



Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study

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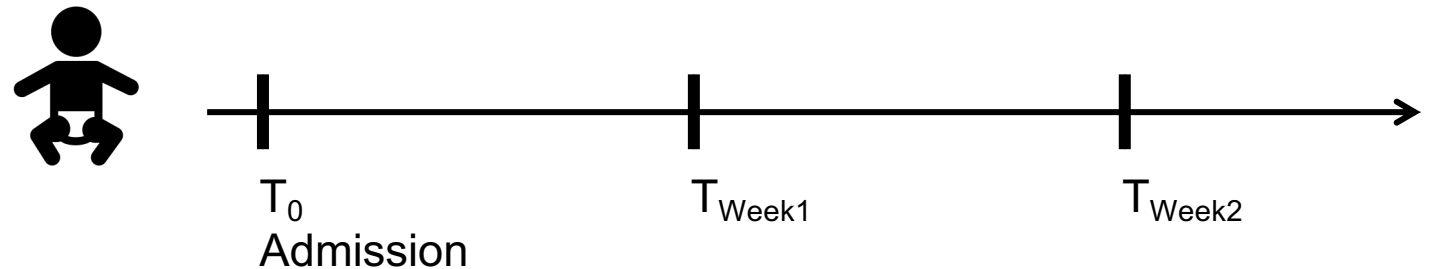
Lancet Infectious Diseases, 2013

Background

- In the Rosie Hospital (UK) all babies are MRSA **screened** on admission and once per week thereafter
- In 2011, an MRSA outbreak in a special care **baby unit** (SCBU) was detected
- Conventional methods, i.e. profiles of antibiotic susceptibility testing identified **12 linked cases** out of 17 tested isolates (6 months retrospectively)
- Ward was deep **cleaned**



cuh.nhs.uk/rosie-hospital/

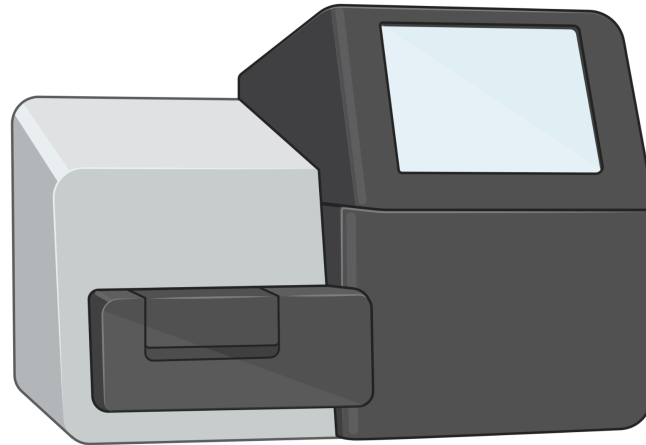


- Aim: Retrospectively compare the current standard procedure to WGS



Assessing the interventions by whole-genome sequencing

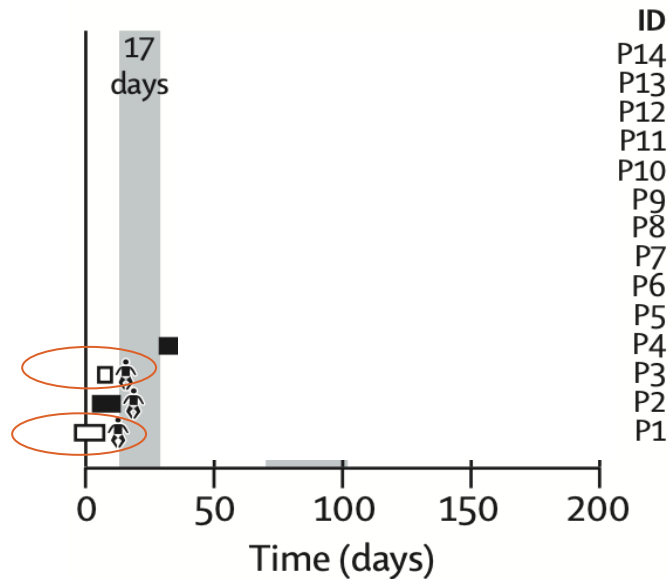
- Restreaked all 17 infant samples
- DNA extracted, then **sequenced** by Illumina MiSeq (150bp paired end reads)
- Compared to a **reference genome**: sequence type 22, 80% of all HA MRSA in the UK



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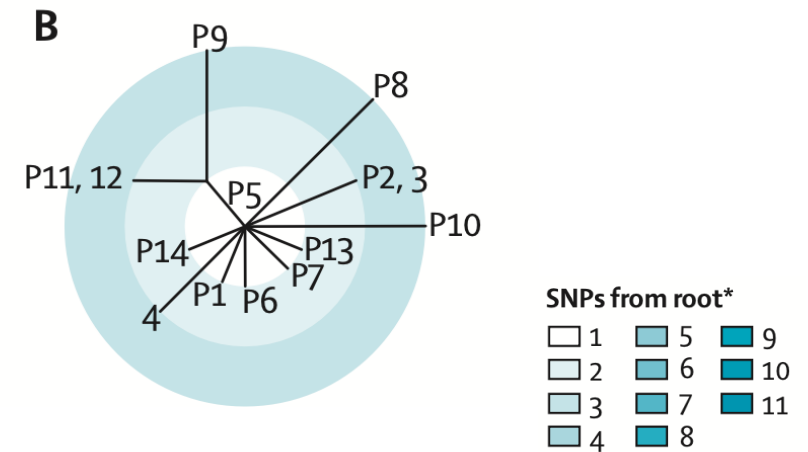
Assessing the interventions by whole-genome sequencing lead to more detected cases in the outbreak and ST2371

- 14/17 were distant to reference (by \approx 550 SNPs) but similar to each other (by max. 20 SNPs)
- New sequence type: **ST2371**
- Panton-Valentine Leucocidin (**PVL**) positive



Epidemiological map

2 additional connected isolates found

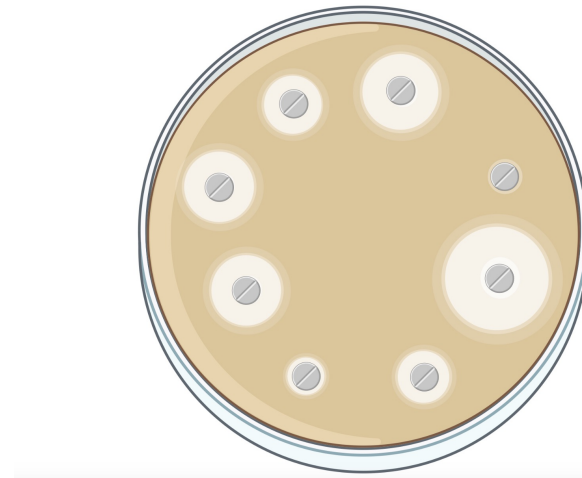


Phylogenetic Tree

Cases are **closely connected**: only a few single nucleotide polymorphisms

Targeted MRSA sequencing from the database and ongoing samples

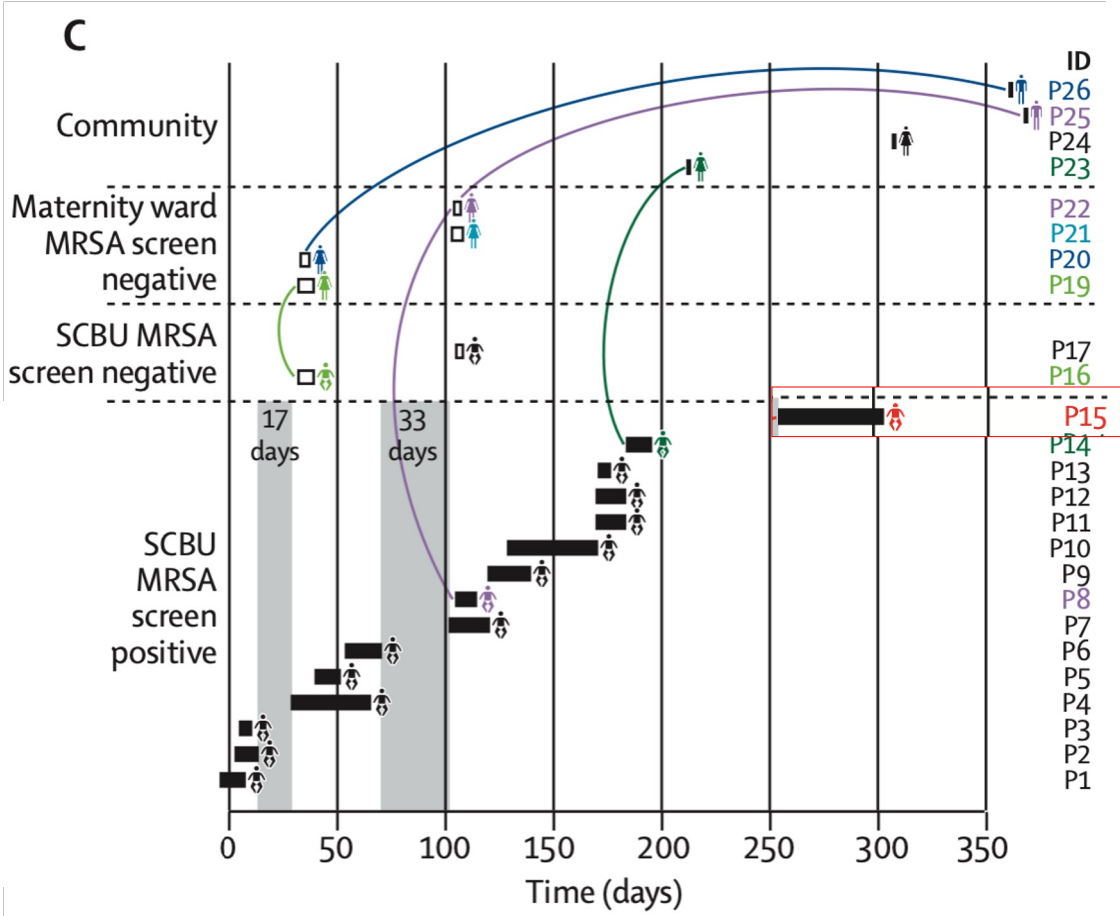
- Tested **stored MRSA samples** of the entire database for **antibiotic susceptibility** by disk diffusion
- **WGS** performed, if antibiogram is max. one antibiotic different from the profile of the outbreak group



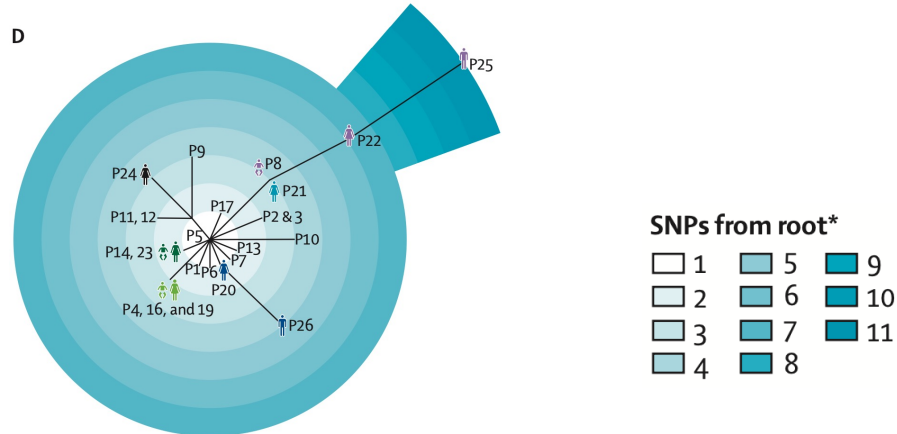
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Targeted MRSA sequencing from the database and ongoing samples lead to even more connected isolates

- 10/19 with the same ST type



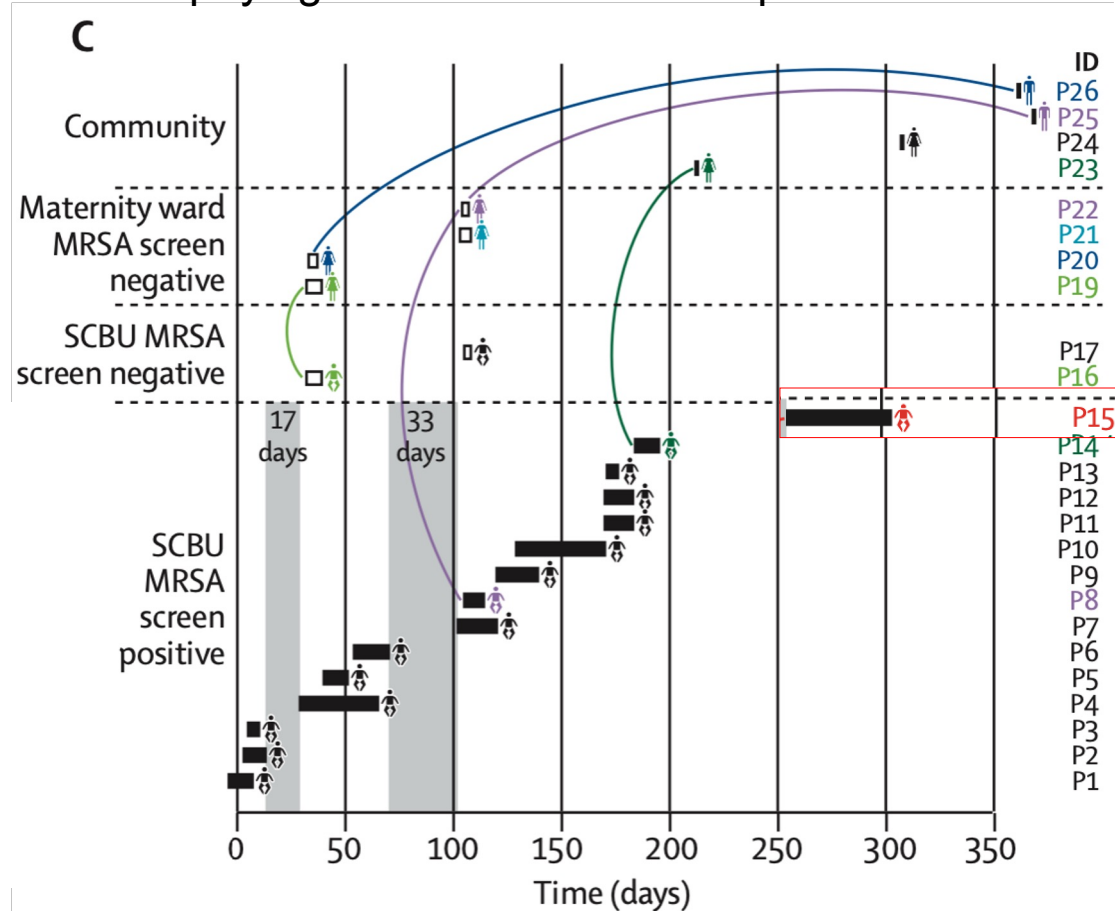
1. Transition to the future
2. Outbreak still ongoing in non-SCBU
3. Connection between HA and CA



Cases are **closely connected**, but no “time corresponding distance” detectable

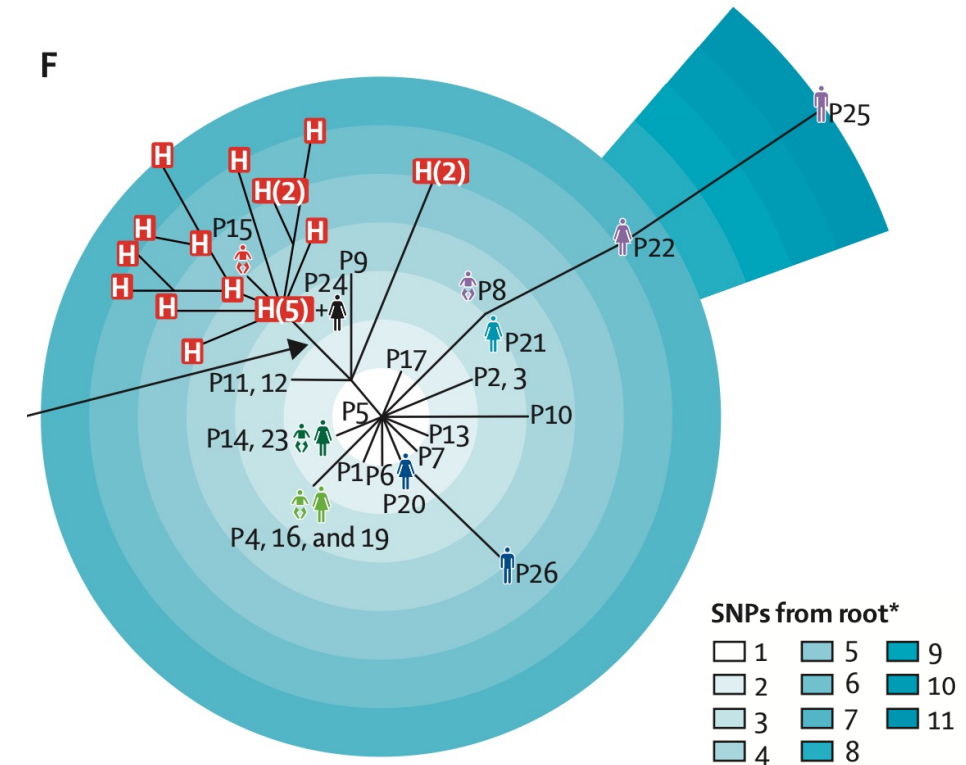
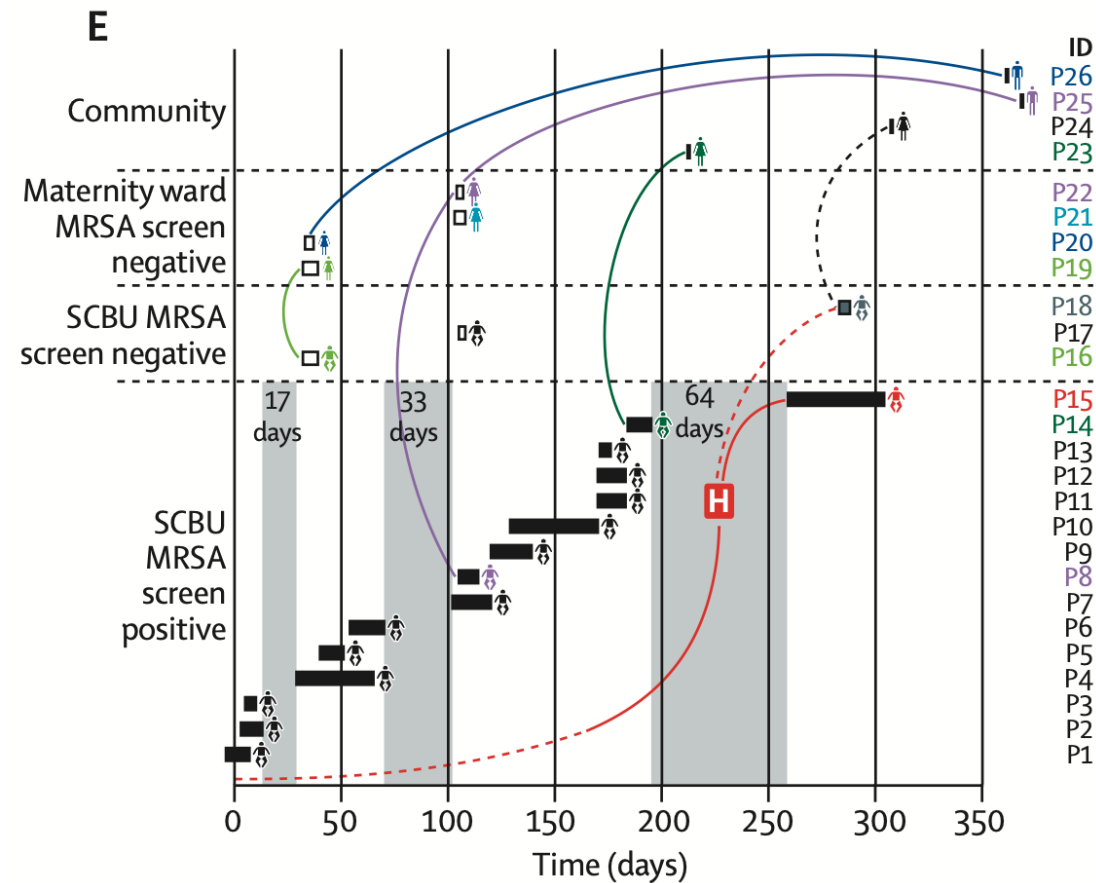
The appearance of yet another MRSA baby lead to a suspicion

- **New MRSA baby** with the same ST after 64 days after the departure of the last known infected baby
- SCBU also deep cleaned in the meantime
- The phylogenetic tree indicates “repeated introduction from an **external source**” was possible



A healthcare worker was MRSA positive and likely the missing link between the babies

- 1/154 **healthcare worker** from the SCBU also had the same ST
- Relieved from work, decolonisation

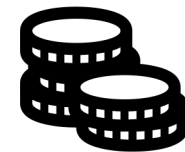
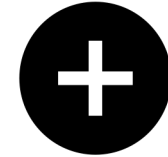


Profiles of HCW and last MRSA baby very similar. Also aligns with mutations/time.

Conclusion

WGS is not only comparable but more precise than standard methods

- **Additional samples discovered** (26 instead of only 12)
- Standard methods wrongly excluded cases based on AST (which was not reproducible → confirmed inclusion)
- Outbreak localised not only at the ward but also within the hospital and community
- Contribution to the identification of the **MRSA reservoir** (source)
- Lower **costs**: The outbreak caused ca. >10'000£, whereas sampling WGS is (was) 95£/sample
 - 36 samples x 95£ = 3'420£



Review

Positives

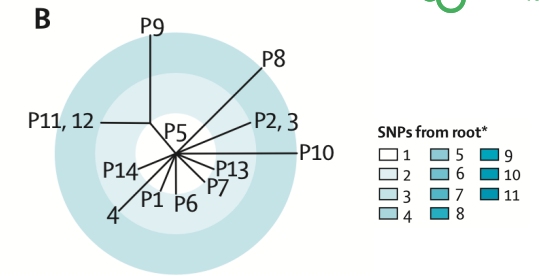
- Good storyline; one conclusion leads to the other, one question to the next
- Intuitive illustration of trees and SNP differences
- Significance: from phenotypic (AST) to genotypic (WGS) characterisation

Limitations

- Definition of an outbreak/cluster? → 2013
- Non-related samples not included to differentiate from clusters
- Unique situation with NHS: samples from family doctors could be assessed as well
- SNP mutation rate calculations for the duration of HW colonisation unclear
- A graphical timeline would facilitate interpretation a lot

Further reading

- “Evolution of *Staphylococcus aureus* and MRSA during outbreaks” Jodi A. Lindsay, 2016
- “A pseudo-outbreak of MRSA due to laboratory contamination related to MRSA carriage of a laboratory staff member”, Karlijn M. G. Houkes *et al.*, 2023
- “Outbreak investigation including molecular characterization of community-associated methicillin-resistant *Staphylococcus aureus* in a primary and secondary school in Eastern Switzerland” Frederike Waldeck *et al.* (incl. Tim Roloff, Adrian Egli), 2022

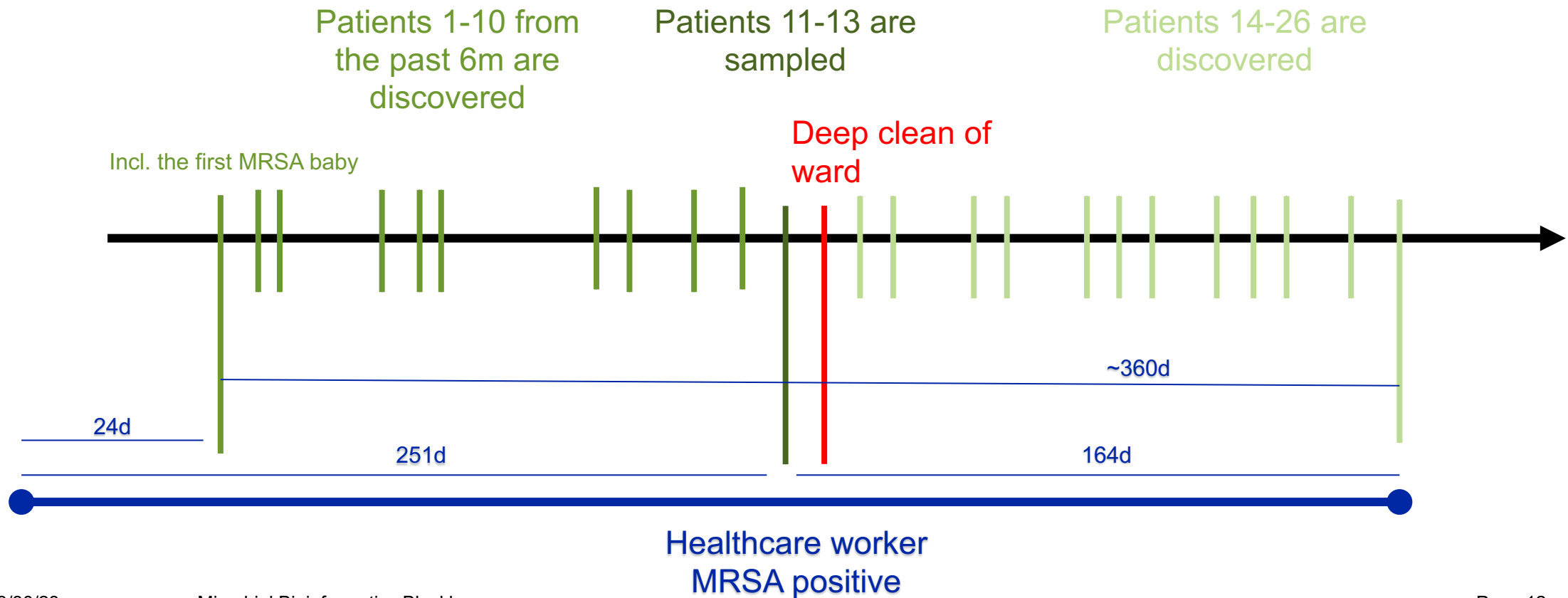


Appendix



Timeline

- Mutation rate of ST22: 1 SNP per 15 week
- HW has been positive for $\sim 251 + 164d = 415d = 59.3w$ dh 3.9SNPs...?
- Difference to last MRSA baby (Pat 15): max. 2SNPs



How are the different patients related? Clinical presentation?

	Description	Type of infection	Presentation	Treatment
Patient 1	Infant (part of outbreak)	Superficial pustules	Inpatient	MRSA decolonisation therapy
Patient 2	Infant (part of outbreak)	Superficial pustules	Inpatient	MRSA decolonisation therapy
Patient 4	Infant (part of outbreak)	Superficial pustules	Inpatient	MRSA decolonisation therapy
Patient 11	Infant (part of outbreak)	Superficial pustules	Inpatient	MRSA decolonisation therapy
Patient 16	Infant on SCBU for 5 days during outbreak, negative MRSA screen	Abscess (chest wall)	13 days after discharge from SCBU	One ED visit, one hospital outpatient clinic visit, four primary care visits, MRSA decolonisation therapy
Patient 17	Infant, on SCBU for 3 days during outbreak, negative MRSA screen	Abscess (cheek)	29 days after discharge from SCBU	One primary care visit, MRSA decolonisation therapy
Patient 19	Mother of patient 16 (a known MRSA carrier on SCBU)	Abscess (breast)	21 days after infant discharged from SCBU	Two breast surgery clinic visits, one ED visit, two primary care visits, MRSA decolonisation therapy
Patient 20	Mother of infant not on SCBU; contact in postnatal ward with mothers of MRSA positive infants in SCBU	Abscess (breast)	17 days after discharged home following delivery	Four breast surgery clinic visits, one ED visit, one primary care visit, 3 days of inpatient treatment, MRSA decolonisation therapy
Patient 21	Mother of infant not on SCBU; contact in postnatal ward with mothers of MRSA-positive infants in SCBU	Abscess (breast)	26 days after discharged home following delivery	Four breast surgery clinic visits, one ED visit, one primary care visit, MRSA decolonisation therapy
Patient 22	Mother of infant patient 8 (known MRSA carrier on SCBU)	Abscess (thigh)	175 days after MRSA carriage detected in own infant	One primary care visit, MRSA decolonisation therapy
Patient 23	Mother of infant patient 14 (known MRSA carrier on SCBU)	Abscess (breast)	18 days after MRSA carriage detected in own infant	One ED visit, one breast surgery clinic visit, MRSA decolonisation therapy
Patient 24	Mother of patient 18 (MRSA-screen negative infant on SCBU)	Abscess (breast)	11 days after own infant was discharged from SCBU	Four breast surgery clinic visits, MRSA decolonisation therapy
Patient 25	Partner of patient 22 and father of infant patient 8	Abscess (ear)	257 days after MRSA carriage detected in infant, and 82 days since partner presented with abscess on thigh	One primary care visit, one ED visit, three hospital outpatient clinic visits, 1 day of inpatient treatment, MRSA decolonisation therapy
Patient 26	Partner of patient 20	Abscess (thigh)	325 days after mother and infant discharged home after delivery	One primary care visit, MRSA decolonisation therapy

MRSA=meticillin-resistant *Staphylococcus aureus*. SCBU=special care baby unit. ED=emergency department.

Table: Clinical infections and transmission of MRSA sequence type 2371

Which difficulties did the previous standard face? Why didn't they find all the connected cases?

Previous standard: compare antibiotic susceptibility patterns

- Gaps in MRSA cases (17, 33, 64d)
- Cases also from outpatients or family doctors → problematic separation from HA and CA MRSA
- Delay between MRSA cases in baby units and community MRSA cases
- MRSA screens only 1xweek; potential transmission in between
- Inconsistent antibiotic susceptibility tests (first different pattern, if redone then also to outbreak)

- use of whole-genome sequencing (WGS) to investigate a hospital outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA).
- The authors used WGS to sequence the MRSA isolates collected during the outbreak and performed a phylogenetic analysis to infer the evolutionary relationships among the isolates.
- compared the WGS data with traditional typing methods, such as pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST).
- utility of WGS for outbreak investigation.
- highlights the limitations of traditional typing methods and the advantages of WGS, such as higher resolution and accuracy.
- descriptive study, and therefore, no causal inference can be made. Unknown impact of WGS on patient outcomes, the generalizability of the findings to other settings (hospitals) is unclear. Cost assessment?
- In conclusion, the paper by Harris et al. provides valuable insights into the use of WGS for outbreak investigation. It demonstrates its potential to improve the accuracy and resolution of epidemiological studies. However, further research is needed to determine the impact of WGS on patient outcomes and to assess the generalizability of the findings to other settings.

Sorry, I was thinking about food, what exactly happened?

We've discussed a paper from 2013 which introduces WGS as a new tool for outbreak detection with an applied example:

- An MRSA outbreak in an infant ward (special care baby unit, SCBU) in Cambridge, UK was detected
- Conventional methods, e.g. profiles of antibiotic susceptibility testing identified 12 linked cases out of 17 tested isolates
- After reviewing these, whole-genome-sequencing WGS identified 14 strains
 - 3 of the sequenced strains were identified as known sequence types (STs)
 - The other 14 sequenced strains were a new sequence type: ST2371 and had a very similar profile to each other (20 SNPs difference)
 - ST2371 isolates differ in 550 single-nucleotide-polymorphisms (SNPs) when compared to the reference genome ST22, the most common ST in UK HA MRSA (80%)
- In addition to these 14 cases in the SCBU, another 10 highly similar cases were detected after expanding the search (mothers, partners, other patients with similar antibiograms etc.)
 - difference by only 34 SNPs!
- Patient 15 was detected 64d after the departure of patient 14 and after a thorough deep clean of the ward and also has ST2371
- Indications of re-infection, as a new patient after deep clean and based on the phylogenetic tree (no time-correlated “evolution”/ similarity)
- After consultation, screening of 154 SCBU staff, of which one was indeed MRSA positive, even with ST2371...
- Staff member could be decolonised, infection chain could be stopped
- Happy end

