



Uncluttering Case Clusters: Use of Rapid Whole Genome Sequencing to Exclude Transmission Events

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BACKGROUND

- Newly identified multi-drug resistant organisms (MDRO) isolated from hospitalized patients with shared epidemiological characteristics can either represent transmission events or independent, unrelated acquisitions, or pseudo-outbreaks.
- Whole genome sequencing (WGS) can improve the efficiency of investigations triggered by MDRO cases with apparent epidemiological linkages by early exclusion of clonality.
- We report an implementation of WGS applied to the investigation of a cluster of methicillin-resistant *Staphylococcus aureus* (MRSA) [Investigation #1] in our Neonatal Intensive Care Unit (NICU) and a cluster of carbapenem-resistant Enterobacterales (CRE) in our Respiratory Acute Care Unit (RACU) [Investigation #2].
- For each respective cluster, these inpatient nosocomial infections were initially considered to represent possible nosocomial transmission based on time and location of identification.

OBJECTIVE/DESIGN

- To investigate whether transmission events occurred between patients.
- WGS was applied to determine clonality.
- Investigations were initiated as part of routine infection control practices.
- The two investigations were conducted as retrospective case studies.

METHODS

- Investigation #1 study participants included five NICU patients with nosocomial MRSA isolates recovered between June and August 2020.
- Investigation #2 involved two RACU patients with nosocomial CRE infections in October 2020.
- Routine unit surveillance activities and characterization using standard epidemiologic criteria identified the isolates as nosocomial to their respective unit.
- The isolates were then submitted for epiXact, a rapid WGS and clonal transmission analysis service offered by Day Zero Diagnostics. The epiXact pipeline uses Illumina high depth whole genome sequencing and a reference mapping approach to quantify single nucleotide polymorphism (SNP) differences between isolates to determine genomic relatedness.

RESULTS: Investigation #1 MRSA

- The MRSA cluster included five neonates with either clinical or surveillance isolates.
- All MRSA isolates were identified over a period of 60 days. (Figure 1)
- WGS identified one of the five isolates as methicillin susceptible *S. aureus* due to the absence of the *mecA* or *mecC* resistance gene, despite growth on chromogenic MRSA screening agar. Follow up re-phenotyping confirmed WGS results.
- WGS revealed each of the five isolates as belonging to a distinct multilocus sequence type (MLST) with thousands of SNP differences between samples. (Figure 2)
- WGS results suggest that a nosocomial transmission linking these infections was highly unlikely.
- WGS returned results within thirty-six hours of sample receipt.

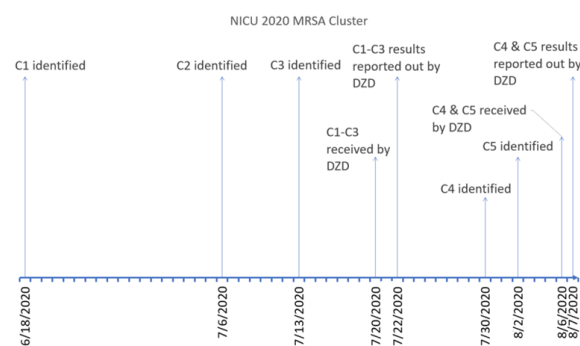


Figure 1- Timeline for NICU MRSA Cluster

Staph. aureus	SA-1	SA-2	SA-3	SA-4	SA-5
SA-1		>40k	>40k	2308	>20k
SA-2			>40k	>40k	>40k
SA-3				>40k	>40k
SA-4					>40k
SA-5					

Figure 2- Panel showing single nucleotide polymorphism (SNP) differences between MRSA isolates

ST-5	ST-45	ST-30	ST-105	ST-8
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RESULTS: Investigation #2 CRE

- The CRE cluster included two patients with *Klebsiella pneumoniae* isolated from clinical cultures within five days of each other on the same clinical unit. (Figure 3)
- The two isolates had identical antimicrobial susceptibility profiles.
- WGS of the two isolates revealed that they belonged to different MLSTs and had tens of thousands of differing SNPs. (Figure 4)
- WGS results suggest that a nosocomial transmission linking these infections was highly unlikely.
- WGS returned results within thirty-six hours of sample receipt.

Figure 3- Timeline for RACU CRE Cluster

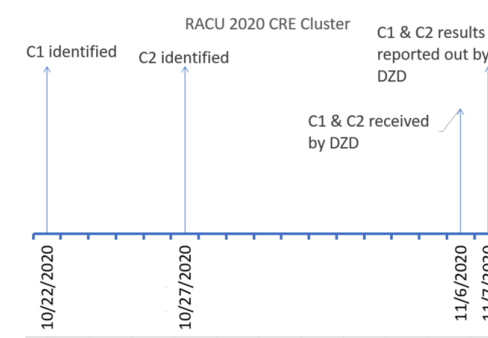


Figure 4- Panel showing single nucleotide polymorphism (SNP) differences between CRE isolates

Kleb. pneumo	KP-1	KP-2
KP-1		>30k
KP-2		
MLST Type	ST-219	ST-11

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Conclusions

- Suspected transmission events can be resource intensive to investigate and manage.
- The application of rapid WGS allowed for early discontinuation of investigations and conservation of resources.

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