UDK 616.98:619
Review article
Received: 5 October 2011
Accepted: 14 December 2011

EMERGENCE AND SPREAD OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

Krešimir Matanović, Selma Mekić, Branka Šeol

Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

Summary

Staphylococcus pseudintermedius is the predominant coagulase-positive species in the normal flora of dogs and cats. It can be isolated from the nares, mouth, anus, groin and forehead of healthy dogs and cats. *S. pseudintermedius* is an opportunistic pathogen most frequently encountered in canine and feline skin and ear infections. Methicillin-resistant *S. pseudintermedius* (MRSP) emerged in Brazil in the late nineties. Today, two different clones dominate in the population of dogs and cats. Dominant European clone ST71 appeared in Germany in 2005 and has rapidly spread around the world, while lineage ST68 dominates in North America. Both clones are multiresistant and present one of the biggest problems of antimicrobial resistance in the veterinary medicine. Besides all beta-lactam antimicrobials, they are typically resistant to aminoglycosides, fluoroquinolones, macrolides, lincosamides, trimethoprim-sulfamethoxazol and in many cases to tetracycline and chloramphenicol. The treatment of MRSP infections is a new challenge in veterinary medicine because of the very limited therapeutic options. The multidrug-resistance pattern results in a potential pressure for veterinarians to use antimicrobials licensed in human medicine, such as vancomycin, mupirocin and rifampicin. This opens ethical questions because of the possible emergence of resistance to these antimicrobials. Although the zoonotic potential is much lower than for MRSA, veterinarians are at a higher risk for becoming colonized and should be aware of the zoonotic risk.

Keywords: methicillin; resistance; methicillin-resistant *Staphylococcus pseudintermedius*; MRSP

Corresponding author: Krešimir Matanović
E-mail: kmatanov@vef.hr

INTRODUCTION

The most clinically relevant staphylococci in veterinary medicine are the coagulase positive *Staphylococcus aureus* and members of the *Staphylococcus intermedius* group, particularly *Staphylococcus pseudintermedius*. The importance of methicillin-resistant *Staphylococcus aureus* (MRSA) in human medicine is well known and, even though MRSA emerged almost 50 years ago, it still presents a very serious problem in intensive care and recently also in the community. Although there are many reports of the colonization and even infection of dogs and cats with MRSA, the proportion of this species in staphylococcal isolates from animals in community is almost negligible compared to methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), at least in Europe. Methicillin-resistant *Staphylococcus pseudintermedius* emerged and rapidly spread around the world and today presents one of the biggest problems of antimicrobial resistance in the veterinary medicine.

DESCRIPTION OF THE SPECIES *S. PSEUDINTERMEDIUS* – RECENT CHANGES IN TAXONOMY

Staphylococcus intermedius was described as a species in 1976 based on G+C content and phenotypic tests [1]. In 2005, Devriese et al. described a novel staphylococcal species, *S. pseudintermedius* [2]. The description was based on 16S rRNA gene sequence analysis of isolates from a cat, a dog, a horse and a parrot. It was later found that those isolates don't represent a new species but that all isolates formerly identified as *S. intermedius* by phenotypic characteristics, and obtained from dogs, are actually *S. pseudintermedius* [3]. The authors proposed a new classification based on the nucleotide sequence analysis of the *sodA* and *hsp60* genes and divided the *S. intermedius* group into three clusters: *S. intermedius*, *S. pseudintermedius* and *S. delphini* [3]. It should be noted that isolates from dogs and cats reported as *S. intermedius* in the literature, are most probably *S. pseudintermedius*.

Phenotypic tests are not sufficient for the discrimination of the species in the *S. intermedius* group. For that purpose it is necessary to use molecular methods such as restriction fragment length polymorphism analysis of the PCR amplified fragment of the *pta* gene [4] or the multiplex PCR method of the *nuc* gene [5], which can differentiate up to seven coagulase positive species of staphylococci. In addition, *S. pseudintermedius* is phenotypicaly very similar to *S. aureus*, and can be easily misidentified if a wide range of biochemical properties is not examined. Both species show double-zone hemolysis on sheep blood agar which is characteristic for staphylococcal beta-hemolysin production. They are tube coagulase and

deoxyribonuclease positive. *S. aureus* has golden-yellow and *S. pseudintermedius* white pigmented colonies, but the pigment production is often very weak after overnight incubation, especially in isolates from dogs [6]. Although *S. pseudintermedius* doesn't produce free coagulase, it can be positive in the latex-agglutination test. These tests often combine detection of both free coagulase and protein A. The latter is present in both species and can lead to misidentification of *S. pseudintermedius* as *S. aureus*, especially in samples from humans, and is a reason why *S. pseudintermedius* is probably underreported in human medicine [7]. However, these two species can be easily distinguished using commercial strips for biochemical identification such as ID32 STAPH (bioMerieux, France) or computerized systems. The tests such as production of acetoin and beta-galactosidase are highly discriminatory. It should be noted that *S. pseudintermedius* will be identified as *S. intermedius* by the API database.

S. pseudintermedius is the predominant coagulase-positive species in the normal flora of dogs and cats. Dogs, and more often cats, can harbor *S. aureus.* However, pets acquire *S. aureus* mostly from their owners, which is also true for the methicillin-resistant strains. *S. pseudintermedius* is an opportunistic pathogen most frequently encountered in canine and feline skin and ear infections but can cause infections in virtually all body tissues and cavities, including septicemia [8, 9, 10]. According to this, we can expect to find methicillin-resistant *S. pseudintermedius* in similar sites as a colonizer or as an infectious agent.

METHODS FOR THE DETECTION OF MRSP

Methicillin resistance in staphylococci is conferred by the *mec*A gene, which encodes for production of an altered penicillin binding protein (PBP2a or PBP2') that has a low affinity for all beta-lactam antibiotics [11]. This gene is located on a staphylococcal chromosomal cassette *mec,* a transmissible genetic element, which can also carry other resistance genes depending on its type and size. For detection of MRSP, phenotypical and/or molecular methods can be used. Current CLSI guidelines for detection of methicillin resistance in *S. pseudintermedius* recommend the use of Müller-Hinton agar with the addition of 2% NaCl and a disk of oxacillin (1 μg). Plates should be incubated at 35ºC for the whole 24 hours, and isolates with the inhibition zone of less than 18 mm are considered resistant [12]. It should be emphasized that the use of cefoxitin disk instead of oxacillin is inappropriate for detection of methicillin-resistance in *S. pseudintermedius,* and will bring an unacceptable level of false negative results [13,14]. Methicillin resistance can be further confirmed by PCR detection of *mecA* gene or latex agglutination test for PBP2a.

EMERGENCE AND SPREAD OF MRSP

Methicillin-resistant *S. pseudintermedius* was first reported in Brazil, and was isolated from a skin of clinically healthy cat [15]. Colombini et al. reported first two isolates of MRSP in the USA obtained from dogs with otitis media [16]. MRSP emerged in Europe in 2005 in Germany where twelve multiresistant isolates were obtained from 11 dogs and one cat at the veterinary dermatology referral clinic [17]. The isolates were resistant to oxacillin, enrofloxacin, gentamicin, macrolides, lincosamides, trimethoprim-sulfamethoxazol and most of them to tetracycline while their pulse field gel electrophoresis profiles showed that they were very closely related. Since then, MRSP was reported in other studies in Europe: Germany [18-20], Italy [21, 22], Switzerland [23], Poland [24], and several other European countries including Sweden, Denmark, Netherlands, Luxemburg and United Kingdom [25-28]. In Croatia, MRSP was confirmed in 2008 [29], although retrospective analysis of antimicrobial susceptibility testing data indicates its possible presence even earlier than it was reported in Germany. Besides dogs and cats, methicillin-resistant *S. pseudintermedius* has been isolated also from horses and a donkey in Germany [30] and a horse in Italy [31]. Studies on the prevalence of MRSP among healthy and diseased dogs show variable percentages depending on the geographical area, type of samples investigated and the method of detection or isolation of the bacterium. MRSP was found in 1.5-2% of healthy dogs in Slovenia and USA [32, 33] and in 3.5–7% of dogs with skin disease in USA [33, 34]. However, no MRSP was isolated from anal swabs of 175 clinically healthy dogs in Canada [35]. In Europe, the prevalence among diseased dogs seems to be lower than in USA. Ruscher and coworkers found MRSP in 0.8% and 0.1% of clinical samples from dogs and cats, respectively. Most isolates were obtained from infected wounds, auditory channel and skin [30]. In Italy, among 590 canine specimens MRSP was found in 2% [21]. On the other hand, prevalence of MRSP in animals admitted to dermatology clinics in USA and Canada ranged from 3.1% in cats to 6.2% in dogs [9]. Approximately 40% of isolates from dogs with recurrent pyoderma in the USA were MRSP. In Japan, MRSP was found in 30% of dogs examined at a veterinary clinic [36]. *Mec*A gene was detected in 66.5% of *S. pseudintermedius* obtained from dogs in two veterinary hospitals in Japan [37]. Studies on the risk factors for MRSP colonization or infection are scarce. Dogs with MRSP infections had more likely been treated with antimicrobials within the 30 days prior to the onset of the infection compared to dogs with MSSP infections [38]. This indicates that antimicrobial use is a risk factor for MRSP infections.

MOLECULAR TYPING OF MRSP

Molecular typing methods for MRSP allow investigation of the possible linkage between isolates from different geographical locations, and are the basis for epidemiological analysis. MRSP can be grouped on the basis of their staphylococcal chromosomal cassette *mec* type (SCC*mec*) which can be determined using a combination of several multiplex PCRs and sequencing [39]. In MRSP, SCC*mec* was found to be of type III, II-III, V and VII [27,40-42]. SCC*mec* II-III is the most prevalent type among European MRSP, and is a combination of SCC*mec* II from *S. epidermidis* and SCC*mec* III from *S. aureus*. SCC*mec* types III and II-III seem to be identical but different criteria were used for classification in different studies [40, 42]. On the other hand, dominant North American clonal lineage harbors SCC*mec* type V [27,40-42].

Besides SCC*mec* typing, several other typing methods were developed and are similar to those used for typing of MRSA. Pulse field gel electrophoresis (PFGE) has been widely used for typing of methicillin-sensitive and methicillin-resistant *S. pseudintermedius* with good typability and resolution, but modern sequence-based typing methods are more convenient due to easier interlaboratory comparison, exchange of data and development of automatic databases. *Spa* typing is a single locus typing method which involves sequencing of the variable X- region of staphylococcal protein A (*spa*) gene and identification of short (30bp) tandem repeats. After that a numerical *spa* type is assigned. *Spa* type t01 was assigned to a methicillin sensitive reference strain ED99 which sequence was used for developing the method. The *spa* typing for MRSP was established by Moodley et al. and consisted of a single PCR reaction using two specific primers for amplification of the X-region of *spa* gene [42]. It was later adapted as a nested PCR for typing of strains that were untypable with the original protocol. In the first PCR reaction the whole *spa* gene is amplified and the product is used for second reaction and amplification of X-region [27]. Ruscher et al. didn't have problems with *spa* typing of MRSP. However, in that study different primers were used and only European strains were analyzed [28]. In all studies conducted so far, *spa* type t02 dominated among European and t06 among North American MRSP. *Spa* types t05 and t06 can also be found in Europe and are closely related to t02 with the main difference in the number of r03 repeats in the central part of the X-region. Other *spa* types were found sporadically, and were often associated to isolates with different resistance phenotypes. On the other hand, *spa* type t06 dominates among North American MRSP [27,28,42].

Multilocus sequence typing (MLST) method for *S. pseudintermedius* was developed by Bannoehr et al. in 2007 [25]. It includes sequencing of five gene loci: 16S rRNA, *cpn60, tuf, pta* and *agrD* and assignment of a sequence type (ST). The analysis of 105

isolates of *S. pseudintermedius* (16 of them were MRSP) revealed that this species has a largely clonal population structure with minor effects of recombination in the evolution of the investigated genes. The study has shown that different MRSP sequence types have evolved by multiple acquisition of *mec*A gene by different clones. Among the 16 MRSP isolates five different STs were found (ST29, ST68, ST69, ST70 and ST71) with the predominance of ST71 among isolates from North and Central Europe, indicating the spread of a very successful clone in European dog population. There was no sharing of STs between European and American MRSP and all MRSP STs but one (ST29) were different from those identified among methicillin-sensitive strains. Later studies have confirmed the domination of ST71 among dogs and cats in Europe and ST68 in North America. Isolates that belong to ST71 can be sporadically found in North American MRSP and are common in dogs in Hong Kong. Compared to *mecA*-negative *S. pseudintermedius*, where numerous different STs can be found, MRSP seems to be less diverse [42,43,26-28]. However, up to now, only a single case of ST68 has been recorded in Europe [44,45].

Analysis of isolates using a combination of *spa* typing, MLST and SCC*mec* typing has shown that the majority of European MRSP belong to ST71, *spa* type t02 and carry a staphylococcal chromosomal cassette *mec* of type II-III. *Spa* types t05 and t06 can also be found within ST71 and carry the same SCC*mec*. On the other hand, the most common North American lineage belongs to ST68, *spa* type t06 and carries a type V SCC*mec* [27].

RESISTANCE OF MRSP TO NON-BETA-LACTAM ANTIMICROBIALS

The most clinically important characteristic of recently emerged dominant MRSP clones is their antimicrobial resistance. Both European and American dominant clonal lineages harbor multiple resistance mechanisms and are resistant to practically all clinically relevant antimicrobials licensed for use on animals. Besides beta-lactams, isolates from the European lineage ST71 are also resistant to macrolides, lincosamides, aminoglycosides, fluoroquinolones and trimethoprim, while approximately 75% and 68% of isolates are resistant to chloramphenicol and tetracycline, respectively. Resistance to macrolides and lincosamides is due to the chromosomally located methylase gene *erm*(B), while chloramphenicol resistant isolates carry chloramphenicol acetyltransferase gene cat_{pC221} which is commonly found on small plasmids. Resistance to gentamicin and kanamycin is associated with the bifunctional acetyltransferase/phosphotransferase gene *aac(6')-aph(2'')* while trimethoprim-resistant isolates carry dihydrofolate reductase gene *dfr*(G). The North American clonal lineage ST68 contains virtually the same resistance genes as

ST71 with the exception of *tet*(M), instead of *tet*(K), the absence of cat_{pC221}, and an additional lincosamide resistance gene *lnu*(A) in some isolates. The mechanism of resistance to fluoroquinolones in MRSP was not investigated in these studies [26,27]. However, very high minimum inhibitory concentrations of ciprofloxacin indicate the possible presence of multiple mutations in the *grlA*, *grlB* and *gyrA* genes which was previously reported in fluoroquinolone resistant MRSP [40]. MRSP still remain susceptible to vancomycin and mupirocin, but resistance to rifampicin has already been detected in several isolates from dogs treated with this antimicrobial [46], and indicates the possibility of further acquisition or development of resistance. In addition, these drugs are used for the treatment or decolonization of MRSA infections or therapy of tuberculosis in human medicine and their use in animals should be avoided unless there is no alternative.

INVESTIGATION OF MRSP IN CROATIA

Methicillin-resistant *Staphylococcus pseudintermedius* was confirmed in Croatia in 2008. Since then, the monitoring of MRSP is carried out in the Bacteriology Laboratory of the Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine, Zagreb. All isolates collected in a two year period, from November 2008 to December 2010, were thoroughly analyzed by phenotypic and molecular methods as a part of a doctoral dissertation [47]. In total, 32 isolates were *spa*-typed and their susceptibility to antimicrobials determined using either disk-diffusion and/or Etest. Resistance genes were detected by PCR. Preliminary results confirm the spread of a dominant European MRSP clone, *spa* type t02, in Croatian dogs and cats. Types t05 and t06 were isolated only sporadically. MRSP isolates from Croatia have similar resistance patterns and carry the same resistance genes as European strains, although the susceptibility to tetracycline seems to be much lower [48]. Croatian isolates are commonly resistant to gentamicin, enrofloxacin, erythromycin, clindamycin, trimethoprim-sulfamethoxazol, and more than half of them to chloramphenicol. The susceptibility is retained to amikacin, minocycline, rifampicin, mupirocin, fucidic acid and vancomycin.

TREATMENT OF ANIMALS INFECTED WITH MRSP

The treatment of MRSP infections is a new challenge in veterinary medicine because of the very limited therapeutic options [23]. The multidrug-resistance pattern results in a potential pressure for veterinarians to use antimicrobials licensed in human medicine which requires careful evaluation of extra-label drug use in vete-

rinary medicine [49]. This also raises ethical questions because the use of last resort antimicrobials in veterinary medicine could lead to their ineffectiveness in human medicine. In addition, there is a possibility of transfer of genetic material coding for additional resistances to bacteria that infect humans. Current recommendations for dealing with MRSP infections, brought by Committee for Medicinal Products for Veterinary Use of the European Medicines Agency, are available online and are periodically updated [50]. As the routine use of antimicrobials is a risk factor for spread of MRSP, it is stated that the unnecessary use of antimicrobials should be eliminated. Many MRSP infections are local, such as pyoderma, otitis externa or post operative wound infections. In those cases the use of topical antibiotics or antiseptics is advisable. Wound debridement and use of chlorhexidine or products containing iodine is beneficial. A commercial ear antiseptic containing chlorhexidine and Tris-EDTA showed good *in vitro* bactericidal activity against MRSP [51]. Systemic use of antimicrobials should be limited to deep seated infections, such as osteomyelitis, septicemia or pneumonia, and urinary tract infections. Even in these instances, the use of last resort antimicrobials should be avoided and limited to selected cases where the disease is life threatening and alternative treatments (including non-antimicrobial) have failed. Use of antimicrobials for decolonization seems to be of limited value and should be avoided, because it can lead to further development of resistance [49].

PREVENTION OF SPREAD BETWEEN DOGS AND CATS, AND THEIR OWNERS

Methods for the prevention of transmission of MRSP between animals are similar to those developed for MRSA [52]. These include hygiene measures such as hand disinfection and adequate wound management which will minimize the spread of MRSP. Veterinary practitioners should have in mind that veterinary clinics are the places very convenient for the transmission of MRSP and that proper cleaning and disinfection of the contaminated environment will reduce the number of infective organisms. Admission of animals with MRSA and MRSP infections to veterinary clinic is of special concern. Known or suspected cases should be taken directly into a consultation room to avoid contamination and contagion in the waiting room. The floor, table and other contact surfaces should then be disinfected before they are used for other patients.

The zoonotic potential of MRSP is much smaller than for MRSA. However, humans in close contact with infected animals seem to have a higher risk of being MRSP-positive. Recent study has shown that approximately 4% of small animal der-

matologists are colonized with MRSP. Therefore, veterinarians should be aware of the zoonotic risk and possibility of acquiring a MRSP infection [53].

References

[1] *Hajek V. Staphylococcus intermedius*, a new species isolated from animals. Int J Syst Bacteriol. 1976;26:401-8.

[2] *Devriese LA, Vancanneyt M, Baele M, Vaneechoutte M, Graef EDe, Snauwaert C, Cleenwerck I, Dawyndt P, Swings J, Decostere A, Haesebrouck F. Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int J Syst Evol Microbiol. 2005;55:1569-73.

[3] *Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K.* Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J Clin Microbiol. 2007;45:2770-8.

[4] *Bannoehr J, Franco A, Iurescia M, Battisti A, Fitzgerald JR.* Molecular diagnostic identification of *Staphylococcus pseudintermedius*. J Clin Microbiol. 2009;47:469-71.

[5] *Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K.* Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol. 2010;48:765-9.

[6] *Quinn PJ, Carter ME, Markey B, Carter GR.* Clinical Veterinary Microbiology. London: Elsevier Health Sciences; 1994.

[7] *Pottumarthy S, Schapiro JM, Prentice JL, Houze YB, Swanzy SR, Fang FC, Cookson BT.* Clinical Isolates of *Staphylococcus intermedius* Masquerading as Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol. 2004;42:5881-4.

[8] *Cox HU, Hoskins JD, Roy AF, Newman SS, Luther DG.* Antimicrobial susceptibility of coagulase-positive staphylococci isolated from Louisiana dogs. Am J Vet Res. 1984;45:2039-42.

[9] *May ER.* Bacterial Skin Diseases: Current Thoughts on Pathogenesis and Management. Vet Clin North Am Small Anim Pract. 2006;36:185-202.

[10] *Morris DO, Boston RC, O'Shea K, Rankin SC.* The prevalence of carriage of meticillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets. Vet Dermatol. 2010;21:400-7.

[11] *Kwon N, Park K, Jung W, Youn H, Lee Y, Kim S, Bae W, Lim J, Kim J, Kim J, Hong S, Park Y.* Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol. 2006;117:304-12.

[12] *Papich MG.* Proposed changes to Clinical Laboratory Standards Institute interpretive criteria for methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs, J Vet Diagn Invest. 2010;22:160.

[13] *Bemis DA, Jones RD, Frank LA, Kania SA.* Evaluation of susceptibility test breakpoints used to predict *mecA*-mediated resistance in *Staphylococcus pseudintermedius* isolated from dogs. J Vet Diagn Invest. 2009;21:53-8.

[14] *Schissler JR, Hillier A, Daniels JB, Cole LK, Gebreyes WA.* Evaluation of Clinical Laboratory Standards Institute interpretive criteria for methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs. J Vet Diagn Invest. 2009;21:684–8.

[15] *Lilenbaum W, Nunes ELC, Azeredo MAI.* Prevalence and antimicrobial susceptibility of staphylococci isolated from the skin surface of clinically normal cats. Lett Appl Microbiol. 1998;27:224-8.

[16] *Colombini S, Merchant SR, Hosgood G.* Microbial flora and antimicrobial susceptibility patterns from dogs with otitis media. Vet Dermatol. 2000;11:235-9.

[17] *Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JM, Winkler M, Weiss R, Lloyd DH.* First report of multiresistant, *mecA*-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Vet Dermatol. 2007;18:412-21.

[18] *Zubeir IE, Kanbar T, Alber J, Lammler C, Akineden O, Weiss R, Zschock M.* Phenotypic and genotypic characteristics of methicillin/oxacillin-resistant *Staphylococcus intermedius* isolated from clinical specimens during routine veterinary microbiological examinations. Vet Microbiol. 2007;121:170-6.

[19] *Schwarz S, Kadlec K, Strommenger B.* Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the BfT-GermVet monitoring programme 2004–2006 in Germany. J Antimicrob Chemother. 2008;61:282-5.

[20] *Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, Kreienbrock L, Schwarz S, Simon D, Nolte I.* Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. Vet Microbiol. 2011;150:191-7.

[21] *De Lucia M, Moodley A, Latronico F, Giordano A, Caldin M, Fondati A, Guardabassi L.* Prevalence of canine methicillin resistant *Staphylococcus pseudintermedius* in a veterinary diagnostic laboratory in Italy. Res Vet Sci. 2011;91:346-8.

[22] *Meucci V, Vanni M, Guardabassi L, Moodley A, Soldani G, Intorre L.* Evaluation of methicillin resistance in *Staphylococcus intermedius* isolated from dogs. Vet Res Commun. 2010;34(Suppl 1):S79-S82.

[23] *Wettstein K, Descloux S, Rossano A, Perreten V.* Emergence of methicillin-resistant *Staphylococcus pseudintermedius* in Switzerland: Three cases of urinary tract infections in cats. Schweiz Arch Tierheilk. 2008;7:339-43.

[24] *Kizerwetter-Swida M, Chrobak D, Rzewuska M, Binek M.* Antibiotic resistance patterns and occurrence of *mec*A gene in *Staphylococcus intermedius* strains of canine origin. Pol J Vet Sci. 2009;12:9-13.

[25] *Bannoehr J, Ben Zakour NL, Waller AS, Guardabassi L, Thoday KL, Van Den Broek AH, Fitzgerald JR.* Population genetic structure of the *Staphylococcus intermedius*

group: insights into *agr* diversification and the emergence of methicillin-resistant strains. J Bacteriol. 2007;189:8685-92.

[26] *Kadlec K, Schwarz S, Perreten V, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, Van Duijkeren E, Scott Weese J, Ross Fitzgerald J, Rossano A, Guardabassi L.* Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. J Antimicrob Chemother. 2010;65:1826-28.

[27] *Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, Van Duijkeren E, Scott Weese J, Ross Fitzgerald J, Rossano A, Guardabassi L.* Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother. 2010;65:1145-54.

[28] *Ruscher K, Lübke-Becker A, Semmler T, Wleklinski C-G, Paasch A, Šoba A, Stamm I, Kopp P, Wieler LH, Walther B.* Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. Vet Microbiol. 2010;144:340-6.

[29] *Matanović K, Šeol B, Mekić S.* First report of methicillin-resistant *Staphylococcus pseudintermedius* in Croatia. Proceedings of the Central European Symposium on Antimicrobial Resistance - CESAR 2009, 2009 Sep 23-26. Zadar, Croatia.

[30] *Ruscher C, Lübke-Becker A, Wleklinski CG, Soba A, Wieler LH, Walther B.* Prevalence of Methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. Vet Microbiol. 2009;136:197-201.

[31] *De Martino L, Lucido M, Mallardo K, Facello B, Mallardo M, Iovane G, Pagnini U, Tufano MA, Catalanotti P.* Methicillin-resistant staphylococci isolated from healthy horses and horse personnel in Italy. J Vet Diagn Invest. 2010;22:77-82.

[32] *Vengust M, Anderson MEC, Rousseau J, Weese JS.* Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Lett Appl Microbiol. 2006;43:602-6.

[33] *Griffeth GC, Morris DO, Abraham JL, Shofer FS, Rankin SC.* Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. Vet Dermatol. 2008;19:142-9.

[34] *Kania SA, Williamson NL, Frank LA, Wilkes RP, Jones RD, Bemis DA.* Methicillin resistance of staphylococci isolated from the skin of dogs with pyoderma. Am J Vet Res. 2004;65:1265-68.

[35] *Rubin JE, Chirino-Trejo M.* Prevalence, sites of colonization, and antimicrobial resistance among *Staphylococcus pseudintermedius* isolated from healthy dogs in Saskatoon, Canada. J Vet Diagn Invest. 2011;23:351-4.

[36] *Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K.* Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol. 2007;45:1118-25.

[37] *Kawakami T, Shibata S, Murayama N, Nagata M, Nishifuji K, Iwasaki T, Fukata T.* Antimicrobial susceptibility and methicillin resistance in *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* subsp. *coagulans* isolated from dogs with pyoderma in Japan. J Vet Med Sci. 2010;72:1615-19.

[38] *Weese J, Frank LA, Reynolds LM, Bemis DA.* Retrospective study of methicilin-resistant and methicilin-susceptible *Staphylococus pseudintermedius* infections in dogs. 43A. ASM. Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications. 2009 Sep 22-25. London, England.

[39] *Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K.* Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother. 2007;51:264-74.

[40] *Descloux S, Rossano A, Perreten V.* Characterization of new staphylococcal cassette chromosome *mec* (SCC*mec*) and topoisomerase genes in fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius*. J Clin Microbiol. 2008;46:1818–23.

[41] *Black CC, Solyman SM, Eberlein LC, Bemis DA, Woron AM, Kania SA.* Identification of a predominant multilocus sequence type, pulsed-field gel electrophoresis cluster, and novel staphylococcal chromosomal cassette in clinical isolates of *mecA*-containing, methicillin-resistant *Staphylococcus pseudintermedius*. Vet Microbiol. 2009;139:333–8.

[42] *Moodley A, Stegger M, Ben Zakour NL, Fitzgerald JR, Guardabassi L.* Tandem repeat sequence analysis of staphylococcal protein A (*spa*) gene in methicillin-resistant *Staphylococcus pseudintermedius*. Vet Microbiol. 2009;135:320-6.

[43] *Boost MV, So SY, Perreten V.* Low rate of methicillin-resistant coagulase-positive staphylococcal colonization of veterinary personnel in Hong Kong. Zoonoses Public Hlth. 2011;58:36-40.

[44] *Couto N, Pomba C, Moodley A, Guardabassi L.* Prevalence of meticillin-resistant staphylococci among dogs and cats at a veterinary teaching hospital in Portugal. Vet Rec. 2011;169:72.

[45] *Pomba C, Couto N, Moodley A.* Treatment of a lower urinary tract infection in a cat caused by a multi-drug methicillin-resistant *Staphylococcus pseudintermedius* and *Enterococcus faecalis*. J Feline Med Surg. 2010;12:802-6.

[46] *Kadlec K, van Duijkeren E, Wagenaar JA, Schwarz S.* Molecular basis of rifampicin resistance in methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs. J Antimicrob Chemother. 2011;66:1236-42.

[47] *Matanović K.* Genotyping and detection of resistance genes in methicillin-resistant *Staphylococcus pseudintermedius* [dissertation]. Zagreb: University of Zagreb; 2011. In Croatian.

[48] *Matanović K, Mekić S, Šeol B.* Tetracycline and chloramphenicol resistance in methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). 4th Congress of European Microbiologists-FEMS 2011 "Advancing Knowledge on Microbes", 2011 Jun 26-30. Geneva, Switzerland.

[49] *Weese JS, Van Duijkeren E.* Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol. 2010;140:418-29.

[50] *European Medicines Agency.* Reflection paper on meticillin-resistant *Staphylococcus pseudintermedius*, 2010.

[51] *Guardabassi L, Ghibaudo G, Damborg P.* In vitro antimicrobial activity of a commercial ear antiseptic containing chlorhexidine and Tris-EDTA. Vet Dermatol. 2010;21:282-6.

[52] http://www.bsava.com/Advice/MRSA/tabid/171/Default.aspx (accessed September 16, 2011).

[53] *Paul NC, Moodley A, Ghibaudo G, Guardabassi L.* Carriage of Methicillin-Resistant *Staphylococcus pseudintermedius* in Small Animal Veterinarians: Indirect Evidence of Zoonotic Transmission. Zoonoses Public Hlth. 2011 Jan 31. doi: 10.1111/j.1863-2378.2011.01398.x. PubMed PMID: 21824350.

Sažetak

Pojava i širenje meticilin-rezistentnih sojeva bakterije *Staphylococcus pseudintermedius*

Staphylococcus pseudintermedius najčešća je koagulaza-pozitivna vrsta stafilokoka u fiziološkoj mikroflori pasa i mačaka. Može se izdvojiti iz nosnica, usne šupljine, anusa i kože slabinskog i čeonog područja zdravih pasa i mačaka. Uvjetno je patogena bakterija i jedan od najčešćih uzročnika upala kože i zvukovoda. Meticilin-rezistentan *Staphylococcus pseudintermedius* (MRSP) prvi je put izdvojen u Brazilu u kasnim devedesetima 20. stoljeća. Danas u populaciji pasa i mačaka prevladavaju dva klona. Dominantni europski klon ST71 pojavio se 2005. godine u Njemačkoj i brzo proširio po svijetu, dok klon ST78 prevladava u Sjevernoj Americi. Oba su klona višestruko rezistentna na antimikrobne lijekove i jedan su od najvećih problema rezistencije u veterinarskoj medicini. Izolati MRSP rezistentni su na sve beta-laktamske antibiotike, aminoglikozide, fluorokinolone, makrolide, linkozamide, kombinaciju sulfametoksazola i trimetoprima i većina na kloramfenikol i tetraciklin. Liječenje životinja inficiranih sojevima MRSP-a vrlo je zahtjevno zbog nedostatka djelotvornih antimikrobnih lijekova. Veterinari su često prisiljeni posegnuti za lijekovima registriranim isključivo za liječenje ljudi, primjerice vankomicinom, mupirocinom i rifampicinom, što otvara brojna etička pitanja zbog opasnosti razvoja rezistencije na te antibiotike. Opasnost od zaraze ljudi sojevima MRSP-a općenito je manja u usporedbi s MRSA-om. Veterinari su zbog rada sa životinjama pod povećanim rizikom i trebaju biti svjesni da postoji mogućnost kolonizacije nosnica takvim sojevima.

Ključne riječi: meticilin; rezistencija; meticilin-rezistentan *Staphylococcus pseudintermedius*; MRSP