

Research and identification of *Staphylococcus Pasteur*, 1880 (Bacillales Staphylococcaceae), potentially zoonotic, isolated from Sicilian dogs

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ABSTRACT

The uncontrolled abuse of antibiotics used in veterinary medicine, has led to the development of some mechanisms of antibiotic-resistance in the bacteria. This event allows them to breed and increase in number inside a host organism. *Staphylococcus* spp. strains (Bacillales Staphylococcaceae) have been isolated from cutaneous swabs of dogs, have been identified through microbiological methodologies on a biochemical basis, and their sensitive profile to various antibiotics, commonly used in the veterinary domain and in human medicine, was valued. Other molecular and microbiological studies on these *Staphylococcus* spp. strains have also been carried.

KEY WORDS

Staphylococcus; Sicilian dog; antibiotic-resistance.

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INTRODUCTION

In the last decades, as a result of the uncontrolled abuse of antibiotics used in veterinary medicine, bacteria have developed some mechanisms of antibiotic-resistance, which allow them to breed and increase in large numbers inside a host organism. In spite of this, pharmaceutical industries continue to launch new types of antibiotic drugs on the market, as soon as new resistances are developed.

Staphylococcus pseudintermedius Devriese, Vancanneyt, Baele, Vaneechoutte, De Graef, Snauwaert, Cleenwerck, Dawyndt, Swings, Decostere et Haesebrouck, 2005, is positive Gram's cocci, that commonly lives inside pets, where it colonize the oral cavity, the nasal mucosa, the abdominal skin, and the arial mucosa, causing abscesses, purulent infections, pyometra, folliculitis, dermati-

tis, and otitis externa as well (Devriese et al., 2005; Weese & Van Duijkeren, 2010).

Since 1990, the emergence of the *Staphylococcus pseudintermedius* strains, resistant to methicillin (MRSP), has been identified in infections of a huge number of species of pets and other animals. These MRSPs have often been isolated from dog specimens, shifting the scientific community attention on pets, as a potential source of resistant bacteria, which are transmissible to humans (Devriese et al., 2005; Bannoehr & Guardabassi, 2012).

The aim of this work is the multidisciplinary study of *Staphylococcus* spp. strains, isolated from cutaneous swabs of dogs. The isolated strains have been identified through microbiological methodologies on a biochemical basis, evaluating their sensitive profile to various antibiotics, commonly used

in the veterinary domain, and which find similar molecules in human medicine.

A molecular analysis results for some strains were compared with those obtained by the classical microbiological methods.

Furthermore, rDNA sequences obtained for various species were studied to reveal differences and genetic distances in the isolated strains. The sequences were compared in the Wu Blast database.

MATERIAL AND METHODS

Sampling

During the study period, 275 swabs were taken from infected animals, 65 of them were positive to the growth of *Staphylococcus* spp.

Microbiological analysis

Each swab sample was employed for the bacterial isolation on Mannitol Salt Agar, that it is selective for *Staphylococcus* spp.

Staphylococcus positive samples were identified by Gram staining (*Staphylococcus* Gram positive), following: catalase test (*Staphylococcus* catalase positive), oxidase test (*Staphylococcus* oxidase negative), coagulase test (*Staphylococcus* coagulase positive), Voges-Proskauer test *S. aureus* Rosenbach, 1884 (VP+), and *S. intermedius* Hájek 1976 (VP-) tests, STAPH API test used to identify the species.

All the isolated bacterial strains were subjected to an exam to evaluate their sensitivity resistance and intermediate reactivity to antibiotics.

Subsequently, the following antibiotics were tested: amoxicillin, enrofloxacin, marbofloxacin, convenia, methicillin, doxycycline, penicillin, and pradofloxacin. These antibiotics are all commonly used in veterinary medicine.

Molecular analysis

Molecular biology investigations were performed by extracting the DNA. The PCR was targeted to a specific 16S rRNA gene fragment 600 bp long. The amplicones were loaded on agarose gel to confirm the PCR results. The samples of amplified DNA were purified with a commercial kit

“GFX PCR DNA and gel band purification kit”, the sequence products were placed in a filtering column containing a particular “Sephadex®” resin using the “Illustra” purification kit AutoSeq G-50 Dye. Sanger sequencing allowed to obtain a sequence of a nucleic acid molecule by identifying the samples on a molecular basis.

Twenty-one strains of *Staphylococcus* spp. were selected for the molecular proof: the results obtained after the sequencing procedure were compared with recorded sequences for the identifications with the online system BLASTn on the GenBank database and then aligned through the Geneious program.

RESULTS AND DISCUSSION

On the base of the microbiological and biochemical tests were obtained the following identifications: n° 38 strains of *S. aureus*, 18 *S. xylosus* Schleifer et Kloos, 1975, 5 *S. lentus* Kloos, Schleifer et Smith, 1976, 2 *S. hycus*, 1 *Staphylococcus lugdunensis* Freney et al., 1988, 1 *S. epidermidis* (Winslow et Winslow 1908) Evans, 1916. Thanks to the analysis done with the VP test: 38 strains were identified by the API system as *S. aureus*: 20 were identified as *S. intermedius* (negative VP).

In line with what previously described by other authors, that associates a greater probability to find a colonization done by *Staphylococcus pseudintermedius* in samples coming from animals (Futagawa-Saito et al., 2006), the VP test allowed to differentiate and identify 30% of the strains as *S. pseudintermedius* against 27% of *S. aureus*. Moreover, the data obtained from the antibiograms show that 3 staphylococcal species have a strong resistance to methicillin and in particular 88% of the *S. aureus* are MRSA, 63% of the *S. pseudintermedius* are MRSP, and only 16% of *S. xylosus* show a resistance to methicillin. These data confirms the recent emergence regarding the MRSP strains diffusion in veterinary field (Perrent et al., 2010).

The antibiograms performed on all the strains of isolated staphylococci, allow us to understand the different degree of resistance/sensitivity to the antibiotics most commonly used in veterinary medicine, as well as in the human domain. So our study

confirms the emergence of the phenomenon of resistance to antibiotics also in the veterinary field, paying particular attention to the high presence of penicillin-resistant strain (90%), methicillin (47.7%), and amoxicillin (27.7%). The data obtained show a strong sensitivity to difluoroquinolones in a higher percentage than the one cited in a previous literature (Humphries et al., 2016; Yarbrough et al., 2018).

As a matter of fact, in the veterinary field, this last class of antibiotics is primarily used for the in-

fections caused by *S. pseudintermedius* (Perreten et al., 2010).

The molecular analysis used as support to the traditional identification techniques have confirmed the identification of isolated staphylococci species. On 17 strains, it has not been possible to confirm strains of *S. pseudintermedius*.

The molecular identification results, obtained in this work, are in line with what is reported in the bibliography (Bannoehr & Guardabassi, 2012) and underline the presence of potentially pathogenic

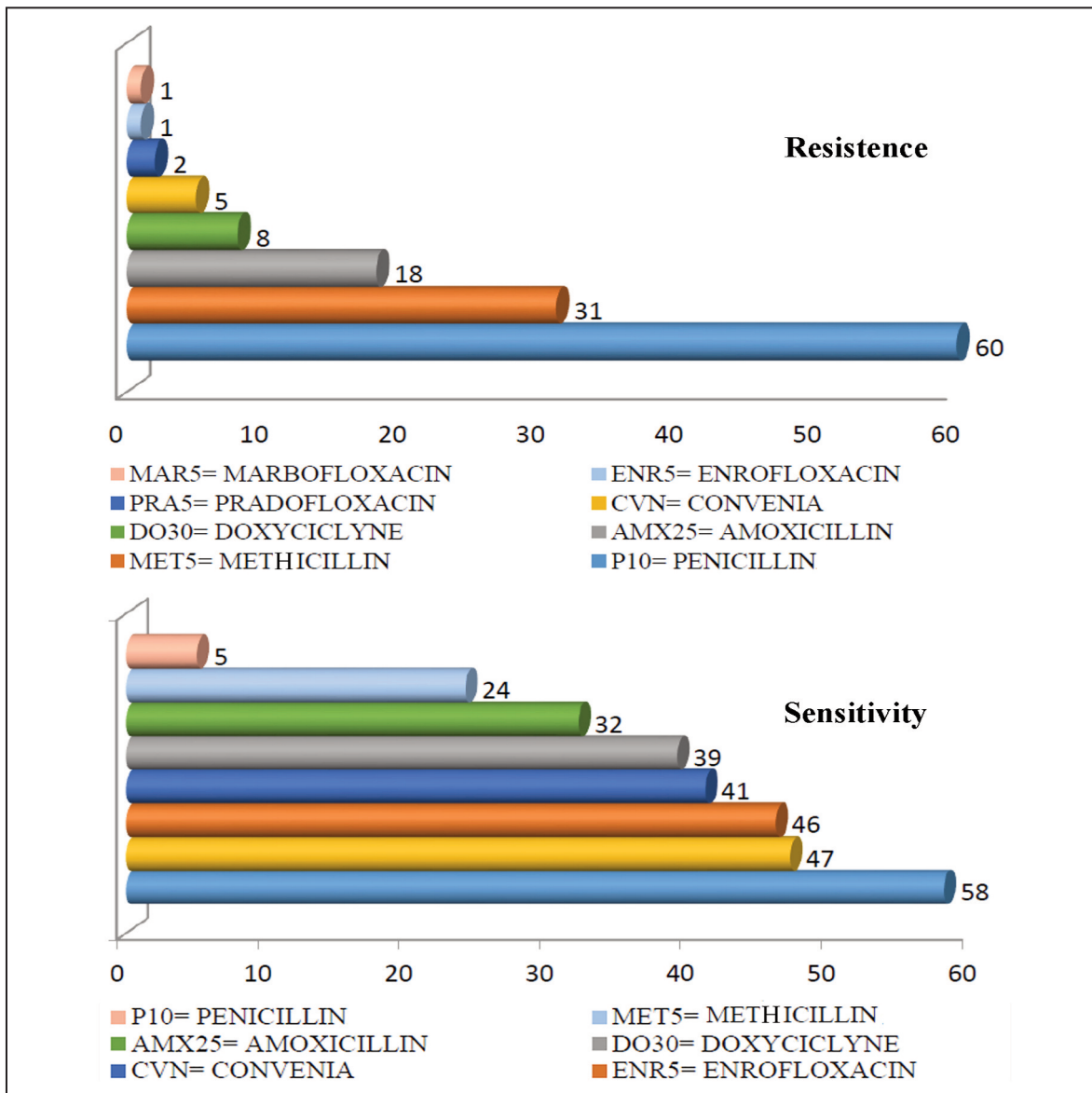


Figure 1. Number of isolated strains for which was detected the antibiotic resistance panel.

positive coagulase strains belonging to the *S. aureus* and *S. pseudintermedius* in pets, and in particular in dogs. The 16S rDNA sequence data allowed us to analyze the genetic distance in the isolated strains and those in the GenBank.

The increasing feasibility of “high-throughput” sequencing suggests that it is promising as a rapid procedure to differentiate a number of pathogens in a biological sample.

This work is proposed as the continuation of a surveillance program, started in 2012, on 100 samples taken from dogs, only 2 of them were detected as resistant to methicillin.

In this work, 20 of 65 isolates were identified as *S. pseudintermedius*, 13 of which were MRSP. Thirty-one strains showed resistance to methicillin. The validated approach could be employed on a large scale for epidemiological studies in regions where antibiotic resistances are very diffused.

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