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GENOTYPIC DIFFERENTIATION OF *S. INTERMEDIUS* AND *S. PSEUDINTERMEDIUS* STRAINS ISOLATED FROM DOGS AND CATS

DIFERENȚIEREA GENOTIPICĂ A UNOR TULPINI DE *S. INTERMEDIUS* ȘI *S. PSEUDINTERMEDIUS* IZOLATE DE LA CÂINI ȘI PISICI

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ABSTRACT | REZUMAT

Coagulase-positive and coagulase-negative staphylococcal infections are common in both dogs and cats. Thus, staphylococci strains included in *S. pseudintermedius* species are frequently isolated from these species, a bacterium with zoonotic potential, which is part of the resident bacterial microflora of dogs and cats, but produces various localized infections.

Twenty-five staphylococci strains isolated from dogs and cats (22 strains from dogs and 3 strains from cats), that based on phenotypic characters were included in the *S. intermedius* species, were subjected to the PCR technique for differentiation between these two species. Considering the fact that *S. pseudintermedius* is dominant in the last years and that these two species cannot be phenotypically distinguished, in this research the differentiation between it was made by the PCR technique.

Using the PCR technique with two specific primers, nine staphylococci strains (36%) were included in *S. intermedius* species, six strains isolated from dogs and three strains isolated from cats, while 16 staphylococci strains (64%) were included in *S. pseudintermedius* species, all of them isolated only from dogs.

Keywords: *S. intermedius, S. pseudintermedius,* staphylococci strains, PCR

Infecțiile cu stafilococi coagulazo-pozitivi și coagulazo-negativi sunt des întâlnite, atât la câini, cât și la pisici. Astfel, de la aceste specii, se izolează, în mod frecvent, tulpini de stafilococi ce aparțin lui *S. pseudintermedius*, o bacterie cu potențial zoonotic, ce face parte din microflora bacteriană rezidentă a câinilor și pisicilor, dar care poate produce și diferite infecții localizate. Au fost supuse tehnicii PCR, pentru diferențiere între speciile *S. intermedius* și *S. pseudintermedius*, un număr de 25 de tulpini izolate de la câini și pisici (22 tulpini de la câini și 3 tulpini de pisici) care, pe baza caracterelor fenotipice, au fost încadrate în specia *S. intermedius.* Având în vedere faptul că, în ultimii ani, este dominantă specia *S. pseudintermedius* și că aceste două specii nu pot fi diferențiate fenotipic, în cadrul acestor cercetări, diferențierea a fost făcută prin tehnica PCR.

Cu ajutorul acestei tehnici și prin utilizarea a doi primeri specifici, nouă tulpini, respectiv 36%, au fost incluse în specia *S. intermedius*, șase tulpini fiind izolate de la câini și trei tulpini de la pisici, iar 16 tulpini, respectiv 64%, au fost incluse în specia *S. pseudintermedius*, toate fiind izolate numai de la câini.

Cuvinte cheie: *S. intermedius, S. pseudintermedius,* tulpini stafilococcice, PCR

Staphylococci are pathogenic bacteria, conditionally pathogenic and opportunistic, depending on the species and the presence of some favourable factors. They have a marked tropism for the skin and mucous membranes and produce localized suppurative infections, septicaemia and infectious entities, well defined, evolving both in animals and humans (6, 11, 13, 21, 27, 28). Both dogs and cats often develop coagulase-positive and coagulase-negative staphylococcal infections. Thus, staphylococci strains included in *S. pseudintermedius* species are frequently isolated from these species, a bacterium with zoonotic potential, which is part of the resident bacterial microflora of dogs and cats, but produces various localized infections (otitis, dermatitis, adenitis) (5, 16, 17, 18, 21, 24).

In recent years, several molecular biology techniques have been used for the definitive identification of staphylococcal strains, providing much safer results than phenotypic tests which give inconclusive results to related species. Thus, three species, namely *S. intermedius*, *S. pseudintermedius* and *S. delphini* constitute the *Staphylococcus Intermedius Group* (SIG), among which the most important in terms of pathogenicity and frequency is *S. pseudintermedius*. These species cannot be phenotypically differentiated, which is why the definitive differentiation is given only by the PCR technique (1, 2, 3, 8, 19, 20, 23).

The aim of this research was to characterize phenotypically and genotypically differentiate strains of *S.*

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pseudintermedius and *S. intermedius*, isolated from dogs and cats.

MATERIALS AND METHODS

Fourty samples of pathological material were taken from healthy dogs and cats or with various localized infections, community or pets and were examined bacteriologically. To obtain the primary cultures, the inseminations were made in peptone water and subsequently, the cultures were introduced to the thermostat, for 18-20 hours at a temperature of 37°C. Gram-stained smears were made from these cultures and after that inseminations on Chapman medium, used to isolate the staphylococcal strains. The isolates were then sorted based on cultural, morphological and tinctorial characters.

The definitive identification of the staphylococcal strains was made by biochemical tests and the isolates were included in *S. intermedius /S. pseudintermedius* species, based on the glucidolytic properties of the strains. Considering that, in recent years, *S. pseudintermedius* species has been dominant and that these two species (*S. intermedius, S. pseudintermedius*) cannot be phenotypically differentiated, in this research, the discrimination between these species was made with PCR technique. Thus, the strains isolated from dogs and cats, which based on phenotypic characteristics were included in *S. intermedius/S. pseudintermedius* species, were subjected to PCR technique.

The staphylococci strains were inseminated in Petri dishes with nutrient agar and incubated at 37°C for 18-20 hours to obtain isolated colonies. A colony was taken from each strain and inseminated in broth tubes required for DNA extraction, which included the following steps: enzymatic and chemical lysis, purification, elution of the DNA, and storage at -20°C to use.

Amplification of gene fragments was performed using 2 primers. The thermal profile was formed from 2 minutes at 95°C for the initial denaturation of the DNA, followed by 35 amplification cycles with the final extension of 7 minutes at 72°C and storage of the amplicons obtained with the size of 320 bp, at 4°C. The amplification products were subjected to a specific enzymatic digestion according to the reaction kit used, after which their electrophoresis was performed and finally the results were interpreted.

RESULTS AND DISCUSSIONS

Following the bacteriological and bacterioscopic examinations and based on the main phenotypic characters tested, 40 staphylococci strains were identified. The definitive identification of the isolates was performed by examining the carbohydrolytic properties and the results were the following: 7 - *S. aureus subsp. aureus* strains, 4 - *S. epidermidis* strains, 2 - *S. felis* strains,25-*S. intermedius/S.pseudintermedius* strains, 2 - *S. sciuri* strains and 2 - *S. xylosus* strains.

Since the phenotypic identification based on biochemical characters, does not allow the differentiation of *S. intermedius* and *S. pseudintermedius* species, the name *S. intermedius/S. pseudintermedius* was used.

Due to this aspect, Schissler Jennifer Ruth in 2009, proposes the generic name of *S. intermedius* group (SIG), which includes 3 species: *S. intermedius, S. pseudintermedius* and *S. delphini*, name accepted also by other authors (8, 20, 22).

Given that in our research, biochemical tests based on the fermentation of monoglycerides and polyalcohol, used to differentiate staphylococci strains, did not allow the classification of the isolated strains in the two species, a number of 25 strains isolated from dogs and cats, were subjected to the PCR test for their classification in the two species.

The results obtained on the differentiation of these two species of staphylococci included in the SIG group are shown in Table 1. For genotypic differentiation, 25 staphylococci strains were tested, of which 22 strains were isolated from dogs and 3 were isolated from cats.

With this molecular biology technique and by using two specific primers, 9 strains (36%), 6 strains isolated from dogs and 3 strains isolated from cats were included in *S. intermedius* species, while 16 strains (64%) isolated only from dogs, were included in *S. pseudintermedius* species.

The results obtained regarding the differentiation of these two species, confirm the fact that, in dogs and cats, the species *S. pseudintermedius* is dominant.

These results are similar to existing data in the literature, which show that strains included in this opportunistic species are frequently isolated, which, under favourable conditions, causes various localized infections in dogs and cats (4, 11, 18, 24, 25, 26).

In Romania, there are relatively few data on the prevalence of *S. pseudintermedius* species in dogs and cats, but in the literature, after Devriese et al. (2005) proposed this new species of staphylococci, there is a lot of data on the prevalence of this species, the virulence factors it carries and the resistance to antibiotics (7, 9, 10, 17, 18).

Existing data in the literature show that phenotypic differentiation of these three species included in SIG group, is almost impossible, which is why, over time, genetic studies have been performed that have shown notable differences between the three species, based mainly on nucleotide substitutions that occur in the form of point mutations (19, 20, 23).

Several research teams, based on Devriese's work, have used various molecular biology techniques such as: sequencing, phylogenetic analysis, PCR technique, RFLP technique, pulsatile field electrophoresis and

DNA-DNA hybridization, in order to differentiate these three species. Depending on their complexity, these molecular biology techniques can be used as methods in routine diagnosis or in more complex researches (1, 2, 3, 10, 23).

Table 1

Results on genotypic differentiation of *S. intermedius*/*S. pseudintermedius* strains

Crt. no.	Strain origin	*S. intermedius*	*S. pseudintermedius*
1	dog	-	+
2	dog	-	+
3	dog	-	+
4	dog	-	+
5	dog	-	+
6	dog	-	+
7	dog	-	+
8	dog	+	-
9	dog	+	-
10	dog	-	+
11	dog	-	+
12	dog	-	+
13	dog	-	+
14	dog	-	+
15	cat	+	-
16	dog	-	+
17	dog	+	-
18	dog	+	-
19	cat	+	-
20	dog	-	+
21	dog	-	+
22	dog	-	+
23	dog	+	-
24	dog	+	-
25	cat	+	-
TOTAL:		9	16

Chrobak et al. (2013) based on the results of their own research and the existing data in the literature, recommend, for the discrimination of these two species, the DNA-DNA hybridization as a routine method in bacteriological diagnosis (8).

Given the fact that *S. pseudintermedius* has been isolated more frequently from dogs and cats, several authors followed the prevalence of this species, using various molecular biology techniques. Thus, Nam et al. (2011) established that this species had a prevalence of 90.3% at the investigated dogs, Paul et al. (2012) demonstrated that *S. pseudintermedius* isolated from dogs had a prevalence of 69%, the authors concluding that *S. pseudintermedius* is a commensal bacterium, adapted to dogs, from where it is easily transmitted to either humans or other animal species. Similar results were obtained by Guardabassi et al. (2012) who based on the results, considered that mustelids are a reservoir for the SIG group and canids (foxes) are a reservoir for *S. pseudintermedius* (12, 14, 15).

In 2014, Vasilescu et al. (2014), in Romania, optimized a molecular biology technique PCR-RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) and subjected to genotypic discrimination a number of 82 strains isolated from dogs. Thus, 79 strains (96.34%) were included in *S. pseudintermedius* species and 3 strains were included in *S. intermedius* species (23). The results obtained in this research, corroborated with the results communicated by the mentioned research teams, highlight the importance of molecular biology tests used to discriminate these two species, as well as the higher frequency of *S. pseudintermedius* species.

CONCLUSIONS

Using this PCR technique, nine strains (36%) were included in *S. intermedius* species, while sixteen strains (64%) were included in *S. pseudintermedius* species. The results confirm the higher frequency of *S. pseudintermedius* species in both dogs and cats. This molecular biology technique can be taken and used in the laboratory diagnosis of staphylococcal infections in dogs and cats for the discrimination of these two staphylococci species.

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