1	Clonal variation in high- and low-level phenotypic and genotypic mupirocin resistance of
2	MRSA isolates in South East London
3	Short Title: MRSA clonal variation in mupirocin resistance.
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25	V588f, IleS.
26	

- 27 Abstract
- 28

Objectives: Both Low-Level Mupirocin Resistance (LMR) and High-Level Mupirocin Resistance
 (HMR) have been identified. The aim of the study was to determine the epidemiology of LMR
 and HMR in MRSA isolates at five hospitals that have used mupirocin for targeted
 decolonization as part of successful institutional control programmes.

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34 Methods: All MRSA identified in three microbiology laboratories serving five Central and 35 South East London hospitals and surrounding communities between November 2011 and 36 February 2012 were included. HMR and LMR were determined by disc diffusion testing. Whole 37 genome sequencing was used to derive MLST type and presence of HMR and LMR resistance 38 determinants.

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40 **Results:** Prevalence of either HMR or LMR amongst first healthcare episode isolates from 795 41 identified patients was 9.69% (95% CI 7.72-11.96); LMR was 6.29% (95% CI 4.70-8.21), and 42 HMR 3.40% (95% CI 2.25-4.90). Mupirocin resistance was not significantly different in isolates 43 identified from inpatients at each microbiology laboratory, but was more common in 44 genotypically defined 'hospital' rather than 'community' isolates (OR 3.17, 95% CI 1.36-9.30, 45 p=0.002). LMR was associated with an inpatient stay, previous history of MRSA and age \geq 65 46 years; HMR was associated with age \geq 65 years and a residential postcode outside London. 47 LMR and HMR varied by clone, with both being low in the dominant UK MRSA clone ST22 48 compared with ST8, ST36 and ST239/241 for LMR, and with ST8 and ST36 for HMR. V588f 49 mutation and *mup*A carriage had high specificity (>97%) and area under the curve (>83%) to 50 discriminate phenotypic mupirocin resistance, but uncertainty around the sensitivity point 51 estimate was large (95% CI 52.50-94.44%). Mutations in or near the mupA gene were found 52 in eight isolates that carried *mupA* but were not HMR.

- 54 Conclusions: Mupirocin resistance was identified in less than 10% of patients, and varied
 55 significantly by clone implying that changes in clonal epidemiology may have an important
 56 role in determining the prevalence of resistance in conjunction with selection due to
 57 mupirocin use.
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61 Mupirocin (pseudomonic acid A) is an antibiotic commonly used for the nasal decolonization 62 of MRSA and MSSA.^{1,2} It has been widely used as part of the successful UK MRSA control 63 programmeme over the last 10 years.³ It has also been shown to reduce the rate of MRSA 64 body site infections when applied universally in conjunction with chlorhexidine to all patients 65 admitted to the ICU.⁴ Mupirocin-resistant Staphylococcus aureus was first reported in 1987 at 66 St. Thomas' Hospital, which now forms part of Guy's and St. Thomas' NHS Trust (GSTT).⁵ 67 Mupirocin binds to the bacterial isoleucyl-tRNA synthetase gene, inhibiting protein 68 replication.^{1,2} Mupirocin resistance is classified as either Low-Level Mupirocin Resistance 69 (LMR) or High-Level Mupirocin Resistance (HMR).¹ LMR is mediated through point mutations 70 in the native isoleucyl-tRNA synthetase gene (ileS) causing a Val-to-Phe change in the 71 mupirocin binding site, at either residue 588 (V588F) or 631 (V631F).⁶ HMR is due to carriage 72 of a distinct plasmid-mediated isoleucyl-tRNA synthetase gene, most commonly mupA, 73 although mupB has been reported.^{2,7,8} HMR is associated with MRSA decolonization failure, 74 and LMR appears to be associated with early re-colonisation and in some reports, 75 decolonization failure.^{1,9–11}

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77 The prevalence of mupirocin resistance (LMR and HMR) and of the underpinning genotypic 78 determinants has been widely reported. In 1998, a survey of MRSA from 19 European 79 hospitals found HMR in 3.6% and LMR in 2.6% of 194 MRSA samples.¹² A Japanese cohort 80 reported LMR prevalence of 0.8% to 4.0 % between 1998 and 2001 with no HMR detected.¹³. 81 A more recent study of 156 MRSA isolates in the United States demonstrated LMR in 18.6%, 82 and HMR in 5.1% of isolates.¹⁴ Similarly, a Singaporean cohort study identified HMR in 11% of 83 307 isolates.¹⁵ Several reports suggest that carriage of *mupA* is more common in some clones, 84 but to our knowledge, the distribution of LMR by MRSA clone has not been reported.¹⁶⁻¹⁸

The concern with increasing use of mupirocin is selection of MRSA isolates that are mupirocinresistant, thus compromising the long term sustainability of decolonization both for the individual patient and as an infection control intervention to prevent transmission. ^{1,2} Recent hospital admission and use of mupirocin have been identified as risk factors of HMR or LMR, implying that exposure to an environment where there is intensive mupirocin use is a risk factor for resistance.^{19,20} It is however unclear whether there is selection for both HMR and LMR and how this relates to carriage of *mupA* and the V558F mutation.^{1,13,14,21-23}

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94 This study reports the prevalence of mupirocin resistance (LMR and HMR) and carriage of 95 mupirocin resistance determinants (V588F/V631F and mupA/mupB) in hospital and 96 community MRSA isolates identified in three laboratories serving five hospital and community 97 healthcare facilities across three adjacent London boroughs. All healthcare facilities in this 98 area have implemented effective infection control programmes over the past 5-7 years 99 involving use of mupirocin for decolonization of patients identified in the universal admission screening programme ("screen and treat approach")²⁴ and seen MRSA levels fall by over 85%. 100 101 ²⁵ The aim of the study was to determine the distribution, risk factors, and clonal variation in 102 LMR and HMR, and their genotypic determinants.

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104 Methods

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From 1st November 2011 to 29th February 2012, we collected all MRSA isolates identified by a hospital cohort that serves a resident population of 867,254 ²⁶ and provides microbiology diagnostic services to all inpatients, outpatients and community patients in London boroughs of Southwark, Lambeth and Lewisham. Participant centres included four acute tertiary hospitals in two NHS Trusts (GSTT, and King's College Hospital NHS Foundation Trust) and an acute district general hospital (Lewisham and Greenwich NHS Trust). All three NHS Trusts had
 polices in place for use of mupirocin for decolonization of MRSA inpatients, although in one
 Trust (GSTT), it was not used on the intensive care units. ²⁷ The number of nasal mupirocin
 tubes prescribed during the study period was obtained from pharmacy electronic systems at
 each Trust.

116

117 MRSA isolates were submitted to the Centre for Clinical Infection and Diagnostics Research 118 (CIDR) at GSTT. Isolates confirmed as MRSA by culture on chromogenic agar (Oxoid Brilliance) 119 and rapid latex agglutination test (Staphaurex, Remel) were included in the study. 120 Anonymised patient-level details were submitted with each specimen and used to construct 121 a database. MRSA isolates were screened for mupirocin resistance using a semi-confluent 122 inoculum ²⁸ on Iso-Sensitest agar with a 200-µg disc (Oxoid Ltd.), incubated at 35-37°C in air 123 for 18–20 hours. NCTC 6571 guality control strain was used for internal validation. HMR was 124 defined by an inhibition zone of <18 mm based on a BSAC Working Party study conducted at 125 St Thomas' Hospital. This breakpoint coincides with that defined by EUCAST. ¹ To define 126 susceptible (i.e. not LMR), harmonization of the 'susceptible' EUCAST breakpoint (\geq 30mm)¹ 127 was conducted under the guidance of BSAC. Susceptible was defined as a zone of inhibition of 128 ≥ 32 mm and LMR as a zone inhibition of 18-31 mm. The 'susceptible' breakpoint was validated 129 by determining MICs with Etest (BioMerieux) using a 0.5 MacFarland standard inoculum on 130 Mueller-Hinton agar (Oxoid). MIC breakpoints were defined as susceptible, $\leq 1 \mu g/mL$; LMR, 131 2–256 µg/mL; and HMR, >256 µg/mL¹. MICs were also determined for all *mupA* positive 132 isolates.

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Whole genome sequencing (WGS) was conducted on eligible isolates using HiSeq 2500
(Illumina UK Ltd). Extracted genomic DNA was quantified using the Qubit High Sensitivity Kit
(Life Technologies, Carlsbad, CA, USA) and 50 ng was taken through 96-plex Nextera DNA

137 sample prep protocol (Illumina Inc, San Diego, CA, USA) following the manufacturer's 138 instructions. Libraries were quantified individually using the Qubit High Sensitivity Kit and 139 equimolar amounts pooled for sequencing. Pooled 96-plex libraries were diluted and 140 denatured ready for paired-end 150 cycle sequencing on the Illumina HiSeg 2500 platform in 141 rapid run mode, running a 96-plex pool in each lane. Contigs were de novo assembled using the trimmed reads and Velvet (version 1.2.10)²⁹ and VelvetOptimiser (version 2.2.5, 142 143 http://bioinformatics.net.au/software.velvetoptimiser.shtml) for each sample. Draft 144 assemblies were analysed in silico to determine the multilocus sequence type (MLST), 145 staphylococcal cassette chromosome mec (SCCmec) type, carriage of the Panton-Valentine 146 leukocidin (PVL) and identify genomic markers of mupirocin resistance using BWA³⁰ and 147 BLAST.³¹ WGS was conducted on the first confirmed MRSA isolate from each individual at each 148 unique healthcare setting (i.e. whenever an individual was admitted as inpatient to a new 149 hospital, or received care in a new outpatient clinic or community service throughout the 150 study period); thus, follow-up genomic information was available for patients who received 151 care at multiple settings.

152

Isolates carrying *mupA* or *mupB* were classified as 'genotypic HMR'. ^{7,8} Isolates with V588F or V631F chromosomal mutations in Ile, respectively, were classified as 'genotypic LMR'. ⁶ Isolates were classified as 'hospital-associated' (HA) if they were PVL-negative and contained SCC*mec* types I, II or III, and 'community associated' (CA) if they were PVL-positive or contained SCC*mec* types IV, V or non-typeable. ^{32,33} Exceptions were ST22-IV isolates and ST5-IV isolates, which were classified as HA unless they were PVL-positive. ^{32,33}

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160 Analysis

162 Univariate logistic regression analyses of the patients' first healthcare episode were used to 163 investigate risk factors for phenotypic HMR and LMR. The patients' first episode was classified 164 as 'inpatient', 'outpatient' or 'community' depending on whether provision of healthcare 165 involved admission to hospital, an outpatient clinic appointment or service from a general 166 practitioner (GP) or other community provider. The first episode was defined as 'HMR' if at 167 least one MRSA isolate during that episode was HMR; an episode was defined as 'LMR' if at 168 least one MRSA isolate was LMR and no HMR isolates were identified during the episode. 169 Potential risk factors for HMR and LMR included in the study were patients' age and gender, 170 type of healthcare episode, MRSA genomic type (HA or CA), previous history of MRSA infection 171 and/or colonisation, history of admission to hospital in the previous year and London 172 residency. Analysis of patients' first healthcare episode, restricted to inpatient stays, was also 173 used to investigate differences in level of phenotypic resistance across participant hospitals.

174

Univariate logistic regression analysis of de-duplicated unique-patient isolates was used to investigate whether genotypic and/or phenotypic mupirocin resistance is dependent on the MRSA MLST. The analyses included all isolates (including those from follow-up healthcare episodes) for which complete phenotypic and genotypic mupirocin resistance and MLST data were available. Within each patient, consecutive samples with identical MRSA MLST, and mupirocin resistance phenotypic and genotypic profile, were assumed to be the same isolate and were de-duplicated accordingly for analysis.

182

The sensitivity, specificity, accuracy, positive and negative predictive values and area under the curve were calculated to examine the reliability of genetic markers to discriminate phenotypic mupirocin resistance. Due to the limited number of isolates, the reliability of genetic markers across MRSA MLSTs was not examined. All analyses and summary statistics were conducted in R-3.1.1 statistical software. ³⁴ 188

189 This research was conducted following approval from the National Research Ethics Service190 (REC reference 11/NW/0733).

191

- 192 **Results**
- 193
- 194 Analysis of risk factors for phenotypic mupirocin resistance

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196 1523 consecutive isolates from 839 patients presenting with one or multiple healthcare 197 episodes (n=1096), were retrieved from the microbiology laboratories serving Lambeth, 198 Southwark and Lewisham (Figure 1). To avoid pseudo-replication, the analysis was based on 199 the characterization of MRSA isolates from the patients' first healthcare episode, leaving 795 200 patients' first episodes (1131 isolates) for analysis. 201 202 Prevalence of any LMR or HMR amongst patients' first episode (n= 795) was 9.69% (95% CI 203 7.72-11.96, n=77). LMR was 6.29% (95% CI 4.70-8.21, n=50), and HMR 3.40% (95% CI 2.25-204 4.90, n=27). Prevalence of any mupirocin resistance (p = 0.84), LMR (p = 0.79) or HMR (p =205 0.74) amongst first inpatient episodes (n=419), was not different across two Trusts and one 206 general district hospital included in the study. Only four episodes had combined LMR and

HMR, and were classified as HMR.

208

Risk factors for LMR or HMR combined, or for LMR or HMR individually are shown in Table 1.
Overall, the odds of any resistance (LMR or HMR) in genetically classified hospital MRSA was
three-fold that of community MRSA (OR 3.17, 95% CI 1.36-9.30, p=0.002); only HMR was
observed in community MRSA. LMR was associated with current (OR 5.23, 95% CI 1.56-32.63,
p=0.003) or recent (last 12 months) inpatient stay (OR 2.03, 95% CI 1.14-3.65, p=0.016),

214 previous history of MRSA (OR 1.94, 95% CI 1.09-3.47, p=0.025) and age \geq 65 years (OR 2.21,

215 95% CI 1.23-4.09, p=0.008). HMR was associated with age \geq 65 years (OR 3.52, CI 1.54-9.08,

p=0.003) and a residential postcode outside London (OR 2.99, Cl 1.25-6.68, p=0.016). The

217 majority of patients from outside London were UK residents (104/113).

218

219 During the study period, the ratio of prescribed mupirocin nasal tubes/number of admitted

colonised MRSA patients was similar amongst two Trusts (2.8 [648/232]; 2.1 [412/197]) and

one general district hospital (2.8 [142/51]) included in the cohort.

222

223 Relationship between genotypic and phenotypic mupirocin resistance

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225 A total of 665 de-duplicated unique-patient MRSA isolates (from 663 episodes and 648 226 patients), with complete data for MLST, genotypic and phenotypic mupirocin resistance, were 227 available for analysis (Figure 1). The prevalence of the V588F chromosomal mutation 228 (conferring LMR) was 8.42% (95% CI 6.42-10.80, n=56) and the prevalence of mupA 229 (conferring HMR) was 3.01% (95% CI 1.85-4.61, n=20). mupB and V631F mutations were not 230 identified in any isolate. The prevalence of any phenotypic mupirocin resistance, phenotypic 231 HMR and LMR in the sub-set of isolates for which genotypic data were available was similar 232 to that reported by episode (any (9.32% [95% CI 7.22-11.79]); LMR (6.62% [95% CI 4.85-8.78]); 233 HMR (2.71% [95% CI 1.61-4.24])).

234

Statistical measures of classification performance to examine the reliability of *mupA* in identifying HMR were based on all 665 de-duplicated isolates, whereas the performance of V588f to discriminate LMR excluded 14/665 isolates with combined V588F and *mupA* carriage (n=651; Table 2). The sensitivity of V588F carriage to predict LMR was 67.50% (95% CI 52.50-82.50 and the specificity was 97.55% (95% CI 96.24-98.69). The sensitivity of *mupA* carriage to predict HMR was 77.78% (CI 55.56-94.44) and the specificity was 99.07 (95% CI 98.3099.69). Area under the curve estimates were high (V588f: 83.21 [95% CI 76.35-90.08]; *mupA*:
88.43% [95% CI 78.54-98.31]). Four out of 14 isolates with combined V588F and *mupA* carriage
(28.57%) were phenotypically LMR and nine were HMR (64.29%). The relationship between
carriage of genetic markers and phenotypic resistance by MRSA MLST is summarised in Figure
24.

246

247 Genome sequence data of all mupA positive isolates (n=23), including same-patient 248 consecutive isolates and isolates with incomplete genetic data, was compared with the pPR9 249 mupA positive reference plasmid (GU237136) to investigate lack of HMR in 8/23 isolates 250 carrying *mupA*. This identified mutations in or near *mupA* likely to result in loss of function, in 251 *mupA* positive isolates that failed to express HMR but not in those with the HMR phenotype 252 (Table 3). Four isolates from three patients, had an INDEL of the internal homopolymeric tract 253 resulting in a frameshift and loss of functionality. Three isolates from two patients, had a wild 254 type *mupA* but had significant genetic loss to the upstream gene (p2) that may have resulted 255 in loss of the *mupA* operon promotor. One susceptible isolate appeared to have a fully 256 functional *mupA* operon but had a non-synonymous SNP within *mupA*.

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258 Genotypic and phenotypic mupirocin resistance and MRSA MLST

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Marked differences in carriage of genotypic markers and phenotypic resistance were observed across MRSA MLSTs. ST8 and ST36 were each in excess of 7, 2 and 16 times more likely to exhibit any resistance, LMR or HMR, respectively, than the most commonly identified endemic MLST (ST22) and other sporadic MLSTs. ST8 and ST36 were more than 10 and 70 times more likely to carry V588F mutation and *mup*A, respectively, than ST22 and sporadic MLSTs. No HMR or *mup*A carriage was detected in the closely related ST239 and ST241, but the odds of LMR and V588F carriage in these MLSTs was more than 20-fold that in ST22 andsporadic MLSTs. See Tables 4 and 5.

268

269 Discussion

This study evaluated phenotypic LMR and HMR and carriage of genotypic markers of resistance in a large series of contemporaneously collected hospital and community MRSA isolates from across three London boroughs and found significant heterogeneity across MRSA clones.

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275 Mupirocin use at each Trust and hospital during the study period, equated to between 1 and 276 3 tubes of mupirocin per admitted colonized MRSA patient and was consistent with adherence 277 to the 'screen and treat' decolonization guidelines ²⁴, given that the vast majority of nasal 278 mupirocin prescribed is used for MRSA decolonisation. In this context, 10% of patients across 279 the three boroughs had MRSA isolates phenotypically either LMR or HMR with the prevalence 280 of LMR (6%) higher than HMR (3%). Previous studies have more often reported that prevalence of LMR is higher, ^{13,14} although one study has reported the reverse.¹² The 281 282 prevalence of LMR and HMR reported elsewhere is variable, ranging from virtually none to 283 almost 20% for LMR and none to 10% for HMR. 12-15

284

Previous studies have generally shown a high concordance between the carriage of *mupA* and HMR ^{15,18,22,35} and one study has demonstrated a high concordance between LMR and the presence of the V588F mutation. ³⁶ In this study, carriage of mupirocin resistance genetic determinants had a high specificity (>97%) and area under the curve (>83%) to discriminate phenotypic resistance, suggesting very good diagnostic accuracy. Despite these findings, the correlation between genetic markers and phenotype was imperfect, and uncertainty around the sensitivity (95% CI 52.50-94.44%) precluded us from reporting a conclusive point estimate. 292 Genomic analysis of discordant isolates identified mutations in or near mupA as a likely 293 explanation for loss of HMR, although a single *mupA* SNP in one susceptible isolate may not 294 have caused loss of function alone. Moreover, four mupA positive isolates that failed to 295 express HMR, had an INDEL of the internal homopolymeric tract that allows for subsequent 296 slip-strand miss-pairing mutation to restore functionality, supporting observations that HMR 297 might be phase variable or transient. ³⁷ Gene carriage, therefore, does not invariably translate 298 into expression of resistance ^{37,38} and this limits the use of genetic markers to infer phenotype 299 unless detailed genetic analysis is undertaken. Discordance between LMR and V588f and an 300 explanation for HMR in four mupA negative isolates is presently lacking and the focus of 301 further research.

302

303 The main finding from this study, with significant clinical implications, was the high 304 heterogeneity in distribution of phenotypic and genotypic markers of resistance across MRSA 305 clones. Phenotypic HMR and mupA were predominantly found in ST8 and ST36, whilst 306 phenotypic LMR and V588F were predominantly in ST239/241 as well as ST8 and ST36. HMR 307 and LMR were low (<4%) in the current dominant UK MRSA clone ST22 and 308 community/sporadic MRSA isolates. To our knowledge, this is the first study to report clonal 309 variation in LMR and V588f mutation from clinical isolates. This supports a recent in-vitro 310 study, which suggests that mutations conferring LMR may be more readily inducible in some 311 clones. ³⁹ Clonal variation in HMR had been shown previously. ^{16–18} A plausible explanation for 312 the latter, may be that particular MRSA clones are more receptive to conjugation with 313 coagulase-negative staphylococci (CoNs)⁴⁰ that commonly carry *mupA*, and which may act as 314 a reservoir for transmission into S. aureus. ⁴¹ An explanation for clonal variation in LMR and 315 V588f is presently lacking.

317 We hypothesise that local variation in dominant MRSA clones may, at least in part, explain 318 why increasing mupirocin resistance associated with intensive mupirocin use, has only been 319 reported in some studies. ^{1,36,42} At least for the case of HMR, there is evidence that a difference 320 in resistance phenotype in the dominant UK clones ST22 and ST36, has existed for many years 321 and at GSTT it pre-dates introduction of intensive decolonisation as part of the successful 322 'screen and treat' infection control campaign that began in 2004. Between 1999 and 2004 323 ST36 caused 50.0% of 498 MRSA bloodstream infections of which 40.1% were HMR, whereas 324 ST22 comprised 29.5% but none were HMR (data extracted from dataset used by Miller et al. 325 ³³). Subsequently, between 2004 and 2009, ST36 accounted for 28.6% of 255 MRSA 326 bloodstream infections - of which 26.0% were HMR - whereas ST22 comprised 39.6% of 327 bloodstream infections and only 2.0% were HMR.

328

329 Lack of selection for mupirocin resistance at GSTT is likely to be multifactorial, with clonal 330 composition playing a pivotal role. Firstly, there may be an intrinsic lower propensity of clones 331 such as ST22 to acquire resistance. Secondly, resistant clones may carry a fitness cost making 332 them less transmissible than susceptible clones. Evidence for the latter has been reported in 333 a recent companion study (Deeny et al submitted), and may help explain the particularly rapid 334 decline of ST36 over the past ten years in the context of improving infection control practice. 335 ⁴³ Thirdly, a conservative approach to MRSA control - where mupirocin prescription is targeted 336 to MRSA carriers only - may not provide significant selection of resistance. Indeed, simulation 337 studies show that prevalence of resistance is expected to remain stable under 'screen and 338 treat' guidelines whilst predicted to increase under 'universal' use (Deeny et al submitted).

339

Our study has a number of strengths. We determined phenotypic and genotypic resistance
 for a large collection of consecutive MRSA isolates from adjacent laboratories covering five
 different London hospitals and their adjacent community. Also, we analysed anonymised

343 patient-level data in order to derive risk factors for LMR and HMR. These findings will prove 344 useful to inform the development of mupirocin resistance transmission models to evaluate 345 the threat that may arise from increasing mupirocin usage. Limitations are that we only 346 evaluated known mechanisms for LMR and HMR and, although we had access to detailed 347 clinical information, we did not have data on use of mupirocin for individual patients.

348

349 In summary, mupirocin resistance varies significantly by clone implying that changes in clonal 350 epidemiology may have an important role in determining the prevalence of resistance in 351 conjunction with selection due to mupirocin use. Low levels of resistance (<10%) across 352 Central / South East London after an extended period of decolonisation linked with a 353 successful UK MRSA control programme, may in part be explained by the MRSA clonal 354 population structure and specifically by ST22 being the dominant clone. We conclude that 355 mupirocin use alone is not sufficient to predict resistance trends and that determining the 356 local population of MRSA MLSTs and monitoring changes in the population structure may be 357 a useful way of guiding mupirocin usage policies.

358

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364

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- 370

371 Transparency Declarations

- 372 None of the authors has any financial conflicts of interest to declare. The funding bodies had
- 373 no role in study design, data collection and analysis, decision to publish, or preparation of the
- 374 manuscript.
- 375

376 Disclaimer

- 377 The views expressed are those of the authors and not necessarily those of the NHS, the NIHR
- 378 or the Department of Health.

			Any Mupirocin Resistance		Low Mupirocin Resistance			High Mupirocin Resistance			
Variable	Levels	Total	OR	95% CI	p-Value	OR	95% CI	p-Value	OR	95% CI	p-Value
Episode Type	Community	110	-	-	0.002	-	-	0.003	-	-	0.317
	Inpatient	419	3.17	1.36-9.30		5.23	1.56-32.63		1.60	0.53-6.95	
	Outpatient	266	1.43	0.55-4.46		2.33	0.61-15.29		0.82	0.21-3.97	
Patient Gender	Male	462	-	-	0.208	-	-	0.393	-	-	0.363
	Female	331	0.73	0.44-1.19		0.77	0.42-1.39		0.69	0.29-1.52	
Patient Age	< 65 years	431	-	-	<0.001	-	-	0.008	-	-	0.003
	≥ 65 years	364	2.71	1.66-4.53		2.21	1.23-4.09		3.52	1.54-9.08	
Genomic MRSA Type	Community	163	-	-	<0.001			NA	-	-	0.908
	Hospital	519	4.29	1.86-12.45		NA	NA-NA		0.94	0.36-2.93	
Previous History of MRSA	No	502	-	-	0.010	-	-	0.025	-	-	0.224
	Yes	293	1.87	1.17-3.01		1.94	1.09-3.47		1.62	0.74-3.51	
Hospital Admission (past 12 months)	No	478	-	-	0.398	-	-	0.016	-	-	0.051
	Yes	314	1.23	0.76-1.97		2.03	1.14-3.65		0.42	0.15-1.00	
Patient Residential Postcode	London	640	-	-	0.003	-	-	0.084	-	-	0.016
	Other	113	2.38	1.35-4.07		1.88	0.91-3.63		2.99	1.25-6.68	

Table 1. Risk factors of phenotypic mupirocin resistance (n=795).

382 Table 2. Classification performance for reliability of V588F and *mupA* genetic markers in

	V588f -> LMR (n=651) ¹		mupA	-> HMR (n=665)
	%	95% CI	%	95% CI
Specificity	97.55	96.24-98.69	99.07	98.30-99.69
Sensitivity	67.50	52.50-82.50	77.78	55.56-94.44
Accuracy	95.70	94.16-97.08	98.50	97.59-99.25
Negative predictive value	97.87	96.92-98.84	99.38	98.77-99.84
Positive Predictive Value	64.58	52.17-77.78	70.59	53.85-88.24
Area Under the Curve	83.21	76.35-90.08	88.43	78.54-98.31

383 predicting low and high phenotypic mupirocin resistance respectively.

384 ¹ 14/665 isolates with combined V588F mutation and *mupA* were excluded to estimate

385 classification performance of V588f.

386

laslata	MICT	MUP200 Disc Diffusion (DD) Test		MIC	mun A Cons Mutations	Cone Deletions compared to pDD0 placmid			
isolate	IVILSI	Category	DD Zone (mm)	µg/mL	mupA Gene Mutations				
1	ST22	HMR	0	>1024		p10, p11, p25-p39			
2	ST45	HMR	0	>1024		p1, p5, p9-p11, p13			
3	ST59	Susceptible	37	0.094	INDEL in polymeric tract	p10-p42			
4a	ST36	HMR	0	>1024		p38, p39			
4b	-	HMR	0	>1024		p38, p39			
5	ST36	HMR	0	>1024		p38, p39			
6	ST36	HMR	0	>1024		p38, p39			
7	ST36	HMR	0	>1024		p38, p39			
8	ST36	HMR	0	>1024		p38, p39			
9	ST36	HMR	0	>1024		p38, p39			
10	ST36	HMR	0	>1024		p38, p39			
11a	ST36	LMR ³	30	6	INDEL in polymeric tract	p38, p39			
11b	ST36	LMR ³	30	12	INDEL in polymeric tract	p38, p39			
12	ST36	LMR ³	31	16	INDEL in polymeric tract	р38, р39			
13	ST36	Susceptible	41	0.125	SNP (C42T)	p30-p35, p38-p42			
14	ST8	HMR	0	>1024		p10, p11, p38, p39			
15	ST8	HMR	13	>1024		p10, p11, p38, p39			
16	ST8	HMR	0 -> 38 ¹	0.125		p10, p11, p38, p39			
17	ST8	HMR	0	>1024		р10-р42			
18	ST8	HMR	0	>1024		р10-р42			
19a	ST8	LMR ³	25	64		p2, p4, p10-p42 ²			
19b	ST8	LMR ³	25	24		p2, p4, p10-p42 ²			
20	ST8	LMR ³	28	8		p2, p4, p10-p42 ²			

Table 3. Phenotypic mupirocin resistance of *mupA* positive isolates.

389 All mupA positive MRSA isolates, including same-patient consecutive isolates and isolates with incomplete genetic data (n=23), are shown in the table. Isolates from 390 the same patient are given the same number ID (e.g. 4a and 4b). MUP200 disc diffusion test (DDT) shows the classification of isolates as HMR, LMR or susceptible 391 according to the susceptibility test conducted in 2011-2012, before storage of live isolates at -80C. Whole genome sequencing was also conducted on DNA 392 extracted before storage of isolates. MICs were conducted on re-cultured isolates in 2015. Presence of plasmid genes was determined by mapping sequence reads 393 against pPR9 reference plasmid (GU237136).¹ A DDZ=0mm (HMR) was observed in 2011-2012 whilst a DDZ=38mm (sensitive) and an MIC = 0.125 µg/µl was 394 observed in 2015, suggesting loss of plasmid during storage.² P2 is the first gene in the operon. Deletion of p2, including the upstream sequence, may result in loss

395 of promotor binding site and loss of downstream *mupA* expression.³ V588f mutation was detected in all LMR isolates.

	MLST	Total	Resistant	OR	95% CI	p-Value
Any Mupirocin Resistance	ST22	404	18	-	-	<.0001
	Other	147	4	0.60	0.17-1.65	
	ST239 / 241	11	5	17.87	4.74-65.35	
	ST36	63	25	14.11	7.10-28.66	
	ST08	40	10	7.15	2.94-16.71	
Low Mupirocin Resistance	ST22	404	15	-	-	<.0001
	Other	147	2	0.36	0.06-1.29	
	ST239 / 241	11	5	21.61	5.65-80.47	
	ST36	63	18	10.37	4.89-22.35	
	ST08	40	4	2.88	0.79-8.47	
High Mupirocin Resistance	ST22	404	3	-	_	<.0001
	Other	147	2	1.84	0.24-11.30	
	ST239 / 241	11	0	NA	NA-NA	
	ST36	63	7	16.71	4.49-79.70	
	ST08	40	6	23.59	5.93-116.39	

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Table 4. Phenotypic mupirocin resistance by MRSA multilocus sequence type (n=665).

410 Table 5. Genotypic markers of mupirocin resistance by MRSA multilocus sequence type

411 (n=665).

	MLST	Total	Positive	OR	95% CI	p-Value
V588f	ST22	404	8	-	-	<.0001
	Other	147	0	NA	NA-NA	
	ST239 / 241	11	9	222.75	48.35-1648	
	ST36	63	32	51.10	22.62-128.59	
	ST08	40	7	10.50	3.47-31.20	
mupA	ST22	404	1	-	-	<.0001
	Other	147	2	5.56	0.52-121.73	
	ST239 / 241	11	0	NA	NA-NA	
	ST36	63	10	76.04	14.09-1428	
	ST08	40	7	85.48	14.54-1645	

425 Figure 1. Study Flow Chart.





432 Figure 2. Relationship between genotypic and phenotypic mupirocin resistance by MRSA multilocus sequence type (n=665).

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