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Proceeding Paper

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 isolate tested for oxacillin n = 6 was MRSP, and for n = 4, it was 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 a major concern worldwide, in both human and veterinary medicine. It has been increasing in recent years and has major implications for health, as the failure of 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 some 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 with the presence of the *mecA* gene encoding the penicillin binding protein 2a (PBP2a). This gene has the ability to reduce the affinity of the bacteria for all b-lactam antimicrobials, therefore making it resistant to this bacterial agent [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. Materials and Methods

Microbiological cultures from skin infections from dogs and cats were submitted to the INNO Veterinary Laboratory between January 2013 to June 2021, from various clinics in Portugal.

Samples were incubated in Columbia agar +5% sheep blood, Columbia CNA agar +5% sheep blood and McConkey Agar (BioMérieux, Paris, France) plates at 37 °C for 24 h. 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, Paris, France) with the Vitek 2 ID card (ref 21341, bioMérieux, Paris, France). For the present study, only samples with *S. aureus* and *S. pseudintermedius* growth were selected. Automated antimicrobial susceptibility testing was performed via the Vitek 2 Compact system method (BioMerieux, Paris, 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, Paris, France) with the Vitek 2 AST-GP80 card (ref 421826, BioMerieux, Paris, France) by oxacillin minimum inhibitory concentration (MIC) testing automatically interpreted 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 isolate tested for oxacillin n = 6 was MRSP, and for n = 4, it was MRSA. In Figure 1, it is possible to observe the evolution in 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 that was most affected was the non-defined breed SRD (n = 6).

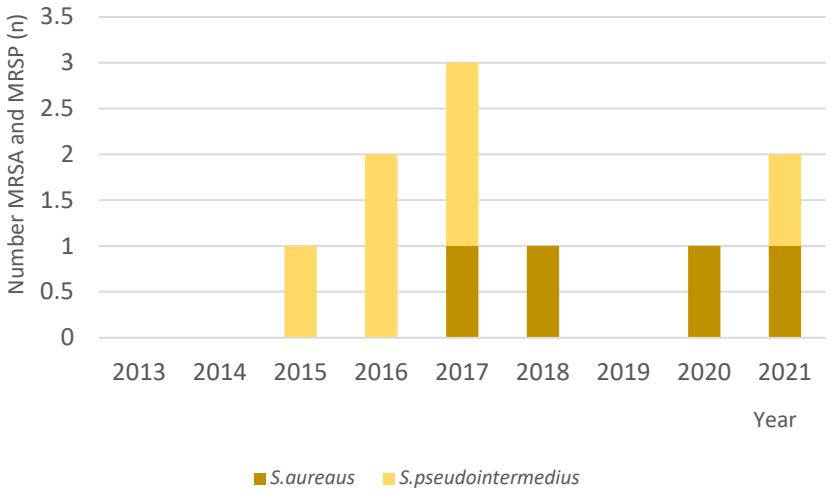

Figure 1. Evolution in the number of MRSA and MRSP from 2013 to 2021.

4. Discussion

Skin infections are among the most common pathologies observed in small animal clinics [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 in many instances, the bacterial infection is only secondary, and antibiotics can be

administered and induce resistance in bacteria present in the flora of the animals, which can later become a problem [3].

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

The origin of these agents is diverse. It 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, as it most likely originates from external sources. The most probable origin of these MRSA is humans. The close contact between pets and their owners offers favorable conditions for the transmission of bacteria via direct contact (petting, licking, etc.) or through the domestic environment (contamination of food, toys, etc.) [8].

The prevalence of methicillin-resistant staphylococcal pyoderma in the present study was low. This study was performed with samples that were collected in clinics, and it is reasonable to suspect that the prevalence of MRSA and MRSP could be higher amongst these samples. It is possible that some bacteria were not identified due to errors in sample collection, misidentification or overlooking a bacterial pathogen in the case of a polymicrobial infection. Culture conditions were not ideal: the bacteria were in a viable but not culturable stage and low prevalence of resistant subpopulations is undetectable using 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* is an uncommon agent to isolate from animals [4]. The most affected animal was the dog, but this was expected: of the 6.7 million pets existing in Portugal, 38% are dogs and 20% are cats [9].

5. Conclusions

The results obtained in this study help us to understand the situation at a national level, where studies in this area are almost non-existent. They prove the importance of an accurate understanding of the prevalence of methicillin-resistant staphylococcal infections in veterinary practice and the value 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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