Infection Control & Hospital Epidemiology (2020), **41**, 1402–1408
doi:10.1017/ice.2020.355

Original Article

Molecular epidemiology of methicillin-susceptible *Staphylococcus aureus* in infants in a neonatal intensive care unit

Hye-Kyung Cho MD, PhD[1], Joshua N. Yang MLS[1], Scott A. Cunningham MS[1], Kerryl E. Greenwood-Quaintance MS[1], Mary L. Dalton RN, CIC[2], Christopher A. Collura MD[3], Jennifer L. Fang MD[3], Angela L. Heinrich MSN, RN[3,4], W. Charles Huskins MD[2,5] and Robin Patel MD[1,6]

[1]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic Rochester, Minnesota, [2]Infection Prevention and Control, Mayo Clinic, Rochester, Minnesota, [3]Division of Neonatal Medicine, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, Minnesota, [4]Department of Nursing, Mayo Clinic, Rochester, Minnesota, [5]Division of Pediatric Infectious Diseases, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, Minnesota and [6]Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota

Abstract

Objective: To investigate the molecular epidemiology of methicillin-susceptible *Staphylococcus aureus* (MSSA) in infants in a neonatal intensive care unit (NICU) using whole-genome sequencing.

Design: Investigation of MSSA epidemiology in a NICU.

Setting: Single-center, level IV NICU.

Methods: Universal *S. aureus* screening was done using a single swab obtained from the anterior nares, axilla, and groin area of infants in the NICU on a weekly basis. Core genome multilocus sequence type (cgMLST) analysis was performed on MSSA isolates detected over 1 year (2018–2019).

Results: In total, 68 MSSA-colonized infants were identified, and cgMLSTs of 67 MSSA isolates were analyzed. Overall, we identified 11 cgMLST isolate groups comprising 39 isolates (58%), with group sizes ranging from 2 to 10 isolates, and 28 isolates (42%) were unrelated to each other or any of the isolate groups. Cases of infants colonized by MSSA were scattered throughout the 1-year study period, and isolates belonging to the same cgMLST group were typically detected contemporaneously, over a few weeks or a few months. Overall, 13 infants (19.7%) developed MSSA infections: bacteremia (n = 3), wound infection (n = 5), conjunctivitis (n = 4), and cellulitis (n = 1). We detected no association between these clinically manifest infections and specific cgMLST groups.

Conclusions: Although MSSA isolates in infants in a NICU showed high diversity, most were related to other isolates, albeit within small groups. cgMLST facilitates an understanding of the complex transmission dynamics of MSSA in NICUs, and these data can be used to inform better control strategies.

(Received 2 March 2020; accepted 16 July 2020; electronically published 16 September 2020)

Staphylococcus aureus is a major pathogen in hospitalized infants and is the second most common cause of late-onset neonatal sepsis.[1,2] These infections are associated with mortality, morbidity, and prolonged lengths of stay.[3–5] *Staphylococcus aureus* commonly exists as a colonizer of the nasal cavity, skin or gastrointestinal tract of humans, being found in ~20%–40% of healthy adults and 5%–55% of infants.[6–10] Neonates colonized with *S. aureus* have an increased risk of subsequent infection.[11–13] Newborns may acquire *S. aureus* during their birth or shortly thereafter, from a colonized mother, the hospital setting, or the community surrounding them.[9,14–16]

Since hospital-associated methicillin-resistant *S. aureus* (MRSA) first emerged in 1980s and became prevalent in neonatal intensive care units (NICUs), attention to *S. aureus* in NICUs has largely focused on MRSA, with relatively little consideration given to methicillin-susceptible *S. aureus* (MSSA).[9,17] Most centers have implemented strategies for *S. aureus* screening and isolation with or without decolonization, again, focused on MRSA.[18–20] However, MSSA infections are 3 times more common than MRSA infections among infants in NICUs, and both are associated with similar morbidity and mortality.[17,21,22]

Molecular epidemiological studies of MRSA in NICUs have shown a few clones to be responsible for spread[23]; in contrast, the molecular epidemiology of MSSA in this setting is less well defined. Traditionally, several methods, such as pulsed-field gel electrophoresis, surface protein A sequence (*spa*) typing, and multilocus sequence typing (MLST), have been used for molecular typing of *S. aureus* isolates. Recent studies have shown that molecular analysis using whole-genome sequencing (WGS) enables higher discrimination than conventional methods, allowing better understanding of molecular epidemiology.[23–25]

Author for correspondence: Robin Patel, E-mail: patel.robin@mayo.edu

Cite this article: Cho H-K, *et al.* (2020). Molecular epidemiology of methicillin-susceptible *Staphylococcus aureus* in infants in a neonatal intensive care unit. *Infection Control & Hospital Epidemiology*, 41: 1402–1408, https://doi.org/10.1017/ice.2020.355

We noted an increase in the MSSA colonization rate in our NICU beginning in May 2018. In this study, we analyzed the clinical and molecular epidemiology of MSSA in NICU infants at Mayo Clinic Rochester to describe clusters of MSSA colonization that occurred over a 1-year period.

Methods

Setting

This study was carried out in a level-4 NICU at Saint Mary's Campus, Mayo Clinic Hospital in Rochester, Minnesota. The NICU is a 34-bed unit caring for ~385 infants per year, with an average daily census of 15–33 patients (median, 24). The unit consists of 5 rooms with 4–6 bed spaces in each room, plus 6 additional single-room bed spaces for patients in need of isolation.

Screening

Beginning in April 2016, universal *S. aureus* screening was in place, using a single swab obtained from the anterior nares, axilla, and groin area of each infant in the NICU every Tuesday on a weekly basis.

Culture and identification

Swabs were plated on trypticase soy agar with 5% sheep blood agar and Columbia nalidixic acid (CNA) with 5% sheep blood agar plates, incubated at 35°C with CO_2, and examined for growth at 16–24 hours and, if negative, at 48 hours. Colonies were identified by gram staining and coagulase testing, and/or by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF-MS). Antimicrobial susceptibility testing was performed using agar dilution with results interpreted using Clinical and Laboratory Standards Institute guidelines.[26] Isolates were archived at −80°C in 0.9% sterile saline (Baxter, Deerfield, IL). When >1 MSSA isolate was detected from the same infant, only the first isolate was subjected to WGS.

Whole-genome sequencing

DNA was extracted from cultured MSSA isolates using the Zymo Research Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. Paired-end sequencing libraries were prepared using Nextera XT DNA Library Prep kits (Illumina, San Diego, CA) and WGS performed on an Illumina MiSeq (Illumina) using V2 2 × 250-bp chemistry targeting 200× depth of coverage. Raw sequencing reads were processed for adapter and index removal using the MiSeq reporter software in real time.

Genome assembly and core genome multilocus sequencing type (cgMLST) determination

FASTQ files were imported into SeqSphere+ version 6.0.2 software (Ridom, Munster, DE) and handled using an automated assembly and typing pipeline. The pipeline includes FastQC read quality control and Velvet de novo genome assembly. Assembled genomes were mapped against an *S. aureus* reference genome (NC_002951.2) to define the core genome. A core genome target threshold of ≥95% was applied to all assemblies. Isolate relatedness was based on the total number of core genome allelic differences between each isolate.

Results

cgMLST of MSSA isolates

Over the 1-year study period (May 2018 to May 2019), 68 MSSA-colonized infant cases were detected. The median (range) gestational age and birth weight were 28 weeks (range, 22–40) and 985 grams (range, 360–4,280), respectively. The proportions of infants born via vaginal delivery, and infants with central venous catheters were 39.7% and 52.9%, respectively. Of 68 isolates, 67 were subjected to cgMLST analysis. (One isolate could not be retrieved for sequencing.) Of the 67 isolates analyzed, cgMLST analysis identified 11 groups of related isolates based on their having ≤9 allelic differences (Fig. 1, groups 1–11) comprising a total of 39 isolates, with group sizes ranging from 2 to 10 isolates. Furthermore, 28 isolates were unrelated to each other and to any of the groups, differing by >100 allelic differences. Cases of infants colonized with MSSA were scattered throughout the study period; isolates belonging to the same relatedness group were detected over periods of a few weeks to a few months (Fig. 2).

Group 1, the largest group, included 10 isolates; group 1 isolates were detected from infants in 4 multibedded rooms (room 1–4) with overlapping stays from May to September 2018 (Fig. 3). Group 2 consisted of 4 isolates, 2 of which were obtained from twin infants (twin pair D) and were isolated after 2 months of their hospital admission. The other 2 isolates in group 2 were detected from 2 infants whose stays overlapped with each other but not with the twins. Group 3 included 4 isolates from infants who were in room 1 on the same day. Group 4 also consisted of 4 isolates; they were detected from infants housed in 3 rooms (rooms 1–3), and 2 of the affected infants were admitted to room 2 but not at the same time. Group 5 also included 4 isolates; these isolates were found in infants in 2 different rooms (rooms 1 and 3) but at the same time. Group 6 included 3 isolates from infants who had been born at different medical centers and who were housed with temporally overlapping stays in room 1 in the NICU. Groups 7, 8, 10, and 11 were comprised of 2 isolates each and were all found in infants in different rooms but who had temporally overlapping hospital stays (ie, for each related pair). The 2 group 9 isolates were from infants who shared room 4.

Among the MSSA-harboring infants, there were 5 pairs of twins (A–E), of which 3 (A, C, and E) had unrelated isolates between each twin pair, even though they stayed in the same room throughout their hospital stays (Figs. 1 and 2). Of the 2 pairs of twins with related isolates (B and D), 1 pair (B) had stayed in the same room and the other pair stayed (D) in different rooms (Fig. 3, room 2 and 6).

Clinical information

Among the colonized infants, 3 (4.5%) died during their hospitalization; no deaths were related to MSSA infection. Overall, 13 infants (19.7%) developed MSSA infection: bacteremia (n = 3), wound infection (n = 5), conjunctivitis (n = 4), and cellulitis (n = 1). The 3 bacteremia events involved isolates from different WGS groups. There was no association between isolates from infants with clinical infection and specific WGS groups. Among infants not colonized with MSSA, no bacteremia occurred through their discharge during the study period.

Antimicrobial susceptibility

Antimicrobial susceptibilities for MSSA isolates according to WGS group are shown in Supplemental Table 1 (online). All MSSA

https://doi.org/10.1017/ice.2020.355 Published online by Cambridge University Press

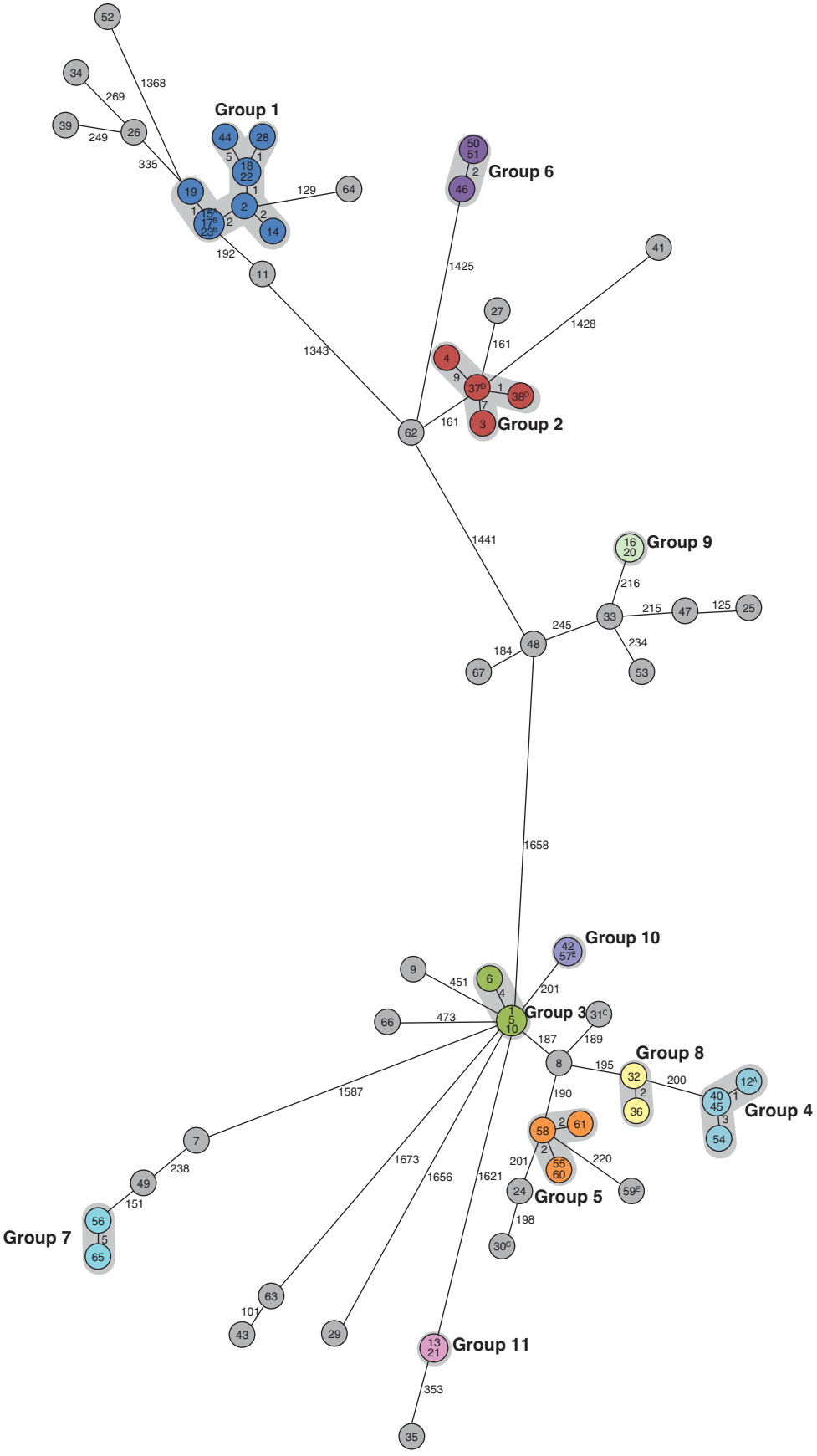

Fig. 1. Core genome multilocus sequence type (cgMLST) minimum spanning tree of methicillin-susceptible *Staphylococcus aureus* isolates from colonized neonatal intensive care unit infants. Numbers in each circle indicate isolate numbers, assigned chronologically by the date of isolation. Numbers next to lines indicate the numbers of allelic differences between isolates, as determined by cgMLST. Superscripted characters indicate pairs of twins (A–E); twin pair A (isolate 12 and 15), twin pair B (isolate 17 and 23), twin pair C (isolate 30 and 31), twin pair D (isolate 37 and 38), and twin pair E (isolate 57 and 59). Line lengths are not proportional to the number of allelic differences and angles between lines are randomly assigned. Background grey shading indicates isolates in the same group.

https://doi.org/10.1017/ice.2020.355 Published online by Cambridge University Press

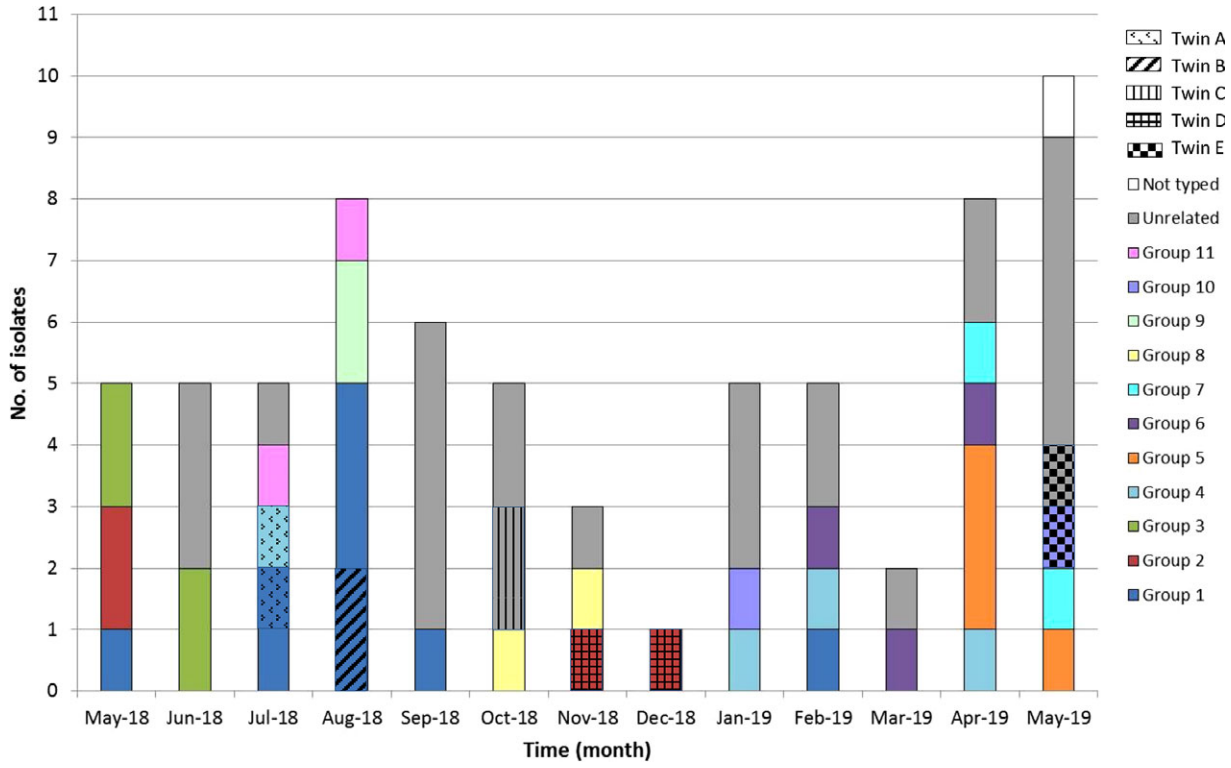

Fig. 2. Monthly distribution of methicillin-susceptible *Staphylococcus aureus* (MSSA) colonization in neonatal intensive care unit infants from May 29, 2018, to May 28, 2019, by cgMLST group. MSSA colonization was detected throughout the study period, with peaks in August–September 2018 and April–May 2019. Isolates in each cgMLST group were serially detected over several months. Among colonized infants, there were 5 pairs of twins, of which 2 pairs (twin pairs B and D) had related isolates, and 3 pairs (twin pairs A, C, and E) had unrelated isolates. Of the 2 pairs of twins with related isolates, 1 pair (twin pair B) had stayed in the same room and the other in different rooms.

isolates in groups 1, 2, 3, 6, 10, and 11 were susceptible to all tested antimicrobials, whereas all isolates in groups 5, 7, and 8 were resistant to clindamycin and all isolates in group 9 were resistant to doxycycline.

Control measures

Several control measures were implemented in an attempt to prevent additional spread of MSSA in the NICU. Infants who were identified as colonized with MSSA were cared for using standard precautions; contact precautions were not used unless there was a separate indication for the use of contact precautions or another isolation precautions category. As a part of standard precautions, staff were encouraged to wear gloves and gowns when they had close direct physical contact with an infant, such as while holding the infant during feeding. MSSA colonized babies were treated with nasal mupirocin twice daily for 5 days and chlorhexidine baths twice, 48 hours apart once they reached 36 weeks of postmenstrual age and were at least 72 hours old. From May 2019 onward, colonized or infected infants were cared for in single rooms or were isolated in cohorts in multibed rooms. Hand hygiene for staff and environmental cleaning were reinforced, including use of audits and adenosine triphosphate testing of high-touch surfaces. Hand hygiene compliance in the NICU during the study period was 40%, 63%, 53%, and 100% in July–September 2018, October–December 2018, January–March 2019, and April–June 2019, respectively. Parents were instructed at the time of their infants' admission and encouraged throughout their hospitalizations to perform hand hygiene prior to entering the NICU and prior to touching or holding their infants. Staff were advised to clean their mobile devices with disinfectant wipes at the beginning and end of each shift, when visibly soiled, after direct contact with patients or patient environments, and after exiting isolation rooms.

Discussion

The molecular epidemiology of MSSA in this study differed from that in our previous study,[23] which analyzed MRSA in the same NICU over 3 years using the same WGS methods. The previous MRSA analysis demonstrated that only a few clones were responsible for spread of MRSA and that spread occurred over a long period of time.[23] In contrast, in this study, 58% of MSSA isolates from NICU infants over the 1-year study period belonged to 11 small groups, with 42% being unique. Although the 2 studies were not conducted during the same periods, this finding suggests that the within-facility clonal diversity of MSSA is higher than that of MRSA. This finding is consistent with a previous molecular surveillance study of MSSA and MRSA colonization in infants, their parents, and medical staff in a NICU.[27] Although the molecular characterization methods differed from those used in our study, 15 patterns were identified among 25 MSSA isolates and 3 patterns were identified among 9 MRSA isolates, indicating higher clonal diversity of MSSA than MRSA.[27] Another study found heterogeneous MSSA colonization, with 16 shared strains and 22 unique strains among 85 isolates.[28] MRSA is likely to be acquired from limited sources and spread through shared spaces, equipment, and healthcare personnel, whereas MSSA likely originates from diverse sources. Notably, however, some studies have reported hospital-associated transmission of MSSA with a few predominant clones associated with infection and colonization in NICUs.[29,30]

https://doi.org/10.1017/ice.2020.355 Published online by Cambridge University Press

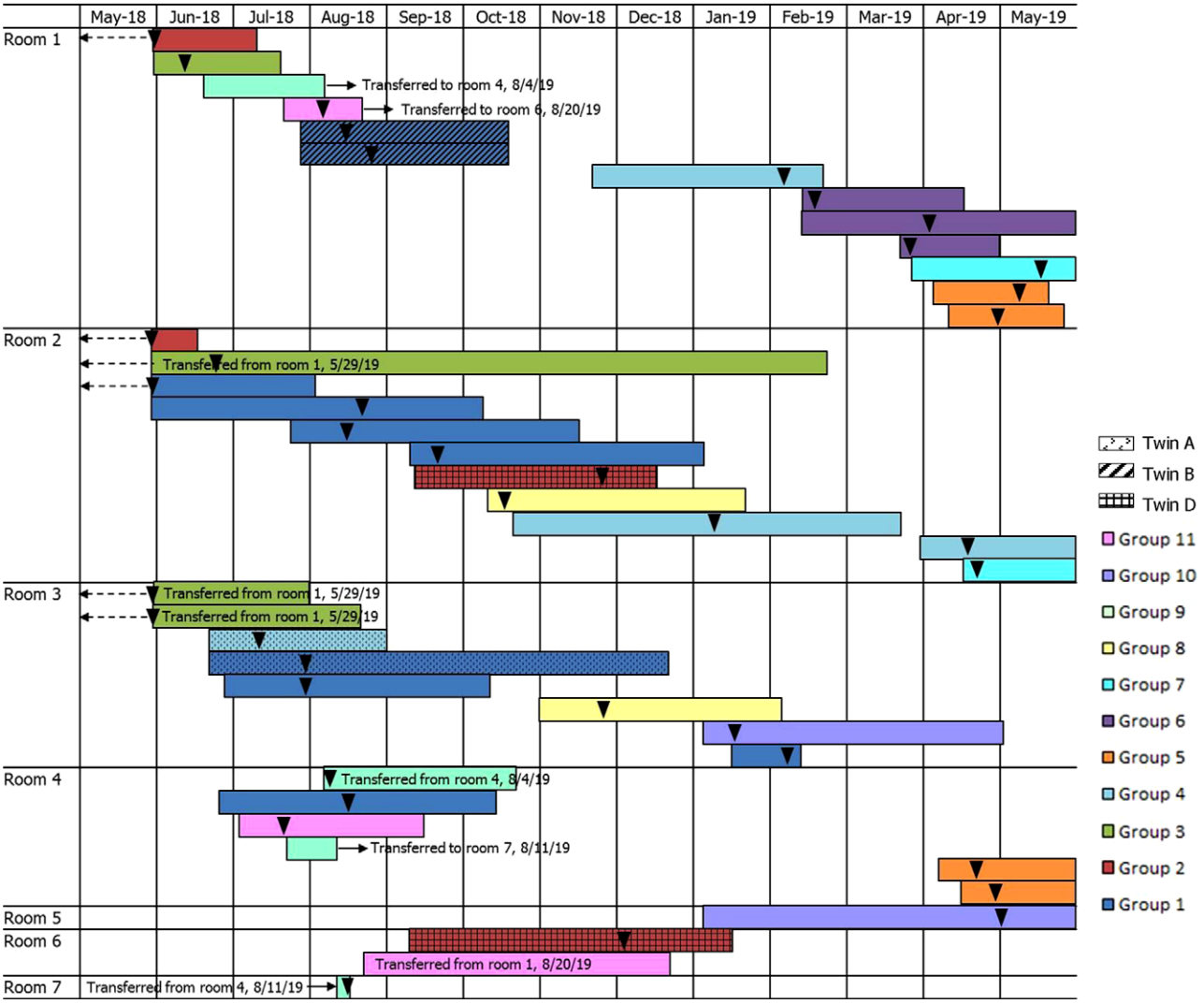

Fig. 3. Timeline of grouped methicillin-susceptible *Staphylococcus aureus* isolates from infants in the neonatal intensive care unit according to the rooms in which they stayed, by core genome multilocus sequence type (cgMLST) group. Bars illustrate hospitalization period of colonized infants. Bar colors represent cgMLST groups, with small inverted triangles indicating dates of isolation. Dotted lines designate those infants who were in the indicated location before the study period began.

In our study, some clones showed spatial and temporal relations, suggesting hospital-associated transmission, but no extensive spread of any single strain occurred; differences may depend on intrinsic properties of specific strains alongside local infection prevention and control practices.[29]

Colonization of family members or visitors may lead to subsequent *S. aureus* acquisition by infants in NICUs. The nasal carriage rate of *S. aureus* is ~20%–30% in the general population, with the rate being ~15%–20% for parents of infants admitted to NICUs.[27,31,32] A recent randomized controlled study of the effect of decolonization of parents colonized with *S. aureus* (MSSA or MRSA) on transmission to neonates in 2 NICUs illustrates the potential magnitude of parent-to-infant spread.[16] The study found that, by 90 days, nearly 60% of colonized infants were colonized with *S. aureus* strains concordant with their parent's baseline strains, although pulsed-field gel electrophoresis (not WGS) was used to determine concordance. In addition, the study found that decolonization of parents reduced the rate of acquisition of a concordant strain by >50%. However, a variety of issues, including generalizability, feasibility, scalability and the impact on family-centered care must be understood better before this intervention is applied routinely.[33]

Another mechanism by which infants may acquire *S. aureus* carriage is perinatal acquisition from colonized mothers. Recent studies have shown colonization with *S. aureus* in 10%–15% of newborns at or shortly after birth, with maternal colonization increasing infant colonization.[9,14,34] Our study could not confirm whether or not perinatal acquisition affected introduction of the bacteria into the NICU because the carriage state of parents was not examined and screening of infants at the time of admission was not performed during the study period. However, since most MSSA was detected in the second or subsequent screenings performed >1 week after admission, most of the infants might be presumed to have acquired MSSA during their hospitalization.

Our data also show that more than half of isolates were related to other isolates. Isolates belonging to groups 1, 3, 5, and 6 were detected serially in the same room or in different rooms with overlapping stays, suggesting at least some within-hospital

https://doi.org/10.1017/ice.2020.355 Published online by Cambridge University Press

transmission. This has been shown in recent MSSA outbreaks in NICUs, in which 2 parallel outbreaks had originated from exogenous sources.[35] Spread of *S. aureus* within hospitals can be mediated by medical staff or hospital environments, including medical equipment, as a reservoir or vector.[19,36,37] Because no systematic surveillance of the medical staff or the hospital environment was carried out in our study, it was not possible to determine whether they might have mediated bacterial transmission. Interestingly, after infants with group 2 isolates were discharged at July 2018, group 2 isolates were not detected until they reappeared in November 2018. One of the infants with group 4 carriage stayed in a different room and did not have overlapping hospital stays with the other group 4 infants (Fig 3). We were unable to determine whether a reservoir mediating transmission might have existed or whether isolates endemic in the community might have been introduced on >1 occasion.

Although MSSA has received less attention than MRSA in NICUs, MSSA is an important cause of morbidity and mortality.[22,38] The incidence of MSSA bacteremia over a year in this study was 3 times higher than that of MRSA bacteremia in our previous MRSA study, a finding consistent with other reports.[13,30] Previous studies show a comparable mortality rate between MSSA and MRSA infections in infants admitted to NICUs.[17,22] Recently, there has been an increasing recognition that hospital infection prevention and control efforts must focus not only on preventing MRSA infections but *S. aureus* infections overall, including those caused by MSSA.[19,22,39] However, additional study is needed to more precisely quantify the association of MSSA acquisition on subsequent infection, morbidity or mortality in infants as well as effects of interventions to prevent *S. aureus* colonization.

Although the cgMLST results were not compared with those of other typing methods for the MSSA isolates from this study, we previously reported that cgMLST provides high resolution for determining relatedness of both MSSA and MRSA.[23,24] In our prior neonatal MRSA study, cgMLST provided more resolution than PFGE, separating, for example, 2 isolates away from 23 that were all included in the same indistinguishable PFGE group.[23] Similarly, in our *S. aureus* bacteremia study, several isolates assigned to the same *spa* type were unrelated to each other by cgMLST.[24] In a neonatal MSSA bacteremia study, cgMLST was able to distinguish outbreak-related isolates from the other isolates of the same sequence type by classical MLST.[40]

This study has several limitations. Cultures from the hospital environment and surveillance of healthcare workers were not performed, so we were unable to determine whether MSSA from infants in different rooms or in the same rooms during nonoverlapping periods of hospitalization was acquired through horizontal transmission. In addition, because family members and visitors were not screened, we were unable to determine whether they were the source of the babies' MSSA. Because MSSA isolates collected from clinical infection sites were not sequenced, we could not definitively show that colonizing isolates caused clinical infections. Finally, since only 1 isolate was sequenced from each infant, colonization with >1 strain of MSSA would not have been detected.

In summary, WGS demonstrated that MSSA isolates colonizing infants in a NICU had a high degree of overall clonal diversity: 42% of isolates were unrelated to each other or any of the isolate group. Nonetheless, most isolates showed relatedness to other isolates, but generally with small numbers of isolates in each group. These results demonstrate that WGS is a useful tool to understand the molecular epidemiology of MSSA in NICUs, although additional study using WGS is needed to understand complex transmission pathways in more detail to better inform infection prevention and control practices.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2020.355

Acknowledgments. We appreciate the physicians, nurses, and other staff in the NICU for their help with this investigation. We also appreciate the staff of the clinical microbiology laboratory for their help with isolate acquisition, storage, processing, and analysis. Finally, we thank the Mayo Clinic Center for Individualized Medicine for assistance with development of the WGS platform.

Financial support. This research was supported by the Mayo Clinic Center for Individualized Medicine.

Conflicts of interest. Dr Patel reports grants from CD Diagnostics, Merck, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, ContraFect, TenNor Therapeutics Limited and Shionogi. Dr Patel is a consultant to Curetis, Specific Technologies, Next Gen Diagnostics, PathoQuest, Selux Diagnostics, 1928 Diagnostics and Qvella; monies are paid to Mayo Clinic. In addition, Dr Patel has a patent on *Bordetella pertussis*/*parapertussis* PCR issued, a patent on a device and method for sonication with royalties paid by Samsung to Mayo Clinic, and a patent on an antibiofilm substance. Dr Patel receives travel reimbursement from ASM and IDSA, an editor's stipend from IDSA, and honoraria from the NBME, Up-to-Date and the Infectious Diseases Board Review Course. Dr Huskins reports fees for serving as a consultant to ADMA Biologics and Pfizer. All other authors declare no conflicts of interest.

References

1. Boghossian NS, Page GP, Bell EF, *et al.* Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. *J Pediatr* 2013;162:1120–1124.
2. Lake JG, Weiner LM, Milstone AM, Saiman L, Magill SS, See I. Pathogen distribution and antimicrobial resistance among pediatric healthcare-associated infections reported to the National Healthcare Safety Network, 2011–2014. *Infect Control Hosp Epidemiol* 2018;39:1–11.
3. Verstraete E, Boelens J, De Coen K, *et al.* Healthcare-associated bloodstream infections in a neonatal intensive care unit over a 20-year period (1992–2011): trends in incidence, pathogens, and mortality. *Infect Control Hosp Epidemiol* 2014;35:511–518.
4. Stoll BJ, Hansen NI, Adams-Chapman I, *et al.* Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA* 2004;292:2357–2365.
5. Song X, Perencevich E, Campos J, Short BL, Singh N. Clinical and economic impact of methicillin-resistant *Staphylococcus aureus* colonization or infection on neonates in intensive care units. *Infect Control Hosp Epidemiol* 2010;31:177–182.
6. Weidenmaier C, Goerke C, Wolz C. *Staphylococcus aureus* determinants for nasal colonization. *Trends Microbiol* 2012;20:243–250.
7. Rana D, Abughali N, Kumar D, Super DM, Jacobs MR, Kumar ML. *Staphylococcus aureus*, including community-acquired methicillin-resistant *S. aureus*, in a level III NICU: 2001 to 2008. *Am J Perinatol* 2012;29:401–408.
8. Datta F, Erb T, Heininger U, *et al.* A multicenter, cross-sectional study on the prevalence and risk factors for nasal colonization with *Staphylococcus aureus* in patients admitted to children's hospitals in Switzerland. *Clin Infect Dis* 2008;47:923–926.
9. Jimenez-Truque N, Tedeschi S, Saye EJ, *et al.* Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Pediatrics* 2012;129:e1252–e1259.
10. Rodriguez EA, Correa MM, Ospina S, Atehortua SL, Jimenez JN. Differences in epidemiological and molecular characteristics of nasal colonization with *Staphylococcus aureus* (MSSA-MRSA) in children from a university hospital and day care centers. *PLoS One* 2014;9:e101417.

11. Delaney HM, Wang E, Melish M. Comprehensive strategy including prophylactic mupirocin to reduce *Staphylococcus aureus* colonization and infection in high-risk neonates. *J Perinatol* 2013;33:313–318.
12. Huang Y, Chou Y, Su L, Lien R, Lin T. Methicillin-resistant *Staphylococcus aureus* colonization and its association with infection among infants hospitalized in neonatal intensive care units. *Pediatrics* 2006;118:469–474.
13. Popoola VO, Milstone AM. Decolonization to prevent *Staphylococcus aureus* transmission and infections in the neonatal intensive care unit. *J Perinatol* 2014;34:805–810.
14. Leshem E, Maayan-Metzger A, Rahav G, *et al.* Transmission of *Staphylococcus aureus* from mothers to newborns. *Pediatr Infect Dis J* 2012;31:360–363.
15. Akinboyo IC, Voskertchian A, Gorfu G, *et al.* Epidemiology and risk factors for recurrent *Staphylococcus aureus* colonization following active surveillance and decolonization in the NICU. *Infect Control Hosp Epidemiol* 2018;39:1334–1339.
16. Milstone AM, Voskertchian A, Koontz DW, *et al.* Effect of treating parents colonized with *Staphylococcus aureus* on transmission to neonates in the intensive care unit: a randomized clinical trial. *JAMA* 2019;323:319–328.
17. Shane AL, Hansen NI, Stoll BJ, *et al.* Methicillin-resistant and susceptible *Staphylococcus aureus* bacteremia and meningitis in preterm infants. *Pediatrics* 2012;129:e914–e922.
18. Dong Y, Glaser K, Speer CP. New threats from an old foe: methicillin-resistant *Staphylococcus aureus* infections in neonates. *Neonatology* 2018;114:127–134.
19. Romano-Bertrand S, Filleron A, Mesnage R, *et al. Staphylococcus aureus* in a neonatal care center: methicillin-susceptible strains should be a main concern. *Antimicrob Resist Infect Control* 2014;3:21.
20. Milstone AM, Song X, Coffin S, Elward A. Identification and eradication of methicillin-resistant *Staphylococcus aureus* colonization in the neonatal intensive care unit: results of a national survey. *Infect Control Hosp Epidemiol* 2010;31:766–768.
21. Carey AJ, Duchon J, Della-Latta P, Saiman L. The epidemiology of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit, 2000–2007. *J Perinatol* 2010;30:135–139.
22. Ericson JE, Popoola VO, Smith PB, *et al.* Burden of invasive *Staphylococcus aureus* infections in hospitalized infants. *JAMA Pediatr* 2015;169:1105–1111.
23. Madigan T, Cunningham SA, Patel R, *et al.* Whole-genome sequencing for methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak investigation in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2018;39:1412–1418.
24. Park KH, Greenwood-Quaintance KE, Uhl JR, *et al.* Molecular epidemiology of *Staphylococcus aureus* bacteremia in a single large Minnesota medical center in 2015 as assessed using MLST, core genome MLST and spa typing. *PLoS One* 2017;12:e0179003.
25. Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol* 2014;52:2365–2370.
26. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing, 28th edition. CLSI supplement M100*. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
27. Mongkolrattanothai K, Mankin P, Cranston J, Gray BM. Molecular surveillance of *Staphylococcus aureus* colonization in a neonatal intensive care unit. *Am J Infect Control* 2010;38:660–663.
28. Ahmed S. Methicillin-susceptible *Staphylococcus aureus* colonization in the neonatal intensive care unit. *Yale Medicine Thesis Digital Library* 2007;321.
29. Achermann Y, Seidl K, Kuster SP, *et al.* Epidemiology of methicillin-susceptible *Staphylococcus aureus* in a neonatology ward. *Infect Control Hosp Epidemiol* 2015;36:1305–1312.
30. Graham PL, 3rd, Morel AS, Zhou J, *et al.* Epidemiology of methicillin-susceptible *Staphylococcus aureus* in the neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2002;23:677–682.
31. Pinter DM, Mandel J, Hulten KG, Minkoff H, Tosi MF. Maternal-infant perinatal transmission of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *Am J Perinatol* 2009;26:145–151.
32. Sakr A, Bregeon F, Mege JL, Rolain JM, Blin O. *Staphylococcus aureus* nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. *Front Microbiol* 2018;9:2419.
33. Zachariah P, Saiman L. Decreasing *Staphylococcus aureus* in the neonatal intensive care unit by decolonizing parents. *JAMA* 2019 Dec 30 [Epub ahead of print]. doi: 10.1001/jama.2019.20784.
34. Maayan-Metzger A, Strauss T, Rubin C, *et al.* Clinical evaluation of early acquisition of *Staphylococcus aureus* carriage by newborns. *Int J Infect Dis* 2017;64:9–14.
35. Roisin S, Gaudin C, De Mendonca R, *et al.* Pan-genome multilocus sequence typing and outbreak-specific reference-based single nucleotide polymorphism analysis to resolve two concurrent *Staphylococcus aureus* outbreaks in neonatal services. *Clin Microbiol Infect* 2016;22:520–526.
36. Eldirdiri S, Lee J, Jack A, Wright A, Findlay A, Phillips G. Outbreak of gentamicin-resistant, meticillin-susceptible *Staphlococcus aureus* on a neonatal unit. *J Hosp Infect* 2018;98:419–424.
37. Price JR, Cole K, Bexley A, *et al.* Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *Lancet Infect Dis* 2017;17:207–214.
38. Dolapo O, Dhanireddy R, Talati AJ. Trends of *Staphylococcus aureus* bloodstream infections in a neonatal intensive care unit from 2000-2009. *BMC Pediatr* 2014;14:121.
39. Carey AJ. War on *Staphylococcus aureus*. *J Perinatol* 2014;34:803–804.
40. Slingerland BCGC, Vos MC, Bras W, *et al.* Whole-genome sequencing to explore nosocomial transmission and virulence in neonatal methicillin-susceptible *Staphylococcus aureus* bacteremia. *Antimicrob Resist Infect Control* 2020;9:39.

https://doi.org/10.1017/ice.2020.355 Published online by Cambridge University Press