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## ■ Ever-increasing emergence of multi-drug resistant *Staphylococcus pseudintermedius* in the dog and its zoonotic potentials

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## ■ Το πρόβλημα της αυξανόμενης εμφάνισης πολυανθεκτικών στα αντιβιοτικά στελεχών του *Staphylococcus pseudintermedius* στο σκύλο και η σημασία για τη Δημόσια Υγεία

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**ABSTRACT.** Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has emerged as an important pathogen in the dog over the past 10 years with an ever increasing incidence worldwide. This review focuses mainly on the epidemiology and detection of MRSP, emphasizing on the interpretation and pitfalls of screening laboratory tests and antimicrobial susceptibility tests. Risk factors for colonization and infection with MRSP and molecular analysis of the dominant clonal lineages are also described. The zoonotic potential and worldwide epidemiology of multidrug resistant *Staphylococcus pseudintermedius* (MDRSP) are presented. Finally, control options for the colonization of healthy dogs as well as infection by MRSP and MDRSP are described emphasizing on the indications for bacterial culture and susceptibility testing and the principles of topical therapy which may prove to be the sole effective treatment in several canine pyoderma cases.

**Keywords:** multi-drug resistant *Staphylococcus pseudintermedius*, dog, Public Health significance

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**ΠΕΡΙΛΗΨΗ.** Ανθεκτικά στη μεθικιλίνη στελέχη του *Staphylococcus pseudintermedius* (MRSP) έχουν εμφανιστεί στο σκύλο τα τελευταία 10 χρόνια, με ολοένα αυξανόμενη συχνότητα σε ολόκληρο τον κόσμο. Η βιβλιογραφική αυτή ανασκόπηση στοχεύει στην επιδημιολογία και ταυτοποίηση του MRSP και ιδιαίτερα στην ερμηνεία και τις παγίδες που ενδέχεται να κρύβουν οι εργαστηριακές δοκιμές ανίχνευσης και αντιμικροβιακής ευαισθησίας. Ακολουθεί η περιγραφή των παραγόντων κινδύνου για τον αποικισμό και την λοίμωξη από τον MRSP και η μοριακή ανάλυση των κυρίαρχων κλώνων. Επιπλέον, επισημαίνεται η σημασία που έχει ο πολυανθεκτικός στα αντιβιοτικά *Staphylococcus pseudintermedius* (MDRSP) για τη Δημόσια Υγεία, από επιδημιολογική άποψη. Αναφέρονται και οι επιλογές ελέγχου του αποικισμού των κλινικά υγιών σκύλων από τον MRSP και των νοσοκομειακών λοιμώξεων από τους MRSP και MDRSP. Τέλος, αναφέρεται το που και πότε πρέπει να γίνεται βακτηριδιακή καλλιέργεια και αντιβιογράμμα και οι αρχές της τοπικής αντισταφυλοκοκκικής θεραπείας η οποία σε πολλά περιστατικά πυοδέρματος του σκύλου είναι η μοναδική θεραπευτική επιλογή του κτηνιάτρου.

**Λέξεις ευρητηριασμού:** ανθεκτικός στη μεθικιλίνη, πολυανθεκτικός στα αντιβιοτικά, *Staphylococcus pseudintermedius*, σκύλος, Δημόσια Υγεία.

## INTRODUCTION

*Staphylococcus pseudintermedius* is a commensal and, by far, the most common and important bacterial pathogen of the canine skin that has mainly been associated with the superficial and deep pyoderma. In many instances, pyoderma has been linked to poorly or even well controlled allergic diseases, most notably canine atopic dermatitis. *S. pseudintermedius* is also a leading cause of ear infections, those of other body tissues and cavities, and post-operative wound infections in dogs (Weese and van Duijkeren, 2010). *S. pseudintermedius* colonizes, in particular, mucocutaneous sites such as nares, mouth, anus, as well as groin and forehead of clinically healthy dogs (Devriese and De Pelsmaecker, 1987; Griffeth et al., 2008; Siak et al., 2014). The reported prevalence of *S. pseudintermedius* carriage in healthy dogs range from 46% to 92% depending on the animal population and methodology applied (Bannoehr and Guardabassi, 2012).

At the present, all staphylococcal strains isolated from dogs is suggested to be reported as *S. pseudintermedius*, unless genomic analysis proves otherwise (Devriese et al., 2009).

## METHICILLIN-RESISTANT *S. PSEUDINTERMEDIUS* (MRSP)

Methicillin-resistance reflects the expression of *mecA* gene coding for a modified penicillin-binding cell wall protein (PBP2a) whose low affinity for β-lactam antibiotics makes penicillins and cephalo-

sporins less effective (Loeffler et al., 2007). *MecA* gene is located within the staphylococcal chromosomal cassette *mec*, (*SCCmec*), a large and mobile genetic element (Bond and Loeffler, 2012). Acquisition of *SCCmec* by *S. pseudintermedius* strains has led to the emergence of MRSP all over Europe (Loeffler et al., 2007; Ruscher et al., 2009; Perreten et al., 2010). An increasing number of studies have documented the rapid spread of MRSP worldwide (Perreten et al., 2010; Ruscher et al., 2010; Feng et al., 2012; Wang et al., 2012; Bardiau et al., 2013).

In the U.S, MRSP has been identified in 57 out of 336 isolates (17%) over one-year (Morris et al., 2006) whereas the figure dropped to 1 out of 25 (4%) in the 1995 to 1998 period (Gortel et al., 1999). In Europe, MRSP accounted for 23% of *S. pseudintermedius* isolates from a dermatology practice (Loeffler et al., 2007). In an Italian study, MRSP accounted for 10 out of 48 *Staphylococcus intermedius* group (SIG) isolates (21%), all of which were resistant to fluoroquinolones, gentamicin, lincosamides, tetracyclines and potentiated-sulphonamides (De Lucia et al., 2011), thus reflecting the acquisition of additional resistance genes. In Greece, the resistance of *S. intermedius* (most likely *S. pseudintermedius* based on the new classification) isolates originating from 53 natural cases of canine pyoderma was found to be 0% for amoxicillin/clavulanate, 11.3% for oxacillin, 7.5% for cefalexin, 5.7% for enrofloxacin, 32.1% for erythromycin, 11.3% for tylosin, 20.8% for lincomycin, 18.9% for clindamycin, 32.1% for doxycycline, 5.7% for ampicillin, 7.5% for chloramphenicol and 92.5% for trimethoprim/

sulfamethoxazole (Saridomichelakis et al., 2002). The authors have also isolated MRSP strains from a few canine pyoderma cases in Athens, Greece, over the last two years.

Human and veterinary diagnostic laboratories commonly use phenotypic methods for the detection of MRSP/MRSA strains. The use of oxacillin disk diffusion along with the interpretative criteria applied to *Staphylococcus aureus* has led to a high percentage of false-negative results thus making it rather inappropriate as a screening tool (Bemis et al., 2009). In 2009, the Clinical and Laboratory Standards Institute (CLSI) of Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee re-evaluated the interpretative criteria, proposing an oxacillin MIC of  $\geq 0.5$  mg/L (agar and broth dilution) and a zone diameter of  $\leq 17$  mm (disc diffusion) as highly reliable for the detection of *mecA* in *S. pseudintermedius* strains (Papich, 2010).

In antimicrobial susceptibility interpretation, the fact that methicillin resistance is mediated by *mecA* gene, that confers resistance to all  $\beta$ -lactam antibiotics, should be taken into account, regardless of the *in vitro* results (Jones et al., 2007). The same applies to macrolides because of their cross-resistance (Ganiere et al., 2005). In inducible clindamycin resistance (iCR) the responsible gene is not expressed until the exposure to this antibiotic, thus complicating the interpretation of susceptibility testing; this has been associated, though uncommonly, with MRSP (Rubin et al., 2011). *S. pseudintermedius* isolates that are resistant to other macrolides (e.g erythromycin) but not to clindamycin should be tested for the presence of iCR either by D-test or genetic testing, because if not there is increased risk for treatment failures (Gold and Lawhon 2013). Before the emergence of MRSP, most of *S. pseudintermedius* infections in dogs were successfully managed with empirical or based on culture and susceptibility testing antibacterial treatment.

Interestingly, PBP2a latex agglutination test, developed for methicillin-resistant *S. aureus* (MRSA) in humans may result in false-positive results when applied to *S. pseudintermedius* isolates, thus making it unacceptable as a sole test to confirm the presence of MRSP (Pottumarthy et al., 2004).

MRSP strains have been isolated from dogs, cats and humans (Wettstein et al., 2008; Hanselman et al., 2009), in situations associated with contamination, colonization or infection (van Duijkeren et al., 2011).

Despite the restricted number of studies on the risk factors for MRSP colonization or infection the use of antibiotics in hospitalization and surgeries have been so far incriminated (Sasaki et al., 2007; Weese et al., 2009; Nienhoff et al. 2011). Busy practice and treatment with ear medications or glucocorticoids are also hold responsible for such an infection (Lehner et al., 2014).

Molecular analyses have greatly facilitated the epidemiological and evolutionary studies regarding the origin and spread of MRSP (van Duijkeren et al., 2011; Chanchaithong and Prapasarakul, 2011). A certain number of MRSP strains are resistant to antibiotics usually applied in the everyday practice, notably ST71 in Europe and ST68 in North America (Osland et al., 2012). *In vitro* conditions, MRSP ST71 strain has an increased adherence to canine and human corneocytes than the other MRSP strains and the methicillin susceptible *S. pseudintermedius* (MSSP) strains (Paul et al., 2011; Latronico et al., 2014). This enhanced adherence of ST71 may contribute to the epidemiological success of the MRSP ST71, most likely due to its higher ability to adapt to human epidermis, that may explain the expanded host spectrum and zoonotic significance (Paul et al., 2011).

#### **MULTI-DRUG RESISTANT *S. PSEUDINTERMEDIUS* (MDRSP)**

In veterinary medicine, multi-drug resistance (MDR) is of major concern (Frank and Loeffler, 2012). MDR is defined as resistance developed to three, at least, different classes of antimicrobials in addition to  $\beta$ -lactams (Coombs et al., 2004). In a recent study on a total of 1069 *S. pseudintermedius* isolates, 4.5% were MRSP and 27.5% MDR (Detwiller et al., 2013). In a large study including 103 staphylococcal isolates and originating from North America and Europe, 90% were resistant to ciprofloxacin, clindamycin, erythromycin, kanamycin, streptomycin and trimethoprim, 70% to gentamicin and tetracycline and 57% to chloramphenicol in addition to their resistance to  $\beta$ -lactam antibiotics (Perreten et al., 2010); in Germany, the resistance rates to fluoroquinolones, aminoglycosides and macrolides have been higher (Ruscher et al., 2009). Also, many MRSP isolates from north America are susceptible to chloramphenicol, rifampicin and amikacin; in Europe they are often resistant to chloramphenicol but susceptible to minocycline (Frank and Loeffler, 2012), although the explanation behind these geographical differences in MRSP is still lacking. These findings may lower the

chances for a successful systemic treatment especially in canine pyoderma cases.

The fact that, MRSP *Staphylococcus pseudintermedius* strains have recently been isolated from various body sites in healthy dogs and cats (Davis et al., 2014), make their role as reservoirs of MDRSP possible as well as their ability to transfer to people interacting with animals.

## ZOONOTIC IMPLICATIONS

It is very uncommon for *Staphylococcus pseudintermedius* to colonize the human skin, but carriage rates are generally higher in people interacting with dogs (Harvey et al., 1994; Goodacre et al., 1997; Guardabassi et al., 2004). *S. pseudintermedius* isolates are an uncommon occurrence in human hospitals (Mahoudeau et al., 1997), although the initial misidentification of *S. pseudintermedius* as *S. aureus* was a matter of concern (Tanner et al., 2000; Potthumathy et al., 2004; Kempker et al., 2009). In particular, *S. pseudintermedius* is normally negative by rapid slide clumping factor test and commercial latex agglutination test, both detecting the clumping factor, protein A and/or surface antigens of *S. aureus* (Bannerman, 2003). Phenotypic tests that help to differentiate *S. pseudintermedius* from the other staphylococcal species in the dog include coagulase, acetoin production, pyrrolidonyl arylamidase, b-galactosidase, polymyxin B resistance and D-mannitol acidification (Bannerman, 2003).

Nasal carriage of *S. pseudintermedius* was not demonstrated in any of 56 healthy human volunteers, in contrast to dental plaques with a figure as high as 8.9% (Ohara-Nemoto et al., 2008). Nasopharyngeal colonization of less than 1.5% has been reported in the academic veterinary staff (Talan et al., 1989; Loeffler et al., 2005). However, in more recent studies the rates were higher among dog owners. In particular, 1 persistent and 4 temporary nasal carriers of *Staphylococcus* “*intermedius*” were identified in 16 owners with atopic dogs and in 13 veterinary practice members (Harvey et al., 1994). The strains recovered from humans were in general correlated with those obtained from the in-contact dogs (Goodacre et al., 1997). *S. pseudintermedius* was also isolated from 9 out of 24 (37,5%) small animal owners, in which 4 carried the strain isolated from their dogs (Hanselman et al., 2009). Also, nasal carriage rates of *S. pseudintermedius* were higher among owners with dogs suffering from deep pyoderma, although this has been

shown to be a temporary colonization (Guardabassi et al., 2004; Frank et al., 2009). Regarding MRSP strains, the recognition of carriage among humans in contact with dogs was lower as well as that of sporadic human infections (Gerstadt et al., 1999, Campanile et al., 2007, Kempker et al., 2009, Stegmann et al., 2010).

Whereas the staphylococcal colonization of humans is transient after their exposure to *S. pseudintermedius* of canine origin, after the establishment of the corresponding infection it is difficult to eradicate (Stegmann et al., 2010). Interestingly, human carriage rates of MRSP are higher than that of MSSP, possibly reflecting the in-contact risk in both veterinarians and dog owners (Loeffler et al., 2010). Furthermore, MRSP of canine origin is considered a potential source of SCC-*mec* transfer and perhaps other mobile determinants of antimicrobial resistance to staphylococcal nomads in human skin and mucosae (Guardabassi et al., 2004).

## CONTROL OPTIONS FOR COLONIZED HEALTHY DOGS

Due to MRSP carriage potential in clinically healthy dogs (Davis et al., 2014), all the necessary precautions should be taken whenever a close contact with them is anticipated. Most likely, dogs colonized with MRSP are of greater risk to develop relevant infections especially when are wounded or undergone antimicrobial therapy. These animals should be washed with chlorhexidine-containing shampoos, in an effort to decontaminate the hair coat and skin. Long-standing colonization with MRSP may occur because dogs are natural hosts of *S. pseudintermedius* (van Duijkeren et al., 2011). Cleaning and disinfection of the house will probably help to prevent re-colonization from contaminated sources. At the present, there is no scientific evidence regarding the effectiveness of antibiotics to clear MRSP colonization in the dog.

## INFECTION CONTROL IN VETERINARY PRACTICE

Successful control of MRSP and/or MDRSP infection/contamination is based on personal and environmental hygiene. Due to the fact that staphylococci are usually transmitted via hand contact, relevant hygiene is critical for the prevention of MRSP spread, as well. Also, environmental cleaning and disinfection play an important role, as MRSA carriage in rescue kennels for dogs has

reportedly been eliminated as soon as regular cleaning of the facilities was instituted (Loeffler et al., 2010a). Environmental hygiene is also important as MRSP was being isolated over a 6 month period from households after the resolution of MRSP infection in the residing pets (Laarhoven et al., 2011). These measures will limit the spread of MRSP from infected patients and unrecognized carriers and help to prevent nosocomial infections in the setting of veterinary practice (Frank and Loeffler, 2012). In addition, isolation procedures within veterinary practices are warranted, because healthy dogs in contact with MRSP-infected dogs showed unusually high (36%) MRSP carriage rates (van Duijkeren et al., 2011).

In general, routine measures aiming at reducing the risk for antimicrobial resistance include a) hand washing and disinfection of surfaces and equipment in-between patient handling. Alcohol gel pouches can be used immediately after any contact with dogs. Alcohol is not effective in the presence of organic material, thus necessitating its removal by hand washing with a detergent. b) avoid materials at hand touch sites that cannot be cleaned (e.g. use washable keyboards or keyboard covers in computers) c) cover skin wounds and excoriations with waterproof dressings d) apply aseptic techniques and high standard cleaning e) dispose all waste and contaminated material and f) make sure that the veterinary staff understand and adhere to infection control measures (Nuttal et al., 2008).

In all cases of poorly responsive canine pyoderma to empiric therapy, bacterial culture and susceptibility testing are recommended along with treatment compliance. Culture is also recommended in patients with deep pyoderma and suspected concurrent infection with Gram-negative bacteria, atypical bacterial infection (e.g. *Mycobacterium*, *Actinomyces*, *Nocardia*), severe or life-threatening infections and frequently relapsing pyoderma (Vitale, 2014). In general, the recommended duration of treatment is approximately 3 weeks for superficial and 4-6 weeks for deep canine pyoderma. The length of antibiotic administration should be 7 days past clinical remission in uncomplicated infections and 10-14 days past clinical remission in complicated infections, such as recurrent or deep pyoderma and those associated with immunosuppression (Frank and Loeffler, 2012). Treatment protocols of shorter duration may contribute to the emergence of MRSP strains.

Topical treatment may be used as a sole therapy in recurrent pyoderma cases and particularly in those

caused by MRSP, where the choices for systemic antibiotic therapy are limited and/or the adverse reactions to systemic medications are prohibiting. Shampoos containing chlorexidine, benzoyl peroxide or ethyl lactate have all demonstrated high efficacy in dogs with staphylococcal infections, although chlorexidine should be preferred in MRSP-induced infections (Siak et al., 2014). Antibacterial bathing should be practiced 3 times per week, with 10 min contact time for better results (Murayama et al., 2011). Focal lesions can be treated with chlorexidine spray, mupirocin ointment, benzoyl peroxide gel, fusidic acid ointment or nisin (Werner and Russel, 1999). As is the case with any antimicrobial, prudent use of topical products is advised to prevent widespread resistance.

## CONCLUDING REMARKS

An increasing number of studies have documented the rapid spread of MRSP, worldwide. Several screening tests, used by human and many veterinary diagnostic laboratories, for the detection of methicillin resistance in staphylococci, may lead to a high percentage of false-negative or false-positive results.

Furthermore, when interpreting antimicrobial susceptibility results it should be kept in mind that methicillin resistance includes cephalosporins and amoxicillin-clavulanate, regardless of the *in vitro* results. *S. pseudintermedius* isolates that are resistant to macrolides, such as erythromycin, but susceptible to clindamycin should be tested for the presence of inducible clindamycin resistance, because the latter phenomenon may result in treatment failures.

Prior hospitalization and antibiotic treatment are usually associated with MRSP colonization and infection in dogs. Canine-derived MRSP should be considered as a potential source for SCC*mec* transfer and possibly other mobile determinants of antimicrobial resistance to susceptible staphylococci on human skin and mucosae. Identification of *S. pseudintermedius* as *S. aureus* is rather common misdeed in medical laboratories, used to deal with the latter species.

Successful control of MRSP contamination or infection are mainly based on hand and environmental hygiene.

## CONFLICT OF INTEREST STATEMENT

None declared. ■

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