Excision of staphylococcal cassette chromosome mec in methicillin-resistant Staphylococcus aureus assessed by quantitative PCR

Miloš Stojanov1,2*, P. Moreillon1 and O. Sakwinska1,3

Abstract

Background: Methicillin-resistance in staphylococci is conferred by the mecA gene, located on the genomic island Staphylococcal Cassette Chromosome mec (SCCmec). SCCmec mobility relies on the Ccr recombinases, which catalyze insertion and excision form the host's chromosome. Although being a crucial step in its horizontal transfer, little is known about the dynamics of SCCmec excision.

Results: A quantitative PCR-based method was used to measure the rate of SCCmec excision by amplifying the chromosome–chromosome junction and the circularized SCCmec resulting from excision. SCCmec excision rate was measured in methicillin-resistant Staphylococcus aureus (MRSA) strain N315 at various growth times in broth cultures. In the present experimental settings, excision of SCCmec occurred at a rate of approximately $2 \times 10^{-6}$ in MRSA N315.

Conclusion: This work brings new insights in the poorly understood SCCmec excision process. The results presented herein suggest a model in which excision occurs during a limited period of time at the early stages of growth.

Keywords: SCCmec, Excision, MRSA, Staphylococcus aureus

Background

Staphylococcus aureus is a successful human pathogen, causing a large variety of diseases that range from minor skin infections to life-threatening bloodstream infections and endocarditis [1]. Many of the virulence factors encoded by S. aureus are located within mobile genetic elements, which come in addition to the well-conserved core genome, and account for the majority of genetic variation between the isolates [2–4]. This is also often the case for antibiotic resistance genes, which can be found on plasmids, transposons or genomic islands [4].

Methicillin-resistant S. aureus (MRSA) arose during the 1960s, due to the acquisition of the Staphylococcal Cassette Chromosome mec (SCCmec) [5]. SCCmec is a staphylococcal genomic island carrying the mecA gene, which encodes the low affinity penicillin-binding protein 2a (PBP2a) that is responsible for cross resistance to virtually all antibiotics of the β-lactam family [5, 6]. It is inserted at a unique location in the chromosome by the Ccr proteins (cassette chromosome recombinases), which catalyze site-specific recombination between a $3'$ end of the rlmH gene (attB), previously known as orfX, and the homologous sequence on SCCmec (attS). Upon integration the attS and the attB sequences form two direct repeats (designated attL and attR) flanking the cassette [7, 8]. In the opposite path, Ccr proteins catalyze the excision of SCCmec from the host's chromosome [8, 9].

Early clues of SCCmec excision rate were reported by Ito et al., who detected excision of the cassette in MRSA N315 at a rate of less than $10^{-4}$ [10]. Nevertheless, precise excision rate and dynamics of SCCmec excision have never been determined. To address this issue, we used a qPCR-based system allowing us to quantify precisely the rate of SCCmec net excision from the chromosome.
of MRSA strain N315, as well as to detect the resulting excised closed circular forms appearing in the cell.

**Methods**

**Bacterial strains, media and culture conditions**

Bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strain DH5α was routinely used for plasmid propagation and cloning experiments, and cultivated on Luria-Bertani (LB) medium (Becton–Dickinson, Sparks, MD, USA) supplemented with 100 mg/L ampicillin (AppliChem, Darmstadt, Germany) at 37 °C. *S. aureus* strains were grown with aeration in trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI, USA) in a rotating incubator (at 180 rpm) at 37 °C. If required, tetracycline and kanamycin (AppliChem) were added at a final concentration of 10 mg/L. Oxacillin (Sigma-Aldrich, Buchs, Switzerland) was used at 4 mg/L, which corresponded to the MICs for strain N315.

**DNA manipulations**

Genomic DNA of *S. aureus* was extracted using a protocol described previously [14]. For genomic DNA, 3 mL of overnight cultures were harvested and resuspended in 50 μL of lysis solution (0.5 μg/mL lysostaphin, 10 mM Tris–Cl, pH 7.5, and 1 mM EDTA). After 30 min of incubation at 37 °C, cell suspension were lysed by adding 300 μL of “Nuclei Lysis Solution” (Promega Corporation, Madison, WI) and heating at 80 °C for 10 min. After RNase treatment, 100 μL of “Protein Precipitation Solution” (Promega Corporation) were added to the samples, followed by incubation on ice for 5 min. Samples were then centrifuged (15,600 × g) at 4 °C for 10 min. Supernatants were recovered and DNA was precipitated using 300 μL of isopropanol, followed by centrifugation (15,600 × g) at 4 °C for 10 min. DNA precipitate was washed with 70 % ethanol and pelleted by centrifugation (15,600 × g) at 4 °C for 10 min. DNA samples were then air-dried and dissolved at 4 °C overnight in 20 μL of nuclease-free H2O. DNA concentrations and qualities were determined using an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Plasmids from *E. coli* were isolated using the QIAprep Spin Miniprep Kit (QIAGEN Inc., Hilden, Germany). The same protocol was used for *S. aureus*, after treatment with lysis solution (0.5 μg/mL lysostaphin, 10 mM Tris–Cl, pH 7.5, and 1 mM EDTA).

Digestions with restriction enzymes (Promega AG, Dübendorf, Switzerland) were carried out according to the manufacturer’s specifications. PCR fragments were purified using the QIAquick PCR Purification Kit (Qiagen Inc.) and gel-bands were purified using QIAquick Gel Extraction Kit (Qiagen Inc.) according to manufacturer’s protocols. Ligations were performed using 1 μL of T4 ligase (Promega AG) according to the manufacturer’s specifications.

**PCR and quantitative PCR (qPCR)**

All primers used in this study are listed in Table 2. GoTaq DNA polymerase (Promega AG) was used for colony PCR screening during cloning experiments and end-point PCR. DNA fragments required for cloning were amplified with KAPA HiFi DNA Polymerase (KAPA Bio-systems, Cape Town, South Africa). All reactions were carried out according to manufacturers’ specifications.

**Artificial excision of SCCmec to obtain N315EX**

SCCmec was cured from *S. aureus* N315 to obtain N315EX using a method from Katayama et al. [7]. Briefly, strain N315 was electroporated with thermosensitive plasmid pSR3-1, containing the ccrAB genes and a tetracycline-resistance marker. Transformants were grown for 24 h at 30 °C in TSB supplemented with tetracycline, serially diluted and plated on TSA supplemented with tetracycline. Single colonies were picked, grown for 24 h at 42 °C TSB to promote cure of thermosensitive pSR3-1, and dilutions were plated on TSA to screen for colonies susceptible to both oxacillin and tetracycline. One

### Table 1 Bacterial strains and plasmids

| Strain or plasmid | Relevant characteristics | References |
|-------------------|--------------------------|------------|
| **Strains**       |                          |            |
| E. coli DH5α      | Host for DNA cloning     | Laboratory collection |
| *S. aureus*       |                          |            |
| RN4220            | Restriction-deficient derivative of *S. aureus* RN450 | [11] |
| N315              | MRSA carrying type II SCCmec | [12] |
| N315EX            | Isogenic MSSA derivative of N315 | This study |
| **Plasmids**      |                          |            |
| pUC28             | CoE1 replicon, high copy number vector for cloning, ampicillin resistance | [13] |
| pSR3-1            | Thermosensitive-replicon plasmid carrying the ccrAB genes of strain N315 (used for SCCmec excision in N315), tetracycline resistance | [7] |
| qPlasmid          | Plasmid used for qPCR analysis | This study |
double-susceptible colony, which lost both the plasmid (i.e. tetracycline-resistance) and kanamycin-resistance (encoded by SCC\textit{mec}), was recovered and absence of SCC\textit{mec} was confirmed by PCR amplification of qEx fragment using primers pair Excision fw and rev (Table 2).

### Co-culturing of N315 and N315EX

Co-culturing assays were performed in triplicates. 10\(^3\) CFU of overnight cultures of parent MRSA N315 and its excisant N315EX were washed with phosphate buffered saline, mixed at 10\(^2\) CFU/mL each in 10 mL of TSB, and incubated at 37 °C. The CFU counts of the two strains were determined by plating dilutions of the cultures at 0, 7, 24, 48 and 72 h. Strain N315 was selected for kanamycin-resistance (encoded by SCC\textit{mec} and more reliable than resistance to oxacillin) on TSA plates supplemented with kanamycin, and total number of cells was determined by plating the competition mixture on TSA plates. Quantification of strain N315EX was calculated by subtracting the number of CFU on kanamycin plates from the number of CFU on plates without antibiotic.

### Designing a qPCR-based system to quantify site-specific excision of SCC\textit{mec} and formation of the circular forms

Specific primers were designed using Primer3 software (version 2.3.4, [15]) and used to amplify the three targets depicted in Fig. 1. qControl amplicon, targets a region of \textit{rlmH} gene, which is present in cells with and without SCC\textit{mec}. This control was used to determine the number of total chromosomes in the samples. Amplicons qEx and qCirc were used to specifically detect excisants and circularized SCC\textit{mec}, respectively. These were compared to the total number of chromosomes, which was determined by targeting a region of the \textit{rlmH} gene, present in both MRSA- and MSSA-like cells.

In order to standardize the qPCR assay, qPlasmid, a plasmid carrying the three targets of the qPCR reactions (qCirc, qControl, and qEx) was constructed (Fig. 1). A 555-bp fragment containing the chromosomal junction formed upon excision, amplified from chromosomal DNA of \textit{S. aureus} N315EX, was cloned in pUC28 using restriction sites Sph\textit{I} and Xba\textit{I}. A second fragment of 411 bp, containing the \textit{attS} site from the SCC\textit{mec} circular form, was amplified from chromosomal DNA of \textit{S. aureus} N315 and cloned using Eco\textit{RI} and Sph\textit{I} restriction sites.

Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo Fisher Scientific AG, Switzerland) was used to determine concentration of qPlasmid, which was subsequently used to optimize the reactions efficiencies of qControl, qEx and qCirc amplicons. Different amounts of qPlasmid (40, 4 \times 10^2, 4 \times 10^4, 4 \times 10^6 and 4 \times 10^8 copies) were used as template for different primer concentration combinations in order to find the optimal conditions (Additional file 1: Supporting data 1).

qPCR reactions were performed in 20 μL volume reactions using the KAPA SYBR FAST qPCR Kit (Kapa

### Table 2 Primers used in the study

| Primer          | Sequence                                | Position\(^{a}\) | Annealing temperature (°C) |
|-----------------|-----------------------------------------|------------------|---------------------------|
| Screening primers |                                          |                  |                           |
| Excision fw     | GTTAGGCCCATAACACAAAGATAGA               | 33768–33792      | 54.7                      |
| Excision rev    | TGCCACATTTAACCCTCCTCC                   | 87194–87173      | 55.1                      |
| Cloning primers |                                          |                  |                           |
| orfXcloning fw  | TTTTTTTGATGCCAATCCATTAGCTGTAGGG         | 33696–33718      | 64.2                      |
|                 | (Sph\textit{I})                         |                  |                           |
| orfXcloning rev | TTTTTTTTCAGATTAATGAACTGTTAGGG           | 87215–87193      | 57.2                      |
|                 | (Xba\textit{I})                         |                  |                           |
| circCloning fw  | TTTTTTTGAAATCTGTGGAAGGTTTGAAGACTACGG   | 86883–86905      | 59.8                      |
|                 | (Eco\textit{RI})                        |                  |                           |
| circCloning rev | TTTTTTTGATCGAAAGACTGCGGGGCTAATAT       | 34316–34294      | 66.6                      |
|                 | (Sph\textit{I})                         |                  |                           |
| qPCR primers    |                                          |                  |                           |
| qControl fw     | CGTTTAGGCCCATAACACAAAGATAGAC            | 33767–33793      | 59.2                      |
| qControl rev    | TGATACATCTAAAATCTTTATGGAAGGC            | 34215–34187      | 60.1                      |
| qEx fw          | CGCAGTAACTAGCGCCTCTATCGAC              | 34049–34075      | 59.4                      |
| qEx rev         | TGAATGAACTGAACTTGAATGCTGAC             | 87213–87187      | 60.8                      |
| qCirc fw        | GGTATATTAATTAGGAGGTGGGAGCTTGA          | 87037–87067      | 60.6                      |
| qCirc rev       | CTTCTATAAAAACATAACAGGAAATACATACAAAA    | 34259–34226      | 60.1                      |

\(^{a}\) Position in the chromosome of \textit{S. aureus} N315 (GenBank:BA000018)
Biosystems). DNA samples extracted from three independent cultures inoculated with 1 mL of overnight culture (approximately 10^9 CFU) in 100 mL of TSB were used as template for the assays. When required, oxacillin was added at the concentration of 4 mg/L before adding the inocula. A total of 5 ng of DNA was used for each analysis. Reactions were carried out in MicroAmp optical tubes (Applied Biosystems, Foster City CA, USA) using an ABI7000 machine (Applied Biosystems). The following run protocol was used: initial denaturation step at 95 °C for 2 min then 40 cycles of 95 °C for 10 s and 60 °C for 40 s. Specificity of the qPCR amplification was confirmed by the sequencing of obtained DNA fragments (data not shown).

Standard curves prepared with different amounts of qPlasmid (40, 4 × 10^2, 4 × 10^4, 4 × 10^6 and 4 × 10^8 copies) were used to determine the number of copies in the samples using a linear regression model. All analyses were performed in triplicates.

**Fig. 1** Schematic representation of SCCmec excision and formation of its circular form. The figure shows the linear SCCmec integrated in the chromosome (a), the circularized SCCmec excised from the chromosome (b) and the religated chromosome after excision (c). Chromosomal DNA is shown in yellow and SCCmec DNA is shown in dark blue. Locations of primers used for qPCR are shown by arrows and their amplicons are highlighted in light blue. qControl amplicon is used to determine the number of chromosomes in the assay, while qEx and qCirc specifically detect excisants and circularized SCCmec, respectively.

Determination of total chromosome copies (qControl amplicons), excised SCCmec (qEx amplicons) and circularized SCCmec (qCirc amplicons)

Genomic DNA samples were quantified using NanoDrop ND-1000 (Thermo Fisher Scientific AG) and diluted in nuclease-free H₂O at a concentration of 10 ng/μL. A total of 10 ng was used for the qPCR assay. Quantification of qControl, qEx and qCirc amplicons was done by extrapolating the results from the standard curve obtained in the standardization experiments (see above), using the following formula:

\[
\text{Sample copy number} = 10^{([C_t - b]/a)}
\]

where \(C_t\) is the threshold cycle; \(a\) and \(b\) are the slope and the y-intercept of the standard curve, respectively. The dynamic range of the assay ranged from 4 × 10^8 to 40 amplicon copies. Proportions of excisants and circular form of SCCmec were calculated by dividing the absolute amount of the qEx and qCirc amplicons by the absolute amount of qControl amplicons. Negative controls consisting in nuclease-free H₂O were included in the analysis.

**Statistical analysis**

Unpaired t test was used to assess statistical differences between excision frequencies of cultures with different inocula (1/100 and 1/10,000). A \(p\) value of <0.05 was considered to indicate statistical significance.


**Results**

**Rate of SCCmec excision in standard experimental conditions**

Quantitative PCR was used to measure the amounts of both unoccupied chromosomal sites and SCCmec circular forms by specific amplification of the reconstituted chromosomal insertion site obtained after SCCmec excision and the junction formed upon SCCmec circularization, respectively (Fig. 1). These were compared to the total number of chromosomes, which was determined by targeting a region of the rlmH gene, present in both MRSA- and MSSA-like cells (Fig. 1).

Prior to the quantification of net excision rates, it was critical to ensure that the parent MRSA N315 and its excised mutant N315EX grew at the same rate in our experimental conditions. Any growth advantage of one of the strains would bias the results, since that strain would become overrepresented in the culture over time. Thus, a co-culturing assay in which 10^2 CFU/mL of N315 and N315EX were inoculated together in 10 mL TSB was performed. Both strains grew at the same rate and did not interfere with each other for up to 72 h (Additional file 1: Supporting data 2).

The ratio of excised forms versus total chromosomes was first measured during growth at 37 °C in TSB. Figure 2 indicates that this ratio was approximately 2 × 10^−6 already at first time point of the culture (i.e. 3 h), and remained constant in spite of further bacterial multiplication. However, our results also show that an overnight culture of N315 contains ca. 10^3/mL excisants. In fact, such culture contains ca. 2 × 10^9/mL cells and the frequency of excisants is 2 × 10^−6/mL (Fig. 2). Knowing this, we noticed that when we were using a 1/100 dilution of an overnight culture as inoculum, 10 excisants/mL (i.e. MSSA cells) were readily introduced at beginning of the experiment. Therefore, our observations might result only from multiplication of the pre-introduced excisants.

To answer this question, we performed the same experiment, except that we used a 1/10,000 dilution of an overnight culture as starting inocula. This ensured a theoretical carryover of less than one excisant (0.1 excisants/mL), in opposition to 10 excisants/mL inoculated with the 1/100 dilution. Figure 3 (grey columns) shows that a constant ratio of approximately 8 × 10^−7 excisants was again present throughout the experiment, which could not result from carryover, indicating that excision occurred de novo. The significantly higher frequency of excisants observed for the 1/100 dilution inocula could be explained by the presence of the carryover from overnight cultures in addition to newly excised cells (Fig. 3).

To confirm our results, we tested the contribution of de novo excision to the total number or excisants in cultures undergoing successive passages. We reasoned that if de novo excision occurred in addition to carry over in new cultures, then repeating successive cultures should demonstrate a progressive accumulation of excisants, resulting from the sum of carry over plus de novo excisions. In contrast, if carry-over was the only source of excisants for new cultures, the frequency of 2 × 10^−6 excisants should remain stable over time. A 1/100 dilution (i.e. 0.1 mL in 10 mL) from an overnight culture containing 10^9 CFU/mL was inoculated into fresh broth, which was left to grow to the stationary phase (Fig. 4a). The new culture was grown to the stationary phase and used to reinoculate the next fresh culture, and the process was repeated for 9 consecutive passages. Figure 4b shows that the ratio of excised forms to total chromosomes increased approximately 10 times over the whole experiment. Thus, each of the 9 passages had contributed for an increase of a little more than 10^−6 de novo excisants, which summed up to a tenfold increase in excisants (i.e. approximately 10^−5 instead 10^−6) at the end of the experiment. This experiment was also performed using 1/10,000 dilutions as inocula for consecutive cultures (Fig. 4a). The increase in excisant frequency was less pronounced and results were not significant as in the case of the 1/100 dilution.

**Detection of the circular form of SCCmec and effect of oxacillin treatment**

In addition to the occupancy of the SCCmec insertion site, our study aimed at detecting the SCCmec circular form created upon excision. Extrachromosomal SCCmec cannot replicate autonomously and must be constantly generated de novo in order to be detectable. Using our experimental settings, quantification of the circular form remained below the limit of detection during all
time-points. On the other hand, using endpoint PCR (40 cycles) with an excess of DNA template (approximately 0.5 µg), the specific junction formed upon SCC\textit{mec} circularization could be detected at 3 h. Similar intensities of the bands suggested that approximately the same amount of PCR product was present at 24 h and 72 h, corroborating our hypothesis excision of SCC\textit{mec} might take place only during the early stages of bacterial growth.

This hypothesis was further supported by the results obtained with oxacillin treatment. We reasoned that if excision occurred only transiently at the beginning of incubation and did not occur later in growth, then the oxacillin-susceptible excisants should be inhibited and become diluted off along growth. In contrast, if excisants would continuously arise at a sizeable rate (e.g. $2 \times 10^{-6}$) from SCC\textit{mec}-positive cells growing in oxacillin, then molecular signatures of de novo excised chromosomes should be detectable even if the resulting methicillin-susceptible excisants could not grow. Figure 3 shows that excisants rapidly disappeared after addition of oxacillin and that no detectable excision occurred thereafter. This supports the hypothesis that excision occurred transiently at the beginning of growth.

**Discussion**

In the present study, we developed a qPCR-based method to monitor the rate of net excision of SCC\textit{mec} in MRSA, taking strain N315 as model system. We found that in standard conditions, i.e. growth at 37 °C in TSB, the ratio of excised versus total chromosomes in N315 was approximately $2 \times 10^{-6}$. This observation suggests that, in our experimental conditions, a culture of N315 is composed of a mixture of a majority of cells carrying SCC\textit{mec} and of rare cells without it. This proportion was
already present at the first checkpoint during growth, i.e. 3 h after inoculation, and remained constant until 72 h in the late stationary growth phase. The consecutive passage experiments further supported the finding that in the present experimental conditions, net excision occurred de novo at the rate of approximately $2 \times 10^{-6}$. By diluting the inocula to 1/10,000, we excluded the possibility that detected excisants were due to carry-over from preceding cultures. We did observe that the excisants accumulated more slowly in the experiment with high 1/10,000 dilution, confirming that the frequency of excisants is the outcome of de novo excision and carry-over.

Net production of excisants in the presence of oxacillin was detected only during the early stages of growth, since the antibiotic was inhibiting the replication of bacteria without SCCmec, as expected. Several studies have assessed the expression of ccrAB genes in the presence of β-lactams [14, 16]. However, it is difficult to compare these results with our observations. First, our experiment measure net excision, and it is a net product of molecular process of excision and reinsertion, both depending on the activity of CcrAB recombinases. Moreover, the knowledge of post-transcriptional and post-translational regulation of CcrAB recombinases is quite limited. Nevertheless, differential expression of ccrAB genes between different MRSA strains could suggest that SCCmec excision might vary between different cassette types and MRSA backgrounds. Moreover, nucleotide variations in ccrAB genes from different SCCmec have been shown to influence the recombination activity and might influence the rates of excision as well [17]. Variation in the excision of SCCmec might be also observed in different ecological niches, as in the case of hospital-acquired MRSA and community-acquired MRSA, especially due to different antibiotic pressures. In the case of community-acquired MRSA, the smaller size of SCCmec might represent a factor facilitating the excision.

With our approach we measured net excision rates, and we could not directly determine how much actual excision and reinsertion occurred. However, the inability to detect the extrachromosomal circular form of SCCmec at all time-points suggested that excision might take place punctually during the early stages of growth. The consecutive passage experiments indicated that SCCmec is lost from cells during several cycles of replication, a situation that could theoretically lead to the fade of a given MRSA population. However, our experiment suggested several reasons why this may never happen. The most obvious is the selection pressure for maintenance of SCCmec and against excisants by antibiotics. Moreover, our experiments suggested that the excision happens mostly during the early growth of bacterial cells. The excision rate is likely much lower among MRSA colonizing human hosts, where the growth rates are certainly much slower. Additionally, because of their low frequency, the excisants might simply be extremely diluted or even lost during consecutive passages, e.g. from one human host to another, as suggested by the experiment with high 1/10,000 dilution, where the excisants accumulated more slowly than 1/100 dilution. These preliminary observations give important clues about the mobilization of SCCmec, a poorly understood process.

**Conclusions**

Taken together, the present results indicate that excision of SCCmec occurred at a rate of approximately $2 \times 10^{-6}$ in MRSA N315. This observation is concordant with a previous study in which the excision rate was evaluated to be lower than $10^{-4}$ [10]. The dynamics of the proportion of excisants during growth suggests that excision occurred rather early on, at a given cell density, and
during a limited period of time. The technique described here for MRSA N315 is amenable to study excision of other types of SCCmec cassettes in other MRSA backgrounds, in order to better understand this crucial step of the horizontal transfer of this mobile element.

**Availability of supporting data**
The data sets supporting the results of this article are included within the article (and its additional files).

**Additional file**

*Additional file 1.* Supplementary data 1 – qPCR reaction efficiencies.

**Abbreviations**

SCCmec: staphylococcal cassette chromosome mec; MRSA: methicillin-resistant *Staphylococcus aureus*; PBP2a: penicillin-binding protein 2a; Ccr: cassette chromosome recombinases; LB: Luria–Bertani broth; TSB: trypticase soy broth; CFU: colony forming units; qPCR: quantitative PCR; SD: standard deviation.

**Authors’ contributions**

MS performed the experiments. MS, PM and OS conceived the study, participated in its design and coordination, and drafted the manuscript. All authors read and approved the final manuscript.

**Author details**

1. Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland. 2 Present Address: Institute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland. 3 Present Address: Nestlé Research Center, Lausanne, Switzerland.

**Acknowledgements**

This work was supported by grant 3200B0-113854 to PM and by Marie Heim Vogtlin Grant PMPDA-106195 to OS, both from the Swiss National Science Foundation. We thank T. Ito for providing plasmid pSR3-1.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 14 April 2015  **Accepted:** 17 December 2015  **Published online:** 29 December 2015

**References**

1. Que Y-A, Moreillon P. *Staphylococcus aureus* (including staphylococcal toxic shock). In: Mandell GL, Dolin R, editors. Mandell, Douglas, and Bennett’s principles and practices of infectious diseases. Philadelphia, PA: Churchill Livingstone Elsevier; 2009. p. 2543–78.

2. Feng Y, Chen CJ, Su LH, Hu S, Yu J, Chiu CH. Evolution and pathogenesis of *Staphylococcus aureus*: lessons learned from genotyping and comparative genomics. FEMS Microbiol Rev. 2008;32(1):23–37.

3. Lindsay JA. Genomic variation and evolution of *Staphylococcus aureus*. Int J Med Microbiol. 2010;300(2–3):98–103.

4. Malachowa N, DeLeo FR. Mobile genetic elements of *Staphylococcus aureus*. Cell Mol Life Sci. 2010;67(18):3057–71.

5. Chambers HF, DeLeo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol. 2009;7(9):629–41.

6. Llamulli LL, Fisher JF, Mobashery S. Molecular basis and phenotype of methicillin resistance in *Staphylococcus aureus* and insights into new beta-lactams that meet the challenge. Antimicrob Agents Chemother. 2009;53(10):4051–63.

7. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcal cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2000;44(6):1549–55.

8. Misirua A, Pigli YZ, Boyle-Vavra S, Daum RS, Boocock MR, Rice PA. Roles of two large serine recombinases in mobilizing the methicillin-resistance cassette SCCmec. Mol Microbiol. 2013;88(6):1218–29.

9. Wang L, Archer GL. Roles of CcrA and CcrB in excision and integration of staphylococcal cassette chromosome mec, a *Staphylococcus aureus* genomic island. J Bacteriol. 2010;192(12):3204–12.

10. Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob Agents Chemother. 1999;43(6):1449–58.

11. Kreiswirth BN, Lofdahl S, Betley MJ, O'Reilly M, Schlievert PM, Bergdoll MS, Novick RP. The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. Nature. 1983;305(5936):709–12.

12. Kuroda M, Ohta T, Uchijima Y, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumura H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Uti Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kaneshita M, Yoshinaga T, Oshima K, Futuya K, Yoshino C, Shiba T, Hattori M, Ogawara N, Hayashi H, Hiramatsu K. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet. 2001;357(9264):1225–40.

13. Benes V, Hostomsky Z, Arnold L, Paces V. M13 and pUC vectors with new unique restriction sites for cloning. Gene. 1993;130(1):151–2.

14. Stojanov M, Sakwinska O, Moreillon P. Expression of SCCmec cassette chromosome recombinases in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. J Antimicrob Chemother. 2013;68(4):749–57.

15. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. Primer3—new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115.

16. Higgins PG, Rosato AE, Seifert H, Archer GL, Wispelinghoff H. Differential expression of ccrA in methicillin-resistant *Staphylococcus aureus* strains carrying staphylococcal cassette chromosome mec type II and Iva elements. Antimicrob Agents Chemother. 2009;53(10):4556–8.

17. Wang L, Ahmed MH, Safa MK, Archer GL. A plasmid-borne system to assess the excision and integration of staphylococcal cassette chromosome mec mediated by CcrA and CcrB. J Bacteriol. 2015;197(17):2754–61.