Increased Overall Antibiotic Susceptibility in Staphylococcus aureus femAB Null Mutants

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The staphylococcal pentaglycine side chain of the peptidoglycan is reduced to one glycine in femAB null mutants. This is associated with increased susceptibility to methicillin and to a whole range of unrelated antibiotics as well. Genetic evidence suggests that femAB null mutants are only viable because of a compensatory mutation in an unlinked site.

The cell wall in Staphylococcus aureus consists of about 40 layers of peptidoglycan strands that are highly cross-linked via the long and flexible pentaglycine side chain that is attached to peptidoglycan-stem peptide (10). This side chain is synthesized by sequential addition of glycines donated by cell-wall-specific glyeryl-tRNAs (12) and at least two factors, FemA and FemB, encoded by the femAB operon (11, 14). Strains lacking FemA (6) and femAB null mutants (14) form only monoglycine side chains and poorly cross-linked cell walls. Such strains are resistant to lysostaphin, a glyerylglycine endopeptidase, and hypersusceptible to β-lactams. The low-affinity penicillin-binding protein PBP2°, which confers methicillin resistance (Mc°) in S. aureus, cannot express Mc° in femAB mutants (8, 14). We show here that femA and femAB null mutants, besides being hypersusceptible to all β-lactams, became more susceptible to other classes of antibiotics as well. We postulate, moreover, that femAB null mutants have to undergo compensatory mutations to survive with such a drastically shortened side chain.

In the original femAB null mutant, AS145 (mec ΔfemAB::tetK), the femAB operon is replaced with a tetK cassette (14) (Fig. 1). Attempts to transduce the tetK marker with transducing phage 80a (3) from AS145 to other strains by selecting for Tc° were unsuccessful, except when 8 μg of lysostaphin ml⁻¹ was added as a second selective agent. We therefore introduced into AS145, close to tetK, another selective marker by transducing transposon Tn551, which confers, due to ermB, high-level constitutive erythromycin resistance (Em°). We used strains BB291 (mec Ω2000chr::Tn551 [3]) and BB413 (mec Ω8chr::Tn551 [4]) as donors for the transduction of the inserts Ω2000 and Ω8, respectively (Fig. 1). Both insertions were known to cause no apparent change in phenotype in complex medium and had no negative effects on methicillin resistance (Table 1). By testing the transductants on 5 μg of methicillin ml⁻¹ by replica plating, we showed that the femAB° wild-type allele was cotransduced with either Ω8 or with Ω2000 with a frequency of 96% when selecting for Em°. One of the few transductants that was still Mc° and that therefore had retained the femAB deletion, strain K14 (Ω8chr::Tn551 ΔfemAB::tetK), was utilized as a donor to transduce ΔfemAB::tetK together with Ω8 into different isogenic recipients. This allowed the use of erythromycin instead of tetracycline as the selective agent.

From the cotransduction frequency determined above, we expected a reciprocal cotransduction frequency of ΔfemAB::tetK with Ω8 close to 96%. Transductants with the femAB null mutation were expected to become lysostaphin resistant and β-lactam hypersusceptible. However, although Ω8 was transduced with a normal frequency of 10⁻² per PFU, none of the several hundred transductants tested had received ΔfemAB::tetK. This lack of cotransduction suggested that the femAB deletion by itself may be lethal to the cell unless some compensatory mutation occurs to allow survival. This compensatory mutation seemed somehow to be favored by the selection for lysostaphin resistance. Therefore, we suspected that our initial femAB null mutant AS145, and possibly also the femA null mutant UK17 [mec femA (ochre) (6)], the latter constructed by chemical mutagenesis with subsequent selection for lysostaphin resistance (9), may have acquired some additional hidden mutation(s) during their construction. To test this hypothesis, we restored in AS145 and UK17 the femAB wild-type allele by phage 80a-mediated cotransduction of Ω2000 with the donor strain BB291. We obtained the strains BB1305 (mec Ω2000chr::Tn551) and BB1308 (mec Ω2000chr::Tn551), respectively. Strains BB1305 and BB1308 regained lysostaphin susceptibility and Mc°; however, their growth rate remained almost as low as before (Table 1). This suggested that they still harbored some defects, although in Southern blots, they showed the expected restriction pattern around the femAB region (Fig. 2). To characterize them further, their susceptibility to other antibiotics was analyzed. The femAB null mutants AS145 and K14 as well as the femA mutant UK17 had a greatly increased susceptibility to all β-lactams and additionally showed increased susceptibility to other cell wall-directed antibiotics.
TABLE 1. Doubling time and MICs.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Doubling time (min)</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild Type</td>
<td></td>
<td>36</td>
<td>0.5</td>
</tr>
<tr>
<td>AS105</td>
<td>mec chr BB2000</td>
<td>36</td>
<td>0.5</td>
</tr>
<tr>
<td>BB1308</td>
<td>mec chr BB2000</td>
<td>49.5</td>
<td>0.125</td>
</tr>
<tr>
<td>BB270</td>
<td>mec chr BB2000</td>
<td>31.5</td>
<td>0.25</td>
</tr>
<tr>
<td>BB291</td>
<td>mec chr BB2000</td>
<td>24</td>
<td>0.004</td>
</tr>
<tr>
<td>BB815</td>
<td>mec chr BB2000</td>
<td>24</td>
<td>0.004</td>
</tr>
<tr>
<td>BB1305</td>
<td>mec chr BB2000</td>
<td>24</td>
<td>0.004</td>
</tr>
<tr>
<td>BB413</td>
<td>mec chr BB2000</td>
<td>48</td>
<td>0.03</td>
</tr>
<tr>
<td>BB255</td>
<td>mec chr BB2000</td>
<td>48</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Antimicrobials:**
- Teicoplanin
- Vancomycin
- Erythromycin
- Tetracycline
- Bacitracin
- Penicillin G
- Fosfomycin
- Methicillin
- Lysostaphin

**Mutations and Genotypes:**
- **femA** mutation and in the mutant with the insert O8::Tn551 in fragment 5, which has moved up by 5.2 kb to the top of the lane. Lane e, strain K14, which is essentially the same as AS145 with the insert O8::Tn551 in fragment 5, which has moved up by 5.2 kb to the top of the lane. Lane f, strain BB1305, which is a backcross of femAB into AS145 by cotransduction of the insert O2000::Tn551 in fragment 1, and in which the wild-type restriction pattern is restored, and fragment 1 is moved up by 5.2 kb. Lane g, strain UK17, with a point mutation in femA that does not appear on the gel. Lane h, strain 1308, femAB+ backcross with insert O2000::Tn551, the same as strain BB1305. Lane i, strain BB413, the donor of O8::Tn551. Lane j, strain BB291, the donor of O2000::Tn551.

**Antimicrobial Susceptibility:**
- Levels of resistance to unrelated antibiotics such as chloramphenicol, gentamicin, ciprofloxacin, trimethoprim, and sulfamethoxazole were reduced by a factor of at least 2 to 4 in the strains with the femAB null mutation and in the femA mutant (data not shown). Although this was but a small difference, the lowered susceptibility was reproducible in repeated measurements done by different methods, such as broth microdilution, E-tests, and gradient plates. The backcrosses BB1305 and BB1308 with the repaired femAB region regained susceptibility values corresponding to those of the parent BB270, except for the bacitracin MIC for strain BB1305, which remained low for yet unknown reasons, but which may be a consequence of the compensatory mutation. Also tetracycline susceptibility (Tc') was higher (the MICs of tetracycline were lower) in femA mutant UK17 than in wild-type BB270, and the tetK resistance marker in the femAB null mutant AS145 was able to increase Tc' only about sixfold compared to UK17. The final Tc' was, therefore, in AS145 and K14 only about three times higher than that in the wild-type strains, too small a difference to allow selection of transductants as in our previous experiments. The resistance mediated by TetK is due to a membrane-bound tetracycline efflux protein (7) and is gene dosage dependent, so that, besides the femAB deletion, other factors may be responsible for the relatively low resistance mediated by TetK in these particular mutants. Furthermore, a drastic reduction in the MIC of erythromycin was seen in the femAB null mutant K14, which harbored the ermB-encoded Em' marker. The MIC dropped from over 256 µg ml⁻¹ in strains BB815, BB413, and BB291 to 16 µg ml⁻¹ in strain BB1305.
in K14 (Table 1). Why ErmB, a ribosomal methylase \( (5) \), produces in a femAB null background such a low level of resistance is unclear. The Em' was again fully restored in the femAB" backcrosses BB1305 and BB1308.

The consequence of femAB inactivation seems to be a generally greater susceptibility to antibiotics, since backcrossing of the wild-type femAB allele into the mutants restored resistance. This increased susceptibility suggests that more than the formation of the pentaglycine side chain may be affected. A higher uncontrolled influx of drugs into the cells may occur, due to the impaired peptidoglycan precursor formation that may also affect the cell membrane organization. Two indications that compensatory mutation(s) may have occurred securing survival of the cells were (i) that the growth rate in the backcrosses remained low and (ii) that transduction of the femAB null mutation was impossible even when selecting for the Tn551 marker introduced nearby. This suggests that deletion of the femAB operon without concomitant compensatory rearrangements may be lethal. Potential inhibitory agents directed against FemA may therefore not only potentiate the effect of ß-lactams, but may ultimately be lethal to the cells. As expected from factors analogous to FemA and FemB found in coagulase-negative staphylococci (1), this concept could be valid for all staphylococci.

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REFERENCES


