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RESEARCH ARTICLE

Multidrug resistant Staphylococcus pseudintermedius isolated from superficial bacterial folliculitis in dogs from Portugal and Spain

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Abstract:

Multidrug resistant Staphylococcus pseudintermedius isolates have been recently increasing, which is alarming and dramatically decreases antimicrobial treatment options.
T he objective of this study was to evaluate the incidence of multidrug resistance (MDR) in meticillin-susceptible and resistant Staphylococcus pseudintermedius isolates (MSSP/MRSP), previously collected from dogs with superficial bacterial folliculitis (SBF) in two referral hospitals in Portugal and Spain.
Sixty S. pseudintermedius isolates were tested for oxacillin susceptibility by the Kirby-Bauer technique and divided into MRSP (30/60) and MSSP (30/60). Isolates were tested for first and second tier antibiotics recommended by the Working Group of the International Society for Companion Animal Infectious Diseases (ISCAID).
All MRSP exhibited resistance to amoxicillin-clavulanic acid, clindamycin and erythromycin. High resistance levels were observed to trimethoprim-sulfamethoxazole (97%), tetracycline and gentamicin (87%), cefalothin and enrofloxacin (83%), pradofloxacin (80%) and minocycline (50%). Low resistance level was observed for chloramphenicol (17%), amikacin (7%) and rifampicin (7%). One MRSP was susceptible to trimethoprim-sulfamethoxazole. Sixty and 57% of MSSP were resistant to tetracycline and minocycline, respectively. Additionally, 43% of the isolates were resistant to clindamycin and erythromycin, 20% to trimethoprim-sulfamethoxazole, 7% to enrofloxacin and pradofloxacin and 3% to chloramphenicol, gentamicin and amikacin. None were resistant to amoxicillin-clavulanic acid, cefalothin and rifampicin. Most isolates were MDR (38/60). All non-MDR isolates were MSSP. Meticillin resistance was associated with MDR to other classes of antibiotics (P=0.001).
Our study showed correlation between MRSP and MDR. The presence of MRSP should alert the practitioner for MDR and limited antibiotic options.

Keywords: Staphylococcus pseudintermedius, Multidrug resistance, Superficial bacterial folliculitis, Antibiotics, Dog

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Introduction

In the nineties, most infections caused by Staphylococcus pseudintermedius in dogs were effectively treated with empiric antibiotherapy with beta-lactams, macrolides or potentiated sulphonamides antibiotics [1, 2]. At least in Europe, multidrug resistance was extremely rare [3-5]. Between 1987 and 1995, resistance to cephalexin, amoxicillin-clavulanic acid, oxacillin/meticillin and enrofloxacin had never been reported in the UK [3].

The first multidrug resistant (MDR) meticillin resistant Staphylococcus pseudintermedius (MRSP) isolates were reported at a dermatology referral centre in Germany in 2005 [6]. Multidrug resistance is considered as resistance to three or more classes of antibiotics (coombs 2004; Schwarz et al 10).

Two MRSP strains developed simultaneously in Europe and USA with different resistant patterns. MRSP isolates were first reported in 1999 in North America and throughout Europe between 2005 and 2006, and are actually recognized as having a worldwide distribution [6-10]. The North America strain is still susceptible to chloramphenicol, rifampicin and amikacin. Regarding the predominant MRSP strain in Europe, resistance to beta-lactams, aminoglycosides, macrolides, lincosamides, tetracyclines, chloramphenicol, trimethoprim and fluoroquinolones is normally observed, although susceptibility to amikacin, fusidic acid, minocycline, rifampicin, vancomycin, teicoplanin and linezolid is still maintained [9,11]. This demonstrates the importance of recognizing MRSP isolates susceptibility in order to apply effective antibiotherapy.

The aim of the present work was to evaluate the MDR profile of 60 S. pseudintermedius isolates from two referral veterinary hospitals in Portugal and Spain.

Material and Methods

Characterization of the isolates

Sixty S. pseudintermedius isolates, previously collected from dogs with superficial bacterial folliculitis presented to the Dermatology Service of the XXXX and XXXX, were used in this study. These isolates were collected between January and December of 2014. Isolates were storedin a mixture of glycerol 30% (Scharlab S.L., Barcelona, Spain) and nutrient broth at -80 ° C. All media used were supplied by Oxoid (Oxoid, Hampshire, UK) unless stated otherwise.

The isolates were previously characterized as Gram-positive cocci, catalase and coagulase positive. They were also purposely chosen as MRSP (30/60) and MSSP (30/60) after disk diffusion susceptibility test to oxacillin by the Kirby-Bauer technique following the Clinical Laboratory Standards Institute (CLSI) guidelines [12].

A multiplex PCR of 16S rRNA (Staphylococcus genus specific), nuc (Staphylococcus aureus species specific), mecA (a determinant of meticillin-resistance) genes was used for identification of the isolates [13]. The isolates were

then identified as S. pseudintermedius by PCR-RFLP assay, as described by Bannoehr and collaborators [14].

Antibiotic-Susceptibility Testing

Antibiotic susceptibility testing was performed in accordance with the Kirby-Bauer methodology described in CLSI-Vet 2014 [12]. Antibiotics were chosen based on the recommendation of the Working Group of the International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the treatment of superficial bacterial folliculitis (SBF) [15]. The inhibition halos were interpreted as susceptible or resistant using the recommended diameters by CLSI-Vet 2014 [12] and, if not available, by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [16]. The following antibiotic disks were tested: oxacillin (1 µg), amoxicillin-clavulanic acid (20/10 µg), cefalothin (30 µg), clindamycin (2 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), minocycline (30 µg), enrofloxacin (5 µg), pradofloxacin (5 µg; Mast Diagnostics, UK), chloramphenicol (30 µg), rifampicin (5 µg), gentamicin (10 UI; Bio-Rad, France) and amikacin (30 µg). Staphylococcus aureus (ATCC® 29213™) strain was used as the quality control recommended by the CLSI. Isolates were classified as MDR or non-MDR according to the number of classes to which they were resistant [17,18]. The following antibiotics were used to determine the MDR pattern: oxacillin (beta-lactam class), clindamycin (lincosamide class), erythromycin (macrolide class), trimethoprim-sulfamethoxazole (sulphonamide class) tetracycline (tetracycline class), enrofloxacin (fluoroquinolone class), chloramphenicol (phenicol class), rifampicin (ansamycin class) and gentamicin (aminoglycoside class) (Table 1).

Table 1: Antibiotic classification according to the Working Group of the International Society for Companion Animal Infectious Diseases (ISCAID) guidelines

FIRST TIER ANTIBIOTICS	SECOND TIER ANTIBIOTICS
Amoxicillin-clavulanic acid	Tetracycline
Cefalothin	Minocycline
Clindamycin	Enrofloxacin
Erythromycin	Pradofloxacin
Trimethoprim-sulfamethoxazole	Chloramphenicol
	Rifampicin
	Amikacin
	Gentamicin

Statistical analysis

Statistical analysis was performed with Statistical Package for the Social Sciences 16.0 (IBM SPSS Chicago, IL).

Results

Identification of MRSP and MSSP isolates

All isolates were identified as S. pseudintermedius. The mecA gene was present in all MRSP isolates and absent in MSSP isolates, confirming the oxacillin-resistance phenotype. From the total of 60 isolates, 38 were of Portuguese origin (19 MSSP/ 19 MSRP) and 22 originated from Spain (11 MSSP/ 11 MRSP).

Antibiotic susceptibility testing

All MRSP isolates displayed resistance to amoxicillin-clavulanic acid. Isolates also exhibited resistance to clindamycin and erythromycin. High level of resistance was observed against trimethoprim-sulfamethoxazole, tetracycline, gentamicin, cefalothin, enrofloxacin, pradofloxacin and minocycline. Low level of resistance was observed for chloramphenicol, amikacin and rifampicin. Within the MSSP group, all isolates were susceptible to amoxicillin-clavulanic acid, cefalothin and rifampicin. A high number of isolates exhibited resistance to tetracycline, minocycline, clindamycin and erythromycin (Figure 1).

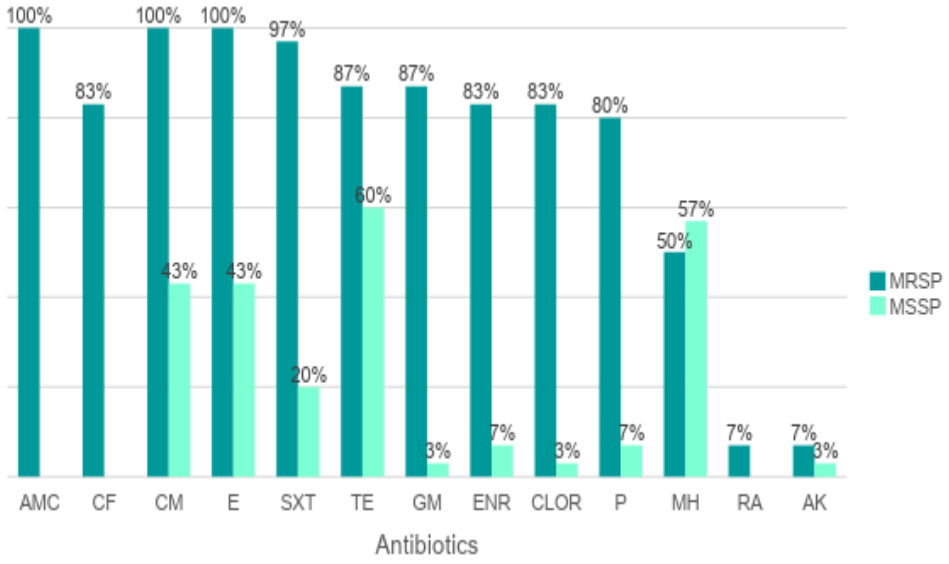

Fig 1: Relative frequency (%) of antibiotic resistance in the MRSP and MSSP group. First tier antibiotics: AMC, Amoxicillin-clavulanic acid; CF, Cefalothin; CM, Clindamycin; E, Erythromycin; SXT, Trimethoprim-sulfamethoxazole. Second tier antibiotics: TE, Tetracycline; GM, Gentamicin; ENR, Enrofloxacin; CLOR, Chloramphenicol; P, Pradofloxacin; MH, Minocycline; RA, Rifampicin; AK, Amikacin.
*, statistically significant differences between MSSP and MRPS groups.

Overall, resistance to oxacillin was associated with resistance to other antibiotics, except for minocycline (P=0.796), rifampicin (P=0.492) and amikacin

(P=1.000). Out of 60 isolates, eleven were only susceptible to rifampicin and/or amikacin, and none of them to chloramphenicol.

Within the tetracycline class, 12 isolates (11 MRSP and 1 MSSP) were susceptible to minocycline but resistant to tetracycline (12/44; P=0.000); amongst the fluoroquinolone class, only 2 (1 MSSP and 1 MRSP) enrofloxacin-resistant isolates were pradofloxacin susceptible (2/27; P=0.000). Within the aminoglycoside group, 24 gentamicin-resistant isolates (all MRSP) were amikacin susceptible (24/27; P=0.085).

Most isolates were MDR (63%, 38/60). All non-MDR isolates belonged to the MSSP group. Meticillin resistance was associated with multidrug resistance to other classes of antibiotics (P=0.001) (Table 2).

Table 2: Association between multidrug resistance and meticillin resistance.

	MDR	Non-MDR
MRSP	79% (30/38)	0% (0/20)
MSSP	21% (8/38)	100% (22/22)

MSSP (meticillin susceptible Staphylococcus pseudintermedius), MRSP (meticillin resistant Staphylococcus pseudintermedius) and MDR (multidrug resistance).

Discussion

This study demonstrates the presence of MDR S. pseudintermedius isolates in dermatology referral patients from the Iberian Peninsula. Multidrug resistance was associated with the presence of meticillin resistance. The percentage of MDR isolates was extremely high within the MRSP isolates obtained in this study, however, multidrug resistance was also observed in MSSP isolates. The presence of these MDR patterns has become a clinical challenge for veterinarians, since it reduces the number of antibiotic alternatives for the successful treatment of canine SBF. This is in accordance with other studies reported in Germany, UK and other European countries [6,19,20]. In Portugal, multidrug resistance has also been detected in colonization and infection cases in dogs [21].

Since the first report in 2005, there has been increased incidence of MRSP across Europe [6,9]. In Spain, MRSP has been reported in healthy dogs with isolates being resistant to beta-lactams, tetracycline, macrolides, lincosamides, aminoglycosides, trimethoprim-sulfamethoxazole and, in some cases, to fluoroquinolones [22]. More recently, a human infection caused by S. pseudintermedius originating in dogs has been described [23]. . Cases of MRSP have been described since 2010 and a trend towards oxacillin resistance has been suggested [21,24-27].

Nowadays, it is recommended to follow the guidelines for the diagnosis and antimicrobial therapy of SBF developed by the ISCAID. These guidelines provide

updated information for adequate treatment of canine SBF and rational use of antibiotics [15].

Staphylococcus pseudintermedius is the most common bacterial pathogen associated with canine SBF [28]. Treatment of these infections typically involves administration of broad-spectrum antibiotics, such as beta-lactams, clindamycin, erythromycin or potentiated sulphonamides [15]. This study demonstrates the presence of high levels of resistance to first line antibiotics, often used empirically, which significantly limits treatment options by the veterinarian, particularly in MRSP isolates. In fact, only one MRSP isolate was susceptible to trimethoprim-sulfamethoxazole. However, MSSP isolates were also resistant to first line antibiotics, such as clindamycin, erythromycin and trimethoprim-sulfamethoxazole. This is one of the reasons why bacterial culture and susceptibility testing should always be performed in case of lack of clinical response after two weeks of appropriate empirical antibiotherapy [15].

Based on the resistance profile to oxacillin, the representative antibiotic for the beta-lactam class, the isolates were divided into two groups: MRSP and MSSP. It is important to evaluate the susceptibility to the oxacillin disk in S. pseudintermedius isolates, since it is an indicator of resistance mediated by the mecA gene, when its detection by PCR, the gold standard method, is not possible. The link between MDR and MRSP has been reported [2,19,20]. In this study a clear association was observed between MRSP and multidrug resistance. Resistance to meticillin causes a major impact on treatment using beta-lactam class antibiotics, which are administered empirically for the treatment of staphylococcal infections. Although some MRSP displayed susceptibility to cefalothin, according to the CLSI recommendations and the ISCAID guidelines, they should be considered and reported as resistant, as meticillin resistance confers resistance to virtually every beta-lactam antibiotic, with the exception of anti-MRSA cephalosporins [29-32].

Lincosamides and macrolides are considered good antibiotic choices for the treatment of canine SBF caused by staphylococci due to its good oral absorption, distribution in tissues and high intracellular concentration [2,15]. However, especially in recurrent infections, their use is limited by a high level of resistance, particularly when there is cross resistance between the two antibiotics [2]. The interpretation of susceptibility to lincosamides should be considered carefully since cross-resistance with macrolides can occur. The presence of cross-resistance should be considered when the isolate demonstrates in vitro resistance to erythromycin and susceptibility to clindamycin [9,33].

Potentiated sulphonamides are first line antibiotics frequently used in the treatment of canine SBF [15,34]. The use of potentiated sulphonamides as first line antibiotics is limited by the relatively high incidence of resistance and potential side effects [29,35].

Since tetracycline is only the marker for resistance to the tetracycline class, doxycycline is usually the administered antibiotic. Even though minocycline is not licensed for use in dogs [36], results of the current study suggest that minocycline could have been used to treat 12 patients which were tetracycline-resistant. Therefore, in addition to its use being recommended in the ISCAID guidelines, minocycline represents a useful alternative.

Fluoroquinolones, particularly in MSSP isolates in which a high susceptibility rate is observed, can be administered for staphylococcal infections. The

association between the use of quinolones and carbapenems and an increased risk for MRSA is reported in a large hospital study among hospitalized people [39].

Chloramphenicol is rarely used in the treatment of S. pseudintermedius infections as it may cause severe adverse effects, particularly in humans [40]. Susceptibility in MSSP isolates was still observed, although resistance to this antibiotic is common in Europe, due to the expression of the chloramphenicol acetyltransferase [2,41,42]. Isolates resistant to chloramphenicol are still susceptible to florfenicol, a derivative of chloramphenicol, so it may be a safer option [2,43,44].

Resistance to rifampicin is normally rare and the presence of resistant isolates can be associated with previous administration of this antibiotic, since mutations for resistance can persist for months [9,45]. Even when the isolate displays susceptibility, the administration of rifampicin as monotherapy or in combination with another antibiotic to treat MDR-MRSP infections, can result in high levels of resistance [45].

Aminoglycosides can be used in the treatment of staphylococcal infection, if no other safer alternatives are available. Against resistant isolates, amikacin is more active than gentamicin and resistance is less likely to occur, which was verified in this study as amikacin could have potentially been used to treat most of the gentamicin-resistant patients [32]. Inactivation by aminoglycoside modifying enzymes is the main resistance mechanism to aminoglycosides [9,46]. These antibiotics are not routinely used in the treatment of staphylococcal infections, particularly due to their nephrotoxic and ototoxic effects and the inconvenience of parenteral administration [15]. Their administration should be avoided in animals with renal insufficiency and in healthy animal monitoring of renal function, according to International Renal Interest Society guidelines for prevention of aminoglycoside-induced acute kidney injury is advised [47]. However, with the increasing prevalence of MRSP, their use is becoming a necessary alternative and they are recommended in the ISCAID guidelines when no other safer alternatives are present [15].

Ideally, second choice antibiotics like fluoroquinolones should only be used in MRSP infection cases [15]. In general, higher susceptibility rates were associated with minocycline, amikacin and rifampicin, but side-effects can deem their use unacceptable in certain patients. MRSP were less likely to be susceptible to doxycycline, enrofloxacin, pradofloxacin and gentamicin.

Although this study was performed in a limited number of animals, the isolates were recovered from dogs attending referral consultation and, therefore, these data further supports the fact that first line antibiotics are extremely limited to treat these patients.

The association between MRSP and the presence of MDR was evident and it was also observed in MSSP isolates.

This problem requires a prudent, targeted use of antibiotics and the development of novel topical treatments to control infections caused by MDR S. pseudintermedius. Additionally, bacterial culture to identify the bacteria and sensitivity testing are essential tools to determine the most appropriate treatment plan, which can include antibiotics with potential severe side-effects.

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