Isolation of *Staphylococcus aureus* from screening swabs: comparison of blood agar and SAID, pre and post-enrichment

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The Centers for Disease Control and Prevention (CDC) identifies pre-operative nares carriage of *Staphylococcus aureus* in healthy humans as a risk factor for the development of surgical site infection (SSI). It is carried in the nares of 20–30% of humans.[1] Infection control and microbiology staff require sensitive and inexpensive methods to facilitate the rapid identification of pathogens such as *S. aureus*.[2]

The present study, which is designed to determine an efficient method for the laboratory isolation of *S. aureus* from screening nasal swabs, precedes a larger study to determine the carriage rate of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). The larger study is being undertaken at the Coombe Women and Infants University Hospital (CWIUH), Dublin, to ascertain if there is a need for a pre-admission surveillance strategy for MSSA and MRSA carriage in at-risk groups. This type of surveillance initiative is in line with the rationale and objectives of the EU Hospital in Europe Link for Infection Control through Surveillance (HELICS) network.

At the hospital level, HELICS states that its objective of lowering the incidence of SSI can be achieved by compliance with existing guidelines and good surgical practice, correcting or improving specific practices, and developing, implementing and evaluating new preventive practices. This study may allow the hospital to develop such a new preventative practice.

The Coombe Women and Infants University Hospital is a standalone obstetrics and gynaecology hospital. It is one of the largest providers of women's and infants' healthcare in Europe. In 2007, more than 8500 babies were born there. The hospital, which has 251 beds, with 14 neonatal intensive care unit (NICU) beds, also provides the largest gynaecological service in the Republic of Ireland.

In total, 1250 swabs will be taken for the larger study. An initial 205 swabs will be used to determine the optimum recovery strategy for *S. aureus*. The isolation rate of *S. aureus* from a non-selective medium, Columbia blood agar, is compared with the rate from bioMérieux *S. aureus*. ID medium (SAID) agar, a selective chromogenic agar. The latter has been found to be highly sensitive and specific in differentiating *S. aureus* from coagulase-negative staphylococci. Direct plating on both agars is compared to pre-enrichment with brain-heart infusion broth (bioMérieux).

Methods to detect *S. aureus* ideally should have high sensitivity and a short time to reporting of results.[3] Chromogenic media allow preliminary identification in a timely manner.

To date, in CWIUH, diagnosis of *S. aureus* infection is achieved through culture of swabs directly on non-selective agars and identification via biochemical tests. The microbiology laboratory screens for MRSA carriage using pre-enrichment with brain-heart infusion broth and plating on MRSA Id agar (bioMérieux). Recently, chromogenic agars for the identification of *S. aureus* have been introduced to laboratories in a bid to increase yield, decrease turnaround times and reduce staff workload.

The aim of this initial study is to evaluate the sensitivity, specificity and ease of use in a routine clinical laboratory setting of *S. aureus* chromogenic agar versus non-selective agar and to determine the effect of pre-enrichment in a broth medium prior to plating on these agars.

Two hundred and four nasal swabs were collected for the detection of *S. aureus*. These were taken from healthy antenatal patients attending the out-patients' department of CWIUH for the first time in their current pregnancy, and in gynaecological patients attending for the first time in this referral.

The swabs were plated on Columbia blood agar (5% sheep blood) streaked for single colonies, and read after 24 h at 35°C. Plates were re-incubated and read again after a further 24-h incubation at 35°C. The swabs were also plated on SAID, a selective prepoured chromogenic medium, and read after 24 h at 35°C. Its performance has not been found to differ significantly between 24-h and 48-h incubation, so optimal results can be obtained after a single day's incubation.[4]

After direct plating, the same swabs were placed in brain-heart infusion broth and incubated for 24 h at 35°C. The broth was then subcultured on blood agar and SAID, and incubated as above.

Colonies of *S. aureus* appear green on SAID agar, due to the production of α-glucosidase. Other staphylococci generally produce white colonies and these were not identified. Other organisms are inhibited or grow poorly. Suspect colonies were chosen from blood agar based on their typical colonial morphology.

All suspect colonies were identified as *S. aureus* using the Staphaurex Plus rapid latex kit (Remel) and DNase production (inoculation on a DNase plate [Opticult]) followed by flooding with HCl (1 mol/L). Staphaurex Plus is a rapid latex agglutination test for the identification of staphylococci which possess clumping factor, protein A and/or surface antigens characteristic of *S. aureus*. Any isolate positive with both Staphaurex Plus and DNase was identified as *S. aureus.* Discrepancies were checked by tube coagulase. *S. aureus* ATCC 29213 was used as a positive control and *S. epidermidis* ATCC 12228 was used as a negative control for both Staphaurex Plus and DNase.

Antibiotic susceptibility to oxacillin and cefoxitin was performed using the CLSI disc-diffusion method on Mueller Hinton agar. *S. aureus* ATCC 25923 was used as a control strain for the antibiotic discs.[5]

Any *S. aureus* isolates resistant to oxacillin and/or cefoxitin had Gram-positive antibiograms performed (Vitek 1, bioMérieux), thus confirming the isolate as MRSA. In this study, no discrepancies were found between disc-diffusion results and the minimum inhibitory concentration (MIC)

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values provided by the Vitek. In the clinical setting, any isolate with discrepant results was referred to the Irish National MRSA Reference Laboratory.

Of the 204 swabs tested, 85 yielded *S. aureus* using one or more of the media. This gave a positivity rate of 41.7%. Of these *S. aureus*, three isolates (4%) were MRSA, giving a positivity rate of 1.5%. One swab yielded both MSSA and MRSA. The number of isolates of MSSA and MRSA yielded by the various methods is summarised in Table 1.

The percentage yield of *S. aureus* from the 204 swabs, using the various media pre- and post-enrichment are presented in graphical form in Figure 1. The relative sensitivities and negative predictive values of the media, with and without pre-enrichment, are summarised in Figure 2.

The sensitivity at 24 h for chromogenic and blood agar was identical; however, while the numbers of *S. aureus* isolated were identical, these did not always come from the same swabs.

At 48 h, there were three sets of cultures to read: blood agar direct plating after 48-h incubation, SAID post-enrichment and blood agar post-enrichment (24-h incubation). The results are presented in Table 2. In this study, all swabs that yielded *S. aureus* were positive in the SAID post-enrichment, giving it a sensitivity of 100%.

Using Fisher's exact test to compare the number of *S. aureus* isolates from blood agar (after 48-h incubation) with those from SAID post-enrichment, $P=0.0095$ was obtained. This is considered to be very statistically significant. Comparing blood agar post-enrichment (88.2% sensitivity) with SAID post-enrichment, $P=0.3615$ was obtained, which is not statistically significant.

The blood agar post-enrichment at 48 h yielded only one more *S. aureus* isolate than did the blood agar post-enrichment at 24 h. This was not statistically significant and the additional 24 h is clinically unacceptable. In addition, at the 48-h stage, SAID post-enrichment results were available and yielded nine more isolates than did the 72-h methodology using non-selective media.

Directly plated SAID yielded 54 *S. aureus* isolates compared with the 85 isolates obtained post-enrichment on SAID. This is considered to be very statistically significant ($P=0.0017$). Directly plated blood agar (24 h) yielded 54 *S. aureus* isolates compared with the 75 isolates obtained post-enrichment on blood agar (24 h). This is statistically significant ($P=0.033$).

The global incidence of *S. aureus* infection is rising.[6] The problems with MRSA are well documented; however, it should not be forgotten that methicillin-sensitive strains can also cause severe infection. Staphylococcal infections cause significant morbidity and mortality in both the community and hospital settings.[7] While it is not a widespread practice, some hospitals routinely screen patients at high-risk for staphylococcal nasal carriage prior to invasive procedures.

S. aureus accounts for approximately 13% of all nosocomial blood infections.[8] Nasal carriage is a risk factor for acquiring nosocomial infection and it has been shown that 80% of nosocomial *S. aureus* bacteraemias are attributable to an endogenous source.[9] In addition, *S. aureus* is a frequent SSI isolate. It has been known for years that the development of SSI involving *S. aureus* is definitively associated with pre-operative nares carriage of the organism in surgical patients.[10] Effective screening, prevention and control of *S. aureus*, particularly MRSA, depends on reliable and timely laboratory results.[11]

Molecular techniques have been developed but are expensive. While they may become more widespread in the future, the use of isolation media that give accurate and fast results is essential at present.[12] The general perception of culture-based methods as being slow and laborious is undergoing rapid change with the introduction of chromogenic agars, as these can produce results efficiently without the added set up, expertise and costs required for molecular tests.[13]

The percentage yield of *S. aureus* from the 204 swabs,

Table 1. MRSA and MSSA isolates (204 nasal swabs).

	MRSA	MRSA and MSSA	MSSA	Total *S. aureus*
BA direct (24 h)	2	1	51	54
BA direct (48 h)	2	1	56	59
SAID direct (24 h)	2	1	51	54
Post-BHIB BA (24 h)	2	1	72	75
Post-BHIB BA (48 h)	2	1	72	76
Post-BHIB SAID (24 h)	2	1	82	85

Table 2. Yield of *S. aureus* (sensitivity) at 48 h.

BA direct	Enrichment + SAID	Enrichment + BA (24 h)
59 (69.4%)	85 (100%)	75 (88.2%)

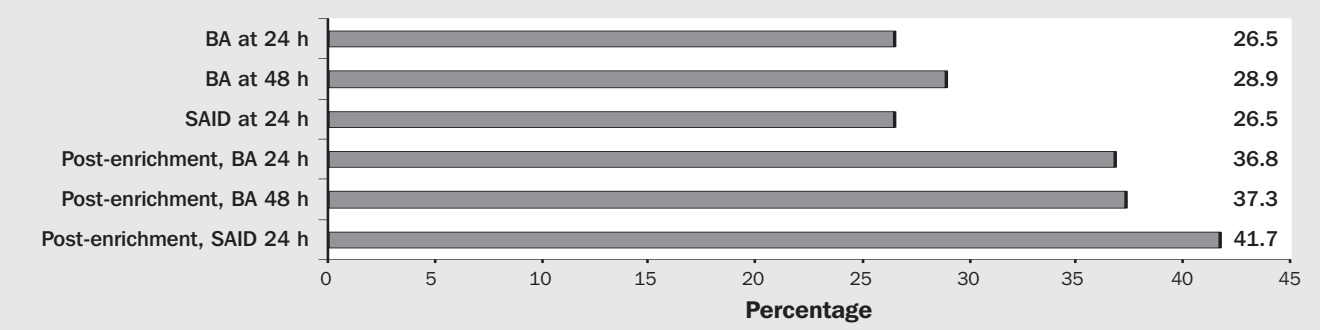

Fig. 1. Percentage yield of *S. aureus* from swabs ($n=204$)

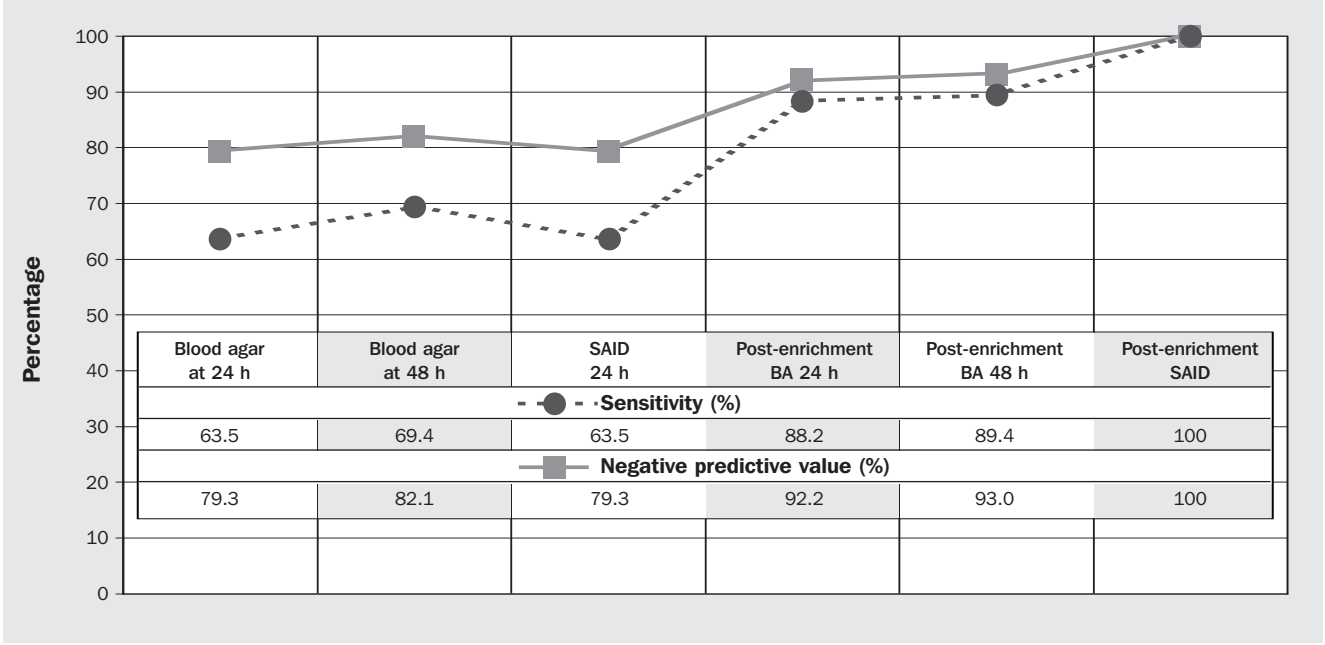

Fig. 2. Sensitivity and negative predictive values.

using the various media pre- and post-enrichment are presented in graphical form in Figure 1. Direct plating on blood agar and SAID yielded the same percentage (26.5%) of *S. aureus* after 24 h; an additional 24-h incubation of the blood agar resulted in 2.4% more *S. aureus* being identified. However, at this 48-h stage, the SAID and blood agar post-enrichment plates were also ready for reading. These gave an increased yield of 12.8% and 7.9%, respectively.

Of the 204 nasal swabs cultured, 85 yielded *S. aureus*. The SAID agar post-enrichment was the only method that achieved 100% sensitivity. Other studies have found varying results with enrichment broths, providing only marginal improvement at best.[14] The results presented here, however, agreed with those studies that found that a strategy of direct inoculation combined with overnight enrichment provided the best balance between rapid results and increased sensitivities.[13,15–18] In this study, pre-enrichment and plating on SAID agar increased the yield of *S. aureus* from 54 to 85 isolates. Not only was this clinically significant, it was statistically very significant ($P=0.0017$). Even using conventional non-selective agar, pre-enrichment increased the yield of *S. aureus* from 54 to 75 isolates ($P=0.033$), which is statistically significant. The pre-enrichment strategy used in this study took 48 h in total.

Employing the more rapid 24-h strategy of direct plating, only 54 *S. aureus* isolates were obtained, giving a sensitivity of 63.5%. This sensitivity was identical for chromogenic and blood agar. As might be expected, use of the non-selective blood agar involved far more work.

While the numbers of *S. aureus* isolated were identical, these did not always come from the same swabs. In six cases, SAID at 24 h yielded *S. aureus* that was not picked up on the blood agar at 24 h. Those strains which were not detected could be explained by the shortcomings of selective media when inocula levels are very low.[14] Overgrowths of coagulase-negative staphylococci were involved in all six cases where isolates grew on blood agar at 24 h but not on SAID. Post-enrichment, there were even more difficulties with overgrowths on the non-selective blood agar plates. Overgrowths with Gram-negative bacilli were seen in 12 cases and with *Bacillus* species in 15 cases. The finding of clearer visualisation on SAID agar, due to the chromogenic and inhibitory properties of the medium, agree with those of a previous study that reported inhibition of Gram-negative organisms as well as nasopharyngeal flora from nasal swabs could be achieved successfully using selective agar.[19]

The colonies selected for further identification from the blood agar resulted in 483 Staphaurex Plus and DNase tests being performed. Only 141 isolates were selected from the corresponding SAID plates for further identification. All colonies resembling staphylococci morphologically on blood agar were tested. The additional flora on the non-selective blood agar meant that many staphylococcal isolates needed to be subcultured before identification and susceptibility testing could be performed. On SAID, colonies were selected for testing on the basis of colour change and morphology. There was a more than three-fold increase in laboratory workload using blood agar, which has implications for staff time and resource use, both of which are costly commodities. From a clinical perspective, the best negative predictive value was obtained using SAID post-enrichment. This is an indicator of the proportion of patients with negative test results who were diagnosed correctly.

The Clinical and Laboratory Standards Institute (CLSI) document M35-A for the abbreviated identification of bacteria and yeast includes chromogenic media among acceptable rapid tests for organism identification as long as the accuracy is greater than 95%.[20] The results from the present study fulfil this criterion. Clearly, SAID has the potential to identify more rapidly and accurately carriers of *S. aureus* for the purposes of surveillance and possible intervention. It also has the potential to be used in a clinical situation where a severe staphylococcal infection is suspected.

The need for surveillance cultures is likely to rise in the future as hospitals attempt to cope with the rise in staphylococcal nosocomial infections, and media attention continues to increase. The present study found that SAID

permits the easy selection of *S. aureus*, reduces laboratory costs and improves patient care.

Therefore, it would appear that the best clinical and laboratory strategy for *S. aureus* screening from nasal swabs is a combination of direct plating on chromogenic agar, read at 24 h, with an enrichment stage that is followed by plating on chromogenic agar. In this study, 63.5% of *S. aureus* isolates were detected at 24 h, with the remaining 36.5% identified at 48 h. Pre-enrichment of swabs in brain-heart infusion broth, followed by plating on SAID agar, will be used as the strategy of choice for screening the remaining 1046 nasal swabs for *S. aureus* for the larger study designed to determine the carriage rate of MSSA and MRSA and the associated risk factors.

The positivity rate for carriage of *S. aureus* in this study of 204 nasal swabs was 41.7%, which is higher than the rate quoted by CDC; however, the number of swabs examined was small and the true positivity rate will only be determined following completion of the main study.

The authors are grateful to Hazel Robbins (CWIUH) for her help in recruiting volunteer patients for this study. They would also like to thank the microbiology staff of the CWIUH for their support.

References

1 Perl TM, Golub JE. New approaches to reduce *Staphylococcus aureus* nosocomial infection rates: treating *S. aureus* nasal carriage. *Ann Pharmacother* 1998; **32**: 7–16.

2 Flayhart D, Hindler JF, Brukner DA *et al*. Multicentre evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *J Clin Microbiol* 2005; **43**: 5536–40.

3 Diederen B, van Leest ML, van Duijn I *et al*. Performance of MRSA ID, a new chromogenic medium for detection of methicillin-resistant *Staphylococcus aureus. J Clin Microbiol* 2006; **44**: 586–8.

4 Diederen BMW, van Leest CM, van Duijn I *et al*. Evaluation of *S. aureus* ID, a chromogenic agar medium, for the detection of *Staphylococcus aureus. Infection* 2006; **34**: 95–7.

5 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 18th informational supplement M100–S18. 2008; 27.

6 Bowers KM, Wren MW, Shetty NP. Screening for methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: an evaluation of three selective media and Mastalex-MRSA latex agglutination. *Br J Biomed Sci* 2003; **60**: 71–4.

7 Cosgrove SE, Sakoulas G, Perencevich EN *et al*. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteraemia: a meta-analysis. *Clin Infect Dis* 2003; **36**: 53–9.

8 Wertheim H, Vos M, Ott A *et al*. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *Lancet* 2004; **364**: 703–5.

9 von Eiff C, Becker K, Machka K *et al*. Nasal carriage as a source of *Staphylococcus aureus* bacteraemia. *N Engl J Med* 2001; **344**: 11–6.

10 Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* 1999; **20**: 250–78.

11 Petersson AC, Miorner H, Kamme C. Identification of *MecA* related oxacillin resistance in staphylococci by the Etest and the broth microdilution method. *J Antimicrob Chemother* 1996; **37**: 445–56.

12 Davies S, McIntyre S, Fowler J *et al*. Methicillin-resistant *Staphylococcus aureus* detection using chromogenic media: the Sheffield experience. *Br J Biomed Sci* 2008; **65**: 13–7.

13 Malhotra-Kumar S, Haccuria K, Michiels M *et al*. Current trends in rapid diagnostics for methicillin-resistant *Staphlyococcus aureus* and glycopeptide-resistant *Enterococcus* species. *J Clin Microbiol* 2008; **46**: 1577–87.

14 Cherkaoui A, Renzi G, Francois P *et al.* Comparison of four chromogenic media for culture-based screening of methicillin-resistant *Staphylococcus aureus. J Med Microbiol* 2007; **56**: 500–3.

15 Perry PL, Coombs GW, Boehm JD *et al.* A rapid (20 h) solid screening medium for detecting methicillin-resistant *Staphylococcus aureus. J Hosp Infect* 1998; **40**: 67–72.

16 Safdar N, Narans L, Gordon B *et al*. Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*: a prospective study comparing 32 methods. *J Clin Microbiol* 2003; **41**: 3163–6.

17 Nahimana I, Francioli P, Blanc DS. Evaluation of three chromogenic media (MRSA-ID, MRSA-Select, CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus. Clin Microbiol Infect* 2006; **12**: 1168–74.

18 Ieven M, Michiels M, Jansens H *et al*. MRSASelect applied on enrichment broths significantly increases the sensitivity of MRSA detection in screening samples (Abstract). *ESCMID* 2007; 1734_103.

19 Flayhart D, Lema C, Borek A *et al*. Comparison of the BBL CHROMagar *Staph aureus* agar medium to conventional media for detection of *Staphylococcus aureus* in respiratory samples. *J Clin Microbiol* 2004; **42**: 3566–9.

20 Clinical and Laboratory Standards Institute. Abbreviated identification of bacteria and yeast; approved guideline. M35-A 2003; **22**.

Long-term antibiotic treatment of patients with cystic fibrosis: a commensal organism's view

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The long-term use of several classes of antibiotic agent for the prophylaxis, maintenance and treatment of bacterial respiratory pathogens causing chronic chest infections in

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