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Case report: Identification of intra-laboratory blood culture contamination with *Staphylococcus aureus* by whole genome sequencing

Anushia Ashokan, *[1,2,3,] Lito E. Papanicolas,[1,2,] Lex E.X. Leong,[1,2,] Maria Theodossi,[4,] Santhosh Daniel,[5,] Steve L. Wesselingh,[1,2,] Geraint B. Rogers,[1,2,] David L. Gordon[4,5.]

[1]Infection and Immunity Theme, South Australia Health and Medical Research Institute(SAHMRI), Adelaide, South Australia, 5000, Australia; [2]SAHMRI Microbiome Research Laboratory, Flinders University School of Medicine, Bedford Park, South Australia, 5042, Australia; [3]University of Adelaide, Adelaide, South Australia, 5000, Australia; [4]South Australia(SA) Pathology, Flinders Medical Centre, Bedford Park, South Australia, 5042, Australia; [5]Department of Microbiology and Infectious Diseases, Flinders Medical Centre, Bedford Park, South Australia, 5042, Australia.

***Corresponding author:** Dr Anushia Ashokan.

Email address: Anushia.Ashokan@sa.gov.au

Mailing address: SAHMRI Microbiome Research Laboratory, Flinders University School of Medicine, Flinders Drive, Bedford Park, South Australia, 5042, Australia

Telephone number: ++61 8 82047504 **Fax number**: ++61 8 82044733

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Abstract

Staphylococcus aureus in blood cultures is rarely considered a contaminant. We report a case of intra-laboratory contamination between blood culture bottles which was confirmed by whole genome sequencing, highlighting the importance of molecular analysis in the clinical laboratory setting.

Case report

Staphylococcus aureus bacteraemia is a common and life-threatening condition, with mortality rates of over 20%, rising to 30% for methicillin resistant strains (MRSA) (1). Detection of *S. aureus* in blood cultures occasionally may not represent true bacteraemia. In one prospective study, *S. aureus* was considered a contaminant or of unknown significance in 12.8% of cases (2). However, in our clinical experience, *S. aureus* is rarely a blood culture contaminant and, due to the severe consequences of *S. aureus* bacteraemia, it is usual to treat all *S. aureus* positive blood culture results. Nevertheless, rare instances of true contamination are difficult to confirm and the absence of a gold standard test for contamination could contribute to unnecessary treatment and investigations. Most of suspected contamination events are thought to result from the introduction of skin commensals during phlebotomy. However, a recent case in our hospital highlights the potential for intra-laboratory contamination between blood cultures and illustrates how these events can be definitively identified through whole genome sequencing (WGS).

A 41-year-old male (Patient A) was admitted with fever, dyspnoea and a productive cough. Examination revealed left-sided crepitations and chest X ray showed left lower lobe consolidation. Blood cultures were obtained and incubated in the Bactec^{TM} FX (BD Diagnostics, New Jersey, USA) blood culture system. Gram-positive diplococci in pairs

resembling streptococci were visualised after 14 hours from the aerobic, then 18 hours from the anaerobic, blood culture bottle. *Streptococcus pneumoniae* (both bottles) and scant growth of MRSA (aerobic bottle only) were isolated on subculture. Further blood cultures were not taken during admission. While the patient had shown improvement on empirical ceftriaxone and azithromycin, intravenous vancomycin was commenced to expand coverage for MRSA. He completed four weeks of vancomycin via a peripherally inserted central venous catheter (PICC), as per guidelines for treatment of community acquired staphylococcal bacteraemia (1). Repeat blood cultures taken after the initial positive culture were negative. Additional cultures collected prior to antimicrobials, might have been informative in this case, but even a single positive blood culture for MRSA would usually be considered significant.

As the Gram stain showed gram-positive cocci resembling streptococci, the isolation of MRSA was unexpected and further laboratory investigations were undertaken. The initial Gram stain slides were reviewed and confirmed the original findings. However, repeat Gram stain (24 hours later) revealed gram-positive cocci resembling both staphylococci and streptococci (aerobic bottle only). Both blood culture bottles were re sub-cultured onto solid media, with the aerobic bottle giving rise to growth of MRSA (increased colonies compared to previous culture) and *S. pneumoniae*. Concurrently, a second patient (Patient B) was noted to have multiple (x12) positive blood cultures for MRSA. Further review revealed that Patient A's positive bottle was processed immediately following the processing of bottles from Patient B. MRSA antimicrobial susceptibility results from both patients were identical.

WGS was performed on Patients A and B isolates, as described previously (3). The two isolates were five days apart due to the unavailability of the same-day culture from Patient B.

Two additional MRSA blood isolates collected from the same hospital within one week (isolates C and D) were also analysed. Multilocus sequence typing against the PubMLST database (4) identified isolates A and B as sequence type 5, while C and D were sequence types 1 and 22, respectively. When mapped against MRSA type strain Mu50 (sequence type 5), isolates A and B were separated by only 16 SNPs and were distinct from comparator strains C and D (separated by >17,240 SNPs; Figure 1). Importantly, patients A and B were housed in separate wards (General Medical and Haematology respectively), located at opposite ends of the hospital, and cared for by different medical, nursing, and allied health providers.

The WGS analysis, co-processing of the positive bottles, and absence of any epidemiological link between the patients, strongly suggest that cross-contamination occurred within the microbiology laboratory. WGS analysis indicated a high degree of similarity between isolates A and B and marked divergence from two other concurrent MRSA laboratory isolates. The 16 SNP difference between the linked isolates is consistent with derivation from a common bacterial population (5). There are previous reports of intra-laboratory blood culture cross contamination reported with earlier radiometric analysers in which growth is detected following regular automated needle sampling and gas flushing of bottles (6). However, current analysis platforms such as the Bactec^{TM} FX, used in our laboratory, utilise fluorescence detection without bottle sampling, removing the potential for contamination within the instrument.

In our laboratory, all blood cultures are routinely incubated for five days, with blood culture processing performed in a Class 2 biosafety cabinet. During Gram stain preparation, each vial top is sterilised with an alcohol-based wipe prior to sampling with a single-use device for

staining and subculture. We hypothesise that contamination between adjacent bottles occurred at this time, with inoculation of MRSA into Patient A's bottle during sampling (hence its recovery on re-subculture). Contamination might have occurred via the gloves during sample handling, or through aerosolisation from Patient B's bottle during the use of the blood transfer device, which can eject a small amount of blood due to pressure build up.

This is, to our knowledge, the first documentation of intra-laboratory contamination involving closed blood culture analysers. The ability to confidently exclude laboratory contamination of blood cultures could have significant impacts on patient care. In this case, vancomycin would have been stopped and no PICC line inserted. Extended vancomycin treatment carries significant risks including nephrotoxicity and thrombophlebitis (7) and insertion of a PICC is associated with overall complication rates of 30% (8).

Despite the availability of rapid "benchtop" platforms and a continuing decline in associated costs, WGS is not routinely applied in most laboratories. The improvement in turn-around time means that WGS results can be available within days, fast enough to influence clinical outcomes. In addition to providing epidemiological information about culture isolates, WGS analysis can also inform the clinician about the carriage of antibiotic resistance genes and virulence factors. As documented in tuberculosis laboratories, contamination events cannot be completely avoided, even with particularly careful handling of cultures (9). Laboratory cross-contamination of blood cultures is likely to occur infrequently, but without routine surveillance with a comprehensive typing method such as WGS, the true rate of these events will remain unknown.

This case highlights the need for diagnostic laboratories to consider incorporating WGS into investigations of suspected laboratory contamination. We envision that the application of WGS to clinically important blood isolates will become more routine, not only facilitating epidemiological investigations of outbreaks, but also promptly identifying episodes of laboratory contamination events.

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Figure legend

Figure 1. Neighbour-Net analysis from core genome SNP alignment (40,424 SNPs) from the four MRSA isolates (A-D) against five type strains (MRSA strain JH1, JH9, Mu50, Mu3, and N315) using uncorrected (observed, "P") distances. Scale bar indicates number of SNPs per base pair of aligned core genome.