Improved detection of *Staphylococcus intermedius* group in a routine diagnostic laboratory

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Abstract

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 *S. pseudintermedius*.

Main text

*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 characterisation has resulted in its re-classification as *Staphylococcus intermedius* group (SIG), which includes *S. intermedius*, *S. pseudintermedius* and *S. delphini* (3, 4). Non-bite associated human isolates were described 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 (JL) undertook a training session for laboratory staff to raise awareness of SIG, 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 October 2010 – September 2013 at the Department of Clinical Microbiology, Royal Cornwall Hospital, Cornwall, UK. 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 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% CO₂ – our standard laboratory procedure. Bacterial colonies typical of SIG - white, entire, convex, glistening colonies 5-6mm in diameter (Figure S1) - were selected for further characterization using a simple algorithm (Figure 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 media (Oxoid). Isolates which were latex negative and produced DNase were processed by the VITEK2 (BioMerieux) 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 analysed with MEGA5 (8). Sensitivity testing was performed on the
VITEK2 using AST-P578 panel, interpreted by EUCAST guidelines. Sensitivity results were compared with those 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 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 impetigo, cellulitis, erythema, pain, purulent exudate, inflammation and post-surgical 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 10777 times respectively, from 39380 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 (Figure 2). One divergent strain, NW1, which came from a visitor to Cornwall, could not be assigned to a recognised SIG species (Figure 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 a *S. intermedius* strain isolated from a pigeon (10) (Figure S2).
The antibiotic susceptibilities of SIG isolates resembled those of local community-acquired *S. aureus* with universal susceptibility to oxacillin, erythromycin, fusidic acid, chloramphenicol, ciprofloxacin, dindamycin, gentamicin, linezolid and rifampicin. Tetracycline resistance was more common (15% vs 4.7%) in SIG isolates than in *S. aureus*. Polymixin MICs were higher (range 8 to 24 mg/l) for *S. pseudintermedius* strains than for strain NW1 (4mg/l).

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 which 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
polymixin, 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 anti-staphylococcal antibiotics was similar to community acquired *S. aureus* isolates but with decreased susceptibility to tetracycline; this may reflect veterinary prescribing practice (12).

In conclusion, SIG are under-reported 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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Figure 1

Phenotypic algorithm for the detection of SIG isolates.

Figure 2

Maximum Likelihood Tree based on the concatenated hsp60 and sodA gene fragments (1000 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.
References


6. Sakamoto M, Suzuki N, Benno Y. 2010. hsp60 and 16S rRNA gene sequence relationships among species of the genus Bacteroides with the finding that...
Bacteroides suis and Bacteroides tectus are heterotypic synonyms of Bacteroides pyogenes. International journal of systematic and evolutionary microbiology 60:2984-2990.


S. aureus-like colony morphology

→

Pro lab Staph Xtra latex DNase

→ Latex-, DNase+

→ Discard

VITEK 2

→ SIG group (S. intermedius)

→ Discard

PCR and sequencing hsp60 and sodA
Strains in This Study, S. pseudintermedius LMG22219T, NVAU02002 and NVAU02003 (Sasaki 2007) (Cat and Dog)

- S. delphini CIP103732T (Sasaki 2007) (Dolphin)
- NW1 This Study
- Staphylococcus sp. 2008-01-1056-2 (Slettemes 2010) (Dog)
- S. intermedius ATCC29663T (Sasaki 2007) (Pigeon)
- S. aureus subsp. aureus MRSA252 (Holden 2004) (Human)