Whole-genome sequencing for analysis of an outbreak of meticillin-resistant \textit{Staphylococcus aureus}: a descriptive study


Summary

Background The emergence of meticillin-resistant \textit{Staphylococcus aureus} (MRSA) that can persist in the community and replace existing hospital-adapted lineages of MRSA means that it is necessary to understand transmission dynamics in terms of hospitals and the community as one entity. We assessed the use of whole-genome sequencing to enhance detection of MRSA transmission between these settings.

Methods We studied a putative MRSA outbreak on a special care baby unit (SCBU) at a National Health Service Foundation Trust in Cambridge, UK. We used whole-genome sequencing to validate and expand findings from an infection-control team who assessed the outbreak through conventional analysis of epidemiological data and antibiogram profiles. We sequenced isolates from all colonised patients in the SCBU, and sequenced MRSA isolates from patients in the hospital or community with the same antibiotic susceptibility profile as the outbreak strain.

Findings The hospital infection-control team identified 12 infants colonised with MRSA in a 6 month period in 2011, who were suspected of being linked, but a persistent outbreak could not be confirmed with conventional methods. With whole-genome sequencing, we identified 26 related cases of MRSA carriage, and showed transmission occurred within the SCBU, between mothers on a postnatal ward, and in the community. The outbreak MRSA type was a new sequence type (ST) 2371, which is closely related to ST22, but contains genes encoding Panton-Valentine leucocidin. Whole-genome sequencing data were used to propose and confirm that MRSA carriage by a staff member had allowed the outbreak to persist during periods without known infection on the SCBU and after a deep clean.

Interpretation Whole-genome sequencing holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

Funding UK Clinical Research Collaboration Translational Infection Research Initiative, Wellcome Trust, Health Protection Agency, and the National Institute for Health Research Cambridge Biomedical Research Centre.
6) The authors initially use a simple phenotypic typing technique to identify isolates, which they suspect to be part of the same outbreak. What method is this? By applying Whole Genome Sequencing (WGS), they then use two different genotypic typing techniques to investigate the isolates further. Which two genotypic typing techniques are these?

7) Based on the phenotypic method above, how many cases does the Infectious control unit finally conclude to be linked to the outbreak in the SCBU (before WGS was performed) and how many cases did they exclude? Did the subsequent WGS analysis agree to this conclusion and if not, where did the two analysis results differ?

8) Why does the primary investigator have difficulties establishing if the putative outbreak had extended over 6 months? Why is this issue important in typing/outbreak investigations?

9) Based on additional sampling in combination with WGS analysis, what do the authors conclude is the most likely source for the MRSA outbreak? How do the authors come to this conclusion? Is this a likely explanation for the spread of MRSA in hospital settings?

10) Suggest a strategy for what the hospital should have done in order to prevent the spread of MRSA before or while the outbreak was occurring ("in real time"). Could WGS have been part of this strategy with the current technology?
6. The authors initially use a simple **phenotypic typing technique** to identify isolates, which they suspect to be part of the same outbreak. **What method is this?** By applying Whole Genome Sequencing (WGS), they then use two different genotypic typing techniques to investigate the isolates further. Which two genotypic typing techniques are these?

**Procedures**

We cultured MRSA from screening swabs and clinical specimens by plating on to MRSA selective medium (Brilliance MRSA chromogenic medium, Oxoid, Basingstoke, UK). We **identified** bacteria with a commercial latex agglutination kit (Pastorex Staph Plus, Bio Rad Laboratories, Hemel Hempstead, UK). We did **antimicrobial susceptibility testing with disk diffusion**\(^2\)
The authors initially use a simple phenotypic typing technique to identify isolates, which they suspect to be part of the same outbreak. What method is this? By applying Whole Genome Sequencing (WGS), they then use two different genotypic typing techniques to investigate the isolates further. Which two genotypic typing techniques are these?

Whole-genome sequencing was done with an Illumina MiSeq (Illumina, San Diego, CA, USA) to generate 150 bp paired end reads, and interpreted by an investigator (SRH) who was masked to all clinical, epidemiological, and microbiological information. The genome data has been deposited in the European Nucleotide Archive (appendix). We aligned sequence reads to chromosome (accession number HE681097) and (CP002148) of a reference isolate (HO 5096) to identify single-nucleotide polymorphisms (SNP) insertions or deletions. This reference isolate was identified by multilocus sequence typing as sequence type (ST)
7. Based on the **phenotypic method above**, how many cases does the Infectious control unit finally conclude to be linked to the outbreak in the SCBU (before WGS was performed) **and how many cases did they exclude**? Did the subsequent WGS analysis agree to this conclusion and if not, where did the two analysis results differ?

**Results**

In 2011, we noted three contemporaneous cases of MRSA carriage on the SCBU at the CUH (patients 11–13; figure A). We regarded these cases as a possible outbreak because of the temporal clustering of the cases and identical (patient 11 and patient 12) or near identical (patient 13) antibiotic susceptibility profiles (appendix). The infection-control team did an investigation in which they completed a systematic review of MRSA isolates from the SCBU during the preceding 6 months. This review identified another 13 infants with one or more positive MRSA screens (patients 1–10 in figure A, and a further three cases not shown). Comparison of the antibiograms (appendix) showed that isolates from eight of the 13 infants (patient 2, patients 4–10, figure A) had an identical pattern to isolates from patient 11 and patient 12, whereas isolates from the five other cases differed by at least two antibiotics and were therefore excluded by the infection control investigation from the suspected outbreak on the basis of usual practice in our hospital (patient 1, patient 3 and the three cases not shown). Thus, 11 infants (patient 2 and patients 4–13) were regarded by the infection-control team to be putatively linked (figure A). Mapping of these 11 cases to a timeline showed
7. Based on the phenotypic method above, how many cases does the Infectious control unit finally conclude to be linked to the outbreak in the SCBU (before WGS was performed) and how many cases did they exclude? **Did the subsequent WGS analysis agree to this conclusion** and if not, where did the two analysis results differ?

which was not identified before sequencing. A phylogenetic tree based on core SNPs identified a unique, highly related cluster made up of all ST2371 isolates (figure B). Only 20 SNPs differentiated these 14 isolates from each other, compared with a mean of 550 SNPs between ST2371 isolates and the reference ST22 genome. In view of the epidemiological data, we regarded this finding as consistent with the involvement of 14 isolates in the SCBU outbreak.

The infection-control team had correctly excluded three isolates (ST1, ST8, and ST22) from the outbreak, but had also incorrectly excluded two isolates (patient 1 and patient 3; figure A) that belonged to the ST2371 clone. This decision had been made because these two isolates differed from the outbreak antibiogram by two antibiotics (appendix). In retrospect, this difference in susceptibility pattern proved to be incorrect because repeat antimicrobial susceptibility testing confirmed these two clones had the same profile as the outbreak. Additionally, whole-genome sequencing suggested that the outbreak spanned the MRSA-free periods on the SCBU.
8. **Why** does the primary investigator have difficulties establishing if the putative outbreak had extended over 6 months? **Why is this issue important** in typing/outbreak investigations?

Because there are two “gaps” in the epidemiological data (grey in Figure A). The current data can not explain how e.g. patient 4 (P4) was infected, as this patient did not have contact with P1-P3.

This is important as typing can not be used alone to define an outbreak. It can be used to detect possible outbreaks, but to define an outbreak, one will also need epidemiological data (Same time, same source, same contact etc).
9. Based on additional sampling in combination with WGS analysis, what do the authors conclude is the most likely source for the MRSA outbreak? How do the authors come to this conclusion? Is this a likely explanation for the spread of MRSA in hospital settings?

introduction from an external source was possible. The combined whole-genome sequencing and epidemiological data were presented at a meeting of the infection-control team, senior clinicians, nurses, and managers on the SCBU, which resulted in the decision to screen staff members. After obtaining written informed consent, the infection-control team screened 154 SCBU staff members for MRSA colonisation. One staff member was positive for MRSA, which was confirmed as ST2371 with whole-genome sequencing. The staff member was relieved from clinical duties and reviewed by the occupational health department. The staff member had no skin lesions and underwent successful MRSA decolonisation.
10. **Suggest a strategy** for what the hospital should have done in order to prevent the spread of MRSA before or while the outbreak was occurring ("in real time"). **Could WGS have been part of this strategy with the current technology?**

Retrospectively, the hospital should have made a continuous monitoring of all staff members for MRSA.

Yes in principle, WGS could be used as it is fast enough to type and analyze isolates within days ("real time"). The data presented in the Harris paper also shows that the typing results are more trustworthy than e.g. antibiograms. However, due to the cost of WGS, simpler typing tools might still be considered for monitoring to minimize the costs.

In fact, the use of NGS for real-time typing is currently being investigated at Hvidovre hospital, which Henrik Westh already presented to us two weeks ago.