AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

70 (2023) 2, 134–141

DOI:
10.1556/030.2023.01899

RESEARCH ARTICLE

Prevalence and clonal diversity of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs and cats with eye discharge

SEYDA CENGIZ[1], SITKICAN OKUR[2], CIHAN OZ[1], FERDA TURGUT[2], BURAK GUMURCINLER[2], NUR SELCEN SEVUK[3], AYSE ILGIN KEKEC[3], HALUK CEPOGLU[4], UMIT SEVIMLI[5] and MEHMET CEMAL ADIGUZEL[1,6*]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Ataturk University, 25240, Erzurum, Turkey

[2] Department of Surgery, Faculty of Veterinary Medicine, Ataturk University, 25240, Erzurum, Turkey

[3] Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpasa, 34320, Istanbul, Turkey

[4] Vetmemorial Veterinary Clinic, 34734, Istanbul, Turkey

[5] Pendik Veterinary Control Institute, Istanbul, 34890, Turkey

[6] Veterinary Vaccine and Biological Product Development Application and Research Center, Ataturk University, 25240, Erzurum, Turkey

Received: October 4, 2022 • Accepted: January 18, 2023
Published online: February 1, 2023

ABSTRACT

Objectives: Methicillin-resistant *Staphylococcus* (MRS) has originated, spread extensively, and become a prominent source of bacterial infections in both human and animal. *Methods:* We report the prevalence, genetic diversity, and antimicrobial resistance pattern of *Staphylococcus pseudintermedius* and *Staphylococcus aureus* strains isolated from dogs and cats with eye discharges. *Results:* A total of 12 (6.0%) coagulase-positives staphylococci were identified as (6/200, 3%) *S. aureus* and (6/200, 3%) *S. pseudintermedius*. The phenotypic methicillin resistance of *S. aureus* and *S. pseudintermedius* were 50.0% (3/6) and 16.7% (1/6), respectively. None of the isolates showed biofilm formation in the microtiter plate assay. The highest resistance (50.0%) for *S. pseudintermedius* strains was detected against clindamycin and tetracycline. 67.0% of *S. aureus* isolates were resistant to penicillin-G. The PCR analysis conducted for detection of *mecA* gene indicated that only one *S. aureus* isolated from a cat was *mecA* gene positive. Phylogenetic analysis based on repetitive sequence-based PCR (rep-PCR) showed that all strains were typable and generated PCR products ranging from 800 bp to 4,400 bp. The lineages ST241 and the novel ST2361 in multi-locus sequence typing (MLST) analysis were detected in one methicillin-susceptible *S. pseudintermedius* and methicillin-resistant *S. pseudintermedius* of dogs, respectively. In addition, the lineages ST4155 and ST7217 of two methicillin-resistant *S. aureus* strains of cats were connected epidemiologically to previously reported cases. *Conclusions:* These results indicate epidemiologically related strains (ST241, ST4155, and ST7217) transferring between animals and humans. Therefore, the strategies to combat the widespread MRS should be based on collaboration between human and veterinary medicine under the One Health concept.

KEYWORDS

cats, dogs, methicillin-resistant *S. aureus*, methicillin-resistant *S. pseudintermedius*

*Corresponding author. Department of Microbiology, Faculty of Veterinary Medicine, Ataturk University, 25240, Erzurum, Turkey.
Tel.: +90 442 231 7043.
E-mail: mcemal.adiguzel@atauni.edu.tr, mcemaladiguzel@gmail.com

1. INTRODUCTION

Bacteria in the genus *Staphylococcus* exist as a member of the cutaneous and mucosal flora of the companion animals [1, 2]. They can be found at the various body site of sick and/or healthy animals, however, some *Staphylococcus* species including *Staphylococcus aureus* and *Staphylococcus pseudintermedius* were generally reported from healthy or clinical cases of animals [3–6]. Especially, once the integrity of the ocular tissue is injured or damaged due to some physical impact, opportunistic bacteria including *Staphylococcus* species may cause the ocular infection [1]. Multi-resistant staphylococci species, especially harbored in companion animals such as dogs and cats, may cause infection in humans. Veterinarians have close contact with these pathogens due to the sick animals and also can transfer to humans via direct contact or contamination of the animal hospital environment [4, 7–9]. Genetic analysis of strains isolated from companion animals, humans, and animal hospital environments in previous studies showed that there is a high similarity between the recovered strains, implying that animals and hospital environments have a significant role in colonization and transmission to humans [10–12].

Staphylococcal infections require antimicrobial therapy and the most preferred antimicrobial agent is beta-lactam antibiotics [13]. However, *Staphylococcus* species including *S. aureus* and *S. pseudintermedius* have developed and/or acquired resistance to almost all antibacterial agents due to the massive misuse of the antimicrobial agents [7]. Recent reports showed that *S. aureus* and *S. pseudintermedius* have developed an antimicrobial resistance to methicillin that indicates a low affinity to all beta-lactams including cephalosporins [14]. In addition, methicillin-resistant *Staphylococcus* spp. (MRS) are considered resistant to all beta-lactams approved for veterinary use by the definition of the Clinical Laboratory Standard Institute [15]. Besides, methicillin-resistant *S. aureus* (MRSA) and *S. pseudintermedius* (MRSP) may also be resistant to non-beta lactam antibiotics, and this further reduces the antimicrobial agent options when the infection occurs. [7].

Different *mec* genes have been reported to represent methicillin resistance by molecular techniques such as polymerase chain reaction (PCR) [16–19]. For instance, the *mecA* gene is potentially located on mobile genetic elements called staphylococcal cassette chromosome *mec* (SCCmec). SCCmec elements are motile and therefore the same elements can be found in different strains of *S. aureus*, *S. pseudintermedius*, and other staphylococci [20].

The purposes of this study were to detect the prevalence of MRS isolates from dogs and cats with eye discharges, characterize the isolates with molecular techniques, and reveal the possible genetic similarity between strains.

2. MATERIALS AND METHODS

2.1. Sample population and collection

A prospective study was conducted on dogs (n = 71) and cats (n = 129) with clinical eye discharge, which might have a history of ophthalmological problems during sampling or before. All patients having an eye discharge were included in this study at the Faculty of Veterinary Medicine Animal Hospital at Ataturk University in eastern Turkey between 2019 and 2021. The sex, breed, age, and clinical signs of the animals were recorded. In addition, physical examination of the eye such as keratitis, chemosis, conjunctivitis, corneal pannus, and corneal edema was recorded by physicians. Each separate sample (one sample per animal) was collected from animals using a sterile cotton swab (Stuart medium, Becton Dickinson and Company, USA) at the site of the medial canthus of the eye. The swab samples were stored at −80 °C till starting laboratory analyses. The study has conducted with the approval and permission of the Local Ethics Council of Animal Experiments (Approved: E-75296309-050.01.04-2200103621).

2.2. Bacterial culture, phenotypic, and molecular identification

Samples were enriched by inoculating into Trypticase soya broth (TSB, Oxoid, Cambridge, UK) and incubated for 24 h at 37 °C. Following enrichment, a homogenate loop was streaked onto Baird-Parker agar (BPA, Cambridge, UK) and Mannitol salt agar (MSA, Cambridge, UK) at the same time. Suspicious colonies on BPA (shiny and black colonies surrounded by a light area) and MSA (yellow colonies) were subcultured onto blood agar plates with 7% sheep blood to obtain pure culture which was then confirmed to species-level identification with the standard operating procedure of the laboratory, which included colony color, deoxyribonuclease activity, a positive catalase and tube coagulase reaction, Gram stain, and polymyxin B susceptibility. Isolates were identified using the BD Phoenix™ PMIC-600 panel (Becton Dickinson, San Jose, CA, USA) identification and susceptibility testing. The confirmed isolates were stored at −80 °C for further laboratory analyses.

The boiling method was used to get genomic DNA. Briefly, a colony was dissolved in 40 µL single-cell lysis buffer (including Tris–HCl, disodium EDTA, and TE Buffer). To lyse bacterial cells, the tube was then heated to 80 °C for 10 min and cooled down to 55 °C for 10 min in a thermocycler. After that, the suspension was diluted with distilled water and centrifuged at 4,500×g for 30 s to eliminate the debris [21].

Using a PCR master mix (Dream Taq Green, Thermo Scientific, Waltham/MA, USA), a PCR reaction for confirmation of suspected isolates based on the *nuc* gene locus were performed with the previously reported protocol [22]. PCR products were run on a 1.5% agarose gel with DNA stain (Applied Biological Materials, British Columbia, Canada) for 45 min at 100 V. Gels were photographed using a gel documentation system (Vilber Lourmat, Cedex, France). The genomic DNA of the laboratory strain of *S. pseudintermedius* and *S. aureus* ATCC 25923 were used as positive control during the experiment.

2.3. The biofilm production on crystal violet microtiter plate assay

The biofilm formation for *S. aureus* and *S. pseudintermedius* isolates was performed as previously reported using a 96-well microplate [23]. Briefly, the strains were incubated aerobically on Mueller Hilton (MH) agar (Oxoid, Cambridge, UK) overnight at 37 °C. After a colony was taken out, it was cultured in MH broth overnight at 37 °C to create a bacterial suspension with 0.5 McFarland standard turbidity. The final salt or sugar concentrations in TSB was prepared as previously reported [23]. The fresh culture was adjusted to a final bacteria concentration of 10^6 CFU mL^{-1} and allocated into the wells (200μL/well). An empty well was used for the negative control. The microtiter plate was kept aerobic condition at 37 °C for 24 h. The bacteria were cultivated from each microtiter plate, and the heat at 65 °C for an hour was used to fix the cells. Non-adherent bacteria were removed by washing the wells twice with 200 μL of phosphate buffered saline (PBS, pH 7.4). The crystal violet 0.1% (w/v) was then used for staining of wells for 5 min. The residual crystal violet was then removed, and it was washed twice with PBS again to eliminate any residual dye before being allowed to dry at room temperature for 30 min. The 150 μL of 33% glacial acetic acid per well was used to dissolve the stain-attaching biofilm for 30 min. The biofilm development was assessed using a 96-well ELISA reader to measure the absorbance at 595 nm (Multiscan FC, Thermo Fisher Scientific, Waltham/MA, USA). Each test was repeated two times. Biofilm formation was confirmed by having the absorbance of 595 nm being three times the mean absorbance standard deviation of the negative control.

2.4. Antimicrobial susceptibility and detection of methicillin resistance

The standard broth microdilution method was performed to detect the antimicrobial susceptibility of isolates according to the European Committee on Antimicrobial Susceptibility Testing [24]. Commercially available BD Phoenix™ PMIC-600 panel (Becton Dickinson) antimicrobial susceptibility test (AST) cards for staphylococci were used for the detection of the minimum inhibitory concentration (MIC). The results were obtained at the end of the whole reaction in the BD Phoenix™ system. The antimicrobial resistance breakpoints were used according to the interpretive standards established by the EUCAST for bacteria isolated from animals [24].

Detection of methicillin-resistant *Staphylococcus* spp. (MRS) was based on the oxacillin MIC result according to AST cards. The confirmation of MRSA and MRSP strains after the BD Phoenix™ panel was performed using a disc diffusion test (cefoxitin 30 μg for *S. aureus* and oxacillin 1 μg for *S. pseudintermedius*) according to EUCAST criteria. The phenotypic oxacillin-resistant strains were analyzed to detect of the *mecA* gene by PCR. A PCR master mix (Thermo Scientific), template DNA, reverse and forward primers (10 pmol μL^{-1}) for *mecA* gene, and deionized water were used in the reactions. To amplify the *mecA* gene of the strains, annealing temperature, and primers were used as in previously reported studies [19]. The laboratory strain detected by PCR and confirmed by sequencing was used as a positive control for the *mecA* gene.

2.5. Repetitive sequence-based PCR (rep-PCR) fingerprinting

The rep-PCR fingerprinting was performed as described previously [25]. Totally, 2 μL template DNA and 23 μL of PCR master mix including a PCR master mix (Thermo Scientific), $(GTG)_5$ primer: 5′-GTGGTGGTGGTGGTG-3′ (10 pmol μL^{-1}), and deionized water were contained into PCR reaction mix. PCR conditions were used following denaturation at 95 °C for 7 min, 35 cycles of amplification (denaturation at 95 °C for 1 min, primer annealing at 40 °C for 1 min, extension at 65 °C for 8 min), and final extension at 72 °C for 10 min in a thermal cycler. PCR products were visualized on a 1% agarose gel containing the SafeView DNA stain (Applied Biological Materials) for 90 min at 75 V. The presence of band patterns was transferred using Microsoft Excel, which was then utilized to create a data matrix [26]. The percentage of similarity and matrix data were analyzed using the unweighted pair group technique with arithmetic mean (UPGMA) and complete linkage algorithms [12]. The ITOL (https://itol.embl.de/) was used to display relationships between the various band patterns.

2.6. MLST analysis

MLST analysis was performed following the methods described at the *S. aureus* and *S. pseudintermedius* PubMLST website [27] based on seven housekeeping genes including *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqi*, and *purA*, *fdh*, *ack*, *sar*, t*uf*, *cpn60*, and *pta,* respectively. The PCR products were sequenced and the results were analyzed in PubMLST *S. aureus* and *S. pseudintermedius* database. Allelic profiles, allelic numbers, and sequence types (STs) were assigned using the different modules of *S. aureus* and *S. pseudintermedius* typing database at the PubMLST website.

2.7. Statistical analysis

The One-Way Anova and Fisher's exact test were used to the comparison of the age of animals and isolated bacteria, and bacteria and animal species, respectively. In addition, Fisher's exact was also used to detect of the relationship between eye discharge and isolated bacteria from samples. The $P < 0.05$ was considered significant.

3. RESULTS

In total two-hundred dogs ($n = 71$) and cats ($n = 129$) were included in this study. Although there were 106 (53.0%) male dogs and cats, the rest of the animals were female ($n = 94$, 47%). The sampled animals in this study had clinically serous (59.0%), mucopurulent (18.5%), purulent (12.5%), seromucous (7.5%), and mucous discharge (2.5%).

The mean age of all dogs and cats were 2.02 ± 2.50 (range; 0.5–9 years) and 1.01 ± 1.28 years (range, 0.3–7 years), respectively. There was no statistical relationship between age and isolated bacteria, type of eye discharge and isolated bacteria tested in this study ($P < 0.05$).

Out of two-hundred collected samples, 12 (6.0%) yielded coagulase-positives staphylococci. In total, six (3%) *S. aureus* and *S. pseudintermedius* were isolated from dogs and cats sampled in this study and confirmed by BD Phoenix™ PMIC-600 panel (Becton Dickinson) and PCR. *S. aureus* strains were isolated from two dogs and four cats, whereas *S. pseudintermedius* strains were from four dogs and two cats.

In vitro phenotypic biofilm formation of *S. aureus* and *S. pseudintermedius* was investigated by the microplate test, which showed that all strains tested in this study were weak biofilm producers. None of the isolates tested in the current study was classified as moderate or strong biofilm producers.

The antimicrobial susceptibility test using the standard broth microdilution method following the European Committee on Antimicrobial Susceptibility Testing instruction was performed for a total of six *S. aureus* and six *S. pseudintermedius* isolates including the antimicrobials in the BD-Phoenix panel (Table 1). All isolates including both *S. pseudintermedius* and *S. aureus* displayed no resistance to amikacin, gentamicin, and nitrofurantoin (Fig. 1). 67% of *S. aureus* isolates were resistant to penicillin G, whereas 50% of *S. pseudintermedius* were resistant. In addition, resistance against penicillin G was detected in nine strains (75%) tested

Table 1. Distribution of MIC values of *S. aureus* and *S. pseudintermedius* isolates ($n = 12$)

Antibiotics	Bacteria	No. of isolates with MIC (μg mL^{-1})[†]												
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64	NS%
Amikacin	*S. aureus*	–	–	–	–	–	–	–	6	–	| 0	–	–	0.0
	S. pseudintermedius	–	–	–	–	–	–	–	–	6	| 0	–	–	0.0
Ciprofloxacin	*S. aureus*	–	–	–	–	–	5	| 0	1[‡]	0	–	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	5	| 0	1[‡]	0	–	–	–	16.6
Clindamycin	*S. aureus*	–	–	–	5	0	| 0	1	–	–	–	–	–	25.0
	S. pseudintermedius	–	–	–	3	1	| 0	2	–	–	–	–	–	50.0
Daptomycin	*S. aureus*	–	–	–	–	–	5	| 0	1[‡]	–	–	–	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	5	| 0	1[‡]	–	–	–	–	–	16.6
Erythromycin	*S. aureus*	–	–	–	4	0	| 0	0	2	–	–	–	–	33.3
	S. pseudintermedius	–	–	–	4	0	| 0	0	2	–	–	–	–	33.3
Fosfomycin w/G6P	*S. aureus*	–	–	–	–	–	–	–	–	6[‡]	0	0	| 0	0.0
	S. pseudintermedius	–	–	–	–	–	–	–	–	5[‡]	0	0	| 1	16.6
Fusidic Acid	*S. aureus*	–	–	–	–	–	6	| 0	0	0	–	–	–	0.0
	S. pseudintermedius	–	–	–	–	–	5	| 0	–	–	1	–	–	16.6
Gentamicin	*S. aureus*	–	–	–	–	–	6[‡]	0	0	| 0	–	–	–	0.0
	S. pseudintermedius	–	–	–	–	–	6[‡]	0	0	| 0	–	–	–	0.0
Levofloxacin	*S. aureus*	–	–	–	–	–	5	| 0	0	1	–	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	5	| 0	0	1	–	–	–	16.6
Linezolid	*S. aureus*	–	–	–	–	–	–	5	0	| 0	1	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	–	5	0	| 0	1	–	–	16.6
Moxifloxacin	*S. aureus*	–	–	–	5	0	| 0	1	–	–	–	–	–	16.6
	S. pseudintermedius	–	–	–	5	0	| 0	1	–	–	–	–	–	16.6
Nitrofurantoin	*S. aureus*	–	–	–	–	–	–	–	–	–	–	6	| 0	0.0
	S. pseudintermedius	–	–	–	–	–	–	–	–	–	–	6	| 0	0.0
Penicillin G	*S. aureus*	–	–	2	| 1	0	3	–	–	–	–	–	–	66.6
	S. pseudintermedius	–	–	3	| 1	0	2	–	–	–	–	–	–	50.0
Rifampin	*S. aureus*	–	–	–	5	0	0	| 1	–	–	–	–	–	16.6
	S. pseudintermedius	–	–	–	5	0	0	| 1	–	–	–	–	–	16.6
Teicoplanin	*S. aureus*	–	–	–	1	0	4	| 0	0	0	1	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	5	| 0	0	0	1	–	–	16.6
Tetracycline	*S. aureus*	–	–	–	–	3	1	0	0	| 2	–	–	–	33.3
	S. pseudintermedius	–	–	–	–	3	0	0	0	| 3	–	–	–	50.0
Trimethoprim-Sulfamethoxazole	*S. aureus*	–	–	–	–	–	–	5[‡]	| 0	1	–	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	–	5[‡]	| 0	1	–	–	–	16.6
Oxacillin	*S. aureus*	–	–	–	3	| 1	1	0	0	1	–	–	–	50.0
	S. pseudintermedius	–	–	–	5	0	0	0	| 0	1	–	–	–	16.6
Vancomycin	*S. aureus*	–	–	–	–	–	5	0	| 0	0	1[‡]	–	–	16.6
	S. pseudintermedius	–	–	–	–	–	5	0	| 0	0	1[‡]	–	–	16.6

[†]Antibiotic concentrations included in the test panel are displayed. [‡]MIC values should be read as ≥ or ≤ to the corresponding concentration. Thick black lines indicate breakpoints (should be read as ≥ to the corresponding concentration) for not susceptible (NS; intermediate + resistant) isolates for each antimicrobial.

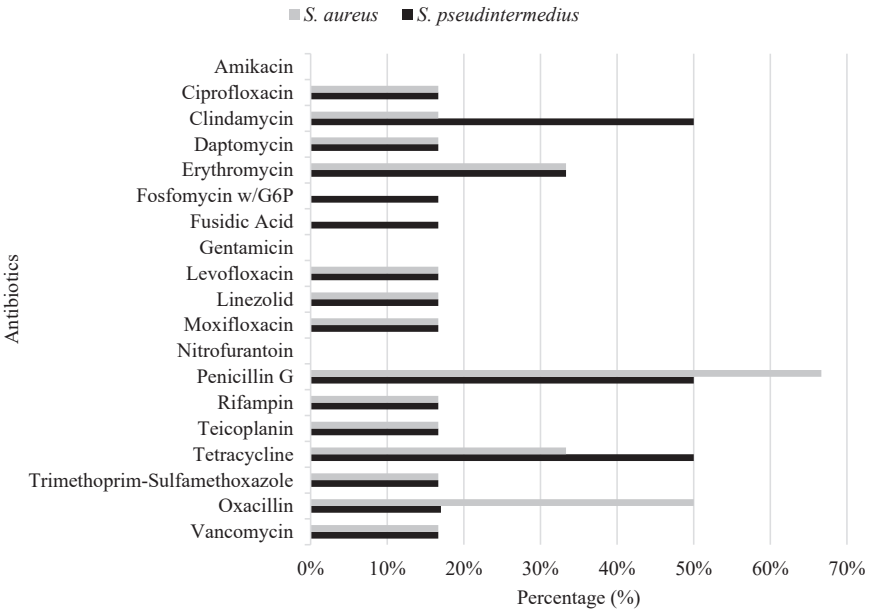

Fig. 1. Percentage of *S. pseudintermedius* ($n = 6$) and *S. aureus* ($n = 6$) isolates resistant to the antimicrobials tested in this study

in this study. The highest resistance for *S. pseudintermedius* strains tested in this study was detected against clindamycin and tetracycline (50%). The low level of antimicrobial resistance (17%) of both *S. pseudintermedius* and *S. aureus* isolated in this study was determined against ciprofloxacin, daptomycin, levofloxacin, linezolid, moxifloxacin, rifampin, teicoplanin, trimethoprim-sulfamethoxazole, and vancomycin (Fig. 1).

Although the three *S. aureus* and five *S. pseudintermedius* isolates were susceptible (MIC value of all strains was ≤0.25 μg mL^{-1}) to oxacillin based on MIC values by Phoenix antimicrobial susceptibility panel, three (3/6, 50.0%) *S. aureus* and one (1/6, 16.7%) *S. pseudintermedius* isolated from dogs and cats with conjunctivitis had >4 μg mL^{-1} MIC value and were evaluated as methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MPSP) in this study, respectively. However, the disc diffusion results of the phenotypic methicillin resistance of the strains showed that three of the *S. aureus* (the zone diameter on the agar plate of resistant strains was 13 mm (#65), 14 mm (#94), and 12 mm (#176), and it was >28 for susceptible strains) (50.0%) were resistant to cefoxitin, and all of the *S. pseudintermedius* strains (the zone diameter on the agar plate of susceptible strains was >19) (0.0%) tested in this study were susceptible to oxacillin (Fig. 2). To detect the *mecA* gene in phenotypic methicillin-resistant three *S. aureus* and one *S. pseudintermedius* strains, PCR was performed using primer sets and protocols previously reported in each respective manuscript. The result showed that only one *S. aureus* isolated from cat was carrying *mecA* gene, whereas the other three strains were negative for all *mecA* gene via PCR tested in this study.

The rep-PCR fingerprint analysis showed that both *S. aureus* and *S. pseudintermedius* strains tested in this study were typable and generated PCR products ranging from 800

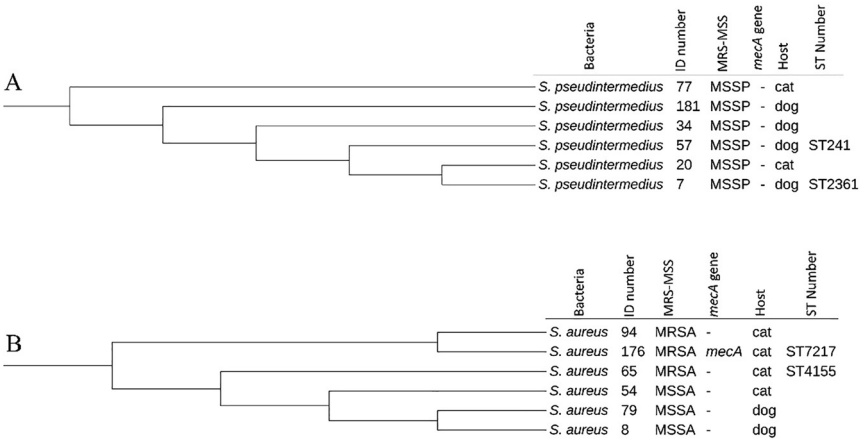

Fig. 2. Unweighted pair group method with arithmetic mean and complete linkage algorithms phylogenetic tree based on rep-PCR harboring *S. pseudintermedius* (A) and *S. aureus* (B) isolated in this study. ID numbers determined for this study are indicated in the figure

bp to 4,400 bp. The phylogenetic tree using the unweighted pair group method with arithmetic mean and complete linkage algorithms displayed two main clusters. The phylogenetic relationship between the strains isolated in this study is presented in Fig. 2.

MLST was performed on selected isolates (one methicillin-susceptible and three methicillin-resistant strains) to represent the genetic diversity. Each of the STs was represented by a single strain. The novel ST (ST2361) was caused by the detection of new allelic profiles for one *S. pseudintermedius* strain (MRSP) tested in this study. Another *S. pseudintermedius* strain belonged to ST241 (methicillin-susceptible *S. pseudintermedius*, MSSP), while two *S. aureus* were ST4155 and ST7217. Of note, ST7217, which is MRSA and *mecA* gene-positive, was determined in MLST clonal complex (CC)1, whereas ST4155 (MRSA strain but *mecA* gene negative) was CC22.

4. DISCUSSION

In this study, we investigated the prevalence and genomic diversity of MRS in dogs and cats with eye discharges between 2019 and 2021 in eastern Turkey. One MRSA strain (from the cat) was shown to carry the *mecA* gene. Further characterization of the isolates was conducted by rep-PCR fingerprint and MLST analysis. MRS strains (3/12, 25.0%) were detected in two dogs and one cat with conjunctivitis in the current study. In contrast to our study, MRS was isolated in seventeen keratitic dogs with corneal stromal ulcer ($n = 14$), corneal incision infection ($n = 2$), and infected corneal rupture ($n = 1$) in the study population in the USA [28]. The coagulase-positive and coagulase-negative MRS species such as *S. aureus*, *S. pseudintermedius*, and *S. epidermidis* were reported in previous studies (in Germany from 2016, in the USA from 2015, in the USA from 2013, and in India from 2012, respectively) as the etiological agent of bacterial infections in ophthalmologic disease in the dogs and other species [1, 5, 28, 29]. A study conducted in Turkey reported that 66.67% prevalence rate of coagulase-positive staphylococci in 142 dog and cat samples. In addition, the prevalence of MRSP and MRSA strains were 25.64% and 28.20% in the same study, respectively [30]. Although the prevalence of *S. pseudintermedius* was reported at 39.6% in dogs and cats in Germany between 2015 and 2016, MRSP prevalence was 7.9% from dog samples in the same study [1]. Another study displayed that the prevalence of MRSP and MRSA were 8.5% (6/71) and 1.4% (1/71) isolated from dogs with keratitis, whereas MSSP and MSSA were 49.3% (35/71) and 2.8% (2/71), respectively [28].

Moderately high-level resistance to clindamycin, penicillin G, and tetracycline was detected in *S. aureus* and *S. pseudintermedius* the isolates. In addition, *S. aureus* and *S. pseudintermedius* displayed low-level resistance to some of the first option antibiotics for anti-MRS drugs used in human medicine such as vancomycin, teicoplanin, linezolid, and daptomycin in the current study. Interestingly, the same isolates were simultaneously resistant to these four antibiotics. These results further indicated that MRS strains from dogs and cats in this study still persist non-resistant to newly used MRS infection in human medicine, indicating these antibiotics may be effective against these agents. Similar to our study findings, the none or low level of resistance to the last resort antibiotics of using in human medicine was reported by other investigators in published articles [1, 7, 31–34]. Low resistance rates against the last resort antibiotics were detected in MRSP and MRSA isolates in the USA [31], in MRSA isolates in Serbia [33], and in MRSP strains in the Netherlands [34]. Of note, according to the MIC result, two strains (one of *S. aureus* and *S. pseudintermedius*) were resistant to vancomycin (MIC≥16 μg mL^{-1}) in the current study. Fifty-two vancomycin resistant *S. aureus* strains have been reported so far from different countries including Pakistan, Iran, Brazil, Portugal, USA. A *vanA* gene cluster, which is acquired from vancomycin resistant enterococcus, mediates vancomycin resistant in *Staphylococcus* species [35]. However, further analyses are needed to definitively ascertain the resistance mechanism of these strains.

PCR analysis of these strains was shown that only one MRSA strain isolated from a cat with conjunctivitis is *mecA* gene-positive. Previous studies indicated that different resistance mechanisms such as mutations in some PBP genes and β-lactamase hyperexpression could play a role in *mec*-genes-negative MRS strains [31, 36]. In addition, a previous study showed that other regulatory *mec* genes such as *mecI* or *mecR* alleles may play a role in developing methicillin resistance, however, those genes were not analyzed in the present study [37]. Further analyses are needed to definitively ascertain the exact resistance genes. EUCAST defined *S. aureus* that is high oxacillin MIC values in absence of *mec* gene-mediated resistance as borderline oxacillin resistant *S. aureus* [24].

Previously, a study reported the detection of MSSP ST241 isolated from lesions of patients and from nasal samples of their dogs living in the same household showed identical PFGE patterns and similar resistance phenotype and genotype in Spain [38]. Interestingly, the MSSP strain in that study shared the same ST as the current study strain, suggesting a potential transmission of MSSP strains between dogs and humans. Another study reported *S. pseudintermedius* ST241 in puppy and dam's milk, which is used for feeding puppies [39]. In addition, the same ST was reported in unrelated studies in humans and dogs in Switzerland and Sweden in the PubMLST database between 2014 and 2016 (https://pubmlst.org/organisms/staphylococcus-pseudintermedius). These findings clearly emphasize that *S. pseudintermedius* ST241 have an epidemiological link between human and animals and as a zoonotic pathogen is potentiated, even if it needs more research.

MRSA ST7217 harboring the *mecA* gene was reported from human sinusitis in Egypt in 2018. In addition, MRSA ST4155 isolated in the current study shared the same ST with human cases in the UK in 2017. These findings re-emphasize the transmission of MRSA strain between animals and humans even animal products as reported by other researchers previously [14, 33, 40, 41].

5. CONCLUSION

In summary, the current study indicates important insights into the prevalence, antimicrobial patterns, and clonal diversity of MRS strains from dogs and cats with eye discharge submitted to the diagnostic laboratory between 2019 and 2021. *S. aureus* and *S. pseudintermedius* might be playing an important role in eye discharge in the current study. Hence, further studies need to perform to understand the role of the bacteria. The coagulase positive staphylococci isolated in this study shared the MLST types of bacteria previously recovered from human clinical infections. Interestingly, one strain each of *S. aureus* and *S. pseudintermedius* isolated in the present study were resistant to vancomycin. These findings underline the importance of the antimicrobial susceptibility test and routine infection control measurements against MRS infections with eye infected dogs and cats, as well as reducing the spread of these bacteria from humans to animals.

REFERENCES

1. Soimala T, Lübke-Becker A, Hanke D, Eichhorn I, Feßler AT, Schwarz S, et al. Molecular and phenotypic characterization of methicillin-resistant *Staphylococcus pseudintermedius* from ocular surfaces of dogs and cats suffering from ophthalmological diseases. Vet Microbiol 2020; 244, 108687.
2. Morris DO, Loeffler A, Davis MF, Guardabassi L, Weese JS. Recommendations for approaches to meticillin-resistant staphylococcal infections of small animals: diagnosis, therapeutic considerations and preventative measures. Clinical consensus guidelines of the world association for veterinary dermatology. Vet Dermatol 2017; 28: 304–e69.
3. Thomson P, García P, Miles J, Isla D, Yáñez C, Santibáñez R, et al. Isolation and identification of *Staphylococcus* species obtained from healthy companion animals and humans. Vet Sci 2022; 9: 79.
4. Gentile D, Allbaugh RA, Adiguzel MC, Kenne DE, Sahin O, Sebbag L. Bacterial cross-contamination in a veterinary ophthalmology setting. Front Vet Sci 2020: 1141.
5. Mouney MC, Stiles J, Townsend WM, Guptill L, Weese JS. Prevalence of methicillin-resistant *Staphylococcus* spp. in the conjunctival sac of healthy dogs. Vet Ophthalmol 2015; 18, 123–6.
6. Varges R, Penna B, Martins G, Martins R, Lilenbaum W. Antimicrobial susceptibility of Staphylococci isolated from naturally occurring canine external ocular diseases. Vet Ophthalmol 2009; 12: 216–20.
7. Feßler AT, Scholtzek AD, Schug AR, Kohn B, Weingart C, Schink AK, et al. Antimicrobial and biocide resistance among feline and canine *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolates from diagnostic submissions. Antibiotics 2022; 11: 127.
8. Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou Elnaga Y, et al. Sharing more than friendship—nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS One 2012; 7, e35197.
9. Sing A, Tuschak C, Hörmansdorfer S. Methicillin-resistant *Staphylococcus aureus* in a family and its pet cat. N Engl J Med 2008; 358: 1200–1.
10. Hemeg HA. Determination of phylogenetic relationships among methicillin-resistant *Staphylococcus aureus* recovered from infected humans and companion animals. Saudi J Biol Sci 2021; 28: 2098–101.
11. Tabatabaei S, Najafifar A, Badouei MA, Salehi TZ, Tamai IA, Khaksar E, et al. Genetic characterisation of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in pets and veterinary personnel in Iran: new insights into emerging methicillin-resistant *S. pseudintermedius* (MRSP). J Glob Antimicrob Resist 2019; 16: 6–10.
12. Garcia-Vallvé S, Palau J, Romeu A. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. Mol Biol Evol 1999; 16: 1125–34.
13. Schwarz S, Feßler AT, Loncaric I, Wu C, Kadlec K, Wang Y, et al. Antimicrobial resistance among staphylococci of animal origin. Microbiol Spectr 2018; 6: 6–14.
14. Baran A, Oz C, Cengiz S, Adiguzel MC. Genomic characterization, antimicrobial resistance profiles, enterotoxin, and biofilm production of methicillin-resistant *Staphylococcus aureus* isolated from clinical and animal products origins in Eastern Turkey. Pesq Vet Bras 2022; 42: e06991.
15. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 5th ed. Wayne, PA, USA: CLSI Supplement VET01S; Clinical and Laboratory Standards Institute, 2020.
16. Becker K, van Alen S, Idelevich EA, Schleimer N, Seggewiß J, Mellmann A, et al. Plasmid-encoded transferable *mecB*-mediated methicillin resistance in *Staphylococcus aureus*. Emerg Infect Dis 2018; 24: 242.
17. Schwendener S, Cotting K, Perreten V. Novel methicillin resistance gene *mecD* in clinical *Macrococcus caseolyticus* strains from bovine and canine sources. Sci Rep 2017; 7, 43797.
18. Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, et al. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue mecALGA251. Clin Microbiol Infect 2012; 18: 395–400.
19. Horie H, Yamahata N, Takahashi N, Yoshida A, Katabami K, Sato H, et al. Enhancement of antimicrobial activities of antibiotics by combination with epigallocatechin gallate against methicillin-resistant *Staphylococcus aureus*. J Hum Nurs Stud 2009; 7: 19–26.
20. Krapf M, Müller E, Reissig A, Slickers P, Braun SD, Müller E, et al. Molecular characterisation of methicillin-resistant *Staphylococcus pseudintermedius* from dogs and the description of their SCC*mec* elements. Vet Microbiol 2019; 233: 196–203.
21. Sahin O, Shen Z, Zhang Q. Methods to study antimicrobial resistance in *Campylobacter jejuni*. Methods Mol Biol 2017; 1512: 29–42.
22. Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, et al. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol 2010; 48: 765–9.
23. Lade H, Park JH, Chung SH, Kim IH, Kim JM, Joo HS, et al. Biofilm formation by *Staphylococcus aureus* clinical isolates is differentially affected by glucose and sodium chloride supplemented culture media. J Clin Med 2019; 8: 1853.
24. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2022: 33–8.

25. Švec P, Pantůček R, Petráš P, Sedláček I, Nováková D. Identification of *Staphylococcus* spp. using (GTG) 5-PCR fingerprinting. Syst Appl Microbiol 2010; 33: 451–6.
26. Albufera U, Bhugaloo-Vial P, Issack MI, Jaufeerally-Fakim Y. Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. Infect Genet Evol 2009; 9: 322–7.
27. Solyman SM, Black CC, Duim B, Perreten V, Van Duijkeren E, Wagenaar JA, et al. Multilocus sequence typing for characterization of *Staphylococcus pseudintermedius*. J Clin Microbiol 2013; 51: 306–10.
28. LoPinto AJ, Mohammed HO, Ledbetter EC. Prevalence and risk factors for isolation of methicillin-resistant *Staphylococcus* in dogs with keratitis. Vet Ophthalmol 2015; 18: 297–303.
29. Kaliamurthy J, Kalavathy CM, Parmar P, Nelson Jesudasan CA, Thomas PA. Spectrum of bacterial keratitis at a tertiary eye care centre in India. Biomed Res Int 2013; 2013, 181564.
30. Göçmen H, Tamakan H, Şükür H, Esendal ÖM. Investigation of multidrug resistance among *Staphylococcus* species isolated from cats and dogs. Atatürk Univ J Vet Sci 2020; 15: 156–66.
31. Adiguzel MC, Schaefer K, Rodriguez T, Ortiz J, Sahin O. prevalence, mechanism, genetic diversity, and cross-resistance patterns of methicillin-resistant *Staphylococcus* isolated from companion animal clinical samples submitted to a veterinary diagnostic laboratory in the midwestern United States. Antibiotics 2022; 11: 609.
32. Shariati A, Dadashi M, Chegini Z, van Belkum A, Mirzaii M, Khoramrooz SS, et al. The global prevalence of daptomycin, tigecycline, quinupristin/dalfopristin, and linezolid-resistant *Staphylococcus aureus* and coagulase–negative staphylococci strains: a systematic review and meta-analysis. Antimicrob Resist Infect Control 2020; 9: 1–20.
33. Asanin J, Misic D, Aksentijevic K, Tambur Z, Rakonjac B, Kovacevic I, et al. Genetic profiling and comparison of human and animal methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from Serbia. Antibiotics 2019; 8: 26.
34. Wegener A, Broens EM, Zomer A, Spaninks M, Wagenaar JA, Duim B. Comparative genomics of phenotypic antimicrobial resistances in methicillin-resistant *Staphylococcus pseudintermedius* of canine origin. Vet Microbiol 2018; 225: 125–31.
35. Cong Y, Yang S, Rao X. Vancomycin resistant *Staphylococcus aureus* infections: a review of case updating and clinical features. J Adv Res 2020; 21: 169–76.
36. Argudín MA, Roisin S, Nienhaus L, Dodémont M, De Mendonça R, Nonhoff C, et al. Genetic diversity among *Staphylococcus aureus* isolates showing oxacillin and/or cefoxitin resistance not linked to the presence of *mec* genes. Antimicrob Agents Chemother 2018; 62: e00091–18.
37. Tyson GH, Ceric O, Guag J, Nemser S, Borenstein S, Slavic D, et al. Genomics accurately predicts antimicrobial resistance in *Staphylococcus pseudintermedius* collected as part of Vet-LIRN resistance monitoring. Vet Microbiol 2021; 254, 109006.
38. Lozano C, Rezusta A, Ferrer I, Pérez-Laguna V, Zarazaga M, Ruiz-Ripa L, et al. *Staphylococcus pseudintermedius* human infection cases in Spain: dog-to-human transmission. Vector-Borne Zoonotic Dis 2017; 17: 268–70.
39. Zakošek Pipan M, Švara T, Zdovc I, Papić B, Avberšek J, Kušar D, et al. *Staphylococcus pseudintermedius* septicemia in puppies after elective cesarean section: confirmed transmission via dam's milk. BMC Vet Res 2019; 15: 1–8.
40. Venugopal N, Mitra S, Tewari R, Ganaie F, Shome R, Rahman H, et al. Molecular detection and typing of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci isolated from cattle, animal handlers, and their environment from Karnataka, Southern Province of India. Vet World 2019; 12: 1760.
41. Rahman MM, Amin KB, Rahman SMM, Khair A, Rahman M, Hossain A, et al. Investigation of methicillin-resistant *Staphylococcus aureus* among clinical isolates from humans and animals by culture methods and multiplex PCR. BMC Vet Res 2018; 14: 1–6.