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Molecular Diagnostic Identification of *Staphylococcus pseudintermedius*▽

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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, which was absent in all *S. intermedius* and *S. delphini* 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 AGC ATT GTA CCG), a 0.2 mM concentration of the de-

TABLE 1. Staphylococcal isolates previously identified to the species level by a DNA sequencing approach[a]

Strain	Species	Host origin	MboI restriction site[b]
ED99	*S. pseudintermedius*	Dog	+
AV8001	*S. pseudintermedius*	Dog	+
95072195	*S. pseudintermedius*	Dog	+
94062394	*S. pseudintermedius*	Dog	+
N900260	*S. pseudintermedius*	Human	+
HT20030686	*S. pseudintermedius*	Dog	+
M72199	*S. pseudintermedius*	Dog	+
3279 (MRSP)	*S. pseudintermedius*	Dog	+
3414	*S. pseudintermedius*	Cat	+
9075	*S. pseudintermedius*	Dog	+
690	*S. pseudintermedius*	Cat	+
8478	*S. pseudintermedius*	Dog	+
HH1	*S. pseudintermedius*	Dog	+
Can6	*S. pseudintermedius*	Dog	+
Can10	*S. pseudintermedius*	Human	+
BH47	*S. pseudintermedius*	Dog	+
AV8024	*S. pseudintermedius*	Dog	+
8016	*S. pseudintermedius*	Dog	+
LMG22219	*S. pseudintermedius*	Cat	+
LMG22220	*S. pseudintermedius*	Horse	+
ATCC 49171	*S. delphini*	Dolphin	−
HT20030680	*S. delphini*	Camel	−
9106	*S. delphini*	Horse	−
8485	*S. delphini*	Horse	−
AV8051	*S. delphini*	Pigeon	−
HT20030677	*S. delphini*	Camel	−
AV8047	*S. delphini*	Pigeon	−
HT20030676	*S. delphini*	Camel	−
HT20030674	*S. delphini*	Camel	−
8086	*S. delphini*	Horse	−
HT20030679	*S. delphini*	Camel	−
AV8061	*S. intermedius*	Pigeon	−
NCTC11048	*S. intermedius*	Pigeon	−
AV8063	*S. intermedius*	Pigeon	−
AV8063	*S. intermedius*	Pigeon	−
ATCC 43808	*S. schleiferi* subsp. *schleiferi*	Human	−
CCUG37248	*S. schleiferi* subsp. *coagulans*	Dog	−
Newman	*S. aureus*	Human	+
N315	*S. aureus*	Human	+

[a] The isolates were previously identified by Bannoehr et al. (1). +, present; −, absent.
[b] *S. pseudintermedius pta* MboI restriction fragments were 107 bp and 213 bp, and *S. aureus pta* MboI restriction fragments were 156 bp and 164 bp.

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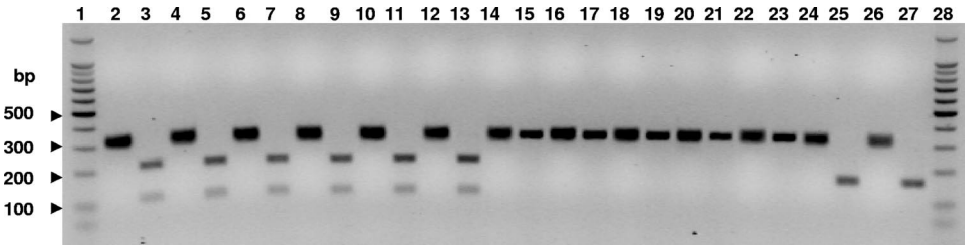

FIG. 1. Agarose gel electrophoresis of MboI restriction digest *pta* PCR products. Lane 1, 100-bp ladder; lane 2, *S. pseudintermedius* ED99; lane 3, *S. pseudintermedius* ED99, MboI digested; lane 4, *S. pseudintermedius* HH1; lane 5, *S. pseudintermedius* HH1, MboI digested; lane 6, *S. pseudintermedius* Can6; lane 7, *S. pseudintermedius* Can6, MboI digested; lane 8, *S. pseudintermedius* Can10; lane 9, *S. pseudintermedius* Can10, MboI digested; lane 10, *S. pseudintermedius* LMG22219; lane 11, *S. pseudintermedius* LMG22219, MboI digested; lane 12, *S. pseudintermedius* 3414; lane 13, *S. pseudintermedius* 3414, MboI digested; lane 14, *S. delphini* ATCC 49171; lane 15, *S. delphini* ATCC 49171, MboI digested; lane 16, *S. delphini* 9106; lane 17, *S. delphini* 9106, MboI digested; lane 18, *S. delphini* HT20030680; lane 19, *S. delphini* HT20030680, MboI digested; lane 20, *S. intermedius* NCTC11048; lane 21, *S. intermedius* NCTC11048, MboI digested; lane 22, *S. intermedius* AV8061; lane 23, *S. intermedius* AV8061, MboI digested; lane 24, *S. aureus* Newman; lane 25, *S. aureus* Newman, MboI digested; lane 26, *S. aureus* N315; lane 27, *S. aureus* N315, MboI digested; lane 28, 100-bp ladder.

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 canoidea, 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

1. **Aarestrup, F. M.** 2001. Comparative ribotyping of *Staphylococcus intermedius* isolated from members of the Canoidea gives possible evidence for host-specificity and co-evolution of bacteria and hosts. Int. J. Syst. Evol. Microbiol. **51:**1343–1347.
1a. **Bannoehr, J., N. L. Ben Zakour, A. S. Waller, L. Guardabassi, K. L. Thoday, A. H. van den Broek, and J. R. Fitzgerald.** 2007. Population genetic structure of the *Staphylococcus intermedius* group: insights into *agr* diversification and the emergence of methicillin-resistant strains. J. Bacteriol. **189:**8685–8692.
2. **Baron, F., M. F. Cochet, J. L. Pellerin, N. Ben Zakour, A. Lebon, A. Navarro, I. Proudy, Y. Le Loir, and M. Gautier.** 2004. Development of a PCR test to differentiate between Staphylococcus aureus and Staphylococcus intermedius. J. Food Prot. **67:**2302–2305.
3. **Descloux, S., A. Rossano, and V. Perreten.** 2008. Characterization of new staphylococcal cassette chromosome mec (SCCmec) and topoisomerase

genes in fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius*. J. Clin. Microbiol. **46:**1818–1823.
4. **Devriese, L. A., K. Hermans, M. Baele, and F. Haesebrouck.** 2008. Staphylococcus pseudintermedius versus Staphylococcus intermedius. Vet. Microbiol. **133:**206–207.
5. **Devriese, L. A., M. Vancanneyt, M. Baele, M. Vaneechoutte, E. De Graef, C. Snauwaert, I. Cleenwerck, P. Dawyndt, J. Swings, A. Decostere, and F. Haesebrouck.** 2005. *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int. J. Syst. Evol. Microbiol. **55:**1569–1573.
6. **Frank, L. A., S. A. Kania, K. A. Hnilica, R. P. Wilkes, and D. A. Bemis.** 2003. Isolation of Staphylococcus schleiferi from dogs with pyoderma. J. Am. Vet. Med. Assoc. **222:**451–454.
7. **Guardabassi, L., S. Schwarz, and D. H. Lloyd.** 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. J. Antimicrob. Chemother. **54:**321–332.
8. **Hajek, V.** 1976. *Staphylococcus intermedius*, a new species isolated from animals. Int. J. Syst. Bacteriol. **26:**401–408.
9. **Morgan, M.** 26 September 2008, posting date. Methicillin-resistant Staphylococcus aureus and animals: zoonosis or humanosis? J. Antimicrob. Chemother. [Epub ahead of print.]
10. **Pottumarthy, S., J. M. Schapiro, J. L. Prentice, Y. B. Houze, S. R. Swanzy, F. C. Fang, and B. T. Cookson.** 2004. Clinical isolates of *Staphylococcus intermedius* masquerading as methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **42:**5881–5884.
11. **Sasaki, T., K. Kikuchi, Y. Tanaka, N. Takahashi, S. Kamata, and K. Hiramatsu.** 2007. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J. Clin. Microbiol. **45:**1118–1125.
12. **Sasaki, T., K. Kikuchi, Y. Tanaka, N. Takahashi, S. Kamata, and K. Hiramatsu.** 2007. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J. Clin. Microbiol. **45:**2770–2778.
13. **Van Hoovels, L., A. Vankeerberghen, A. Boel, K. Van Vaerenbergh, and H. De Beenhouwer.** 2006. First case of *Staphylococcus pseudintermedius* infection in a human. J. Clin. Microbiol. **44:**4609–4612.