



Whole Genome Sequencing Enabled Identification of Undetected Putative Infection Transmission in a NICU

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Introduction

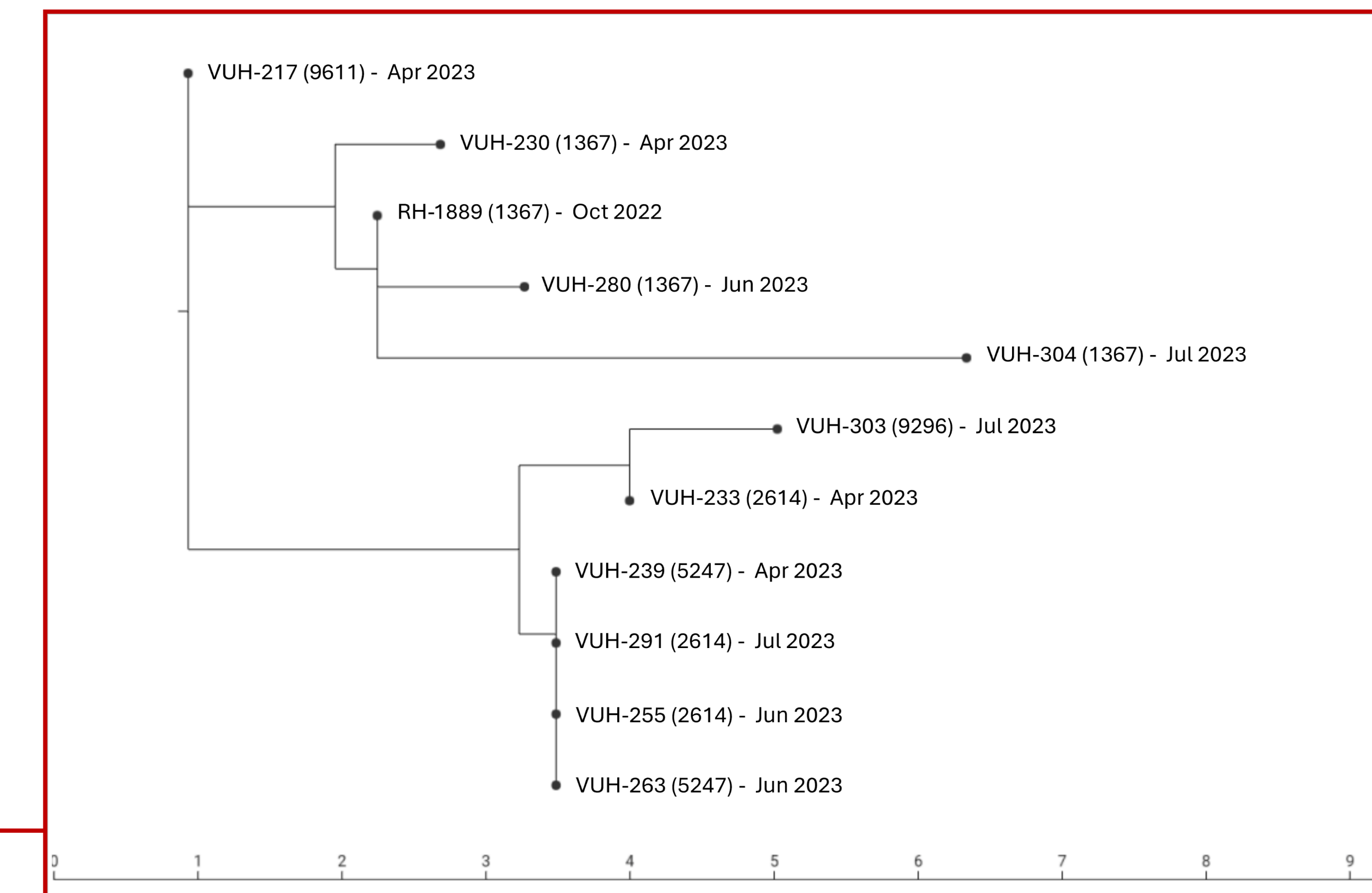
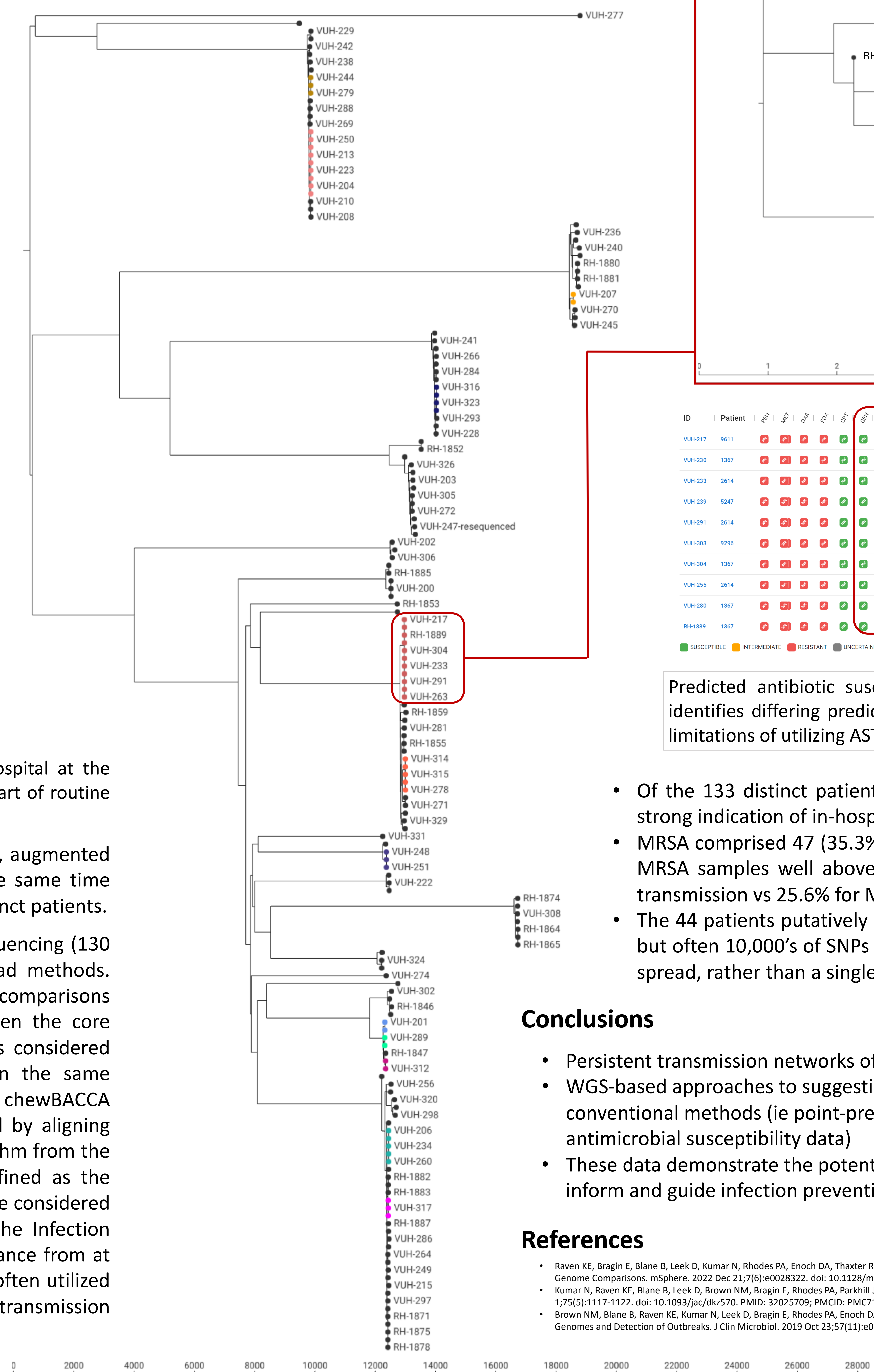
- *Staphylococcus aureus* colonization of infants admitted to a neonatal intensive care unit (NICU) can lead to invasive infection in this highly vulnerable population. As patients admitted to a NICU have generally not received care outside of the hospital environment, colonization with *S. aureus* is presumed to occur via in-hospital transmission. Such transmission can occur from family members or caregivers, yet more concerning is possible transmission from healthcare providers or the hospital environment itself.
- Infection prevention efforts employed by hospital epidemiology services are aimed at detecting colonization and preventing in-hospital transmission. Contemporary methods utilize culture-based and/or molecular-based screening. These methods can detect the presence or absence of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in a single patient, as well as provide measures of point-prevalence for colonization in a NICU. However, these methods cannot establish relatedness to suggest transmission and aid hospital epidemiologists in prevention efforts.
- Whole genome sequencing (WGS) of cultured isolates allows relatedness determination and suggests transmission events if isolates from different patients are closely related. WGS studies are often retrospective and triggered by suspected clusters of infection; it is not clear how much transmission is undetected. Prospective screening with WGS can potentially meet this need by providing evidence for relatedness and potential transmission. Here we report the results of using WGS to detect transmission, in a 100-bed NICU of a major medical center.

Methods

- **Screening:** All infants admitted to the NICU of Monroe Carell Jr. Children's Hospital at the Vanderbilt University Medical Center were tested for *S. aureus* colonization as part of routine unit-based active surveillance cultures on 3 occasions: April, June and July 2023.
- **Specimens:** 135 *S. aureus* screening isolates (46 MRSA, 34%; 30 MSSA, 66%), augmented with 41 *S. aureus* isolates from sterile site infections in the NICU over the same time period, were submitted for sequencing. Isolates were obtained from 133 distinct patients.
- **Sequencing and Bioinformatics:** 171 isolates met quality thresholds for sequencing (130 screening, 41 invasive). Isolates were sequenced using standard short read methods. Relatedness between all 171 samples was computed (171 * 170 / 2 = 14,535 comparisons were made) based on the number of SNP's and indel's observed between the core genomes; thus cgSNP rather than cgMLST was utilized. This method was considered preferable to cgMLST because it takes account of multiple SNPs within the same gene. Core genes in the assemblies of each strain were identified using chewBACCA software and core gene lists from cgmlst.org. Relatedness was computed by aligning corresponding alleles of core genes with the Striped Smith-Waterman algorithm from the scikit.bio package. The resulting distance between two samples was defined as the number of SNPs plus the number of nonterminal indels. Sets of samples were considered putatively connected by transmission, subject to further assessment by the Infection Prevention team, if each sample within the set was 6 or fewer SNPs in distance from at least one other sample in the set. A conservative 6 SNP cutoff, lower than often utilized in the literature, was chosen to enhance the likelihood of correlation with transmission when pairs of samples met that relatedness requirement.

Results

Dendrogram of all sequenced isolates. "VUH indicates screening isolate, "RH" indicates sterile-site isolate.



Focused MRSA transmission network. De-identified patient IDs are in parenthesis, followed by month and year of collection. Transmission of related isolates spans patients and collection dates.

ID	Patient	Apr	Jun	Jul	Apr	Jun	Jul	Apr	Jun	Jul	Apr	Jun	Jul	Apr	Jun	Jul	Apr	Jun	Jul	Apr	Jun	Jul
VUH-217	9611	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-230	1367	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-233	2614	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-239	5247	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-291	2614	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-303	9296	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-304	1367	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-255	2614	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VUH-280	1367	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
RH-1889	1367	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Predicted antibiotic susceptibility of focused MRSA transmission network. Boxed-section identifies differing predicted AST profiles between related isolates, demonstrating potential limitations of utilizing AST to determine relatedness.

- Of the 133 distinct patients, 44 (33.1%) were found to have strains separated by <=6 SNPs, a strong indication of in-hospital transmission.
- MRSA comprised 47 (35.3%) of the patient-unique samples, with putative transmission amongst MRSA samples well above the frequency of MSSA (46.8% probability of MRSA connected by transmission vs 25.6% for MSSA).
- The 44 patients putatively connected by transmission hosted 14 distinct strains, sometimes 10's but often 10,000's of SNPs apart, suggesting frequent distinct entry of infection and then limited spread, rather than a single widely spread outbreak.

Conclusions

- Persistent transmission networks of MRSA and MSSA are present in this NICU.
- WGS-based approaches to suggesting relatedness provide detailed information surpassing that of conventional methods (ie point-prevalence measures, multi-locus sequence typing, and comparison of antimicrobial susceptibility data)
- These data demonstrate the potential of WGS to identify ongoing transmission, thereby helping to inform and guide infection prevention efforts.

References

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