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Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power

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Summary

Nothing documents better the spectacular adaptive capacity of *Staphylococcus aureus* than the response of this important human and animal pathogen to the introduction of antimicrobial agents into the clinical environment. The effectiveness of penicillin introduced in the early 1940s was virtually annulled within a decade due to the plasmid epidemics that spread the β -lactamase gene through the entire species of *S. aureus*. In 1960 within one to two years of the introduction of penicillinase resistant β -lactams (methicillin), methicillin resistant *S. aureus* (MRSA) strains were identified in clinical specimens. By the 1980s, epidemic clones of MRSA acquired multidrug resistant traits and spread worldwide to become one of the most important causative agents of hospital acquired infections. In the early 2000s, MRSA strains carrying the Tn1546 transposon-based enterococcal vancomycin resistant mechanism were identified in clinical specimens, bringing the specter of a totally resistant bacterial pathogen closer to reality. Then, in the late 1990s, just as effective hygienic and antibiotic use policies managed to bring down the frequency of MRSA in hospitals of several countries, MRSA strains began to show up in the community.

Introduction

The purpose of this brief overview is to generate interest among readers in MRSA – a fascinating and dangerous human pathogen continually evolving under our eyes to cope with a constantly changing human environment. In this overview, a special emphasis was paid to the unique staphylococcal chromosome cassettes the structure of which plays a critical role in the epidemiology of both the hospital associated and community acquired MRSA.

The globally spread epidemic clones of MRSA are the product of convergence of several unusual evolutionary processes [1].

- i. The resistance mechanism itself is unusual: it involves acquisition of the *mecA* gene, the determinant of a unique penicillin binding protein PBP2A which has low affinity for β -lactam antibiotics and which can function as a surrogate of the native staphylococcal PBPs – cell wall synthetic enzymes – that are inactivated by β -lactam antibiotics [2].

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- ii. The resistance gene *mecA* appears to have evolved from a domestic gene of the fully β -lactam susceptible *Staphylococcus sciuri*, a frequent colonizer of the skin of both wild and domestic animals and one of the most abundant staphylococcal species on this planet [3].
- iii. Before “arriving” to *S. aureus* the *mecA* gene has to be incorporated into a unique molecular vector called the staphylococcal chromosome cassette (SCC) which has its own independent evolutionary history and which is capable of delivering a variety of different resistance or virulence determinants to *S. aureus*, including *mecA*.
- iv. Successful “grafting” of the SCC_{*mec*} into an *S. aureus* cell requires still additional factors: a unique and apparently rare genetic background that allows maintenance and expression of *mecA*. The relatively few successful SCC_{*mec*} acquisition events that give rise to an MRSA strain appear to require the absence of a genetic “barrier” that is frequent in the genetic background of many *S. aureus* lineages [4].
- v. Of the MRSA strains that have managed to maintain and express the SCC_{*mec*}, only a handful of lineages seem to be able to spread globally. Genetic determinants that define the superior epidemicity of these clones are not known at the present time but may be associated with genes that are responsible for the effective colonization of the host [5].

For the first three decades after their appearance, MRSA strains typically have remained hospital associated pathogens (HA-MRSA). Then, in an unexpected epidemiological “move”, MRSA strains also began to appear in the community among people who had none of the usual risk factors for such infections. Such community acquired MRSA (CA-MRSA) has since been recognized as a major and disturbing reality in many countries, but, the precise scenario that has led to its appearance is still a matter of speculation.

The origin of *mecA*

Screening of isolates from different staphylococcal species with a DNA probe internal to the *mecA* of MRSA has led to a staphylococcal species, *S. sciuri* in which each one of up to 200 epidemiologically unrelated isolates gave strong hybridization signal [3]. The large majority of these isolates were fully susceptible to all β -lactam antibiotics including penicillin. The gene homologue of *mecA* was identified as a determinant showing linear structure and conserved motifs typical of a bacterial penicillin binding protein [6], with a transpeptidase (TPase) domain that showed 92% identity with the amino acid sequence of the TPase domain of *mecA* from MRSA strains. Recent work identified the *mecA* homologue as the genetic determinant of an 84 kDa PBP in *S. sciuri*. Up-regulation of the promoter of this gene was shown to provide wide spectrum β -lactam resistance to the *S. sciuri* strain and transfer of the up-regulated *S. sciuri* *mecA* homologue on a plasmid to a fully β -lactam susceptible *S. aureus* caused substantial increase in the oxacillin MIC value of the *S. aureus* recipient (Zhou et al. *In preparation*). It was also possible to show that the plasmid-born *S. sciuri* gene was able to participate in the synthesis of peptidoglycan of the *S. aureus* transductant growing in the presence of β -lactam antibiotics and under these conditions, the *S. sciuri* *mecA* homologue produced a peptidoglycan the composition of which was typical of the host strain *S. aureus* and was completely different from the peptidoglycan of *S. sciuri* [7].

More recently, the protein product of the *S. sciuri* *mecA* homologue was purified to homogeneity and compared to the properties of PBP2A, the product of the *S. aureus* *mecA*. The two proteins showed very similar properties which included inhibition by a wide range of β -lactam antibiotics; the existence of an allosteric site for binding of *S. aureus* peptidoglycan precursors; both PBP2A and the *S. sciuri* protein contained a sheltered active site, the access

of which to both substrates and β -lactam inhibitors required a conformational change in the proteins [8].

The first MRSA type mechanism was identified in clinical specimens within a very short – 1-2 years – period after the introduction of the first penicillin resistant – oxazolidine type – β -lactam into clinical use. Given the complexity of the *mecA*-based resistance mechanism, the notion that MRSA emerged under the selective pressure of these new β -lactamase resistant antibiotics is unlikely. It was proposed recently that the evolution of *mecA* may have occurred over a much longer span of time in a staphylococcal species free of penicillinase under the selective pressure of penicillin [1] which began to be used extensively as a prophylactic agent in veterinary medicine in 1949, i.e., shortly after the introduction of penicillin into human clinical practice [9]. The staphylococcal species involved may have been *S. sciuri*, which is free of the penicillinase plasmid and is a very frequent inhabitant of the skin of domestic animals. In this scenario, an up-regulated version of the native *S. sciuri mecA* homologue may have emerged among colonizers of the skin of domestic animals under the selective pressure of prophylactic use of penicillin.

SCCmec core structure and definitions

The *mecA* gene, is embedded in a large heterologous chromosomal island – staphylococcal cassette chromosome (SCCmec) [10], that integrates into the *S. aureus* chromosome at a site-specific location (*attBsc*), located near the origin of replication [11]. The genetic organization of the close *mecA* vicinity defines the *mec* gene complex and, in *S. aureus*, three major classes have been described: class A containing the complete *mecA* regulon (*mecI-mecR1-mecA*) and classes B and C containing the *mecA* regulatory genes disrupted by insertion sequences, Ψ IS1272- Δ *mecR1-mecA* and IS431- Δ *mecR1-mecA*, respectively [12].

The mobility of SCCmec is in part due to the presence of fully functional recombinases of the invertase/resolvase family and encoded by the *ccr* gene complex [13]. The *ccr* gene complex may have two genes, *ccrAB*, with four known allotypes [14,15], or a single gene not closely related to the *ccrAB* genes, *ccrC* [16].

SCCmec types are defined by combining the class of the *mec* gene complex with the *ccr* allotype [10,14,16-18] and based on this definition a new nomenclature has been recently proposed [19], so that SCCmec type definitions are as follows (proposed new names in parenthesis): type I – *mec* class B and *ccrAB* allotype 1 (1B); type II – class A and *ccrAB2* (2A); type III – class A and *ccrAB3* (3A); type IV – class B and *ccrAB2* (2B); type V – class C and *ccrC* (5C); and type VI – class B and *ccrAB4* (4B).

The remaining parts of SCCmec are called junkyard (J) regions (J1, J2 and J3), which constitute nonessential components of SCCmec; although, in some cases, these regions carry additional antibiotic resistance determinants [20]. Starting clockwise from the origin of replication of *S. aureus* chromosome, the J3 is the region between the chromosomal left junction and the *mec* complex; J2 is the region between the *mec* complex and the *ccr* complex; and J1 is the region between the *ccr* complex and the chromosomal right junction. Therefore, SCCmec structural organization may be summarized as: J3-*mec*-J2-*ccr*-J1 – see Figure 1. Variations in the J regions (within the same *mec-ccr* combination) are used for defining SCCmec subtypes or variants.

CA-MRSA are predominantly characterized by SCCmec type IV or by type V [21](the later described only in 2004 [16]. SCCmec type IV is the smallest structural type of SCCmec and is believed to be the most mobile version [22]. Perhaps as a consequence of its enhanced mobility, SCCmec type IV is also more variable than other SCCmec types and eight subtypes (types IVa through IVh) differing mainly in the J1 region have been described so far [17,20,

23-25]. Besides frequently associated to CA-MRSA, *SCCmec* type IV is also characteristic of some HA-MRSA clones, such as the EMRSA-15, an endemic clone in the UK, which is also spreading in several European countries [26].

For the sake of the extension of this review we will focus on *SCCmec* typing strategies based on multiplex PCR assays, which are very practical and, as consequence, the most commonly used. Multiplex PCR approaches have a limited number of interrogated *loci* which may restrict the resolution of the typing strategy but, as they came in different flavors, they can cover different discriminatory needs. Still, no single multiplex PCR reaction can include the 22 out of 34 binary targets required, according to Stephens *et al* [27], to genotype properly all the 46 *SCCmec* variants.

In 2006, Kondo *et al* have proposed a *SCCmec* typing scheme based on six multiplex PCR reactions [28]. This strategy is very complete and while being able to detect the great majority of *SCCmec* it also identifies new ones. Still, due the high number of required assays it may not be feasible for routine purposes.

SCCmec typing scheme: a proposal

The choice of the best *SCCmec* typing strategy depends on the purpose of the study and its setting, such as local versus global surveillance of MRSA clones, clinical or research laboratory, etc. A flexible and feasible *SCCmec* typing scheme that could provide suitable discriminatory power for most study purposes would have three (sequential) stages and the following design.

- i. *ccrB* sequencing [29]. This tool, based on the DNA sequencing of an internal fragment of the *ccrB* gene amplified using a single pair of degenerate primers has the advantage to be easily integrated into the *spa* and MLST protocols, and, likewise, is amenable for data deposition and analysis in a central web-based database, which is currently being developed (<http://www.ccrbtyping.net/>). The sequence data generated may be used for tracing the dissemination of *SCCmec* elements within the MRSA population or even between *S. aureus* and coagulase-negative staphylococci (CoNS).
- ii. “*SCCmec* multiplex PCR” [30]. This strategy is able to identify in a single multiplex PCR assay using 20 primers *SCCmec* types I – VI, which are assigned through the presence of a specific amplification pattern of 2 – 5 bands.
- iii. “*SCCmec* IV multiplex PCR” [24]. This is a multiplex PCR assay for the subtyping of *SCCmec* type IV strains. *SCCmec* type IV is highly polymorphic and so far eight variants, differing mainly in the J1 region, have been described (IVa – IVh). Since type IV predominates among CA-MRSA, subtyping this *SCCmec* element may be critical to trace properly the dissemination of particular MRSA clones. Under this rationale, we have recently developed a single multiplex PCR assay based on the detection of subtype specific J1 sequences able to discriminate most subtypes of *SCCmec* IV – see Figure 2.

Non-mec SCC elements

The SCC element, defined as a mobile chromosomal cassette with dedicated recombinase genes (*ccr*) and characteristic flanking short sequence repeats, is not restricted to the dissemination of the *mecA* gene, as several non-*mec* SCC and ψ SCC (without or no functional *ccr* genes) elements have been described carrying other genetic determinants (for a recent review see [31]. Some of these non-*mec* SCC elements encode for traits that may contribute to the survival or pathogenic potential of the bacteria, such as resistance to heavy metals (SCC *mer* [19] and SCC CI [32] or fusidic acid (SCC *MSSA476* [33], capsule biosynthesis (SCC *cap1* [34] and SCC *15305cap* [35], potassium transport (ψ SCC *h1* [36], cell wall cross-linking

(*pbb4*) and teichoic acid biosynthesis (*tagF*) (*SCC_{pbb4}* [32], DNA protection by restriction-modification systems (*SCC_{CI}* [32], or arginine deiminase and oligopeptide permease (arginine catabolic mobile element, ψ *SCC_{ACME}* [37]. Many of these non-*mec* SCC or ψ SCC elements have been described in coagulase-negative staphylococci (CoNS), a finding which further implicates these species as responsible for the assembling and dissemination of SCC elements, including *SCC_{mec}*.

The ACME element is a striking example of SCC dissemination routes and also how these elements may contribute to the phenotypic plasticity of *S. aureus* clones. In *S. epidermidis*, a ubiquitous commensal of the human skin, the ACME element is highly prevalent, whereas in *S. aureus* it is restricted to a single lineage circulating mainly in the USA community but also in Canada and Europe (CA-MRSA clone USA300, ST8-IVa) [37,38] This clone has been implicated in several epidemiologically unassociated outbreaks of skin and soft tissue infections in otherwise healthy individuals and has also been associated with unusually invasive disease [37]. Although, all *S. aureus* carry a native arginine deiminase metabolic pathway (*arc* gene cluster), the acquisition of the ACME element was shown to increase the gene dosages, which was suggested to contribute to the unique virulent phenotype of USA300 [37]. The arginine deiminase has been established in *Streptococcus pyogenes* as a virulence factor involved in the invasion and intracellular survival of host cells [39]. Moreover, since the depletion of L-arginine by arginine deiminase inhibits nitric oxide production, the ACME element is likely to suppress both innate and adaptive host immune responses [37]. The fact that ACME has non-functional *ccr* genes and is inserted into the chromosome in tandem with *SCC_{mec}*, suggests that its acquisition was mediated by the *SCC_{mec}* resident recombinase genes. Since very few MSSA isolates positive for the ACME element have been detected [40], the USA300 MRSA clones may have originated in two sequential steps: first acquiring the *SCC_{mec}* and then the ACME element.

Number of *SCC_{mec}* acquisition events and factors that limit their occurrence

While the primary mode of spread of MRSA is clearly clonal expansion, the “birth” of each MRSA strain involves the acquisition of an *SCC_{mec}* element. Only speculations exist concerning how these genetic elements enter an *S. aureus* cell. A favored model assumes transduction by one or the other of the enormous number of phages that have been identified in staphylococci. Using the e-BURST method, the types of *SCC_{mec}* and the genetic backgrounds (sequence types) of MRSA strains, it has been estimated that the number of times *SCC_{mec}* has been acquired by the species of *S. aureus* is around 20 [22]. The same method resulted in a substantially higher number of acquisition events (around 54) for the case of methicillin resistant *S. epidermidis* [41].

One should remember that such acquisition events would only be registered if they lead to the production of a MRSA lineage that can be detected in surveillance studies. Recent experiments by Katayama and Chambers suggest that not all *S. aureus* genetic backgrounds are hospitable to maintaining the *mecA* gene in an active form. It was proposed that many *S. aureus* genetic backgrounds have a “barrier” against maintaining, transcribing and translating a plasmid born *mecA* gene and bypassing such a barrier appears to be the property of a select group of *S. aureus* genetic backgrounds [4]. The mechanism and genetic basis of the barrier effect is not known at the present time but its existence could certainly contribute to the relatively low number of MRSA lineages identified in epidemiological studies. A role for the restriction modification system in the spread of various *SCC_{mec}* types have recently been proposed by Lindsay et al. [42].

Few epidemic MRSA clones cause the largest number of MRSA disease worldwide

One of the surprising findings of the first international molecular epidemiological study on MRSA was the recognition that a handful of MRSA lineages (initially defined by shared PFGE patterns) were responsible for MRSA infections in hospitals located in Europe, USA and the far East in as many sixteen different participating countries [43]. Subsequent and more extensive international surveillance studies confirmed this finding using additional molecular typing techniques, such as MLST and SCCmec typing [44]. These observations indicate the importance of yet another evolutionary process that MRSA clones need in order to successfully compete with drug susceptible lineages of the species. It is interesting that the globally spread and highly epidemic MRSA clones often share a common genetic background with epidemic lineages of MSSA suggesting that critical determinants for epidemicity reside in the genetic background of MRSA clones [45].

Emergence of MRSA in the community

Just as the combination of strict antibiotic use and infection control procedures succeeded in reducing the level of MRSA infections in several hospitals in Nordic countries and the Netherlands, *S. aureus* “pulled” another surprise: patients from the community without any of the usual risk factors for MRSA infections began to appear in emergency rooms of hospitals most often with skin lesions caused by methicillin resistant *S. aureus* strains. Initially, and in contrast with the MRSA clones spreading in hospitals (HA-MRSA), these community acquired MRSA strains (CA-MRSA) showed diverse genetic backgrounds; carried – invariably – the relatively small SCCmec type IV or V; were rarely multi-drug resistant and exhibited less defective “fitness” (i.e., grew faster in vitro), than the typical HA-MRSA strains. Soon afterwards, unique clonal lineages of CA-MRSA began to take over, the most prominent ones being clones with the genetic backgrounds of ST30, ST80 and ST8 and often the CA-MRSA lineages also carried the genetic determinant of the PVL toxin which seems to be the critical factor for the overwhelmingly skin and soft tissue infections caused by CA-MRSA strains. (For a recent review see [46].

Conclusion

Contrasting scenarios for the evolution of hospital acquired and community acquired MRSA

In the 1960s the first MRSA was “born” in the hospital environment as an already multidrug resistant pathogen and for over 17 years spread extensively in hospitals as a single clonal type (Archaic clone) [47]. It was only after this period that new clonal types began to appear and spread globally. These epidemic HA-MRSA clones had different genetic backgrounds (defined by MLST) and carried a variety of different SCCmec types (mainly I, II and III).

In contrast, when MRSA was “born again” in the 1990s, it first appeared in the community carrying initially only a single resistance trait (*mecA*) and exclusively in the form of SCCmec types IV or V and in many diverse genetic backgrounds. Subsequently, a few highly epidemic clones began to emerge, acquire additional resistance traits and spread in the community.

These contrasting features of HA-MRSA and CA-MRSA are intriguing and raise questions about the nature of environmental and microbial factors that contributed to the surprisingly different epidemiological and molecular profiles of the “old” HA-MRSA and the “new” CA-MRSA strains. The scenario of evolution of HA-MRSA has been reviewed recently in some detail [1], and several recent reviews have addressed the various issues involving the CA-MRSA [48,49]. Nevertheless, factors that contributed to the emergence of CA-MRSA remain enigmatic.

On the molecular level the initial appearance of CA-MRSA may have been the consequence of an increased frequency in the encounters between potential SCCmec donors (most likely methicillin resistant coagulase negative staphylococci) and MSSA recipients that most frequently colonize individuals in the community. MRSE strains – frequent colonizers of the human skin – were shown to carry each one of the major SCCmec types identified in various MRSA clones including SCCmec IV [41,50] – which, together with SCCmec type V represents one of the two chromosomal cassettes identified in all CA-MRSA examined so far. The genetic background of MSSA that most frequently colonize humans in the community (such as ST30, ST8) are the same as the genetic backgrounds of the rapidly spreading clones of CA-MRSA (ST30 in the “Oceania” clone; ST8 in the USA300 clone), which eventually emerged from the initially diverse genetic backgrounds of CA-MRSA.

A new and frequent molecular marker of many – but certainly not all – CA-MRSA is the *pvl* toxin gene which seems to correlate with the high frequency of skin and soft tissue infections caused by these bacteria [46].

A second important factor that may have contributed to the rise of CA-MRSA in our era could be the emergence outside the hospital environment of some new high risk human populations and/or lifestyles that facilitate transmission of infectious agents including MRSA.

The first reports on appearance of CA-MRSA came from Australia from studies on Aboriginal communities [51] followed by reports on CA-MRSA strains spreading in Algerian immigrant populations in Southern France [52] and Indian Reservations in the USA [53]. The crowdedness, lack of hygienic culture and misuse and overuse of antimicrobial agents in such communities has been documented repeatedly.

In the mid 1990s, the carriage rate of MRSA among healthy individuals in the community was exceedingly low: less than 0.1 % in a country where over 50% of *S. aureus* hospital isolates were resistant to methicillin during the same time period [54]. A more recent survey by the CDC has confirmed that the nasal carriage of MRSA has remained extremely low among healthy individuals in the USA community [55]. On the other hand, surprisingly high carriage rate of MRSA (6-8%) was reported when “under-served” groups were assayed for MRSA carriage such as the homeless, drug addicts [56], inmates in crowded prisons of the USA and attendees of Day Care Centers. A compromised immune system (HIV positive individuals), drug addicts sharing drug paraphernalia, men having sex with men (MSM), but also the proliferation of contact sports combined with inappropriate hygienic conditions may have created an environment in which both the transfer of SCCmec from MRSE to MSSA and also the person to person transmission of infectious agents – including MRSA – is amplified. Thus when MRSA was “born again” it was not in the hospital but in some enclaves of the human community from where such CA-MRSA clones could emerge and spread and were eventually able to make their way back into hospitals both in the USA [57] and in Europe [38]. The entry into the hospitals of such MRSA clones with their relatively small “fitness” burden and frequent association with the PVL toxin may increase the seriousness of drug resistant *S. aureus* infections and make effective chemotherapy more complex and problematic [49].

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Figure 1.
Genetic organization of SCC*mec* types I – VI (Maria Miragaia, Ph.D. thesis, Universidade Nova de Lisboa, 2006).

Figure 2.

“SCCmec IV multiplex PCR” (Adapted with permission from reference [24].

Pannel A - Genetic organization of the subtypes of SCCmec type IV. Pannel B – Amplification patterns obtained with the SCCmec multiplex IV PCR for the prototype strains of SCCmec IV subtypes. M, DNA molecular size marker (1 Kb DNA Ladder Plus, Invitrogen Life Technologies, Carlsbad, USA).