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Antimicrobial susceptibility of *Staphylococcus pseudintermedius* isolated from dogs and cats in Croatia during a six-month period

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ABSTRACT

Staphylococcus pseudintermedius is part of the normal flora of dogs but is also commonly found as an opportunistic pathogen. Antimicrobial resistance in *S. pseudintermedius* is changing over time and is generally rising steadily for those antimicrobials that are frequently used. In this work, the susceptibility of 106 canine and feline isolates of *S. pseudintermedius* was determined by the disk-diffusion method. Isolates were collected from various body sites of diseased dogs and cats during a six-month period in 2011. Most isolates were susceptible to amoxycillin/clavulanic acid, cephalexin, enrofloxacin and oxacillin (92.5%), followed by trimethoprim-sulfamethoxazole (90.6%) and gentamicin (82.1%). Lower susceptibility was found for minocycline (67.0%), erythromycin, clindamycin and tetracycline (62.3%) and kanamycin (58.5%). Only 21.7% of the isolates were susceptible to ampicillin. Susceptibilities of isolates were compared from different isolation sites and from different temporal origins. The possible association of resistances to different antimicrobial agents is discussed.

Key words: *Staphylococcus pseudintermedius*, antimicrobial resistance, disk-diffusion method

Introduction

Staphylococcus pseudintermedius belongs to the *Staphylococcus intermedius* group (SIG) together with the closely related species *Staphylococcus intermedius* and *Staphylococcus delphini* (BANNOEHR et al., 2007). It is one of the most common pathogens isolated from skin and ear infections in dogs (PETERSEN et al., 2002). This bacterial species commonly occurs as part of the normal flora of dogs, but can also be an opportunistic pathogen. Antimicrobial resistance in *S. pseudintermedius* is changing

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over time and is generally rising steadily for those antimicrobials that are frequently used (AUTHIER et al., 2006; ONUMA et al., 2012).

The susceptibility of SIG isolated from dogs and cats in Croatia was investigated 15 years ago and showed rather low resistance rates except for ampicillin, macrolides and chloramphenicol (NAGLIĆ et al., 1998). The investigation of susceptibility to cefovecin in Croatia showed that this third-generation cephalosporin was active against 92.8% *S. pseudintermedius* isolates (ŠEOL et al., 2011). In 2009, we reported the emergence of methicillin-resistant *S. pseudintermedius* (MRSP) in Croatia and determined its susceptibility to antimicrobials (MATANOVIĆ et al., 2009).

The aim of the present study was to examine the occurrence of antimicrobial resistance in *S. pseudintermedius* isolated from diseased dogs and cats in Croatia.

Materials and methods

During a six-month period, from April to September 2011, specimens originating from diseased cats and dogs were submitted for microbiological examination to the bacteriology laboratory at the Department for Microbiology and Infectious Diseases with Clinic of the Faculty of Veterinary Medicine, University of Zagreb. The majority of samples were collected from animals examined at the Clinics of the Faculty of Veterinary Medicine, University of Zagreb, while a smaller portion originated from private veterinary clinics throughout Croatia. History on previous antimicrobial chemotherapy was not available. Samples were streaked on Columbia agar with 5% sheep blood (Bio-Rad, France) and incubated at 37 ºC for 18 h. Isolates were identified as members of the SIG on the basis of beta-hemolysin production and biochemical properties as described by SASAKI et al. (2007), and were stored at -20 ºC until further analysis. Replicate isolates from the same animal were excluded. DNA was isolated from overnight cultures grown on Columbia agar (Bio-Rad, France). Briefly, several bacterial colonies were picked from agar and suspended in 200 µl of 2% Chelex-100 solution (Bio-Rad, USA). After thermal lysis at 100 ºC for 10 min suspensions were chilled on ice, centrifuged at 13000×g for 10 min, and supernatant was used as DNA template. Identification of *S. pseudintermedius* to the species level and detection of *mecA* gene were performed by polymerase chain reaction, using a Promega Core System I (Promega, USA) kit and the primers previously described (ZUBEIR et al., 2007; SASAKI et al., 2010). Only isolates that were confirmed as *S. pseudintermedius* were included in susceptibility testing.

Susceptibility to antimicrobials was determined on Mueller-Hinton agar (Bio-Rad, France) using the disk-diffusion procedure according to recommendations by the Clinical and Laboratory Standards Institute (ANONYMOUS, 2008). The following antimicrobial disks (Bio-Rad, USA) were used: amoxycillin/clavulanic acid (20/10 µg), ampicillin (10 µg), chloramphenicol (30 µg), clindamycin (2 µg), enrofloxacin (5 µg), erythromycin

(15 μg), gentamicin (10 μg), kanamycin (30 μg), minocycline (30 μg), tetracycline (30 μg) and trimethoprim-sulfamethoxazole (1.25/23.75 μg). Cephalotin (30 μg) was used to predict the susceptibility to cephalexin (ANONYMOUS, 2008). Inhibition zone diameters were interpreted according to criteria recommended in CLSI documents M31-A3 and, for kanamycin and minocycline, M100-S20 (ANONYMOUS, 2008; ANONYMOUS, 2010). *S. aureus* ATCC 25923 was used as a control strain. Susceptibility to oxacillin was determined on Mueller-Hinton agar (Bio-Rad, France) supplemented with 2% NaCl using 1 μg oxacillin disks (Bio-Rad, USA), and zone diameters were interpreted as recommended by the CLSI subcommittee on Veterinary Antimicrobial Susceptibility Testing (PAPICH, 2010). Inducible clindamycin resistance was tested using the D-test (ANONYMOUS, 2010).

Statistical analysis was performed using Statistica (Statsoft, USA) and MedCalc (MedCalc Software, Belgium) software. The proportions of susceptible and resistant categories between isolates obtained from the skin and ear canal were compared by Chi-square test. Association of resistances between different antimicrobial agents was examined by Cochran's Q test. Statistical significance was set at $P<0.05$. For statistical analysis all intermediate sensitive isolates were included in the resistant category.

Results

In this work 106 isolates of *S. pseudintermedius* were isolated from 102 dogs and four cats. All isolates were associated with clinical disease and were recovered from the affected sites: skin (40), external ear canal (39), conjunctival sac (8), nostrils (7), infected wounds (6), urine (4) and the female genital tract (2).

A 960 bp DNA fragment of the *nuc* gene was amplified by PCR in all *S. pseudintermedius* isolates (Fig. 1).

The results of susceptibility testing are presented in Table 1. In this study 8 (7.5%) isolates of *S. pseudintermedius* were resistant to oxacillin. MRSP was isolated from the skin (2), ear canal (2), infected wounds (2) and urine (1) of dogs and from a nostril (1) of a cat. All isolates resistant to oxacillin carried the *mecA* gene, and were also resistant to amoxycillin/clavulanic acid and cephalexin. All oxacillin-sensitive isolates were susceptible to amoxycillin/clavulanic acid and cephalexin. Out of 40 isolates resistant to erythromycin, 32 were also resistant to clindamycin. The remaining eight erythromycin-resistant isolates, which were intermediate susceptible (six isolates) or resistant (two isolates) to clindamycin in disk-diffusion testing, were positive in the D-test and classified as inducible resistant to clindamycin. When these data are taken together, all 40 (37.7%) isolates resistant to erythromycin were also resistant to clindamycin. No isolates were susceptible to clindamycin and positive in the D-test.

Table 1. Antimicrobial susceptibility of 106 isolates of S. *pseudintermedius* determined by disk-diffusion method

Antimicrobial agent	Number (%) of sensitive, intermediate sensitive and resistant isolates of S. *pseudintermedius*											
	Skin (n = 40)			Ear canal (n = 39)			Other sites (n = 27)			Total (n =106)		
	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	10 (25.0)	-	30 (75.0)	9 (23.1)	-	30 (76.9)	4 (14.8)	-	23 (85.2)	23 (21.7)	-	83 (78.3)
Amoxycillin /clav. acid	38 (95.0)	-	2 (5.0)	37 (94.9)	-	2 (5.1)	23 (85.2)	-	4 (14.8)	98 (92.5)	-	8 (7.5)
Cephalexin	38 (95.0)	-	2 (5.0)	37 (94.9)	-	2 (5.1)	23 (85.2)	-	4 (14.8)	98 (92.5)	-	8 (7.5)
Chloramphenicol	25 (62.5)	-	15 (37.5)	31 (79.5)	-	8 (20.5)	22 (81.5)	-	5 (18.5)	78 (73.6)	-	28 (26.4)
Clindamycin	24 (60.0)	1 (2.5)[a]	15 (37.5)	27 (69.2)	2 (5.1)[a]	10 (25.6)	15 (55.6)	3 (11.1)[a]	9 (33.3)	66 (62.3)	6 (5.7)[a]	34 (32.1)
Erythromycin	24 (60,0)	-	16 (40.0)	27 (69.2)	-	12 (30.8)	15 (55.6)	-	12 (44.4)	66 (62.3)	-	40 (37.7)
Enrofloxacin	37 (92.5)	2 (5.0)	1 (2.5)	38 (97.4)	-	1 (2.6)	23 (85.2)	-	4 (14.8)	98 (92.5)	2 (1.9)	6 (5.7)
Gentamicin	32 (80.0)	-	8 (20.0)	34 (87.2)	-	5 (12.8)	21 (77.8)	-	6 (22.2)	87 (82.1)	-	19 (17.9)
Kanamycin	24 (60.0)	-	16 (40.0)	25 (64.1)	-	14 (35.9)	13 (48.1)	-	14 (51.9)	62 (58.5)	-	44 (41.5)
Minocycline	29 (72.5)	8 (20.0)	3 (7.5)	24 (61.5)	10 (25.6)	5 (12.8)	18 (66.7)	5 (18.5)	4 (14.8)	71 (67.0)	23 (21.7)	12 (11.3)
Oxacillin	38 (95.0)	-	2 (5.0)	37 (94.9)	-	2 (5.1)	23 (85.2)	-	4 (14.8)	98 (92.5)	-	8 (7.5)
Sulfa/ trimethoprim[b]	38 (95.0)	-	2 (5.0)	36 (92.3)	-	3 (7.7)	22 (81.5)	-	5 (18.5)	96 (90.6)	-	10 (9.4)
Tetracycline	28 (70.0)	-	12 (30.0)	21 (53.8)	-	18 (46.2)	17 (63.0)	-	10 (37.0)	66 (62.3)	-	40 (37.7)

[a]All isolates intermediately sensitive to clindamycin showed inducible resistance in D-test. [b]Sulfamethoxazole/trimethoprim. S=sensitive, I=intermediate sensitive, R=resistant

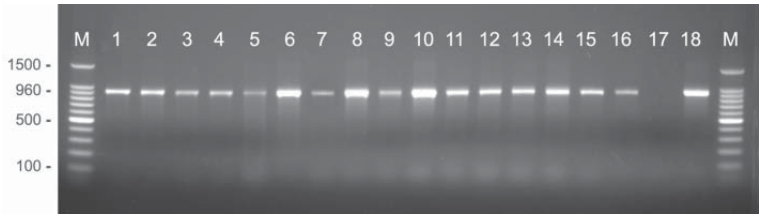

Fig. 1. Agarose gel-electrophoresis showing 960 bp fragment of the *nuc* gene for 16 representative *S. pseudintermedius* isolates. M = Promega BenchTop 100bp DNA Ladder (Promega, USA); Lanes 1-16 = *S. pseudintermedius* isolates; Lane 17 = negative control; Lane 18 = positive control (methicillin-resistant *S. pseudintermedius* ST71-t02).

Table 2. Antimicrobial resistance patterns detected among 106 isolates of *Staphylococcus pseudintermedius*

Antimicrobial agents	No. of isolates
No resistance	23
AM	30
AM + GM + K	1
AM + MNO + TE	8
AM + MNO + SXT + TE	1
AM + GM + K + TE	1
AM + K + MNO + TE	1
AM + GM + K + MNO + TE	1
AM + C + CM + E + K	5
AM + C + CM + E + GM + K	2
AM + C + CM + E + K + TE	3
AM + CM + E + K + MNO + TE	5
AM + C + CM + E + K + MNO + TE	7
AM + CM + E + GM + K + MNO + TE	1
AM + CM + E + K + MNO + SXT + TE	1
AM + C + CM + E + GM + K + MNO + TE	7
AM + C + CM + E + ENR + K + MNO + TE	1
AM + AMC + CM + CN + E + ENR + GM + K + OX + SXT	3
AM + AMC + CM + CN + E + K + MNO + OX + SXT + TE	1
AM + AMC + C + CM + CN + E + ENR + GM + K + OX + SXT	3
AM + AMC + CM + CN + E + ENR + K + MNO + OX + SXT + TE	1
Total	106

AM = ampicillin; AMC = amoxycillin/clavulanic acid; C = chloramphenicol; CM = clindamycin; CN = cephalexin; E = erythromycin; ENR = enrofloxacin; GM = gentamicin; K = kanamycin; MNO = minocycline; OX = oxacillin; SXT = sulfamethoxazole/trimethoprim; TE = tetracycline.

There was no statistically significant difference in susceptibility to antimicrobials between isolates obtained from the skin and external ear canal (Chi-square test, $P>0.05$). Cochran's Q test revealed a statistically significant association between resistance to erythromycin, kanamycin, gentamicin and chloramphenicol ($P<0.05$). Resistance to tetracycline was associated with resistance to erythromycin, kanamycin and chloramphenicol (Cochran's Q test, $P<0.05$) but not with gentamicin and ampicillin.

Table 2 shows the different resistance patterns based on the number of antimicrobials to which the isolates were resistant. Only 23 (21.7%) isolates were susceptible to all antimicrobial agents tested. All isolates resistant to at least three antimicrobials were always resistant to ampicillin, i.e. resistance to any antimicrobial was always associated with resistance to ampicillin. There was a high proportion (37.7%) of multiresistant isolates (resistant to four or more classes of antimicrobials). Among them, the two most common patterns included resistance to ampicillin, macrolides, lincosamides, aminoglycosides, tetracyclines and chloramphenicol.

Discussion

Resistance to beta-lactamase sensitive penicillins is widespread among staphylococci of human and animal origin. The resistance rate of 78.3% for ampicillin determined in this study is similar to that found in SIG isolates from first-time and recurrent cases of pyoderma in dogs in Sweden (HOLM et al., 2002) and diseased cats and dogs in Switzerland (BOERLIN et al., 2001). Other studies conducted on diseased dogs (FUTAGAWA-SAITO et al., 2007; PENNA et al., 2010) detected lower resistant rates. Differences in locality and investigation period might be among the possible reasons for the variations in the reported resistance rates. Our findings show that beta-lactamase sensitive penicillins can no longer be used for treatment of canine staphylococcal infections in Croatia due to the very high number of resistant isolates.

The emergence of MRSP poses significant problems to veterinarians treating infected animals. The first isolates of MRSP in Croatia were detected in 2008 (MATANOVIĆ et al., 2009). Two studies conducted in Italy, the closest country to Croatia with reports on MRSP, reported half the oxacillin resistance rate in *S. pseudintermedius* than found in this work (VANNI et al., 2009; MEUCCI et al., 2010). However, in another Italian study, 21% MRSP was found among canine isolates from the *Staphylococcus intermedius* group (DE LUCIA et al., 2011). Nevertheless, our data is similar to the 7.4% MRSP prevalence detected among SIG isolates from canine diagnostic samples in Germany (RUSCHER et al., 2009). According to a CLSI document M100-S20 (ANONYMOUS, 2010), isolates susceptible to oxacillin can be considered susceptible to penicillinase-stable penicillins, such as amoxicillin/clavulanic acid, and cephalosporins, such as cephalexin, the two most commonly used beta-lactams in dogs. This was also confirmed in our study.

In this work, 37.7% of isolates were simultaneously resistant to erythromycin and clindamycin (inducible-resistant isolates included). This finding is quite surprising if we bear in mind that these antimicrobials are not commonly used in dogs and cats in Croatia. In addition, the percentage of erythromycin-resistant isolates in this study is almost identical to that reported by NAGLIĆ et al. (1998) in Croatia 15 years ago, where of 36 SIG isolates examined 38.9% were resistant to erythromycin. In a similar study conducted in Canada, only 5% and 4% of *S. pseudintermedius* isolates from canine otitis externa were resistant to erythromycin and clindamycin, respectively (HARIHARAN et al., 2006). Lower resistance rates for these antimicrobials were also reported for isolates from diseased dogs in the USA (HARTMANN et al., 2005), Denmark (PEDERSEN et al., 2007) and France (GANIERE et al., 2005) but in these studies susceptibility testing was performed by the broth microdilution method rather than disk-diffusion. The relatively high resistance rate determined in this work may be a result of co-selection of erythromycin resistance during therapy with other antimicrobials (BOERLIN et al., 2001). In the latter study the authors found that the *erm*B gene, conferring resistance to macrolides, lincosamides and streptogramin B, is physically linked to the *aadE-sat4-aphA-3* gene cluster in SIG isolates from dogs and cats. This cluster confers resistance to kanamycin, neomycin, streptothricin and streptomycin so therapy with any of these antimicrobials, such as neomycin, included in many topical formulations, will also select for resistance to macrolides and lincosamides.

The prevalence of resistance to tetracycline in our study was 37.7%, which is similar or lower to that determined by the broth microdilution method for canine clinical isolates in France (GANIERE et al., 2005), USA (HARTMANN et al., 2005), Norway (NORSTRÖM et al., 2009) and Japan (ONUMA et al., 2012), but higher than that found in isolates from diseased dogs in Denmark (PEDERSEN et al., 2007). In addition, the prevalence of resistance to tetracycline in our study was the same as erythromycin resistance, which is quite surprising considering the rare use of tetracycline in dogs and cats (ESCHER et al., 2011; MATEUS et al., 2011). It has been suggested that tetracycline-resistant isolates may be co-selected by therapy with other antimicrobial agents and so further increase the overall level of resistance to tetracycline (HOLM et al., 2002).

Of the 40 tetracycline-resistant isolates, 35 (87.5%) were also minocycline-resistant (intermediate isolates included in the resistant category). This phenotype is usually associated with the presence of the *tet*M gene, encoded on the transposon, which confers resistance to tetracycline and minocycline *via* the ribosomal protection mechanism. Five out of 40 (12.5%) tetracycline-resistant isolates were susceptible to minocycline. These isolates probably carry a *tet*K gene, previously found on plasmids, and encoding efflux pump-mediated resistance (SCHWARZ et al., 1998).

Although gentamicin showed the highest activity among the tested aminoglycosides, almost 18% of isolates were resistant to this agent. This is in contrast to other studies, where the gentamicin resistance rate was significantly lower, and ranged from 0% to 9.1% (GANIERE et al., 2005; HARTMANN et al., 2005; HARIHARAN et al., 2006; FUTAGAWA-SAITO et al., 2007; NORSTRÖM et al., 2009; VANNI et al., 2009). However, in those studies the prevalence of MRSP was low, while in this study gentamicin-resistant MRSP significantly contributed to the observed gentamicin resistance rate. Even though gentamicin is not a common choice for systemic therapy in dogs and cats, it is often used in Croatia for the topical treatment of localized skin infections, otitis externa and conjunctivitis. This may explain the relatively high resistance level to this antibiotic. However, a significantly lower resistance level was found in the study conducted by NAGLIĆ et al. (1998) in Croatia, where only one out of 180 (0.6%) SIG isolates was resistant to this agent. This increase is alarming and might lead to the ineffectiveness of this important antibiotic in the future.

Although chloramphenicol use is banned in food producing animals, it is still used in Croatia for topical treatment in pets. This might be the reason why more than a quarter of isolates was resistant to this antibiotic. Interestingly, 33 out of 46 (28.3%) SIG isolates from dogs were found to be resistant to chloramphenicol in a previous study (NAGLIĆ et al., 1998), so it seems that the resistance to this drug has remained stable in Croatia over the last 15 years. Similar resistance rates were also observed in France and Japan (GANIERE et al., 2005; ONUMA et al., 2012) but resistance was lower in North America, Norway and Sweden (HOLM et al., 2002; HARTMANN et al., 2005; HARIHARAN et al., 2006; NORSTRÖM et al., 2009) which probably reflects the local policies or recommendations for chloramphenicol use.

Resistance to trimethoprim-sulfamethoxazole combination in *S. pseudintermedius* is relatively low. Several studies have reported from 0% to 2.5% resistant strains (HOLM et al., 2002; GANIERE et al., 2005; PEDERSEN et al., 2007). However, resistance rates from 18% to 28% were found among methicillin-sensitive isolates from dogs in Canada and the USA (HARTMANN et al., 2005; HARIHARAN et al., 2006) and might be a result of its frequent use. In this work, resistance to the trimethoprim-sulfamethoxazole combination was found in two out of 98 (2.04%) methicillin-sensitive strains. This proportion is understandable considering the relatively low frequency of its use in dogs in Croatia, and reveals a potentially useful agent which might not select for resistance to other antimicrobials.

In this study, the lowest resistance was observed to enrofloxacin (5.66%). This is higher than found by ŠEOL (2005), where out of 50 isolates of the *S. intermedius* group examined in Croatia, only one was resistant to enrofloxacin. All isolates resistant to enrofloxacin were multi-resistant MRSP and its emergence probably accounts for the rise in resistance to enrofloxacin in Croatia. In addition, there were two isolates with

an intermediate sensitive phenotype, one was MRSP and the other was a multi-resistant methicillin-sensitive strain. These results are in agreement with other studies and show that resistance to fluoroquinolones, despite its frequent topical and systemic use in dogs, is rare among methicillin-sensitive *S. pseudintermedius* in Europe and North America (HOLM et al., 2002; HARTMANN et al., 2005; HARIHARAN et al., 2006; PEDERSEN et al., 2007; NORSTRÖM et al., 2009; VANNI et al., 2009; MEUCCI et al., 2010). The high prevalence of enrofloxacin resistance among MRSP is understandable since six out of eight showed a multi-resistant pattern typical for the dominant European MRSP clone (PERRETEN et al., 2010).

The spread of MRSP exerts pressure for the use of antimicrobials not licensed in veterinary medicine and may induce the development of resistance to those antimicrobial agents (VAN DUIJKEREN et al., 2010). MRSP has been recognized as an emerging zoonotic agent and veterinarians seem to be at higher risk of being colonized (PAUL et al., 2011). In order to reduce the selection of methicillin-resistant staphylococci during antimicrobial therapy, it would be beneficial to use agents with a narrow spectrum. In addition, appropriate antimicrobial therapy can reduce the length of overall treatment and minimize its effect on microbial flora. The results of the present study may be used for the development of guidelines for empirical therapy of skin and ear infections in dogs in Croatia. Antimicrobials that may be appropriate for such treatment are amoxicillin/clavulanic acid, cephalexin and sulfamethoxazole-trimethoprim. Although the overall resistance to enrofloxacin was low, this antimicrobial should not be used as a first choice drug due to its potential for selection of antimicrobial resistance in other bacterial species (TROTT et al., 2004). In particular, the efficacy of fluoroquinolones should be preserved for cases of recurrent or deep pyoderma, and for severe, life-threatening infections associated with Gram-negative organisms (GUARDABASSI et al., 2008). Due to the high percentage of strains resistant to ampicillin, kanamycin, erythromycin, clindamycin and tetracyclines, these drugs should not be used for therapy without prior susceptibility testing. Although 17.9% and 26.4% of isolates were resistant to gentamicin and chloramphenicol, respectively, these agents are mainly used topically (otitis externa and/or conjunctivitis) and usually achieve concentrations well above MIC, and may be effective even against resistant isolates. Pet owners should be advised to wear gloves when treating animals with chloramphenicol. However, use of gentamicin and chloramphenicol in situations with documented resistance may lead to prolongation of therapy and should be discouraged and replaced by some antistaphylococcal agents, such as fucidic acid or bacitracin. In addition, the marked increase in gentamicin resistance rate during the last 15 years in Croatia is alarming and might lead to the ineffectiveness of this important antibiotic in the future.

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SAŽETAK

Bakterija *Staphylococcus pseudintermedius* dio je fiziološke mikroflore pasa, ali i čest uzročnik oportunističkih zaraza. Kod ove je bakterije primjetan trend porasta rezistencije na antimikrobne lijekove koji se učestalo koriste u liječenju pasa. U ovom radu istražena je osjetljivost na antimikrobne lijekove 106 izolata bakterije *S. pseudintermedius* izdvojenih iz pasa i mačaka. Osjetljivost je određena disk-difuzijskim postupkom. Izolati su potjecali od bolesnih pasa i mačaka i prikupljeni su u razdoblju od šest mjeseci tijekom godine 2011. Većina izolata bila je osjetljiva na amoksicilin s klavulanskom kiselinom, cefaleksin, enrofloksacin i oksacilin (92,5%), sulfametoksazol s trimetoprimom (90,6%) i gentamicin (82,1%). Niži postotak osjetljivosti utvrđen je za minociklin (67,0%), eritromicin, klindamicin i tetraciklin (62,3%) te kanamicin (58,5%). Samo 21,7% izolata bilo je osjetljivo na ampicilin. U radu je uspoređena učestalost rezistencije između izolata izdvojenih s kože i iz zvukovoda, a istražene su i razlike u rezistenciji u odnosu na prijašnje istraživanje provedeno u Hrvatskoj. Raspravlja se i o mogućoj povezanosti između rezistencija na različite antimikrobne lijekove.

Ključne riječi: *Staphylococcus pseudintermedius*, rezistencija, disk-difuzijski postupak