

Current knowledge of the very low methicillin resistant MRSA spreading among injection drug users in Zürich, Switzerland

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Introduction

A very rapidly growing, extremely low-level methicillin resistant *Staphylococcus aureus* clone spreads among injection drug users in Zurich, Switzerland. This so called "drug clone" is indistinguishable from methicillin susceptible *S. aureus* (MSSA) by oxacillin minimum inhibitory concentration (MIC) tests, but can cause treatment failure due to the segregation of highly resistant subclones during β -lactam therapy. This strain contains a new type VI SCCmec (staphylococcal chromosome cassette mec), which encodes methicillin (*mecA*), fusidic acid (*fusB1*) and trimethoprim (*dfrA*) resistance genes. Its extremely low-level oxacillin resistance was partially traced back to a point mutation in the -10 promoter region of *mecA*, leading to reduced *mecA* transcription and reduced PBP2a content. However, the genetic background of the drug clone had a much higher impact on resistance, as low-level oxacillin resistance was maintained regardless of the amount of PBP2a produced.

A novel DNA-binding protein (SA1665) was found to bind to the *mecR1-mecA* promoter region and to increase methicillin resistance upon inactivation in this clone and in other unrelated MRSA. Trans complementation of SA1665 deletion mutants restored low-level resistance, ruling out polar effects and confirming its effect on oxacillin resistance. Since SA1665 affected neither *mecA* transcription nor PBP2a content, we postulate that this protein modulates oxacillin resistance levels indirectly, through the regulation of other genomic factors.

SCCmec_{N1}

The drug clone was found to carry a new SCCmec element of ~ 45.7 kb. It contained a class B mec complex (*mecA*- Δ *mecR1*::IS1272), a truncated Tn4003 harbouring the *dfrA* gene and a *fusB1* gene, conferring methicillin, trimethoprim and low level fusidic acid resistance, respectively. In addition to the two insertion site sequences (ISS) framing SCCmec, a third internal ISS (ISS*) was also present (FIG. 1). Uniquely, there were two *ccrAB* type-4 recombinase loci (*ccrAB4-1* and *ccrAB4-2*), responsible for the site- and orientation-specific chromosomal integration and excision of SCCmec, with the *CcrA* and *CcrB* amino acid sequences differing by 11.3% and 4.6%, respectively. Both recombinase loci were functional and able to excise SCCmec_{N1} or parts thereof, resulting in excision variants at the ISS loci or in regions of high nucleotide sequence similarity (FIG. 1).

Differences in the size of SCCmec_{N1} in diverse clinical drug clone isolates could be explained by small variations in the regions framed by primer pair 4/6, primer pair 7/9, or in presence or absence of the right-end 6.4-kb fragment.

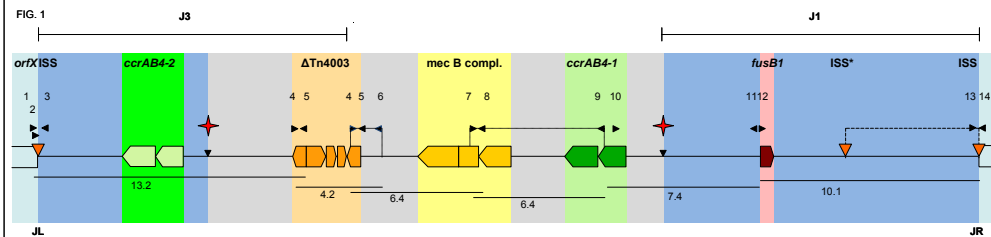


FIG. 1. Schematic organisation of the SCCmec_{N1} of strain CHE482, the drug clone type strain. Primers are indicated by black arrows. Regions coloured in blue represent the core chromosome. Red stars indicate the excision sites of the partially cured strain. Orange arrows indicate the ISS sites and the internal ISS*. The *ccrAB4-1* and *ccrAB4-2* complexes are highlighted in dark and light green, respectively. The truncated Tn4003 in orange comprises IS431-*orf140-dfrA-thyE*-IS431. The class B mec complex in yellow includes *mecA*- Δ *mecR1*-IS1272. The fusidic acid resistance gene *fusB1* is shown in pink. Dotted lines indicate regions of variability.

Total: 45.7 kb

Point mutation in the -10 *mecA* promoter region

A point mutation present in the -10 box of the *mecA* promoter in the drug clone (low MRSA) created a perfect palindrome compared to the wildtype MRSA consensus sequence (FIG. 2). Plasmids containing the *mecA* genes and promoters from the drug clone (pLow) and the wildtype promoter of a highly resistant MRSA (pHigh) were constructed. Plasmids were then introduced into variants of the drug clone (Δ low MSSA) and the high MRSA (Δ high MSSA), which had been cured of SCCmec. In both strains pLow conferred a slightly lower cefoxitin resistance than pHigh, however, the genetic background of the strains had a much larger impact on final resistance levels than the promoter sequences (FIG. 3).

pHigh produced more *mecA* and PBP2a than pLow in both backgrounds (FIG. 4). The presence of a penicillinase plasmid in Δ low MSSA conferred inducible *mecA* expression, while expression in Δ high MSSA was constitutive. The penicillinase plasmid had no influence on the final resistance levels of the strains (data not shown).

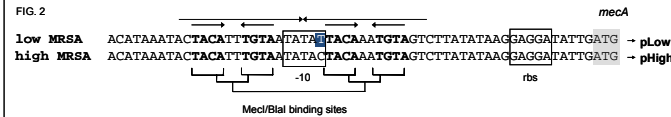


FIG. 2. Partial *mecA* promoter sequence of a low and high resistant MRSA. The mutation is highlighted in blue. Binding sites of MecI/BlaI are in bold, direct and indirect inverted repeats are indicated by arrows. The -10 promoter sequence and ribosomal binding site are framed. The start codon of *mecA* is highlighted in grey. Cloning of the whole *mecA* promoter and its associated *mecA* gene results in pLow and pHigh.

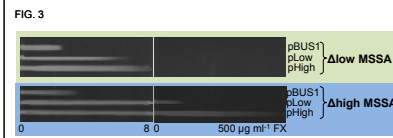


FIG. 3. Cefoxitin gradient plates of the cured strains Δ low MSSA and Δ high MSSA containing the empty plasmid pBUS1, pLow or pHigh.

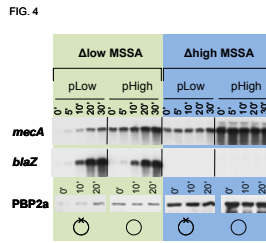


FIG. 4. Northern and Western blot analysis. Cultures were induced at OD600nm 1.0 with 4 μ g/ml cefoxitin. RNA and protein were extracted at time points indicated.

Resistance levels are only slightly affected by the point mutation, leading to the conclusion that resistance levels are governed by strain-specific genomic factors and not by *mecA* expression levels or amounts of PBP2a.

Identification of SA1665, a new factor modulating methicillin resistance levels

A DNA-protein binding assay using the *mecA* promoter region as bait, identified SA1665, a putative DNA binding protein with a helix-turn-helix motif (FIG. 5 and 6). Non-polar deletion of SA1665 increased oxacillin resistance in different MRSA and wild type resistance was subsequently restored by trans complementation (FIG. 7). Northern blots showed that SA1665 transcription was repressed by oxacillin. Deletion of SA1665, however, had no apparent effect on *mecA* transcription (FIG. 8) or PBP2a content (data not shown).

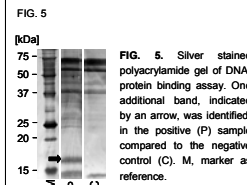


FIG. 5. Silver stained polyacrylamide gel of DNA-protein binding assay. One additional band, indicated by an arrow, was identified, in the positive (P) sample compared to the negative control (C). M, marker as reference.

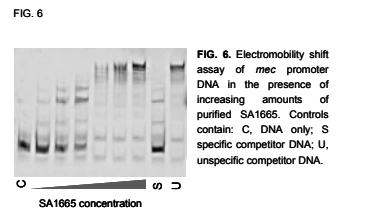


FIG. 6. Electromobility shift assay of *mec* promoter DNA in the presence of increasing amounts of purified SA1665. Controls contain: C, DNA only; S, specific competitor DNA; U, unspecific competitor DNA.

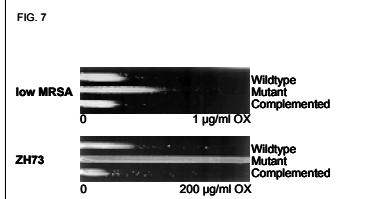


FIG. 7. Oxacillin gradient plates of the wildtypes (drug clone, ZH73), their SA1665 deletion mutants and complemented strains.

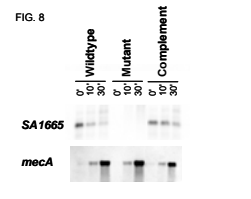


FIG. 8. Northern blot analysis of strains induced at OD600nm 1.0 with 4 μ g/ml cefoxitin.

Although SA1665 was shown to bind to the *mecA* promoter region, neither *mecA* transcription nor PBP2a production were affected in the SA1665 deletion mutants. Therefore β -lactam resistance levels must be modulated in a *mecA* independent manner.