**Clonal variation in high- and low-level phenotypic and genotypic mupirocin resistance of MRSA isolates in South East London**

**Short Title:** MRSA clonal variation in mupirocin resistance.

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**Abstract**

**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.

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

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

**Conclusions:** Mupirocin resistance was identified in less than 10% of patients, and varied significantly by clone implying that changes in clonal epidemiology may have an important role in determining the prevalence of resistance in conjunction with selection due to mupirocin use.

**Introduction**

Mupirocin (pseudomonic acid A) is an antibiotic commonly used for the nasal decolonization of MRSA and MSSA.1,2 It has been widely used as part of the successful UK MRSA control programmeme over the last 10 years.3 It has also been shown to reduce the rate of MRSA body site infections when applied universally in conjunction with chlorhexidine to all patients admitted to the ICU.4 Mupirocin-resistant *Staphylococcus aureus* was first reported in 1987 at St. Thomas’ Hospital, which now forms part of Guy’s and St. Thomas’ NHS Trust (GSTT).5 Mupirocin binds to the bacterial isoleucyl-tRNA synthetase gene, inhibiting protein replication.1,2 Mupirocin resistance is classified as either Low-Level Mupirocin Resistance (LMR) or High-Level Mupirocin Resistance (HMR).1 LMR is mediated through point mutations in the native isoleucyl-tRNA synthetase gene (*ileS*) causing a Val-to-Phe change in the mupirocin binding site, at either residue 588 (V588F) or 631 (V631F).6 HMR is due to carriage of a distinct plasmid-mediated isoleucyl-tRNA synthetase gene, most commonly *mupA*, although *mupB* has been reported.2,7,8 HMR is associated with MRSA decolonization failure, and LMR appears to be associated with early re-colonisation and in some reports, decolonization failure.1,9–11

The prevalence of mupirocin resistance (LMR and HMR) and of the underpinning genotypic determinants has been widely reported. In 1998, a survey of MRSA from 19 European hospitals found HMR in 3.6% and LMR in 2.6% of 194 MRSA samples.12 A Japanese cohort reported LMR prevalence of 0.8% to 4.0 % between 1998 and 2001 with no HMR detected.13. A more recent study of 156 MRSA isolates in the United States demonstrated LMR in 18.6%, and HMR in 5.1% of isolates.14 Similarly, a Singaporean cohort study identified HMR in 11% of 307 isolates.15 Several reports suggest that carriage of *mup*A is more common in some clones, 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 mupirocin-resistant, 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

This study reports the prevalence of mupirocin resistance (LMR and HMR) and carriage of mupirocin resistance determinants (V588F/V631F and *mupA*/*mupB*) in hospital and community MRSA isolates identified in three laboratories serving five hospital and community healthcare facilities across three adjacent London boroughs. All healthcare facilities in this area have implemented effective infection control programmes over the past 5-7 years 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%. 25 The aim of the study was to determine the distribution, risk factors, and clonal variation in LMR and HMR, and their genotypic determinants.

**Methods**

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.

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

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 sample prep protocol (Illumina Inc, San Diego, CA, USA) following the manufacturer’s instructions. Libraries were quantified individually using the Qubit High Sensitivity Kit and equimolar amounts pooled for sequencing. Pooled 96-plex libraries were diluted and denatured ready for paired-end 150 cycle sequencing on the Illumina HiSeq 2500 platform in 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, http://bioinformatics.net.au/software.velvetoptimiser.shtml) for each sample. Draft assemblies were analysed *in silico* to determine the multilocus sequence type (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*)type, carriage of the Panton-Valentine leukocidin (PVL) and identify genomic markers of mupirocin resistance using BWA30 and BLAST.31 WGS was conducted on the first confirmed MRSA isolate from each individual at each unique healthcare setting (i.e. whenever an individual was admitted as inpatient to a new hospital, or received care in a new outpatient clinic or community service throughout the study period); thus, follow-up genomic information was available for patients who received care at multiple settings.

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

*Analysis*

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

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.

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

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

**Results**

*Analysis of risk factors for phenotypic mupirocin resistance*

1523 consecutive isolates from 839 patients presenting with one or multiple healthcare episodes (n=1096), were retrieved from the microbiology laboratories serving Lambeth, Southwark and Lewisham (Figure 1). To avoid pseudo-replication, the analysis was based on the characterization of MRSA isolates from the patients’ first healthcare episode, leaving 795 patients’ first episodes (1131 isolates) for analysis.

Prevalence of any LMR or HMR amongst patients’ first episode (n= 795) was 9.69% (95% CI 7.72-11.96, n=77). LMR was 6.29% (95% CI 4.70-8.21, n=50), and HMR 3.40% (95% CI 2.25-4.90, n=27). Prevalence of any mupirocin resistance (p = 0.84), LMR (p = 0.79) or HMR (p = 0.74) amongst first inpatient episodes (n=419), was not different across two Trusts and one general district hospital included in the study. Only four episodes had combined LMR and HMR, and were classified as HMR.

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), previous history of MRSA (OR 1.94, 95% CI 1.09-3.47, p=0.025) and age ≥65 years (OR 2.21, 95% CI 1.23-4.09, p=0.008). HMR was associated with age ≥65 years (OR 3.52, CI 1.54-9.08, p=0.003) and a residential postcode outside London (OR 2.99, CI 1.25-6.68, p=0.016). The majority of patients from outside London were UK residents (104/113).

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.

Relationship between genotypic and phenotypic mupirocin resistance

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

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 *mup*A carriage to predict HMR was 77.78% (CI 55.56-94.44) and the specificity was 99.07 (95% CI 98.30-99.69). 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 2.

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

Genotypic and phenotypic mupirocin resistance and MRSA MLST

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 and sporadic MLSTs. See Tables 4 and 5.

**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.

Mupirocin use at each Trust and hospital during the study period, equated to between 1 and 3 tubes of mupirocin per admitted colonized MRSA patient and was consistent with adherence to the ‘*screen and treat*’ decolonization guidelines 24 , given that the vast majority of nasal mupirocin prescribed is used for MRSA decolonisation. In this context, 10% of patients across the three boroughs had MRSA isolates phenotypically either LMR or HMR with the prevalence 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 prevalence of LMR and HMR reported elsewhere is variable, ranging from virtually none to almost 20% for LMR and none to 10% for HMR. 12–15

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. Genomic analysis of discordant isolates identified mutations in or near *mupA* as a likely explanation for loss of HMR, although a single *mupA* SNP in one susceptible isolate may not have caused loss of function alone. Moreover, four *mupA* positive isolates that failed to express HMR, had an INDEL of the internal homopolymeric tract that allows for subsequent slip-strand miss-pairing mutation to restore functionality, supporting observations that HMR might be phase variable or transient. 37 Gene carriage, therefore, does not invariably translate into expression of resistance 37,38 and this limits the use of genetic markers to infer phenotype unless detailed genetic analysis is undertaken. Discordance between LMR and V588f and an explanation for HMR in four *mupA* negative isolates is presently lacking and the focus of further research.

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

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

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

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

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

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**Transparency Declarations**

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

**Disclaimer**

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

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

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **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 2.** Classification performance for reliability of V588F and *mupA* genetic markers in predicting low and high phenotypic mupirocin resistance respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **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 |
|  |  |  |  |  |

**1** 14/665 isolates with combined V588F mutation and *mupA* were excluded to estimate classification performance of V588f.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Isolate** | **MLST** | **MUP200 Disc Diffusion (DD) Test** | | **MIC** | ***mupA* Gene Mutations** | **Gene Deletions compared to pPR9 Plasmid** |
| **Category** | **DD Zone (mm)** | **µg/mL** |
| 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** | p38, p39 |
| 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 |  | p10-p42 |
| 18 | ST8 | HMR | 0 | >1024 |  | p10-p42 |
| 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.

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 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 according to the susceptibility test conducted in 2011-2012, before storage of live isolates at -80C. Whole genome sequencing was also conducted on DNA extracted before storage of isolates. MICs were conducted on re-cultured isolates in 2015. Presence of plasmid genes was determined by mapping sequence reads 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 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 of promotor binding site and loss of downstream *mupA* expression.3V588f mutation was detected in all LMR isolates.

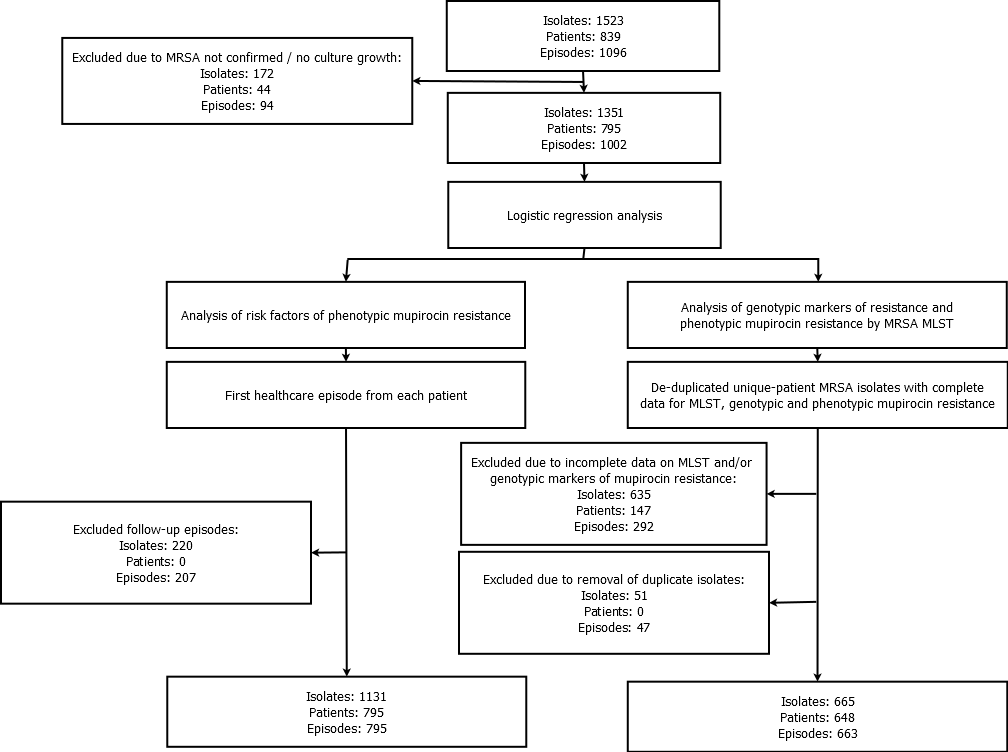
**Table 4.** Phenotypic mupirocin resistance by MRSA multilocus sequence type (n=665).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **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 |  |
|  |  |  |  |  |  |  |

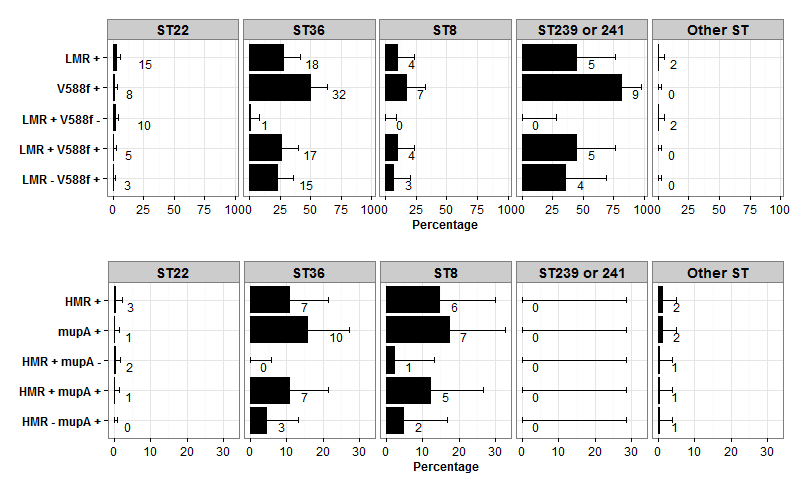
**Table 5.** Genotypic markers of mupirocin resistance by MRSA multilocus sequence type (n=665).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **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 |  |
|  |  |  |  |  |  |  |

**Figure 1. Study Flow Chart.**



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



**References**

1. Hetem DJ, Bonten MJM. Clinical relevance of mupirocin resistance in Staphylococcus aureus. *J Hosp Infect* 2013; **85**(4): 249–56.

2. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. *Clin Infect Dis* 2009; **49**(6): 935–41.

3. Coia JE, Duckworth GJ, Edwards DI et al. Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities. *J Hosp Infect* 2006; **63** Suppl 1: S1–44.

4. Huang SS, Septimus E, Kleinman K et al. Targeted versus universal decolonization to prevent ICU infection. *N Engl J Med* 2013; **368**(24): 2255–65.

5. Rahman M, Noble WC, Cookson B et al. Mupirocin-resistant Staphylococcus aureus. *Lancet* 1987; **330**(8555): 387–8.

6. Antonio M, McFerran N, Pallen MJ. Mutations affecting the Rossman fold of isoleucyl-tRNA synthetase are correlated with low-level mupirocin resistance in Staphylococcus aureus. *Antimicrob Agents Chemother* 2002; **46**(2): 438–42.

7. Hodgson JE, Curnock SP, Dyke KG et al. Molecular characterization of the gene encoding high-level mupirocin resistance in Staphylococcus aureus J2870. *Antimicrob Agents Chemother* 1994; **38**(5): 1205–8.

8. Seah C, Alexander DC, Louie L et al. MupB, a new high-level mupirocin resistance mechanism in staphylococcus aureus. *Antimicrob Agents Chemother* 2012; **56**(4): 1916–20.

9. Walker ES, Vasquez JE, Dula R et al. Mupirocin-resistant, methicillin-resistant Staphylococcus aureus: does mupirocin remain effective? *Infect Control Hosp Epidemiol* 2003; **24**(5): 342–6.

10. Harbarth S, Liassine N, Dharan S et al. Risk factors for persistent carriage of methicillin-resistant Staphylococcus aureus. *Clin Infect Dis* 2000; **31**(6): 1380-5.

11. Robicsek A, Beaumont JL, Thomson RB et al. Topical therapy for methicillin-resistant Staphylococcus aureus colonization: impact on infection risk. *Infect Control Hosp Epidemiol* 2009; **30**(7): 623–32.

12. Schmitz FJ, Lindenlauf E, Hofmann B et al. The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 European hospitals. *J Antimicrob Chemother* 1998; **42**(4): 489–95.

13. Fujimura S, Watanabe A. Survey of high- and low-level mupirocin-resistant strains of methicillin-resistant Staphylococcus aureus in 15 Japanese hospitals. *Chemotherapy* 2003; **49**(1-2): 36–8.

14. Mongkolrattanothai K, Mankin P, Raju V et al. Surveillance for mupirocin-resistance among methicillin-resistant Staphylococcus aureus clinical isolates. *Infect Control Hosp Epidemiol* 2008; **29**(10): 993–4.

15. Choudhury S, Krishnan PU, Ang B. Prevalence of high-level mupirocin resistance among meticillin-resistant Staphylococcus aureus isolates in a tertiary care hospital in Singapore. *J Hosp Infect* 2012; **82**(1): 56–7.

16. Desroches M, Potier J, Laurent F et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistan Staphylococcus aureus (MRSA) in France: Emergence of a mupirocin-resistant MRSA clone harbouring mupA. *J Antimicrob Chemother* 2013; **68**(8): 1714–7.

17. Cadilla A, David MZ, Daum RS et al. Association of high-level mupirocin resistance and multidrug-resistant methicillin-resistant Staphylococcus aureus at an academic center in the midwestern United States. *J Clin Microbiol* 2011; **49**(1): 95–100.

18. Chaves F, García-Martínez J, De Miguel S et al. Molecular Characterization of Resistance to Mupirocin in Methicillin-Susceptible and -Resistant Isolates of Staphylococcus aureus from Nasal Samples. *J Clin Microbiol* 2004; **42**(2): 822–4.

19. Jones JC, Rogers TJ, Brookmeyer P et al. Mupirocin resistance in patients colonized with methicillin-resistant Staphylococcus aureus in a surgical intensive care unit. *Clin Infect Dis* 2007; **45**(5): 541–7.

20. Caffrey AR, Quilliam BJ, LaPlante KL. Risk factors associated with mupirocin resistance in meticillin-resistant Staphylococcus aureus. *J Hosp Infect* 2010; **76**(3): 206–10.

21. Torvaldsen S, Roberts C, Riley T V. The continuing evolution of methicillin-resistant Staphylococcus aureus in Western Australia. *Infect Control Hosp Epidemiol* 1999; **20**(2): 133–5.

22. Vasquez JE, Walker ES, Franzus BW et al. The epidemiology of mupirocin resistance among methicillin-resistant Staphylococcus aureus at a Veterans’ Affairs hospital. *Infect Control Hosp Epidemiol* 2000; **21**(7): 459–64.

23. Hogue JS, Buttke P, Braun LE et al. Mupirocin resistance related to increasing mupirocin use in clinical isolates of methicillin-resistant Staphylococcus aureus in a pediatric population. *J Clin Microbiol* 2010; **48**(7): 2599–600.

24. Department of Health UK 2006. *Screening for Meticillin-resistant Staphylococcus aureus (MRSA) colonisation: A strategy for NHS trusts: a summary of best practice*. http://webarchive.nationalarchives.gov.uk/20130107105354/http:/www.dh.gov.uk/prod\_consum\_dh/groups/dh\_digitalassets/@dh/@en/documents/digitalasset/dh\_063187.pdf.

25. Edgeworth JD. Has decolonization played a central role in the decline in UK methicillin-resistant Staphylococcus aureus transmission? A focus on evidence from intensive care. *J Antimicrob Chemother* 2011; **66** Suppl 2: 41–7.

26. UK Data Service Census Support. *Office for National Statistics, 2011 Census: Aggregate data (England and Wales)*. http://infuse.mimas.ac.uk. Licensed under the terms of the Open Government Licence [http://www.nationalarchives.gov.uk/doc/open-government-licence/version/2].

27. Batra R, Cooper BS, Whiteley C et al. Efficacy and limitation of a chlorhexidine-based decolonization strategy in preventing transmission of methicillin-resistant Staphylococcus aureus in an intensive care unit. *Clin Infect Dis* 2010; **50**: 210–7.

28. Andrews JM. BSAC standardized disc susceptibility testing method. *J Antimicrob Chemother* 2001; **48** Suppl 1: 43–57.

29. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 2008; **18**(5): 821–9.

30. Langmead B, Trapnell C, Pop M et al. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 2009; **10**(3): R25.

31. Altschul SF, Gish W, Miller W et al. Basic local alignment search tool. *J Mol Biol* 1990; **215**(3): 403–10.

32. Otter JA, French GL. Community-associated meticillin-resistant Staphylococcus aureus: The case for a genotypic definition. *J Hosp Infect* 2012; **81**(3): 143–8.

33. Miller CE, Batra R, Cooper BS et al. An association between bacterial genotype combined with a high-vancomycin minimum inhibitory concentration and risk of endocarditis in methicillin-resistant Staphylococcus aureus bloodstream infection. *Clin Infect Dis* 2012; **54**(5): 591–600.

34. R Foundation for Statistical Computing Vienna Austria 2013. *R: A Language and Environment for Statistical Computing*. http://web.mit.edu/r\_v3.0.1/fullrefman.pdf

35. Udo EE, Jacob LE, Mathew B. Genetic analysis of methicillin-resistant Staphylococcus aureus expressing high- and low-level mupirocin resistance. *J Med Microbiol* 2001; **50**(10): 909–15.

36. Lee AS, MacEdo-Vinas M, Franois P et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant staphylococcus aureus carriage after decolonization therapy: A case-control study. *Clin Infect Dis* 2011; **52**(12): 1422–30.

37. Driscoll DG, Young CL, Ochsner UA. Transient loss of high-level mupirocin resistance in Staphylococcus aureus due to MupA polymorphism. *Antimicrob Agents Chemother* 2007; **51**(6): 2247–8.

38. Fritz SA, Hogan PG, Camins BC et al. Mupirocin and chlorhexidine resistance in Staphylococcus aureus in patients with community-onset skin and soft tissue infections. *Antimicrob Agents Chemother* 2013; **57**(1): 559–68.

39. Lee AS, Gizard Y, Empel J et al. Mupirocin-induced mutations in ileS in various genetic backgrounds of methicillin-resistant Staphylococcus aureus. *J Clin Microbiol* 2014; **52**(10): 3749–54.

40. Hurdle JG, O’Neill AJ, Mody L et al. In vivo transfer of high-level mupirocin resistance from Staphylococcus epidermidis to methicillin-resistant Staphylococcus aureus associated with failure of mupirocin prophylaxis. *J Antimicrob Chemother* 2005; **56**(6): 1166–8.

41. Bathoorn E, Hetem DJ, Alphenaar J et al. Emergence of high-level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use. *J Clin Microbiol* 2012; **50**(9): 2947–50.

42. Fawley WN, Parnell P, Hall J et al. Surveillance for mupirocin resistance following introduction of routine peri-operative prophylaxis with nasal mupirocin. *J Hosp Infect* 2006; **62**(3): 327–32.

43. Ellington MJ, Hope R, Livermore DM et al. Decline of EMRSA-16 amongst methicillin-resistant Staphylococcus aureus causing bacteraemias in the UK between 2001 and 2007. *J Antimicrob Chemother* 2010; **65**(3): 446–8.