Horizontal Gene Transfer of Mobile Genetic Elements by *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is an opportunistic pathogen that is the major cause of hospital- and community acquired infections. The misuse of antibiotics is the most prominent reason for bacterial resistance and resulted in the emergence of Methicillin Resistant *S. aureus* (MRSA) strains. For these reasons, studying horizontal gene transfer systems in *S. aureus* is highly important to elucidate the molecular systems underlying the acquisition of resistance and toxin genes, and thereby reducing the risk of a post antibiotic era. Here, the most recent literature is reviewed to elucidate the recently acquired knowledge about horizontal gene transfer of mobile genetic elements by *S. aureus*.

Introduction

Staphylococcus aureus is an opportunistic pathogen that is the major cause of hospital- and community acquired infections (NCBI). Penicillin resistant *S. aureus* strains where already found, shortly after the first production of pharmaceutical graded penicillin, and its resistance was shown to be inheritable (Demerec, 1945). Nowadays, Methicillin Resistant *S. aureus* (MRSA) causes invasive infections that occurred, in the US, 80,451 times in 2011 (Dantes *et al,* 2013). Also other *S. aureus* strains, resistant to vancomycin (VRSA) were found during the last decade; however these infections continue to be a rare occurrence (according to CDC).

The misuse of antibiotics is the most prominent reason for bacterial resistance. Since the first use of antibiotics, bacterial resistance developed and is developing with newly introduced antibiotic drugs (Bell M, 2014). Antibiotics are also used for our livestock and in 2003 the first livestock associated MRSA (LA-MRSA) was identified. It was found that LA-MRSA transmitted to the veterinarians working with the livestock and sequentially transmitted it further to their household (Bosch *et al,* 2014).

Methicillin sensitive *S. aureus* (MSSA) retrieves its resistance by acquiring a mobile genetic element (MGE) called *Staphylococcal* Cassette Chromosome *mec* (SCC_{mec}) encoding for methicillin resistance (Katayama *et al,* 2000). Also virulence factors are most often coupled to MGEs (Novick, 2003), e. g. the Panton-Valentine Leukocidin, a toxin that lyses leukocytes (Lina *et al,* 1999). The way for transferring MGEs is horizontal (lateral) gene transfer (HGT). The most prominent system in *S. aureus* is transduction by bacterial phages, because this is most probably the major system of gene transfer (Lindsay, 2014). Other HGT systems are conjugation and transformation, where the significant contribution of the latter is still unclear (Fagerlund *et al,* 2014).

Because resistant *Staphylococcal* bacteria will be a continuous problem, studying HGT systems in *S. aureus* is highly important to elucidate the molecular systems underlying the acquisition of resistance and toxin genes, and thereby reducing the risk of a post-antibiotic era. Acquisition of antibiotic resistance via HGT is not solely restricted to one species; as cross-species spread of the SCC_{mec} MGEs has been observed between *S. epidermidis* and *S. aureus*. The similar occurrence of SCC_{mec} MGEs in the two *Staphylococcal* species is due to HGT between the species rather than convergent or parallel evolution within the species (Smyth *et al,* 2011).

Antibiotic resistant *Staphylococcal* bacteria will be a continuous problem; therefore we must require fundamental insights on how the molecular basis of obtaining antibiotic resistance is achieved. For this reason, this essay is focused on reviewing the most recent literature to elucidate the recently acquired knowledge about horizontal gene transfer of mobile genetic elements by *S. aureus*.

Mobile genetic elements

Mobile genetic elements (MGEs) play an important role in the transfer of genetic information in *S. aureus*. There are various MGEs (discussed below) such as, *Staphylococcal* Cassette Chromosomes (SCC), Phage Related Chromosomal Islands (PRCIs), Transposons, and Plasmids (Malachowa & DeLeo, 2010).

Staphylococcal Cassette Chromosomes *mec* (SCC_{mec})

The SCC_{mec} element was first designated as a MGE more than a decade ago. Besides the already identified *mecA* gene, two site-specific recombinases were found, CcrA and CcrB (Chromosome Cassette Recombinase A and B) (Katayama *et al,* 2000). The gene encoding for methicillin resistance is *mecA*, which encodes for a Penicillin Binding Protein (PBP2a) that has a low binding affinity for methicillin or other ß-lactams (Ubukata *et al,* 1989). SSC_{mec} elements are highly diverse and there are many classes of these various elements. Therefore, the IWG-SCC (International Working Group on the Classification of *Staphylococcal* Cassette Chromosome Elements (IWG-SCC), 2009) published guidelines on the classification of already existing and newly found SCC_{mec} elements. SCC_{mec} elements are classified on **(1)** the type of *mec* complex, **(2)** the type of *ccr* complex, and **(3)** the presence of J (joint)-regions. An example is given where a SCC_{mec} type $2A_1$ element is depicted containing *mec* complex A, *ccr* complex 2, and 3 J-regions **(Figure 1)**. In SCC_{mec} type $2A_1$ a plasmid (pUB110) and a transposon (Tn554) are located in the J-regions, which is only specific for this type.

Diagnostic classification of MRSA strains is done by multiplex PCR systems commercially available; however these systems generate false-positive results and therefore lead to misidentification of MRSA infections. The false-positive results are generated by strains that carry SCC elements without the *mecA* gene (Blanc *et al,* 2011). When misidentified MRSA strains were investigated it was found that only a small portion of the strains could have lost the *mecA* gene because the greater portion (87%) was not SCC_{mec} related. This indicates that the SCC elements are much more complex than previously thought (Stojanov & Blanc, 2014).

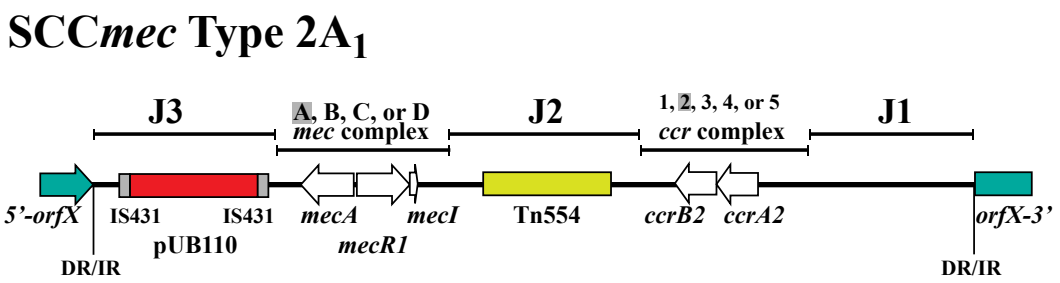


Figure 1 – SCC_{mec} Type $2A_1$

Adapted from Chongtrakool *et al*, (2006)**.** Genes from SCC_{mec} Type $2A_1$ element are depicted as example for SCC_{mec} elements. This element has three J-regions (J1; J2; J3) with *mec* complex A (highlighted in grey) and *ccr* complex 2 (highlighted in grey). Both on the 5'- and 3'-ends direct repeats (DR) or inverted repeats (IR) are located which serve as target for *ccr* mediated recombination. *orfX* is the location on the genome of *S. aureus* where the SCC_{mec} element is inserted. This type of SCC_{mec} element has plasmid pUB110 (red) in the J3-region flanked by two IS431 insertion sequences (grey) which, for example, SCC_{mec} Type $2A_2$ does not have. In the J2-region the Tn552 transposon (yellow) is located, which is possessed by both Type 2 and 3.

Phage Inducible Chromosomal Islands (PICI)

In contrast to SCC elements, *S. aureus* Pathogenicity Islands (SaPIs) are phage related and associated with pathogenic features. However, SaPIs do not encode for a horizontal transfer machinery, but need helper-phages to transduce **(Figure 2A)**. It was shown that SaPIs interfered with helper-phage infections by 'hijacking' their capsid proteins to encapsulate itself (Ruzin *et al,* 2001). Nevertheless, all SaPIs have conserved genes encoding for, intergrase, primase, a replication initiator, and Stl the major repressor of the excision-replication-encapsulation cycle (Novick *et al,* 2010) **(Figure 2B)**. Besides these conserved genes, SaPIs transfer pathogenic features such as the superantigens —A protein family that triggers excessive and abnormal T-cell activation (Llewelyn & Cohen, 2002)— TSST1, enterotoxin, and other toxins (Novick *et al,* 2010). Recently, studies revealed that SaPIs additionally (to capsid hijacking) use

also genetic phage interference mechanisms (Ram *et al,* 2012;2014). This suggests that SaPIs, initially derived from (proto)phages, have undergone an evolutionary differentiation including the acquisition of phage-resistance mechanisms (Ram *et al,* 2014) by 'hijacking' the helper-phage capsid proteins resulting in interference with the encapsulation of the helper-phage **(Figure 2A).**

A

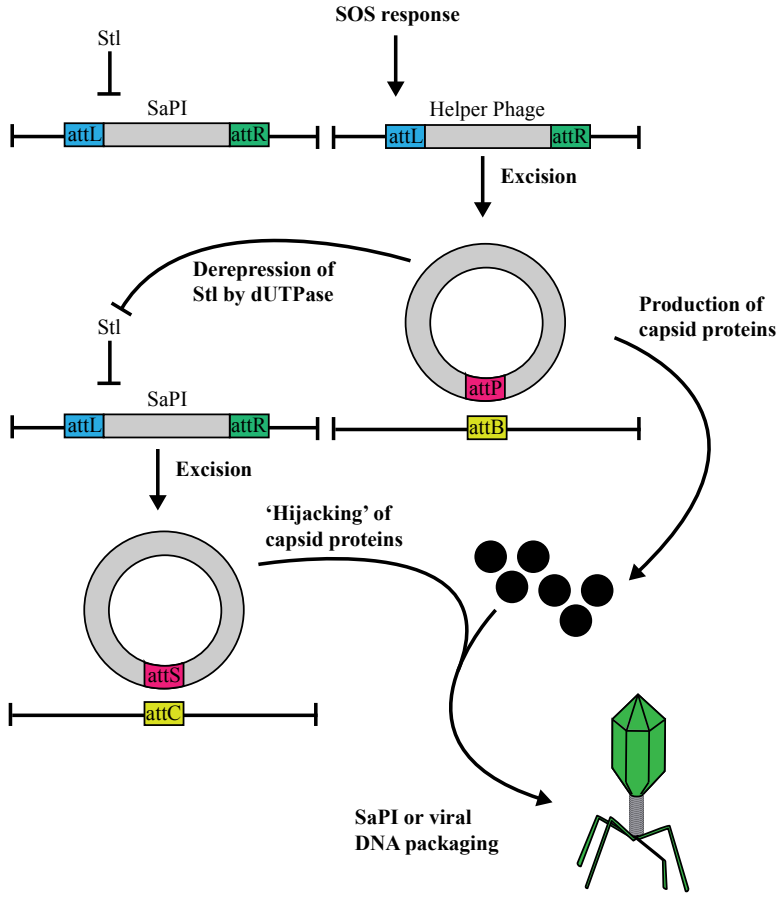
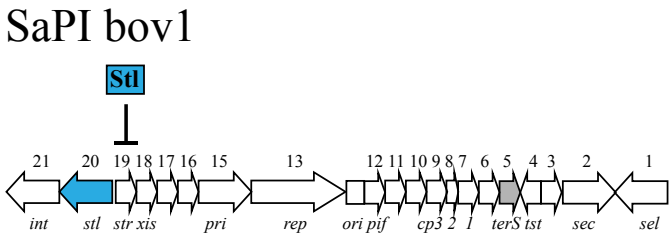


Figure 2 – SaPI transduction
Adapted from Novick *et al*, (2010). In **(A)** a model of SaPI transduction is depicted, where an SOS-response triggers the excision of the helper phage DNA which encodes a dUTPase that derepresses the major SaPI repressor Stl. Both SaPI and Phage DNA have their specific attachment sites *attS* and *attP* which are corresponding to locations on the chromosome *attC* and *attB*. When Stl is derepressed by dUTPase, the SaPI DNA is excised and 'hijacks' the capsid proteins from the helper phage which results in competition between SaPI and phage transduction (SaPI interference). In **(B)** the genes of SaPI bov1 are depicted with Stl as major repressor of the SaPI excision machinery (blue). SaPI uses its own TerS protein (grey) for transduction and does not need the TerS from its helper phage (discussed below). Other gene annotations or functions: *int*, intergrase; *str*, regulatory protein; *pri*, DNA primase; *rep*, replication initiator; *ori*, replication origin; *pif*, phage interference; *cp123*, capsid size determinants; *terS*, terminase small subunit; *tst*, toxic shock syndrome toxin; *sec*, enterotoxin type C; *sel*, enterotoxin type L.

B



Plasmids

One difference between *Staphylococcal* plasmids and *Staphylococcal* Cassette Chromosomes is that the SCC elements integrate at a specific site in the *Staphylococcal* genome at the Integrate Site Sequence (ISS), i.e. at 3'-side of *orfX* (IWG-SCC, 2009). The *Staphylococcal* plasmids were initially divided in three classes based on their incompatibility; two plasmids with the same replication initiator sequence (*rep*) would be incompatible within one host and therefore grouped into one class (Ruby & Novick, 1975). Class I consists of small plasmids ranging from 1-5kbp, with a high copy number often carrying one antibiotic resistance gene, class II and III consist of larger plasmids ranging from 20-60kbp with lower copy numbers and often carry multiple antibiotic resistance genes. Wherein class III differs from class II with the presence of conjugative transfer genes (*tra*) (Novick, 1989). Nowadays a more sophisticated classification is proposed using alignments of the *rep* sequences for classification

of mainly *Enterococcal* and *Staphylococcal* plasmids (Jensen *et al,* 2010). The *Staphylococcal* plasmid classification was later extended with 13 more classes. It was found that 51% of the plasmid groups carry more than one *rep* sequence, indicating that recombination between plasmids occurs frequently. Interestingly, only 5% of the plasmids analyzed possessed *tra* genes, indicating that the distribution of the majority of the plasmids in *S. aureus* is lineage associated (McCarthy & Lindsay, 2012).

Transposons and Insertion Sequences

Transposons (Tn) and insertion sequences (IS) share similar features such as inverted repeats at the flanking regions for transposition, however there is one major difference; insertion sequences seem to encode for no other genes than for the transposase machinery (Siguier *et al,* 2006a). In contrast, transposons carry, besides the transposase machinery, other varying genes encoding for, resolvases, antibiotic resistance, and other accessory proteins. Interestingly, some transposons have *tra,* encoding for conjugative transfer machinery, as accessory gene and these hybrid transposons are designated as conjugative transposons which can transfer within the bacterial genome and between bacterial species (Kleckner, 1990). To keep track of all the identified transposable elements, guidelines for the nomenclature of transposable elements, for transposons (Tn*1*, *2*, *3*, *etc.*) and insertion sequences (IS*1*, *2*, *3*, *etc.*), were proposed in 1979 (Campbell *et al,* 1979). However, in that time there were no online databases and after the retirement of the person who administrated the allocated numbers, the numbering stopped. Later, it was proposed to keep the original nomenclature system and to allocate the data in an online database separately for Tn sequences (Roberts *et al,* 2008) and IS sequences (Siguier *et al,* 2006b).

Horizontal Gene Transfer Systems

All the mobile genetic elements described above can be transferred from one strain to another, by transduction, conjugation or transformation (see Figure 3). That MGEs are transferred via HGT was already suggested for a long time, however recently experimental evidence showed a high frequency of HGT between two *S. aureus* strains *in vivo*. Experimental evolution was used with gnotobiotic piglets, where a human associated MSSA strain (H398, white colony, 2 MGEs) was co-colonized with a livestock associated MRSA strain (S0385, grey colony, 8 MGEs) on the piglets' skin. Using multiplex PCR and whole genome sequencing, isolates from piglets were tested for their MGEs (4 h, 2, 4, 12, 16 days) and their acquired or lost MGEs (mobilomes) were mapped. The minimum MGE transfer events on the 16th day were significantly higher in H398 then in S0385, however importantly, high frequency MGE transfer was already detected after 4 hours. Comparisons between *in vivo* and *in vitro* experiments revealed that HGT between the strains occured significantly more in the *in vivo* experiments. Because transformation is inefficient in *S. aureus* and there were no *tra* genes for conjugation involved, the authors concluded that transduction was the main mechanism used for HGT *in vivo* (McCarthy *et al,* 2014).

Transduction

Transduction is a mechanism where bacteriophages transfer DNA from one cell to another. There are two types of transduction, generalized and specialized transduction. In generalized transduction, any part of the host's DNA can be packaged, whereas in specialized transduction only DNA from a specific region is packaged, which only occurs in temperate phages (Madigan *et al,* 1997).

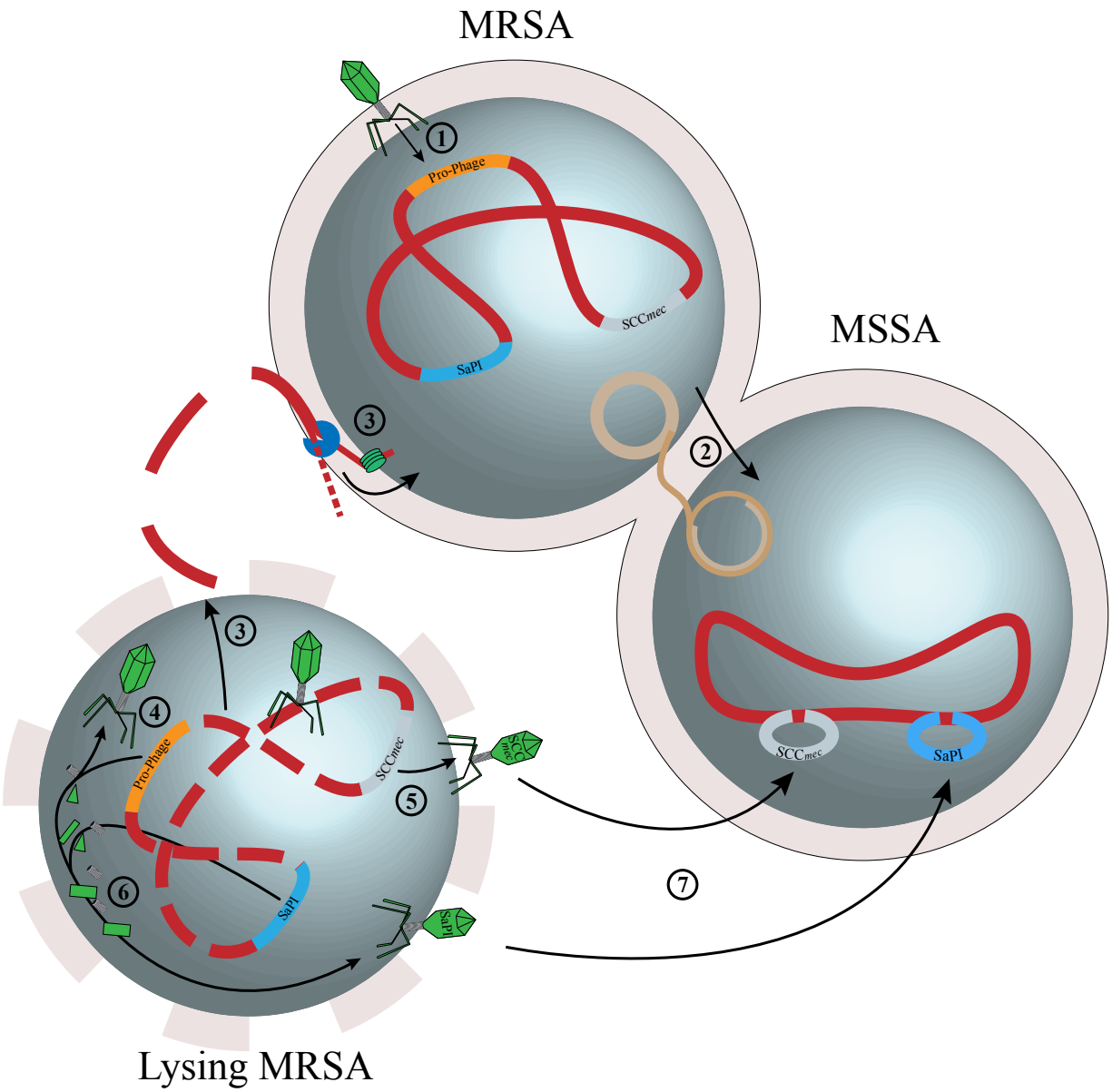


Figure 3 – Mobile genetic elements transferred by horizontal gene transfer systems.
Three *S. aureus* cells are depicted, where the numbers associate with various horizontal gene transfer systems that are transferring mobile genetic elements from cell to cell. In **(1)**, a phage is inserted in the genomic DNA from the host (red) and entered the lysogenic cycle. In **(2)**, a conjugative plasmid is transferred via conjugation to the MSSA recipient cell that is in close proximity. In **(3)**, $_{DS}$DNA released from a lysing MRSA cell and is taken up, as $_{SS}$DNA, by the recipient cell via transformation, where the proteins depicted are form the competence machinery. In **(4)**, the pro-phage is activated and packages its DNA inside capsids. In **(5)**, due to generalized transduction non-phage DNA is packaged inside the phage capsids. In **(6)**, the SaPI element is activated by a helper-phage infection, and hijacks the capsids from the helper-phage. In **(7)**, the SCC$_{mec}$ and SaPI MGEs are transferred via transduction to the recipient MSSA cell.

Virtually any type of MGE described above can be transferred via transduction which is also the major mechanism of HGT in *S. aureus* (Lindsay, 2014;McCarthy *et al,* 2014). In fact this has been proven in a recently published study, which aimed to show that phages, as generally accepted, are indeed able to package various MGEs as a prerequisite for their potential transfer by transduction. A new method was employed, using quantitative PCR (qPCR), to establish the bacterial gene copy number per nanogram of DNA isolated from phage particles. Two phage-serogroups (A; φ81, and B; φ80, φ80α, φ11) were used in the study and statistical analysis showed that there was a significant difference in the amount of bacterial

DNA packaged, between serogroup A and B (P≤0.001). Serogroup B phages efficiently packed various MGEs in their capsids, notably two segments of the SCC_{mec} I element were simultaneously packed, which indicated that generalized transduction plays an important role in SCC_{mec} transfer (Mašlaňová *et al,* 2013). The larger SCCmec V (5C2&5) element was also able to be encapsulated by the capsids of bacteriophage φ53, however the transduction of SCCmec V was not detectable with φ53 and neither with identified prophages from the host cell. It was not entirely clear why SCCmec V could not be detected in the recipient strains, but it could rely on specific conditions for (homologous) recombination (Chlebowicz *et al,* 2014).

Conditions required for the transduction of the SCC_{mec} elements were recently further elaborated (Scharn *et al,* 2013) from early reports. These reports showed that the highest transfer frequencies with phages φ80α or φ29, for methicillin resistance, were achieved at 30°C for 48 to 72 hours using a recipient strain harboring a plasmid (possibly with a prophage) encoding for a penicillinase (Cohen & Sweeney, 1970;1973). An additional condition was recently added to the requirements of transduction. The arginine catabolic metabolic element (ACME), adjunct to the integrated SSC_{mec} or *orfX* on the chromosome, revealed that, transduction of the SSC_{mec} elements was only observed from $_{ACME}$+ donor to $_{ACME}$+ recipient or from $_{ACME}$− donor to $_{ACME}$− recipient strains. This led to the speculation that the acquisition of SSC_{mec} was rather related to homologous recombination than on Ccr-mediated recombination. Another aspect of this study showed that during transfer, via transduction, interesting SCC_{mec} rearrangements occur, where deletions of the *mecA* gene were observed (Scharn *et al,* 2013).

Crucial elements from phage φ11 have been investigated recently in relation with SaPI interference (i.e. transduction) and plasmid transduction (Quiles-Puchalt *et al,* 2014). Systematic gene deletions were used to characterize the phage forming units (PFU/mL), number of SaPI transductions, and number of plasmid transductions. Note that SaPIs are transduced by 'hijacking' the capsid proteins from a phage **(Figure 2A)** and may need different proteins from the phage than the phage itself. Therefore, in order to investigate this, the packaging elements had to be identified. Because the authors showed previously that the gene *rinA* is responsible for packaging and lysing the cell (Ferrer *et al,* 2011;Quiles-Puchalt *et al,* 2013), it was speculated that genes under control of RinA are required for the formation of transduction particles **(Figure 4)**. After conformation of this speculation, 28 packaging genes were systematically deleted and their phenotype and the number of transductions were investigated. One of these proteins was a terminase, terminases are hetero-oligomers of small (TerS) and large (TerL) subunits, where TerS is involved in DNA recognition and TerL is necessary for translocation of the viral DNA, cleavage of DNA, and docking the DNA portal of the phage-head (Rao & Feiss, 2008). Interestingly, *terS* encoding for the small terminase was only found to be required for phage and plasmid packaging and not for the packaging of SaPIs (Quiles-Puchalt *et al,* 2014). This confirms that SaPIs utilizes its own TerS (DNA recognition) protein **(Figure 2B)** as suggested in an earlier report (Úbeda *et al,* 2007). Besides *rinA* both SaPI and phage require the same amount, with a few exceptions, of packaging elements for transduction **(Figure 4)** (Quiles-Puchalt *et al,* 2014).

Transduction, in this case of SaPIs, does not constrain between spiecies transfer, in fact it has been shown that helper-phage (φ12 and φSLT) mediated SaPI transfer occured inter- and intra-generically between *S. aureus* and *Listeria monocytogenes*, *Staphylococcus xylosus*, *Staphylococcus epidermidis* (Chen *et al,* 2014).

Phage Φ11

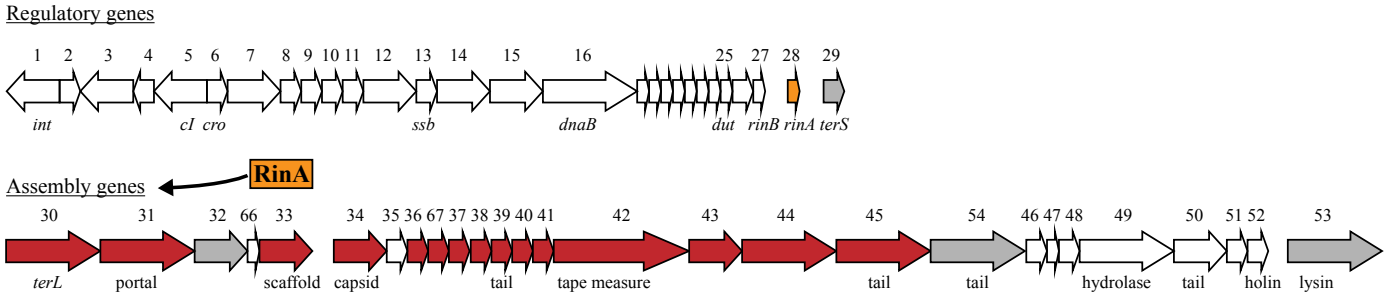


Figure 4 – Phage φ11
Adapted from Quiles-Puchalt *et al*, (2014). Here the genome of phage φ11 is depicted, one part contains the regulatory genes (1-28) and the other part contains the assembly genes (30-54, 66, 67) which are under control of RinA (orange). The genes colored red are essential for SaPI transduction. The regulatory genes, the genes colored red, and the genes colored grey are essential phage transduction. Gene annotations or functions: *int*, Intergrase; *cI*, major repressor; *cro*, regulatory protein; *ssb*, singe stranded DNA binding protein; *dnaB*, DNA primase/helicase; *dut*, dUTPase; *rinB*, regulatory protein; *rinA*, phage transcriptional activator; *terS*, terminase small subunit; *terL*, terminase large subunit.

Transformation

The acquisition of extracellular DNA from the environment is defined as transformation, where the ability to bind and take-up extracellular DNA is defined as competence. The acquisition or uptake of the extracellular DNA is a process where DNase-sensitive DNA is transferred into a DNase-protected environment; in gram positive bacteria this is equivalent to the transfer across the cytoplasmic membrane. Only single stranded DNA is transported over the cytoplasmic membrane, where the other strand is degraded in single nucleotides and released into the extracellular environment (Chen & Dubnau, 2004).

To date, actual evidence for transformation of extracellular DNA in *S. aureus* by natural genetic transformation has not yet been shown (Morikawa *et al,* 2012), despite the presence of orthologous genes with the competence genes from *Bacillus subtilis* and *Streptococcus pneumoniae* (known for their natural competence). Orthologous genes of the competence-related DNA transformation transporter system from the model organism *B. subtilis* are compared with *S. pneumoniae* and *S. aureus* in Table 1.

Table 1 – DNA transformation transporter systems

		Bacillus subtilis subsp. *subtilis* 168	*Staphylococcus aureus*	*Streptococcus pneumoniae*	
Gene	**Locustag**	**Orthologues**			**Function**
comC	BSU2807	✓		✓	Leader peptidase (prepilin peptidase)[2]
comEA	BSU2559	✓		✓	Competence protein, membrane DNA receptor[1]
comEB	BSU2558	✓		✓	dCMP deaminase[2]
comEC	BSU2557	✓		✓	Channel protein for DNA binding and uptake[1]
comER	BSU2560	✗		✗	Late competence protein, pyrroline-5'-carboxylate reductase[3]
comFA	BSU3547	✓		✓	Helicase competence protein[3]
comFB	BSU3546	✗		✗	Function unknown
comFC	BSU3545	✓		✓	Putative component of the DNA transport machinery[3]
comGA	BSU2473	✓		✓	Late competence protein, traffic ATPase, binds and transports transforming DNA[1]
comGB	BSU2472	✓		✓	Polytopic membrane protein, DNA transport machinery[1]
comGC	BSU2471	✓		✓	DNA uptake, major component of the transformation pilus[1]
comGD	BSU2470	✓		✓	DNA transport machinery, minor pilin[1]
comGE	BSU2469	✓		✗	DNA transport machinery, minor pilin[1]
comGF	BSU2468	✓		✓	DNA transport machinery, minor pilin[1]
comGG	BSU2467	✗		✗	Putative component of the DNA transport machinery[3]

[1] Fagerlund *et al*, 2014; [2] KEGG Genes; [3] Caspi *et al,* 2014

Studies have been performed to test whether *S. aureus* is naturally competent, and this started with the identification of an alternate sigma factor H (σ^H) that associates with RNA polymerase to transcribe the *comG* and *comE* operons in *S. aureus* **(Figure 5)** (Morikawa *et al,* 2003). A decade later, active σ^H cells were generated and were analyzed for further investigation of σ^H. By means of Southern blot, it was revealed that some strains had a tandem repeat/fusion of *sigH* in their chromosome, this led the authors believe that tandem duplications resulted in higher expression levels of *sigH*. Further investigation of the activation mechanism of σ^H showed that only a small fraction of the cells expressed *sigH*, indicating that there would be a stochastic gene expression system involved. To show that σ^H results in natural competence various *S. aureus* strains, where *sigH* was absent or overexpressed, were transformed under specific conditions. This resulted in 10^{-8} – 10^{-9} transformants per CFU when the *comEG* operons were present and *sigH* was overexpressed (Morikawa *et al,* 2012). This transformation efficiency indicates that σ^H is clearly not solely responsible for natural competence in *S. aureus* populations (Fagerlund *et al,* 2014). To elucidate this potency of *S. aureus* to take-up DNA, the *sigH* and competence regulons were investigated by whole transcriptome sequencing. Two *B. subtilis* putative orthologs of *comK* (competence transcription factor ComK) were found in *S. aureus*, *comK1* and *comK2*. Luciferase assays showed that ComK1 induces the *comGA* promoter at low-level expression, however when *sigH* was overexpressed together with ComK1 the *comGA* promoter was highly expressed. The co-overexpression of σ^H and ComK1 resulted in a synergistic activation of the *comGA* promoter **(Figure 5)** with a fold change of 1613×. Which is significantly higher in contrast to the overexpression of σ^H only (392×) and ComK1 only (8×). Furthermore, the sequence data confirmed that the *sigH* regulon includes *comG* and *comE* operons, including two additional operons necessary for competence, *drpA*, *coiA, oppF,* and *ssB*. These observations led to the conclusion that σ^H is clearly involved in *S. aureus* competence regulation however, σ^H is not a master regulator of competence. The development of competence depends most likely on two or more competence regulators (Fagerlund *et al,* 2014).

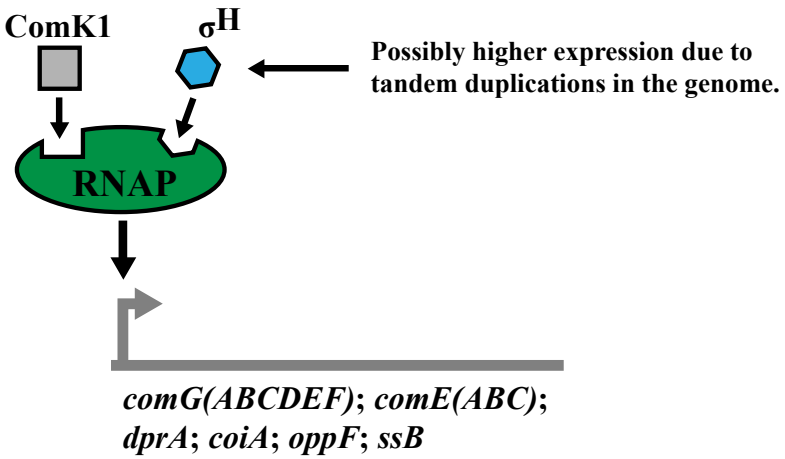


Figure 5 – Model of σ^H and ComK1 transcriptional regulation.
Both ComK1 and σ^H act synergistically on the transcription of the DNA transformation transporter system operons *comG* and *comE* (see table 1), by binding the RNA polymerase (RNAP). Other genes regulated by ComK1 and σ^H are *dprA* (DNA recombination-mediator protein A), *coiA* (unknown function), *oppF* (oligoendopeptidase F), and *ssb* (Single-strand DNA-binding protein).

A proteomic approach for investigating the pseudopilus, necessary to take up DNA, in *S. aureus* was performed on ComGC. Whereby in *B. subtilis* posttranslational signal cleavage by ComC, and posttransitional disulfide bond formation by BdbD (in *S. aureus* DsbA) is necessary. Using Western Blot and immunofluorescence microscopy, it was revealed that, also in *S. aureus*, ComGC proteins are processed by ComC, stabilized by DsbA, localized in the cell wall and membrane, and faced the cell surface (van der Kooi-Pol *et al,* 2012).

Another study showed that Atl, the major peptidoglycan hydrolase in *S. aureus* that has bifunctional properties such as amidase and endo-*ß*-*N*-acetylglucosaminidase activity, binds to DNA *in vitro* unspecific for the sequence. Atl harbors an N-terminal signal peptide and is localized and processed extracellular. Deletion mutants of *atl* show a disordered division pattern, defects in cell separation and impairment in biofilm formation. First, the authors speculated that Atl may be involved in the process of anchoring chromosomal DNA to the cell envelope to ensure proper cell division (Grilo *et al,* 2014), however *atl* mutants show no abnormalities in cell division (Sugai *et al*, 1995) (except for defects in cell separation). This led to a speculation that the DNA binding capacities of Atl could be involved in the take-up of extracellular DNA (Grilo *et al,* 2014). However, the authors do not have any structural evidence to support this speculation, nonetheless it could be interesting to verify this *in vivo*.

Conjugation

Conjugation in bacteria is a process where DNA is transferred from a donor cell to a recipient cell by a conjugation system (*tra*) encoded by a plasmid or by a conjugative transposon, which only can be used when cells are in close proximity from each other. In gram negative bacteria the cell to cell contact is established by sex pili; however these have not been identified in gram positive bacteria yet (Grohmann *et al,* 2003;Lindsay, 2014). Nevertheless, conjugational transfer in *S. aureus* and *S. epidermidis* has already been identified for some plasmids that contained genes for conjugational transfer (*tra*), which could also mediate transfer of *tra* negative plasmids (McDonnell *et al,* 1983). Thus, it is presumed that *S. aureus* cells in close proximity generate pores for conjugative transfer of plasmids (Lindsay, 2014).

McDonnell *et al*. (1983) showed that conjugational transfer was established in both mixed cultures and filter-mating cultures (donor and recipient strains are mixed and inoculated on a filter that is incubated on broth agar). However, a much more recent study investigated conjugational transfer in *S. aureus* biofilms because of the efficient conjugation in filter mating (i.e. on a surface) conditions. Conjugative transfer frequencies were investigated in planktonic, filter-mating, and biofilm forming conditions. In planktonic cultures the conjugation frequency was low but detectable, however in both filter-mating and biofilm forming conditions the conjugation frequencies were significantly higher (up to ~16,000 fold). Thus conjugational transfer is significantly enhanced during biofilm formation. However, this enhancement occurs also in filter-mating conditions, therefore, anti-biofilm formation agents were used on filter-mating experiments and showed that the conjugational transfer decreased significantly. This indicates that the efficient conjugational transfer in filter-mating conditions is partly due to the consequence of biofilm formation on the filter (Savage *et al,* 2013). This is in agreement with the notion that *S. aureus* cells have to be in close proximity to each other (*i.e.* in biofilms) in order to efficiently perform conjugational transfer.

Barriers of HGT

Restriction modification systems

The difficulty to perform genetic manipulation in *Staphylococci* is because *Staphylococci* have a strong restriction modification (RM) barrier (Monk & Foster, 2012). Naturally, this barrier also limits the frequency of horizontal gene transfer in *S. aureus*. In fact, *Staphylococcal* cells are protected to a certain extent from phage lysis and for the acquisition of foreign DNA, by means of the RM barrier in *S. aureus* (Waldron & Lindsay, 2006).

There are four major types of RM systems described in literature. RM system type 1 (RM-1) is multisubunit protein that acts as one protein that contains, two restriction subunits (R), one modification (i.e. methyltransferase) subunit (M), and one specificity subunit (recognition of DNA sequence) (S). RM-1 enzymes act on unmethylated DNA and most often act as restriction enzyme but in rare cases as methyltransferase, they cleave the DNA at variable positions adjacent to their recognition sequence until they are physically hindered. RM-2 enzymes cleave at a specific site and act independently from their methyltransferase subunit and normally recognize palindromic sequences. Deviations of these enzymes, e.g. asymmetric sequence recognition and cleavage sites, are divided in subclasses. RM-3 enzymes consist of two subunits that function either in DNA recognition and modification or restriction. For restriction, they interact with two non-palindromic sequences that are in inverted orientation in the substrate DNA, subsequently it cleaves the DNA at variable positions adjacent to their recognition sequence as RM-1. RM-4 enzymes consist most likely of one or two proteins that only cleave modified DNA, however their recognition sequence is not known (Roberts *et al,* 2003).

In *S. aureus*, a RM-1 enzyme, Sau1, was identified as a lineage-specific horizontal gene transfer barrier. While investigating the *S. aureus* strain that is able to accept foreign plasmid DNA (RN4220), a stop mutation in the R gene of Sau1 was found, explaining its tolerance for foreign DNA. Besides the R and M genes, which were highly conserved among strains, several variants of the S gene (necessary for DNA recognition) were found among 161 *S. aureus* isolates. Interestingly, S gene sequences from strains from the same lineage were highly conserved. This indicates that the cell is protected from foreign DNA from other lineages which suggests that, HGT within lineages occurs at a higher frequency than between lineages, and could explain why the SCC_{mec} element is found in only six *S. aureus* lineages (Waldron & Lindsay, 2006). Later, Sau1 specific recognition sites were identified for four different *S. aureus* lineages. The lack or the methylated forms of these target recognition sequences on plasmids, e.g. the conjugative plasmid pUSA03, showed that plasmids were able to bypass the Sau1 RM-1 system, resulting in efficient transfer of resistance genes by HGT (Roberts *et al,* 2013). Also another restriction barrier was found, a gene (SAOUHSC_02790) encoding for a RM-3 like restriction enzyme. Deletion of SAOUHSC_02790 and ORFs corresponding to this gene in other lineages resulted in susceptible strains for the acquisition of foreign DNA (Corvaglia *et al,* 2010).

CRISPR

A relatively recent, new discovery revealed a different type of HGT barrier in *Staphylococci*. This new barrier is the the CRIPSR-Cas system (Clustered Regularly Interspaced Short Palindromic Repeat-CRISPR associated proteins), which is an adaptive immune system in prokaryotes necessary for immunity against phages. The CRISPR locus is a recollection of specific DNA sequences (spacers), homologous to DNA from previously encountered phages. However, authors found a spacer in the CRISPR locus of *S. epidermidis*, homologous to the *nes* gene found in all conjugational plasmids of *Staphylococci*. In order to investigate if this spacer prevents conjugation in *S. epidermidis* the authors disrupted the sequence match with *nes*, by silent mutations in the sequence of *nes* on the conjugational plasmid pG0400. Conjugational experiments with pG0400 (wt) and pG0400*nes** between a donor strain and a CRISPR negative (–) or a CRISPR positive (+) recipient strain were performed. Experiments showed that both plasmids were able to be transferred to the CRISPR– strain as expected. In contrast, only the pG0400*nes** plasmid was able to be transferred to the CRISPR+ strain which led to the conclusion –after more genetic experiments– that CRISPR systems can interfere with plasmid transfer (Marraffini & Sontheimer, 2008).

Conclusion

That *S. aureus* acquires its antibiotic resistance by transfer of mobile genetic elements via horizontal gene transfer is already known for a long time. However, the understanding of the underlying mechanisms, which *S. aureus* uses to transfer these MGEs are still being investigated today. Despite the fact that *S. aureus* is protected against the acquisition of foreign DNA by RM systems and the CRIPSR system, the frequency of HGT is still enough to acquire virulent and antibiotic resistance genes. Only a short while ago it has been found that HGT between two lineages was "unexpectedly high" *in vivo* compared towards *in vitro* experiments (McCarthy *et al,* 2014). This suggests that perhaps the HGT barrier is not that strict during host colonization for yet unknown reason.

The ongoing bacterial acquirement of antibiotic resistance could also be explained by the usage of antibiotics such as ß-lactams and ciprofloxacin which promote horizontal gene transfer (Goerke *et al*, 2006;Maiques *et al*, 2006). In the Netherlands only, ß-lactams were used by ~1.5 million users (amoxicillin, 1,095,000 users; flucloxacillin 280,180 users; feneticillin 151,090 users) and ciprofloxacin was used by 263,330 users in 2013 (The Drug Information System of National Health Care Institute).

In 2003 the first model on how antibiotics can promote the spread of bacterial antibiotic resistance genes was presented. The authors showed that Mitomycin C, a DNA damaging agent which induces the SOS-response, enhanced the conjugational transfer of a MGE called SXT in *E. coli* (Beaber *et al*, 2003). The SOS-response is also known as inducer of temperate phages which is well studied in *E. coli* (Little & Mount, 1982). Thus, when a DNA damaging agent is used, such as ciprofloxacin or Mitomycin C, against a bacterial infection, it will trigger the SOS-response and subsequently will induce temperate phages which promote HGT. Experimental evidence in *S. aureus* for this hypothesis was published almost decade ago. A lysogenic *S. aureus* strain challenged with various concentrations (until 1 MIC) of ciprofloxacin showed that the increase of the ciprofloxacin concentration correlated with an increasing amount of phage forming units and newly synthesized phage DNA after induction (quantified by qPCR) (Goerke *et al*, 2006). Similar results were published the same year with subinhibitory concentrations of ß-lactams, which are known for their inhibitory effect on cell wall synthesis. The subinhibitory concentrations of the tested ß-lactams also induced an SOS-response and therefore were able to induce phage and SaPI transductions (without a functioning SOS-response system (a *recA* mutant) no transductions were observed) (Maiques *et al*, 2006).

The equilibrium between the acceptance of foreign DNA and the protection against foreign DNA, in *S. aureus*, is not well understood yet. To control virulent and antibiotic resistant bacteria, it would be of importance to investigate how *S. aureus* regulates the acceptance or rejection of foreign DNA. However, for a fact is that (mis)use of antibiotics at (sub)inhibitory concentrations promotes HGT of MGEs in *S. aureus* and other (opportunistic) pathogenic bacteria. Therefore it is of great importance to use non SOS-response promoting antibiotics for treatment of bacterial infections, possibly together with biofilm-inhibiting agents to prevent conjugational transfer.

References

Beaber JW, Hochhut B, Waldor MK (2003) SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427:**72-74

Bell M (2014) Antibiotic misuse: A global crisis. *JAMA Internal Medicine* . 2014.3289

Blanc DS, Basset P, Nahimana-Tessemo I, Jaton K, Greub G, Zanetti G (2011) High proportion of wrongly identified methicillin-resistant *Staphylococcus aureus* carriers by use of a rapid commercial PCR assay due to presence of *Staphylococcal* cassette chromosome element lacking the mecA gene. *J Clin Microbiol* **49:**722-724.

Bosch T, Verkade E, van Luit M, Landman F, Kluytmans J, Schouls LM (2014) Transmission and persistence of livestock-associated MRSA among veterinarians and their household members. *Appl Environ Microbiol* . 02803-14

Campbell A, Berg D, Lederberg E, Starlinger P, Botstein D, Novick R, Szybalski W (1979) Nomenclature of transposable elements in prokaryotes. *Plasmid* **2:**466-473

Caspi R, Altman T, Billington R, Dreher K, Foerster H, Fulcher CA, Holland TA, Keseler IM, Kothari A, Kubo A, Krummenacker M, Latendresse M, Mueller LA, Ong Q, Paley S, Subhraveti P, Weaver DS, Weerasinghe D, Zhang P, Karp PD (2014) The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Research* **42:**D459-D471.

Chen I, Dubnau D (2004) DNA uptake during bacterial transformation. *Nature Reviews Microbiology* **2:**241-249

Chen J, Carpena N, Quiles-Puchalt N, Ram G, Novick RP, Penadés JR (2014) Intra-and inter-generic transfer of pathogenicity island-encoded virulence genes by cos phages. *The ISME journal.* 1751-7370/14

Chlebowicz MA, Mašlaňová I, Kuntová L, Grundmann H, Pantůček R, Doškař J, Dijl JMv, Buist G (2014) The *Staphylococcal* Cassette Chromosome *mec* type V from *Staphylococcus aureus* ST398 is packaged into bacteriophage capsids. *International Journal of Medical Microbiology.* 304 (2014) 764–774

Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, Jamklang M, Chavalit T, Song JH, Hiramatsu K (2006) *Staphylococcal* cassette chromosome mec (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. *Antimicrob Agents Chemother* **50:**1001-1012.

Cohen S, Sweeney HM (1973) Effect of the prophage and penicillinase plasmid of the recipient strain upon the transduction and the stability of methicillin resistance in *Staphylococcus aureus*. *J Bacteriol* **116:**803-811

Cohen S, Sweeney HM (1970) Transduction of Methicillin Resistance in *Staphylococcus aureus* Dependent on an Unusual Specificity of the Recipient Strain. *J Bacteriol* **104:**1158-1167

Corvaglia AR, Francois P, Hernandez D, Perron K, Linder P, Schrenzel J (2010) A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical *Staphylococcus aureus* strains. *Proc Natl Acad Sci U S A* **107:**11954-11958.

Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, Lessa FC, Lynfield R, Nadle J, Petit S (2013) National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA internal medicine* **173:**1970-1978

Demerec M (1945) Production of Staphylococcus Strains Resistant to Various Concentrations of Penicillin. *Proc Natl Acad Sci U S A* **31:**16-24

Fagerlund A, Granum PE, Håvarstein LS (2014) *Staphylococcus aureus* competence genes: mapping of the SigH, ComK1 and ComK2 regulons by transcriptome sequencing. *Mol Microbiol.* 94(3), 557-579.

Ferrer MD, Quiles-Puchalt N, Harwich MD, Tormo-Mas MA, Campoy S, Barbe J, Lasa I, Novick RP, Christie GE, Penades JR (2011) RinA controls phage-mediated packaging and transfer of virulence genes in Gram-positive bacteria. *Nucleic Acids Res* **39:**5866-5878.

Goerke C, Koller J, Wolz C (2006) Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **50:**171-177.

Grilo IR, Ludovice AM, Tomasz A, Lencastre H, Sobral RG (2014) The glucosaminidase domain of Atl–the major *Staphylococcus aureus* autolysin–has DNA-binding activity. *MicrobiologyOpen* **3:**247-256

Grohmann E, Muth G, Espinosa M (2003) Conjugative plasmid transfer in gram-positive bacteria. *Microbiol Mol Biol Rev* **67:**277-301.

International Working Group on the Classification of *Staphylococcal* Cassette Chromosome Elements (IWG-SCC) (2009) Classification of *Staphylococcal* cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother* **53:**4961-4967.

Jensen LB, Garcia-Migura L, Valenzuela AJS, Løhr M, Hasman H, Aarestrup FM (2010) A classification system for plasmids from enterococci and other Gram-positive bacteria. *J Microbiol Methods* **80:**25-43

Katayama Y, Ito T, Hiramatsu K (2000) A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **44:**1549-1555

Kleckner N (1990) Regulation of transposition in bacteria. *Annu Rev Cell Biol* **6:**297-327

Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* **29:**1128-1132.

Lindsay JA (2014) *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. *International Journal of Medical Microbiology* **304:**103-109

Little JW, Mount DW (1982) The SOS regulatory system of Escherichia coli. *Cell* **29:**11-22

Llewelyn M, Cohen J (2002) Superantigens: microbial agents that corrupt immunity. *The Lancet infectious diseases* **2:**156-162

Madigan MT, Martinko JM, Parker J, Brock TD (1997) *Biology of microorganisms*. Prentice Hall, Upper Saddle River, NJ

Maiques E, Ubeda C, Campoy S, Salvador N, Lasa I, Novick RP, Barbe J, Penades JR (2006) beta-lactam antibiotics induce the SOS response and horizontal transfer of virulence factors in *Staphylococcus aureus*. *J Bacteriol* **188:**2726-2729.

Malachowa N, DeLeo FR (2010) Mobile genetic elements of *Staphylococcus aureus*. *Cellular and molecular life sciences* **67:**3057-3071

Marraffini LA, Sontheimer EJ (2008) CRISPR interference limits horizontal gene transfer in *Staphylococci* by targeting DNA. *Science* **322:**1843-1845.

Mašlaňová I, Doškař J, Varga M, Kuntová L, Mužík J, Malúšková D, Růžičková V, Pantůček R (2013) Bacteriophages of *Staphylococcus aureus* efficiently package various bacterial genes and mobile genetic elements including SCCmec with different frequencies. *Environmental microbiology reports* **5:**66-73

McCarthy AJ, Lindsay JA (2012) The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. *BMC Microbiol* **12:**104-2180-12-104.

McCarthy AJ, Loeffler A, Witney AA, Gould KA, Lloyd DH, Lindsay JA (2014) Extensive Horizontal Gene Transfer during *Staphylococcus aureus* Co-colonization *In vivo*. *Genome Biol Evol* **6:**2697-2708.

McDonnell RW, Sweeney HM, Cohen S (1983) Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and Staphylococcus epidermidis. *Antimicrob Agents Chemother* **23:**151-160

Monk IR, Foster TJ (2012) Genetic manipulation of *Staphylococci*—breaking through the barrier. *Frontiers in cellular and infection microbiology* **2:**49.

Morikawa K, Takemura AJ, Inose Y, Tsai M, Ohta T, Msadek T (2012) Expression of a cryptic secondary sigma factor gene unveils natural competence for DNA transformation in *Staphylococcus aureus*. *PLoS pathogens* **8:**e1003003

Morikawa K, Inose Y, Okamura H, Maruyama A, Hayashi H, Takeyasu K, Ohta T (2003) A new *Staphylococcal* sigma factor in the conserved gene cassette: functional significance and implication for the evolutionary processes. *Genes to Cells* **8:**699-712

Novick RP (2003) Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of *Staphylococcus aureus*. *Plasmid* **49:**93-105

Novick RP (1989) *Staphylococcal* plasmids and their replication. *Annual Reviews in Microbiology* **43:**537-563

Novick RP, Christie GE, Penadés JR (2010) The phage-related chromosomal islands of Gram-positive bacteria. *Nature Reviews Microbiology* **8:**541-551

Quiles-Puchalt N, Martínez-Rubio R, Ram G, Lasa Í, Penadés JR (2014) Unravelling bacteriophage ϕ11 requirements for packaging and transfer of mobile genetic elements in *Staphylococcus aureus*. *Mol Microbiol* **91:**423-437

Quiles-Puchalt N, Tormo-Mas MA, Campoy S, Toledo-Arana A, Monedero V, Lasa I, Novick RP, Christie GE, Penades JR (2013) A super-family of transcriptional activators regulates bacteriophage packaging and lysis in Gram-positive bacteria. *Nucleic Acids Res* **41:**7260-7275.

Ram G, Chen J, Ross HF, Novick RP (2014) Precisely modulated pathogenicity island interference with late phage gene transcription. *Proc Natl Acad Sci U S A* **111:**14536-14541.

Ram G, Chen J, Kumar K, Ross HF, Ubeda C, Damle PK, Lane KD, Penades JR, Christie GE, Novick RP (2012) *Staphylococcal* pathogenicity island interference with helper phage reproduction is a paradigm of molecular parasitism. *Proc Natl Acad Sci U S A* **109:**16300-16305.

Rao VB, Feiss M (2008) The bacteriophage DNA packaging motor. *Annu Rev Genet* **42:**647-681

Roberts AP, Chandler M, Courvalin P, Guédon G, Mullany P, Pembroke T, Rood JI, Jeffery Smith C, Summers AO, Tsuda M (2008) Revised nomenclature for transposable genetic elements. *Plasmid* **60:**167-173

Roberts GA, Houston PJ, White JH, Chen K, Stephanou AS, Cooper LP, Dryden DT, Lindsay JA (2013) Impact of target site distribution for Type I restriction enzymes on the evolution of methicillin-resistant *Staphylococcus aureus* (MRSA) populations. *Nucleic Acids Res* **41:**7472-7484.

Roberts RJ, Belfort M, Bestor T, Bhagwat AS, Bickle TA, Bitinaite J, Blumenthal RM, Degtyarev SK, Dryden DT, Dybvig K, Firman K, Gromova ES, Gumport RI, Halford SE, Hattman S, Heitman J, Hornby DP, Janulaitis A, Jeltsch A, Josephsen J, Kiss A, Klaenhammer TR, Kobayashi I, Kong H, Kruger DH, Lacks S, Marinus MG, Miyahara M, Morgan RD, Murray NE, Nagaraja V, Piekarowicz A, Pingoud A, Raleigh E, Rao DN, Reich N, Repin VE, Selker EU, Shaw PC, Stein DC, Stoddard BL, Szybalski W, Trautner TA, Van Etten JL, Vitor JM, Wilson GG, Xu SY (2003) A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes. *Nucleic Acids Res* **31:**1805-1812

Ruby C, Novick RP (1975) Plasmid interactions in *Staphylococcus aureus*: nonadditivity of compatible plasmid DNA pools. *Proc Natl Acad Sci U S A* **72:**5031-5035

Ruzin A, Lindsay J, Novick RP (2001) Molecular genetics of SaPI1–a mobile pathogenicity island in *Staphylococcus aureus*. *Mol Microbiol* **41:**365-377

Savage VJ, Chopra I, O'Neill AJ (2013) *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. *Antimicrob Agents Chemother* **57:**1968-1970.

Scharn CR, Tenover FC, Goering RV (2013) Transduction of *Staphylococcal* cassette chromosome mec elements between strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother* **57:**5233-5238.

Siguier P, Filée J, Chandler M (2006a) Insertion sequences in prokaryotic genomes. *Curr Opin Microbiol* **9:**526-531

Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M (2006b) ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* **34:**D32-6.

Smyth DS, Wong A, Robinson DA (2011) Cross-species spread of SCC *mec* IV subtypes in *Staphylococci*. *Infection, Genetics and Evolution* **11:**446-453

Stojanov M, Blanc D (2014) Characterization of the *Staphylococcal* cassette chromosome mec insertion site in 108 isolates lacking the mecA gene and identified as methicillin-resistant *Staphylococcus aureus* by the Xpert MRSA assay. *European Journal of Clinical Microbiology & Infectious Diseases* 1-5

Sugai M, Komatsuzawa H, Akiyama T, Hong YM, Oshida T, Miyake Y, Yamaguchi T, Suginaka H (1995) Identification of endo-beta-N-acetylglucosaminidase and N-acetylmuramyl-L-alanine amidase as cluster-dispersing enzymes in *Staphylococcus aureus*. *J Bacteriol* **177:**1491-1496

Úbeda C, Maiques E, Tormo M, Campoy S, Lasa Í, Barbé J, Novick RP, Penadés JR (2007) SaPI operon I is required for SaPI packaging and is controlled by LexA. *Mol Microbiol* **65:**41-50

Ubukata K, Nonoguchi R, Matsuhashi M, Konno M (1989) Expression and inducibility in *Staphylococcus aureus* of the mecA gene, which encodes a methicillin-resistant *S. aureus*-specific penicillin-binding protein. *J Bacteriol* **171:**2882-2885

van der Kooi-Pol MM, Reilman E, Sibbald MJ, Veenstra-Kyuchukova YK, Kouwen TR, Buist G, van Dijl JM (2012) Requirement of signal peptidase ComC and thiol-disulfide oxidoreductase DsbA for optimal cell surface display of pseudopilin ComGC in *Staphylococcus aureus*. *Appl Environ Microbiol* **78:**7124-7127.

Waldron DE, Lindsay JA (2006) Sau1: a novel lineage-specific type I restriction-modification system that blocks horizontal gene transfer into *Staphylococcus aureus* and between *S. aureus* isolates of different lineages. *J Bacteriol* **188:**5578-5585.