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SHEA

Original Article

Molecular concordance of methicillin-resistant *Staphylococcus aureus* isolates from healthcare workers and patients

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant nosocomial pathogen in the ICU. MRSA contamination of healthcare personnel (HCP) gloves and gowns after providing care to patients with MRSA occurs at a rate of 14%–16% in the ICU setting. Little is known about whether the MRSA isolates identified on HCP gown and gloves following patient care activities are the same as MRSA isolates identified as colonizing or infecting the patient.

Methods: From a multisite cohort of 388 independent patient MRSA isolates and their corresponding HCP gown and glove isolates, we selected 91 isolates pairs using a probability to proportion size (PPS) sampling method. To determine whether the patient and HCP gown or gloves isolates were genetically similar, we used 5 comparative genomic typing methods: phylogenetic analysis, *spa* typing, multilocus sequence typing (MLST), large-scale BLAST score ratio (LSBSR), and single-nucleotide variant (SNV) analysis.

Results: We identified that 56 (61.5%) of isolate pairs were genetically similar at least by 4 of the methods. Comparably, the *spa* typing and the LSBSR analyses revealed that >75% of the examined isolate pairs were concordant, with the thresholds established for each analysis.

Conclusions: Many of the patient MRSA isolates were genetically similar to those on the HCP gown or gloves following a patient care activity. This finding indicates that the patient is often the primary source of the MRSA isolates transmitted to the HCP, which can potentially be spread to other patients or hospital settings through HCP vectors. These results have important implications because they provide additional evidence for hospitals considering ending the use of contact precautions (gloves and gowns) for MRSA patients.

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Staphylococcus aureus, including methicillin-resistant *S. aureus* (MRSA), is a common cause of healthcare-associated infections that increase patient morbidity, length of stay, and mortality.[1,2] Transmission of MRSA from patient to patient in healthcare settings is often an indirect transmission due to limited, or no, direct patient-to-patient contact; however, transmission in the healthcare setting is thought to most often occur via environmental or healthcare personnel (HCP) vectors.[3] Particularly, MRSA transmission from patient to HCP has been demonstrated to occur at a rate of 14%–20%, often through contamination of HCP gown or gloves after performing patient care activity on a patient with confirmed MRSA colonization and/or infection.[3–5] These studies surmised that the isolates identified on the gown or gloves of the HCP are the same as those found on the patient; however, this was not directly demonstrated in these previous studies using genomic epidemiology.

Prior studies have used pulsed field-gel electrophoresis to determine MRSA relatedness between patients who were part of the hospital and outbreak investigations.[6–8] However, this traditional typing method has limited ability to discriminate between closely related isolates when compared to newer, more comprehensive genomic methods, such as whole-genome sequencing (WGS), which has been demonstrated in recent studies characterizing MRSA transmission in the healthcare setting.[9–11]

Although genomic epidemiology approaches have been used to study the interactions among patients, healthcare workers, and the environment, no study to our knowledge has reported whether isolates on gloves or gowns of the HCP acquired after patient care activity are genetically similar or identical to isolates from the patient.

In this study, we sought to determine whether MRSA isolates on HCP gown or gloves after patient care are genetically similar to the

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MRSA isolates recovered from patients. We used multiple molecular typing schema to demonstrate the genetic relatedness between HCP gown and glove isolates and patient isolates.

Materials and methods

Isolate selection

Our cohort contained clinical or surveillance MRSA isolates from 388 independent patients from the intensive care unit (ICU) and the paired isolates from the corresponding HCP's gown or gloves, as part of a previously described study.[5,12,13] In the parent study by O'Hara et al,[5] the MRSA isolates from each patient were defined as high, mid-level, or low transmitters, with the low transmitters having no transmission events that occurred from the patient to HCP. The low-transmitting isolates were not included in this analysis.[5] This study was conducted across 4 hospitals, 2 in Maryland and 1 each in New York and California. Clinical cultures were defined as cultures ordered by HCP to determine whether patients had an active infection, and in comparison, surveillance cultures were cultures used to screen patients for colonization with MRSA and were taken at the time of admission and, depending on the unit, weekly until discharged. These patients were on contact precautions for MRSA; thus, HCP were required to don a new pair of gloves and a gown prior to entering a patient's room. After an HCP entered the patient's room and performed patient-care activities, the HCP gown and gloves were swabbed. Swabs were cultured onto a CHROMagar MRSA (Becton Dickinson, Sparks, MD) and incubated overnight.[5,12,13]

From the 388 independent patient isolates, we selected 96 paired isolates using stratified sampling. We selected the patient isolates in proportion to the number of the isolates that were identified as part of the different genomic clades identified in our previous study.[14] We then selected a paired HCP sample at random, either a glove or gown isolate from an HCP who provided care to the patient whom we selected previously. Figure 1 outlines how isolates were selected through the various steps of the current study.

Genome sequencing

The genome sequencing and assembly used to analyze the patient isolates and HCP glove and gown isolates was described in Adediran et al.[12,13] After 5 pair of isolates were removed from the analysis due to failing quality control metrics after sequencing or molecular typing issues, 91 pairs of isolates remained. Thus, 182 total isolates were included in the genomic epidemiology studies. All genome assembly metrics and accession numbers for isolates included in the comparative analysis are included in Supplementary Table 1 (online).

Comparative genomics

Phylogenetic analysis

The In Silico Genotyper (ISG) was used to infer the whole-genome phylogeny.[15] Sequence data from the patient isolates and HCP gown and glove isolates were aligned to the USA300-ISMMS reference genome (GenBank Assembly Accession: GCA_000568455.1).[16] Gaps in 1 or more genomes were removed to create the core alignments for the isolates.[17] A phylogenetic tree was created using FastTree as previously described[18–20] and visualized with FigTree version 1.4.0 software (http://tree.bio.ed.ac.uk/software/figtree/). Genetic concordance was defined as paired isolates within the same phylogenetic group (Fig. 2).

MLST analysis

The 7 conserved housekeeping loci (*arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) of the MLST scheme previously developed were identified in each of the genomes.[21] The allele numbers of each locus and the sequence types (STs) of each genome were determined using BIGSdb software (https://pubmlst.org/saureus/).[22] We identified the STs and clonal complex (CC) for each patient isolate and HCP gown or glove isolate. We defined genetically similar isolates as patient isolates with the same ST and CC as the HCP gown or glove isolates.

spa typing analysis

A *spa*-typing analysis was performed on the 182 MRSA isolates of interest using spaTyper version 1.0 software (Center for Genomic Epidemiology, Denmark, https://cge.cbs.dtu.dk/services/spatyper/) with default parameters.[23] Genomes were examined to identify the *spa* types for each patient and HCP gown or glove isolates.[23] We defined the isolates as genetically similar when the patient isolate exhibited the same *spa* type as the corresponding paired HCP gown or glove isolate.

Large-scale BLAST score ratio (LSBSR)

LSBSR analyses were performed on the isolates as previously described.[17] The LSBSR uses predicted coding sequences from all query genomes to align each coding sequence to each genome. Each alignment generates a query bit score.[24] The query bit score is divided by the reference bit score to obtain a final BSR value. We completed a gene-by-gene pairwise comparison of the genomic content of the paired isolates (ie, patient isolates and HCP gown or glove isolates). We defined overall genetic similarity as the paired isolates having genomic content that was 90% similar, which was calculated by the number of genes that had the same LSBSR value divided by the total number of genes within the genomes.[25]

SNV analysis

We conducted a single-nucleotide variant (SNV) analysis using ParSNP (https://github.com/marbl/parsnp). We conducted pairwise comparisons for each pair of isolates with the patient isolate as the reference. We determined the number of SNVs between each of the paired isolates. Isolates were defined to be the same if they differed by <40 SNVs, a threshold previously utilized when examining genetic similarities of MRSA isolates.[9,11,27,28] Bee-swarm plots were created to visually examine the threshold for the defining number of SNVs.[9–11] We calculated the summary statistics using R version 4.02 software (R Foundation for Statistical Computing, Vienna, Austria).[29]

Results

Phylogenetic analysis of MRSA paired isolates

Phylogenetic analysis was performed on the 91 paired MRSA isolates. We identified 4 main phylogenetic groups among the paired isolates, which corresponded to the 4 main phylogenetic groups identified in the parental genomic study.[14] The most frequent transmission type among the patient isolates was midlevel transmitters (n = 64 of 91, 70%), which were defined as MRSA transmitted to the HCP at rates between 1% and 49%, based on the examination of 10 HCP–patient interactions. The remaining 27 patient isolates (30%) were considered high transmitters, defined as a transmission rate >50%, based on the examination of 10 HCP-patient interactions. Of the 91 patient isolates, 47 (52%) were

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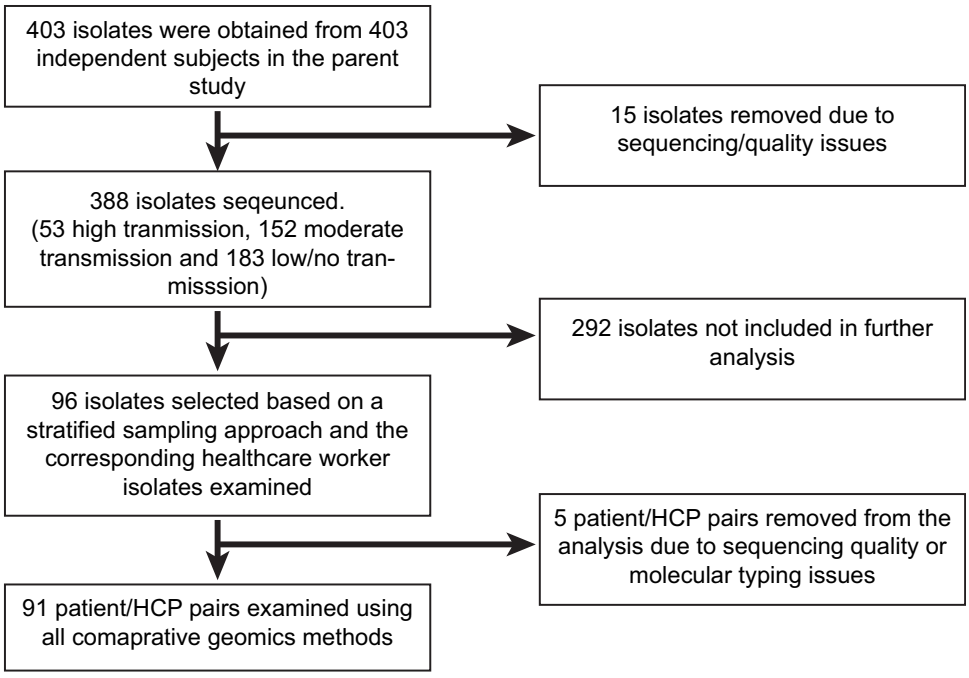

Fig. 1. Study flow diagram for the paired isolates used in the study.

obtained from clinical cultures; the remaining isolates were obtained from surveillance cultures. We detected no statistical difference between these groups and phenotypic transmission type. Also, 71 (81%) of the examined isolates came from Maryland. Comparing the transmission and isolate type (ie, clinical vs surveillance or high vs midlevel transmitters) by genomic group, we identified no significant association between these groups ($P = .34$ and .33, respectively). However, we identified geographic location to be the most significant association with the genomic groups ($P < .001$). We identified 76 (83.5%) paired isolates that were genetically similar (Table 1).

MLST typing

In total, 10 MLST types were identified from the 91 paired isolates. Among the typable isolate pairs, the MLST sequence types were the same between the patient and HCP gown and glove isolates in 54 (59.3%) of the 91 isolate sets. Additionally, 57 (62.6%) of the 91 paired isolates shared the same clonal complex. (Table 1)

spa typing

We identified a total of 18 different *spa* types among 91 paired isolates. Among both the patient isolates and HCP gown or glove isolates, the most common *spa* types were t008 (n = 33 of 91, (36.8%) and t002 (n = 17 of 91, 18.7%). We defined genetic concordance as paired isolates with the same *spa* type. Based on our definition by this analysis, 71 (78%) of 91 paired isolates were genetically similar. (Table 1)

LSBSR

The genome content of the patient and HCP gown or glove isolates were analyzed using LSBSR.[17] The LSBSR matrix is composed of 8,523 potential coding sequences. Of the 91 paired isolates, 77 (84.6%) were considered genetically similar based on our definition. Among the discordant pairs, the range of gene content concordance was 34%–53.4% (Table 1).

SNV analysis

The minimum number of SNVs between the paired isolates was zero, and the maximum number of SNVs was 62,464, with a median value of 48.5 SNVs between the paired isolates. Among the 91 paired isolates, 45 (48%) were genetically similar by this metric (Fig. 3).

Summary of genomic epidemiology results

We examined the frequency of paired isolates being considered genetically similar based on all the typing methods used. Only 28 (30.7%) of the 91 paired isolates were considered to be genetically similar using all 5 typing mechanisms, followed by 28 paired isolates (30.7%) that were genetically similar in 4 of 5 typing schemas (Fig. 4). The most frequent discordant typing schema was SNV, with 49 samples being discordant.

Discussion

The objective of this study was to determine whether MRSA isolates identified from HCP gown or gloves were genetically similar to MRSA isolates from the patient. Our phylogenetic analysis identified 83% of the paired isolates as genetically similar. Similarly, the *spa* typing and the LSBSR analysis indicated that >75% of the examined isolate pairs were concordant. Among the 5 typing schemes, 56 pairs (61.5%) were considered concordant based on criteria of being concordant on 4 typing schemes. We utilized several typing methods of varying discriminatory power to convey genomic differences between the paired isolates. This is the first study to our knowledge that has employed genomic epidemiology to understand patient-to-HCP transmission in multiple-ICU setting.

Few previous studies have used WGS to determine whether MRSA transmission occurred in the healthcare setting[9–11]; however, each of the previous studies differs significantly from our study. Stine et al[10] focused on direct acute patient-to-patient transmission rather than patient–HCP transmission, and they used an

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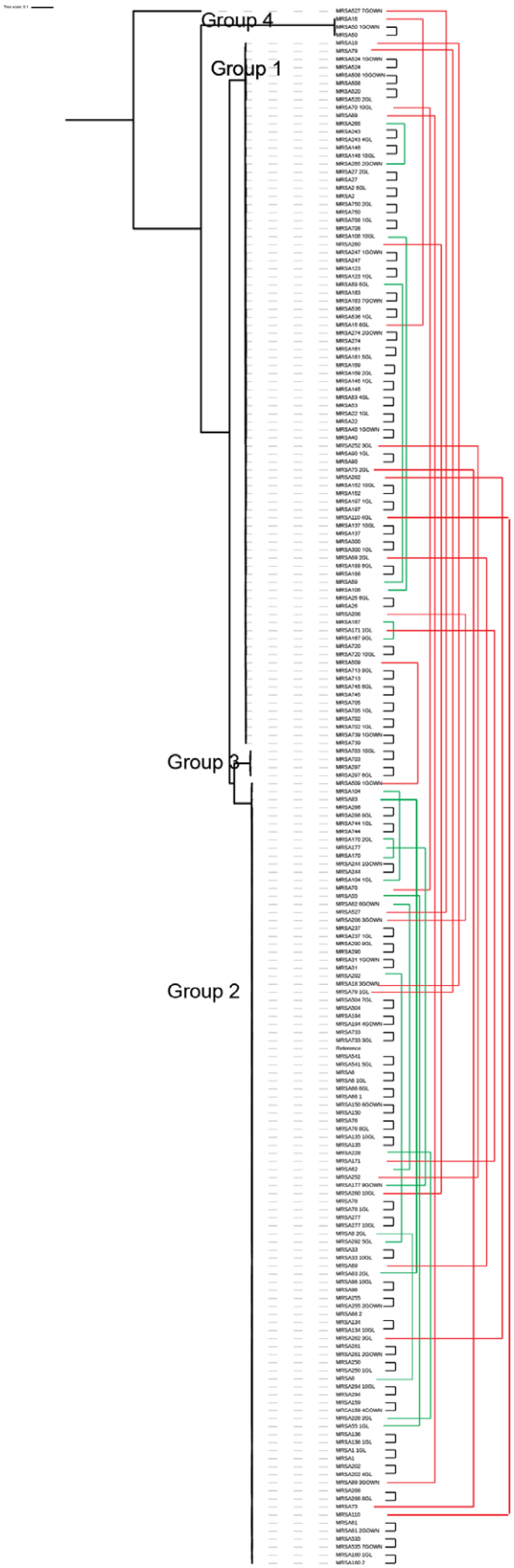

Fig. 2. Phylogenetic analysis of newly sequenced methicillin-resistant *Staphylococcus aureus* (MRSA) paired isolates. Genomes were aligned to one another, and 102,599 single-nucleotide polymorphisms (SNPs) were identified using ISG.[15] RAxML[40] was used to create the phylogenetic tree using 100 bootstrap replicates, and FigTree (http://tree.bio.ed.ac.uk/software/figtree/) was used for visualizations.[15,40] Black brackets represent paired isolates neighboring each other on the tree and are within the same group. Green brackets represent paired isolates that are within the same phylogenetic group. Red brackets represent paired isolates that are not within the same group and do not neighbor each other.

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Table 1. Typing Schema Among Paired Patient and HCP Gown or Glove Isolates (N=91)

Paired Isolates	Patient Isolate *spa* Type	HCP Isolate	Genetic Relatedness[a]	HCP ST/CC	Patient ST/CC	Genetic Relatedness[b]	No. of Genes the Same Between the paired Isolates	% of Genes That Are Genetically Similar	Genetic Relatedness[c]	SNV	Genetic Relatedness[d]	Genotypic Concordance[e]
MRSA1 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,488	99.6	Concordant	13	Concordant	Concordant
MRSA104 pairs	t064	t008	Discordant	8 (CC8)	8 (CC8)	Concordant	7,437	87.3	Concordant	2,440	Discordant	Concordant
MRSA106 pairs	t242	t002	Discordant	225 (CC5)	5 (CC5)	Discordant	7,829	91.9	Concordant	866	Discordant	Concordant
MRSA110 pairs	t008	t002	Discordant	5 (CC5)	8 (CC8)	Discordant	4,326	50.8	Discordant	24,806	Discordant	Discordant
MRSA123 pairs	t045	t045	Concordant	225 (CC5)	225 (CC5)	Concordant	8,423	98.8	Concordant	0	Concordant	Concordant
MRSA134 pairs	t121	t121	Concordant	8 (CC8)	8 (CC8)	Concordant	8,481	99.5	Concordant	10	Concordant	Concordant
MRSA135 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,500	99.7	Concordant	22	Concordant	Concordant
MRSA136 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,492	99.6	Concordant	10	Concordant	Concordant
MRSA137 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8,365	98.1	Concordant	184	Discordant	Concordant
MRSA145 pairs	t002	t002	Concordant	105 (CC5)	105 (CC5)	Concordant	8,451	99.2	Concordant	32	Concordant	Concordant
MRSA146 pairs	t2302	t2302	Concordant	5 (CC5)	5 (CC5)	Concordant	8,358	98.1	Concordant	11	Concordant	Concordant
MRSA15 pairs	t1081	t045	Discordant	105 (CC5)	45 (CC45)	Discordant	3,205	37.6	Discordant	62,464	Discordant	Discordant
MRSA150 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,450	99.1	Concordant	72	Discordant	Concordant
MRSA152 pairs	t105	t105	Concordant	105 (CC5)	105 (CC5)	Concordant	8,490	99.6	Concordant	0	Concordant	Concordant
MRSA159 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,461	99.3	Concordant	12	Concordant	Concordant
MRSA161 pairs	t002	t002	Concordant	ND (ND)	ND (ND)	Concordant	8,489	99.6	Concordant	4	Concordant	Concordant
MRSA163 pairs	t002	t002	Concordant	105 (CC5)	105 (CC5)	Concordant	8,482	99.5	Concordant	1	Concordant	Concordant
MRSA167 pairs	t242	t242	Concordant	5 (CC5)	5 (CC5)	Concordant	8,175	95.9	Concordant	79	Discordant	Concordant
MRSA169 pairs	t2308	t2308	Concordant	105 (CC5)	105 (CC5)	Concordant	8,467	99.3	Concordant	1,672	Discordant	Concordant
MRSA170 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,364	98.1	Concordant	39	Concordant	Concordant
MRSA171 pairs	t008	t242	Discordant	5 (CC5)	8 (CC8)	Discordant	4,359	51.1	Discordant	23,169	Discordant	Discordant
MRSA177 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,224	96.5	Concordant	261	Discordant	Concordant
MRSA18 pairs	t088	t211	Discordant	8 (CC8)	840 (CC5)	Discordant	4,486	52.6	Discordant	21,607	Discordant	Discordant
MRSA188 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8,489	99.6	Concordant	2	Concordant	Concordant
MRSA194 pairs	t024	t024	Concordant	8 (CC8)	8 (CC8)	Concordant	8,446	99.1	Concordant	20	Concordant	Concordant
MRSA197 pairs	t105	t105	Concordant	105 (CC5)	105 (CC5)	Concordant	8,482	99.5	Concordant	5	Concordant	Concordant

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Table 1. (*Continued*)

Paired Isolates	Patient Isolate *spa* Type	HCP Isolate	Genetic Relatedness[a]	HCP ST/CC	Patient ST/CC	Genetic Relatedness[b]	No. of Genes the Same Between the paired Isolates	% of Genes That Are Genetically Similar	Genetic Relatedness[c]	SNV	Genetic Relatedness[d]	Genotypic Concordance[e]
MRSA2 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8,464	99.3	Concordant	2	Concordant	Concordant
MRSA202 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,350	98	Concordant	90	Discordant	Concordant
MRSA206 pairs	t242	t008	Discordant	8 (CC8)	5 (CC5)	Discordant	4,527	53.1	Discordant	23,252	Discordant	Discordant
MRSA228 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,095	95	Concordant	1,374	Discordant	Concordant
MRSA237 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,445	99.1	Concordant	73	Discordant	Concordant
MRSA243 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8,458	99.2	Concordant	160	Discordant	Concordant
MRSA244 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,461	99.3	Concordant	15	Concordant	Concordant
MRSA25 pairs	t242	t242	Concordant	5 (CC5)	5 (CC5)	Concordant	8,160	95.7	Concordant	41	Discordant	Concordant
MRSA250 pairs	t008	t024	Discordant	8 (CC8)	8 (CC8)	Concordant	8,183	96	Concordant	88	Discordant	Concordant
MRSA252 pairs	t008	t002	Discordant	105 (CC5)	8 (CC8)	Discordant	4,465	52.4	Discordant	22,335	Discordant	Discordant
MRSA255 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,464	99.3	Concordant	16	Concordant	Concordant
MRSA260 pairs	t045	t008	Discordant	8 (CC8)	225 (CC5)	Discordant	4,358	51.1	Discordant	23,523	Discordant	Discordant
MRSA265 pairs	t002	t450	Discordant	5 (CC5)	5 (CC5)	Concordant	7,417	87	Concordant	3,112	Discordant	Concordant
MRSA268 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,289	97.3	Concordant	452	Discordant	Concordant
MRSA27 pairs	t450	t450	Concordant	5 (CC5)	5 (CC5)	Concordant	8,514	99.9	Concordant	2	Concordant	Concordant
MRSA274 pairs	t002	t002	Concordant	ND (ND)	ND (ND)	Concordant	8,470	99.4	Concordant	10	Concordant	Concordant
MRSA277 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,479	99.5	Concordant	22	Concordant	Concordant
MRSA281 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,480	99.5	Concordant	7	Concordant	Concordant
MRSA286 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,328	97.7	Concordant	229	Discordant	Concordant
MRSA290 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,478	99.5	Concordant	61	Discordant	Concordant
MRSA292 pairs	t211	t008	Discordant	8 (CC8)	8 (CC8)	Concordant	7,887	92.5	Concordant	453	Discordant	Concordant
MRSA294 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,466	99.3	Concordant	30	Concordant	Concordant
MRSA297 pairs	t148	t148	Concordant	770 (CC8)	72 (CC8)	Discordant	8,484	99.5	Concordant	0	Concordant	Concordant
MRSA300 pairs	t010	t010	Concordant	72 (CC8)	5 (CC5)	Discordant	8,428	98.9	Concordant	80	Discordant	Concordant
MRSA31 pairs	t211	t211	Concordant	3081 (CC5)	8 (CC8)	Discordant	8,475	99.4	Concordant	19	Concordant	Concordant
MRSA33 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,466	99.3	Concordant	19	Concordant	Concordant

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Table 1. (*Continued*)

Paired Isolates	Patient Isolate *spa* Type	HCP Isolate	Genetic Relatedness[a]	HCP ST/CC	Patient ST/CC	Genetic Relatedness[b]	No. of Genes the Same Between the paired Isolates	% of Genes That Are Genetically Similar	Genetic Relatedness[c]	SNV	Genetic Relatedness[d]	Genotypic Concordance[e]
MRSA40 pairs	t002	t002	Concordant	770 (CC8)	105 (CC5)	Discordant	8,330	97.7	Concordant	25	Concordant	Concordant
MRSA50 pairs	t1081	t1081	Concordant	ND (ND)	45 (CC45)	Discordant	8,155	95.7	Concordant	115	Discordant	Concordant
MRSA504 pairs	t530	t530	Concordant	617 (CC45)	8 (CC8)	Discordant	8,470	99.4	Concordant	87	Discordant	Concordant
MRSA508 pairs	t002	t002	Concordant	770 (CC8)	5 (CC5)	Discordant	8440	99	Concordant	95	Discordant	Concordant
MRSA509 pairs	t242	t451	Discordant	3081 (CC5)	5 (CC5)	Discordant	4,168	48.9	Discordant	22,106	Discordant	Discordant
MRSA520 pairs	t002	t002	Concordant	770 (CC8)	5 (CC5)	Discordant	8,464	99.3	Concordant	20	Concordant	Concordant
MRSA524 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8,472	99.4	Concordant	13	Concordant	Concordant
MRSA527 pairs	t008	t355	Discordant	ND (ND)	8 (CC8)	Discordant	2,930	34.4	Discordant	61.351	Discordant	Discordant
MRSA534 pairs	t002	t002	Concordant	ND (ND)	105 (CC5)	Discordant	8,303	97.4	Concordant	569	Discordant	Discordant
MRSA535 pairs	t008	t008	Concordant	ND (ND)	8 (CC8)	Discordant	8,474	99.4	Concordant	26	Concordant	Concordant
MRSA536 pairs	t002	t002	Concordant	770 (CC8)	105 (CC5)	Discordant	8,479	99.5	Concordant	63	Discordant	Concordant
MRSA541 pairs	t008	t008	Concordant	ND (ND)	ND (ND)	Concordant	8,482	99.5	Concordant	12	Concordant	Concordant
MRSA55 pairs	t008	t008	Concordant	8 (CC8)	1750 (CC8)	Discordant	7,594	89.1	Concordant	2,282	Discordant	Concordant
MRSA595 pairs	t242	t002	Discordant	ND (ND)	5 (CC5)	Discordant	8,035	94.3	Concordant	980	Discordant	Concordant
MRSA6 pairs	t008	t008	Concordant	ND (ND)	8 (CC8)	Discordant	8,474	99.4	Concordant	20	Concordant	Concordant
MRSA61 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,210	96.3	Concordant	34	Concordant	Concordant
MRSA62 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,056	94.5	Concordant	417	Discordant	Concordant
MRSA66 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,342	97.9	Concordant	58	Discordant	Concordant
MRSA69 pairs	t008	t002	Discordant	ND (ND)	8 (CC8)	Discordant	4,549	53.4	Discordant	23,762	Discordant	Discordant
MRSA70 pairs	t008	t2235	Discordant	5 (CC5)	8 (CC8)	Discordant	4,483	52.6	Discordant	21,757	Discordant	Discordant
MRSA702 pairs	t242	t242	Concordant	5 (CC5)	5 (CC5)	Concordant	8,421	98.8	Concordant	54	Discordant	Concordant
MRSA705 pairs	t242	t242	Concordant	ND (ND)	5 (CC5)	Discordant	8,430	98.9	Concordant	25	Concordant	Concordant
MRSA708 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8440	99	Concordant	98	Discordant	Concordant
MRSA713 pairs	t242	t242	Concordant	5 (CC5)	5 (CC5)	Concordant	8,480	99.5	Concordant	34	Concordant	Concordant
MRSA720 pairs	t242	t242	Concordant	5 (CC5)	5 (CC5)	Concordant	8,496	99.7	Concordant	2	Concordant	Concordant
MRSA733 pairs	t008	t008	Concordant	ND (ND)	8 (CC8)	Discordant	8,279	97.1	Concordant	20	Concordant	Concordant

(*Continued*)

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Table 1. (*Continued*)

Paired Isolates	Patient Isolate *spa* Type	HCP Isolate	Genetic Relatedness[a]	HCP ST/CC	Patient ST/CC	Genetic Relatedness[b]	No. of Genes the Same Between the paired Isolates	% of Genes That Are Genetically Similar	Genetic Relatedness[c]	SNV	Genetic Relatedness[d]	Genotypic Concordance[e]
MRSA739 pairs	t242	t242	Concordant	ND (ND)	5 (CC5)	Discordant	8,441	99	Concordant	43	Discordant	Concordant
MRSA744 pairs	t008	t008	Concordant	ND (ND)	8 (CC8)	Discordant	8,463	99.3	Concordant	15	Concordant	Concordant
MRSA745 pairs	t242	t242	Concordant	ND (ND)	5 (CC5)	Discordant	8,503	99.8	Concordant	8	Concordant	Concordant
MRSA75 pairs	t008	t105	Discordant	105 (CC5)	8 (CC8)	Discordant	4,440	52.1	Discordant	24,746	Discordant	Discordant
MRSA750 pairs	t002	t002	Concordant	5 (CC5)	5 (CC5)	Concordant	8,495	99.7	Concordant	151	Discordant	Concordant
MRSA76 pairs	t008	t008	Concordant	ND (ND)	8 (CC8)	Discordant	8,474	99.4	Concordant	9	Concordant	Concordant
MRSA78 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,467	99.3	Concordant	7	Concordant	Concordant
MRSA79 pairs	t2666	t008	Discordant	8 (CC8)	840 (CC5)	Discordant	4,404	51.7	Discordant	21,611	Discordant	Discordant
MRSA8 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	7,408	86.9	Concordant	1,294	Discordant	Concordant
MRSA83 pairs	t008	t008	Concordant	8 (CC8)	8 (CC8)	Concordant	8,003	93.9	Concordant	1,992	Discordant	Concordant
MRSA89 pairs	t002	t008	Discordant	8 (CC8)	5 (CC5)	Discordant	4,517	53	Discordant	22,184	Discordant	Discordant
MRSA90 pairs	t002	t002	Concordant	ND (ND)	105 (CC5)	Discordant	8,511	99.9	Concordant	1	Concordant	Concordant
MRSA96 pairs	t008	t008	Concordant	ND (ND)	8 (CC8)	Discordant	8,316	97.6	Concordant	64	Discordant	Concordant

[a]Concordance was defined as paired isolates with same *spa* type.
[b]Concordance was defined as paired isolates with same CC type.
[c]Concordance was defined as paired isolates with a genetically similar ≥90%.
[d]Concordance was defined as paired isolates with <40 SNVs.
[e]Concordance was defined by the phylogenomic similarity in Figure 2.

SNV-based analysis that identified 3 transmission clusters in nursing home over a 12-week period. Two additional WGS-based studies focused on MRSA transmission by examining patient, HCP, and environmental surfaces such as computers and mobile devices in the ICU setting.[9,11] Price et al[9] and Popovich et al[11] each examined how the HCP or environment could be potential vectors of transmission to patients in the ICU setting using a longitudinal cohort. Both studies identified transmission events between patient and the HCP; acquisition occurred 7 of 25 times in the study by Price et al and 4 of 6 times in the study by Popovich et al.[9,11] However, Price et al focused on HCP nasal carriage as a proxy of potential transmission, which is significantly different than our study, which used HCP gown or gloves as a measure of transmssion. Nasal carriage suggests potential colonization and does not consider transient contamination and short-term carriage that fails to result in colonization.

In contrast, our study focused on the acute transient transmission of MRSA from the patient to HCP gown and gloves. We obtained isolates from the gown and gloves of HCP immediately after patient-care activity, suggesting an acute transmission event directly or indirectly from the patient to the HCP. Due to the longitudinal focus and the time between patient contact and measurement of the HCP, Price et al may not have ascertained direct acute transient transmission, which has been demonstrated to be a frequent occurrence (16.2% of the time in MRSA) in the ICU setting among HCP- and MRSA-positive patients.[5,6] Additionally, we are the first researchers, to our knowledge, to employ multiple genomic epidemiology techniques to ascertain transmission of MRSA from the patient to HCP.

We anticipated that many paired isolates would be genetically similar; however, we identified several isolate pairs that were not genetically similar depending on the molecular typing schema used (20%–48%). Several hypotheses may explain these results. First, HCP may have picked up isolates from the patient room environment when performing healthcare activities; thus, the identified isolate may not be directly from the current patient but rather from other patients or sources, such as the HCP themselves or equipment within the ICU.[6,7,9,11]

Another possible explanation of why HCP gown and glove isolates differed from the patients isolate following patient-care activity is that the patient may harbor multiple MRSA strains that were not detected in the clinical sample. We did not capture the genomic diversity among the patient isolates because we examined only a single MRSA isolate per patient for WGS; however, patients may have

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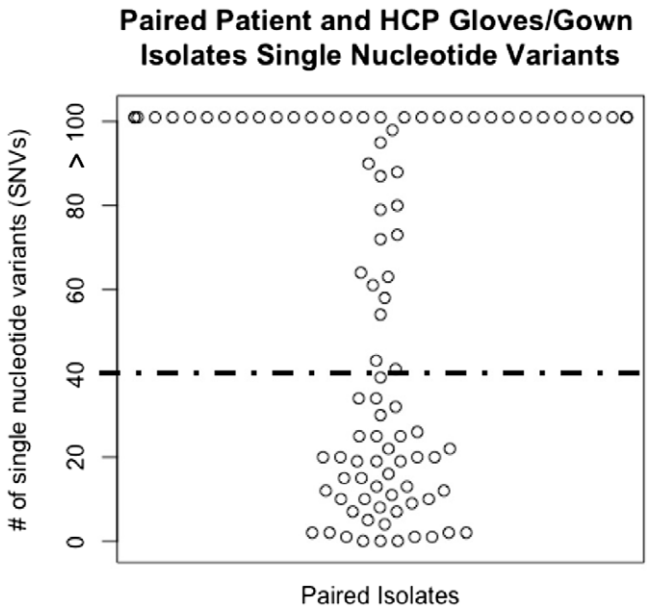

Fig. 3. Single nucleotide variant differences within paired isolates paired methicillin-resistant *Staphylococcus aureus* (MRSA) isolates using Parsnp.[26] A bee-swarm plot was used to plot single-nucleotide variant (SNV) differences and was generated using R version 4.02.[29] Genetic concordance was defined as paired isolates differing by <40 SNVs as previously defined in the literature.[9,10]

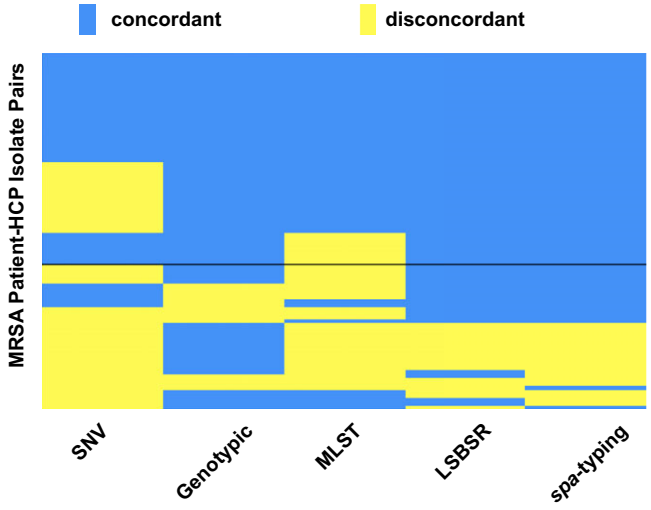

Fig. 4. A heatmap of the frequency of genetic concordance among the paired isolates using the 5 comparative genomic techniques in the study. The line on the figure is the line of concordance. Paired isolates below the line are considered discordant based on the 4 of the typing methods.

multiple MRSA isolates from a single swab.[10,30] Previous studies have demonstrated that some patients have >1 MRSA isolate, with the prevalence of multiple isolates in patient samples being as high as 38%.[10,30] Additional studies that examine multiple diverse isolates per sample with WGS may be required for a complete understanding of the diversity of the patient and HCP samples.

Third, isolates identified on gowns and gloves of HCP could be from HCP nasal or hand carriage. The prevalence of MRSA carriage among HCP has been previously measured at 4.6%.[31,32] Studies have demonstrated the HCP as a possible source of MRSA transmission through possible shedding from HCP nasal carriage.[33,34] HCP may have unknowingly contaminated their gown and gloves with MRSA while performing routine daily duties, which might have facilitated spread to the patient environment and, subsequently, the patient.

Lastly, HCP gowns and gloves can be contaminated in the common areas where gowns or gloves are housed. HCP don new gowns and gloves from the communal supply area before entering the patient's room. Diaz et al[35] identified that 75% of gloves tested from examination rooms were positive for bacterial pathogens including coagulase-negative *Staphylococcus*, *Bacillus* spp, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. However, gloves from a newly opened box were not contaminated, suggesting that contamination occurred after opening.[35] However, additional studies have demonstrated that there is little contamination found in glove boxes.[36] Further studies are needed to demonstrate the risk of contamination of glove boxes to determine whether this hypothesis explains some of the observed discordance between the paired isolates.

Despite its novelty, this study had several limitations. First, neither the HCP gown nor gloves were fully cultured to examine the total genomic diversity of the MRSA. We examined a sample from the gown and gloves using a standardized technique described in previous studies, which were the most likely areas that came into contact with the patient.[4,5,36,37] Additionally, we did not find an association of the genotypes isolated or diversity observed with the origin of the HCP sample (glove or gown). Second, we did not culture the patient environment; therefore, we did not determine whether the isolates found on the gown and gloves of HCP were also common in the environment. Distinguishing between environmental and patient isolates may be difficult because patient-care activities require interaction with the environment (eg, blood pressure cuffs, IV tubing) as well as the patient. Third, we did not swab HCP hands and nasal carriage before patient-care activities to determine the MRSA burden and genomic diversity on the HCP. Finally, we did not attempt to assess the possible transmission from the HCP to secondary patients. Although it is an important aspect of organismal transmission, this study was not designed to assess secondary transmission; we examined the primary transmission events. Establishing secondary transmission patterns from the primary HCP would be interesting, but it was beyond the scope of analysis.

Overall, our results demonstrate that transmission of MRSA from the patient to HCP does occur when HCP care for patients, and most paired isolates were genetically similar. Comparative genomics has increased our understanding of the isolates identified on the gown and gloves of HCP. These findings strengthens our knowledge regarding the extent to which MRSA patients contaminate the HCP gown and gloves following HCP–patient interaction. These data suggest that if healthcare workers were not wearing gloves and gowns, their hands and clothing would frequently become contaminated with MRSA, resulting in subsequent transmission to other patients and the hospital environment. Our results provide important data related to the debate about the pros and cons of glove and gown use (ie, contact precautions) as part of hospital MRSA control programs.[38,39]

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2022.159

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Conflicts of interest. The authors declare no conflicts of interest relevant to this article.

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