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Improved culture detection of *Staphylococcus aureus* from sputum of patients with cystic fibrosis (CF)

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Staphylococcus aureus (SA) is a facultative anaerobic Gram-positive coccus, found in 30% to 50% of the healthy adult population.[1] It is considered to be a commensal organism of humans and around 20% of individuals are 'persistent carriers'. The organism resides most commonly in the anterior nares, where there is a relative absence of immunological defences and colonisation is achieved through bacterial adherence to the surface membrane of the host cells.[2] Other common sites include the axillae, vagina and pharynx. SA is responsible for a diverse range of diseases; from skin and soft tissue infection, pneumonia and gastrointestinal (GI) poisoning, to fatal conditions of bacteraemia, endocarditis, sepsis and toxic shock syndrome.[1] Within cystic fibrosis (CF), SA is the most commonly isolated organism, where more than half of US individuals had at least one culture positive for methicillin sensitive SA in 2017 and its occurrence, was highest in those younger than 10.[3] Controversies remain ongoing regarding its clinical significance in early and adult lung pathology, as well as optimal treatments regimens in these populations. In a seminal review by Wong and colleagues,[4] the authors highlighted the need for further investigations to help understand (i). the early host immune response that enables SA to reside within the CF lung, (ii) to determine if there are organism specific factors that are associated with CF lung disease and (iii) to clarify the utility of anti-staphylococcal antibiotic prophylaxis and/or eradication in the treatment of this patient population. Therefore, robust and reliable methods for the laboratory detection of SA are needed to support the evidence for SA involvement in lung disease. It was the aim of this study to (i). compare the optimum agar to recover SA from CF sputum and (ii). compare selective enrichment versus non-enrichment for the recovery of SA from CF sputa.

(i). Comparison of non-selective, selective and differential agars for the detection of *Staphylococcus aureus*

Freshly expectorated sputum was obtained from CF patients (n=47) and processed with , sputasol (SR0233, Oxoid Ltd., UK), as previously described.[5] Five agar media were compared by reconstituting in accordance with the manufacturers' instructions, including (i) nutrient agar (CM003), (ii) Columbia blood agar (CM0331), supplemented with 5% (v/v) defibrinated horse blood, (iii). colomycin agar [Columbia blood agar base (CM0331) 39g/L; mannitol 10g/L; NaCl 25g/L, bromocresol purple (1.6 [w/v]% solution) 4mL/L, colomycin (1 Million I.U. dissolved in 10mL of Sterile Water) 0.5mL/L, (iv). Baird-Parker agar (Oxoid CM1127) and (v) Brilliance Staph agar (LIP W11494). Lysed CF sputum (10µl) was inoculated onto each plate and incubated aerobically at 37ºC for 48h, prior to examination of plates for the typical presence of SA. Presumptive SA colonies were subsequently subcultured and confirmation testing. Two positive SA controls were also included, namely NCTC 6571 WDCM 00035 and a well-characterised CF clinical SA isolate, NISA1.

(ii) Comparison of selective enrichment and non-enrichment for the detection of *Staphylococcus aureus*

Freshly expectorated sputum was obtained from CF patients (n=19) and processed with , sputasol (SR0233, Oxoid Ltd., UK), as previously described .[5] For the non-enrichment arm of the study, Baird-Parker agar (Oxoid CM1127) and Brilliance Staph agar (LIP W11494) were inoculated with lysed CF sputum (10µL) and incubated aerobically at 37ºC for 48h. For the selective enrichment arm of the study, mannitol salt broth (MSB) was prepared as follows:-nutrient broth No.2 Base (Oxoid CM0085) 25g/L, supplemented with mannitol (10g/L) and NaCl (70g/L). MSB was dispensed in 10mL amounts into disposable universal bottles, capped and autoclaved at 121°C/20min.

CF sputum (500µL) was inoculated into MSB (10mL) and incubated at 37°C for 48 hours. Following this period, 10 µL was inoculated onto Baird-Parker agar (Oxoid CM1127) and Brilliance Staph agar (LIP W11494) and incubated as above. Two positive SA controls were also included in this study, as detailed above. All isolates examined were methicillin-sensitive *Staphylococcus aureus* (MSSA).

Isolation of SA varied depending on agar type. Highest recovery of SA was from Baird-Parker agar (24/47; 51.1%) > Brilliance Staph agar (23/47; 48.9%) > colomycin agar (6/47; 12.8%) > nutrient agar (1/47; 2.1%), Columbia Blood agar (1/47; 2.1%). All five media successfully grew both SA control organisms, namely WDCM 00035 & NISA1. Selective enrichment recovered more SA from CF sputum on Baird Parker agar (6 SA positives/19 CF sputa v 3/19 for non-enrichment) and on Brillance Staph agar (6/19 v 4/19 for non-enrichment).

This study showed that both Baird Parker agar and Brilliance Staph agar were the best agars for recovery SA from CF sputum, with the others, namely nutrient agar, Columbia blood agar and colomycin agar, being poor at isolating SA (<10% recovery). This was probably due the overgrowth of SA on these media with contaminating organisms from the rich diversity of bacteria found in CF sputum. Current guidelines for the isolation of SA from CF sputum include (i). UK CF Trust Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis [6] (ii). Public Health England's UK Standards for Microbiology Investigations: Investigation of bronchoalveolar lavage, sputum and associated specimens [7] and (iii). the US Cumitech protocol.[8] All of these methods recommend inclusion of mannitol salt agar/chromogenic agar for the isolation of SA from CF respiratory specimens. However, none of these include a selective enrichment step, prior to selective plating. This

current study has shown that in addition to the plating stage with selective/differential agar, inclusion of a selective enrichment step helped increase the number of positive SA specimens, compared to not having incorporated an enrichment stage. Selectivity for SA was enhanced by including the MSB enrichment step, as it added a preliminary selection phase for SA based on high salt concentration [7.5% w/v], followed by the selective components of either Baird Parker agar or Brilliance Staph chromogenic agar. Higher recovery rates were seen when using enrichment prior to selective plating. We believe that the reason for the difference is due to bacterial numbers, namely that enrichment was able to allow for the proliferation of small numbers in CF sputum to detectable levels that were able to be seen on subsequent selective culture.

Where SA is present in smaller numbers, i.e. during early colonisation/infection or during intermittent infection, we therefore endorse the isolation method, as detailed in Figure 1, for the optimal recovery of SA from CF sputum.

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COMPETING INTERESTS: None declared.

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FIGURE LEGEND

Figure 1: Improved methodology for the isolation of *Staphylococcus aureus* from the sputum of patients with cystic fibrosis (CF)