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Impact of *mecA* promoter mutations on *mecA* expression and β-lactam resistance levels

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Abstract

The reason for the extremely low-level oxacillin resistance in a so-called 'drug clone', a methicillin-resistant *Staphylococcus aureus* circulating among injection drug users in Zurich, Switzerland, could be traced back to the *mecA* promoter sequence and particularly to the strain's genetic background. Sequencing of its mec complex identified a point mutation (TATA**C**T to TATA**T**T), creating a perfect palindrome in the −10 region of the *mecA* promoter/operator region containing the binding sites for the *mecA* repressors MecI and BlaI. Two strains with vastly different β-lactam resistance phenotypes, the low-level resistant drug clone type strain CHE482 and the highly homogeneously resistant strain COLn, were cured of their SCC*mec* elements and subsequently transformed with plasmids containing *mecA* under the control of either the wild-type or mutant promoter. Expression studies showed that this mutation had significant effects on both *mecA* transcription and corresponding PBP2a production, but only small effects on β-lactam resistance levels within a given genetic background. A further mutation in the *mecA* ribosomal binding site (GGAG**G** to GGAG**T**), common to SCC*mec* type IV strains, was found to have no discernable effect on *mecA* transcription and PBP2a content, and only minimal effects on β-lactam resistance. Factors associated with the genetic backgrounds into which these differently controlled *mecA* genes were introduced had a much higher impact on β-lactam resistance levels than the rates of *mecA* transcription. The tight repression of *mecA* expression in this drug clone in the absence of β-lactams could contribute to the apparent fitness of this fast growing strain.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA); *mecA*; Promoter mutation; β-Lactam resistance levels

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) can express highly variable levels of β-lactam resistance. Oxacillin minimal inhibitory concentrations (MIC) can vary from as low as $1\,\mu g\,ml^{-1}$ up to values over $1000\,\mu g\,ml^{-1}$. Extremely low-level resistant MRSA strains are dangerous as they often evade phenotypic detection. While they appear phenotypically susceptible, these strains still carry the *mecA* gene which encodes the β-lactam resistance protein penicillin binding protein (PBP) 2a, and express resistance heterogeneously. This means that upon β-lactam exposure they can segregate highly resistant subpopulations, at frequencies well above spontaneous mutation rates, and cause β-lactam treatment failure.

β-Lactam resistance relies on the presence of *mecA* and the expression of PBP2a; however, several other factors are known to influence resistance. Chromosomal

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mutations or specific genomic factors are required for expression of high-level resistance (Murakami and Tomasz, 1989; Ryffel et al., 1994). Resistance levels are also influenced by environmental conditions such as temperature, pH, osmolarity, divalent cations, and aerobiosis (Matthews and Steward, 1984). The actual genetic basis governing low-level, heterogeneous, or high homogeneous resistance is unknown.

Expression of *mecA* is inducible and can be controlled by either its cognate regulators MecI (DNA binding repressor protein) and MecR1 (sensor/signal transducer) or by the structurally and functionally similar β-lactamase regulators BlaI and BlaR1, respectively. Because of structural and functional similarity, both MecI and BlaI can bind as homodimers to the promoter/operator region of both *mecA* and *blaZ* (Gregory et al., 1997; Sharma et al., 1998). Repression of transcription by these two inhibitors is even stronger when both repressors are present (Rosato et al., 2003a). In the presence of β-lactams the MecR1/BlaR1 sensor-transducers promote the cleavage of their respective repressors allowing transcription of *mecA*. In the absence of both regulatory loci, *mecA* is constitutively expressed.

High conservation of the *mecA* gene sequence (Chambers, 1997), the promoter region, and the preferred presence of regulatory genes (*blaI*/*blaR1* or *mecI*/*mecR1*) (Rosato et al., 2003a) highlights the need for controlled expression of *mecA*. In strains such as N315, a so-called pre-MRSA (Niemeyer et al., 1996; Weller, 1999), carrying both complete regulatory systems, *mecA* is repressed by both MecI and BlaI. This tight repression can prevent *mecA* transcription and lead to inhibition of β-lactam resistance and to the misinterpretation of these strains as methicillin-sensitive *S. aureus* (MSSA).

The amount of PBP2a produced has been shown to have no direct correlation with resistance level; however, mutations in *mecA*, *mecI*, the *mecI* ribosomal binding site, or *mecI* deletion can lead to increased resistance (Katayama et al., 2004; Rosato et al., 2003a; Niemeyer et al., 1996), whereas interruption of *blaR1* results in constitutive repression and therefore decreased resistance (Hackbarth et al., 1994).

An MRSA strain with very low-level oxacillin resistance with the ability to generate highly resistant subclones is spreading among injection drug users in Zurich, Switzerland (Qi et al., 2005). Isolates of this clone having oxacillin MICs of 0.5–4 μg ml^{-1} are susceptible or just at the breakpoint of resistance for oxacillin, according to CLSI guidelines. They fall within the classification of the recently described OS-MRSA (oxacillin-susceptible, methicillin-resistant *S. aureus*), defined as being oxacillin-sensitive but *mecA*-positive strains with MICs of 2 μg ml^{-1} or below (Hososaka et al., 2007). A recent analysis of the SCC*mec* element of the drug clone type strain CHE482 identified a composite element carrying a mecB complex (IS*1272*–Δ-*mecR1*–*mecA*) (Ender et al., 2007). Further investigation of the mec complex revealed that the *mecA* promoter differed from the consensus by a 1-bp substitution in the −10 region. Here we determined the influence of this nucleotide substitution on *mecA* transcription and β-lactam resistance levels in different genetic backgrounds, using MRSA cured from their SCC*mec* element as recipients.

Materials and methods

Bacterial strains and growth conditions

All strains and plasmids used in this study are listed in Table 1. Strains were grown in Luria Bertani broth (LB, Difco) at 37 °C, with shaking at 180 rpm. Media were supplemented with 10 μg ml^{-1} tetracycline for strains harbouring the plasmids pME1, pME2, or pME3, and with 0.8 μg ml^{-1} ampicillin for those containing the penicillinase plasmids pBla or pI524.

Curing of SCC*mec*

Strains CHE482 and COLn were cured of SCC*mec* using the method described by Katayama et al. (2000), resulting in strains ME21 and ME131, respectively.

Cloning and transformation

The *mecA* gene, together with its upstream promoter region, was amplified from strains CHE482, COLn, and ZH47 using the primer pair (5′-ATTAGGATCCC-CAAATCTTATGTGACATAA-3′/5′-ATTAGGATC-CATCCTCAATATATGCATATAG-3′) and subsequently cloned into the BamHI site of the *E. coli*–*S. aureus* shuttle vector pBUS1. The resulting plasmids pME1, pME2, and pME3 as well as pBla, the native penicillinase plamid of CHE482, and pI524, a penicillinase plasmid described in Murphy and Novick (1980), were electroporated directly into ME21 and ME131. Direct electroporation was done as described by Katayama et al. (2003), whereby cells were mixed with 500 ng of plasmid DNA, incubated for 10 min on ice and electroporated using following settings: 25 μF, 2.0 kV, and 100 Ω.

Sequence analysis

The plasmids pME1, pME2, and pME3 were sequenced with an ABI Prism 310 genetic analyser (Applied Biosystems) using the ABI PRISM BigDye Terminator Cycle sequencing reaction kit (US Biochemicals). Thermal