

1 Whole genome SNP typing to investigate methicillin-resistant *Staphylococcus aureus* carriage in  
2 a health-care provider as the source of multiple surgical site infections.

3

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9 Running title: WGST for tracking MRSA SSI point source.

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23

24 *ABSTRACT*

25 *Background.* Prevention of nosocomial transmission of infections is a central responsibility in  
26 the healthcare environment, and accurate identification of transmission events presents the first  
27 challenge. Phylogenetic analysis based on whole genome sequencing provides a high-resolution  
28 approach for accurately relating isolates to one another, allowing precise identification or  
29 exclusion of transmission events and sources for nearly all cases. We sequenced 24 methicillin-  
30 resistant *Staphylococcus aureus* (MRSA) genomes to retrospectively investigate a suspected  
31 point source of three surgical site infections (SSIs) that occurred over a one-year period. The  
32 source of transmission was believed to be a surgical team member colonized with MRSA,  
33 involved in all surgeries preceding the SSI cases, who was subsequently decolonized. Genetic  
34 relatedness among isolates was determined using whole genome single nucleotide polymorphism  
35 (SNP) data.

36  
37 *Results.* Whole genome SNP typing (WGST) revealed 283 informative SNPs between the  
38 surgical team member's isolate and the closest SSI isolate. The second isolate was 286 and the  
39 third was thousands of SNPs different, indicating the nasal carriage strain from the surgical team  
40 member was not the source of the SSIs. Given the mutation rates estimated for *S. aureus*, none of  
41 the SSI isolates share a common ancestor within the past 14 years, further discounting any  
42 common point source for these infections. The decolonization procedures and resources spent on  
43 the point source infection control could have been prevented if WGST was performed at the time  
44 of the suspected transmission, instead of retrospectively.

45

46 *Conclusions.* Whole genome sequence analysis is an ideal method to exclude isolates involved in  
47 transmission events and nosocomial outbreaks, and coupling this method with epidemiological  
48 data can determine if a transmission event occurred. These methods promise to direct infection  
49 control resources more appropriately.

50

51 Keywords: nasal carriage, surgical site infections, MRSA, transmission, whole genome  
52 sequencing

53

54 *Background*

55

56 Surgical site infections (SSIs) account for 31% of all healthcare-associated infections in  
57 the United States [1]. A critical risk factor for the development of a SSI is the severity of wound  
58 contamination during surgery [2, 3]. As one of the most predominant organisms colonizing the  
59 skin, *Staphylococcus aureus* is a common causative agent of SSI [4], and methicillin-resistant *S.*  
60 *aureus* (MRSA) accounts for 15% of reported SSIs [5]. The most significantly associated  
61 independent circumstance for the development of a *S. aureus* SSI is nasal carriage by the patient  
62 [3]. Studies that examined perioperative decolonization of patients using intranasal mupirocin  
63 showed a significant decrease in the risk of developing a SSI [6–8]. The risk of a *S. aureus* SSI  
64 associated with *S. aureus* nasal carriage or colonization of surgical team members is unknown.

65

66 The implementation of whole genome sequencing (WGS) in the clinical setting to closely  
67 analyze isolates has allowed for precise identification and characterization of putative  
68 transmission events among patients. WGS also allows for high resolution analysis of source

69 tracing in hospitals which can have the potential to impact hospital-based outbreak investigations  
70 and infection prevention practices [9–12]. To our knowledge, however, no study has investigated  
71 MRSA carriage among surgical staff members as a source of MRSA transmission to surgical  
72 patients using WGST.

73

74 In this study, a hospital infection control team identified a cluster of MRSA SSIs from a  
75 single surgical team. Antibiotic susceptibility profiles suggested that the cases were related. The  
76 surgical team in question was screened for nasal carriage by the infection control team and one  
77 member tested positive for MRSA by PCR, as well as by culture. The surgical team member was  
78 decolonized per hospital protocol, and the MRSA decolonization was documented as successful,  
79 based on further nasal screening. In this study, we retrospectively examine the MRSA strain  
80 from the colonized surgical team member along with patient and control strains to determine  
81 whether it was the source of the cluster of SSIs using whole genome single nucleotide  
82 polymorphism (SNP) typing (WGST) [13–15]. These results demonstrate the utility of WGST  
83 for hospital molecular epidemiology and provide confirmation that the source of infection for  
84 three patients was not related to the surgical team member isolate.

85

86 *Methods*

87 *Surveillance*

88

89 From February 2010 to August 2011, 24 MRSA isolates were obtained from 18 patients  
90 and one surgical team member in a rural 280-bed hospital (Table 1). Isolates were obtained from  
91 leftover, in-house testing as approved by the Institutional Review Board of the participating

92 institution. No patient consent was needed as samples obtained were isolates only. The isolates  
93 fell into three groups; the first group comprised the surgical team member's isolate (suspected  
94 point source) and three patients' SSI isolates. The three isolates were from patients who  
95 developed MRSA surgical site infections following surgical procedures by the same medical  
96 team. The surgical team in question was screened for nasal carriage and one member tested  
97 positive for MRSA by the GeneXpert Infinity MRSA PCR (Cepheid), and subsequently MRSA  
98 was cultured from the original swab providing the surgical team member isolate. The surgical  
99 team member's isolate was tested and confirmed positive for *mecA*, an assay included in the  
100 GeneXpert PCR assay.

101

102 The second group consisted of 10 background isolates considered to have no association  
103 with the first group of samples but were also from SSIs, collected randomly from the regular  
104 sample flow at the hospital. The third group comprised 10 background samples, also randomly  
105 collected, from outpatients and patients admitted to the hospital with MRSA infections. These  
106 isolates were sourced from blood, sputum, wound and urine specimens. In all three groups of  
107 isolates, multiple samples from individual patients, labeled as "a" and "b", were taken over time  
108 to serve as a control threshold for genetic similarity and relatedness.

109

#### 110 *DNA sequencing*

111

112 Isolates were grown on trypticase soy agar media and DNA was prepared using DNeasy  
113 Blood & Tissue Kit as described by the manufacturer (Qiagen, Valencia, CA) with the addition  
114 of lysostaphin to the gram-positive extraction protocol from Qiagen. The DNA samples were

115 prepared for multiplexed, paired-end sequencing with a 500 base pair insert using Library  
116 Preparation Kit with Standard PCR Library Amplification (KAPA Biosystems, Woburn, MA). A  
117 100 bp read paired-end run was used for all twenty-four isolates on the GAIIX sequencing  
118 platform (Illumina, Inc. San Diego, CA).

119  
120 *SNP Phylogenetic Analysis*

121  
122 A phylogeny of all the MRSA isolates was constructed based on a matrix that tabulates  
123 all SNPs among the genomes of the isolates by their locus within a reference genome. The  
124 matrix was generated using the Northern Arizona SNP Pipeline (NASP)  
125 (<http://github.com/TGenNorth/NASP/releases/tag/v0.9.6>). NASP aligns DNA sequencing reads  
126 to a reference, identifies SNPs and filters SNP loci based on user-defined parameters as  
127 previously described[14–18]. NASP generated a matrix for this study by aligning sequence reads  
128 from the 24 isolates to the reference genome, FPR3757 (NCBI accession number: NC\_007793),  
129 using Novoalign (Novocraft.com). Reads that mapped to multiple locations within the reference  
130 genome were excluded from the alignments as were reads containing insertions or deletions.  
131 SNPs were identified in the alignments using the Genome Analysis Toolkit (GATK) [19]. Only  
132 SNP loci found throughout all samples were used for the phylogenetic analysis. Additionally,  
133 SNPs had to be in >90% of the reads and have minimum 10X coverage to be included in a final  
134 matrix. MUMmer version 3.22 [20] was used to identify duplicated regions within FPR3757 and  
135 SNP loci within these regions were removed from the final analysis. The phylogenetic analysis  
136 of the high-quality core genome SNP matrix was performed with *MEGA* version 5.05 [21] using  
137 the maximum parsimony algorithm and bootstrap analysis with 1,000 replicates. Read depth

138 statistics and reference coverage were determined from NASP. The whole genome sequence read  
139 files were deposited in the NCBI Sequence Read Archive under BioProject ID PRJNA255788.  
140 Read data were also used to identify the multi-sequence locus type (MLST) using SRST2 [22].

141

## 142 *Results*

### 143 *Genomic Investigation*

144

145 The sequence analysis provided appropriate depth and breath of sequence coverage  
146 across all isolates to conduct robust WGST analysis. Mean sequence read coverage of the  
147 reference genome FPR3757 for all samples was  $\geq 95\%$  with a mean read-depth of 103X. The  
148 phylogeny constructed was based on 18,520 parsimony informative SNPs and revealed two  
149 distinct clades (Figure 1). MLST analysis using WGS data identified these clades as two distinct  
150 sequence types: ST5, which is known to include a major clone (commonly referred to as PFGE  
151 type USA100) that is historically been associated with healthcare-acquired infections; and ST8, a  
152 major clonal group of largely community-acquired strains (containing the virulent clone that is  
153 commonly referred to as PFGE type USA300). ST5 and ST8 represent two distantly related  
154 lineages of *S. aureus* that are common in hospitals across the country [23, 24].

155

156 WGST analysis demonstrated that no obvious transmission event occurred between the  
157 colonized surgical team member and patients. First, the two distinct clades in the phylogenetic  
158 tree, ST5 and ST8, are separated by 17,042 SNPs. The surgical team member isolate and one of  
159 the MRSA SSI cluster isolates, Case 3, are located in ST5 and ST8 respectively. Second, the  
160 surgical team member isolate is 283 SNPs different from the closest case isolate, Case 2, and

161 even more distant from the third, Case 4. Estimates of the mutation rate for *S. aureus* have been  
162 calculated repeatedly at a rate of approximately  $3.3 \times 10^{-6}$  mutations per site per year, or one core  
163 genome SNP every 6 to 7 weeks [25–27]. Using this estimate, there should be approximately 8 to  
164 10 SNPs per year after strains diverge from a common ancestor due to baseline mutation alone.  
165 Given their 283 SNP separation, the isolates from the surgical team member and Case 2 had a  
166 common ancestor more than 14 years prior to the surgery. Case 4, the other suspected related  
167 isolate, was as distant from the surgical team member isolate as Case 2, with a 285 SNP  
168 differentiation.

169  
170 Additionally, in order to determine epidemiologically related and unrelated isolates using  
171 WGST, a threshold was established from a set of related isolates for our study, using multiple  
172 samples taken from individual patients over time (Healthcare Control 9a and 9b, Case 3, 3a and  
173 3b, Hospital Control 8a and 8b and Hospital Control 4a and 4b). The average pairwise distance  
174 between each set of related isolates was 2.3 SNPs with the exception of Case 3. Case 3, 3a and  
175 3b were from the same patient over the span of one year and taken from multiple body sites.  
176 These three isolates contained 21 SNPs among them, with no more than 10 SNPs between any  
177 two isolates; however, this number is still within the predicted baseline mutation rate of *S.*  
178 *aureus*. Healthcare control samples 9a, 9b and 11 had two SNP differences separating them,  
179 indicating a likely recent transmission event, probably within the long-term care facility where  
180 both patients resided.

181  
182 *Discussion*

183



184 Our investigation determined that the surgical team member isolate was not the source of  
185 a transmission event and that no evidence of transmission occurred among the suspect cases. It is  
186 well established that humans are commonly colonized with *S. aureus*, with a fraction of the  
187 population being carriers of MRSA; it is estimated that 0.2-7% of inpatient hospital populations  
188 and upwards of 42.2% of hospital staff are MRSA carriers [28–30]. A recent study estimates *S.*  
189 *aureus* carriage among surgeons as high as 45.4% [31]. A high incidence of both methicillin-  
190 susceptible *S. aureus* (MSSA) and MRSA nasal carriage are exacerbating the increasing number  
191 of healthcare-associated *S. aureus* infections in patient care facilities [32]. Reducing patient  
192 colonization has been hypothesized to help in the prevention of MRSA transmission and  
193 infection [28, 29]. However, the impact of MRSA carriage by medical providers and hospital  
194 ancillary staff when compared to the MRSA nasal carriage rate of patients, in association with  
195 the incidence of MRSA nosocomial infections, is still not well understood but has been  
196 speculated as a source of patient infection[31, 33, 34]. Such provider to patient transmission  
197 would have significant liability, reimbursement and risk management implications for both  
198 providers and healthcare facilities. In this study, we used high-resolution techniques to compare  
199 a MRSA isolate carried by a surgical team member to the MRSA isolates of that surgical team  
200 member’s patients who had post-operative SSIs. WGST confirmed that transmission events did  
201 not occur between a healthcare provider and the surgical patients examined. In order to  
202 determine accurate epidemiological association, background control isolates from the hospital  
203 and community were compared to the suspected cluster isolates. By including these background  
204 isolates, if a transmission event had occurred, we would have observed a clear phylogenetic  
205 distinction between the background isolates and the isolates involved in the transmission event.  
206 However, we identified more than 17,000 parsimony informative SNPs among the 24 isolates

207 and we did not observe a distinct grouping of the isolates that were originally thought to be  
208 surgery-associated transmission events.

209  
210         WGST has been successfully used as a molecular epidemiology tool for numerous  
211 outbreak investigations [13–18, 35]. Our study further establishes the effectiveness of WGST for  
212 epidemiological investigations within a hospital setting, which is especially critical for rapid  
213 analysis during an ongoing outbreak. Interestingly, we identified a previously unrecognized  
214 transmission event outside the hospital that likely would not have been discovered without  
215 WGST. Furthermore, this study highlights the need for additional investigation to identify the  
216 source of hospital-acquired MRSA infections, including but not limited to medical providers,  
217 hospital staff members and the patient’s own nasal colonization, as well as the importance of  
218 source tracing to correctly identify an outbreak.

219         Implementing the reported mutation rate within our data disqualifies our hypothesis of a  
220 healthcare provider-related transmission, with at least 14 years of evolutionary time between the  
221 isolates from the surgical team member and the respective patients. Furthermore, the high  
222 resolution of WGST provides a genetic threshold to gauge relatedness between isolates collected  
223 from a single patient from multiple body sites and over time in comparison to isolates from  
224 unrelated patients. This genetic relatedness framework also allowed for the detection of a  
225 previously unrecognized MRSA transmission event. These data also suggest importation of  
226 strains circulating in other care facilities is a potential source for hospital-acquired infections.

227  
228         A limitation of this study is the sequencing of a single colony from the suspected source.  
229 Recent research shows the bacterial diversity within a host can impede accurate depiction of

230 transmission events [36]. Pathogen genetic diversity within a host can be widespread and may be  
231 due to numerous transmissions from multiple sources [36]. Furthermore, *S. aureus* studies reveal  
232 carriage of numerous sequence types within a single host likely due to multiple independent  
233 transmissions [37, 38]. In order to account for within-host diversity and reduce the risk of falsely  
234 interpreting results, it will be important to increase the sampling of isolates from the suspected  
235 source in future investigations. However, the fact that none of the three SSI isolates were related  
236 to one another provides evidence against a point source regardless of the single isolate sampling  
237 from the surgical team member.

238

239 *Conclusions.*

240

241 The lack of a MRSA transmission event between the colonized surgical team member  
242 and the surgical patients shown here has implications in the healthcare field, from infection  
243 prevention to hospital epidemiology and healthcare reimbursement. These findings were only  
244 apparent through the use of WGST. Low resolution strain typing methods like antibiogram  
245 profiles may lead to erroneous conclusions, and may instigate inappropriate, costly, and  
246 ineffective infection control measures as was the case here, as well as limit reimbursement for  
247 inpatient services from third-party payers (e.g., CMS) [39]. The accurate determination or  
248 repudiation of a healthcare-associated outbreak or cluster will prevent unnecessary, disruptive  
249 and costly infection prevention procedures and allow the implementation of these control  
250 measures when they are appropriate. With the increasing reality of clinical laboratory bacterial  
251 sequencing and accessible analysis tools, healthcare and public health systems would do well to

252 maintain isolate and/or sequence repositories of local critical nosocomial pathogens to quickly  
253 detect and analyze possible hospital associated infection (HAI) outbreaks.

254

255 *Abbreviations.*

256 **MRSA:** Methicillin resistant *Staphylococcus aureus* **SSI:** Surgical site infection **SNP:** Single  
257 nucleotide polymorphism **WGST:** Whole genome SNP typing **WGS:** Whole genome  
258 sequencing **PCR:** Polymerase chain reaction **NASP:** Northern Arizona SNP pipeline **GATK:**  
259 Genome analysis toolkit **MLST:** Multi-locus sequence type **PFGE:** Pulse-field gel  
260 electrophoresis **ST:** sequence type **MSSA:** Methicillin resistant *Staphylococcus aureus* **HAI:**  
261 Hospital associated infection

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263 *Conflict of interest statement*

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265 All authors on this manuscript do not have an association that might pose a conflict of interest.

266

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271

272 *Author's Contributions*

273

274 CR analyzed the data and contributed to writing and revising the manuscript. KH contributed to  
275 the design of the study and sample collection. ED contribute to the design of the study, the  
276 analysis and writing and revising the manuscript. JB contributed to study design, processing  
277 samples and writing and revising the manuscript. JT contributed to writing the manuscript and  
278 the interpretation of the data. PK contributed to the study design, writing and revising the  
279 manuscript. DE contributed to the study design, writing and revising the manuscript. All authors  
280 read and approved the manuscript.

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451 *Figure Legends*

452 Figure 1. **Phylogenetic SNP analysis.** Unrooted phylogenetic SNP analysis based on whole  
453 genome sequence data of 24 MRSA isolates using the maximum parsimony algorithm.

454 Table 1. **Clinical histories of patients with MRSA infections, including suspected index case.**

455 Multiple samples taken from individuals are labeled as “a” and “b.”

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478 Table 1. Clinical histories of patients with MRSA infections, including surgical team member  
479 isolate.

<b>Patient</b>	<b>Isolates</b>	<b>Source</b>	<b>Year Collected</b>
Surgical Team Member	1	Nasal	February 2011
Case 2	1	Blood	February 2011
Case 3a	3	Abdominal fluid	September 2010
Case 3b	-	Anal fistula	August 2011
Case 3c	-	Sputum	August 2011
Case 4	1	Deep wound	July 2010
Hospital Control 1	1	Tissue	March 2010
Hospital Control 2	1	Incision	December 2010
Hospital Control 3	1	Joint fluid	September 2010
Hospital Control 4a	2	Tissue	March 2010
Hospital Control 4b	-	Deep wound	March 2010
Hospital Control 5	1	Tissue	November 2010
Hospital Control 6	1	Tissue	October 2010
Hospital Control 7	1	Deep wound	December 2010
Hospital Control 8a	2	Abdominal fluid	February 2011
Hospital Control 8b	-	Abdominal fluid	February 2011
Healthcare Control 9a	2	Head	February 2011
Healthcare Control 9b	-	Head	February 2011
Healthcare Control 10	1	Right stump	March 2011
Healthcare Control 11	1	Urine	February 2011
Community Control 12	1	Blood	March 2011
Community Control 13	1	Boil	October 2010
Community Control 14	1	Abscess groin	February 2011
Community Control 15	1	Excision site abdominal	March 2011

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