Are *Staphylococcus intermedius* Infections in Humans Cases of Mistaken Identity? A Case Series and Literature Review

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***Staphylococcus intermedius* and *Staphylococcus pseudintermedius* are difficult to distinguish using conventional microbiological methods. Molecular diagnostic tools change our understanding of the epidemiology of these 2 organisms. In this study, we present (1) a detailed review of the current literature on molecular diagnostics and (2) a case series in which misidentification was proven in 1 case. We conclude that *S pseudintermedius* is a more common human pathogen than previously recognized.**

Keywords. molecular diagnostics; skin and soft tissue infections; *Staphylococcus intermedius*; *Staphylococcus pseudintermedius*; zoonotic infection.

Staphylococcus intermedius and *Staphylococcus pseudintermedius* are zoonotic pathogens found in a variety of wild and domestic animals [1]. Both organisms occasionally cause disease in humans, sometimes due to transmission by dogs or other household pets [1, 2]. Based upon their production of coagulase, DNases, and similar growth characteristics, initial classification methods grouped *S intermedius*, along with other coagulase-positive staphylococci, together with *Staphylococcus aureus* [3]. In 1976, differences in cell wall composition and guanine-cytosine content permitted *S intermedius* to be separated from *S aureus* [4]. By 2005, molecular diagnostics enabled more precise characterizations, dividing bacteria previously classified as *S intermedius* into 3 species: *S intermedius*, *S pseudintermedius*, and *Staphylococcus delphini* [4]. In this study, we present a case report in which molecular diagnostics determined that a clinical isolate initially identified at our institution as *S intermedius* was actually *S pseudintermedius.*

We conducted a retrospective review of 9 other cases from our institution and reviewed all prior literature, using the following search terms to query PubMed: "*Staphylococcus intermedius*" and "*Staphylococcus pseudintermedius*". We included all 10 cases at our institution and all cases in the reviews that described humans with infections caused by these organisms. Species identification using molecular diagnostic studies suggests a change in the current understanding of the epidemiology of *S intermedius* and *S pseudintermedius*. In this review, following the convention of Sasaki et al [5], we consider bacteria previously identified as *S intermedius* to be part of the *S intermedius* group (SIG) unless molecular methods were used to confirm the isolate's species.

CASE (INCLUDING MOLECULAR DIAGNOSTIC STUDIES)

Case 1

A 78-year-old man with severe peripheral neuropathy due to diabetes and a prior burn injury to his lower extremities presented to the podiatry clinic with new onset of blood-filled blisters on his left foot for 2 days. Plain radiographs showed a fracture of the proximal phalanx of the hallux. After 1 month of immobilization and topical treatment, he presented with cellulitis, underwent debridement, and was started on an empiric course of oral doxycycline. Superficial cultures grew *S aureus* (susceptible to methicillin and doxycycline), *Enterococcus faecalis* (susceptible to ampicillin and vancomycin), and *Proteus vulgaris* (susceptible to ciprofloxacin, ceftriaxone, and trimethoprim/sulfamethoxazole).

Despite 1 month of antibiotic therapy, his wound progressed. Imaging studies raised concerns for osteomyelitis, and the patient underwent surgical debridement. Histopathological examination of bone biopsies confirmed osteomyelitis. Cultures from a bone biopsy grew oxacillin-susceptible SIG and *Enterococcus faecalis*, for which the patient received 6 weeks of vancomycin. His affected foot healed well. The patient did not report any recent contact with dogs and was not a pet-owner.

Methods and Results

The isolate was subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS)

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system (Vitek MS Plus, bioMerieux, Durham, NC). We extracted DNA (UltraClean Microbial DNA Isolation Kit, MO BIO, Carlsbad, CA) and amplified a fragment of the catalase gene (*kat a*) using known primers [6]. Amplicons were sequenced at a commercial facility (MCLAB, South San Francisco, CA). Amplicons were then assembled and compared with sequences deposited into GenBank.

The SIG isolated was confirmed as *S pseudintermedius* by MALDI-TOF MS with 99% confidence. Sequencing of the *kat* gene showed a 99% similarity to *S pseudintermedius* ED99 (GenBank Accession ID: CP002478.1).

Cases 2–10

For the remaining cases, identified from a microbiology database at our institution (2005–2014), bacterial isolates were unavailable. Accordingly, we attribute all of these infections to SIG (as reported in Table 1).

Case 2

A 74-year-old man with congestive heart failure presented to the dermatology clinic with a lesion on his left hand consistent with a squamous cell carcinoma in situ. After 6 weeks of topical fluorouracil, he developed pain and a clear exudate at the same site. He stopped using the fluorouracil and continued to have symptoms for the next month. A wound culture grew oxacillin-susceptible SIG for which he was started on topical silver sulfadiazine. He was lost to follow up.

Case 3

A 77-year-old man with diabetes, liver cirrhosis, and heart failure who had undergone a revision of his pacemaker 2 years prior presented with a 2-week history of purulent drainage from the pacemaker insertion site. Cultures grew oxacillin-susceptible SIG. After removal of his pacemaker, he received antibiotics for 14 days, had a new pacemaker placed, and recovered uneventfully.

Case 4

A 59-year-old man with poorly controlled diabetes and a chronic foot ulcer presented with a 2-day history of cellulitis and fever. Plain radiographs showed soft tissue swelling and bone deformities of the fourth and fifth metatarsals. On admission, his white blood cell count was 21×10^3 cells/mm^3; blood cultures grew coagulase-positive *Staphylococcus* that was eventually identified as oxacillin-susceptible SIG. After surgical debridement of his foot ulcer, he received a 4-week course of ceftriaxone. His foot ulcer resolved. The patient reported having 2 healthy dogs.

Cases 5–10

Cased 5–10 all involved diabetic men, aged 45–79, with new onset foot infections. None were bacteremic. Osteomyelitis afflicted 4 of the patients; 3 of them required toe amputation followed by 10 days of antibiotics (cefazolin or amoxicillin-clavulanic acid), whereas 1 received debridement followed by 6 weeks of intravenous vancomycin. The 5th patient had a heel eschar which improved after 1 month of clindamycin. The 6th patient underwent multiple bedside debridements for 6 months before the ulcer healed. He also used topical bacitracin. Three of the patients grew oxacillin-susceptible SIG alone, whereas 3 had SIG as part of a polymicrobial infection.

Table 1. Summary of *Staphylococcus intermedius* Group Cases Reported in the Literature and in This Report

			Identification Method			
			Biochemical Methods[c]	Molecular Methods[d]		
Clinical Syndrome[a]	Cases Reported	Animal Exposure[b]	SIG	SI	PSIM	References[e]
Skin and soft tissue	22	9 (3 animal bites)	13	4	5	This report and [1, 13, 20]
Bacteremia without endocarditis	3	3	3	0	0	This report and [1, 7]
Bone/joint infection	4	2	3	0	1	This report and [1]
Endocarditis	3	1	1	0	2	[1, 8]
Central nervous system	2	0	1	1	0	[1, 15]
Sinusitis/mastoiditis	2	2	2	0	0	[1]
Pneumonia	3	1	1	0	2	[1, 14]
Device related	2	1	1	0	1	This report and [1]

Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; SIG, *Staphylococcus intermedius* group; SI, *Staphylococcus intermedius*; PSIM, *Staphylococcus pseudintermedius*.

[a] Most severe clinical syndrome reported (ie, patient with skin infection and bacteremia, reported as bacteremia and not skin and soft tissue).

[b] History of animal exposure was not available in all reports.

[c] Includes automated and manual systems.

[d] Includes gene sequencing (rpoB, hsp60, 16s DNA) and MALDI-TOF.

[e] Multiple cases per reference.

DISCUSSION

Current clinical laboratory methods do not readily permit differentiating between infections caused by *S intermedius* and *S pseudintermedius*. A positive β-galactosidase test can distinguish SIG isolates from *S aureus* [3]. However, not all laboratories perform this test routinely, which may lead to SIG isolates being incorrectly classified as *S aureus* [1, 7]. Although their interpretation is not reliable, arginine dehydrolase and mannitol fermentation may further differentiate *S intermedius* from *S pseudintermedius* [8]. Thus, in the absence of molecular diagnostics, SIG isolates may simply be identified as *S intermedius*.

In contrast, molecular-based methods permit definitive identification of *S intermedius* and *S pseudintermedius*. In 2006, Van Hoovels et al [9] reported the first case of human infection cause by *S pseudintermedius*, describing bacteremia with endocarditis. Multiplex polymerase chain reaction (PCR) and 16S rRNA gene sequence analysis was used to confirm the pathogen's identity. Molecular identification techniques used in subsequent case reports uncovered important epidemiologic differences between infections caused by *S intermedius* and *S pseudintermedius* (Table 1).

Some *S pseudintermedius* isolates have acquired *mecA*, the same gene that encodes methicillin-resistance in *S aureus* [10]. Cefoxitin is often used to detect methicillin-resistance because it induces expression of the *mecA* gene more efficiently than oxacillin or methicillin. For *S pseudintermedius*, however, testing with cefoxitin may be inadequate because its *mecA* gene is less inducible than that of *S aureus* [11]. Although antibiotic failures have not been reported in humans, Perreten et al [12] reported an increased incidence of human/pets pairs colonized or infected with methicillin-resistant *S pseudintermedius* in Europe and North America. In addition, Savini et al [13] described a bone marrow transplant patient with a wound infection due to cefoxitin-susceptible *S pseudintermedius* determined to be *mecA* positive upon molecular testing. The patient was successfully treated with topical gentamicin.

Before molecular identification, approximately half of reported SIG cases were skin and soft tissue infections; the remainder included endocarditis, osteomyelitis, and pneumonia [1, 8, 14]. More recent reports use 16S rRNA gene sequence analysis, multilocus sequence typing, ribotyping, and multiplex PCR to definitively identify *S intermedius* in 4 cases of skin and soft tissue infection and 1 case of meningitis [1, 13, 15]. Reports using similar techniques reveal that *S pseudintermedius*, although still predominantly a pathogen of skin and soft tissue (5 cases), also causes invasive disease (6 cases) and, overall, appears to be a more common human pathogen than *S intermedius* [16]. The severity of *S pseudintermedius* infections ranges from superficial skin infections treated by topical therapy to bacteremia, endocarditis, pneumonia, and septic arthritis [1, 8, 14, 15]. Previous work by Talan et al [17] and Laarhoven et al [18] indicates that SIG isolates rarely colonize humans. As demonstrated by Case 2 above and the report by Savini et al [13], even superficial skin infections with SIG isolates may warrant topical therapy.

Initial descriptions characterized SIG isolates as zoonotic pathogens, with several infections after animal bites. In our series, however, none of the patients reported animal-related trauma, and many reports do not comment on animal exposure. All 10 of our patients were diabetics, as were 4 Swedish patients with *S pseudintermedius* infections [2]. This raises the possibility that diabetes mellitus may be a risk factor for SIG infections. All 10 of our patients were veterans; the prevalence of diabetes in veterans is approximately 3-fold greater than that in the general population (~25% vs 8.3% in 2010) so this may represent selection bias [19].

CONCLUSIONS

Given that bacteria from SIG have the potential to cause serious and systemic infections, we recommend that coagulase-positive, Gram-positive cocci not identified as *S aureus* using routine microbiological methods should undergo identification with molecular methods when available. When molecular methods are not feasible, clinical laboratories may consider an approach recently described by Lee et al [16] to detect SIG isolates using routine microbiological methods. Given the predominance of methicillin resistance reported for *S pseudintermedius* strains in North America and Europe [12], combined with the diminished accuracy of cefoxitin testing to detect methicillin resistance in *S pseudintermedius* [11], we recommend that clinical microbiological laboratories consider using molecular methods to detect *mecA* genes in SIG isolates. Furthermore, to improve the understanding of the epidemiology of infections caused by these bacteria, we favor using molecular diagnostics to accurately distinguish *S intermedius* from *S pseudintermedius*.

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