Genomic and Phenotypic Characterization of Mupirocin Resistant *Staphylococcus aureus* Clinical Isolates

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35 **Key points (40 words):**

36 • In a survey of clinical isolates in Florida, 34.4% of MRSA strains were mupirocin
37 resistant.

38 • Mupirocin resistance correlated with mutations in *ileS* or carriage of *mupA*.

39 • We found evidence for emergence of MRSA mupirocin-resistant strains that were
40 sequence type ST3390.

41 **Word count:** 3499

42 **Author contributions:** AMV and EAF performed all experiments, analyzed the data and wrote
43 the manuscript; JKJ and SJK performed genomic analysis of ST8 sequence types. DNB, AL and
44 SS provided strains and guided MIC studies/interpretation; KA provided prior mupirocin
45 surveillance data and advised on current infection prevention practices; EC and TA sequenced
46 the first 80 strains; LNS and KK supervised the project and edited the final manuscript. All
47 authors provided input into the manuscript content and interpretation of data.

48

Abstract

Background: Colonization with *Staphylococcus aureus* is a risk factor for subsequent infection. Decolonization with the topical antibiotic mupirocin is effective and reduces the risk of subsequent *S. aureus* infection for both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains but may select for mupirocin-resistant isolates.

Methods: We characterized oxacillin and mupirocin susceptibility amongst 384 *S. aureus* strains isolated from clinical samples isolated 2017-2023 in Tampa, Florida, spanning strains collected before and after the onset of the COVID-19 pandemic. Whole genome sequencing of bacterial isolates was conducted in parallel and correlated with drug susceptibility profiles.

Results: Mupirocin resistance (MupR) was nearly exclusively present in MRSA strains (103/106 97.1% of MupR; 103/299 34.4% of MRSA). Although our hospital protocol for decolonization shifted to povidone iodine in the Post-COVID period, the overall prevalence of MupR did not change in Pre-COVID and Post-COVID samples (28.9% vs 26%). Genotype correlated with antibiotic susceptibility with low level MupR (MupLR), linked to mutations in *ileS* and high level MupR (MupHR), linked to the presence of *mupA*. Genome analysis revealed that most MupR strains fell into three sequence types (ST) falling into two major clonal complexes (CC): CC8 ST8 (including Community-Associated MRSA strains USA300 and USA500), CC5 ST5 (associated with Healthcare-Associated MRSA such as USA100), and CC5 ST3390. ST3390 isolates had the highest prevalence of MupR (30/36 83%; MupHR 20/36 55.6%; MupLR 10/36 27.8%).

Conclusions: Mupirocin resistance was prevalent in our hospital MRSA strains. We also found evidence for emergence and persistence of ST3390 MRSA-MupR strains in Florida.

Keywords: Staphylococcus aureus, MRSA, mupirocin, antimicrobial resistance, decolonization

71 **Abbreviations:** methicillin resistant *Staphylococcus aureus* (MRSA); methicillin sensitive
72 *Staphylococcus aureus* (MSSA); Mupirocin Resistant (MupR); clonal complexes (CC); sequence
73 type (ST)

74 **Introduction**

75 Infections with *Staphylococcus aureus* are major causes of skin and soft tissue infections, as well
76 as invasive infections such as endocarditis, pneumonia, and osteomyelitis. Colonization of skin
77 and mucosal surfaces with *S. aureus* is a risk factor for subsequent clinical infections [1].
78 Decolonization is an effective method for reducing risk of subsequent *S.aureus* infections,
79 including those caused by methicillin resistant *S. aureus* (MRSA) [2]. Universal decolonization
80 with mupirocin and chlorhexidine body washes has been shown in randomized control trials as
81 being more effective than targeted decolonization [3]. Decolonization of *S. aureus* prevents post-
82 surgical infections, ventilator associated pneumonias and blood stream infections [4].

83 Mupirocin inhibits bacterial protein synthesis by competitively binding to the isoleucine specific
84 site of the isoleucyl-tRNA synthetase preventing the conversion of isoleucine and tRNA to
85 isoleucyl-tRNA. As this is an essential process, this inhibition leads to bacterial cell death. There
86 have been reports of increasing prevalence of mupirocin resistance, particularly amongst MRSA
87 strains, hypothesized due to increased usage of decolonization protocols designed to prevent
88 MRSA infections [5].

89 Mupirocin resistance (MupR) can be categorized as mupirocin susceptible (MupS, ≤4 μg/mL),
90 low-level resistant (MupLR, 8-64 μg/mL), and high-level resistant (MupHR, ≥ 512 μg/mL) [6] [7]
91 [8] [9] [10] [11]. Isolates demonstrating MupLR typically have a chromosomal mutation altering the
92 Rossman fold of the native isoleucyl-tRNA synthetase (IleS) protein sequence [12]. The most
93 prevalent mutations are V588F and V631F, which result in bulkier residue within the enzymatic
94 binding site, blocking association with mupirocin. The major determining factor behind high-level

resistance is the gene *mupA*, although exceptions have been noted, including *mupA*-negative MupHR-isolates that possess a different gene, *mupB*, which is 65% similar to *mupA* [13]. Whilst mupirocin binds discriminately to the substrate binding sites of bacterial isoleucyl-tRNA synthetases, it has low affinity for eukaryotic isoleucyl-tRNA synthetases. Both MupA and MupB have altered sequences that are closer to that of eukaryotes, whilst still functional in prokaryotes. Importantly, both genes can be found on multi-resistant plasmids, facilitating the spread of resistance.

In this study we apply a combination of Whole Genome Sequencing (WGS) and phenotypic characterization to investigate changes in the molecular epidemiology of *S. aureus* mupirocin resistance within a large metropolitan hospital in Florida. Our study period includes isolates before and after the COVID-19 pandemic, which coincided with a switch in hospital protocols from mupirocin to povidone iodine for decolonization.

Methods

SA Strain Sampling: A random convenience sample of clinical *S. aureus* isolates was collected from discarded residual clinical samples at Tampa General Hospital (TGH) between 2017-2023. TGH is a 1040 bed not-for-profit academic medical center serving a population exceeding six million across western Florida. The TGH research lab archives more MRSA than MSSA isolates and randomly archives samples from different body sites.

Patient Consent Statement: Collection of samples for this study was reviewed by our local IRB and was deemed not human subjects research and did not require patient consent.

MIC Testing: Samples were struck to TSA for isolation overnight and transferred to TSB for overnight growth at 37°C in a shaking incubator. MIC testing was performed using a top agar method. Clinical susceptibility data were verified using E-tests MupR was designated as

119 sensitive (MIC <4 µg/mL), low-level or intermediate resistant (MupLR, MIC = 8-256 µg/mL), or
120 high level resistant (MupHR, MIC >512 µg/mL). Bacteria were suspended to 0.5 McFarland in
121 Mueller Hinton II agar for mupirocin testing and Muller Hinton II Agar + 2% NaCl for oxacillin
122 testing. Inoculated agar (10mL) was poured over premade uninoculated plates with their
123 corresponding agar. Test strips from Liofilchem were placed on dried agar corresponding to the
124 antibiotic being tested. Muller Hinton II agar with mupirocin test strips were incubated inverted at
125 37°C for 18 hours. Muller Hinton II +2% NaCl agar with oxacillin test strips were incubated
126 inverted at 37°C for 24 hours. Mupirocin plates were read with the guidance of "MIC Test Strip
127 Photographic Guide" with interpretation as in current literature. Oxacillin plates were read using
128 CLSI breakpoints provided by Liofilchem. Analysis for statistical differences between groups
129 was performed with SPSS using χ^2 for significance (p=0.05).

130 **Whole Genome Sequencing (WGS)**: Overnight cultures were grown in TSB at 37ºC with
131 shaking. Genomic DNA was collected using the DNeasy Blood and Tissue kit (Qiagen) following
132 the gram-positive specific pretreatment method, with the addition of 20mg/ml lysostaphin. For 80
133 strains (Run 1), quality and quantity was assessed using a Qubit 4 Fluorometer, and sequencing
134 occurred on an Illumina MiSeq instrument with a Nextera Flex library preparation kit and V3 flow
135 cell chemistry. For the remaining 304 strains, quality and quantity was assessed using a Quant-it
136 fluorescent assay kit (Invitrogen) and a Biotek Citation5 microplate reader. Libraries were
137 prepared using a Hackflex [14] adapted version of the Illumina DNA-seq library preparation kit.
138 Sequencing of 70 of the Pre-COVID samples (Run 2) was performed using a Nextseq550 Mid-
139 Output v2.5 Kit (300 cycles) and 234 Post-COVID samples were sequenced using a Nextseq2000
140 with P1 flow cell chemistry (Run 3). Runs 2 and 3 were performed at the USF Genomics Center
141 Core facilities. Strains are listed in Supplementary Table 1.

142 For long read sequencing of TGH462, DNA was extracted using the QIAGEN genomic tip 100/G
143 and genomic DNA buffer kits. Genomic DNA integrity and concentration were accessed via qubit

144 4 (Invitrogen) and Genomic DNA Tapestation (Agilent) QC checks. Samples were then treated
145 with the short fragment eliminator (Nanopore) and prepared with the Rapid Barcoding library prep
146 kit (SQK-RBK114.24). Libraries were sequenced on an R10.4.1 flowcell using a MinION Mk1B
147 with default settings of 72 hour run length and 200 bp minimum read length.

148 **Genomic assembly and molecular characterization**: The raw reads for all samples were
149 trimmed via Trimmomatic v0.39 [15] with SLIDINGWINDOW:10:20 MINLEN:31 TRAILING:2
150 settings [16]. Trimming quality checks were performed with FastQC v0.12.0. The reads were then
151 *de novo* assembled with SPAdes v3.15.5 using default settings [17], and assemblies were
152 checked for quality via Quast v5.2.0 [18]. Genomes were then sequence-typed with pyMLST [19],
153 annotated with prokka, and antimicrobial resistance (AMR) genes identified using AMRFinderPlus
154 v3.10.45 [20]. The presence of DNA and translated Protein sequences for AMR genes of interest
155 were confirmed and aligned using BLASTncbi-BLAST v2.13.0+ [21]. The staphtyper workflow as
156 part of Bactopia v3.0.1 was used to further characterize isolates based on Agr group [22],
157 SCCmec type [23], and Spa type [24].

158 **Phylogenetic analysis:** To explore relatedness of the cluster of ST8-t008 that harbor the novel
159 R112Q *ileS* mutation, Snippy was used to generate an initial core genome single-nucleotide
160 polymorphism alignment. Following this, Gubbins was used to create a recombination masked
161 alignment. From this alignment RAxML was used to infer a maximum-likelihood mid-point rooted
162 tree. The subsequent tree was visualized and annotated using iTOL webserver.

163 **Results**

164 **Mupirocin Resistance (MupR) is Common Amongst Tampa MRSA isolates:** To explore the
165 prevalence of MupR in our hospital system, we curated and characterized a collection of 384 *S.*
166 *aureus* strains. The collection was isolated between February 2017 and September 2023, and
167 comprised of 299 MRSA isolates (77.9%) and 85 MSSA strains (22.1%) (**Figure 1**). Many infection

168 control practices changed during the COVID-19 pandemic, and there have been reports of
169 increased *S. aureus* incidence during the pandemic [25, 26] [27]. Therefore, we designated the
170 collection as being collected Pre-COVID (**Table 1;** 2017-2020, PreC = 211 strains, 54.9%) and
171 Post-COVID (2021-2023, PostC = 173 strains, 45.1%). There were 161 Blood samples making
172 up 41.9% of the sample set (**Table 1)**. Non-Blood strains (223 strains, 58.1%) were isolated from
173 infections of: bone (4 samples, 1%), eye (12 samples, 3.1%), fluid (29 samples, 7.6%), respiratory
174 (58 samples, 15.1%), urine (37 samples, 9.6%), and wound (83 samples, 21.6%) (**Table 1**).

175 The Pre-COVID and Post-COVID periods coincided with switch from a mupirocin-based
176 decolonization protocol to povidone-based protocol in January 2020. Our hospital had found
177 >40% prevalence of mupirocin resistance in 2019 in amongst MRSA isolates from surveillance
178 nasal swabs and a random sampling of MRSA blood cultures (46% of MRSA nares n=127; 43%
179 of MRSA blood cultures n=46). The first case of COVID-19 in the Tampa Bay area was March 1,
180 2020 with Post-COVID cases collected more than a year after change in decolonization protocol.
181 We determined the prevalence of mupirocin resistance amongst both MRSA and MSSA isolates
182 and whether specific genetic or phenotypic features correlated with resistance over time.

183 All strains were screened for oxacillin and mupirocin resistance using Etest strips. From this, we
184 determined that 278 of 384 strains (72.4%; **Figure 1A, Table 1**) were susceptible to mupirocin.
185 Of the 106 (27.6%) resistant strains, 34 (8.9%) had low-level resistance, whilst 72 (18.8%) had
186 high level resistance.

187 **Mupirocin Resistance Prevalence was Stable:** We observed significant correlation between
188 oxacillin resistance and mupirocin resistance (**Supplemental Table 2 and 3**). Most MupR strains
189 were MRSA (**Figure 1A**). From Pre-COVID samples, 2.1% of MSSA samples were also MupR,
190 whilst 36.6% of MRSA samples were MupR ($p<0.001$). 30.7% of MupS samples were oxacillin
191 susceptible, whilst 98.4% of MupR samples were oxacillin resistant. ($p<0.001$). Post-COVID strain

192 susceptibilities were similar. For example, 5.3% of MSSA samples were MupR and 31.9% of
193 MRSA samples were MupR (p<0.001).

194 When comparing levels of MupR between the Pre- and Post-COVID time points, there was no
195 significant difference between the two strain sets (p=0.460). In the Pre-COVID population, 71.1%
196 of strains were susceptible, whilst 28.9% displayed resistance (MupLR = 8.1%, MupHR = 20.9%).
197 In the Post-COVID strains, 74.0% were susceptible, whilst 26% displayed resistance (MupLR =
198 9.8%, MupHR = 16.2%).

199 **Pre-COVID Non-Blood Strains Had Higher Levels of MupR:** We found a significant difference
200 for strains isolated from blood versus non-blood. 21.7% of blood isolates displayed some level of
201 MupR, compared with 31.8% of non-blood samples (p=0.029; **Supplemental Table 4 and 5**).
202 This was driven by the Pre-COVID population, where 20.7% of blood samples displayed resistant
203 versus 34.1% of non-blood samples (p=0.037), whilst Post-COVID had no significant correlation
204 between isolate site and resistance (22.8% of blood samples were resistant versus 28.7% of non-
205 blood samples, p=0.375). When the non-blood strains were parsed more deeply, 46.2% of the
206 Pre-COVID respiratory isolates were MupR (p=0.016), which accounts for 29.5% of all Pre-COVID
207 MupR strains. Amongst our Post-COVID cohort, 26.3% respiratory strains were MupR, making
208 up 11.1% of MupR samples in the Post-COVID era (p=0.651).

209 **Clonal Clusters and Sequence Types Associated with MupR:** All strains from our collection
210 were subject to WGS and initially parsed into CC and then ST (**Figure 1B**). Strains that share six
211 of the seven conserved sequences used for multilocus sequence typing (MLST) are grouped into
212 clonal complexes (CC). MupR was most common in three sequence types (**Figure 2**). CC8 ST8
213 was the most abundant, with 173 strains (PreC = 96, 45.5% vs. PostC = 77, 44.5%), followed by
214 61 CC5 ST5 strains (PreC = 35, 16.6% vs. PostC = 26, 15%) and 36 CC5 ST3390 strains (PreC
215 = 24, 11.4% vs. PostC = 12, 7.5%). CC8 ST8 encompass USA300 and USA500, the most
216 common CA-MRSA in the US. There were 92 USA300 (ST8 t008) isolates (PreC = 51, 55.4% vs.

217 PostC = 41, 44.6%), and 31 USA500 (ST8 t064) strains (PreC = 19, 61.3% vs. PostC = 12,
218 38.7%). CC5 ST5 includes USA100, the CC/ST type classically associated with HA-MRSA) in the
219 US [28]. ST5, ST3390 and ST105 are closely related strains that are members of CC5.

220 When we explored MupR as a function of population structure, the three most common STs ST8,
221 ST5, and ST3390, made up the majority of the MupHR (88.9%) and MupLR (79.4%) strains
222 (**Figure 3**). MupHR strains comprised eight different STs, with the highest proportion being ST8
223 (43.1%) with 31 strains (PreC = 19, 43.2% vs. PostC = 12, 42.9%), then ST3390 (27.8%) with 20
224 strains (PreC = 13, 29.5% vs. PostC = 7, 25%) and 13 ST5 strains (PreC = 8,18.2% vs. PostC =
225 17.9%). ST3390 made up the highest group of MupLR overall with 10 strains (PreC = 6, 35.3%
226 vs. PostC = 4, 23.5%), followed by ST8, the majority of which are PostC (PreC = 2, 11.8% vs.
227 PostC = 7, 41.2%), and then finally, ST5 with most being PreC (PreC = 7, 41.2% vs. PostC = 1,
228 5.9%). Other MRSA-MupHR were ST105, ST1, and ST59. Of the three MSSA-MupR, two were
229 ST121 (one MupHR, one MupLR) and one ST1157 (MupHR).

230 **High Level Mupirocin Resistant Strains Carry the *mupA* Gene:** From our collection, 70 of 72
231 MupHR isolates contained the *mupA* gene, whilst no isolates were found to be *mupB* positive
232 (**Figure 3A**). MupHR strains were most commonly classified in the top three STs within two CCs
233 CC8 (ST8, n=31, 44.3%) and CC5 (ST5, n=13, 18.6% and ST3390, n=20, 28.6%). There was an
234 overall decrease of *mupA* carriage over time (20.4% PreC vs. 15.6% PostC) that was not
235 statistically significant (p=0.228). Within the pre-COVID strain collection (**Figure 3B**), we see a
236 higher number of *mupA*$^+$ isolates (43 strains) from a less diverse mix of the population (four STs
237 and two untypeable strains in total), whereas in the Post-COVID collection (**Figure 3B**) we see
238 27 strains distributed across six STs. The percentage of *mupA*$^+$ ST8 (PreC = 19, 44.2% vs. PostC
239 = 12, 44.4%) and ST5 isolates (PreC = 8, 18.6% vs. PostC = 5, 18.5%) remained largely stable,
240 however there was a slight decrease in the percentage of ST3390 isolates (PreC = 13, 30.2% vs.
241 PostC = 7, 25.9%, p-value = 0.264). The remaining *mupA*$^+$ isolates made up a relatively small

242 proportion of each population (PreC = 3, 6.9% vs. PostC = 3, 11.8%) and did not have any ST in
243 common. The rates of *mupA* prevalence across the Pre and Post pandemic era were not found
244 to be significantly different.

245 In general, the genotype and antibiotic susceptibility phenotype of our *mupA*$^+$ strains conformed,
246 e.g. *mupA*$^+$ strains were MupHR and *mupA*$^-$ strains were MupS (**Figure 3C**). However, there
247 were exceptions. Isolate TGH406, was *mupA*+ but MupS, and no variation found in *mupA*
248 sequence to indicate it had become a pseudogene. We also detected a cluster of five *mupA*$^+$
249 isolates that were phenotypically MupLR (**Figure 3C**). We know from the literature that this is
250 likely due to *mupA* integration onto the chromosome [8]. There were six samples that were found
251 to have mutations within *mupA*, leading to varying degrees of protein truncations. Two truncations
252 were found that resulted in 957/1024 AA (n=2) and 997/1024 AA (n=1) variants that did not have
253 an impact on the function of *mupA* resistance measured phenotypically. Conversely, three isolates
254 had larger truncations, all of 137 AA, resulting in a shorter protein sequence of 887 AA. This
255 variant completely eliminated MupA function, resulting in a MupS phenotype.

256 **Mutations in *IleS* Correlate with Low Level Mupirocin Resistance:** Low level mupirocin
257 resistance is associated with a mutation (V588F) in the native isoleucyl-tRNA synthetase, *ileS*.
258 This mutation was identified in 27/34 MupLR isolates (PreC = 14, 6.6% vs. PostC = 13, 7.5%).
259 Three strains had the V588F mutation in *ileS* and also carried *mupA* and were MupHR as a result.
260 There was also a single MupLR isolate (TGH365) that had a novel *ileS* mutation (W436G) that to
261 our knowledge has never been reported. We also had four MupLR samples that did not contain
262 any mutations in the *ileS* sequence.

263 From a population perspective, there was a notable switch in strains bearing the V588F mutation
264 Pre-COVID and Post-COVID. Pre-COVID, there are no ST8 strains with MupLR that bear *ileS*
265 mutations; instead, the most prevalent groups are ST5 and ST3390, represented by six isolates,
266 each making up 42.9% of the population (**Figure 4A)**. In the Post-COVID population, ST8

267 represents 46.2% of the observed MupLR population, driven by the acquisition of the *ileS* (V588F)
268 mutation (**Figure 4B)**. There are no ST8 samples with this mutation prior to this shift in the Pre-
269 COVID population. Afterwards, Post-COVID ST8 made up 61.5% of all the strains with the
270 (V588F) mutation. The discrepancy between the percentage of Post-COVID MupLR and Post-
271 COVID (V588F) mutation ST8 strains is due to two ST8 strains in which the chromosomal
272 mutation effect is masked by the presence of *mupA*, resulting in a MupHR phenotype. Thus, we
273 observed CA-MRSA strains acquiring mutations in *ileS* and the *mupA* plasmid associated with
274 MupHR.

275 In addition, we identified another conserved novel MupLR *ileS* mutation (R112Q) that has never
276 been reported in the literature. This only appears within 7 of the 41 PostC USA300 strains,
277 indicating that this cluster may represents an outbreak within the ST8 clone (**Figure 4C**). Over
278 time the ST3390 population showed an increase in the presence of MupHR (+25.5% PostC), with
279 decrease in samples with *ileS* mutations (-16.7% PostC), suggesting evolution to a more highly
280 resistant population.

281

282 **Discussion**

283 Prior studies have shown that the rate of MupR varies by geographic region and clinical
284 characteristics of the patient populations sampled. The increasing use of decolonization protocols
285 designed to reduce the incidence of MRSA transmission creates selection pressure for
286 emergence of MupR. MupR has been reported in both MRSA and MSSA strains [9] [10], and
287 genome sequencing has implicated transmission of strains within healthcare settings [9] [10].
288 Amongst our strain collection, prevalence of MupR was higher in MRSA isolates than MSSA
289 isolates, as was also reported in strains from Stamford, CT [9]. For MRSA MupR strains, nasal
290 povidone-iodine protocols for decolonization should be effective [29], although recent clinical trials

291 have shown that nasal povidone iodine is inferior to nasal mupirocin [30]. Thus, optimal
292 management of MRSA colonization is not clear and will be influenced by local molecular
293 epidemiology of *S. aureus* and the rate of emergence of MupR.

294 The mechanism of MupHR is transmission of plasmid mediated *mupA* whilst MupLR is associated
295 with accumulation of chromosomal point mutations. Amongst the risk factors reported for MupR
296 are 1) prior exposure to mupirocin in the past year 2) *Pseudomonas aeruginosa* infection in the
297 year prior to admission and 3) cefepime use in the year prior to culture [31]. Ceasing mupirocin
298 exposure is reported to result in less MupR [8], although we did not see significant differences
299 between rates of MupR Pre-COVID and Post-COVID. The Pre-COVID strain collection was less
300 diverse than the Post-COVID collection so balancing selective pressures may be present. A
301 limitation of our retrospective study is that we did not have a method to determine mupirocin
302 exposure outside the inpatient setting. Other selective pressures that may be operative include
303 exposure to other antibiotics that favor maintenance of the resistance plasmid. Interestingly, many
304 of the isolates that carry *mupA* (58 out of 70), were also found to have a gene encoding for
305 resistance to aminoglycoside antibiotics, ANT(4')-Ia. Therefore, changing our decolonization
306 protocol to povidone iodine may have slowed emergence of MupR, but this trend may have been
307 masked by other factors affecting the population structure of MRSA in our hospital system.

308 The isolates examined were convenience samples, and our laboratory archives a random subset
309 of clinical isolates. We noted differences MupR in some of the subpopulations of isolates tested,
310 including respiratory samples. This phenotypic change in the Non-Blood category isolates may
311 be due to a population shift over time from being predominantly CC5 (HA-MRSA) to CC8 (CA-
312 MRSA), however, we cannot rule out that these differences were due to our small sample size. A
313 more in-depth characterization of samples from different clinical sites is needed to determine if
314 the changes we observed represent changes in population structure of clinical isolates, outbreaks
315 or selection of specific clones.

316 There were two isolates (Blood/Respiratory) from the same patient that had MupHR with no
317 identifiable *mupA* or *mupB* present. Both samples are ST105 SCC*mec* type II *Spa*-type t002, a
318 relative of the New York/Japan clone (USA100/ST5). European ST105-II-t002 has been linked to
319 skin and soft tissue infections, while the more recent South American ST105-II-t002 has been
320 heavily associated with blood stream infections [32, 33].

321 A major theme in *S. aureus* pathogenesis has been the emergence of waves of virulent clones
322 carrying genetic determinants conferring resistance to antibiotics [34]. In the 1990's strains were
323 either HA-MRSA or CA-MRSA, but gradually virulent CA-MRSA strains such as USA300 became
324 dominant [34]. We show that MupR in western Florida is associated with MRSA strains and
325 common US MRSA types such as ST8 (USA300 and USA500) and ST5 (USA100) are highly
326 represented within our clinical strains. Our collection also revealed the emergence of an unusual
327 ST3390 group with unexpected high prevalence of MupR (83%). Our ST3390 strains show
328 greater toxicity to neutrophils and are more virulent in mice than reference ST5 strain (Felton, *et*
329 *al* submitted). The genomes of ST3390 strains show evidence of horizontal exchange of genetic
330 traits that could promote maintenance in the population, increased virulence, and multi-drug
331 resistance (Felton, *et al* submitted).

332 Decolonization protocols for *S. aureus* utilizing antibiotics and antiseptics such as mupirocin or
333 povidone-iodine with chlorhexidine have shown to decrease surgical site infections and invasive
334 infections, but only result in temporary decolonization. These agents could promote the selection
335 of resistant organisms through selective pressure or broad disruption of the microbiome. The
336 incidence of mupirocin resistance and the molecular epidemiology of resistant strains in local
337 hospital populations is an important factor in selecting the appropriate agents used for
338 decolonization.

339

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346 **Data availability:** Whole genome sequence data for all 384 isolates can be accessed at SRA
347 with accession numbers PRJNA785927 (Run1 80 isolates) and PRJNA1125331 (Run 2 and 3;
348 304 isolates). Sequenced strains are listed in Supplementary Table 1.

349 **Conflicts of Interest:** KK is a member of the Editorial Board of the Sanford Guide, has consulted
350 for Shionogi and Regeneron, and has received clinical research grants from Regeneron, Abbott,
351 Astra Zeneca, Pfizer, Romark, and the NIH. The other authors report no conflicts of interest.

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430

431

432 **Table 1**

433 **Samples**

	Strains	*Ox R(MRSA)*	*Mup R*
Pre-COVID	211	164	61
Post-COVID	173	135	45
Blood	161	100	35
Non-Blood	223	199	71
Total	384	299	106

434

435 **Strain Source**

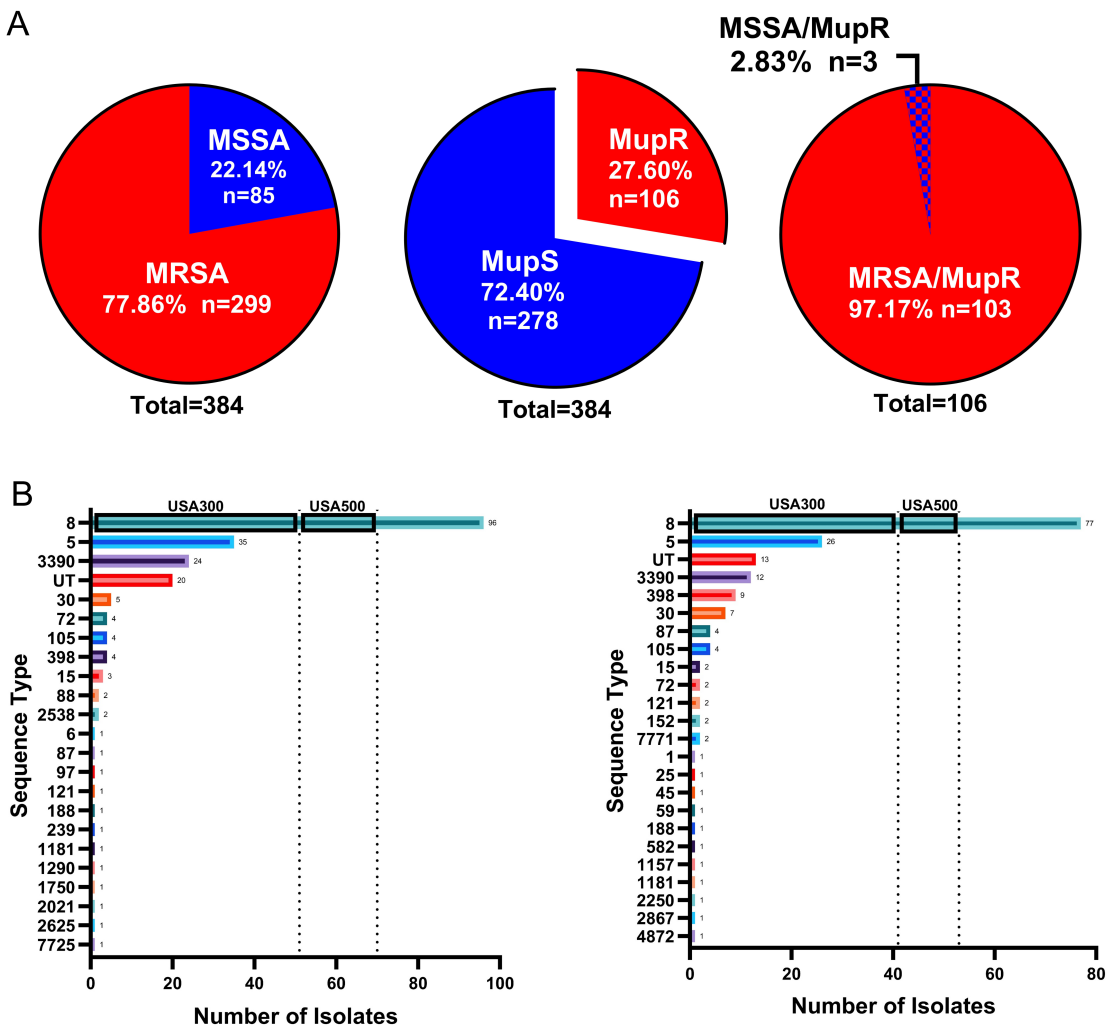
	PreC			***PostC***			***Total***
	MupHR	MupLR	MupS	MupHR	MupLR	MupS	No (%)
Total #	44	17	150	28	17	128	384 (100)
Blood	12	5	65	9	9	61	161 (41.9)
Non-Blood	32	12	85	19	8	67	223 (58)
Bone	1	0	2	0	0	1	4 (1)
Eye	1	0	1	6	0	4	12 (3.1)
Fluid	3	1	12	1	0	12	29 (7.6)
Respiratory	13	5	21	2	3	14	58 (15.1)
Urine	5	1	12	4	2	13	37 (9.6)
Wound	9	5	37	6	3	23	83 (21.6)

436

437 ***S. aureus* strains used in this study.** Numbers collected Pre-COVID (2017-2020) and Post-
438 COVID (2021-2023) are indicated, as are the numbers of strains in each group with oxacillin
439 resistance and mupirocin resistance as defined in the methods. Sites of collection of the strains
440 are also indicated. All strains underwent whole genome sequencing (details in supplementary
441 table 2)

442

443 **Figures:**

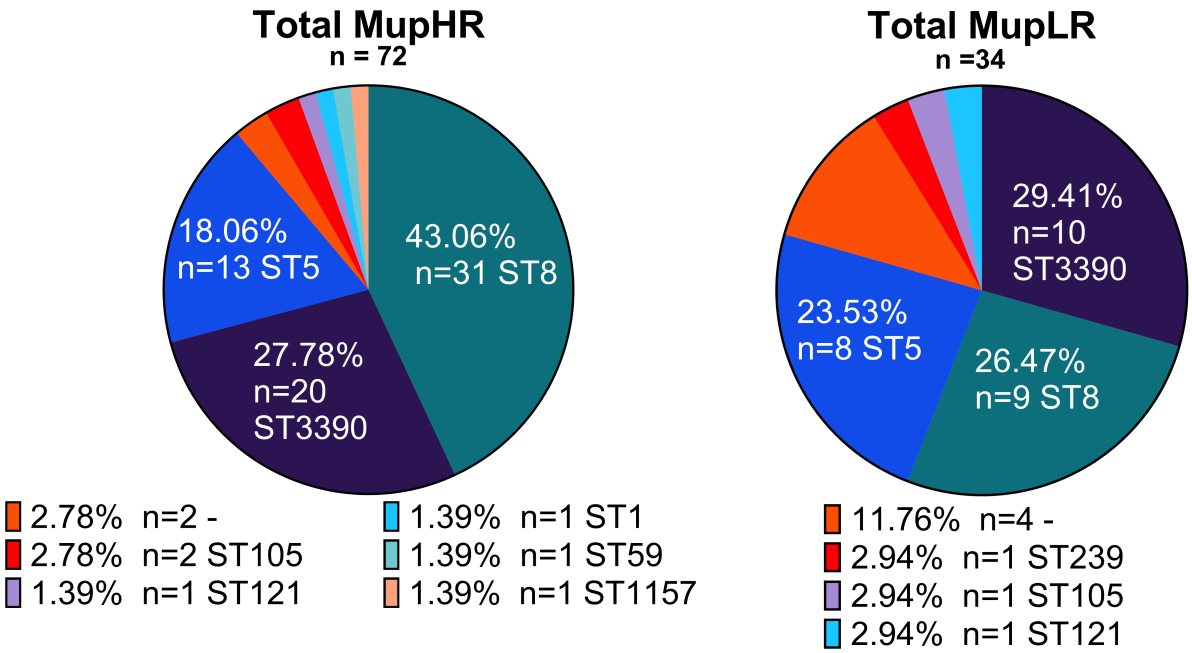


444

445 **Figure 1. Mupirocin Resistance and Sequence Typing of Tampa *S. aureus* isolates.** A. 384
446 *S.aureus* clinical strains from 2017-2023 were characterized. Oxacillin sensitivity (MSSA, n=85),
447 mupirocin sensitivity (MupS, n=278) and the distribution of MRSA and MSSA strains amongst
448 mupirocin resistant (MupR n=106) strains is shown. B. Results of classification of strains into
449 sequence types (ST) are shown with numbers of Pre-COVID isolates (2017-2020) and Post-
450 COVID isolates (2021-2023) within each ST. Numbers of USA300 and USA500 within ST8 are
451 shown. ST5 includes USA100 strains. “UT” signifies strains that were untypeable using the

452 methods deployed herein. ST was determined by analysis of whole genome sequence of each

453 strain.



454

455 **Figure 2. Mupirocin Resistance as a Function of Sequence Type.** Shown are the sequence

456 types for high-level mupirocin resistance (MupHR) and low/intermediate-level mupirocin

457 resistance (MupLR) of the 106 MupR strains as determined by analysis of whole genome

458 sequence. Mupirocin susceptibility was determined by Etests. Two MupHR and four MupLR

459 strains were untypeable.

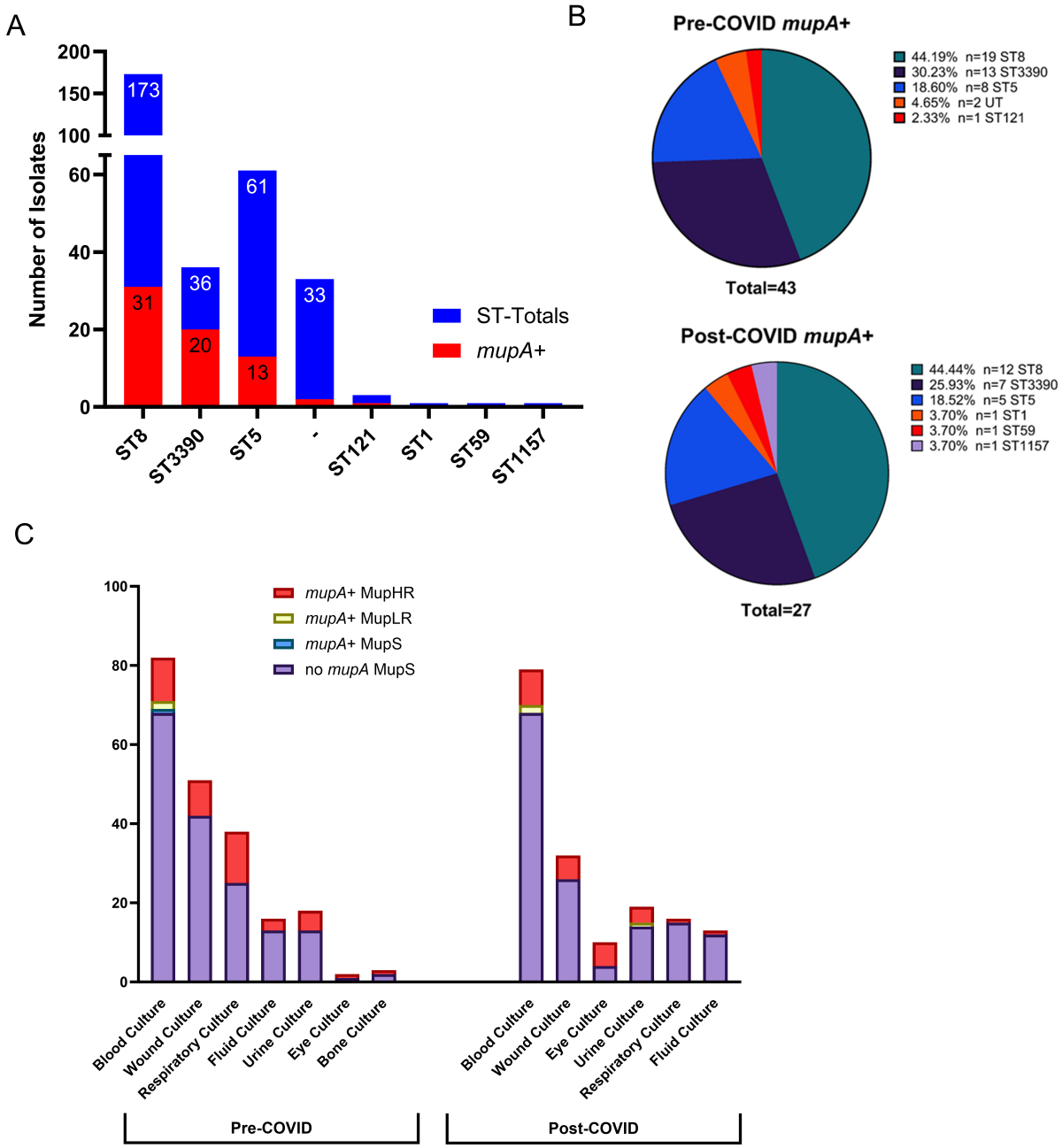
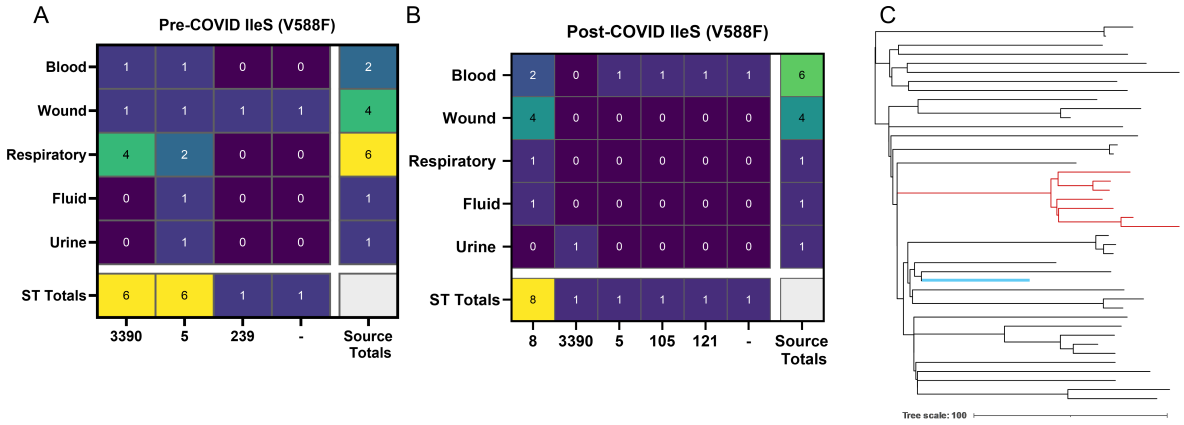


Figure 3. Distribution of *mupA* across Sequence Types and Correlation with Mupirocin Resistance. Summary of the distribution of *mupA*$^+$ isolates and ST across: (A) the entire 384 strain collection, (B) the Pre-COVID strains and the Post-COVID strains. Shown are the percentages and number of strains for each ST. C. The distribution of clinical strains with and without *mupA* and its phenotypic impact are shown. Most *mupA*$^+$ strains were MupHR as

466 determined by eTest. Strains are arrayed by isolation site, isolation period, the presence-absence
467 of *mupA*, and its phenotypic impact on mupirocin resistance/sensitivity. Strains with mutations in
468 *ileS* that are phenotypically MupLR are not included in this depiction.



469

470 **Figure 4. Correlation of MupLR and *ileS* (V588F) as a Function of Sequence Type and**
471 **Isolation Site.** Summary of the distribution of *ileS* (V588F) positive isolates **(A)** Pre-COVID and
472 **(B)** Post-COVID. **(C)** Shown is a recombination masked core SNP alignment maximum-likelihood
473 tree of Post-COVID USA300 isolates (ST8). The clade of 7 mupirocin resistant USA300 isolates
474 that possess the R112Q *IleS* mutation are designated in red. The reference USA300 strain
475 FPR_3757 used for SNP alignment is shown in blue.

476

477 **Supplemental Table 2**

478 **Mupirocin Resistance Comparisons**

479

Pre-COVID vs Post-COVID * Mup _Resistance

			Mup _Resistance			
			Susceptible	Intermediate	Resistant	Total
Group	Pre-COVID	Count	150	17	44	211
		%	71.1%	8.1%	20.9%	100.0%
		% of Total	39.1%	4.4%	11.5%	54.9%
	Post-COVID	Count	128	17	28	173
		%	74.0%	9.8%	16.2%	100.0%
		% of Total	33.3%	4.4%	7.3%	45.1%
Total		Count	278	34	72	384
		% of Total	72.4%	8.9%	18.8%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.551[a]	2	0.460

480

481

482

483 **Supplemental Table 3**

Ox_Resistance vs Mup_Resistance

Group			Mup _Resistance Susceptible	Mup _Resistance Resistant	Total
Pre-COVID	MSSA	Count	46	1	47
		% of MSSA group	97.9%	2.1%	100.0%
		% within Mup _Resistance Group	30.7%	1.6%	22.3%
		% of Total	21.8%	0.5%	22.3%
	MRSA	Count	104	60	164
		% within MRSA group	63.4%	36.6%	100.0%
		% within Mup _Resistance Group	69.3%	98.4%	77.7%
		% of Total	49.3%	28.4%	77.7%
	Total	Count	150	61	211
		% within Ox_Resistance	71.1%	28.9%	100.0%
		% within Mup_Resistance Group	100.0%	100.0%	100.0%
		% of Total	71.1%	28.9%	100.0%
Post-COVID	MSSA	Count	36	2	38
		% within MSSA	94.7%	5.3%	100.0%
		% within Mup _Resistance Group	28.1%	4.4%	22.0%
		% of Total	20.8%	1.2%	22.0%
	MRSA	Count	92	43	135
		% within MRSA	68.1%	31.9%	100.0%
		% within Mup _Resistance Group	71.9%	95.6%	78.0%
		% of Total	53.2%	24.9%	78.0%
	Total	Count	128	45	173
		% within Ox_Resistance	74.0%	26.0%	100.0%

	% within Mup _Resistance Group	100.0%	100.0%	100.0%
	% of Total	74.0%	26.0%	100.0%

484

Chi-Square Tests

Date Code		Value	df	Asymptotic Significance (2-sided)
Pre-COVID	Pearson Chi-Square	21.105[a]	1	0.000
Post-COVID	Pearson Chi-Square	10.893[c]	1	0.001

485

486

487 **Supplemental Table 4**

Blood v. Non-blood * Mup _Resistance

Date Code			Mup _Resistance		
			Susceptible	Resistant	Total
Pre-COVID	Blood	Count	65	17	82
		% within Blood vs. Non-blood	79.3%	20.7%	100.0%
		% of Total	30.8%	8.1%	38.9%
	Non-Blood	Count	85	44	129
		% within Blood v. Non-blood	65.9%	34.1%	100.0%
		% of Total	40.3%	20.9%	61.1%
	Total	Count	150	61	211
		% within Blood v. Non-blood	71.1%	28.9%	100.0%
		% of Total	71.1%	28.9%	100.0%
Post-COVID	Blood	Count	61	18	79
		% within Blood v. Non-blood	77.2%	22.8%	100.0%
		% of Total	35.3%	10.4%	45.7%
	Non-Blood	Count	67	27	94
		% within Blood v. Non-blood	71.3%	28.7%	100.0%
		% of Total	38.7%	15.6%	54.3%
	Total	Count	128	45	173
		% within Blood v. Non-blood	74.0%	26.0%	100.0%
		% of Total	74.0%	26.0%	100.0%

Chi-Square Tests

Date Code		Value	df	Asymptotic Significance (2-sided)
Pre-COVID	Pearson Chi-Square	4.365[a]	1	0.037
Post-COVID	Pearson Chi-Square	.787[c]	1	0.375

488

489

Supplemental Table 5

Blood v. Non-blood * Mup _Resistance, Respiratory broken out

Group			Mup_Resistance Susceptible	Mup_Resistance Resistant	Total
Pre-COVID	Blood	Count	65	17	82
		% of group	79.3%	20.7%	100.0%
		% of Total Mup_Resistant	43.3%	27.9%	38.9%
		% of Total	30.8%	8.1%	38.9%
	Respiratory	Count	21	18	39
		% of group	53.8%	46.2%	100.0%
		% of Total Mup_Resistant	14.0%	29.5%	18.5%
		% of Total	10.0%	8.5%	18.5%
	Non-Blood	Count	64	26	90
		% of group	71.1%	28.9%	100.0%
		% of Mup_Resistant	42.7%	42.6%	42.7%
		% of Total	30.3%	12.3%	42.7%
	Total	Count	150	61	211
		% of group	71.1%	28.9%	100.0%
		% of Mup_Resistant	100.0%	100.0%	100.0%
		% of Total	71.1%	28.9%	100.0%
Post-COVID	Blood	Count	61	18	79
		% within Source Code	77.2%	22.8%	100.0%
		% within Mup_Resistance	47.7%	40.0%	45.7%
		% of Total	35.3%	10.4%	45.7%
	Respiratory	Count	14	5	19
		% within Source Code	73.7%	26.3%	100.0%
		% within Mup_Resistance	10.9%	11.1%	11.0%

		% of Total	8.1%	2.9%	11.0%
	Non-Blood	Count	53	22	75
		% within Source Code	70.7%	29.3%	100.0%
		% within Mup_Resistance	41.4%	48.9%	43.4%
		% of Total	30.6%	12.7%	43.4%
	Total	Count	128	45	173
		% within Source Code	74.0%	26.0%	100.0%
		% within Mup_Resistance	100.0%	100.0%	100.0%
		% of Total	74.0%	26.0%	100.0%

Chi-Square Tests

Group		Value	df	Asymptotic Significance (2-sided)
Pre-COVID	Pearson Chi-Square	8.311[a]	2	0.016
Post-COVID	Pearson Chi-Square	.858[b]	2	0.651