MiniReview

Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant Staphylococcus aureus clones

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) emerged in the early 1960s after the acquisition of the methicillin resistance gene \textit{mecA}, which is carried by the staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}). MRSA seemed to have arisen by multiple introductions of SCC\textit{mec} into successful methicillin-susceptible \textit{S. aureus} (MSSA) lineages. MRSA is one of the most common agents of nosocomial infections worldwide increasing the cost and mortality compared to MSSA infections. Little by little, MRSA has acquired resistance to all antibiotics available in clinical practice, which complicates treatment. This situation was further aggravated by the recent reports of \textit{vanA}–mediated vancomycin-resistant \textit{S. aureus}. As a reaction to the emergence and spread of multidrug-resistant MRSA worldwide, international surveillance systems such as the CEM/NET initiative have been created. The characterization of over 3000 MRSA isolates from different regions of the world evidenced the existence of only a few epidemic clones spread worldwide, namely the Iberian, Brazilian, Hungarian, New York/Japan, Pediatric and EMRSA-16 clones. It was found that in surveillance or evolutionary studies strains should be characterized by a combination of different typing methods, namely pulsed-field gel electrophoresis, multi-locus sequence typing and SCC\textit{mec} typing. In recent years, community-acquired MRSA (CA-MRSA) has become a growing public health concern. However, although many authors reported the emergence of CA-MRSA isolates, a standard definition has not been created and the prevalence of MRSA among persons without risk factors seems to remain very low. CA-MRSA has distinct properties compared to epidemic nosocomial MRSA clones and its origin is still unclear. Certain authors suggest there is MRSA transmission from the hospital setting to the community, namely transfer of nosocomial MRSA minor clones or sporadic isolates showing a high degree of similarity with CA-MRSA; others believe CA-MRSA strains represent new acquisitions of SCC\textit{mec} DNA in susceptible backgrounds. Many questions concerning this extraordinarily versatile and threatening pathogen remain unanswered, needing future investigation.

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1. Introduction

\textit{Staphylococcus aureus} has long been recognized as one of the major human pathogens and is by far one of the most common nosocomial organisms, being responsible for most post-surgical infections. \textit{S. aureus} is an opportunistic bacterium, frequently part of the human microflora, causing disease when the immune system becomes compromised. Although \textit{S. aureus} can be found in different parts of the body, anterior nares are the primary ecological niche in humans. Nasal carriage differs between individuals and is one of the major risk factors for \textit{S. aureus} infection [1]. In the healthy population \~{}20% of the individuals carry \textit{S. aureus} persistently, \~{}60% intermittently and \~{}20% never carry this bacterium [1]. \textit{S. aureus} can cause a wide variety of infections, showing three general types: (i) superficial lesions such as wound infections; (ii) systemic and life-threatening conditions such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis, and bacteremia; and (iii) toxins such as food poisoning, scalded skin syndrome and toxic shock syndrome [2]. \textit{S. aureus} has a great adaptive power to antimicrobial agents, and little by little it has been acquiring resistance to all antibiotics available in clinical practice. Due to an increasing number of infections caused by multidrug-resis-
2. Evolutionary origin of MRSA

2.1. The mecA gene

MRSA was ‘born’ at the moment it acquired the methicillin-resistant gene mecA, a 2.1-kb exogenous DNA fragment, by horizontal transfer. MRSA was first described in 1961 in England [3] and, since then, has gradually disseminated, reaching epidemic proportions in some European countries in the 1960s and in the USA in the 1970s [4]. The mecA gene encodes an additional methicillin-resistant penicillin-binding protein (PBP2A), which has very low affinity for β-lactam antibiotics [5]. PBP2A is a transpeptidase that, assisted by the transglycosidase domain of the native PBP2 of S. aureus, takes over the function of cell wall biosynthesis in the presence of β-lactam antibiotics [6].

The origins of the mecA gene are still unclear. A mecA homologue with a close sequence similarity (overall deduced amino acid similarity of 88%) to the mecA homologue with a close sequence similarity (overall de- nity for β-lactam antibiotics and investigational drugs.

2.2. The staphylococcal cassette chromosome mec

The mecA gene is carried on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), inserted into the chromosome at a site-specific location [11]. For transfer, SCCmec carries two specific genes designated cassette chromosome recombinase A and B (ccrA and ccrB). In addition to the mecA gene, SCCmec contains transposons and integrated copies of plasmids that carry various resistant genes against non-β-lactam antibiotics [12]. To date, four types of SCCmec (I–IV) and a few variants have been described, differing in size and genetic composition [13–15]. A fifth type, designated so far ‘New type’, was recently identified among the community-acquired MRSA (CA-MRSA) and is still under investigation [15].

The origins of the SCCmec are unknown and so far, no bacterial isolates of any other genera have been reported to carry this element. The presence of IS1272, an insertion sequence prevalent in Staphylococcus haemolyticus, in the SCCmec types I and IV led to the proposal that SCCmec type I was transferred from this species to S. aureus in the past [16].

The detection of divergent MRSA lineages [17–20] as well as the identification of different SCCmec types within a MRSA genotype [17,20] evidenced that MRSA has arisen by multiple independent introductions of mec into successful MSSA lineages.

3. Contemporary scene

3.1. Prevalence of MRSA

Initially, MRSA nosocomial infections were mainly detected in large tertiary hospitals and in intensive care units, where colonized and infected patients as well as colonized health care workers were a significant source of cross-infection. Currently, MRSA is one of the most common pathogens in hospitals of all sizes worldwide.

The prevalence of MRSA in hospitals continues to increase worldwide. In the USA, the National Nosocomial Infections Surveillance (NNIS) system reported a 51.3% methicillin rate among S. aureus strains from 18 397 intensive care unit patients between January 1998 and June 2002 [21], which corresponds to an increase of 25% relative to the rates reported for 1995–1999 [22]. In Central Europe (Austria, Germany and Switzerland), the prevalence of MRSA increased from 1.7% in 1990 to 8.7% in 1995 [23]. Data from the SENTRY surveillance study reported a nosocomial prevalence of MRSA of 34.2% in the USA, 26.3% in Europe and 34.9% in Latin America during 1997–1999 [24], and 40.4% in South Africa, 66.8% in Japan and 22.4% in Australia during 1998–1999 [25].

International comparisons indicate that there is considerable variation in the prevalence of MRSA among hospitals and countries within a region. The European Antimicrobial Resistance Surveillance System reported significant differences in the proportion of MRSA in blood isolates between European countries. The prevalence during the period 1999–2001 was much lower (< 3%) in Scandinavia, The Netherlands and Iceland, and higher (> 30%) in Southern Europe as well as in the UK and Ireland [26].
3.2. Vancomycin resistance

Vancomycin, the first glycopeptide antibiotic, was isolated in the mid-1950s and introduced into clinical practice in 1958 [27]. Due to an increasing number of infections caused by multi-resistant MRSA worldwide, vancomycin has been the drug of choice for treatment of staphylococcal nosocomial infections for the last 20 years.

In 1996, the first clinical vancomycin intermediate-resistant S. aureus (VISA) with a minimum inhibitory concentration (MIC) of 8 mg l⁻¹ was documented [28]. Although VISA strains have now been isolated in many countries around the world, they remain rare [29].

Hetero-vancomycin intermediate-resistant S. aureus (hVISA), first described by Hiramatsu et al. [30], display a MIC of ≤ 4 mg l⁻¹, but possess stable subpopulations (ca. 1/10⁶) that can grow in the presence of > 4 mg l⁻¹ of vancomycin. A number of studies have aimed to determine the prevalence of hVISA, which is largely dependent on the definition and methodologies employed for screening and confirmation [31]. Taking all available evidence, it seems that the prevalence of hVISA in most parts of the world is approximately 0–5% of MRSA, although it does appear to be more common in some areas of France, Germany, Spain and possibly Japan [29].

The potential importance of hVISA is that it may be associated with treatment failure and it may be a precursor of VISA, as proposed in the first description by Hiramatsu et al. [30]. Subsequently, evidence of the evolution of hVISA to VISA during an infection has been reported in a New York VISA strain [32].

Data reported from Japan [30], New York [32], Spain [33], France [34], Poland [35], Germany [36], Brazil [37] and Greece [38] indicate that VISA and hVISA strains emerged from MRSA clones prevalent in those areas (i.e., the New York/Japan MRSA [39,40], the Iberian [41,42] and the Brazilian [43] clones).

The molecular mechanism of vancomycin resistance has not been fully elucidated. However, thickening of the cell wall through accumulation of increased amounts of peptidoglycan with reduced levels of cross-linking, either by increased synthesis or by reduction of the turnover, seems to be common to all VISA and hVISA strains. This results in an increase of free d-Ala-d-Ala side chains to which vancomycin can bind, and more vancomycin molecules are trapped in the peptidoglycan layers before reaching the cytoplasmic membrane where the peptidoglycan synthesis occurs (for a review see [29]).

Since the recognition of vancomycin-resistant enterococci in 1988 [44], the emergence of vancomycin-resistant S. aureus (VRSA) (MIC ≥ 32 mg l⁻¹) has been anticipated. Although the transfer of the genetic element containing the vancomycin resistance gene vanA from Enterococcus faecalis to S. aureus was demonstrated in the laboratory in 1992 [45], the first clinical infection with VRSA harboring vanA was reported only 10 years later in the USA. In June 2002, a VRSA displaying a MIC of > 128 mg l⁻¹ was isolated from a patient in Michigan [46]. A vancomycin-resistant E. faecalis was isolated from the same infection site, suggesting vanA transfer from enterococci to staphylococci. Alarming, a second VRSA clinical isolate, containing the vanA gene (MIC = 64 mg l⁻¹), was detected 3 months later in Pennsylvania, USA [47]. Fortunately, to date, no VRSA transmission has been reported.

3.3. Community-acquired MRSA

MRSA, besides having established itself as a major hospital pathogen, is now beginning to appear in the wider community as well. CA-MRSA strains were first reported in Western Australia in 1993 from hospital patients who resided in remote communities [48]. However, it was the 1999 report of four pediatric deaths in Minnesota and North Dakota, USA, resulting from CA-MRSA infection [49] that generated great interest in this organism and raised important questions about the prevalence and origin of CA-MRSA.

Although many authors reported the emergence of CA-MRSA isolates, a standard definition does not exist and at least eight different classifications have been used to classify MRSA infections as community-acquired [50]. The commonly used term CA-MRSA implies that it is known that the organism was acquired in the community. However, this term is often used to refer to the detection of colonization or infection in the community, rather than to actual acquisition of MRSA in the community. MRSA colonization can persist for months to years [51] and the acquisition of MRSA frequently goes unrecognized unless clinical infection develops, making it difficult to know with certainty the true site of acquisition [50]. The presence of risk factors known to be associated with acquisition of MRSA, i.e., recent hospitalization, recent surgery, recent outpatient visit, recent nursing home admission, recent antibiotic exposure, chronic illness, injection drug use and close contact with a person with risk factor(s) for MRSA acquisition [50], should be evaluated before classifying an MRSA isolate as CA-MRSA.

Although several studies have reported a high prevalence of CA-MRSA, a global analysis that examined the results of 57 studies on the prevalence of CA-MRSA among hospital patients or among community members reported that most persons with CA-MRSA had more than one health care-associated risk, which suggests that the prevalence of MRSA among persons without risk factors still remains very low (≤ 0.24%) [50]. The high MRSA colonization rates reported among members of ‘closed populations’, such as Australian aboriginal [48] and Native American [52] communities, may be associated with risk factors for spread in the community, such as overcrowding, high rates of skin infections and frequent use of broad-spectrum antibiotics [53].

Salgado et al. [50] reported that the increase in CA-
MRSA among non-hospitalized patients seems to be mainly due to the introduction of health care-associated strains into the community. Furthermore, Aires de Sousa and de Lencastre [54] found interesting similarities between CA-MRSA and sporadic nosocomial MRSA isolates, raising the possibility that at least some of the MRSA strains described as community-acquired may actually originate in hospitals. By contrast, other authors reported that the clones found among the CA-MRSA are different from any of the major hospital-acquired MRSA clones [15,55], suggesting CA-MRSA may arise de novo through horizontal acquisition of the mecA gene [56]. Moreover, an Australian study reported the introduction of a strain (originated in the community) into the hospital setting [57].

3.4. Increased costs, mortality and preventive measures

According to the NNIS, as many as 80,000 patients per year get an MRSA infection after entering hospital. Infection with MRSA increases the cost and the risk of mortality [58]. The higher cost of treating MRSA infections is due to a variety of factors. First, vancomycin is more expensive than the drugs normally used to treat S. aureus infections. Second, it is often necessary to isolate the patients to keep them from infecting other patients. Third, patients with MRSA infection stay longer in the hospital [59].

In a meta-analysis study, the death rate for patients with MRSA bacteremia was estimated to be about two times higher than the death rate due to bacteremia caused by MSSA [60]. Moreover, in comparison to patients with MSSA surgical site infections (SSI), patients with MRSA SSI had five additional days of hospitalization, a 1.9-fold increase in hospital charges and a 3.4-fold increase in mortality during the 90-day post-operative period [61]. Verhoef et al. [62] estimated that the costs to bring an outbreak of MRSA (in which three to five patients are infected) under control in the Utrecht University Hospital, The Netherlands, can amount to US$250,000. This would involve closure of the intensive care unit (ICU) or ward, postponing operating programs, surveillance cultures, etc.

Reducing the incidence of methicillin-resistant and -sensitive nosocomial infections would reduce the mortality rate and societal costs of S. aureus infections [63]. Many different infection control strategies have been used by hospitals, but no single strategy has been accepted as appropriate for all hospitals. In The Netherlands, the very low MRSA incidence rate may be explained by the stringent antibiotic policy and the so-called Search and Destroy strategy practised in Dutch hospitals. The key elements of this strategy are quarantine in a single room of patients from hospitals ‘abroad’ for 48 h until MRSA cultures are negative, screening of all other patients and health care workers once a patient is found to carry MRSA, and closure of the ward or ICU when two or more patients or one health care worker are found positive with the same MRSA strain until complete disinfection of the ward, decolonization of the personnel and isolation of MRSA-positive patients [62].

3.5. Rebirth of multidrug-susceptible MRSA

New epidemic MRSA strains susceptible to several, or virtually all, non-β-lactam antibiotics have first emerged in several European countries, presenting additional therapeutic options for staphylococcal infections when antibiotics effective against MRSA were becoming scarce. In 1992, a new phenotype arose in French hospitals, characterized by the unexpected reappearance of heterogeneous expression of methicillin resistance and susceptibility to various antibiotics including gentamicin, tetracycline, minocycline, lincomycin, pristinamycin, co-trimoxazole, rifampicin and fusidic acid [64,65]. During the following 7 years, the incidence of isolation of strains of this phenotype has increased steadily throughout France, often replacing the classical multidrug-resistant MRSA [65]. A similar clone was recently reported to have replaced the usual multi-resistant MRSA clones and to predominate (69.1%) in a Greek hospital with a high incidence of MRSA [66]. A marked decrease in the use of gentamicin was suspected to be a factor contributing to the emergence of gentamicin-susceptible MRSA from predominantly gentamicin-resistant MRSA populations in France [64]. However, changes in aminoglycoside consumption alone cannot explain the increase in susceptibility to other antibiotics and the reappearance of heterogeneous resistance to methicillin observed with the new MRSA phenotypes. Rather, fitness benefit, namely growth advantage, may be related to the spread of these more susceptible clones [67].

4. Characterization of MRSA

Monitoring and limiting the intra- and interhospital spread of MRSA strains requires the use of efficient and accurate epidemiologic typing systems that allow the discrimination between unrelated isolates and the recognition of isolates descending from a common ancestor (i.e., belonging to the same clone).

During the last four decades, multiple phenotypic and genotypic typing methods have been developed to type MRSA. The choice of a typing method depends upon the needs, skill level, resources of the laboratory and the type of question to be answered (short- or long-term analysis). An optimal typing method should show high type-ability, adequate stability, high technical reproducibility and high discriminatory power. Additionally, ease of use, ease of interpretation, rapidity, accessibility and low costs may be considered convenient criteria [68].

Phenotypic typing methods such as phage typing, antimicrobial susceptibility testing and multi-locus enzyme
electrophoresis are often limited in reproducibility, as the expression of different genes is often influenced by environmental factors. Moreover, some of these methods lack typeability or discriminatory power and, consequently, are not the most adequate approaches for bacterial comparison.

The shortcomings of phenotype-based typing methods have led to the development of typing methods based on the microbial genotype or DNA sequence. The main genotypic techniques used for MRSA typing include: (i) plasmid analysis; (ii) Southern hybridization analysis of digested chromosomal DNA, such as ribotyping, ClaI-mecA::Tn554 polymorphisms, and binary typing; (iii) polymerase chain reaction (PCR)-based techniques, such as random amplified polymorphic DNA (RAPD), repetitive element sequence based-PCR (rep-PCR), amplified fragment length polymorphism, and SCCmec typing; (iv) pulsed-field gel electrophoresis (PFGE); and (v) sequence typing techniques such as spaA typing and multi-locus sequence typing (MLST). These genotypic techniques minimize problems with typeability and reproducibility and, in some cases, enable the establishment of large databases of characterized organisms.

5. International surveillance of MRSA

As a response to the emergence and worldwide spread of antibiotic-resistant bacterial pathogens there was an urgent need for the creation of international surveillance systems with harmonized methodologies that could help hospital infection prevention and control of such organisms.

The Center for Molecular Epidemiology and International Network (CEM/NET) was created in 1995 to keep track of the movement and to identify the reservoirs of major multidrug-resistant clones of *S. aureus* and other Gram-positive pathogens [69]. The identification of clones was based on a combination of different typing methods such as DNA hybridization with the meca and Tn554 probes, PFGE, RAPD, SCCmec typing, spaA typing and MLST.

HARMONY, another international network, established a collection of European epidemic and other important MRSA strains and developed a standardized PFGE protocol, facilitating international comparisons and tracking of isolates [70].

5.1. Identification of few pandemic clones

Under the CEM/NET initiative, over 3000 MRSA isolates from Europe (Portugal, Spain, Italy, Greece, Turkey, Scotland, Belgium, Germany, Denmark, Poland, Czech Republic and Hungary), Latin America (Brazil, Argentina, Chile, Uruguay, Colombia and Mexico), North America (USA) and Asia (Japan, Taiwan, and China) were characterized by different molecular typing techniques. Six clonal types were identified (the Iberian, the Brazilian, the Hungarian, the New York/Japan, the Pediatric and the EMRSA-16 clones) that seemed to be spread in different regions of the world. These studies contributed to the evidence of the existence of only a few epidemic clones spread worldwide [17]. The combination of the CEM/NET results with the information available in the literature allowed the construction of a map with the distribution of these six pandemic clones (Fig. 1).

The Iberian clone was first identified as the strain responsible for the massive 1989 outbreak of MRSA disease in a hospital in Barcelona, Spain (for reference see table 1 of [14]), but seemed to have been already present in Belgium and France at least since 1984 [71]. Subsequently, it was detected in several Portuguese hospitals (for references see table 1 of [14]) and in many other European countries such as the Czech Republic (for reference see table 1 of [14]), Poland [35], Sweden [70], Italy and Scotland (for reference see table 1 of [14]). This clone was also associated to epidemics in Germany and The Netherlands [71] and found in a few isolates among the CA-MRSA in Finland in persons who had contacts with hospitals [56]. Moreover, the Iberian clone was detected in one hospital in New York (for reference see table 1 of [14]).

The Brazilian clone was shown to be widely disseminated in Brazilian hospitals [43] and to have spread to neighboring countries in South America: Argentina (for references see table 1 of [14]), Uruguay and Chile [72], and to Europe: Portugal, the Czech Republic (for references see table 1 of [14]) and one hospital in Greece [73], where it displaced the local major clone. It was also found in other European countries such as Finland, Germany, Ireland, The Netherlands, Poland, Sweden and the UK [20].

The Hungarian clone has been widely spread in Hungarian hospitals since 1993 (for references see table 1 of [14]) and was recently described as the major clone in two hospitals in Taiwan and China [74].

The New York/Japan clone was identified as the major clone in different states of the USA, namely in New York, Connecticut, New Jersey and Pennsylvania (for references see table 1 of [14]), in several hospitals across Canada [75] and in a hospital in Tokyo [39]. Besides, it was also detected in Europe, in Finland, Ireland and the UK [20].

Clone EMRSA-16 is one of the dominant types of MRSA found in UK hospitals [76]. This clone was widely disseminated in Greece [73], Mexico [72] and Canada [75]. EMRSA-16 was responsible for the largest single-strain outbreak in Scandinavia that occurred in Sweden during the period 1997–2000 [77] and was found among the CA-MRSA in Finland in persons who had contacts with hospitals [56]. This clone was also found in other European countries such as Denmark, Switzerland and Belgium [70].

The Pediatric clone was first reported in 1991 in a pediatric hospital in Portugal (for reference see table 1 of...
(14]) and since then has been found in Poland (for reference see table 1 of [14]), France and the UK [20], the USA, Argentina and Colombia (for references see table 1 of [14]).

5.2. Evolution of the clonal profile of MRSA in Portugal (1990–2000)

The nosocomial prevalence of MRSA in Portugal was estimated as close to 50% in 1996–1997 [78], remaining one of the highest in Europe [26]. The characterization of nosocomial MRSA isolates recovered from different cities of Portugal allowed the construction of a temporal scheme for the evolution of MRSA during the period 1990–2000 in Portuguese hospitals (Fig. 2). At least two epidemiologically important events were recorded: (i) in 1992–1993, the replacement of the Portuguese clone (PFGE type C, ST239-SCCmec type III variant), widespread in Portuguese hospitals in the mid-1980s and early 1990s, by the Iberian clone (PFGE type A, ST247-1A), and (ii) in 1994–1995, the appearance of the Brazilian clone (PFGE type B, ST239-III/IIIA) and its rapid increase in representation since then. These studies indicate a selective advantage of the Iberian clone relative to the Portuguese clone as well as a selective advantage of the Brazilian clone relative to the Iberian clone.

It is noteworthy that the Iberian and Brazilian clones were imported from two countries with a strong connection with Portugal: Spain, the sole country that shares borders with Portugal, and Brazil, a Portuguese-speaking country. The absence of effective control barriers in Portuguese hospitals for colonized or infected patients prior to hospitalization may play a critical role in the introduction of new clones in the hospitals.

5.3. Origins and evolution of minor nosocomial MRSA clones

In contrast to these epidemic clones, there are also clones that are clearly dominant in single hospitals but not seen in others (minor clones), and isolates recovered only from one or a few patients in a single hospital (sporadic isolates). The characterization of nosocomial MRSA isolates of rare occurrence by different typing methods demonstrated extensive diversity among these isolates. Nevertheless, the isolates could be grouped into restricted clonal complexes by using the BURST (i.e., based upon related sequence types) program algorithm, which predicted that most sporadic MRSA isolates have evolved from pandemic MRSA clones [54].
6. CA-MRSA versus sporadic hospital isolates of MRSA

CA-MRSA were reported to be genetically very diverse and to harbor preferentially SCCmec type IV [15]. Most CA-MRSA isolates harboring SCCmec type IV appear to be resistant to β-lactam antibiotics only and have a heterogeneous methicillin resistance phenotype that is consistent with the lack of any antibiotic genes other than meca in this type of mec cassette [14,15]. Besides the relatively simple antibiotic resistance profile, CA-MRSA strains displaying SCCmec type IV were also reported to show several additional differences from most hospital-acquired MRSA strains. CA-MRSA seemed to grow faster in vitro [15] and to carry additional virulence genes [79].

Our recent studies documented several interesting similarities between CA-MRSA and MRSA of rare occurrence present in hospitals [54]. Both CA-MRSA and the nosocomial sporadic MRSA are genetically diverse, frequently carry SCCmec type IV, are susceptible to more antimicrobial agents and may contain the Panton–Valentine leukocidin genes. These observations raise the possibility that at least some of the MRSA strains described as community-acquired may actually originate in hospitals. Presumably, because of their relatively limited antibiotic resistance, sporadic MRSA isolates have a reduced capacity for spread and maintenance in the hospital environment, which is dominated by the multidrug-resistant MRSA clones. After having ‘escaped’ from the antibiotic-saturated hospital environment into the community, such a sporadic MRSA may be in an advantageous position, since it is free of the biological cost of maintaining multidrug resistance mechanisms.

7. Comparison of different typing methods for the differentiation of MRSA clones

Due to the enormous selective pressure associated with widespread antibiotic usage in contemporary hospitals, most MRSA isolates representing different clones demonstrated the same phenotypic pattern of multiple antibiotic resistance, and cannot be differentiated by antimicrobial susceptibility testing. Exceptionally, resistance to trimethoprim-sulfamethoxazole (SXT) was demonstrated to be an important phenotypic marker that can be easily and rapidly used to discriminate two important international clones, the Iberian (SXT-susceptible) and the Brazilian MRSA (SXT-resistant) [80].

Southern hybridization analysis with the meca and Tn554 probes of digested chromosomal DNA combined with PFGE was successfully used for the identification of MRSA clones in several surveillance studies [17]. However, this method is time-consuming and is too discriminatory for isolates belonging to some pandemic clones.

Due to the generation of low-intensity bands it is difficult to compare results of independent RAPD and PCR assays and therefore these methods are not adequate for surveillance or evolutionary studies. However, both of these PCR-based techniques, which are fast and feasible in most molecular biology laboratories, may be used efficiently as a screening method in the investigation of suspected outbreaks, allowing a prompt intervention of the infection control committees and implementation of appropriate measures for controlling a possible outbreak.

PFGE has many of the features associated with the ideal typing method and is generally accepted as the current ‘gold standard’ for typing MRSA. To improve its reproducibility and the comparison of results, several groups focused on the standardization of the method [70,81]. In surveillance studies, PFGE is found to be the most discriminative method. However, in the case of pandemic clones, which have a relatively ‘long’ evolutionary history associated with its successful adaptation in many different hospital ecosystems, the interpretation should be cautious, since a high degree of genetic variation is expected, leading to the appearance of multiple subtype variations. For example, the 151 isolates from Brazil [72], the 67 isolates from the Hospital de Santo António [82], Portugal, and the 71 isolates from Greece [73] belonging to the Brazilian clone showed 30, 24, and 23 PFGE subtypes, respectively. Therefore, in studies involving clones that have a relatively long existence, or in evolutionary studies, interpretation of banding patterns based on the current guidelines [83] may be inadequate.

spaA typing shows rapidity and ease of use, but since it involves a single polymorphic gene the discriminatory power is limited. For instance, this method alone could not differentiate between the Brazilian, Hungarian and Portuguese clones (KAOMQ motif), nor the New York/Japan and the Pediatric clones (DMGMYK motif) [17]. In addition, clone EMRSA-16 showed a quite similar motif (KAOMQQQ) to the Brazilian and related clones [73]. Despite its limited resolution, spaA typing represents an alternative method, which provides useful information as the first approach in investigating an epidemic outbreak [84].

The main advantage of MLST is the unambiguous ability to compare the results obtained in different laboratories via the Internet (www.mlst.net). This method alone exhibits a similar resolution as the spaA typing in the discrimination of the major clones; the Brazilian, Hungarian and Portuguese clones showed ST239, the New York/Japan and Pediatric clones showed ST5, whereas the Iberian clone and EMRSA-16 showed ST247 and ST36, respectively [17].

SCCmec typing, based on a multiplex PCR reaction [85] or on the amplification of the ccr gene and meca gene complexes [13,15], has been recently combined with MLST for MRSA typing. This association was able to distinguish between the Pediatric and the New York/Japan clones (ST5-IV and ST5-II, respectively), but could not distinguish between the ST239 clones (all SCCmec type III,
IIIa or IIIvariant). Recently, Enright et al. proposed a new nomenclature for MRSA clones, based on a combination of their multi-locus sequence type and their SCCmec type [20].

In surveillance studies involving clones that have a relatively long existence or in evolutionary studies, the application of a single method may be inappropriate (poorly or too discriminatory). In these cases we believe strains should be considered related or unrelated, based on the combination of results obtained by different typing methods, namely PFGE, MLST and SCCmec typing.

8. Future perspectives

Although multiple typing techniques have been developed and used during the last four decades to characterize MRSA, to date there is no consensus on the optimal method that permits both answering all types of epidemiological questions and inter-center data exchange. The availability of the whole genome sequence of several MRSA strains will provide useful information to enlarge the number of DNA targets for MRSA typing. We believe, in the future, procedures based on the generation of DNA banding patterns will probably be replaced by procedures producing binary output, facilitating interpretation, comparison and exchange of results. A DNA chip-mediated typing system, using different immobilized probes such as not only the seven MLST housekeeping genes [86] but also repeat elements, plasmid-specific genes, insertion sequences, transposons and binary probes, is a promising candidate for a well-accepted method for short- and long-term analysis as well as large multi-center studies.

An epidemic clone is known for its ease of transmission, long-term persistence, rapid intra- and inter-hospital spread and ability to cross geographical and continental boundaries [87]. Elucidation of the factors that contribute to the superior epidemicity of the pandemic MRSA clones, namely levels of expression of certain virulence genes, ability to survive in the environment and vehicles through which MRSA spread, will be of major importance with respect to the control of MRSA dissemination.

MRSA are spread person-to-person, by direct patient-to-patient transmission or by indirect transmission via contact with the hands of the hospital staff [88]. The spread of MRSA between hospitals and countries seems to be mainly linked to inter-hospital transfers of either S. aureus clones and/or hospital staff and associated risks. Clin. Microbiol. Rev. 10, 505-520.


References
teins in methicillin-resistant strains of *Staphylococcus aureus*. Anti-

and a native penicillin-binding protein cooperate in building the cell
wall of drug-resistant staphylococci. Proc. Natl. Acad. Sci. USA 98,
10886–10891.

ing the evolutionary origin of the methicillin resistance gene: cloning
and sequencing of a homologue of mecA from a methicillin suscep-

[8] Couto, L., de Lencastre, H., Severina, E., Kloo, W., Webster, J.A.,
Hubner, R.J., Sanches, I.S. and Tomasz, A. (1996) Ubiquitous pres-
ence of a mecA homologue in natural isolates of *Staphylococcus sciuri*.

mechA gene homologue of *Staphylococcus sciuri* into a resistance
determinant and expression of the resistant phenotype in *Staphy-

In-vivo transfer of mecA DNA to *Staphylococcus aureus* [cor-
rected]. Lancet 357, 1674–1675.

ootide sequence determination of the entire mecA DNA of pre-methi-
cillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Che-
mother. 43, 1449–1458.

 genetic element, staphylococcus cassette chromosome mec, encodes
44, 1549–1555.

[13] Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K.,
Tienasitorn, C. and Hiramatsu, K. (2001) Structural comparison of
three types of staphylococcal cassette chromosome mec integrated
in the chromosome in methicillin-resistant *Staphylococcus aureus*.

tion of pandemic clones of methicillin-resistant *Staphylococcus au-
reus*: identification of two ancestral genetic backgrounds and the

[15] Okuma, K., Ikawaka, K., Turnidge, J.D., Grubb, W.B., Bell, J.M.,
O’Brien, F.G., Coombs, G.W., Pearman, J.W., Tenover, F.C., Kapi,
methicillin-resistant *Staphylococcus aureus* clones in the com-

Characterization of IS1272, an insertion sequence-like element from

success of a human pathogen: molecular evolution of pandemic
clones of methicillin-resistant *Staphylococcus aureus*. Lancet Infect.
Dis. 2, 180–189.

resistant *Staphylococcus aureus* strains from intercontinental sources:
association of the mec gene with divergent phylogenetic lineages im-
ples dissemination by horizontal transfer and recombination. J. Clin.
Microbiol. 30, 2058–2063.

Musser, J.M. (2001) Evolutionary genomics of *Staphylococcus au-
reus*: insights into the origin of methicillin-resistant strains and the
toxic shock syndrome epidemic. Proc. Natl. Acad. Sci. USA 98,
8821–8826.

[20] Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann,
H. and Spratt, B.G. (2002) The evolutionary history of methicillin-
99, 7687–7692.


