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Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *methicillin-resistant Staphylococcus pseudintermedius* (MRSP) in Skin Infections from Company Animals in Portugal (2013–2021) †

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Abstract: This study aimed to investigate *methicillin-resistant Staphylococcus aureus* (MRSA) and *methicillin-resistant Staphylococcus pseudintermedius* (MRSP) in pyodermas admitted to the INNO Veterinary Laboratory (Braga, Portugal), in the period 2013-2021. From a total of 730 samples that tested positive for bacterial growth, 101 (13.8%) were *S. pseudointermedius* and 27 (3.7%) were *S. aureus*. The isolates tested for oxacillin n = 6 was MRSP and n = 4 MRSA. The presence of MRSA or MRSP in small animals indicates that they are part of the animal-human-environment transmission 'triangle', which should lead us to think of this issue as a public health problem.

Keywords: MRSA; MRSP; small animals; antibiotic resistance; pyoderma

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1. Introduction

Antimicrobial resistance among bacterial pathogens is one of the major concerns worldwide, in both human and veterinary medicine. They have been increasing in the last years and have major implications in health, as the failure in treatment leads to enhanced morbidity, mortality and costs associated with disease treatment [1,2].

Bacterial skin infections are among the most common pathologies present in small animal practice and are one of the most common reasons for antibiotic prescription [3]. Staphylococcus pseudintermedius and, to a less significant extent, *Staphylococcus aureus*, are important causes of skin infections [4].

Methicillin-resistance in Staphylococci is associated to the presence of the *mecA* gene encoding the penicillin binding protein 2a (PBP2a). This gene has the ability of reduce the affinity of the bacteria for all b-lactam antimicrobials, and therefore making her resistant to this bacterial agents [4,5].

Methicillin-resistant Staphylococcus aureus (MRSA) and *methicillin-resistant Staphylococcus pseudintermedius* (MRSP) is now a significant concern in veterinary medicine [4]. MRSA and MRSP pose a major clinical challenge in the treatment of bacterial pyoderma of companion animals [3,4].

The main objective of this work was to determine the prevalence of methicillin-resistance in staphylococci isolated from pyoderma admitted to INNO Veterinary

Laboratory (Braga, Portugal) in the years 2013 to 2021, and evaluate the prevalence of MRSP and MRSA in the same time period.

2. Material and Methods

Microbiological cultures from skin infections from dogs and cats were submitted to the INNO Veterinary Laboratory between January 2013 to June 202, from various clinics in Portugal.

Samples were incubated in Columbia agar + 5% sheep blood, Columbia CNA agar + 5% sheep blood and McConkey Agar (BioMérieux, France) plates during 24 h at 37 °C. Gram coloration of the colonies was made to identify pure colonies of cocos Gram positive.

Phenotype identification of the agents was performed using the automated system Vitek 2 Compact system (BioMerieux, France) with the Vitek 2 ID card (ref 21341, bioMérieux, France). For the present study, only samples with *S. aureus* and *S. pseudintermedius* growth were selected. Automated antimicrobial susceptibility testing was performed with the Vitek 2 Compact system method (BioMerieux, France), using the Vitek 2 AST-GP71 card (bioMérieux, Inc., Durham, NC, USA) in accordance with the manufacturer's specifications [6].

The methicillin resistance was phenotypically detected using the automatized VITEK 2 Compact System (BioMerieux, France) with the Vitek 2 AST-GP80 card (ref 421826, BioMerieux, France) by oxacillin minimum inhibitory concentration (MIC) testing automatically interpreted by using *S. pseudintermedius* and *S. aureaus* specific breakpoints. These guidelines can be found in the CLSI VET01-S2 document.

3. Results

From a total of 730 samples that tested positive for bacterial growth, 101 (13.8%) were S. pseudointermedius and 27 (3.7%) were S. aureus.

The isolates tested for oxacillin n = 6 was MRSP and n = 4 was MRSA. In Figure 1 it is possible to observe the evolution on the number of MRSA and MRSP from 2013 to 2021. The origin of this sample was from one cat and nine dogs. The majority of the animals were females (n = 6). The breed more affect was non defined breed SRD (n = 6).

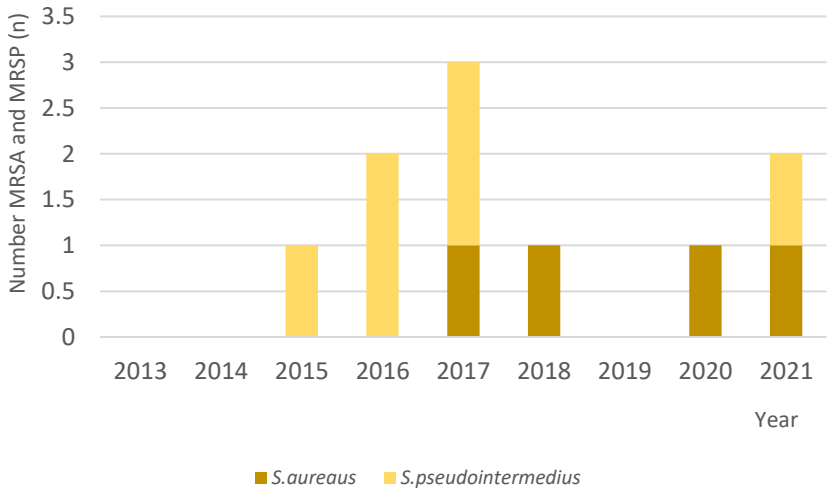

Figure 1. Evolution on the number of MRSA and MRSP from 2013 to 2021.

4. Discussion

Skin infections is one of the most common pathologies observed in small animal clinic [4]. The identification of bacteria should always be performed before applying systemic therapy [3]. It is also important to discover the primary cause of the pyoderma, since

many times the bacterial infection is only secondary and antibiotics can be administered and induce resistance in bacteria present in the flora of the animals that later can become a problem [3].

The isolation of an organism from a certain body location does not mean that agent is responsible by the process. Unfortunately, pathogenic organisms as *S. pseudointermedius* or *S. aureaus* are also frequent colonizers of the normal microbiological flora of the animals, which can lead to confusion as to their significance in cultures from clinical specimens. Some studies have demonstrated that strains of MRSA in animals in the majority of the cases is colonization, with a very small percentage of the cases being the responsible for the infection [7]. It is important that the veterinary clinician is informed of these situations.

The origin of these agents is diverse. Is difficult to determine whether the presence of the organism on the skin is the result or cause of colonization from other body sites. MRSP are commonly isolated from the nose or rectum [4,5]. So, we can assume that the pyoderma in those animals was caused by opportunistic pathogens that are part of the commensal flora [4]. In the case of the MRSA there is no association with colonization, most likely to had origin in external sources. The most probable origin of these MRSA are humans. The close contact between pets and their owners offers favorable conditions for the transmission of bacteria by direct contact (petting, licking, etc.) or through the domestic environment (contamination of food, toys etc.) [9].

The prevalence of methicillin-resistant staphylococcal pyoderma in the present study was low. This study was performed with samples that were collect in clinics, and it is reasonable to suspect that the prevalence of MRSA and MRSP could be higher amongst. There is possible that some bacteria where not identify due to the errors in sample collection, misidentification or overlooking a bacterial pathogen in the case of a polymicrobial infection, culture conditions not ideal, bacteria's viable but not culturable stage and the low prevalence of resistant subpopulations undetectable by standard diagnostic tests.

MRSP were the prevalent isolates in this study. This is in agreement with previous studies that report high incidence rates of S. *pseudointermedius* in dogs and cats, while *S. aureus* in not very common agent to isolated from animals [4]. The most affected animals as the dog, but it was expected, because of the 6.7 million pets existent in Portugal, 38% are dogs and 20% cats [8].

5. Conclusions

The results obtained in this study help to understand the situation at a national level, where studies in this area are almost non-existent. It proves the importance of an accurate understanding of the prevalence of methicillin resistant staphylococcal infections in veterinary practice and the worth of routine culture and susceptibility testing, particularly in practices where methicillin resistance rates are high or are increasing in the population. The presence of MRSA or MRSP in small animals indicates that they are part of the animal-human-environment transmission 'triangle', which should lead us to think of this issue as a public health problem.

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