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The Brief Case: *Staphylococcus intermedius* Group—Look What the Dog Dragged In

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CASE

A 46-year-old man with a history of diabetes mellitus (type I), hypertension, congestive heart failure, and coronary artery disease presented to his primary care physician with a 3-week history of ulceration on his left, great toe. The patient reported poor compliance with blood glucose level checks and insulin use. Upon examination, the physician noted a 1- by 1.5-cm ulcer on the underside of the patient's toe. The ulcer was debrided, and a specimen for microbiological culture was collected on a flocked swab. As part of this routine work-up, the physician took a detailed history and noted that the patient lived alone with his two dogs.

A specimen Gram stain revealed no polymorphonuclear leukocytes but abundant Gram-positive cocci. The specimen was plated to sheep blood agar (SBA), chocolate agar, and MacConkey agar. The next day, abundant white beta-hemolytic colonies resembling *Staphylococcus* spp. were noted on the blood agar plate (BAP) and white colonies were noted on the chocolate plate. No other organisms grew in this culture. No growth was observed on the MacConkey agar plate. Upon further testing, the isolate was positive for catalase, coagulase (StaphTEX Blue; Hardy Diagnostics, Santa Maria, CA), and pyrrolidonyl arylamidase (PYR [Oxoid biochemical identification system]; Thermo Scientific, Hampshire, United Kingdom). A rapid immunochromatographic assay for penicillin-binding protein 2a was positive (PBP2a culture colony test; Alere Inc., Waltham, MA). The isolate was identified by matrix-assisted laser desorption–ionization time of flight (MALDI-TOF) mass spectrometry (MS) as *Staphylococcus intermedius*, a member of the *S. intermedius* group (SIG), with a score of 2.022. Susceptibility testing was notable for discrepant results for methicillin resistance testing by Kirby-Bauer disk diffusion: the isolate was oxacillin resistant (zone size of 17 mm) and cefoxitin susceptible (zone size of 35 mm). Otherwise, the isolate exhibited intermediate resistance to trimethoprim-sulfamethoxazole and was susceptible to vancomycin, linezolid, doxycycline, clindamycin, and erythromycin, as per Clinical and Laboratory Standards Institute document M100 (1). The patient was treated empirically with oral doxycycline and topical gentamicin cream and recovered well.

DISCUSSION

Members of the *Staphylococcus intermedius* group (SIG) include *S. intermedius*, *S. pseudintermedius*, and *S. delphini*. SIG members are common colonizers of animal mucosal surfaces, especially those of dogs (2, 3). Though it can be a component of the normal flora of the mouth, nose, and skin, the SIG, particularly *S. pseudintermedius*, has been implicated as a common cause of pyoderma in dogs (4, 5). In studies assessing carriage rates among dogs and cats, *S. pseudintermedius* was found to colonize up to 68% of dogs and up to 22% of cats, whereas *S. aureus* was a colonizer of up to 14% of dogs and 20% of cats (2). Other species of animal-adapted staphylococci include *S.*

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.00840-17 in this issue.

TABLE 1 Biochemical characteristics used to differentiate *Staphylococcus aureus* from animal-adapted, coagulase-positive *Staphylococcus* species[a]

Staphylococcus species	Presence of indicated characteristic						
	Clumping factor	Free coagulase	PYR	Urease	Heat-stable nuclease	D-Mannitol fermentation	Polymyxin B resistance
S. aureus	+	+	−	V	+	+	+
S. intermedius	V	+	+	+	+	V	−
S. pseudintermedius	−	+	+	+	+	+	V
S. delphini	−	+	ND	+	−	+	−
S. schleiferi subsp. *schleiferi*	+	−	+	−	+	−	−
S. schleiferi subsp. *coagulans*	−	+	V	+	+	V	−

[a]Data are adapted from references 4 and 11. +, 90% or more strains were positive; V, results were varied; −, 90% or more strains were negative; ND, not determined.

schleiferi (canine), *S. felis* (feline), *S. lutrae*, *S. hyicus* (porcine, coagulase variable), and *S. agnetis* (bovine, coagulase variable).

Members of SIG were recently separated into individual species, with the most recent taxonomic change occurring in 2005 with the creation of the *S. pseudintermedius* species (4). *Staphylococcus delphini*, originally isolated from a dolphin specimen, is associated with many types of animals, including birds, cats, horses, and other mammals (4). Despite taxonomic differences, the members of this group are difficult to differentiate phenotypically, suggesting that these species may be interchangeably misidentified (4).

In humans, SIG infections are increasingly recognized. Prior to the advent of MALDI-TOF MS for the identification of bacterial isolates from clinical specimens, SIG members were thought to uncommonly infect humans and were generally associated with dog bites (3–5), foreign-body-related infections, food poisoning, and invasive infections in immunocompromised patients (4). In one study of 39 patients with SIG infections, only 4 had a history of contact with a dog, 2 of which had a SIG member isolated from a dog bite (3). Additionally, SIG isolates were recovered from specimens, including wounds, ears, diabetic ulcers, and cutaneous ulcers (3). In other reports, the SIG was implicated in various other infections, including infective endocarditis and hardware/device infections (6). Differentiating infection from colonization when treating patients with a SIG member can be difficult. For example, the primary-specimen Gram stain of this case showed no polymorphonuclear leukocytes but abundant Gram-positive cocci, suggesting colonization with a SIG member since no inflammatory cells were present. However, this patient was treated because, clinically, this looked like an infection and the swab specimen from this open ulcer grew abundant SIG bacteria.

SIG species are very similar to *S. aureus* in both clinical presentation and microbiologic characteristics, including coagulase positivity, and it is likely that SIG isolates from human clinical specimens were identified as *S. aureus* prior to MALDI-TOF MS (6). Historically, coagulase-positive staphylococci isolated from veterinary specimens were identified as SIG, whereas these isolates were identified as *S. aureus* in human specimens (5). Very few systematic studies have been conducted to evaluate the ability of MALDI-TOF MS instruments to identify SIG isolates. In one study using the Vitek MS (bioMérieux, Durham, NC), four SIG isolates were tested, resulting in three isolates of *S. intermedius* identified to the genus level and one isolate of *S. pseudintermedius* identified to the species level (no MALDI-TOF database was specified in the publication). No *S. delphini* isolates were tested (7). In contrast to that of the Vitek MS, the performance of the Biotyper MALDI-TOF MS in identifying members of the SIG has been more rigorously evaluated. In a study focused on identification of human SIG isolates with the Bruker Biotyper MALDI-TOF MS (database version 3.0), all SIG isolates achieved a score of ≥1.7 (genus-level identification), and 78.9% (15/19) of the *S. delphini* isolates, 76.5% (13/17) of the *S. intermedius* isolates, and 89.5% (145/162) of the *S. pseudintermedius* isolates achieved a score of ≥2.0 (species-level identification) (8).

Like *S. aureus*, SIG species are catalase positive, coagulase positive, Gram-positive cocci in clusters (Table 1). Like *S. aureus*, SIG isolates may grow on mannitol salt agar,

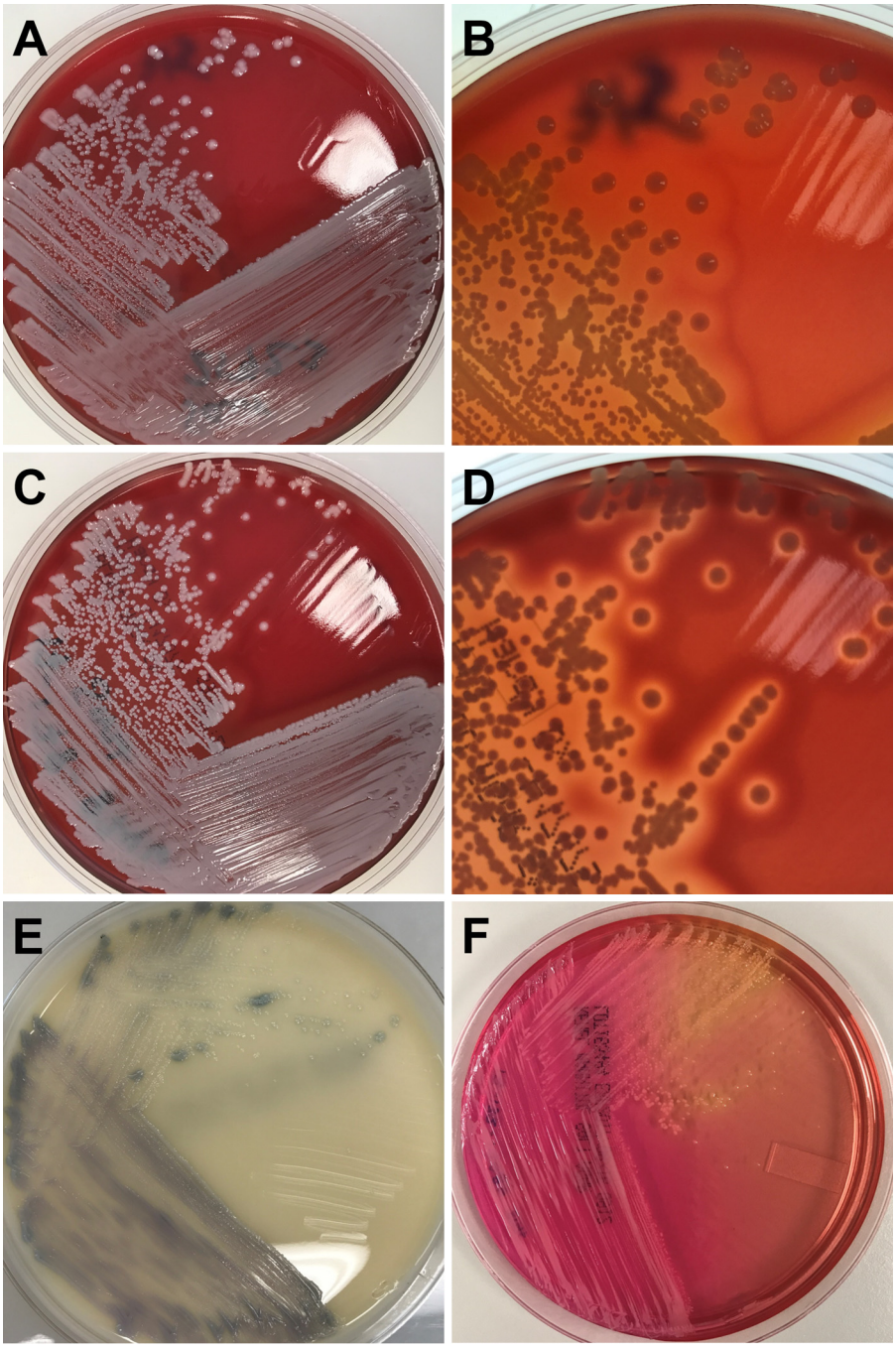

FIG 1 Colony morphologies of *Staphylococcus intermedius* group members and *Staphylococcus aureus* at 24 h of growth. (A) Colony morphology of *S. aureus* on a sheep blood agar plate. (B) Beta-hemolysis caused by *S. aureus*. The photo was taken using backlighting to highlight the hemolysis pattern. (C) Colony morphology of *S. intermedius* group on a sheep blood agar plate. (D) Double zone of beta-hemolysis caused by an *S. intermedius* group member. The photo was taken using backlighting to highlight the hemolysis pattern. (E) Growth of an *S. intermedius* group member on a Spectra MRSA chromogenic agar plate (Remel Microbiology Products, Lenexa, KS). (F) Growth of an *S. intermedius* group member on mannitol salt agar (Remel Microbiology Products).

which is often used in *S. aureus* colonization screening cultures, and many will ferment D-mannitol (Table 1), producing a yellow colony that might be suggestive of *S. aureus* (Fig. 1F) (4). However, colonies of SIG isolates can be whiter than *S. aureus* on SBA plates (Fig. 1A and C). Additionally, SIG isolates are beta-hemolytic, with a double zone of hemolysis (see the clearing and darkening of the agar in Fig. 1D), compared to *S. aureus*,

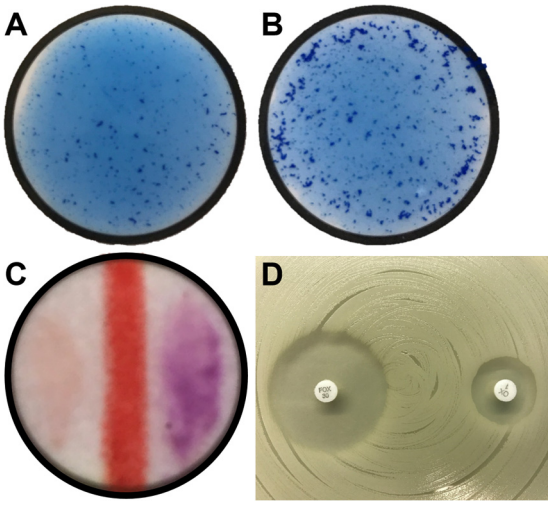

FIG 2 Biochemical characteristics of *Staphylococcus intermedius* group members compared to those of *Staphylococcus aureus*, and susceptibility testing for *S. intermedius* group members. (A) Latex agglutination test (coagulase) result for *S. aureus*; (B) latex agglutination test (coagulase) result for *S. intermedius* group members; (C) PYR testing results for *S. aureus* (left, negative) and *S. intermedius* group members (right, positive); (D) discrepant cefoxitin (FOX 30; 26-mm diameter) and oxacillin (OX 1; 15-mm diameter) results for an *S. intermedius* group isolate.

which has a single zone of beta-hemolysis (Fig. 1B). Despite both being coagulase positive (4), SIG isolates exhibit a slightly different result from *S. aureus* isolates in latex agglutination testing (i.e., see the more chunky and stringy clumping in Fig. 2A and B), and instances of coagulase-positive *S. pseudintermedius* misidentification due to negative latex agglutination tests have been documented (4). This misidentification can happen because current *Staphylococcus* latex agglutination tests employ antibodies to clumping factor and staphylococcal protein A (product of the *spa* gene) (4). Whereas all SIG species are coagulase positive, only *S. intermedius* has been shown to have clumping factor (Table 1). Regardless of similar morphologies and characteristics, SIG members and *S. aureus* can be efficiently differentiated using a variety of biochemical tests (Table 1), including the PYR test (*S. aureus* negative, SIG positive) (Fig. 2C) (4).

Until the early 2000s, very little methicillin resistance (~5% of isolates) was observed in veterinary SIG isolates, but methicillin resistance in SIG members is on the rise (9). As in *S. aureus*, methicillin resistance in SIG species is conferred by alternative penicillin binding proteins (PBPs) through the acquisition of the *mecA* gene, which encodes PBP2a (10). Unlike in *S. aureus* and the coagulase-negative staphylococci, cefoxitin has proven to be a poor surrogate indicator of methicillin resistance in SIG isolates; compared to the presence of *mecA* as determined by PCR, the use of cefoxitin as a surrogate indicator of methicillin resistance resulted in both major and very major errors (5). Rather, oxacillin is the appropriate surrogate for methicillin resistance in SIG isolates (Fig. 2D) (5, 10). Oxacillin susceptibility breakpoints for SIG members have been added to CLSI supplement M100 for this purpose (1). It is recognized that this testing can be problematic for some clinical laboratories, since automated identification and susceptibility systems (such as the BD Phoenix and bioMérieux Vitek 2 systems) may not routinely report an oxacillin MIC result (5) and since some clinical laboratories do not use oxacillin for antimicrobial susceptibility testing. For this reason, immunochromatographic testing for the presence of PBP2a has been studied in SIG isolates and was shown to have 100% sensitivity and 100% specificity for the detection of methicillin resistance in a comparison with *mecA* PCR (10), particularly when induced PBP2a testing with cefoxitin was used (5). Isolates of the SIG can also grow on chromogenic media for the detection of methicillin-resistant *S. aureus* (MRSA), such as Spectra MRSA agar (Remel Microbiology Products, Lenexa, KS). While SIG isolates do not produce the characteristic "denim blue" colony color of MRSA on these plates (Fig. 1E), the colonies may be considered suspicious for MRSA.

Members of the SIG are human pathogens that may be misidentified when we rely on biochemical tests such as coagulase and latex agglutination for identification of catalase-positive, Gram-positive cocci. Accurate identification has important implications for the interpretation of disk diffusion susceptibility testing, including the determination of methicillin resistance. However, identification using MALDI-TOF MS is reliable for identifying these isolates as members of the SIG.

SELF-ASSESSMENT QUESTIONS

1. Which biochemical test can be helpful as a screen to differentiate SIG species from *Staphylococcus aureus*?
   A. Catalase
   B. Pyrrolidonyl arylamidase (PYR)
   C. Coagulase
   D. Ornithine decarboxylase
2. Disk diffusion testing with which antimicrobial agent should be tested as a surrogate for methicillin resistance testing with members of the *Staphylococcus intermedius* group?
   A. Cefazolin
   B. Methicillin
   C. Oxacillin
   D. Cefoxitin
3. Which staphylococcal species is not a member of the *Staphylococcus intermedius* group?
   A. *Staphylococcus pseudintermedius*
   B. *Staphylococcus delphini*
   C. *Staphylococcus lugdunensis*
   D. *Staphylococcus intermedius*

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