported a difference in the phenotypic properties and biochemical test results of *S. canis*. However, no significant differences were observed in this study. The CAMP-like reaction, an important phenotypic feature of *S. canis*, was positive in all isolates (n=12). Although this result is consistent with the findings of Hassan et al. (2005), it differs from those of Devriese et al. (1986) and Lysková et al. (2007), who reported that a CAMP-like reaction was not present in all *S. canis* isolates (n=31) and 36 of 86 *S. canis* isolates, respectively. Another investigated phenotypic feature of *S. canis* was esculin hydrolysis. In the current study, contrary to the results of other researchers (Devriese et al. 1986; Hassan et al. 2005; Lysková et al. 2007), all the isolates (n=12) were negative for esculin hydrolysis Devriese et al. (1986) found that all *S. canis* isolates (n=31) were positive for esculin hydrolysis, and Hassan et al. (2005) reported that esculin hydrolysis was positive in subcultures, despite being negative at first isolation. On the other hand, Lyskova et al. (2007) showed that esculin hydrolysis was positive in only 14 of 86 *S. canis* isolates. In addition, while Devriese et al. (1986), Hassan et al. (2005), and Lam et al. (2007) consistently obtained negative results for hippurate hydrolysis in *S. canis* isolates, Lysková et al. (2007) reported that only one of 86 *S. canis* isolate was hippurate-positive. In this study, hippurate hydrolysis was negative in all *S. canis* strains consistently with the results of Devriese et al. (1986), Hassan et al. (2005), and Lam et al. (2007).

When the carbohydrate fermentation test results were examined, it was found that lactose and sucrose fermentation was positive and raffinose and sorbitol fermentation was negative, which is in agreement with the results of previous studies (Devriese et al. 1986; Hassan et al. 2005; Lyskova et al. 2007). On the other hand, Hassan et al. (2005) reported that the fermentation of salicin was negative, but in the current study, consistent with Devriese et al. (1986), we determined all S. canis isolates (100%) were positive for salicin fermentation. In addition, previous researchers reported different results in relation to mannitol and trehalose fermentation (Devriese et al. 1986; Hassan et al. 2005; Lyskova et al. 2007). Hassan et al. (2005) found mannitol and trehalose fermentation to be negative; Devriese et al. (1986) reported that trehalose fermentation was positive in only four of 31 S. canis isolates but mannitol fermentation was negative in all isolates, and Lysková et al. (2007) detected trehalose fermentation in 13 of 86 S. canis isolates and mannitol fermentation in only one sample. In the current study, similar to the findings of Lysková et al. (2007), nine of 12 isolates were positive for trehalose fermentation and four for mannitol fermentation. We consider that these variations reported in biochemical tests may be due to the epidemiological differences between the countries in which the studies were undertaken. In addition, it has been reported that streptococcal isolates may vary according to the characteristics of the host and disease from which the streptococcal isolates are isolated (Devriese et al. 1986; Jensen and Kilian 2012; Richards et al. 2012). In this study, the lack of significant differences between the streptococcus isolates in terms of phenotypic and biochemical properties may be similarly related to host or disease characteristics or the low number of isolates.

It has been reported that *S. canis* have biochemical and genetic similarities to *Streptococcus pyogenes*, *Streptococcus dysgalactiae* subsp. *equisimilis*, and *S. dysgalactiae* subsp. *dysgalactiae* (Efstratiou et al. 1994; Jensen and Kilian 2012; Lefébure et al. 2012), and there may be genetic transmission between different species (Richards et al. 2012; Pinho et al. 2013). In the literature (Hassan et al. 2003, 2005), it has been suggested that ISR as a species-specific gene can be used in the identification of *S. canis* whereas the $sodA_{int}$ gene is not found in non-*S. canis* streptococcus strains, and therefore can be an important indicator in distinguishing *S. canis* from other *Streptococcus* species. However, Moriconi et al. (2017) reported the presence of the ISR gene region in one *S. dysgalactiae* subsp. *equisimilis* strain and one *S. pyogenes* strain. The authors also noted that the $sodA_{int}$ gene could be used in the accurate identification of *S. canis* strains (Moriconi et al. 2017). In the current study, considering that the use of one primer pair could lead to suspicions or misidentification, the presence of ISR and $sodA_{int}$ was investigated in order to verify the *S. canis* isolates by PCR, and both gene regions were detected in all isolates. This result supports the findings of previous studies (Hassan et al. 2003, 2005), reporting that these genes can be used in the fast and reliable identification of *S. canis*. However, based on the research results reported by Moriconi et al. (2017), we concluded that the presence of ISR and $sodA_{int}$ gene should be investigated in a higher number of *S. canis* isolates for an accurate identification by the PCR test.

Consistent with the literature, the results of this study revealed the susceptibility of *S. canis* isolates to amoxicillin-clavulanic acid, cefoperazone (Hassan et al. 2005), ampicillin (Lysková et al. 2007), and penicillin G (Chaffer et al. 2005; Hassan et al. 2005; Lysková et al. 2007; Pedersen et al. 2007). In contrast, in accordance with the results of other research (Bagcigil et al. 2004; Chaffer et al. 2005; Hassan et al. 2005), all of our isolates (100%) were found to be resistant to gentamicin and neomycin, which is an expected finding for *Streptococcus* sp., although Olufemi et al. (2017) detected gentamicin resistance in only 50% of their isolates. In addition, in the current study, only two (16.66%) of the 12 *S. canis* isolates were found to be resistant to oxytetracycline in contrast to Pedersen et al. (2007), who reported the highest level of resistance to tetracycline (27% of 37 *S. canis* isolates) and Lysková et al. (2007), who calculated the rate of tetracycline resistance as 29.2% in 86 *S. canis* isolates. In another study, Diren Sığırcı et al. (2012) reported that the *S. canis* strain isolated from a dog's urinary tract infection was susceptible to oxytetracycline. Another finding of the current study was that 25% of the *S. canis* isolates were susceptible to enrofloxacin, 83.33% to chloramphenicol, 58.33% to lincomycin, 91.66% to rifamycin, and 75% to trimethoprim-sulfamethoxazole, while erythromycin susceptibility was seen in 50% of the isolates and moderate erythromycin susceptibility in the other half of the sample. Although these results are generally compatible with the findings of other researchers (Chaffer et al. 2005; Hassan et al. 2005; Lysková et al. 2007; Pedersen et al. 2007; Diren Sığırcı et al. 2012),

the susceptibility of the isolates to enrofloxacin and erythromycin were lower than those previously reported. This result also supports the result of a previous study, which reported that the macrolide/lincosamide resistance rates have been increasing rapidly in *S. canis* strains (Fukushima et al. 2019).

Conclusion

We conclude that the identification of *S. canis* can be made more accurately and faster by detecting species-specific ISR and $sodA_{int}$ genes. In addition, unlike *Staphylococci* and *Enterobacteriaceae*, pets infected with *S. canis* were still susceptible to antibiotics commonly used in veterinary medicine. However, in this study, the sensitivity of *S. canis* isolates to enrofloxacin and erythromycin was found to be much lower than previously reported in the literature, indicating their potential to develop resistance to macrolides and quinolones, which warrants periodical monitoring of antibiotic resistance development in *S. canis* isolates. In addition, this result may be an epidemiological indicator of antibiotic use. Therefore, antibiotic susceptibility should be determined before treatment in order to apply the correct treatment protocol for infections caused by *S. canis*. In critical cases where this may not be possible, we suggest that amoxicillin-clavulanic acid, ampicillin, cefoperazone, and penicillin G should be among the first antimicrobial agents to be preferred in the treatment of *S. canis* infections. Considering that *S. canis* is a zoonotic bacterium, possible human infections can be eliminated by preventing the transmission of *S. canis* to animal owners through a rapid and identification of related infections and their treatment with sensitive antibiotics.

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Ethic statement: This study does not present any ethical concerns.

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