



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

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ARTICLE INFO

Article history:

Received 16 January 2019

Received in revised form 14 February 2019

Accepted 14 February 2019

Available online 23 February 2019

Keywords:

Clinical isolates

Metagenomics

MRSA

Bacteremia

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.

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1. Case report

Staphylococcus aureus bacteremia is a common and life-threatening condition, with mortality rates of over 20%, rising to 30% for methicillin resistant strains (MRSA) (Turnidge et al., 2009). Detection of *S. aureus* in blood cultures occasionally may not represent true bacteremia. In one prospective study, *S. aureus* was considered a contaminant or of unknown significance in 12.8% of cases (Weinstein et al., 1997). However, in our clinical experience, *S. aureus* is rarely a blood culture contaminant and, due to the severe consequences of *S. aureus* bacteremia, 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, dyspnea 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™ FX (BD Diagnostics, New Jersey, USA) blood culture system. Gram-positive diplococci in pairs resembling streptococci were visualized 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 4 weeks of vancomycin via a peripherally inserted central venous catheter (PICC), as per guidelines for treatment of community acquired staphylococcal bacteremia (Turnidge et al., 2009). 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.

* Funding information: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

☆☆ Declarations of interest: None.

★ Ethical statement: Not applicable.

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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-subcultured 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 ($\times 12$) 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 (Leong et al., 2017). The two isolates were 5 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 1 week (isolates C and D) were also analyzed. Multilocus sequence typing against the PubMLST database (Jolley et al., 2018) 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; Fig. 1). Importantly, patients A and B were housed in separate wards (General Medical and Hematology 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 (Young et al., 2012). There are previous reports of intra-laboratory blood culture cross contamination reported with earlier radiometric analyzers in which growth is detected following regular automated needle sampling and gas flushing of bottles (Centre for Disease Control, 1982). However,

current analysis platforms such as the Bactec™ FX, used in our laboratory, utilize fluorescence detection without bottle sampling, removing the potential for contamination within the instrument.

In our laboratory, all blood cultures are routinely incubated for 5 days, with blood culture processing performed in a Class 2 biosafety cabinet. During Gram stain preparation, each vial top is sterilized with an alcohol-based wipe prior to sampling with a single-use device for staining and subculture. We hypothesize 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 aerosolization 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 analyzers. 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 (Sorrell and Collignon, 1985) and insertion of a PICC is associated with overall complication rates of 30% (Grau et al., 2017).

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 (Braden et al., 1997). 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

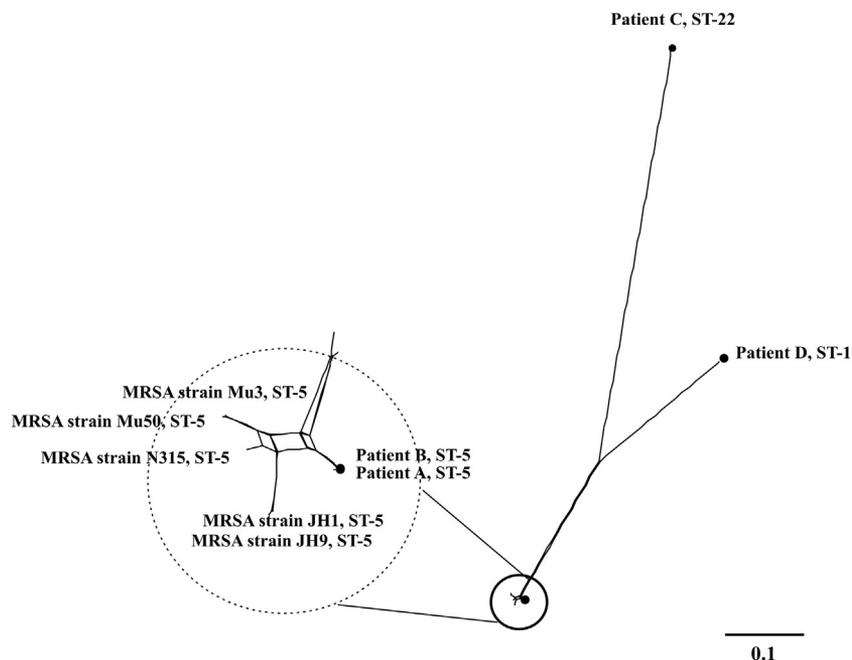


Fig. 1. Neighbor-Net analysis from core genome SNP alignment (40,424 SNPs) from the four MRSA isolates (A–D) against five type strains (MRSA strain JH1, JH9, Mu50, Mu3, and N315) using uncorrected (observed, “P”) distances. Scale bar indicates number of SNPs per base pair of aligned core genome.

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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