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Improved Detection of *Staphylococcus intermedius* Group in a Routine Diagnostic Laboratory

John Lee,[a] Aimee Murray,[b] Richard Bendall,[a] William Gaze,[b] Lihong Zhang,[b] Michiel Vos[b]

Clinical Microbiology, Royal Cornwall Hospital, Truro, Cornwall, United Kingdom[a]; European Centre for Environment and Human Health, University of Exeter Medical School, University of Exeter, Exeter, United Kingdom[b]

The *Staphylococcus intermedius* group (SIG) includes zoonotic pathogens traditionally associated with dog bites. We describe a simple scheme for improved detection of SIG using routine laboratory methods, report its effect on isolation rates, and use sequencing to confirm that, apart from one atypical SIG strain, most isolates are *Staphylococcus pseudintermedius*.

Staphylococcus intermedius sensu lato is a veterinary pathogen occasionally reported from infected-animal (particularly dog) bites in humans (1, 2). Originally thought to be a single species, molecular characterization has resulted in its reclassification as *Staphylococcus intermedius* group (SIG), which includes *S. intermedius*, *S. pseudintermedius*, and *S. delphini* (3, 4). Non-bite-associated human isolates were reported in 1994 (5), but little has been documented since. Our aim was to improve the detection and identification of SIG from human samples using a simple phenotypic algorithm. This initial identification was confirmed by sequencing fragments of two housekeeping genes which were also used to determine diversity within SIG isolates.

Initially, one of us (J. Lee) undertook a training session for laboratory staff to raise awareness of SIG and their colonial morphology and explain the algorithm. This was supplemented by posters in the laboratory and regular feedback. Following this, wound swabs (principally from skin and soft-tissue infections) were screened for SIG over the period from October 2010 to September 2013 at the Department of Clinical Microbiology, Royal Cornwall Hospital, Cornwall, United Kingdom. The specimens had been submitted for routine investigation from local hospitals and primary care physicians. Clinical details were taken from laboratory request forms accompanying the specimens from which SIG bacteria were isolated. No extra information was sought from requesting clinicians.

Swabs were inoculated onto horse blood agar (Oxoid) and examined after overnight incubation in 5% CO_2, our standard laboratory procedure. Bacterial colonies typical of SIG—white, entire, convex, glistening colonies 5 to 6 mm in diameter (see Fig. S1 in the supplemental material)—were selected for further characterization using a simple algorithm (Fig. 1). Briefly, suspect colonies were tested with a commercial latex reagent for clumping factor/protein A detection (Prolab StaphXtra) and for DNase production using commercial medium (Oxoid). Isolates that were latex negative and produced DNase were processed by the VITEK2 (bioMérieux) automated identification/susceptibility system using GP/AST cards. Strains identified by VITEK2 as "*Staphylococcus intermedius*" underwent PCR amplification of fragments of the housekeeping genes *hsp60* and *sodA* using published primer pairs (6, 7). Sequences were trimmed, aligned, and analyzed with MEGA5 (8). Sensitivity testing was performed by using the VITEK2 system and the AST-P578 panel, interpreted by EUCAST guidelines. Sensitivity results were compared with those

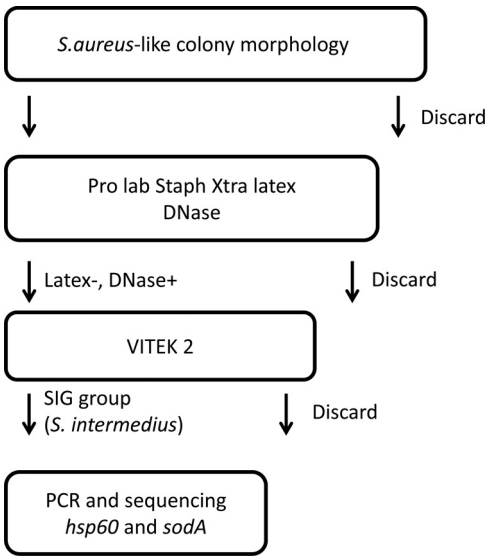

FIG 1 Phenotypic algorithm for the detection of SIG isolates.

of 40 consecutive community-acquired *Staphylococcus aureus* isolates from our laboratory.

No SIG isolates were recovered in our laboratory in the year preceding the study. SIG bacteria were isolated on 40 occasions from 39 patients during the study period. Sites of isolation included wounds (57%), ears (19%), diabetic ulcers (12%), dog bites (7%), and cutaneous ulcers (5%). Clinical details included

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Address correspondence to John Lee, John.Lee@rcht.cornwall.nhs.uk.

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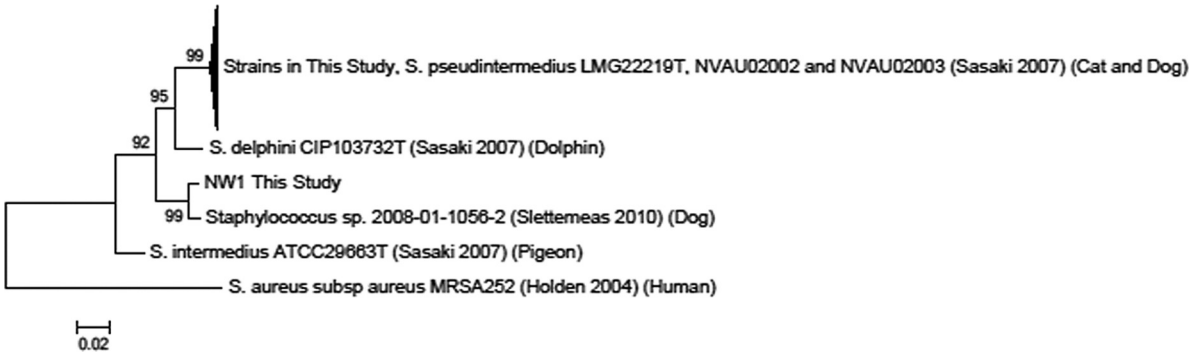

FIG 2 Maximum likelihood tree based on the concatenated *hsp60* and *sodA* gene fragments (1,000 bootstrap replicates). The collapsed branch contains all *S. pseudintermedius* strains in this study. Selected SIG strains isolated from different hosts were used for reference, and *S. aureus* was used as an outgroup.

impetigo, cellulitis, erythema, pain, purulent exudate, inflammation, and postsurgical infection. A history of animal contact was given in 4 of 40 requests; 2 of these were identified as bite wounds. The algorithm was fully operational between May 2012 and September 2013. During this 16-month period, SIG and *S. aureus* were isolated 32 and 10,777 times, respectively, from 39,380 specimens.

Sequencing confirmed that all but one SIG isolate identified by the algorithm belonged to *S. pseudintermedius*. Analysis of *sodA* and *hsp60* sequences revealed that the *S. pseudintermedius* strains, all isolated from local residents, were closely related (Fig. 2). One divergent strain, NW1, which came from a visitor to Cornwall, United Kingdom, could not be assigned to a recognized SIG species (Fig. 2). The *sodA* sequence of this strain was identical to a "staphylococcal species" described previously by Slettemeås and colleagues (9). The *hsp60* sequence from this strain had 95% sequence similarity to an *S. intermedius* strain isolated from a pigeon (10) (see Fig. S2 in the supplemental material).

The antibiotic susceptibilities of SIG isolates resembled those of local community-acquired *S. aureus* bacteria with universal susceptibility to oxacillin, erythromycin, fusidic acid, chloramphenicol, ciprofloxacin, clindamycin, gentamicin, linezolid, and rifampin. Tetracycline resistance was more common (15% versus 4.7%) in SIG isolates than in *S. aureus*. Polymyxin MICs were higher (range, 8 to 24 mg/liter) for *S. pseudintermedius* strains than for strain NW1 (4 mg/liter).

Our results show that it is possible to identify SIG isolates using standard laboratory procedures. Recognition of the colonial morphology of SIG is essential for successful detection. Where this is followed by a simple algorithm, SIG can be identified reliably in clinical specimens. Before this method was introduced in our laboratory, SIG isolates were undetected and almost certainly underreported. Our study was limited in scope and did not attempt to identify colonies that were morphologically atypical, did not seek SIG among "latex-positive" strains, and did not perform genotyping on staphylococcal isolates which VITEK2 identified as non-SIG strains. It is therefore likely that some SIG strains were missed and that the true prevalence of these organisms is higher than our results suggest.

In our population, *S. pseudintermedius* is readily detected, unlike *S. intermedius sensu stricto*. This confirms recent findings that "*S. intermedius* infections" described in earlier reports were likely to be *S. pseudintermedius* infections (11). We also isolated a SIG isolate belonging to a distinct, uncharacterized lineage that has been reported only once before (9). This isolate, NW1, had reduced susceptibility to polymyxin, which is a feature of *S. intermedius sensu stricto* and *S. delphini* (4). It may be a new species in the SIG group (9).

The clinical details given by requesting clinicians were of little use in predicting SIG isolation; very few noted animal contact. This probably reflects the limited information supplied by clinicians rather than an absence of zoonotic risk. The pattern of samples yielding SIG resembled that for *S. aureus*, which supports a pathogenic role for *S. pseudintermedius* in humans. A number of SIG isolates came from diabetic patients, an association which warrants further study. Susceptibility to antistaphylococcal antibiotics was similar to that of community-acquired *S. aureus* isolates but with decreased susceptibility to tetracycline; this may reflect veterinary prescribing practice (12).

In conclusion, SIG bacteria are underreported and often found where there is no stated history of animal contact to guide investigations. A simple modification of routine laboratory methods results in better detection of these organisms. Reporting of clinical isolates as "*Staphylococcus intermedius* group" when identified by phenotype is accurate and should be adopted by clinical laboratories.

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