The isoleucyl-tRNA synthetase mutation V588F conferring mupirocin resistance in glycopeptide-intermediate *Staphylococcus aureus* is not associated with a significant fitness burden

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**Objectives and methods:** Failure to eradicate nasal carriage of a glycopeptide-intermediate *Staphylococcus aureus* (strain GISA-2) with mupirocin was recently attributed to a mutation that confers low-level mupirocin resistance. To identify this mutation the *ileS* genes of GISA-2 and its mupirocin-susceptible progenitor GISA-1 were sequenced. For comparison, the *ileS* genes of 10 laboratory-derived mupirocin-resistant mutants of the GISA strain Mu50 were also examined. The fitness of GISA-2 and mupirocin-susceptible GISA-1, as well as Mu50 and its mupirocin-resistant derivatives, were compared by evaluation of growth rates and performance in mixed-culture competition assays.

**Results:** The point mutation V588F in the isoleucyl-tRNA synthetase was identified from the *ileS* sequences of GISA-2 and mupirocin-resistant mutants of Mu50. The V588F mutation was not associated with a significant fitness burden.

**Conclusions:** The low fitness cost of the V588F substitution in isoleucyl-tRNA synthetase is consistent with the frequent appearance and maintenance of this mutation in mupirocin-resistant clinical isolates, including GISA-2.

**Keywords:** GISA, mupirocin-resistant *Staphylococcus aureus*

**Introduction**

The topical antimicrobial agent mupirocin has been used routinely to eradicate nasal carriage of *Staphylococcus aureus* and in the management of skin infections caused by staphylococci. Mupirocin is a potent inhibitor of the bacterial isoleucyl-tRNA synthetase and has good activity against strains of *S. aureus* including methicillin-resistant *S. aureus* (MRSA) and glycopeptide-intermediate *S. aureus* (GISA). However, the widespread use of this agent has resulted in the emergence of strains expressing mupirocin resistance. Both high-level (MIC > 256 mg/L) and low-level (MIC 8–256 mg/L) mupirocin resistance has been described in the clinic. High-level resistance is mediated by acquisition of the *mupA* determinant, whereas low-level resistance usually results from mutations in the chromosomal *ileS* gene.

Chromosomal mutations conferring antibiotic resistance in bacteria can cause loss of fitness in the mutant organisms. The majority of mutants exhibiting mupirocin resistance in the clinic carry the point mutation V588F. Since this mutation is prevalent in the clinic, it is plausible that this genotype is associated with a low fitness cost.

Previously, the occurrence of mutants expressing low-level mupirocin resistance has been regarded as clinically insignificant. However, the number of cases associating mupirocin treatment failure with the emergence of mutants is increasing. Most recently, failure to eradicate nasal carriage of a GISA with mupirocin was caused by the emergence of a mutant with low-level resistance. In this study we have identified the mutation conferring mupirocin resistance in this clinical GISA isolate and evaluated the fitness cost of this resistance allele. These data were compared with those for in vitro-selected mupirocin-resistant mutants of the GISA strain Mu50.

**Materials and methods**

**Bacterial strains, growth media and chemicals**

Clinical mupirocin-resistant GISA-2 isolate and its mupirocin-susceptible progenitor, GISA-1, were kindly provided by the Department of Hospital Health, Centre Hospitalier de Versailles (Le Chesnay Cedex, France). Mu50, a well-characterized GISA strain, was used for con-
parison. Strains were grown in Iso-Sensitest broth (ISB) or agar (ISA) (Oxoid, Basingstoke, UK). The lithium salt of mupirocin was a gift from GlaxoSmithKline Pharmaceuticals (Harlow, UK).

**Determination of susceptibility to mupirocin and mutation frequency determination**

MICs were determined by agar dilution in ISA with an inoculum in ISB of 10⁶ cfu/spot. The MIC was defined as the lowest concentration of mupirocin that inhibited visible growth after 18 h incubation at 37°C. Mupirocin-resistant mutants were recovered by plating cultures onto ISA selection plates containing the antibiotic at levels four-fold higher than the respective MIC for the parental starting strain. Mutants were recovered following incubation for 18 h at 37°C. Mutation frequencies were determined exactly as described previously using Iso-Sensitest growth media.

**Determination of bacterial fitness**

(i) **Growth rate determinations.** Generation times in ISB were determined from absorbance readings taken at 600 nm. Absorbance was recorded continuously in a Molecular Device Spectra Max Plus 384 microplate reader (Sunnyvale, CA, USA) using 96-well plates. Incubation at 37°C was performed with automated shaking periods of 30 s every 5 min and before each absorbance reading. A minimum of three independent cultures were sampled and maximum generation times were calculated using Prism 3.0 (GraphPad Software, Inc., San Diego, CA, USA).

(ii) **Relative competitive fitness.** The relative competitive fitness of mupirocin-resistant mutants was measured in pair-wise competition experiments. ISB was inoculated with a mixture of overnight cultures of mupirocin-susceptible parental strain and a mupirocin-resistant mutant in a 10:1 ratio. Dilutions of the mixed cultures were plated onto both non-selective ISA and selective ISA containing mupirocin at a concentration of 4 × MIC for the parental strain at time 0 and after 24 h incubation at 37°C. A minimum of three independent cultures were used and the relative competitive fitness was calculated according to Lenski.

**PCR amplification and sequencing of the ileS gene**

PCR amplification of the entire ileS was performed using the following oligonucleotide primers: forward, ileS83, 5'-AGCCTAGTAAAGACGCTATGGTTATATCAC, and reverse, ileS272, 5'-GCCTACTGTATGATGATATTCAATTAA. PCR conditions were used and the relative competitive fitness was calculated according to Lenski.

**Results**

**Mupirocin resistance in GISA-2 and the mutants selected from Mu50**

The mupirocin MIC for the clinical isolate GISA-2 was 32 mg/L, as previously reported. This represents a 128-fold increase in resistance to mupirocin compared with the progenitor strain GISA-1, for which the MIC was 0.25 mg/L. Sequencing the ileS genes of GISA-1 and GISA-2 revealed that GISA-2 differed by one nucleotide with a change of G to T at position 1762. This mutation resulted in an amino acid change of valine to phenylalanine at position 588 in the isoleucyl-tRNA synthetase of GISA-2 compared with GISA-1. This mutation has been widely detected in other mupirocin-resistant clinical isolates, including MRSA.

Twenty mupirocin-resistant mutants, recovered from Mu50 (mupirocin MIC = 0.03 mg/L) in three independent selections, were also examined. Mupirocin MICs for the mutants were 8–16 mg/L, and were recovered at a frequency of 4.9 × 10⁻³, which is consistent with single mutational events. The ileS genes of 10 mutants were PCR-amplified and sequenced. The V588F mutation was found in all 10 strains. The ileS sequences of mupirocin-susceptible GISA and Mu50 were found to be identical.

**Fitness costs of the V588F mutation**

Mixed-culture competition assays and growth rate determinations were performed to examine the effect of the V588F mutation. No significant fitness costs were associated with emergence of this mutation, since the fitness data for GISA-2 and the mutants derived from Mu50 did not differ significantly from their respective parental strains.

Thus the generation times for GISA-1 and GISA-2 were 50 (± 2) and 51 (± 3) min, respectively, whilst the relative competitive fitness of GISA-2 was 0.98 (± 0.03). Similarly, the relative fitness of the 10 mupirocin-resistant mutants of Mu50 ranged from 0.95 (± 0.02) to 0.99 (± 0.01), whereas the generation times varied from 54 (± 4) to 61 (± 6) min, compared with 54 (± 2) min for mupirocin-susceptible Mu50.

**Discussion**

In this study we have demonstrated that chromosomal resistance to mupirocin resulting from the mutation V588F in the isoleucyl-tRNA synthetase did not significantly affect the fitness of a recent glycopeptide-resistant S. aureus isolate and 10 laboratory-derived mupirocin-resistant mutants of Mu50. This conclusion stems from the findings that the generation times and competitive fitness of the resistant mutants were not significantly different from their wild-type progenitor strains. Therefore, this resistance allele (V588F), which has been widely detected in the clinic, appears not to affect the fitness of the resistant mutants in vivo, and it is possible that this mutation has similar low-cost effects in vivo.

The emergence of mupirocin-resistant GISA-2 resulted in the failure of mupirocin to eradicate nasal carriage of a teicoplanin-resistant MRSA, and it is suggested that this mupirocin-resistant strain may have been involved in the development of nosocomial pneumonia in the infected patient. It seems likely, therefore, that mupirocin resistance did not affect the virulence of GISA-2, which is in agreement with the observation that the V588F ileS mutation is not associated with a significant loss of fitness.

Whilst the acquisition by S. aureus strains of mupA, which confers high-level mupirocin resistance, is of great clinical concern, the potential threat offered by low-level resistance to mupirocin acquired by mutation cannot be ignored. The expression of low-level mupirocin resistance that may aid survival in subinhibitory concentrations of the drug could provide a gateway for the subsequent development of high-level resistance through horizontal acquisition of mupA. Indeed, the mutation V588F has already been identified in an MRSA also expressing the mupA gene.
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References


