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(25) The evolution and dynamics of methicillin-resistant *Staphylococcus aureus*

Mohamed M. H. Abdelbary[1], Patrick Basset[1], Dominique S. Blanc[1], Edward J. Feil[2]

[1] Hospital Preventive Medicine, Centre Hospitalier Universitaire Vaudois and University hospital of Lausanne, Lausanne, Switzerland.

[2] The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.

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Table of Contents

1. Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) have been first isolated in 1961, soon after the introduction of methicillin for therapeutic treatments. Since then, the rate of MRSA isolates among *S. aureus* has continuously increased to reach 60% in some health care institutions. The resistance to methicillin is conferred by the acquisition of the *mecA* gene, which is located on a large mobile genetic element called the Staphylococcal chromosome cassette *mec* (SCC*mec*) and so far eleven types of SCC*mec* elements have been described (types I to XI). The population structure of *S. aureus* has been shown to be highly clonal and previous genomic comparisons showed that about 75% of the *S. aureus* genome is highly conserved (core genome), while the remaining 25% varies significantly among strains (accessory genome). The accessory genome consists mostly of mobile genetic elements, such as bacteriophages, pathogenicity islands, genomic islands, plasmids, transposons, and the SCC*mec* element. The importance of understanding the patterns of evolution of MGEs is illustrated by the SCC*mec* element, which has been acquired and/or transferred on multiple occasions during the evolution of the species.

The study of the population genetics of *S. aureus* has been addressed using multilocus sequence typing (MLST). Based on these data, the population structure of *S. aureus* was classified into related groups of strains defined as clonal complexes and their isolated sequence types. Extensive typing showed that the *S. aureus* population associated with humans consists of ten major lineages and most MRSA belong to six of them. The presence of the same clones in different geographical regions suggested their rapid dissemination. Moreover, the epidemiology of MRSA is highly dynamic, and clonal replacement of predominant clones within a given locale has been widely documented. Although the reasons why some clones replace others are unclear, the emergence and replacement of clones might have significant consequences for public health, as different clones possess differing resistance and virulence attributes.

2. Introduction

Staphylococcus aureus is a Gram-positive bacterium that typically resides asymptomatically in the anterior nares and the skin of mammals. Since its discovery in the 1880's, it has been recognized as a major opportunistic pathogen in humans, responsible for various diseases, ranging from minor skin infections to severe bacteraemia and necrotising pneumonia. Before the era of antibiotics, the mortality rate of patients infected with *S. aureus* exceeded 80% [1]. The introduction of penicillin in the early 1940s saved the lives of tens of thousands of wounded allied troops in the Second World War and dramatically improved the prognosis of patients with staphylococcal infections. However, as early as 1942, penicillin-resistant staphylococci were recognized, and these strains arose via the acquisition of a plasmid carrying a gene encoding a penicillinase (β-lactamase). Although the spread of penicillin-resistant *S. aureus* was initially confined to hospital settings, this was quickly followed by the wider dissemination of resistance in the community. By the late 1960s, more than 80% of both community- and hospital-associated *S. aureus* isolates were resistant to penicillin [2]. This pattern is being repeated for methicillin, an alternative semisynthetic β-lactam antibiotic that was designed to resist β-lactamase. Since the introduction of this antibiotic in the 1960s, various hospital-associated methicillin-resistant *S. aureus* (HA-MRSA) clones disseminated worldwide, and virulent

community-associated MRSA (CA-MRSA) and life stock associated (LA-MRSA) have continued to emerge and spread from the mid-1990s onwards.

3. The Staphylococcal Cassette Chromosome *mec*

Staphylococcus aureus is naturally susceptible to most antibiotics, and resistance is often acquired by the horizontal transfer of genes from intrinsically resistant coagulase-negative staphylococci. These genes are generally located on mobile genetic elements (MGEs) such as plasmids or cassettes.

The resistance to methicillin and all other β-lactam antibiotics is conferred by the acquisition of the methicillin resistance gene *mecA* [3]. This gene is carried on a MGE called the staphylococcal chromosome cassette *mec* (SCC*mec*) [4]. This MGE is likely to have been introduced into the *S. aureus* population on multiple occasions from related staphylococcal species [5, 6]. Several structural variants of SCC*mec* have been described, which differ in their gene content and size (21-67 kb), but share four characteristics. First, they carry the *mec* gene complex (*mec*) that made up of the methicillin resistance determinants *mecA*, its expression regulatory genes (*mecR1* [promoter] and *mecI* [repressor]), and the insertion sequence(s). Second, they carry the cassette chromosome recombinase gene complex (*ccr*), which consists of genes that are responsible for the mobility of the element. Third, they have characteristic repeated sequences at both ends. Fourth, they integrate into the *S. aureus* chromosome at a site-specific location (*attBscc*), located within *orfX* near the origin of replication [7-10]. Despite these common characteristics, the detailed structure of SCC*mec* elements is highly divergent. In particular, several allotypic differences have been identified in *ccr* and *mec* complexes [11], as described below.

***ccr* gene complex.** So far, three distinct *ccr* genes have been described (*ccrA*, *ccrB*, and *ccrC*) in *S. aureus*. Whereas *ccrC* is usually found alone, *ccrA* and *ccrB* are generally found adjacently on the same element. In addition, several allotypes of *ccrA* and *ccrB* have been identified. The presence of these genes and allotypes has been used to distinguish among the eight different *ccr* types that are currently observed (Table 1).

***mec* gene complex.** The region of the *mec* gene complex differs among SCC*mec* elements in its composition of regulatory genes (*mecI* and *mecR1*) and/or insertion sequences (IS431 and IS1272). So far, six classes of *mec* gene complexes have been described (A, B, C1, C2, D and E) in *S. aureus* (Table 1).

The typing of *SCCmec* elements has become essential for several reasons. First, in combination with the genotype of the *S. aureus* chromosome, the SCC*mec* type is an important characteristic for defining MRSA clones in epidemiological studies and to understand the evolution of these clones [12]. Second, the various SCC*mec* elements also differ in their patterns of antibiotic susceptibility, which have important clinical implications. For instance, SCC*mec* type I as well as type IV-VIII cause only resistance to β-lactam antibiotics. In contrast, the largest SCC*mec* types II and III cause resistance to multiple classes of antibiotics due to the integration of plasmids or transposons carrying multiple resistance genes within these elements.

Table 1. Major SCC*mec* elements identified in *S. aureus* from (Ito et al., 2009).

ccr gene complex			*mec* gene complex		SCCmec
ccr genes		*ccr* type	*mec* genes	*mec* class	type
ccrA1	and				
ccrB1		1	IS*1272-ΔmecR1-mecA*-IS*431*	B	I
ccrA2	and				
ccrB2		2	*mecI-mecR1-mecA*-IS*431*	A	II
ccrA3	and				
ccrB3		3	*mecI-mecR1-mecA*-IS*431*	A	III
ccrA2	and				
ccrB2		2	IS*1272-ΔmecR1-mecA*-IS*431*	B	IV
ccrC		5	IS*431-ΔmecR1-mecA*-IS*431*	C1[a]	V
ccrA4	and				
ccrB4		4	IS*1272-ΔmecR1-mecA*-IS*431*	B	VI
ccrC		5	IS*431-ΔmecR1-mecA*-IS*431*	C2[a]	VII
ccrA4	and				
ccrB4		4	*mecI-mecR1-mecA*-IS*431*	A	VIII
ccrA1	and				
ccrB1		1	IS*431-ΔmecR1-mecA*-IS*431*	C2[a]	IX
ccrA1	and				
ccrB6		7	IS*431-ΔmecR1-mecA*-IS*431*	C1[a]	X
ccrA1	and		blaZ-*mec*A*LGA251-mec*R1*LGA251-*		
ccrB3		8	*mec*I*LGA251*	E	XI

[a] mec class C1 and C2 differ in the orientation of IS431 upstream of mecA

Several SCC*mec* typing methods have been developed, among which the most widely used are based on multiplex PCR assays that identify the different *ccr* types and *mec* classes [12-16]. These have a limited number of targets, which may restrict their resolution but can be combined according to the level of discrimination required by the study. Two additional sequence-based typing methods based on the *ccr* gene complex have also been proposed [14, 17], and these are likely to provide further useful data. Although SCC*mec* typing is essential for the characterization of MRSA clones in epidemiological studies, it is only recently that a rationalized nomenclature for the SCC*mec* has been proposed [7, 11].

***mecA* gene homologue (*mecC*).** Recently, a novel *mecA* gene homologue, called *mecC*, has been discovered in the genome of *S. aureus* strain LGA251 that was isolated from bovine mastitis [18]. MRSA strains harbouring *mecC* have subsequently been reported from several European countries, and are associated with multiple host species including humans [19-23]. Similar to the *mecA* gene, *mecC* is located within the SCC*mec* element (SCC*mec* type XI) and inserted into the 3' region of *orfX*. In addition, several *S. aureus* virulence factors, such as adhesions, and toxins were detected among *mecC* MRSA strains [24, 25]. The *mecC* gene has been detected in several staphylococcal and other related bacterial species, although the origin of *mecC* in *S. aureus* remains unclear.

Currently, a broad range of commercial and PCR-based approaches are available for the detection of *mecC* MRSA strains, and these have significant diagnostic value for both human and veterinary public health [26-29].

4. Evolution of *Staphylococcus aureus* and MRSA

Most detailed studies on the population genetics of *S. aureus* have been performed using MLST (Box 1). Based on MLST data, the population of *S. aureus* was classified into related groups of strains defined as clonal complexes (CCs) and isolated sequence types (STs) [30]. These CCs are considered as different genetic lineages within the *S. aureus* population and only few differences are detected within groups although the characteristics of MGEs (e.g. SCC*mec*) may vary substantially [31].

Extensive typing showed that the *S. aureus* population associated with humans consists of ten major lineages (i.e. CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45 and CC51), as well as several other minor lineages (Figure 1) [15, 30, 32]. These lineages have not only been identified using MLST but also using other categories of genes [33] confirming the biological reality of the CCs. These CCs generally have a radial genetic structure with a founder ST surrounded by numerous single locus variants of the founder. This observation highlights that with the exception of MGEs the genetic diversity within each lineage is remarkably low [31, 34]. For example, the non-mobile genome of two strains belonging to CC1 (MW2 and MSSA476) differ at only 285 single nucleotide polymorphisms (SNPs) despite one was a PVL positive MRSA isolated in the USA and the other a PVL negative MSSA isolated in the UK [35]. The low variability observed within CCs might be explained by recent expansion and/or strong purifying selection. Although the relative contribution of each of these factors is difficult to disentangle, purifying selection was described for several categories of genes such as the seven housekeeping genes used for MLST typing, core and accessory adhesion genes [36] as well as many others [37-39]. This suggests that purifying selection is an important factor that acts on the chromosome of *S. aureus* and it probably affects the diversity observed within CCs.

Another important factor is the low rate of homologous recombination within the core genome of *S. aureus*. Although several chromosomal replacement events were described for *S. aureus* [40, 41], this species has been shown to be highly clonal using a variety of genes: MLST [32], cell surface *sas* genes [33], cell surface core and accessory (i.e. not present in all the strains) adhesion genes [36] , accessory exotoxin-like genes [42]. For example, using MLST data, it was shown that genetic differences between a single locus variant and its ancestral strains were created 15 times more frequently by a point mutation than by a recombination event [32]. This low rate of recombination can help to explain why the clonal complexes have remained discrete and coherent in the *S. aureus* population, and why the same basic groups tend to be defined regardless of the genes used for typing (with the notable exception of *agr* [43]).

The genetic diversity of MRSA is also known to be much smaller than MSSA [44] (and the most common MRSA isolates belong to only six CCs (i.e. CC1, CC5, CC8, CC22, CC30 and CC45). In contrast to MSSA, the genetic diversity of MRSA differs considerably among countries and dominant MRSA lineages form distinctive geographical clusters, at least in Europe [44]. This largely reflects the recent origin of many MRSA clones, that is, since the first administration of methicillin in 1961. This means that there has been insufficient time for the MRSA clones to fully homogenize geographically.

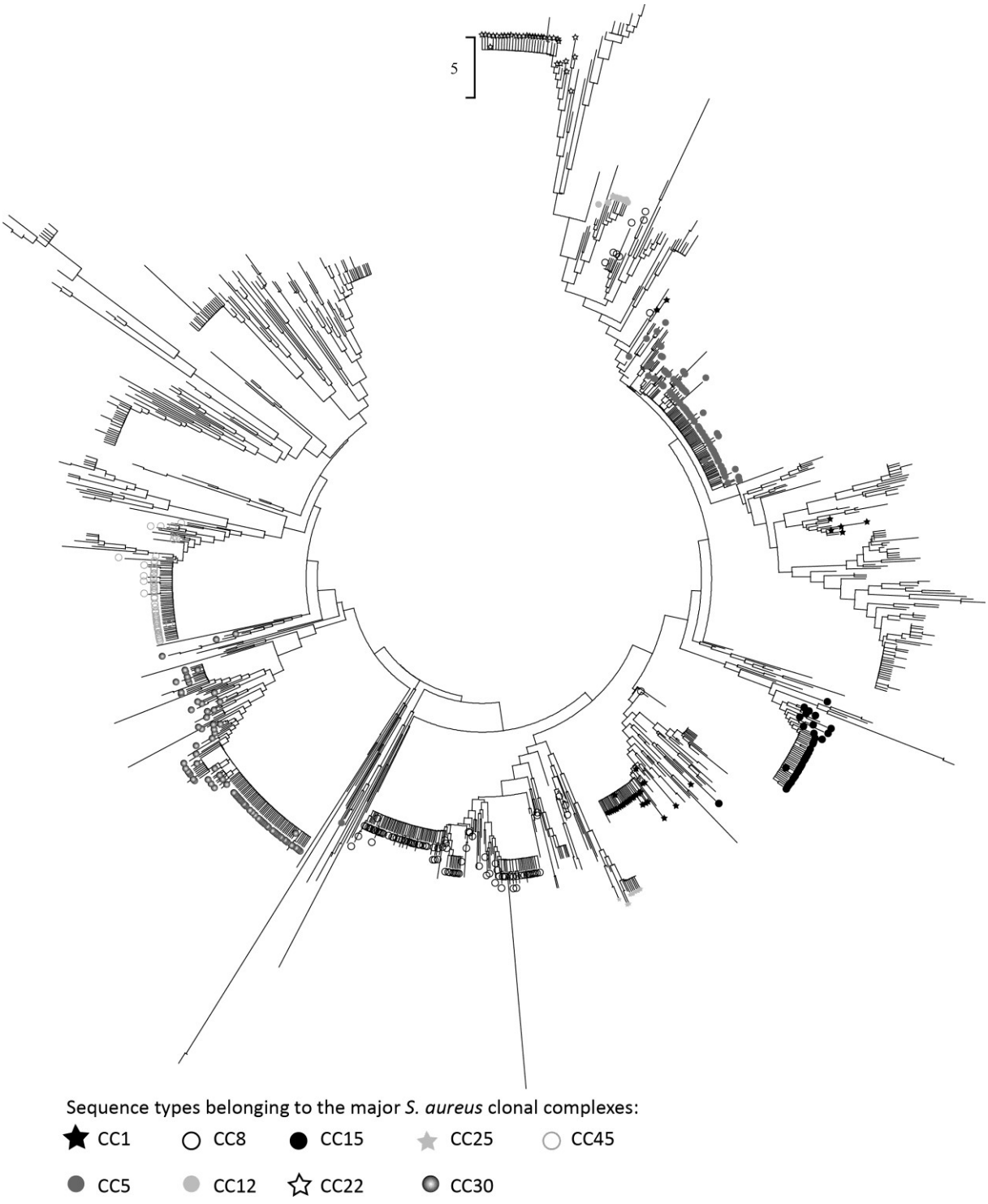

Figure 1 Neighbor-Joining tree of the concatenated sequence of all the STs available in the *S. aureus* MLST database (http://saureus.mlst.net/). The position of the sequence types belonging to major *S. aureus* clonal complexes is indicated by a colored dot.

4.1 Mobile genetic elements

Bacterial genomes can be viewed as two compartments of genes, one comprising "core" genes that are ubiquitously present in all clones of a given species, and the other comprising "accessory" or "non-core" genes that are not present in all isolates in the population, and that have a propensity for horizontal transfer [45]. Whole genome sequence and microarray data have revealed that about 75% of a typical *S. aureus* genome is present in more than 95% of the strains (*i.e.* core genome) [46-48]. As expected, the majority of genes comprising the core genome are composed of species-specific genes and genes associated with central metabolism and other housekeeping function. In contrast, the gene content of the remaining 25% of the genome varies significantly among strains (*i.e.* accessory genome).

The accessory genome mostly consists of MGEs, such as bacteriophages, pathogenicity islands, genomic islands, staphylococcal chromosome cassettes (SCCs), plasmids or transposons. Many of these genetic elements carry virulence genes (*e.g. tst and PVL which are carried on bacteriophages*) [48, 49] and resistance to antibiotics (e.g. *mecA carried in SCCmec*) [30]. The gene content of a particular *S. aureus* strain is thus a combination of (i) vertical inheritance of its core genome, and (ii) horizontal transfer of MGEs, allowing rapid adaptation by loss or gain of virulence and/or resistance genes [48]. Thus, there is a considerable proportion of the genome that is not essential for survival and that contributes to genetic differences between strains. The distribution and horizontal spread of these elements can have important clinical implications and the characterization of these elements is providing insights into how *S. aureus* is evolving and cause diseases.

Whole genome comparisons indicated important variation in the distribution of genomic islands. This suggests that MGEs are readily exchanged in the *S. aureus* population. For example, genome comparison of one MRSA strain with one MSSA strain showed at least 5 different acquisition/loss events involved in differences in virulence factors and drug resistance [48]. Horizontal transfer of MGEs is also suggested by the phylogenetic distribution of these elements, which does not correlate with the genetic relatedness inferred by MLST. This lack of correlation suggests that mobile elements facilitate the exchange of virulence and antibiotic resistance determinants between *S. aureus* lineages and may lead to rapid changes in the pathogenic potential or drug resistance of strains. In contrast, the sequence of the core genome is remarkably constant [10, 50-53].

Several studies suggested that some toxin genes (e.g. toxic shock syndrome toxin 1 (*tst*), leukocidin DE (*lukDE*) and superantigens (*sea*, *seg* and *sei*)) are associated with particular lineages (MLST CCs) [54, 55], and there is evidence of frequent acquisition and loss of particular elements that is restricted to particular CCs. Variability of accessory genes such as resistance, toxin or virulence genes, has recently been described within two STs (ST5 and ST228) of CC5 [56]. However, the biological significance and modalities of this intra-strain variability still need to be clarified.

The importance of understanding the patterns of evolution of MGEs is illustrated by the evolution of the SCC*mec* element. The evolution from MSSA to MRSA involves the acquisition of a SCC*mec* element by an MSSA strain. The exact mechanisms explaining how the SCC*mec* elements enter the *S. aureus* cell are not clearly known. However the transduction by phages is often postulated [8]. The frequency of transfer of SCC*mec* elements as well as their geographical history is also poorly known. Identical clones have been sampled in different countries suggesting a single *SCCmec* acquisition, followed by clonal spread. Yet, the presence of multiple SCC*mec* types in MRSA suggests multiple introductions into *S. aureus*. Moreover, the

occurrence of isolates with identical ST but with different *SCCmec* types indicates that horizontal transfer of SCC*mec* elements is relatively frequent within *S. aureus* [30]. Using MLST, it has been shown that the SCC*mec* element must have been acquired on multiple occasions (at least 20 times) during *S. aureus* evolution [17, 30, 33]. A previous study based on SNPs discovery on a worldwide collection of ST 5 showed a close association between phylogenetic lineages and geography [31]. These data suggest that geographical spread of MRSA over long distance is a rare event compared with the frequency with which the *SCCmec* is imported locally. Moreover, MSSA strains genetically identical to the predominant MRSA strains have been observed at a local level [57, 58], confirming the possibility of local acquisitions of the SCC*mec*.

5. Molecular epidemiology of MRSA

Epidemiological surveillance of MRSA has been greatly facilitated by the development of molecular typing procedures. The grouping of isolates into clones depends on the typing method used (e.g. PFGE, MLST, *spa* typing, and recently whole-genome sequencing) (Box 1). MLST provides a robust-typing system by grouping related *S. aureus* strains into distinct sequence types (STs) based on the sequences of internal fragments of seven housekeeping genes [30]. However, as MLST is only based on the variation within a very small proportion of the genome, much of the fine molecular microevolutionary detail such single nucleotide polymorphisms (SNPs), genome re-arrangements and small INDELS remains undetected. Even before the advent of whole genome sequencing, it was clear that single MLST genotypes often encompassed multiple types as defined by other techniques. For example, ST 239 includes E-MRSA 1, 4, 11 and the Brazilian, Portuguese, Viennese and Hungarian clones, and ST 5 includes the New York/Japan and the Paediatric clones, as defined by PFGE, *spa*- and/or SCC*mec*-typing [59]. Similarly, two Swiss clones (clone D and G) were indistinguishable by MLST, exhibited identical STs (ST228), SCC*mec* type I and virulence gene content as determined by PCR yet differed by 16 bands by PFGE [60]. These differences were likely the result of the gain or loss of mobile genetic elements (MGEs) such as phage, which would not be detected by other approaches. In contrast, in other cases micro-variation is detected by MLST and other methods (such as individual SNPs) but not by PFGE. None of the traditional typing methods provided optimal resolution in all cases. The advent of whole-genome sequencing has provided such a "once size fits all" approach, in that it provides unprecedented discriminatory power for epidemiological surveillance, outbreak investigations and for better understanding of the evolutionary dynamics of both the core and non-core genome of MRSA. [Please Insert Box 1 about here]

Many studies have demonstrated that high frequencies of MRSA within a given location tend to reflect the clonal spread of only one or two clones (e.g. [59-67]). The domain of dominance of specific clones can range in size from a single hospital, single country, or even neighbouring countries [68-70]. Analysis of more than 3,000 isolates from Southern Europe, USA and South America showed that nearly 70% of them belong to 5 major pandemic clones, namely the Iberian (ST247-SCC*mec* I), Brazilian (ST239-SCC*mec* III), Hungarian (ST239-SCC*mec* III), New York/Japan (ST5-SCC*mec* II), and Pediatric (ST5-SCC*mec* IV) clones [15, 59, 71]. The addition of three more clones would essentially encompass Northern Europe: the EMRSA-15 (ST22-SCC*mec* IV), EMRSA-16 (ST36-SCC*mec* II) and Berlin (ST45-SCC*mec* IV) clones [30]. Therefore, it was hypothesized that these clones are particularly transmissible and/or well adapted to the hospital environment [72, 73].

The epidemiology of MRSA is highly dynamic, and clonal replacement of predominant clones within a given locale has been widely documented. Whereas cross-sectional studies showed the

predominance of one or two clones in a defined setting in the 90's, several longitudinal studies showed the replacement of the predominant clones by others within a decade [59, 74]. A very early example was the replacement in England of EMRSA-1 (ST239) by EMRSA-15 and –16 [73] Other ST239 variants (e.g. in particular the Brazillian clone and the Hungarian clone) have subsequently become very widespread throughout South America, Eastern Europe and mainland Asia (including both China and the middle East), where this genotype may account for at least 90% of all cases of HA-MRSA. Additionally, another pandemic clone replaced the Iberian clone on at least two occasions. It was first replaced by EMRSA-16 in one Spanish hospital while the rate of MRSA among *S. aureus* remained constant [74], and by the Brazilian clone in one Portuguese hospital [75]. The fact that on both occasions the Iberian clone was replaced might suggest that it lost its epidemic potential during the last decade. Other examples are the complete replacement in a 2-year period of a local clone (ST5-SCC*mec* IV) by the New York/Japan clone (ST5-SCC*mec* II) in a Mexico City hospital (Velazquez-Meza et al., 2004) and the replacement of the Berlin clone by a variant from the New York/Japan clone (ST105-SCC*mec* II) and by the South Germany clone (ST228- SCC*mec* I) in an area of low MRSA incidence in Western Switzerland [60]. Although the reasons why some clones replace others are typically unclear, the emergence and replacement of clones might have significant public health consequences as different clones possess differing resistance and virulence attributes [74-80]. For instance, during the 1990s in France, the replacement of the Iberian clone (ST 247- SCC*mec* I) by the Lyon clone (ST8- SCC*mec* IV) resulted in a change of the susceptibility profile to antibiotics, the Iberian clone being less susceptible than the Lyon clone (e.g. to gentamicin and co-trimoxasole) [76].

For regions outside of Europe, North America and Australia the picture may be different. For example, ST239 that probably emerged in the mid 1960s [34] is a probable major cause of HA-MRSA infection throughout mainland Asia and South America, a geographical region that holds more than 50% of the world's human population [81]. This sequence type always exhibits a variant of the large SCC*mec* type III; however, four cases of HA-MSSA ST239 were detected in China [82]. ST239 is rarely found outside of the hospital setting, which makes its rapid global dissemination, which must have occurred largely through very short transmission chains between hospitals, even more remarkable.

Perhaps of greatest concern is the emergence of specific MRSA clones within the community. Up until the 1990s, MRSA was found to be restricted to hospitals, but the 2000s have witness a dramatic increase in virulent MRSA clones in the community (CA-MRSA) [83]. These clones are generally characterized by the presence of a SCC*mec* type IV or V and the phage-borne genes encoding the Panton-Valentine leukocidin (PVL) toxin. This toxin is widely considered to be an important virulence factor, particularly for paediatric infection. Molecular typing has revealed that CA-MRSA clones are distinct from those noted in hospital settings [67, 84]. ST80-SCC*mec* IV provides a notable example of an emerging CA-MRSA clone, which is currently restricted to several European communities with low social status (e.g. homeless people). Although the widespread HA-MRSA does not appear to have adapted to the community, it seems that clones that emerge in the community may be able to spread in hospitals. For example, ST8-SCC*mec* IV (generally called the USA300 clone) spread mainly in the USA, initially in the community but is currently also causing a major burden in hospital settings [85, 86]. In countries with low incidence of hospital MRSA such as northern European countries, CA-MRSA has become a major concern [87].

The spread of community-acquired MRSA clones is possibly related to the small size of the SCC*mec* types IV and V. There is a trade-off to the acquisition of resistance, which is that it

imparts of fitness cost which may render the strain uncompetitive against susceptible strains when antibiotics are not present in the environment. This is thought to be the reason why infection, and carriage, of HA-MRSA clones have remained largely confined to health-care settings. The smaller type IV and V SCC*mec* cassettes do not confer multiple resistances, but may also result in a smaller fitness cost.

Although the epidemiological distinctions between CA-MRSA and HA-MRSA can be largely explained in terms of the fitness cost of resistance, the more general question of why a single MRSA clone can predominate in a given area, or the forces underlying clonal replacement, are far less well understood. It is probable that genetic differences underlie increased or decreased fitness (transmissibility) [61, 72, 73, 88, 89], and some general traits have been identified, which may account for epidemic spread. These include the ability to survive in the environment, to colonize the host, to multiply on epithelial and mucosal surfaces, to "detach" from the host, and to resist to various antimicrobials. However, stochastic effects and extrinsic factors, such as local compliance to infection control measures and local use of antibiotics, may also have unpredictable consequences for the local composition of circulating MRSA clones. Furthermore, the specific genetic differences corresponding to fitness effects are very difficult to identify due to extensive gene redundancy and the possibility of subtle epistatic or regulatory effects playing a major role. The precise relationship between the “spread” (epidemicity) of a clone, and its virulence potential, is also unclear.

These complications can perhaps explain why a number of studies drawing comparisons between epidemic and sporadic MRSA have not generated clear experimental evidence consistent with the different epidemiological patterns [90-96]. An exception is a study demonstrating differences in biofilm production and adhesion to epithelial cells within epidemic variants of the Brazillian clone (ST239-III) [97]. Although these laboratory comparisons were carried out on a small sample of strains, an epidemiological study also found evidence for increased virulence of an ST239 variant (TW20) which caused a recent outbreak in a London hospital [98].

Molecular approaches have also not provided a clear understanding of epidemiological differences between clones. Population genetic analyses based on nucleotide sequence data of both housekeeping (MLST) genes and cell surface adhesion genes (which play a key role in host invasion) have also largely failed to detect robust links between genotype and epidemic phenotype [36]. Comparative genome hybridization and WGS have also been used to compare epidemic and sporadic strains but this approach also failed to identify any genes likely to play a major role in increased transmission [99, 100]. These findings are strong evidence against the presence or absence of a single common specific factor differentiating epidemic from sporadic *S. aureus* clones.

Although the evidence linking genotype and epidemiological phenotype is in many cases weak, there are tantalizing clues. For example, the CA-MRSA strain USA300 has disseminated widely throughout the United States. Genome sequencing of this strain revealed a novel genetic element, the arginine catabolic mobile element (ACME), which contained the gene for the arginine deiminase that may play a crucial role in the growth and survival of the bacterial cells [50, 101]. However, recent studies have shown that among 9% to 15% of the USA300 strains do not carry the ACME genomic region [102]. In addition, genome sequencing of 10 other isolates from the same disseminating clone confirmed its recent expansion [103]. Similarly, for the multidrug-resistant ST59 strains, a clone that is predominant in Taiwan has truncated *hsdM* and *hsdS* genes that encode the restriction-modification system. Hence, it was suggested that this

deficiency in the restriction-modification system might have assisted the acquisition of mobile genetic elements from enterococci, which confer multi-drug resistance [104].

Besides being a human pathogen, *S. aureus* also colonizes the skin of animals and can cause a wide range of infections [105-107]. Livestock-associated MRSA (LA-MRSA) strains attract particular attention as the potential for zoonotic transmission raises the concern for public health. Previous reports have shown that distinct MRSA genotypes are associated with specific animal species. However, several studies have documented the transmission of LA-MRSA among different host species (e.g. from animals to humans and vice versa) [108-110]. For instance, the LA-MRSA CC398 was first detected in pig farms and farmers from Europe, and has since been discovered to colonise and cause infections in other animals species (e.g. poultry, horses, dogs and cattle) and humans worldwide [111-115]. Furthermore, CC398 was reported from humans lacking direct contact with livestock or livestock workers [116-118]. A study based on the whole-genome sequencing approach, demonstrated that CC398 originated in human as MSSA and was transmitted to livestock, where it subsequently acquired methicillin resistance [119]. Likewise, phylogenetic analysis of the MRSA CC5 poultry strains revealed that they have originated in humans and later transmitted to poultry, where it subsequently acquired avian-specific MGEs [110]. In contrast, it was shown that the human pandemic MRSA CC97 strains recently made a bovine-to-human jump [120]. Taken together, these findings indicate that host-switches have been a feature in the evolution of a number of MRSA clones.

6. Conclusion

The widespread occurrence of MRSA in hospitals is recognized as a major challenge, especially with the recent emergence of strains with intermediate susceptibility to glycopeptides and of community-acquired MRSA. Given the difficulties to control MRSA, a thorough understanding of the processes underlying the emergence and spread of MRSA may help designing new strategies to counteract this evolution. Several major pandemic clones have been identified and their epidemiology may change rapidly at a regional scale. Changes in clones have significant medical consequences, since the new clones often display different antibiotic susceptibility and/or virulence patterns.

The advance of recent sequencing technologies and the development of associated bioinformatics tools will provide a superior depth in the understanding of MRSA evolutionary history. These data will allow addressing many important questions about the evolution and epidemiology of MRSA and will bridge the gap left by the low discriminatory power of MLST. However, certain challenges concerning whole-genome sequencing still need to be addressed including choosing the proper strains collection, the development of standardized analysis pipeline, and the large-scale data management.

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Box 1. Common typing methods for *S. aureus*

The epidemiology of *S. aureus* has been analyzed by an array of genotypic and phenotypic typing methods. Here, we review the methods that are currently the most widely used:

Pulsed-field gel electrophoresis – Pulsed-field gel electrophoresis (PFGE) is considered as the gold standard for *S. aureus* typing because it shows the highest discriminatory power. This method is based on the restriction of whole DNA with an enzyme that cuts only rarely. The enzyme *SmaI* is generally used for *S. aureus*. Digestion with this enzyme gives between 20 and 50 large fragments (between 10 and 700kb) that can only be separated using a pulsed gel electrophoresis. Although this method is reproducible within a laboratory, the data can be ambiguous [121] and inter-laboratory studies have highlighted the problem of standardization [122]. PFGE standardization can only be obtained with a strict control of all parameters. For example, standardized protocols have been developed for PFGE typing by the American and Canadian CDCs to build nationwide databases [123, 124].

Multi-locus sequence typing – Multi-locus sequence typing (MLST) is a typing method that combines the sequence of several housekeeping genes, and is essentially a sequenced-based version of multi locus enzyme electrophoresis (MLEE) [125]. MLST has been designed to analyze and compare genetic variation in worldwide collections of bacterial pathogens. It gives important information about the nucleotide divergence of the core genome, the clonal origin of one group of strains, the recombination rate and the phylogenetic relationship among strains. The main advantage of this method is that it gives unambiguous data that are reproducible among laboratories. Its limitations are its cost and its relatively low discriminatory power that prevent its use for local epidemiology. For *S. aureus*, the amplification and the sequencing of 450-500bp of the seven genes *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL* have been retained [57]. Alleles at each locus are assigned according to differences in nucleotide sequences. The allelic profile of the seven loci defines the sequence type (ST). For example, isolates with the profile 2-3-1-1-4-4-3 belong to ST 239, of which the Brazilian clone is an example. An international database containing more than 3,000 isolates and 1,600 STs is available at http://www.mlst.net.

spa-typing – *spa*-typing is based on polymorphism of the *spa* locus of *S. aureus,* which codes for the protein A. This locus is highly polymorphic due to an internal variable region of short tandem repeats. It varies not only in numbers but also because of nucleotide substitutions within individual repeats. A *spa* profile is identified by a succession of number representing each individual repetition of the X region. An international database has been created to standardize the nomenclature of the *spa* types (http://spaserver.ridom.de). Several studies have shown the value of this method for *S. aureus* typing [126-128]. However, this method might reflect homoplasy [31], its discriminatory power is below PFGE [129-131] and the analysis of *spa* data is not simple.

Double Locus Sequence typing – We developed a new typing method called Double Locus Sequence Typing (DLST) based on the analysis of partial sequences (ca. 500 bp) of the highly variable *clfB* and *spa* genes [130]. This method was shown to be far more discriminatory than *spa*-typing and matched the high resolution of PFGE. In addition, the combination of high typeability and reproducibility with low cost, ease of use and unambiguous definition of types makes this method promising for epidemiological analyses. It is important to note that although *spa*-typing and DLST investigate polymorphisms in the *spa* gene, these methods do not analyse the same regions of the gene. Therefore the *spa* alleles determined by these two methods are not identical.

Whole-genome sequencing (WGS) – Recently, high-throughput or whole-genome sequencing technologies have provided a significantly improved discriminatory power to study the complete genomes of various bacterial pathogens. WGS techniques generate from bacterial samples multiple short reads that can be assembled based on overlapping regions (*de novo* assembly), and/or mapped to a previously published reference genomes, which then enable the comparison between bacterial strains that genetically diverge at a single nucleotide. Such precise identification and classification of bacterial strains, as well as the parallel sequencing of different bacterial strains in single runs at low costs with a quicker turn-around times have made WGS the most convenient tool for clinical diagnostic investigations in real-time and for tracking disease outbreaks.