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Review article

Multi-drug-resistant *Staphylococcus aureus* and future chemotherapy

K. Hiramatsu*, Y. Katayama, M. Matsuo, T. Sasaki, Y. Morimoto, A. Sekiguchi, T. Baba

Research Center for Infection Control Science, Juntendo University, 2-1-1 Hongo, Bunkyo-Ku, Tokyo, Japan

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ABSTRACT

Staphylococcus (S.) aureus silently stays as our natural flora, and yet sometimes threatens our life as a tenacious pathogen. In addition to its ability to outwit our immune system, its multi-drug resistance phenotype makes it one of the most intractable pathogenic bacteria in the history of antibiotic chemotherapy. It conquered practically all the antibiotics that have been developed since 1940s. In 1961, the first MRSA was found among *S. aureus* clinical isolates. Then MRSA prevailed throughout the world as a multi-resistant hospital pathogen. In 1997, MRSA strain Mu50 with reduced susceptibility to vancomycin was isolated. Vancomycin-intermediate *S. aureus* (VISA), so named according to the CLSI criteria, was the product of adaptive mutation of *S. aureus* against vancomycin that had long been the last resort to MRSA infection. Here, we describe the genetic basis for the remarkable ability of *S. aureus* to acquire multi-antibiotic resistance, and propose a novel paradigm for future chemotherapy against the multi-resistant pathogens.

1. Introduction

Among all the antibiotic resistance achieved by *Staphylococcus aureus*, two most remarkable ones are methicillin and vancomycin resistance. The methicillin resistance was achieved by interspecies transfer of *mecA* gene from an ancestral *Staphylococcus* species to *S. aureus* mediated by a unique staphylococcal mobile genetic element. Vancomycin resistance was achieved by horizontal transfer of a plasmid-born *vanA*-gene transposon from vancomycin-resistant *Enteriococcus* to *S. aureus* across the genus barrier. The other type of vancomycin resistance is expressed by VISA, which is acquired by adaptive mutations incorporated in the genes encoding regulation of bacterial cell physiology. We shall describe below the genetic strategies underlying the organism's admirable adaptability to antimicrobial pressures, and propose the development of 'reverse antibiotics (RA)' as a new paradigm for drug discovery development in the future chemotherapy against the threat of multi-resistant *S. aureus* infection.

2. Great genetic competence of *S. aureus* to acquire antibiotic resistance

2.1. Methicillin resistance in S. aureus

Practically all *S. aureus* isolates were methicillin susceptible until 1961, when Jevons found three MRSA strains among 5440 clinical *S. aureus* strains in England [61]. Then the situation changed as humans started to use methicillin. MRSA became prevalent all over the world, and after five decades, more than half of *S. aureus* clinical strains became methicillin resistant. MRSA is born when methicillin-susceptible *S. aureus* (MSSA) has acquired the methicillin-resistance gene *mecA* by horizontal gene transfer mediated by a mobile genetic element staphylococcal cassette chromosome (SCC) [2]. SCC is a site-specific transposon-like element exclusively used among staphylococcal species [3]. The SCC elements carrying *mecA*, designated SCC*mec*, are integrated in the chromosomes of MRSA strains [2,4]. Fig. 1 illustrates the basic structure of SCC*mec* [5]. The element is composed of *mec*-gene complex encoding methicillin resistance gene *mecA*, and its regulator genes (*mecR1* and *mecI*) and *ccr*-gene complex encoding cassette chromosome recombinase (CCR) that mediates the

Abbreviation: MRSA, methicillin-resistant *Staphylococcus (S.) aureus*; PBP, penicillin-binding protein; VISA, vancomycin-intermediate *S. aureus*; hVISA, heterogeneously vancomycin-intermediate *S. aureus*; sVISA, slow VISA; VRSA, vancomycin-resistant *S. aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; TCRS, two-component regulatory systems; RA, reverse antibiotic; RNAP, RNA polymerase.

* Corresponding author. Tel.: +81 3 5802 1040; fax: +81 3 5684 7830.
E-mail addresses: khiram06@juntendo.ac.jp, khiram@aol.com (K. Hiramatsu).

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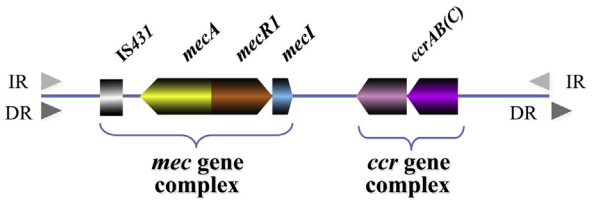

Fig. 1. The structure of SCC*mec*. SCC*mec* is composed of two essential gene complexes. One is *mec*-gene complex, encoding methicillin resistance (*mecA* gene) and its regulators (*mecI* and *mecR1*), and the other is *ccr*-gene complex that encodes the movement, (integration to and precise excision form the chromosome), of the entire SCC element. Abbreviations: IR, inverted repeat; DR, direct repeat.

element's integration into, as well as its precise excision from, the staphylococcal chromosome [3]. There are many structurally distinguishable types and subtypes in SCC*mec*. Detailed description is available elsewhere [5].

1) *oriC* environ as the storage system for useful exogenous genes

SCC is a vehicle for staphylococcal species to exchange genes that are useful for their adaptation to the niches with adverse environmental condition including antibiotic pressure. In the *S. aureus* chromosomal region downstream of the origin of replication (*oriC*), a gene named *orfX* is present. The gene is reported to encode a ribosomal RNA methyltransferase [6]. The *orfX* contains a copy of the direct repeat sequences (DR) that bracket an SCC element (Fig. 1), thus it serves as the unique integration site for SCC elements. Moreover, after the first SCC element is integrated, the second SCC can be integrated at the DR sequence present in the distal side of the first SCC element. In this way, multiple elements can be integrated in tandem forming a cluster of foreign genes downstream of *orfX*. As a result, unique chromosomal region called '*oriC* environ' is formed [5,7].

The *oriC* environ is the most diverged region among *Staphylococcus* chromosomes in terms of its length, GC content, and function of the acquired genes and their integrity. Many transposons and insertion sequences (IS) are found in the *oriC* environ, and they frequently cause deletion, recombination and even a large chromosome inversion across *oriC* [7]. In this way staphylococci can maintain only the genes needed for the survival in the on-going environmental change. Evidently, *mecA* has been the most useful gene ever since the clinical introduction of methicillin in 1960, when a few *S. aureus* strains already seem to have acquired *mecA* [1].

Various functional genes of diverse metabolic pathways are found carried by SCC in the staphylococcal *oriC* environ. Some examples are; *pbp4*, encoding penicillin-binding protein 4 (PBP4) in the cell-wall synthesis pathway [8], arginine catabolic pathway genes (ACME) [9], and *hdc* encoding histidine decarboxylase [10]. However, the genes much more frequently found in the *oriC* environ are drug-resistance genes. Besides *mecA*, such drug-resistance genes against mercury, cadmium, kanamycin, bleomycin, erythromycin, spectinomycin, and fusidic acid have been found in association with SCC elements in *oriC* environ [4,11]. Evidently, the *oriC* environ serves as the storehouse in support for achieving the multidrug-resistance phenotype. *S. aureus* quickly acquired β-lactamase plasmids soon after the penicillin G was introduced in 1940s, but no plasmid carrying *mecA* has been found. Although the reason is not clear, SCC-mediated acquisition of a single copy of *mecA* gene on the chromosome might have been less effective against penicillin-G as compared to the plasmid-born multiple copies of beta-lactamase encoding *blaZ* genes. On the other hand, *mecA* encodes cell-wall synthesis enzyme PBP2' [12]. PBP2' is a homolog of intrinsic *S. aureus* PBPs and considered to have inefficient transpeptidase activity [13,14]. As such, overproduction of PBP2' may cause turbulence in the cell-wall synthesis and a big fitness cost especially during the growth in the absence of β-lactam antibiotics. Storage of *mecA* as a single gene copy in *oriC* environ and multiple gene doses of *blaI* on the penicillinase plasmid would be the best way to maintain *mecA* in the repressed status in the drug-free growth condition. (Here, note that *blaI* gene is the cognate repressor gene of *blaZ*. The BlaI also cross-represses *mecA* gene because the cognate *mecA*-gene repressor gene *mecI* is usually deleted or inactivated by mutations [15].) Apparently, *oriC* environ is suitable for the storage of foreign genes in single copies that may have a hazardous effect on the cell physiology if overexpressed.

2) The origin of *mecA* gene

We previously identified a *mecA*-gene homolog *mecB* on the plasmids and chromosomes of *Macrococcus caseolyticus* isolates [16,17]. Macrococcal species, disseminated in nature as animal commensals, are immediate antecedents of staphylococcal species (Fig. 2) [17]. The macrococcal *mecB* was distantly related to *mecA* (61.7% nucleotide identity), and was found disseminated among the macrococcal strains as a transposon, designated Tn*6045* [16]. No complete form of SCC*mec* was found in macrococcal strains. However, many *ccr* genes are found on the plasmids and chromosomes of the macrococci, and tandem integration of an SCC element and a *mecB* transposon was observed in the *oriC* environ of a macrococcal strain [16]. Spontaneous excision of an SCC and the *mecB* transposon as a closed circle DNA from the *oriC* environ was observed, suggesting *de novo* synthesis of SCC*mec* is on-going in macrococcal species [16].

Recently, the third *mecA* gene homolog *mecC*, which exhibits 68.7% nucleotide identity with *mecA*, was found in *S. aureus* isolates from cattle and a human by using next generation sequencing technology [18]. The SCCs carrying *mecC* were also found in *Staphylococcus sciuri* [19], and *Staphylococcus xylosus* [20]. Previously, *mecA* was the exclusive genetic marker for MRSA. Now, however, we have to worry about overlooking *mecB* or *mecC*-carrying MRSA in the clinical laboratory. According to recent reports, prevalence of *mecC*-mediated methicillin resistance ranges from 0 to 2.8% among human MRSA isolates [21–25]. There is no report yet of *mecB*-carrying *S. aureus*.

Phylogenetic distribution of the *mecA* homologs illustrated in Fig. 2 suggests that *mecA* had been vertically transmitted as an ortholog for some time during the course of speciation of *sciuri*-group staphylococcal species such as *Staphylococcus fleurettii, Staphylococcus vitulinus, S. sciuri* subspecies *sciuri,* and *Staphylococcus carnaticus*. As the vertically transmitted ortholog, *mecA, mecA1,* and *mecA2* are located at the corresponding loci on the chromosomes of the *sciuri*-group species; *S. fleurettii*, *S. sciuri*, and *S. vitulinus*, respectively. They have 99.8%, 80%, and 91% nucleotide identities, respectively, to the *mecA* gene carried by SCC*mec* on the MRSA chromosome [26]. Thus, apparently, *S. fleurettii mecA* was the original *mecA,* which was adopted as the methicillin-resistance determinant of the SCC*mec* that converted *S. aureus* into MRSA. The comparative structural analysis of the *mecA* loci on the chromosomes of *sciuri*-group species corroborated this historical event [26]. Curiously, however, the *mecA* locus was not preserved intact in certain strains of *sciuri* group. Some of them possessed SCC*mec* elements carrying either *mecA* or *mecC* in the *oriC* environ instead of the functional *mecA* ortholog (Fig. 2) [27]. They seem to have had lost methicillin resistance by either deletion or mutations incorporated in the coding region or promoter sequence of the original *mecA* gene [28].

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