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Research article

MOLECULAR PREVALENCE OF *MecA* AND *MecC* GENES IN COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM DOGS WITH DERMATITIS AND OTITIS IN BELGRADE, SERBIA: A ONE YEAR STUDY

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The escalating global concern of antimicrobial resistance in human and veterinary medicine is exacerbated by the inappropriate prescription of antibiotics for bacterial infections in companion animals. This study aimed to determine the distribution of coagulase-positive staphylococci causing clinical skin and ear infections in dogs and to determine methicillin-resistant isolates. A total of 78 staphylococcal strains were isolated from clinical samples taken from patients at the Dermatology Clinic at the Faculty of Veterinary Medicine in Belgrade, Serbia. Multiplex PCR was used for species-specific identification, and *mecA* and *mecC* genes were used to determine methicillin resistance, in addition to phenotypic determination, MIC values and detection of PBP2a. Out of the 78 samples analyzed, 65.8% were identified as *Staphylococcus pseudintermedius*, 22.4% as *S. aureus*, 7.9% as *S. coagulans*, and 3.9% as *S. intermedius*. Four *S. aureus* isolates exhibited methicillin resistance confirmed by cefoxitin disk diffusion, while five were confirmed with MIC testing and latex agglutination. *MecA* gene was detected in 29.4% of *S. aureus* and 30% of *S. pseudintermedius* isolates. These isolates were classified as methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP), respectively. No isolates carried the *mecC* gene. This study provides insights into the prevalence of CoPS species and methicillin resistance in isolates from dogs. Continued surveillance is essential to monitor and understand the emergence and dissemination of antimicrobial resistance in veterinary medicine and the results of this study accent the need for establishment of a continuous antimicrobial resistance surveillance program in the Republic of Serbia.

Keywords: dogs, ear infections, methicilin resistance, MRSA, MRSP, skin infections

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INTRODUCTION

Antimicrobial resistance (AMR) is projected to become the leading cause of death worldwide in the coming decades [1]. Consequently, it has become a major global concern in both human and veterinary medicine [2]. Antibiotic prescriptions are frequently given due to skin and ear infections, which are some of the most prevalent pathologies affecting companion animals [2]. The primary causative agents for these conditions are coagulase-positive staphylococci (CoPS) of which most common in veterinary medicine are *S. aureus* and *S pseudintermedius* [2-4]. In addition to pyodermas and otitis, CoPS can cause a range of infections, including serious infections with bacteremia that can be fatal for both humans and animals. The emergence of methicillin-resistant strains of CoPS has further compounded these infections, as they are often resistant to multiple classes of critically important antimicrobials, thereby limiting therapeutic options and posing a significant clinical challenge in the treatment of bacterial pyoderma in companion animals [5-7]. The most common methicillin-resistant CoPS strains that cause clinical infections are methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP), which have emerged as zoonotic pathogens significant from both animal and public health perspectives [7,8,10,11]. MRSA is responsible for a wide range of infections, from skin and soft tissue infections and due to multidrug-resistant pattern to severe life-threatening conditions [9-11]. *S. pseudintermedius* is a commensal bacterium found on the skin and mucosa of dogs and cats. It can be isolated from healthy animals but also acts as an opportunistic pathogen, commonly causing skin infections, otitis externa, and post-operative wound infections [7]. Furthermore, it has been identified as the causative agent of numerous other infections [12,13]. The frequency of MRSP infections in dogs and cats has been increasing globally, and MRSP is now considered one of the most important pathogens in small animal medicine [7]. It poses a significant challenge in veterinary medicine due to its multidrug-resistant nature [14,15]. Although MRSP infections were initially associated mainly with animals, an increasing number of studies recognize *S. pseudintermedius* and MRSP strains as opportunistic human pathogens and emerging zoonotic agents, albeit to a lesser extent than MRSA [4,7,16]. Apart from *S. aureus* and *S. pseudintermedius*, other CoPS species that can colonize dogs and cats include *S. coagulans* (formerly known as *S. schleiferi* subsp. *coagulans*) and *S. intermedius,* which can also cause diseases in certain situations, although less frequently [7,17]. Over the past few decades, much attention has been focused on the role of pet animals as reservoirs of antimicrobial-resistant bacteria, particularly due to the significant increase in the number of people, especially in developing countries, living with pets, particularly dogs [18,19]. Many individuals consider their pets to be integral parts of their families, and the close physical contact, shared environments, and administration of antibiotics similar to those recommended for humans pose a potential risk of transferring resistant bacteria and resistance genes between humans and animals [20,21]. In recent years, studies have demonstrated the emergence and clonal spread of methicillin-resistant staphylococci (MRS) that can

occur between animals and humans and vice versa [22]. The most frequent mechanism of resistance in MRS is mediated by the *mecA* gene, which is part of a mobile genetic element called the staphylococcal cassette chromosome (SCC*mec*) [23]. This gene encodes the production of an altered penicillin-binding protein, PBP2a, which has extremely low affinity for methicillin. The production of PBP2a renders staphylococci resistant to all beta-lactam antibiotics, including penicillins, beta-lactams with beta-lactamase inhibitors, most cephalosporins and carbapenems. Additionally, the SCC*mec* often carries multiple resistance genes to other antibiotics, resulting in MRS strains' resistance to aminoglycosides, fluoroquinolones, tetracyclines, macrolides and chloramphenicol. Consequently, infections caused by these strains often have fatal outcomes [24-26]. A variant of *mecA*, known as *mecC*, has been detected in MRS from animal and environmental samples [27-30].

The objectives of this study were to determine the prevalence of CoPS species causing clinical skin and ear infections in dogs and to identify methicillin-resistant CoPS isolates in Belgrade, Serbia, during the period 2021-2022. Periodic surveys of this nature are crucial for understanding the trends in the emergence and dissemination of antimicrobial resistance in companion animals, particularly in the Republic of Serbia, where data on this subject are still lacking despite many countries having established antimicrobial resistance surveillance programs recommended by the World Organization for Animal Health.

MATERIALS AND METHODS

A total of 79 staphylococcal strains were isolated from clinical samples collected from dogs with suspected bacterial skin (n=60) and ear (n=19) infection from the Dermatology clinic at the Faculty of Veterinary Medicine, University of Belgrade during the period between October 2021 and December 2022. These samples were collected as part of routine diagnostics. The samples were inoculated in Columbia agar with 5% sheep blood (Becton Dickinson, USA) and MacConkey agar (Becton Dickinson, USA) and incubated at 37°C for 24 hours. Identification of the isolates was conducted using standard bacteriological testing methods, which included assessing colony morphology, hemolysis, Gram stain, catalase test, coagulase production, O-nitrophenyl-β-d-galactopyranoside test and sensitivity to polymyxin B. Once characterized and identified, the isolates were preserved in 20% glycerol media at -20°C until further use.

DNA extraction

After cryopreservation, the isolates were inoculated on Blood agar to confirm the absence of contamination. During this phase, one ear sample was discarded. DNA extraction was performed following the protocol proposed by the European Union's Reference Laboratory for Antimicrobial Resistance located at the Faculty of Veterinary

Medicine in Lisbon, Portugal [31]. Briefly, after incubation on blood agar, colonies were suspended in phosphate-buffered saline (PBS), vortexed, and centrifuged for 5 minutes at 13000 rpm. The pellet was resuspended in 100 µl of Tris-EDTA (TE buffer). A small hole was made in the tube's cap using a hot needle, and the tubes were transferred to a floating Styrofoam rack, boiled for 10 minutes in a water bath, and then incubated on ice for one minute. The boiled suspension was then resuspended in 900 µl of TE buffer. Subsequently, 100 µl of the resuspended solution was transferred to a new tube and stored at -20°C until further use. The quality and quantity of the samples were confirmed using a BioSpec-nano UV-VIS Spectrophotometer.

PCR detection of Staphylococcus strains

For the purpose of differentiating staphylococcal strains, a multiplex PCR was employed for species-specific identification of CoPS based on the sequence diversity of the *nuc* gene, which encodes thermonuclease. The total reaction volume was 30 µl, comprising 3 µl of DNA extract, 0.75 µl of each forward and reverse primer at a concentration of 10 µM, 8.5 µl of nuclease-free water (Thermo Scientific, USA), and 12.5 µl of PCR Master mix (Thermo Scientific, USA). The primer sets used are listed in Table 1. Thermal cycling reactions consisted of an initial denaturation step at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and elongation at 72°C for 30 seconds, with a final elongation step at 72°C for 2 minutes. The specific oligonucleotide primers and the PCR protocol were described by Sasaki et al. [32]. Nuclease-free water served as the negative control, while in-house staphylococcal strains were used as positive control. DNA fragments were analyzed by electrophoresis in 0.5× TBE buffer on a 1.5% agarose gel stained with 0.5 µg/ml ethidium bromide.

Detection of *mecA* and *mecC* genes

Phenotypic detection and interpretation of results of methicillin resistance was performed according to guidelines proposed by CLSI and EUCAST recommendations [24,25] using disc-diffusion method on Mueller Hinton agar (HiMedia, India) and test discs of cefoxitin (30 mg) (Becton Dickinson, USA) for *S. aureus* and oxacillin (2 mg) (Becton Dickinson, USA) for other *Staphylococcus* species given that it has been proven that oxacillin disk diffusion test a superior method for detecting *mecA*-mediated methicillin resistance in *S. pseudintermedius*, *S. coagulans*, and *S. intermedius* [17,24,33]. MIC values were generated using E-test strips with cefoxitin according to the manufacturers' instructions. The test was performed on Mueller Hinton agar (HiMedia, India). Isolates with MIC values equal to and/or larger than 4µg/ml for cefoxitin were considered to be MRS. The presence of PBP2a in *S. aureus* was detected using latex agglutination Slidex®MRSA Detection test (bioMérieux, France) according to manufacturers' instructions. PCR detection of the *mecA* gene was performed according to the protocol from Isenberg et al. [34]. Primers are listed

in Table 1. The cycling conditions were: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94° C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 1 minute, and final elongation at 72°C for 5 minutes in the thermocycler (Eppendorf, Germany). For detection of the *mecC* gene we used specific oligonucleotide primers and cycling conditions described by Becker et al. [35], except the change in annealing temperature. The primers are listed in Table 1 and cycling conditions were: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, elongation at 72°C for 2 minutes and final elongation at 72°C for 7 minutes. The total reaction volume for both reactions was 25 μl containing 4 μl of DNA extract, 1 μl 10 μM forward primer, 1 μl 10 μM reverse primer, 6.5 μl nuclease-free water (Thermo Scientific, USA), and 12.5 μl PCR Master mix (Thermo Scientific, USA). DNA fragments were analyzed by electrophoresis in 0.5´ TBE buffer on a 1.5% agarose gel stained with 0.5 mg/ml ethidium bromide.

Table 1. Primer sequences used in study

Primer	Sequence (5'–3')	Species/gene
au-F3 **au-nucR**	TCGCTTGCTATGATTGTGG GCCAATGTTCTACCATAGC	*S. aureus*
in-F **in-R3**	CATGTCATATTATTGCGAATGA AGGACCATCACCATTGACATATTGAAACC	*S. intermedius*
sch-F **sch-R**	AATGGCTACAATGATAATCACTAA CATATCTGTCTTTCGGCGCG	*S. coagulans*
pse-F2 **pse-R5**	TRGGCAGTAGGATTCGTTAA CTTTTGTGCTYCMTTTTGG	*S. pseudintermedius*
MecAF **MecAR**	AAAATCGATGGTAAAGGTTGGC AGTTCTGCAGTACCGGATTTGC	*mecA* gene
MecCF **MecCR**	TCAAATTGAGTTTTTCCATTATCA AACTTGGTTATTCAAAGATGACGA	*mecC* gene

RESULTS

Out of the 79 samples classified as *Staphylococcus* spp. based on phenotypic characteristics, one ear sample was excluded from the study due to contamination. The presence of CoPS was confirmed in 76 (60 skin and 16 ear samples) out of the total 78 samples using multiplex PCR. Among the 76 CoPS isolates, 50/76 (65.8%) were identified as *S. pseudintermedius*, 17/76 (22.4%) as *S. aureus*, 6/76 (7.9%) as *S. coagulans*, and 3/76 (3.9%) as *S. intermedius*. Cefoxitin resistance, determined by disk diffusion, was confirmed in 4 out of the total 17 *S. aureus* isolates. Additionally, among the 50 isolates identified as *S. pseudintermedius*, oxacillin resistance was confirmed in 15 isolates by disk diffusion. *S. coagulans* and *S. intermedius* isolates were found to be sensitive to oxacillin. MIC values for cefoxitin, determined using E test strips, revealed resistance

in 20 out of the 76 samples (these 20 samples included the 4 isolates of *S. aureus* that were previously proven to be resistant to cefoxitin by disk diffusion and one *S. aureus* isolate that was cefoxitin sensitive, as well as the 15 *S. pseudintermedius* isolates that were shown to be resistant to oxacillin by disk diffusion). PBP2a was detected in 5 out of the 18 *S. aureus* isolates, which were the same isolates that showed resistance to cefoxitin by MIC testing. The prevalence of the *mecA* gene among *Staphylococcus* species was 26.3% (20/76). Among the *S. aureus* and *S. pseudintermedius* isolates, 5/17 (29.4%) and 15/50 (30%) tested positive for the *mecA* gene, respectively (these positive isolates corresponded to the same 5 *S. aureus* isolates that exhibited resistance to cefoxitin by MIC testing and latex agglutination, as well as the same 15 *S. pseudintermedius* isolates that showed resistance to oxacillin by disk diffusion and MIC testing). Consequently, they were classified as MRSA and MRSP, respectively. No isolates were found to carry the *mecC* gene in the tested samples. The amplified multiplex PCR products for the differentiation of staphylococci and the PCR products for the *mecA* gene are shown on Figure 1 and 2, respectively.

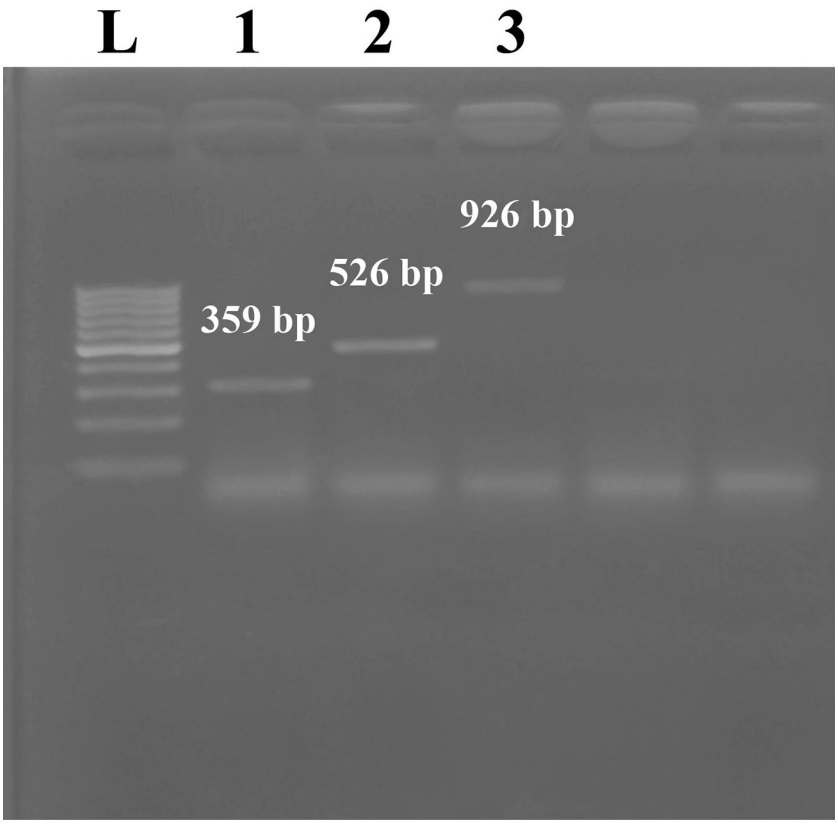

Figure 1. Multiplex PCR products for staphylococci differentiation
* L - ladder*;* 1 – *S. aureus;* 2 - *S. coagulans;* 3 – *S. pseudintermedius;*

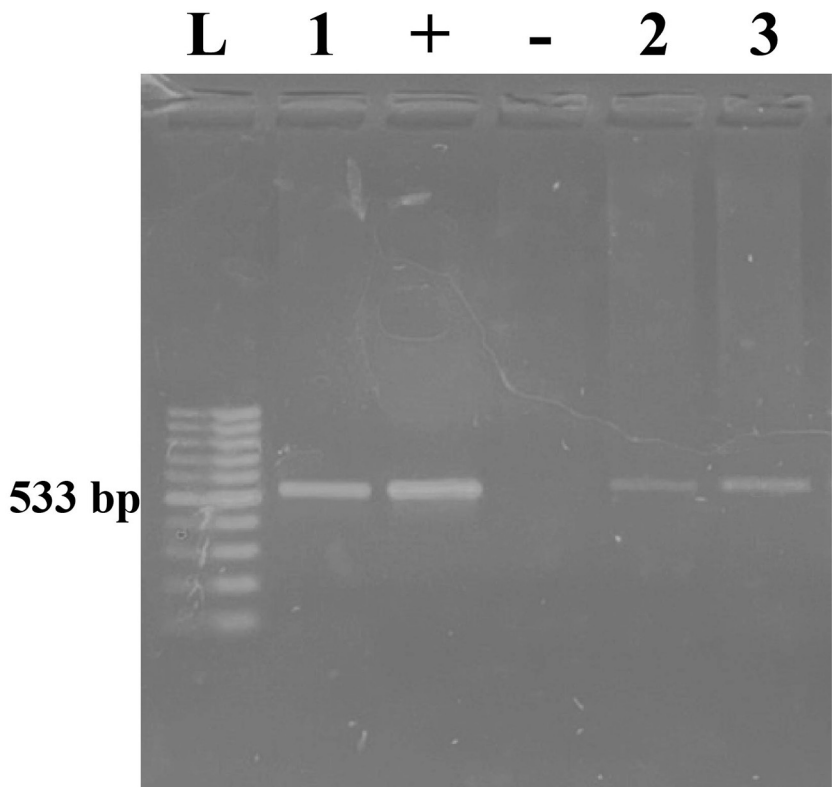

Figure 2. PCR products for the *mecA* gene
* L – ladder; 1, 2, 3 – positive samples; - and + - negative and positive control

DISCUSSION

To accurately differentiate between CoPS members in samples from dogs with clinically manifested bacterial skin and ear infections, we employed multiplex PCR considering that conventional microbiological diagnostic techniques often fail to distinguish between these species due to underdeveloped diagnostic protocols, leading to potential misidentification, particularly of *S. pseudintermedius* [32]. Out of the 76 CoPS samples, 50/76 (65.8%) were identified as *S. pseudintermedius*, 17/76 (22.5%) as *S. aureus*, 6/76 (7.9%) as *S. coagulans*, and 3/76 (3.9%) as *S. intermedius*. These results are not surprising, as *S. pseudintermedius* is the most commonly isolated pathogen in canine dermatological patients, accounting for nearly 90% of canine pyoderma cases [36]. The presence of *S. aureus* in companion animals, observed in 22.5% of the samples, aligns with a recent study confirming its occurrence in 26.6% (40/150) of dogs with otitis in Iraq [10]. This prevalence exceeds the findings of Tarazi et al. who reported 12.7% (19/150) in dogs from Jordan [37], and Cuny et al., who recorded 7.8% (10/112) in Germany [38]. However, it is lower than the prevalence reported by Ma et al. in New

South Wales, Australia, where *S. aureus* was found in 67.3% of dogs (204/303) [39]. Additionally, it is lower than the prevalence reported by Rana et al. at 16% (24/150) [40], Vincze et al. at 16% (24/150) [41], and Saputra et al. at 13.2% (117/877) [6]. The prevalence of *S. pseudintermedius* at 65.8% surpasses the findings of Rana et al. at 45.3% (68/150) [40] and falls below the prevalence reported by Saputra et al. at 70.8% (629/877) [6], Burke and Santoro in canine and feline dermatological patients at 76.9% (575/748) [42], and Platenik et al. at 78.5% (182/232). The prevalence of *S. coagulans* at 7.9% is comparable to the findings of Saputra et al. at 5% (44/877) and lower than the prevalence reported by Platenik et al. at 20.3% (47/232) [43]. The prevalence of *S. intermedius* at 3.9% exceeds the 0.22% (2/877) reported by Saputra et al. [6].

In order to confirm the identification of staphylococcal strains as MRS, it is necessary to perform PCR and/or latex agglutination, which is considered the gold standard for MRS confirmation [26,44]. In terms of MRSA detection, cefoxitin outperforms oxacillin disk diffusion in detecting *mecA*-mediated resistance, aligning with CLSI recommendations [24]. However, when used as a screening test for methicillin resistance in CoPS (except *S. aureus*), cefoxitin disk diffusion testing has been reported to yield unacceptably high percentages of false-negative results and is considered inappropriate [24,45]. CLSI recommendations for *in vitro* determination of MRSP isolates from animals advise the use of the oxacillin test [24]. In our study, there was perfect agreement between the oxacillin test and *mecA* gene detection via PCR. However, discordant results were obtained for the cefoxitin test and *mecA* gene detection in *S. aureus*. Similar discrepancies between the results obtained from the disk diffusion test and PCR detection of *mecA* have been reported previously [17,46]. This discordance between cefoxitin susceptibility and the presence of *mecA* is clinically relevant and can be attributed to the heterogeneous expression of *mecA* [47]. Based on the results of our study, it can be concluded that classifying CoPS from animals as methicillin-resistant was not possible based on a single phenotypic test and that E-test strips and Slidex®MRSA were the methods that yielded results matching 100% with PCR results. Similar observations were made by Asanin et al. [26]. These findings also align with Wu et al. [47], highlighting the challenge laboratories face in accurately identifying *mec* genes when relying solely on one test. Latex agglutination testing, developed for MRSA, can yield false-positive reactions when applied to *S. pseudintermedius* isolates and is therefore not recommended as the sole test for confirming methicillin resistance in *S. pseudintermedius* [49].

The presence of MRSA in animals, especially household pets, can be contributed to increased exposure to MRSA in humans, including individuals who have been previously considered at low risk [50]. This carries significant implications for public health, as animals can serve as reservoirs for MRSA and potentially transmit it to humans. Recent reports indicate a rise in MRSA infections among companion animals [14]. Surveillance data from European countries show a general trend of increasing MRSA prevalence from northern to southern regions. In northern Europe, around 5% of *S. aureus* isolates from invasive infections are MRS, compared to 25-50% in

southern Europe [30]. These variations are likely influenced by differences in infection control practices and antimicrobial usage. Moreover, some researchers have reported greater occurrence of *mecA* positive staphylococci in humans in comparison with animals [51]. In our study, 5 out of 17 (29.4%) *S. aureus* isolates tested positive for the *mecA* gene, thus classifying them as MRSA. This prevalence is lower compared to the findings of Rana et al. [40], who reported a MRSA prevalence of 46.4% in dogs. It is also lower than the study conducted in Germany, where canine and feline isolates showed a prevalence of 62.7% [41]. Conversely, it is higher than the MRSA prevalence reported by Kasper et al., who found rates of 2.6% in dogs and 2.7% in cats [52]. While our study supports the high incidence of MRSA in dogs, it is important to interpret the data cautiously due to the small number of isolates tested. Although there are numerous reports of MRSA colonization and infection in companion animals, the proportion of this species within staphylococcal isolates from the animal community is almost negligible compared to MRSP [17,33].

Recent studies have shown a continuous increase in the incidence of MRSP in companion animals [7,50]. The treatment of MRSP infections presents a new challenge in veterinary medicine due to the limited therapeutic options available. Reports of isolates not susceptible to any authorized veterinary antimicrobials have been published [53,54] which have raised concerns and may lead to the use of antimicrobials authorized for human medicine. The potential transfer of new SCC*mec* elements from MRSP to other staphylococcal species, such as *S. aureus*, and subsequent clonal spread of a new MRSA clone could pose a threat to human health in the future [7]. Several cases of zoonotic transmission of *S. pseudintermedius* between companion animals and humans have been reported [7,50]. In our study of the *S. pseudintermedius* isolates, 15 out of 50 (30%) were positive for the *mecA* gene, classifying them as MRSP. The prevalence of MRSP in companion animals has been studied in various countries, with rates ranging from 0% to 4.5% in community dogs [7] and 0% to 8.1% in dogs with skin disease [7,40]. High prevalence, up to 30%, has been reported in dogs at veterinary clinics in Japan [55]. In one study, 66% of *S. pseudintermedius* isolates from dogs with pyoderma were found to be MRSP based on *mecA* detection [56]. Prevalence rates of MRSP in neighboring countries include 7.5% in Croatia [4] and 29% in Bosnia and Herzegovina [57]. The high MRSP prevalence in our study could be explained by the fact that other studies have used more varied samples, while we focused on otitis and dermatitis cases, which are common sites of MRS infections. Furthermore, strains coming from clinical samples, particularly those with a history of previous antibiotic treatment, are often multiresistant [15].

Although *mecA* is the most common gene associated with methicillin resistance in staphylococci, the investigation of the *mecC* gene is important due to diagnostic challenges and the potential for misdiagnosis as methicillin-sensitive staphylococci, which can have significant consequences for individual patients and the surveillance of MRS [58]. In our study, no *mecC* gene was detected in the samples. This finding is not surprising, considering that other studies have also reported a low prevalence of

the *mecC* gene. Platenik et al. found a prevalence of 0% for *mecC* in canine and feline samples [43]. Similarly, a screening of 565 *S. aureus* isolates in Switzerland did not identify any *mecC* MRSA isolates [58]. The prevalence of *mecC* in MRSA was found to be 1.9% and 2.8% in Denmark [59]. A large-scale collection and characterization of human MRSA in Germany found only two *mecC* MRSA isolates among 3207 MRSA isolates, with a prevalence of 0.06% [60]. To the best of our knowledge, this is the first reported prevalence of the *mecC* gene in Serbia in companion animals. Although no isolates tested positive for *mecC* in this study, the recent increase in some country highlights the need to monitor *mecC* MRSA. Moreover, larger studies are needed to confirm the results presented here. Assessing the prevalence of *mecC* MRSA among different animal species, understanding their role in veterinary disease, and assessing the risk of zoonotic transmission are important areas for future research.

The One Health approach to MRS infections requires a multidisciplinary strategy because their presence in small animals indicates their involvement in the transmission triangle between animals, humans, and the environment, which is considered a public health threat [2]. The results of this study indicate a high prevalence of MRSA and MRSP in companion animals in Belgrade, Serbia. Studies like this one are important because the spread of AMR has serious consequences for both humans and animals. Quality data of resistance prevalence, temporal variations, along with regular updates, is required to assess potential threats to public health and design efficient control strategies [61]. Continuous monitoring of the presence of MRS in certain populations is the first step in combating the high prevalence of MRS and is key for infection treatment [11,30]. Furthermore, the detection and diagnosis of MRS in clinical microbiology settings are essential for informing appropriate treatment of individual patients and for the surveillance of MRSA [57].

Considering the high prevalence of MRS, it can be concluded that better measures are needed in the future to control zoonotic MRS reservoirs and limit their spread. Antimicrobial therapy should be based on in vitro susceptibility testing. Additionally, the application of alternative treatment options should be considered. General hygiene practices, routine infection control, and environmental disinfection are also necessary [11]. Since the early 2000s, MRSA prevalence has been increasing for years, however some countries have observed stable or declining rates, likely due to the implementation of appropriate and timely measures, such as improved national control interventions [30]. Our findings demonstrate the need for ongoing screening studies to gather information from a larger number of samples and different animals. Further investigations should be more comprehensive, focusing on the emergence of certain genetic lineages, molecular typing, virulence factors, whole genome sequencing, and multi-locus sequence analysis.

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Authors' contributions

IP, MI, MR, and NMM carried out the primary bacteriology analysis, including sample collection, culturing bacteria, and biochemical analysis. AR, KA, and VG performed the molecular analysis. DK, NM, NMM, and JN conceived the study, participated in its design and coordination, and revised the final version of the manuscript. IP, DK, and AR came up with the draft of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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MOLEKULARNA PREVALENCIJA *MecA* I *MecC* GENA KOD KOAGULAZA-POZITIVNIH STAFILOKOKA IZOLOVANIH OD PASA SA ZAPALJENJEM KOŽE I UŠIJU: JEDNOGODIŠNJA STUDIJA U BEOGRADU, SRBIJI

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Globalni problem antimikrobne rezistencije u humanoj i veterinarskoj medicini dodatno se pogoršava nesavesnim prepisivanjem antibiotika za lečenje bakterijskih infekcija kućnih ljubimaca. Ovo istraživanje ima za cilj da utvrdi zastupljenost koagulaza-pozi-

tivnih stafilokoka koji uzrokuju kliničke infekcije kože i uha kod pasa kao i utvrđivanje prevalencije rezistencije na meticilin. Ukupno je izolovano 78 sojeva stafilokoka iz kliničkih uzoraka prikupljenih od pacijenata sa Klinike za dermatologiju na Fakultetu veterinarske medicine u Beogradu. Metodom multiplex PCR-a izvršena je identifikacija stafilokoka do nivoa vrste. Pored fenotipske rezistencije, određivanja MIC vrednosti i otkrivanja PBP2a, rezistencija na meticilin potvrđena je detekcijom *mecA* i *mecC* gena. Od ukupno 78 analizirana uzorka, 65,8% je identifikovano kao *Staphylococcus pseudintermedius*, 22,4% kao *S. aureus*, 7,9% kao *S. coagulans*, a 3,9% kao *S. intermedius*. *MecA* gen je detektovan kod 29,4% izolata *S. aureus* i 30% izolata *S. pseudintermedius* i ovi izolati su klasifikovani kao meticilin-rezistentni *S. aureus* (MRSA) i meticilin-rezistentni *S. pseudintermedius* (MRSP). Prisustvo *MecC* gena nije utvrđeno. Ovo istraživanje pruža uvid u prevalencu CoPS, kao i prevalencu *mecA* i *mecC* gena kod izolata poreklom od pasa. Kontinuiran nadzor je od suštinske važnosti za praćenje pojavljivanja i širenja rezistencije na antimikrobne lekove u veterinarskoj medicini, a rezultati ovog istraživanja naglašavaju potrebu za uspostavljanjem kontinuiranog nadzora antimikrobne rezistencije na antibiotike u Republici Srbiji.