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# The Staphylococcal Cassette Chromosome *mec* type V from *Staphylococcus aureus* ST398 is packaged into bacteriophage capsids

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ABSTRACT

The Staphylococcal Cassette Chromosome *mec* (SCC*mec*) confers methicillin resistance to *Staphylococcus aureus*. While SCC*mec* is generally regarded as a mobile genetic element, the precise mechanisms by which large SCC*mec* elements are exchanged between staphylococci have remained enigmatic. In the present studies, we observed that the clinical methicillin-resistant *S. aureus* (MRSA) isolate UMCG-M4 with the sequence type 398 contains four prophages belonging to the serological groups A, B and Fa. Previous studies have shown that certain serological group B bacteriophages of *S. aureus* are capable of generalized transduction. We therefore assessed the transducing capabilities of the phages from strain UMCG-M4. The results show that some of these phages can indeed transduce plasmid pT181 to the recipient *S. aureus* strain RN4220. Therefore, we also investigated the possible involvement of these transducing phages in the transmission of the large SCC*mec* type V (5C2&5) element of *S. aureus* UMCG-M4. While no transduction of the complete SCC*mec* element was observed, we were able to demonstrate that purified phage particles did contain large parts of the SCC*mec* element of the donor strain, including the methicillin resistance gene *mecA*. This shows that staphylococcal phages can encapsulate the resistance determinant *mecA* of a large SCC*mec* type V (5C2&5) element, which may lead to its transfer to other staphylococci.


## Introduction

The Staphylococcal Cassette Chromosome *mec* (SCC*mec*) is a mobile genetic element that confers methicillin resistance to various staphylococcal species, including *Staphylococcus aureus*. A conserved core feature of all SCC*mec* elements is the *mec* gene complex that contains the *mecA* gene responsible for methicillin resistance. In addition, SCC*mec* elements contain other conserved regions (Chlebowicz et al., 2010, 2011, 2013). In methicillin-resistant *S. aureus* (MRSA), the known SCC*mec* elements are inserted in the conserved *orfX* gene, which is located close to the chromosomal origin of replication. This gene contains a specific integration site sequence known as *attB* (Ito et al., 2001). The SCC*mec* can be excised from this position, but this does not always occur precisely (Chlebowicz et al., 2010; Noto et al., 2008a,b). Furthermore, transposons, insertion sequences, complete plasmids, and other SCC elements can insert in SCC*mec* elements, resulting in an overall mosaic structure.

Bacterial determinants for antibiotic resistance can be transferred from one strain to another via (i) natural transformation through binding and uptake of free extracellular DNA by competent cells, (ii) conjugation facilitated by conjugative plasmids, and (iii) transduction by phages (Mašlaňová et al., 2013; Morikawa et al., 2012; Morse, 1969; Novick, 1991; Skippington and Ragan, 2011; Udo and Grubb, 1996). From genome sequencing studies it can be concluded that especially phages have had a major impact on the evolution of *S. aureus* (Holden et al., 2010; Price et al., 2012; Xia et al., 2013). This is evidenced by the presence of phage genome sequences that have inserted at different genomic positions (Goerke et al., 2009; Kwan et al., 2005). These prophages can be induced again by DNA damage, resulting in the synthesis and dissemination of many phage particles by the host cells. Importantly, not only phage DNA is packaged into phage particles, but also plasmids or chromosomal DNA of the bacterial host

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(Mašlaňová et al., 2013; Stewart and Rosenblum, 1980; Townsend et al., 1986; Udo and Grubb, 1996; Varga et al., 2013). This encapsulation depends on the presence of *pac* or *pseudopac* sequences and the specificity of phage packaging proteins that recognize these sequences (Wirtz et al., 2010). Like phage DNA, encapsulated plasmid or chromosomal DNA can be delivered to a new bacterial host cell upon infection. This transduction process has been shown to direct the efficient transfer of plasmids, pathogenicity islands and transposons between different *S. aureus* strains (Bukhari and Froshauer, 1978; Tormo et al., 2008; Townsend et al., 1986; Udo and Grubb, 1996), and it is therefore often used as a tool for the genetic modification of this pathogen (Novick, 1991).

Bacteriophages contribute to the virulence of *S. aureus* by carrying a range of toxin genes (Chen and Novick, 2009; Coleman et al., 1989; Goerke et al., 2009; Shafer and Iandolo, 1979; Xia et al., 2013). For example, the Panton-Valentine Leukocidin (PVL)-containing phages seem to be at least partially responsible for enhanced virulence of some *S. aureus* lineages (Lina et al., 1999). Also, the genes for important immune evasion factors, such as the bacterial plasminogen activator (SAK), the staphylococcal complement inhibitor (SCIN) and the chemotaxis inhibitory protein of *S. aureus* (CHIPS), are encoded by a phage (Coleman et al., 1989; van Wamel et al., 2006).

The mobilization of staphylococcal pathogenicity islands (SaPIs) requires specific SaPI-encoded recombinases (Int and Xis), and is controlled by the master SaPI regulator Stl (Mir-Sanchis et al., 2012). Interestingly, particular phages can mobilize SaPIs not only from one *S. aureus* strain to another (Christie et al., 2010; Stewart and Rosenblum, 1980), but also from *S. aureus* to *Listeria monocytogenes* (Chen and Novick, 2009). The mobilization of SaPIs seems phage-specific as, for example, phage φ80α can mobilize various different SaPIs whereas phage φ80 is specific for SaPI2 (Christie et al., 2010). This relates to the fact that, on one hand, particular phage genes are required for successful transfer of incorporated DNA (Quiles-Puchalt et al., 2014), while on the other hand particular SaPIs can interfere with phage reproduction (e.g. SaPI2 inhibits φ80; Ram et al., 2012). These findings raise the question how SCC*mec* elements are exchanged between cells of *S. aureus*. Recently, it was shown that serogroup B phages can package genes of the SCC*mec* type I into their capsids (Mašlaňová et al., 2013). This suggests a potential role of this mechanism in evolution and emergence of methicillin-resistance among *S. aureus*. A recent report of Scharn et al. (2013) indicates that transduction of the relatively small SCC*mec* types I and IV is possible, but occurs with low frequencies and requires specific conditions.

Molecular analysis of 21 *S. aureus* isolates with the sequence type (ST) 398 revealed the presence of two sub-types, which are characterized by the presence of different prophages (van der Mee-Marquet et al., 2013). The generally livestock-associated ST398 isolates mostly contain the phages Sa2 and Sa6, or a φAvb prophage, whereas some clinical ST398 isolates from humans contain variants of the Sa3 prophage that encode the staphylococcal immune evasion proteins SCIN and CHIPS.

In the present study, we investigated the transducing capabilities of phages from a clinical ST398 MRSA isolate that had caused a severe infection (Chlebowicz et al., 2010). This isolate named UMCG-M4 carries four prophages, including the PVL-encoding prophage Sa2 and the SAK-, CHIPS- and SCIN-encoding prophage Sa3. Specifically, we assessed whether the phages of UMCG-M4 can package this strain's relatively large SCC*mec* type V (5C2&5) element of 39 kb. Our results show that the induced phages are indeed capable of transducing plasmid DNA from strain UMCG-M4 to another recipient. More importantly, our results also show that the SCC*mec* type V (5C2&5) of the UMCG-M4 strain is packaged into phage particles, indicating that this mobile genetic element can be exchanged between staphylococci via transduction.

## Materials and methods

### *Bacterial strains, phages and growth conditions*

The bacterial strains and phages used in this study are listed in Table 1. *S. aureus* UMCG-M4 cells from which DNA was extracted for genome sequencing were grown in tryptic soy broth at 37 °C under vigorous shaking (250 rpm). For phage induction and propagation, bacterial cells were cultivated at 37 °C with aeration in meat peptone broth (MPB), which was prepared as described before (Mašlaňová et al., 2013). To select transductants, *S. aureus* was grown on tryptic soy agar (TSA) plates at 37 °C.

### *Prophage propagation and induction*

Wild-type φ53 formed only very few plaques on *S. aureus* UMCG-M4, and it was therefore necessary to adapt φ53 for propagation on this strain. This was achieved by collecting phages from initially obtained plaques and subsequent passage (5 times) through strain UMCG-M4 grown on meat peptone agar plates until plaques were obtained at high frequency. The identity of modified and wild-type φ53 phages was verified by restriction of isolated phage DNAs with *Hin*dIII. To obtain a lysate with high phage titers, strain UMCG-M4 was infected with the modified φ53 phage and cultivated in MPB for 2 h at 37 °C. Subsequently, the cells were incubated overnight at 4 °C after which most cells had lysed. The plaque-forming units (PFU) per ml were determined by titration on the restriction-deficient strain RN4220 or the prophage-less strain 1039. Logarithmically growing cells of strain UMCG-M4 (pT181) were used for phage induction. The cells were harvested by centrifugation, washed twice in saline solution (0.85% NaCl) and resuspended in 10 ml of saline solution to an optical density at 600 nm of 0.15. The cells were then irradiated for 40 s with an UV lamp (260 nm, 16 W) at a distance of 50 cm and incubated for 2 h at 37 °C under aeration. After overnight bacterial lysis at 4 °C, the remaining bacteria and bacterial debris were removed by centrifugation (15 min, 6500 × *g*) followed by filtration with 0.45 mm L.E. nylon syringe filters (Millex-HV Filter Unit, Merck Millipore). The phage titer was determined by determining the PFU per ml through phage propagation on the restriction-deficient strain RN4220.

### *Phage sensitivity test*

Phage sensitivity testing was performed by the double agar overlay plaque assay at 100× routine test dilution (RTD) of each phage estimated by titration against its specific propagation strain (Blair and Williams, 1961).

### *Transduction experiments*

Phage φ53 propagated on strain UMCG-M4 was used to determine the possible transduction of SCC*mec* to the recipient strains, UMCG-M2 (an MSSA derivative of the MRSA strain UMCG-M4), RN4220, SH1000 or HG001. The transduction procedure was performed as described by Varga et al. (2012).

### *Purification of phage particles, phage DNA isolation, PCR and transformation*

Phage particles from 300 ml of phage lysates were purified by CsCl density gradient centrifugation as described by Sambrook and Russell (2001). To remove non-encapsulated bacterial DNA and RNA, the purified phages were treated with DNase I and RNase (Sambrook and Russell, 2001). As a control for the removal of contaminating non-encapsulated bacterial DNA, genomic DNA



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