# The multifaceted resources and microevolution of the successful human and animal pathogen methicillin-resistant *Staphylococcus aureus*

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*Methicillin-resistant* Staphylococcus aureus *(MRSA) is one of the most important bacterial pathogens based on its incidence and the severity of its associated infections. In addition, severe MRSA infections can occur in hospitalised patients or healthy individuals from the community. Studies have shown the infiltration of MRSA isolates of community origin into hospitals and variants of hospital-associated MRSA have caused infections in the community. These rapid epidemiological changes represent a challenge for the molecular characterisation of such bacteria as a hospital or community-acquired pathogen. To efficiently control the spread of MRSA, it is important to promptly detect the* mecA *gene, which is the determinant of methicillin resistance, using a polymerase chain reaction-based test or other rapidly and accurate methods that detect the* mecA *product penicillin-binding protein (PBP)2a or PBP2'. The recent emergence of MRSA isolates that harbour a* mecA *allotype, i.e., the* mecC *gene, infecting animals and humans has raised an additional and significant issue regarding MRSA laboratory detection. Antimicrobial drugs for MRSA therapy are becoming depleted and vancomycin is still the main choice in many cases. In this review, we present an overview of MRSA infections in community and healthcare settings with focus on recent changes in the global epidemiology, with special reference to the MRSA picture in Brazil.*

Key words: methicillin resistance - *Staphylococcus aureus* - MRSA - HA-MRSA - CA-MRSA

In the early 1940s, before the introduction of penicillin for the treatment of *Staphylococcus aureus* diseases, bloodstream infections (BSI) caused by this pathogen were often lethal. A study carried out at the Boston City Hospital reported a mortality rate of more than 80% for a group of 122 cases of *S. aureus* BSI (Skinner & Keefer 1941). In 1942, shortly after the introduction of natural penicillin in clinical therapy, the emergency of *S. aureus* isolates displaying resistance to this β-lactam drug was first reported (Rammelkamp & Maxon 1942). In the 1960s, approximately 80% of all clinical isolates of *S. aureus* were β-lactamase producers (Jessen et al. 1969). Thus, the need for new drugs to treat staphylococcal infections motivated the development of semi-synthetic penicillins containing a β-lactamase-resistant β-lactam ring, such as methicillin and oxacillin. However, in the early 1960s, the first isolates of methicillin-resistant *S. aureus* (MRSA) were detected in the United Kingdom (UK) (Jevons 1961).

The starting point for the MRSA evolution was the acquisition of the *mecA* gene, which encodes an exogenous penicillin-binding protein (PBP) (78-kDa), known as PBP2a or PBP2', which confers resistance to methicillin and cross-resistance to other β-lactam drugs. This protein is an alternative transpeptidase that has low affinity for β-lactam antibiotics. Therefore, PBP2a is able to catalyse cell-wall synthesis, even when normal PBPs are covalently linked to β-lactams (Brown & Reynolds 1980). The *mecA* gene is a DNA segment of 2.1 kb that is non-native to *S. aureus* and is inserted in a large block of exogenous DNA, known as the staphylococcal cassette chromosome *mec* (SCC*mec*) (Katayama et al. 2000).

The SCC*mec* is integrated in the MRSA chromosome at the 3' end of open reading frame X at the specific site attBSCC, which is located near the origin of replication in the staphylococcal chromosome and flanked by direct and repeated sequences. SCC*mec* carries the *mec* gene, a region encompassing the *mecA* gene and its regulators *mecRI* and *mecI* and the *ccr* complexes, the recombinase genes region, which is responsible for SCC*mec* mobility (Katayama et al. 2000). A number of genetic elements may be present in the SCC*mec,* including insertion elements such as IS*257* and IS*431*, plasmids such as pUB110 and pT181 and transposons such as Tn*554*. Thus, some SCC*mec* can carry genes conferring erythromycin, tetracycline, kanamycin, spectinomycin and tobramycin resistance, in addition to heavy metal detoxification genes. Because of these characteristics, SCC*mec* can be considered a resistance island. In addition to SCC*mec*, the presence of copies of IS*256* distributed in the *S. aureus* genome may also provide hot spots for the insertion of other resistance elements (Katayama et al. 2000).

Due to the increasing number of publications reporting new types, subtypes and variants of SCC*mec*,

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an international working group was created in 2009 to standardise the nomenclature and classification of new SCC*mec* types [International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC)]. All of this information, as well as the characteristics of each type of SCC*mec,* can be accessed on the IWG-SCC website (sccmec.org). Eleven types and several variants of SCC*mec* elements have been identified (Table I) and each displays characteristic combinations of the *ccr* and *mec* complexes (Turlej et al. 2011).

The first SCC*mec* that were described were types I, II and III. Currently, these types are commonly detected in MRSA strains associated with nosocomial infections (Becker et al. 2012, Yamamoto et al. 2012, David et al. 2013, El-Mahdy et al. 2013). The SCC*mec* type I was initially found in MRSA isolates predominantly in the UK in the 1960s. This SCC*mec* type contains no antibiotic resistance genes other than *mecA* (Ito et al. 2001). SCC*mec* types II and III were first associated with strains that were prevalent during the 1980s and carry multiple resistance genes (Ito et al. 1999, 2001). The subsequently described SCC*mec* type IV does not carry an antibiotic resistance gene, except for *mecA* and presents a specific combination of the *ccr* and *mec* complexes (Ma et al. 2002). Furthermore, type IV has predominantly been found among MRSA strains associated with community infections. However, isolates of paediatric and EMRSA-15 clones can also harbour SCC*mec*IV and are typically associated with nosocomial infections (de Miranda et al. 2007, Conceição et al. 2013). SCC*mec*V was first described in 2004 to be associated with MRSA involved in community-acquired infections. This element has no resistance gene other than *mecA* and carries *ccrC* (Ito et al. 2004). The SCC*mec* type VI was found in some a paediatric isolates from Portugal and France (Oliveira et al. 2006, Dauwalder et al. 2008). SCC*mec*VII was initially found in an isolate belonging to sequence typing (ST) 5, which was collected from a community-acquired infection in Sweden (Berglund et al. 2008). This last type is similar to SCC*mec*V in the sense that both carry one copy of *ccrC*, while all other SCC*mec* types harbour two copies of *ccrA* and *ccrB* (Table I). SCC*mec*VIII was detected among typical hospital isolates in Canada and has a unique combination of *mec* and *ccr* regions (Zhang et al. 2009). Finally, three more types of SCC*mec* have recently been detected. Li et al. (2011) described types IX and X, which were carried by isolates of ST398 [multilocus sequence typing (MLST) clonal complex (CC) 398 (CC398)] and both presented a number of genes encoding resistance to heavy metals. The last SCC*mec* described thus far, type XI (harbouring an arsenic resistance operon), was initially reported by Sanger Institute (Hinxton, UK) in ST425 (CC425) MRSA from bovine isolates and subsequently in ST130 (CC130) MRSA from human hospital isolates in the UK (Shore et al. 2011) (Table I).

Four main classes of *mec* complexes (A, B, C and E) were found among the MRSA strains (Turlej et al. 2011). Class A is the prototype complex and was detected in SCC*mec*II, III and VIII. This class contains complete *mecRI* and the *mecI* regulatory genes located upstream of *mecA* and the hypervariable region (HVR) and IS*431* located downstream of *mecA*. Class B was found in SCC*mec*I, IV and VI and is composed of a truncated *mecRI* (due to the insertion of IS*1272*) upstream of *mecA* and HVR and IS*431* downstream of *mecA*. Two types of class C have been described (C1 and C2) and both contain a truncated *mecRI* (due to insertion of IS*431*) upstream of *mecA* and HVR and IS*431* downstream of *mecA*. In complex C1 (SCC*mec*VII and X), the upstream and downstream copies of IS*431* are in the same orientation, while, in complex C2 (SCC*mec*V and IX), these insertion sequences are reversed. Class E (SCC*mec*XI) harbours a new *mecA* allotype known as *mecC* (formerly *mec*$A_{LGA251}$) and novel allotypes of the *mec* regulatory genes that are located upstream of *mecC*, in addition to a new allotype of *blaZ* (β-lactamase-encoding gene) downstream of *mecC* (Turlej et al. 2011) (Table I).

TABLE I

Main characteristics of the staphylococcal cassette chromosome *mec* (SCC*mec*) of *Staphylococcus aureus*

| SCC*mec* types | Main sequence typing (ST) involved | Resistance traits | *ccr*/*mec* complexes |
|---|---|---|---|
| I | ST5, ST228, ST247 | Only *mecA* | A1B1/B |
| II | ST5, ST36 | Antimicrobial resistance genes | A2B2/A |
| III | ST93, ST239 | Antimicrobial resistance genes | A3B3/A |
| IV | ST1, ST5, ST8, ST22, ST30, ST36, ST45, ST59, ST80 | Only *mecA* | A2B2/B |
| V | ST1, ST8, ST59, ST152, ST398 | Only *mecA* | C1/C2 |
| VI | ST5 | Only *mecA* | A4B4/B |
| VII | ST59 | Only *mecA* | C1C1 |
| VIII | ST8, ST59 | Only *mecA* | A4B4/A |
| IX | ST9, ST398 | Heavy metal resistance genes | A1B1/C2 |
| X | ST398 | Heavy metal resistance genes | A1B6/C1 |
| XI | ST425, ST130 | Arsenic resistance operon | A1B3/E |

Despite effective antimicrobial therapy, the incidence of this alarming pathogen has continuously increased. In 1970, it was estimated that MRSA accounted for only 2% of the *S. aureus* isolates found in hospitals of different countries (O'Toole et al. 1970). The late 1980s and early 1990s were marked by a rapid, worldwide spread of multiresistant MRSA (Sanches et al. 1995, Teixeira et al. 1995, Mato et al. 1998, Kahla-Clemenceau et al. 1999). By the 2000s, approximately 60% of *S. aureus* isolates collected from hospitalised patients in the United States of America (USA) and other countries, including Brazil, were resistant to methicillin (NNIS 2004, Amaral et al. 2005). It is likely that this intensive spread of MRSA has mainly occurred due to selective pressure exerted by the high use of antimicrobial drugs in hospitals around the world (Taubes 2008). However, other factors, including (i) invasive approaches for the diagnostic and therapeutic management of infections, (ii) intrinsic characteristics of patients (including populations with age extremes, immunosuppressive conditions or other underlying diseases) and (iii) virulence traits and fitness advantages of the microorganisms, are expected to have contributed to the leadership position of MRSA as a global hospital pathogen (Amaral et al. 2005, Planet et al. 2013). In Brazil, MRSA was ranked as the first etiologic agent of hospital-associated BSI and second in nosocomial pneumonia (Gales et al. 2009).

In October 2007, it was estimated by the Centers for Disease Control and Prevention (CDC) that the number of infections caused by MRSA should be approximately 100,000 per year in the USA, with nearly 19,000 reported deaths, which is a number that was greater than the total deaths attributed to human immunodeficiency virus/acquired immune deficiency syndrome in the same year (CDC 2007, Taubes 2008). In Europe, it was reported that MRSA has caused more than 170,000 infections per year, corresponding to 44% of all infections related to healthcare (ECDC/EMEA 2009). Another study published in 2011 that involved 31 European countries showed that the 27,711 episodes of MRSA BSI were associated with 5,503 deaths and 255,683 days of hospitalisation. The cost related to this length of hospital stay was estimated as 44 million Euros (de Kraker et al. 2011). These data reinforce the importance of these microorganisms as a global public health, social and economic problem.

Remarkably, MRSA has the potential for long-distance clonal spreading (Enright et al. 2000) and specific international clones have been associated with nosocomial [hospital-acquired MRSA (HA-MRSA)] or community infections [community-acquired MRSA (CA-MRSA)]. For this reason, genotyping techniques are widely used in epidemiological studies involving these bacteria. Thus, MRSA isolates with similar or even identical backgrounds can be allocated within the same clone or type according to the results of several typing methods. Among these methods, the most used are the analysis of pulsed-field gel electrophoresis patterns (Sanches et al. 1995), MLST, which defines ST and CC (Enright et al. 2000), SCC*mec* typing (Turlej et al. 2011) and analysis of the polymorphism of the *spa* gene that codes for protein A (Frénay et al. 1996). Nevertheless, MRSA lineages are commonly defined by ST associated with the SCC*mec* type.

The spread of MRSA among pets (including dogs and cats) and farm animals (such as pigs, calves, dairy cattle and horses) has also been reported (Armand-Lefevre et al. 2005, Cuny et al. 2008, Haenni et al. 2012, Quitoco et al. 2013). CC398 MRSA has initially been described among livestock-associated MRSA (LA-MRSA) in Europe (Armand-Lefevre et al. 2005) and has now been detected in different continents from Oceania (Williamson et al. 2013) to South America (Peru) (Arriola et al. 2011). More importantly, infections by CC398 have been reported in humans (Armand-Lefevre et al. 2005, Krziwanek et al. 2009). In contrast to LA-MRSA, the majority of MRSA isolates that have been detected in pets have clustered into the ST observed in CA-MRSA strains commonly detected in humans (Ferreira et al. 2011, Haenni et al. 2012, Loeffler et al. 2013).

Since it was first described in the UK and Denmark in MRSA isolates from cattle harbouring SCC*mec* type XI, MRSA isolates carrying the *mecC* gene have been detected in a number of European countries (Cuny et al. 2011, Garcia-Alvarez et al. 2011, Shore et al. 2011, Laurent et al. 2012, Sabat et al. 2012). More recently, a fatal case of BSI caused by a MRSA harbouring the *mecC* gene was described in Spain. This strain was associated with the CC130 lineage and *spa* type t843 (García-Garrote et al. 2013). In Europe, the *mecC*-MRSA isolates from a range of ST have been found to be associated with infections in a variety of animal species and less frequently in humans (Cuny et al. 2011, Laurent et al. 2012, Paterson et al. 2012). A number of findings have supported the link between humans and livestock animals, strongly suggesting the occurrence of cross-transmission of *mecC* isolates between these two populations (Garcia-Alvarez et al. 2011, Shore et al. 2011, Sabat et al. 2012, Harrison et al. 2013, Vandendriessche et al. 2013). The rapid spread of the novel *mecC* gene in different MRSA strains reinforces the need of control measures to prevent *mecC* dissemination. In addition, *mecC*-positive isolates represent a potential public health problem because the sequence differences between *mecA* and *mecC* can lead to failure in *mec* gene detection when the current tests for detecting methicillin resistance are used (i.e., polymerase chain reaction assays for detecting *mecA* and PBP2a latex agglutination tests) (Ito et al. 2012). To the best of our knowledge, no report on the isolation of *mecC*-harbouring MRSA in Brazil has been published.

*HA-MRSA* - The highest rates of MRSA in hospitals have been reported in North America, South America and Asia (> 50%) and intermediate rates (25-50%) have been found in China, Australia, African countries and in some European countries, for example, Portugal (49%), Greece (40%), Italy (37%) and Romania (34%). Other European countries (Netherlands and Scandinavian countries) have shown low rates of HA-MRSA that are generally ≤ 1% (Deurenberg & Stobberingh 2008, Stefani et al. 2012).

Worldwide, the most frequent CCs found among HA-MRSA isolates are CC5, CC8, CC22, CC30 and CC45. Some CCs may encompass different MRSA clones

and are distributed in diverse countries and/or regions (Deurenberg & Stobberingh 2008, Moore et al. 2010, Menegotto et al. 2012, Machuca et al. 2013). The current MRSA lineages and their corresponding clones are shown in Table II. CC5 and CC8 are currently the most prevalent CCs globally (Deurenberg & Stobberingh 2008, Song et al. 2011, Shittu et al. 2012, Caiaffa-Filho et al. 2013, David et al. 2013, He et al. 2013), while CC30 (ST36) is more commonly detected in the UK (McAdam et al. 2012) and CC45 (ST45) is generally found in the USA and Northern Europe (Moore et al. 2010, Menegotto et al. 2012). However, the most common (typical) HA-MRSA isolates in the USA belong to USA100 clone, which is a CC5 MRSA (ST5) (David et al. 2013). In Asian hospitals, MRSA from CC8 (ST239), CC5 (ST5) and CC22 (ST22) are among the most frequently detected isolates (D'Souza et al. 2010, Ghaznavi-Rad et al. 2010, Song et al. 2011, He et al. 2013). Epidemiological data are still limited in Africa; however, the predominance of CC8 (ST239 and ST612), CC5 (ST5) and CC30 (ST36) was suggested (Moodley et al. 2010, Breurec et al. 2011, Van Rensburg et al. 2011, Shittu et al. 2012). In Latin America, isolates belonging to CC5 (ST5) and CC8 (ST239) are frequently reported in hospitals (Silva-Carvalho et al. 2009, Rodríguez-Noriega et al. 2010, Caiaffa-Filho et al. 2013) and, in Brazil, the nosocomial lineages ST5-SCC*mec*I (Cordobes/Chilean clone), ST5-SCC*mec*IV (USA800; paediatric clone) and ST5-SCC*mec*II (USA100; clone NY/Japan) are prevailing in some hospitals (de Miranda et al. 2007, Silva-Carvalho et al. 2009, Becker et al. 2012, Caiaffa-Filho et al. 2013). Moreover, MRSA isolates of the lineage ST239-SCC*mec*III [Brazilian epidemic clone (BEC)] are still predominantly detected in various regions of this country (Silva-Carvalho et al. 2009, Caboclo et al. 2013, Rodrigues et al. 2013).

TABLE II

Geographic distribution of main methicillin-resistant *Staphylococcus aureus* (MRSA) lineages and clones[a]

| CC | Lineage | Clone | Geographic distribution |
|---|---|---|---|
| CC1 | ST1-SCC*mec*IV | USA400 (CA-MRSA), Brazilian USA400 (HA-MRSA) | Australia, Canada, Europe, South America, USA |
| CC5 | ST5-SCC*mec*II | USA100, NY/Japan (HA-MRSA) | Australia, Canada, Europe, Japan, South America, South Korea, USA |
| | ST5-SCC*mec*IV | USA800, paediatric (HA-MRSA), USA800-related (CA-MRSA) | Europe, South America, USA |
| | ST5-SCC*mec*I | UK-EMRSA-3 or Cordobes/Chile (HA-MRSA) | Asia, Europe, South Africa, South America |
| | ST228-SCC*mec*I | Italian/Southern German (HA-MRSA) | Europe |
| CC8 | ST8-SCC*mec*IV | USA500, UK-EMRSA-2/6 (HA-MRSA) | Australia, Canada, Europe, USA |
| | ST8-SCC*mec*IV | USA300 (CA-MRSA) | Asia, Australia, Europe, USA |
| | ST247-SCC*mec*I | Iberian, UK-EMRSA-5 (HA-MRSA) | Europe, USA |
| | ST239-SCC*mec*III | Brazilian/Hungarian (HA-MRSA), Russian-variant (urethritis-related CA-MRSA) | Asia, Australia, Europe, South Africa, South America |
| | ST612-SCC*mec*IV | - | Australia, South Africa |
| CC22 | ST22-SCC*mec*IV | UK-EMRSA-15 (HA-MRSA) | Australia, Asia, Canada, Europe |
| | ST36-SCC*mec*IV | USA200, UK-EMRSA-16 (HA-MRSA) | Australia, Canada, UK, USA |
| CC30 | ST36-SCC*mec*II | - | Asia, Australia, Europe, South Africa |
| | ST30-SCC*mec*IV | USA1100, OSPC (CA-MRSA) | South America, USA |
| CC45 | ST45-SCC*mec*IV | USA600, Berlin | Europe, USA |
| CC59 | ST59-SCC*mec*IV/VII | USA1000 (CA-MRSA) | Asia, Australia, Europe, USA |
| CC80 | ST80-SCC*mec*IV | Europe (CA-MRSA) | Asia, Australia, Europe |

*a*: adapted of Deurenberg and Stobberingh (2008) and Stefani et al. (2012); CA-MRSA: community-acquired MRSA; CC: clonal complex; HA-MRSA: hospital-acquired MRSA; SCC*mec*: staphylococcal cassette chromosome *mec*; ST: sequence typing.

Studies of single nucleotide polymorphisms (SNP) of ST5 MRSA isolates have suggested that their spread have most likely occurred by various SCC*mec* insertions into different methicillin-susceptible *S. aureus* (MSSA) strains in different regions of the world and not by the global spread of a ST5-MRSA strain over extensive geographical regions (Nübel et al. 2008). Conversely, phylogenetic evidence, also based on SNP analyses, has suggested the intercontinental spread of isolates of the ST239-SCC*mec*III lineage (Harris et al. 2010, Gray et al. 2011).

It is well known that MRSA differs considerably from MSSA strains, whose associated infections are heteroclonal in nature. In a comparative study, MRSA was responsible for higher in-hospital mortality (23.9% vs. 8.9%; p = 0.003) and longer bacteraemia (4.7 ± 6.5 days vs. 2.7 ± 2.9 days; p = 0.01) when compared with MSSA and it was concluded that SCC*mec*-associated virulence factors seem to play a role in the outcome of *S. aureus* BSI infection (Ganga et al. 2009). It was previously suggested that the increased ability of the high-level resistant ST239 lineage to adhere to and invade human epithelial cells and to accumulate large amounts of biofilm might have contributed to its fitness as the most successful MRSA pathogen worldwide (Amaral et al. 2005). In fact, recent studies have implicated the presence of *mecA* and PBP2a in poly-N-acetylglucosamine-independent biofilm formation. In addition, coherent with the increased production of biofilm by the homogeneously, high-level resistant *mecA* construction (HoR 8325-4 derivative), it was demonstrated that its protease level was decreased concomitantly with impairment of the Agr virulence regulatory system (Pozzi et al. 2012).

In infections associated with multiresistant MRSA clones, such as those associated with ST239 isolates or in cases of severe MRSA infections, vancomycin is still the major therapeutic choice. However, because vancomycin is administered in intravenous form, the use of this drug requires patient hospitalisation and, thus, significantly increases treatment costs. Although still rare, vancomycin-resistant MRSA isolates [vancomycin-resistant *S. aureus* (VRSA;)] have been reported in the USA and some countries in Asia (Tenover et al. 2004, Whitener et al. 2004, Tiwari & Sen 2006, Weigel et al. 2007, Saha et al. 2008, Askari et al. 2013, CDC 2013). In December 2012, the microbiology laboratory of the Clinics Hospital of the São Paulo University, Brazil, detected the first case of VRSA in Latin America: a methicillin and vancomycin-resistant strain that was isolated from blood culture (PAHO/WHO 2013). An increased incidence of vancomycin-intermediate *S. aureus* (VISA) or heterogeneous VISA (hVISA) has been documented in different countries (Richter et al. 2011, Hu et al. 2013, Oksuz et al. 2013). Despite the fact that some studies have reported the detection of VRSA and VISA isolates in Brazil (Lutz et al. 2003, Marques et al. 2013, PAHO/WHO 2013), vancomycin is still extremely active against MRSA isolates in this country (Gales et al. 2009).

In spite of the declining interest of the pharmaceutical industry in the development of new drugs, new antimicrobial agents have been discovered for the treatment of MRSA infections (Rivera & Boucher 2011). Some studies using pneumonia animal models have demonstrated that linezolid was more effective than vancomycin toward this type of infection due to its better penetration into the lung. Therefore, it was suggested that linezolid might be chosen for MRSA pneumonia cases with increased vancomycin minimum inhibitory concentration (Docobo-Pérez et al. 2012, Martinez-Olondris et al. 2012, Rodvold & McConeghy 2014). Daptomycin, which is a cyclic lipopeptide, was launched in the USA in 2003 for the treatment of skin/soft tissue infections (SSTI) caused by MRSA and other microorganisms and good results have been reported for this drug for the treatment of BSI and endocarditis caused by these microorganisms (Rodvold & McConeghy 2014). Tigecycline, which is a semisynthetic cyclin-glycyl that was released for use in the USA, Europe and Brazil, was not included in recent guidelines due to US Food and Drug Administration (FDA) safety issues (fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm370170.htm; last access in January 13, 2014). As reported by the FDA, a higher risk of death was found among patients receiving tigecycline compared with other antimicrobials: 2.5% (66/2640) vs. 1.8% (48/2628), respectively. In most cases, the deaths resulted from worsening infections, complications of infections or other underlying medical conditions.

Another new drug, telavancin (once-daily parenteral lipoglycopeptide), was approved by the FDA in June 2013 for limited use when no other options are available. Ceftaroline fosamil is the first FDA-approved cephalosporin with activity against MRSA; however, clinical experience with the use of this antibiotic for invasive infections by MRSA has been limited. For more detailed information on MRSA antimicrobial therapy, see the Guidelines of the Infectious Diseases Society of America (Liu et al. 2011) and the recent review by Rodvold and McConeghy (2014).

*CA-MRSA* - Community-acquired infection by MRSA is classically defined as an infection that affects community patients who have no history of previous MRSA infection or colonisation, surgery or hospitalisation, have not have been admitted into a long-term care facility or submitted to a percutaneous device, indwelling catheter or dialysis, within the year before infection (Maree et al. 2007). Generally, when compared with HA-MRSA, CA-MRSA lineages have different characteristics (Tristan et al. 2007). Frequently, these strains are isolated from SSTI; however, although less frequent, more serious infections such as bacteraemia, necrotising pneumonia and fasciitis, endocarditis and osteomyelitis have been reported in different countries (CDC 1999, Dauwalder et al. 2008, Bassetti et al. 2010, Sola et al. 2012), including Brazil (Ribeiro et al. 2005, Rozenbaum et al. 2009, Ferreira et al. 2012). Moreover, these isolates commonly carry SCC*mec* type IV, V or VII, often harbour *lukS* and *lukF* (*lukSF*$_{pvl}$) genes that encode the S and F subunits of Panton-Valentine leukocidin (PVL) and are usually more susceptible to non-β-lactam antibiotics than HA-MRSA (Tristan et al. 2007, Sun et al. 2013).

The first CA-MRSA isolates that were detected were described at the end of the 1970s and beginning of the

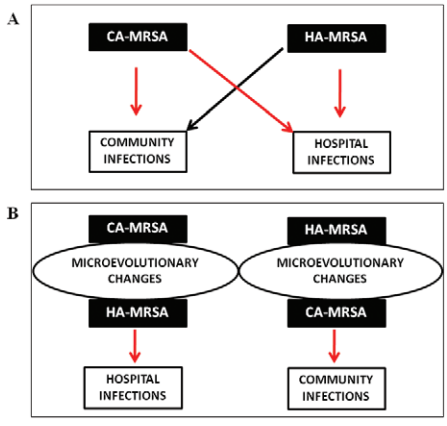
1980s in Western Australia and caused infections in the Aboriginal population. The clone in question was termed Western Australia-1 (WA-1) (CC1-ST1-SCC*mec*IV, PVL negative). Afterward, two other CA-MRSA clones emerged in Australia: the Queensland (CC93-ST93-SCC*mec*IV, PVL positive) and Oceania Southwest Pacific (OSP) (CC30-ST30-SCC*mec*IV and PVL positive) (Udo et al. 1993, Nimmo et al. 2000, Munckhof et al. 2003). In 1999, the Minnesota Department of Health and the CDC reported the deaths of four children due to pulmonary infections that were characterised as necrotising pneumonia with septicaemia and were associated with a specific CA-MRSA clone, named mid-western 2 (MW2), which belonged to the ST1-SCC*mec*IV lineage that is to WA-1, but is a PVL producer (CDC 1999).

In the early 2000s, a rapid increase in the detection of CA-MRSA isolates occurred globally, including Brazil and a number of clones were documented (Naas et al. 2005, Ribeiro et al. 2005, Takizawa et al. 2005, Fleming et al. 2006, Klevens et al. 2006) (Table II). In the USA, the ST1-SCC*mec*IV isolates spread over different regions and the MW2 clone was renamed USA400. Two other CA-MRSA clones have spread in the USA, USA1100 (related to OSP) and USA300 (CC8-ST8-SCC*mec*IV, PVL positive), with USA300 being the predominant CA-MRSA isolate (Mishaan et al. 2005, Klevens et al. 2006, Casey et al. 2013). The main CA-MRSA isolates that are circulating in Brazil are related to the OSPC/USA1100 clone. However, isolates of the USA300 clone have been reported in two Brazilian cities (Ribeiro et al. 2005, 2007). The first case of severe infection (generalised osteomyelitis) caused by CA-MRSA in Brazil was reported in 2009. The recovered isolate was related to USA1100 clone (ST30 lineage) and showed susceptibility to all non-β-lactam antibiotics (Rozenbaum et al. 2009). Shortly after, a rare and severe case of pyomyositis, that was associated with compartment syndrome, was also described in Rio de Janeiro city, Brazil, in a previously healthy child (de Araújo et al. 2010). Unfortunately, few studies regarding the prevalence of CA-MRSA have been reported in Brazil; therefore, little is known about the lineages involved and their prevalence in humans and animals (Ribeiro et al. 2007, Silva-Carvalho et al. 2009, de Araújo et al. 2010, Ferreira et al. 2012, Camargo et al. 2013). Recent data from Argentina have reported the predominance of ST30-SCC*mec*IV in community-acquired invasive infections (Fernandez et al. 2013). Similarly, studies carried out in Uruguay have described the spread of OSP/USA1100 in that country (Pardo et al. 2009).

The CA-MRSA lineage ST80-SCC*mec*IV is common in Europe (European clone) (Goering et al. 2009, Lamy et al. 2012, Rolo et al. 2012). However, in Asian and Pacific regions, including Australia and Taiwan, the ST59-SCC*mec*IV lineage has often been detected. Together, these data show that MRSA lineages may vary in different geographic regions and that, not rarely, a predominant strain can be supplanted by the expansion of a new lineage. In most countries, cases of CA-MRSA infections have been linked to small outbreaks. In the USA, Taiwan, Canada and Australia, CA-MRSA has become endemic in certain populations (O'Brien et al. 1999, Huang et al. 2008, Hung et al. 2012, Marra et al. 2012, Casey et al. 2013). A high rate (42%) of CA-MRSA colonisation of different anatomical sites was found in a Western Australian village (O'Brien et al. 1999) and, in Taiwan, the rate of CA-MRSA isolation in children increased significantly from 9.8% in 1999-2000 to 56% in 2004-2005 (Huang et al. 2008). The average annual increase in CA-MRSA incidence in the USA was 34% (range 6-94%) from 2005-2009 (Casey et al. 2013).

Despite the fact that CA-MRSA isolates are generally susceptible to non-β-lactam antibiotics, these strains may eventually acquire a multidrug resistance (MDR) phenotype. In Japan, for example, a CA-MRSA strain (ST30-SCC*mec*IV, PVL positive) was described to harbour various antimicrobial resistance genes that were carried by an MDR plasmid (Takizawa et al. 2005). A study in a paediatric hospital in mainland China found rates of MDR > 50% among CA-MRSA isolates belonging to ST59, ST338, ST45, ST910 and ST965 (Wang et al. 2012).

Another significant change in the epidemiology of MRSA was the entrance of CA-MRSA lineages into hospital settings in different regions (Donnio et al. 2004, Maree et al. 2007, Song et al. 2011, Campanile et al. 2012) (A in Figure). The incidence of isolates carrying SCC*mec*IV (CA-MRSA sign) in hospitals in the USA increased from < 20% (1999) to > 50% (2004) (Maree et al. 2007) and similar results were reported in a French hospital between 1992-2002 (Donnio et al. 2004). Another study from Italy confirmed the migration of MRSA isolates carrying SCC*mec*IV from the community to hospitals (Campanile et al. 2012). In East Asia, the CA-MRSA




The multifaceted epidemiological scenarios of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Red arrows indicate endemic and black arrow transient infections. A: some typical community-acquired MRSA (CA-MRSA) strains can became endemic in both community and hospital settings. On the other hand, a number of hospital-acquired MRSA (HA-MRSA) clones can transiently circulate in the community; B: as consequence of the impact of the broad use of antimicrobial drugs and widespread of MRSA clones, microevolutionary changes are accumulating in the MRSA genome, occasionally leading to the emergence of novel variants of CA-MRSA or HA-MRSA lineages, presenting distinct epidemiological characteristics from the previous clone.

lineages ST59-SCC*mec*IV, ST30-SCC*mec*IV and ST72-SCC*mec*IV have also spread in hospitals (Song et al. 2011). In addition, reports indicating the USA300 and USA400 isolates causing infections associated with healthcare services have periodically been published (El-Mahdy et al. 2013, Machuca et al. 2013, Oksuz et al. 2013). In Brazil, studies have revealed that PVL-positive CA-MRSA isolates of the lineage ST30-SCC*mec*IV were also involved in hospital-associated infections (Scribel et al. 2009, Silva-Carvalho et al. 2009).

The emergence of a novel variant of the ST1-SCC*mec*IV lineage that was related to the USA400/MW2/WA-1 clones was reported in Rio de Janeiro city in 2009. These isolates were able to overcome the previously predominant MRSA lineage, ST239-SCC*mec*III (BEC), at the studied hospitals. Surprisingly, contrary to USA 400 CA-MRSA, the Brazilian ST1-SCC*mec*IV variant had typical characteristics of a HA-MRSA lineage, showing MDR traits, the absence of $lukSF_{PVL}$ and some enterotoxin genes. Moreover, most of these ST1 isolates from Brazil had tropism for nosocomial-related BSI (Silva-Carvalho et al. 2009) and the ability of these isolates to accumulate moderate/strong amounts of biofilm (in vitro and in vivo) was subsequently demonstrated (Ferreira et al. 2013). The acquisition of antibiotic resistance determinants, loss of toxin-associated genes and increased biofilm accumulation are most likely results of microevolutionary changes that could provide fitness advantages for the emergence of these USA400-related variants in nosocomial environments (B in Figure, left panel). To our knowledge, no report showing typical community-acquired infections caused by the Brazilian USA400-variant has been published. The only report involving the USA400 variant in the community was a community-onset of healthcare-associated endocarditis in a patient in Rio de Janeiro city who had an implanted haemodialysis catheter (Damasco et al. 2013).

A new epidemiological change was the emergence in the community of some MRSA lineages that were found for years to only be linked to hospital-associated infections (B in Figure, right panel). For example, epidemiological surveillances in Argentina have revealed the emergence of community-acquired infections caused by CA-MRSA isolates related to the hospital-associated paediatric clone (ST5-SCC*mec*IV); however, in contrast to the paediatric clone, this clone had acquired $lukSF_{pvl}$ (Gardella et al. 2011, Sola et al. 2012). It was suggested that the acquisition of $lukSF_{pvl}$ and *sea* (encoding enterotoxin A) has contributed to the fitness of this novel clone as a major cause of community onset among children in Argentina (Gardella et al. 2011). Recently, ST5-SCC*mec*IV harbouring $lukSF_{pvl}$ was also reported in Brazil and was isolated from a case of sepsis in a 16-year-old male who had no history of exposure to healthcare or recent travel. More important was the fact that, as the Argentinean isolates, the hVISA phenotype was also detected in the Brazilian ST5 (Camargo et al. 2013). In Russia, isolates of the lineage ST239-SCC*mec*III (related to the BEC), both *spa*3 and its variant *spa*351, have recently been isolated from urethritis in the community (Yamamoto et al. 2012). Although the genomic background of the Russian variant is similar to the hospital-associated ST239, named TW20, it has some marked diversity. What is striking is that the Russian variant incorporated isolates with high ability to transfer MDR elements by conjugation to *S. aureus* 16K, including a chloramphenicol resistance plasmid and the Tn*554* transposon (Yamamoto et al. 2012). These findings demonstrate the dynamic microevolutionary mechanisms of the ST239 isolates in Russia and their potential as a drug resistance disseminator in community settings (Yamamoto et al. 2012). A study carried out in two major cities in Portugal found that public buses are often contaminated with MRSA clones that are currently found in hospitals of the particular geographic area. Therefore, MRSA contamination of public transportation and the transfer of bacteria to the hands of passengers may represent a route through which HA-MRSA isolates may spread to the community (Conceição et al. 2013).

As a consequence of the accelerated MRSA evolution, the epidemiology of MRSA infections has become increasingly complex, with some CA-MRSA gaining access to hospitals and a number of lineages that are typically found among HA-MRSA circulating in the community (Figure). Thus, establishing a clear and accurate definition between CA-MRSA and HA-MRSA strains has not been a simple task in some cases. Until now, molecular characterisation with epidemiological analyses has been considered the best criteria for differentiating the various MRSA lineages as hospital or community origin.

Experimental data using animal models have suggested that CA-MRSA strains were more virulent when compared to HA-MRSA (Day et al. 2012). While not all CA-MRSA carry genes for PVL, this neutrophil cytolysin is known as a virulence determinant related to the epidemiology of CA-MRSA infections because its detection is rare among HA-MRSA (Borghi et al. 2010, Portillo et al. 2013). However, its direct association with the severity of infections caused by this pathogen remains controversial in the scientific literature (Lina et al. 1999, Gillet et al. 2002, Wardenburg et al. 2008, Montgomery & Daum 2009). Some experimental results using animal models for SSTI, sepsis and pneumonia have demonstrated a minimal to no effect when mutants defective for PVL were compared with the respective isogenic wild-type strain (Voyich et al. 2006, Wardenburg et al. 2007, 2008, Diep et al. 2008a, Montgomery & Daum 2009). However, other studies have shown that PVL contributes to tissue damage, skin infections and the severity of necrotising pneumonia (Lina et al. 1999, Gillet et al. 2002, Labandeira-Rey et al. 2007, Tseng et al. 2009).

A number of other factors, including α-haemolysin or α-toxin (Hla), phenol-soluble modulins (PSMs) and arginine catabolic mobile element (ACME), have been identified as important for CA-MRSA virulence and the latter was only detected in USA300 isolates (Wang et al. 2007, Wardenburg et al. 2007, Thurlow et al. 2013). Hla (encoded by the *hla* gene) is a proinflammatory, pore-forming, cytolysin that acts in the lysis of immune system cells, such as macrophages, erythrocytes and lymphocytes (Bartlett et al. 2008). A study using an animal model of pneumonia, i.e., mice infected with *hla* knock-

outs derived from CA-MRSA isolates of the USA300 or USA400 clones, suggested the involvement of Hla in this type of infection (Wardenburg et al. 2007). In addition, data from a recent study using *hla* and *lukSF*$_{pvl}$ knockouts derived from USA300 CA-MRSA have suggested that Hla, and not PVL, impacts rabbit mortality from severe bacteraemia in a model of osteomyelitis. However, in this study, it was shown that PVL and Hla seemed to be required for early lung involvement via haematogenous spread (Crémieux et al. 2013).

The PSMs belong to a class of α-helical, cytolytic peptides (20-25 amino acids) of the surfactant and amphipathic types that are produced by many species of *Staphylococcus*. All PSMs, mainly PMSα3, have proinflammatory activity, which includes activation and induction of chemotaxis and cytokine release from human neutrophils (Forsman et al. 2012). In 2007, Wang et al. (2007) found that removal of the *psmα* operon from two USA300 and USA400 isolates caused a strong, negative impact on neutrophil recruitment and lysis and in the evolution of skin infection and BSI. These results suggested that PSMα appears to contribute significantly to the pathogenesis of CA-MRSA infections.

It was suggested that the 31-kb genomic island, known as the ACME, is particularly important for the fitness of USA300. The ACME *locus* is identical to a genomic region in *Staphylococcus epidermidis* isolates, which are well known as skin surface colonisers (Barbier et al. 2011). The ACME *locus* of USA300 is composed of at least 33 putative genes and two operons, *arc* and *opp*. The *arc* operon is involved in arginine catabolism and it seems important for the ability of USA300 to succeed in acidic environments that mimic that found in the skin. It is thought that once USA300 *S. aureus* penetrates the epidermis, the Arc system leads to the syntheses of polyamines, which are the products of arginine metabolism. Polyamines are also made in human tissues and participate in wound healing and the inflammatory process (Barbier et al. 2011). It was also demonstrated that the ACME *speG* gene, which encodes a spermidine acetyltransferase (SPeG) could determine spermidine and spermine tolerance and such polyamines are lethal to *S. aureus* strains (Joshi et al. 2011). The *opp* operon seems to encode an oligopeptide and it was suggested that homologous genes were implicated in the virulence of *Streptococcus pyogenes* (Wang et al. 2005). It was recently demonstrated that *speG* also enhanced biofilm formation, adherence to fibrinogen/fibronectin and keratinocyte-mediated killing (Planet et al. 2013). Indeed, it was found that the absence of ACME significantly decreased the in vivo fitness of USA300 isolates (Diep et al. 2008b).

Although some progress has been made, the mechanisms involved in the pathogenesis of CA-MRSA are not clearly determined. We still do not understand why some CA-MRSA clones, primarily those related to SSTI, have quickly emerged as successful pathogens in certain regions and what are the key determinants for the establishment of serious CA-MRSA infections among immunocompetent individuals.

*Concluding remarks* - *MRSA* remains one of the most challenging infection-control issues. This formidable pathogen is included in the group of so-called ESKAPE pathogens that is formed by *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species. These bacteria are responsible for approximately 40% of nosocomial infections and represent the vast majority of isolates whose antimicrobial resistance represents serious therapeutic challenge (Hidron et al. 2008).

The global epidemiology of MRSA has continually been changing. Severe MRSA infections that were universally restricted to hospitals for decades are increasingly being reported among immunocompetent patients from the community. Additionally, studies on the molecular characterisation of MRSA have revealed epidemic clones of CA-MRSA causing community and hospital-acquired diseases. Moreover, the cross-transmission of CA-MRSA between humans and pets has also been a concern because these animals may serve as reservoir for human infections and *vice versa*.

More recently, the medical and veterinary communities were surprised by the emergence of LA-MRSA and *mec*C MRSA. Furthermore, some typical HA-MRSA lineages have gained fitness advantages and emerged as CA-MRSA pathogens and the reverse is true: MRSA lineages previously detected only in the community have become more fit to survive in hospital settings.

The multiple epidemiological facets of this old pathogen was, at least in part, built up by the accumulation of microevolutionary changes in the MRSA genome and accelerated by the rapid spread of these bacteria in worldwide hospital and community settings, consequently leading to better fitness of the microorganism for surviving in different environmental scenarios. However, some small resources of antimicrobial agents that work against MRSA are still available.

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