ORIGINAL ARTICLE

Transmission Clusters of Methicillin-Resistant *Staphylococcus Aureus* in Long-Term Care Facilities Based on Whole-Genome Sequencing

O. Colin Stine, PhD;[1] Shana Burrowes, MPH;[1] Sophia David, BSc;[2] J. Kristie Johnson, PhD;[3] Mary-Claire Roghmann, MD, MS[1,4]

OBJECTIVE. To define how often methicillin-resistant *Staphylococcus aureus* (MRSA) is spread from resident to resident in long-term care facilities using whole-genome sequencing.

DESIGN. Prospective cohort study.

SETTING. A long-term care facility.

PARTICIPANTS. Elderly residents in a long-term care facility.

METHODS. Cultures for MRSA were obtained weekly from multiple body sites from residents with known MRSA colonization over 12-week study periods. Simultaneously, cultures to detect MRSA acquisition were obtained weekly from 2 body sites in residents without known MRSA colonization. During the first 12-week cycle on a single unit, we sequenced 8 MRSA isolates per swab for 2 body sites from each of 6 residents. During the second 12-week cycle, we sequenced 30 MRSA isolates from 13 residents with known MRSA colonization and 3 residents who had acquired MRSA colonization.

RESULTS. MRSA isolates from the same swab showed little genetic variation between isolates with the exception of isolates from wounds. The genetic variation of isolates between body sites on an individual was greater than that within a single body site with the exception of 1 sample, which had 2 unrelated strains among the 8 isolates. In the second cycle, 10 of 16 residents colonized with MRSA (63%) shared 1 of 3 closely related strains. Of the 3 residents with newly acquired MRSA, 2 residents harbored isolates that were members of these clusters.

CONCLUSIONS. Point prevalence surveys with whole-genome sequencing of MRSA isolates may detect resident-to-resident transmission more accurately than routine surveillance cultures for MRSA in long-term care facilities.

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Antibiotic resistance is a major public health problem and is amplified by patient-to-patient transmission in the healthcare setting. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic bacterial pathogen and an important cause of both community- and hospital-acquired infections. Efforts to control multidrug-resistant pathogens like MRSA depend on rapid identification and treatment of patients combined with epidemiologic investigations and interventions to prevent ongoing transmission. Surveillance for multidrug-resistant pathogens with molecular typing can aid in identifying ongoing transmission and in developing new interventions to prevent spread; however, current typing methods often lack sufficient resolution to allow accurate inferences regarding transmission events.

More effective containment of MRSA in the healthcare and community settings may be possible if rapid and accurate identification of transmission can be accomplished by whole-genome sequencing (WGS). WGS of MRSA has been used to examine outbreaks in a neonatal intensive care unit,[1,2] 3 intensive care units,[3,4] several acute-care hospitals,[4] and community settings.[5,6] In each case, WGS was able to more precisely define and extend the boundaries of the outbreak by capturing additional transmissions that included other patients, individuals, and a healthcare worker who would not have been included using traditional methods. The extended boundaries included isolates with different antibiograms and different *spa* types. These studies were conducted in settings with a low prevalence of MRSA. WGS has not been used in a setting with a high prevalence of MRSA in which multiple patients are colonized with MRSA for long periods of time, such as long-term care facilities (LTCFs).

Approximately 1.5 million people in the United States reside in ~15,000 LTCFs.[7] In US LTCFs, multidrug-resistant organisms such as MRSA are increasingly important causes of colonization and infection.[8] Approximately 22%–28% of LTCF residents are colonized with MRSA,[9–13] which can be

Affiliations: 1. Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, United States; 2. Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 3. Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland, United States; 4. Geriatrics Research Education and Clinical Center, VA Maryland Health Care System, Baltimore, Maryland, United States.

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spread from resident to resident by healthcare workers. From 2005 to 2008, we performed a prospective cohort study to investigate MRSA transmission in multiple LTCFs.[14] Thus, we have a unique and well-characterized collection of MRSA isolates and swabs from MRSA-colonized LTCF residents. In this study, we examined the genetic variation (1) between multiple MRSA isolates from 2 body sites per resident to assess the genetic variation within and between body sites and (2) between single MRSA isolates from residents in the same unit to assess the proportion of MRSA colonization attributable to transmission clusters.

METHODS

Study Population and Design

The study population consisted of LTCF residents from the Perry Point VA Medical Center. As part of a larger prospective cohort study conducted from March 2005 to September 2008,[14] a single long-term-care unit was followed over two 12-week study periods approximately 1 year apart. This long-term-care unit had a dedicated nursing staff, and its residents were only on this unit during the 12-week study cycle. Residents were stratified by MRSA colonization status based on past clinical and surveillance cultures, which were routinely performed on admission. Cultures for MRSA were obtained weekly from multiple body sites (ie, anterior nares, perineum, axillary skin, and wounds) from residents with known MRSA colonization over 12-week study periods. Simultaneously, culture swabs for MRSA were obtained weekly from the anterior nares and wounds in all residents without known MRSA colonization to detect acquisition. All swab samples, including those from wounds, were cultured to detect colonization. Acquisition was defined as a new positive culture in a resident with no prior history of MRSA whose admission cultures were negative for MRSA by culture and PCR. From the first 12-week cycle, we sequenced 8 MRSA isolates per swab from each of 2 body sites for 6 residents to gain insight into the amount of variation at a single site. The nares comprised 1 body site in each pair of swab samples; the other body sites were wounds (n = 2), axilla (n = 2), and perineum (n = 2). In the present study, 3 pairs of swab samples were selected to have concordant *spa* types (t008), and 3 pairs of swab samples were selected to be discordant (t008 and either t045 or t002) based on previously typed single isolates. For the second 12-week cycle, we sequenced 1 MRSA isolate from each body site of the all residents who had or acquired MRSA to define transmission clusters and to estimate resident-to-resident transmission.

Laboratory Methods

All study cultures for research were collected by a research nurse using a rayon-tipped swab with Amies transport medium (BactiSwab; Remel, Lenexa, KS). All swabs were streaked for isolation onto tryptic soy agar containing 5% sheep blood agar (Remel, Lenexa, KS). Plates were incubated at 37°C for 48 hours. Isolates were characterized as *S. aureus* by catalase and coagulase production (Pastorex, Bio-Rad Laboratories, Hercules, CA). *S. aureus* isolates were plated on oxacillin (6 μg/mL) agar screen plates and incubated at 37°C. Growth on the oxacillin agar screen was classified as oxacillin resistant (ie, MRSA).

Molecular Typing

When multiple MRSA cultures were available from the same resident and same body site, the earliest date with a positive culture was chosen for analysis. Chromosomal DNA was extracted from cells after growth in an overnight culture of tryptic soy broth at 37°C using previously described methods.[15] In brief, cells were lysed using 1:5 ratio of lysostaphin to cell suspension incubated at 37°C for 2–3 hours. DNA isolation was performed using the Prepman Ultra DNA extraction kit (Applied Biosystems, Foster City, CA). Each MRSA isolate was also characterized by the DNA sequence of the protein A (*spa*) gene hypervariable region.[16] The *spa* repeats were defined based on comparison to the sequences in the database at http://www.ridom.de/spaserver.

Whole-Genome Sequencing

Frozen isolates and the original swabs frozen in glycerol were used for both analyses. The selected isolates were streaked; single colonies were selected and cultured overnight in brain-heart infusion (BHI) broth. DNA was then isolated using Qiagen columns (Qiagen, Valencia, CA). A ~350-bp library was prepared from DNA, sheared by a Covaris S2 sonicator (Covaris, Wolburn, MA), using the Nextera kit (Illumina, San Diego, CA). Libraries quantified using Picogreen (Nanodrop Fluorimeter 3300, Wilmington, DE) from 48 isolates were mixed at equal concentrations. The mixed libraries were sequenced using Illumina Hiseq 2500 (Illumina, San Diego, CA) using 100-bp paired-end reads. The reads were separated based on the barcodes indicative of the library.

Whole-Genome Assembly and Phylogenetic Analysis

Isolates were mapped to the reference genome of MRSA USA300 using SMALT v0.7.4. Regions containing mobile genetic elements were excluded from the analysis. An in-house pipeline using SAMtools and BCFtools was used to call bases. To call only high-quality bases, our method required at least 75% of high-quality mapped reads on each strand to agree with the base call, a base quality of at least 50, and a mapping quality of at least 30. Sequence reads that mapped equally well to >1 region were discarded to avoid mapping repetitive regions. Single-nucleotide variants (SNVs) were identified using a standard approach.[17] Maximum likelihood trees were constructed using RAxML based on variable positions in the whole-genome alignment.[18] Tree files were viewed using FigTree v1.4.2.

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RESULTS

MRSA Diversity Within and Between Body Sites on Residents

During the first 12-week study cycle, we examined 6 residents (labelled A-F) who were positive for MRSA at 2 of 4 different body sites. A frozen swab from each body site was streaked on selective media and 8 isolates were picked and sequenced for a total of 96 (Supplemental Table). With 2 exceptions, the isolates at each body site were very closely related to other isolates from the same site, differing at 7 or fewer SNVs (Table 1). For 6 of 12 body sites, the 8 isolates per body site differed at 1 or 0 sites. The 2 exceptions were both swabs from wounds. The wound from resident C revealed 2 closely related groups of isolates; 13 of 28 pairwise comparisons of the genotypes differed by 1 or 0 SNV, while the other 15 pairwise comparisons differed by 9 to 17 SNVs. The wound from resident D had 2 distinct genetic strains; 6 isolates differed by less than 4 SNVs and differed by >19,700 SNVs from the other 2 isolates, which did not differ from each other. Similar large differences (>19,700 SNVs) were seen among residents D, E, and F, who were chosen because a previously typed MRSA isolate from their body sites had distinct *spa* types. In contrast, those isolates with the same *spa* type from different body sites on different residents showed smaller differences (6–108 SNVs), but these differences were still generally larger than those from different sites within an individual (0–17 SNVs).

MRSA Genetic Diversity Between Residents on a Unit

During the second 12-week study cycle, there were 52 residents on the unit; 51 participated in the study. We examined frozen MRSA isolates from all residents with MRSA colonization including 3 residents with newly detected MRSA acquisition. Among the 51 residents who were sampled, 16 residents (labelled 1–16) with MRSA colonization were positive at a total of 30 body sites (Table 2). All 30 isolates were successfully sequenced (Supplemental Table), and the SNVs between genotypes were determined; 28 isolates were *spa* type t008. The remaining 2 isolates were *spa* types t002 and t242. WGS confirmed that these isolates were distinct from each other (differing at 174 nucleotides) and from the rest (differing by >16,515 nucleotides).

As in the first study cycle, SNVs were observed between t008 isolates from distinct body sites on the same individual. We identified 8 residents with t008 MRSA colonization at >1 body site (Table 2). The MRSA isolates may have differed only a few nucleotides (≤3 SNVs, as seen in residents 1, 5, and 8) or by a small number of nucleotides (11–36 SNVs, as seen in residents 3, 4, and 11) or by a larger number of nucleotides (96–102 SNVs, as seen in residents 6 and 9). The majority of isolates within individuals differed by <40 SNVs (Figure 1 and Supplemental Figure 1).

The genetic relatedness of the 28 t008 MRSA isolates is shown in a phylogenetic tree in Figure 2. Each of the 3 circles indicates a cluster of multiple isolates with a genotype that differs by <40 SNVs. The 2 larger clusters each contain 1 isolate that was epidemiologically identified as an acquisition. Cluster A has 12 isolates from residents 1–5. The maximum distance between genotypes in this cluster is 34 SNVs between 2 isolates from different body sites on resident 4. Resident 4 is also resident D and was present on the unit during both study cycles. Resident 4 antedates all of the other second study-cycle residents. Furthermore, 2 of the residents (1 and 2) present at the start of the cycle had isolates whose genotypes differed by 2 SNVs from the isolate of resident 5 (which was an epidemiological acquisition). Cluster B comprised 2 wound isolates from residents 6 and 7; the distance between them was 33 SNV. Although the nares isolate of resident 6 might be expected to be in this cluster, it had a distinct *spa* type (t242 instead of t008). Cluster C had 6 isolates from 3 residents; the maximum difference between genotypes in this cluster was 11 nucleotides. Resident 8 was present at week 0 and had

TABLE 1. Single-Nucleotide Variant (SNV) Differences Within and Between 2 Body Sites on the Same Resident for 6 Residents From the First 12-Week Study Cycle[a]

Resident/ID/ Body Sites	*spa* Types From Single Isolate	SNV Difference Within MRSA Isolates From Nares	SNV Difference Within MRSA Isolates From Other Body Site (Single *spa* Type)	SNV Difference Within MRSA Isolates Between Nares and Other Body Site
A/N, P	t008, t008	3	0	3
B/N, A	t008, t008	1	0	92
C/N, W	t008, t008	0	17	16
D/N, W[b]	t008, t008	3	0 (t008)	15
	t045		4 (t045)	>19,700
E/N, A	t002, t008	7	0	>19,700
F/N, P	t008, t045	1	4	>19,700

NOTE. ID, identification; MRSA, methicillin-resistant *Staphylococcus aureus*; N, nares; A, axilla; P, perineum; W, wound without active infection.
[a]8 MRSA isolates were sequenced from each body site. Maximum number of SNV differences recorded except for alternative *spa* types when a lower bound was recorded.
[b]2 *spa* types in 1 body site.

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table 2. Residents With MRSA Colonization by Week During the Second 12-Week Study Cycle

Resident ID	Cluster	Site[a]	*spa* Type	Week 1	2	3	4	5	6	7	8	9	10	11	12	SNVs Within Resident
1																1, 1, 2
	A	Nares	t008	+	−	−										
	A	Perineum	t008		+	−										
	A	Wound[c]	t008	+												
2																
	A	Nares	t008	+												
3																11, 14, 17
	A	Nares	t008	+		+		+		+		+			−	
	A	Perineum	t008	−		+		−		+		−				
	A	Wound[c]	t008	+								+				
4																28, 32, 34
	A	Axilla	t008	−	−	−		−	−	+						
	A	Nares	t008	−	−	−		+	+	+						
	A	Perineum	t008	−	+	−		−	+	+						
5[b]																1
	A	Nares	t008		−	−	+		+	−		−	−			
	A	Perineum	t008[d]							+		+	−			
6																96
		Nares	t008	+	+											
	B	Wound[c]	t008	+												
7																19,432
		Nares	t242	−	−	−	−	−	−	−	−	−	−	−	+	
	B	Wound	t008									+				
8																1, 2, 3
	C	Axilla	t008[d]	−	−	−	−	−	−	−		−	+			
	C	Nares	t008	−	−	−	−	+	+	+		+	+		+	
	C	Perineum	t008[d]	−	−	−	−	−	−	+		+	+			
9																2, 99, 102
		Nares	t008						−	−	+	−	−	−	−	
	C	Perineum	t008						−	−	+	−	−	−		
	C	Wound[c]	t008 [d]						−			+			−	
10[b]																
	C	Nares	t008									−	−		+	
11																12
		Nares	t008[d]	−	−		−		+	−						
		Throat	t008	+												
12																
		Nares	t008	+	+	−	+	+								
13																
		Nares	t008				+									
14																
		Nares	t002				−	−	+	+	+	−	−	+		
15																
		Nares	t008[d]								+	−	−			
16[b]																
		Nares	t008											−	+	

note. ID, identification; SNV, single-nucleotide variant; MRSA, methicillin-resistant *Staphylococcus aureus*. MRSA-positive swabs are indicated by +; MRSA-negative swabs are indicated by −; missing values indicate that the resident was not on the unit for swab collection or the swab was not taken; MRSA samples used in the analysis are indicated by bold font.
[a]Only body sites with positive cultures are shown.
[b]Residents acquired MRSA.
[c]Without active infection.
[d]Inferred *spa* type t008 based on positive *pvl* and *acme* PCR tests.

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3 positive body sites. Resident 9 was admitted in week 5 and also had MRSA at 3 positive body sites; 2 of these differed from those of resident 8 by 2–11 SNVs. The third isolate 9N was not in the cluster; it differed by >250 SNVs. The last isolate in this cluster was from resident 10, who was admitted in week 8 and acquired MRSA in the nares in week 12. This isolate differed from the perineal isolate of resident 9 by 6 SNVs.

DISCUSSION

Our WGS analyses of selected MRSA isolates from residents in LTCFs produced systematic patterns of genetic relatedness that may be interpreted for improved understanding of transmission clusters on a unit. The cluster identified the patients associated with likely transmission events and unambiguously defined patients who were not part of the cluster, but it did not define specific patient-to-patient transmission events.[19] Traditional epidemiological evidence is required to define patient-to-patient transmission. The inability to define specific transmission events stems from the significant amount of variation observed within and between body sites.[20] Despite this limitation, the presence of a transmission cluster shows that patient-to-patient transmission did occur.

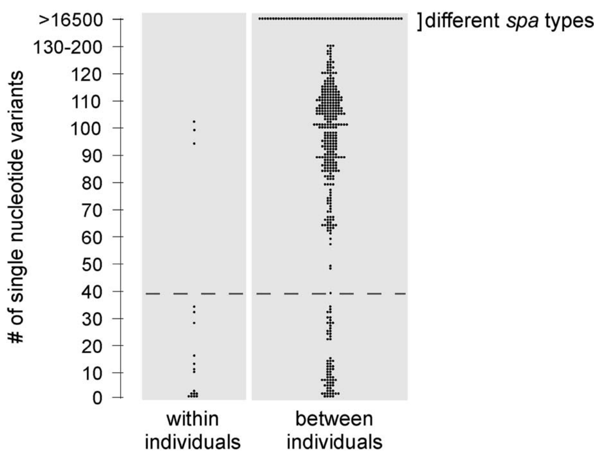

FIGURE 1. Single-nucleotide variant differences within the same resident and between residents for 30 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from 16 residents during the second 12-week study cycle.

Genetic variation in a single body site tended to be very small. In general, the variation was <7 different nucleotides. In our sample, there were 2 exceptions; both were isolates from wound cultures. Resident C's wound had isolates that differed by 9–17 nucleotides, which may have resulted from a single older colonization event or from 2 separate colonization events. Resident D's wound had genotypes that differed by >19,000 SNVs and were characteristic of 2 unrelated *spa* types (t002 and t008). If the isolates from each wound were divided into groups based on the isolate genotypes, then there were 4 genotype groups and the variation within the genotype group was <4 SNVs. Because the colonization with MRSA isolates that had different *spa* types must have resulted from 2 independent acquisitions (or 1 acquisition of 2 genetically distinct strains), the same explanation can be applied to resident C's wound, ie, it was the result of either 2 acquisitions or a single acquisition with 2 genetically distinct strains. Because of our small sample size and sampling methods in the first study cycle (in which we selected for cultures with >1 *spa* type), we cannot say whether multiple colonizations in a single body site were more common in wounds or how frequently multiple colonizations occurred at other body sites.

Colonization at each body site on the same individual represents at least 1 colonization event and perhaps more.

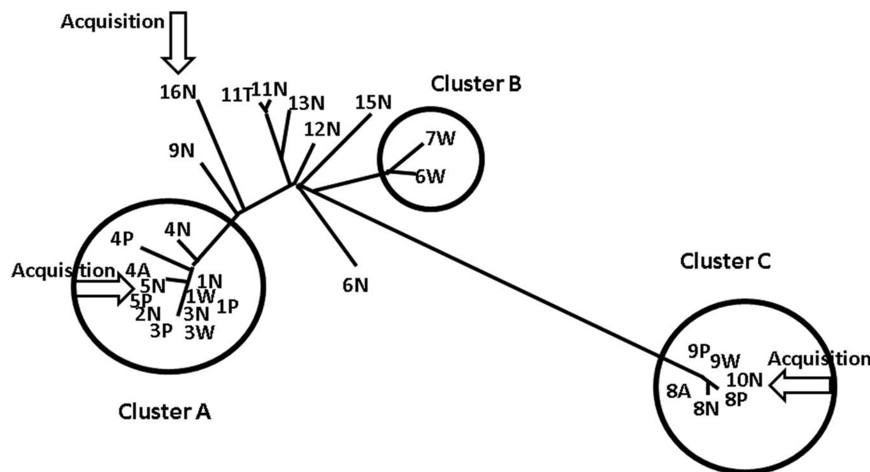

FIGURE 2. Genetic relatedness of 28 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates of t008 *spa* type from 15 residents assessed using whole-genome sequencing. Isolates are labelled with resident identification (ID) and body site of MRSA isolation: N, nares; W, wound; T, throat; P, perineum; and A, axilla. Circles enclose clusters of isolates from >1 individual that are <40 SNVs apart.

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These results extend the observation of 2 genetically distinct strains in 2 distinct wound samples, as discussed above. Although it has been described previously that 2 body sites may carry MRSA from 2 genetically distinct strains, ie, distinct *spa* types exemplified by resident 7 (t008 and t242 differing by 17,265 SNVs), our observation extends the number by adding genetically distinct strains from the same *spa* type. For example, residents 6 and 9 had isolates that differed by approximately 100 SNVs, even though all of the isolates were *spa* type t008.

Genetic variation of isolates from distinct body sites on the same resident and from different body sites on different residents ranged across the entire spectrum from a single SNV to >19,000 SNVs. Although a wide range of values was observed, the variations seemed to fall into 3 categories: <40 SNVs, 60–130 SNVs and different spa types, or >19,000 SNVs. A decision to use 40 SNVs as a cutoff for being from the same individual was also proposed by Price et al[21] for determining patient-to-patient transmission in an ICU. Our data are consistent with previous data; isolates that differ by <40 SNVs can be considered to come from the same transmission cluster, whereas those isolates that are genetically distinct came from separate sources.

When we assessed the 30 MRSA isolates with *spa* type t008 from 16 residents on the unit during the second study cycle (Figure 2), 3 clusters of isolates from multiple residents were apparent. All isolates within a single cluster can be considered to be the same transmission cluster. Cluster A included 12 body sites on 5 residents, 1 of whom (resident 4) was present during the first study cycle. However, the first-cycle isolates of resident 4 differed from those of the second cycle by 57 to 67 SNVs and thus belonged to different clusters. Examining the epidemiologic data, of these 5 residents, only resident 5 arrived on the unit after the study cycle started. We infer that he was infected by 1 of these 4 residents; however, the actual transmitter cannot be identified because sequenced isolates may not represent all of the genetic variants at each site. The most obvious cases in point are the examples of 2 genetically distinct strains in a single infection. Another example of inferred transmission from epidemiological data occurred in transmission cluster B. The genotypes of isolates from wounds on 2 different residents are consistent with a resident-to-resident transmission event. Based on the dates of stay, we can infer the likely transmission from resident 6 to 7. Transmission cluster C contains 6 body sites on residents 8, 9, and 10. Examining the epidemiologic data, resident 8 was previously colonized and was present on the unit from the beginning of the study cycle; this resident was the likely source for resident 9, who arrived on week 5, but the source for the colonization of resident 10, who arrived in week 8, is uncertain.

Of the 3 acquisitions detected by surveillance cultures, 2 acquisitons were due to resident-to-resident transmission events. This rate was substantially higher than the rate recently reported by Price et al[21] in an intensive care unit. We used a very restrictive definition of acquisition, which required no prior history of MRSA colonization and negative admission surveillance tests for MRSA by culture and PCR. Our definition minimizes new acquisitions that are due to false-negative admission tests; however, it discounts MRSA acquisitions of new strains that occur in residents with pre-existing MRSA colonization. An advantage of WGS is that it readily detects these acquisitions.

In our study, isolates from many residents occurred in transmission clusters of MRSA, which implies substantial levels of resident-to-resident transmission. We found that 63% of the residents colonized with MRSA during the second study cycle shared 1 of 3 strains, where the SNV differences were <40 between isolates from different individuals. We caution that this is a small sample size from a single site in need of expansion and replication. However, if the initial findings are confirmed, it may be more accurate to perform point prevalence surveys for MRSA and then analyze them using WGS to detect which residents have transmitted MRSA to other residents. This could move us closer to a future in which WGS is a routine component of public health surveillance.

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Address correspondence to Mary-Claire Roghmann, MD, MS, University of Maryland School of Medicine, 10 South Pine Street, MTSF Room 336, Baltimore, MD 21201 (mroghman@epi.umaryland.edu).

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/ice.2016.41

REFERENCES

1. Koser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N Engl J Med* 2012;366:2267–2275.
2. Harris SR, Cartwright EJ, Torok ME, et al. Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis* 2013;13:130–136.
3. Eyre DW, Golubchik T, Gordon NC, et al. A pilot study of rapid benchtop sequencing of *Staphylococcus aureus* and *Clostridium difficile* for outbreak detection and surveillance. *BMJ Open* 2012;2. 10.1136/bmjopen-2012-001124.

https://doi.org/10.1017/ice.2016.41 Published online by Cambridge University Press

4. Miller RM, Price JR, Batty EM, et al. Healthcare-associated outbreak of methicillin-resistant *Staphylococcus aureus* bacteraemia: role of a cryptic variant of an epidemic clone. *J Hosp Infect* 2014;86:83–89.
5. Uhlemann AC, Dordel J, Knox JR, et al. Molecular tracing of the emergence, diversification, and transmission of S. aureus sequence type 8 in a New York community. *Proc Natl Acad Sci U S A* 2014;111:6738–6743.
6. Alam MT, Read TD, Petit RA, 3rd, et al. Transmission and microevolution of USA300 MRSA in U.S. households: evidence from whole-genome sequencing. *MBio* 2015;6:e00054–15.
7. Nursing Home Care. Centers for Disease Control and Prevention website. http://www.cdc.gov/nchs/fastats/nursing-home-care.htm. Published 2015. Accessed February 11, 2016.
8. Smith PW, Bennett G, Bradley S, et al. SHEA/APIC guideline: infection prevention and control in the long-term care facility, July 2008. *Infect Control Hosp Epidemiol* 2008;29: 785–814.
9. Roghmann MC, Johnson JK, Sorkin JD, et al. Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) to healthcare worker gowns and gloves during care of nursing home residents. *Infect Control Hosp Epidemiol* 2015;36:1–8.
10. Mody L, Kauffman CA, Donabedian S, Zervos M, Bradley SF. Epidemiology of *Staphylococcus aureus* colonization in nursing home residents. *Clin Infect Dis* 2008;46:1368–1373.
11. Hudson LO, Reynolds C, Spratt BG, et al. Diversity of methicillin-resistant *Staphylococcus aureus* strains isolated from residents of 26 nursing homes in Orange County, California. *J Clin Microbiol* 2013;51:3788–3795.
12. Evans ME, Kralovic SM, Simbartl LA, et al. Nationwide reduction of health care-associated methicillin-resistant *Staphylococcus aureus* infections in Veterans Affairs long-term care facilities. *Am J Infect Control* 2014;42:60–62.
13. Crnich CJ, Duster M, Hess T, Zimmerman DR, Drinka P. Antibiotic resistance in non-major metropolitan skilled nursing facilities: prevalence and interfacility variation. *Infect Control Hosp Epidemiol* 2012;33:1172–1174.
14. Furuno JP, Shurland SM, Zhan M, et al. Comparison of the methicillin-resistant *Staphylococcus aureus* acquisition among rehabilitation and nursing home residents. *Infect Control Hosp Epidemiol* 2011;32:244–249.
15. Miller LG, Diep BA. Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* 2008;46:752–760.
16. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol* 2003;41:5442–5448.
17. Harris SR, Feil EJ, Holden MT, et al. Evolution of MRSA during hospital transmission and intercontinental spread. *Science* 2010;327:469–474.
18. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
19. Didelot X, Gardy J, Colijn C. Bayesian inference of infectious disease transmission from whole-genome sequence data. *Mol Biol Evol* 2014;31:1869–1879.
20. Worby CJ, Lipsitch M, Hanage WP. Within-host bacterial diversity hinders accurate reconstruction of transmission networks from genomic distance data. *PLoS Comput Biol* 2014;10: e1003549.
21. Price JR, Golubchik T, Cole K, et al. Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for acquisition of *Staphylococcus aureus* in an intensive care unit. *Clin Infect Dis* 2014;58:609–618.

https://doi.org/10.1017/ice.2016.41 Published online by Cambridge University Press