Molecular Diagnostic Identification of *Staphylococcus pseudintermedius* \(^7\)

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We report the first diagnostic test for the identification of *Staphylococcus pseudintermedius* involving a simple PCR-restriction fragment length polymorphism approach. The method allows discrimination of *S. pseudintermedius* from the closely related members of the *Staphylococcus intermedius* group and other important staphylococcal pathogens of humans and dogs.

Until recently, *Staphylococcus intermedius* was considered responsible for most cases of canine pyoderma, a major skin disease of dogs (8). However, using a multilocus sequencing approach, independent research groups have demonstrated that isolates phenotypically identified as *Staphylococcus intermedius* consist of three distinct species, including *S. intermedius*, *Staphylococcus pseudintermedius*, and *Staphylococcus delphini*, which together represent the *S. intermedius* group (SIG) (1a, 4). Importantly, it was discovered that *S. pseudintermedius*, not *S. intermedius*, is the common canine pyoderma pathogen and that *S. delphini*, isolated from a variety of different animals, may be more clinically important than was previously thought (1a, 4). The recently identified *S. pseudintermedius* (5) is occasionally isolated from serious human infections, and the emergence and spread of methicillin-resistant *S. pseudintermedius* strains are major veterinary and public health issues (1a, 3, 4, 7, 11–13). Sasaki et al. could biochemically differentiate *S. intermedius* from the other SIG species but was unable to identify phenotypic markers to discriminate *S. pseudintermedius*, the most clinically relevant species, from *S. delphini* (12), and DNA sequencing is currently required to identify *S. pseudintermedius* (1a, 12). In order to address the need for a method of discriminating clinical isolates of *S. pseudintermedius*, we have developed a rapid, simple, and robust PCR-restriction fragment length polymorphism (RFLP) approach which has been validated independently in laboratories in separate countries.

Our previous population genetic study of SIG isolates examined sequence diversity at five gene loci among 104 isolates (1a). In the current study, sequence analysis of one of the loci, *pta*, which encodes the enzyme phosphoacetyltransferase, revealed the presence of an *MboI* restriction site in all *S. pseudintermedius* isolates examined. Based on this discovery we have designed a simple, robust, and inexpensive PCR-RFLP diagnostic test for the identification of *S. pseudintermedius*. Staphylococcal genomic DNA isolation was carried out as previously described (1a). PCR amplification of a 320-bp fragment of the *pta* gene was carried out in a 50-μl volume with a 0.2 μM concentration of each oligonucleotide primer (pta_f1, AAA GAC AAA CTT TCA GGT AA, and pta_r1, GCA TAA ACA GTC ATT GTA CCG), a 0.2 mM concentration of the de-
oxynucleoside triphosphates, 1.5 mM MgCl$_2$, 0.5 U Taq DNA polymerase, and 50 ng DNA template, in a 1× reaction buffer. Thermocycling conditions included a 95°C incubation for 2 min followed by 30 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 1 min, with a final incubation of 72°C for 7 min. Twenty-five-microliter samples of the PCR mixtures were incubated with 5 U of MboI and 5 µl of 5× digestion buffer for 2 h, and the digestion products were resolved in 2% (wt/vol) agarose by electrophoresis. We investigated the efficacy of the technique by the analysis of well-characterized isolates of S. pseudintermedius, S. intermedius, and S. delphini that represented the breadth of diversity identified within the SIG and that included the type strains (1). In addition, representative isolates of Staphylococcus aureus, Staphylococcus schleiferi subsp. coagulans, and Staphylococcus schleiferi subsp. schleiferi, which are associated with human and canine infections, were included (Table 1). A pta PCR product of 320 bp was successfully amplified from all strains examined. S. pseudintermedius PCR products all contained a single MboI site, resulting in two restriction fragments of 213 bp and 107 bp, respectively (Fig. 1). In contrast, SIG species S. delphini and S. intermedius and the S. schleiferi strains did not contain an MboI restriction site (Fig. 1). S. aureus isolates contained a unique MboI site, resulting in restriction fragments of 156 bp and 164 bp that appeared as a single band after agarose electrophoresis; this band was readily distinguishable from the S. pseudintermedius restriction fragment profile (Fig. 1). Our data indicate that the PCR-RFLP assay is an effective approach to S. pseudintermedius identification, allowing discrimination from the other SIG species and important staphylococcal pathogens of dogs (6, 9).

In order to test the reproducibility of the method, independent investigators applied the approach in a diagnostic laboratory in Italy to a panel of 112 coagulase-positive staphylococcal field isolates which had been isolated from dogs, cats, cows, horses, wild foxes, and a captive bear and identified as either SIG or S. aureus (2). Of the 86 isolates from dogs, 85 were identified as S. pseudintermedius by the PCR-RFLP approach, consistent with our previous findings that S. pseudintermedius is a common commensal of dogs and the major canine pyoderma pathogen. The remaining isolate resulted in a pta restriction profile which indicated an S. aureus identity. All but 1 of the 14 isolates from cats, all 3 isolates from wild foxes, and the single isolate from a captive bear were identified as S. pseudintermedius. The remaining isolate of feline origin had an S. aureus-specific restriction profile. A previous study reported the isolation of S. intermedius from different species of the canidea, including bears and foxes (1). Our data suggest that S. pseudintermedius may be the most common SIG species associated with these animals. All five isolates from equid genital swabs did not contain a pta MboI site, consistent with our previous findings that S. delphini is the SIG species commonly identified among horses, and three isolates from equine respiratory tracts and a skin lesion were identified as S. aureus. Finally, of the seven isolates from cows, two were S. pseudintermedius and five were identified as S. aureus. To our knowledge, this represents the first reported identification of S. pseudintermedius in association with cows.

Until now, the lack of unique phenotypic markers for S. pseudintermedius in comparison to the other SIG members has precluded its identification without DNA sequencing. Importantly, due to the presence of common phenotypic markers, S. pseudintermedius is occasionally misidentified as S. aureus in human clinical diagnostic laboratories (10). The simple PCR-RFLP approach presented here represents the first reported diagnostic method which is effective for the identification of S. pseudintermedius and for its discrimination from other SIG species and several other important staphylococcal pathogens. As such, the method should prove extremely useful in routine veterinary diagnostic and clinical microbiology laboratories.

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REFERENCES

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