Review article

Genetic nature and virulence of community-associated methicillin-resistant Staphylococcus aureus

Tatsuo Yamamotoa,b,*, Wei-Chun Hunga,b, Tomomi Takanoß, Akihito Nishiyamab

a International Medical Education and Research Center, Niigata, Japan
b Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form 27 November 2012
Accepted 27 November 2012
Available online 24 January 2013

Keywords:
community-acquired methicillin-resistant Staphylococcus aureus (MRSA)
drug resistance evolution
MRSA community-acquired diseases virulence factor

ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) emerged in 1961, just after the introduction of methicillin as a countermeasure against penicillinase-producing “multidrug-resistant” S. aureus, a threat at that time. Since then, MRSA has posed a continuous threat to medical care as a major multidrug-resistant pathogen in hospitals. In 1997–1999, severe invasive infection with MRSA occurred in the community, and this attracted attention as community-associated MRSA (CA-MRSA). The evolutionary steps include species-to-species transfer and salvage of key genetic structures, responsible for community spread, virulence, and resistance. The MRSA epidemic, including invasive diseases, in the community is dynamic.

1. Introduction

The history of modern chemotherapy, which started with the introduction of the antibiotic penicillin in a clinical setting in 1941 [1] is, however, a series of fierce battles with resistant bacteria. Penicillin-resistant (penicillinase-producing) Staphylococcus aureus emerged immediately after its introduction, followed by the further emergence of penicillinase-producing “multidrug-resistant” S. aureus, exhibiting resistance to penicillin G, chloramphenicol, tetracyclines, macrolides (e.g., erythromycin), and aminoglycosides (e.g., streptomycin and kanamycin) [2,3]. Semi-synthetic penicillins, such as methicillin (followed by oxacillin and cloxacillin), which were stable against hydrolysis by penicillinase (penicillinase resistant), were clinically introduced in 1960 as a countermeasure against penicillinase-producing “multidrug-resistant” S. aureus at that time [1]; however, methicillin-resistant S. aureus (MRSA) emerged in the United Kingdom in 1961, again just...
after the introduction of methicillin [1,4]. Therefore, historically, MRSA is multidrug resistant [3,4]. Since then, MRSA has continued to be a life-threatening multiple drug-resistant bacterium in hospitals, albeit with hospital outbreak peaks (e.g., “hospital panics” in Japan) during the 1980s–1990s [3,5,6].

Severe invasive infection with MRSA in the community first occurred in the United States in 1997-1999 [7], slightly after the hospital outbreak peak of MRSA in the 1980s-1990s; and this attracted attention as community-associated MRSA (CA-MRSA). The term CA-MRSA was used earlier (in 1981) [8], but more commonly on this occasion.

After the emergence of CA-MRSA, traditional MRSA has come to be called healthcare-associated MRSA (HA-MRSA), starting the new era of two classes of MRSA. Update, there have been several open questions, such as why did MRSA emerge in the community? Is CA-MRSA more virulent than HA-MRSA? What are the major virulence factors of CA-MRSA? In this review, attempts were made to summarize accumulated knowledge on MRSA that originated and/or selected in the community, focusing on these questions.

2. MRSA lineages and epidemics

2.1. Mechanism of MRSA generation

MRSA is generated from methicillin-susceptible S. aureus (MSSA) by the acquisition of a mobile genetic element called staphylococcal cassette chromosome mec (SCCmec) at the 3’ end (i.e., 15-bp SCCmec insertion site, att) of orfX of the chromosome [9,10]. SCCmec carries the mecA gene, encoding a penicillin-binding protein called PBP2a (or PBP 2a), which shows low affinity to β-lactam agents [10–12]; therefore, for MRSA, the Clinical and Laboratory Standards Institute (CLSI) currently instructs that minimal inhibitory concentration (MIC) results for β-lactam agents, including penicillins, cephalosporins, and carbapenems, should be reported as resistant (R), for example [13].

2.2. HA-MRSA

Historically, the origin of MRSA is “healthcare-associated” methicillin-susceptible S. aureus (MSSA), and the acquisition of SCCmec by these pre-existing MSSA strains has occurred only a limited number of times, resulting in MRSA epidemics in hospital settings [3,14–16]. Major epidemic clones include the archaic clone (ST250/SCCmecI; reported in the UK in the 1960s), Iberian clone (ST247/SCCmecA; reported in Spain in 1989), New York/Japan clone (ST5/SCCmecII; reported in the United States in 1998, but actually much earlier in Japan, in 1992), EMRSA-16 clone (ST36/SCCmecII; reported in the UK in 1993), Brazilian clone (ST239/SCCmecIII; reported in Brazil in 1992), Hungarian clone (ST239/SCCmecIII; reported in Hungary in 1993), EMRSA-15 clone (ST22/SCCmecIV; reported in the UK in 1993), pediatric clone (ST35/SCCmecIV or IVa; reported in Portugal in 1992), and Berlin clone (ST45/SCCmecIVa; reported in Germany in 1998). Some MRSA clones have spread globally and adapted to each region with microevolution. MRSA epidemics have been dynamic and include replacement by a distinct clone.

2.2.1. ST5/SCCmecII lineage

This is one of the most worldwide-disseminated MRSA lineages [14–16]; it became dominant in Tokyo, Japan in 1992 [17] by replacing previous type IV coagulase MRSA. This Japanese MRSA clone was originally characterized by type II coagulase, a ribotype pattern similar to N315, toxin type positive for toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxin C (SEC), and multiple antibiotic resistance, including fluoroquinolone (ciprofloxacin). In the United States, a dominant MRSA clone, which was characterized by mecA polymorph I, Tn554 pattern A and pulsed-field gel electrophoresis (PFGE) pattern A (clonal type I:A:A) was isolated in New York in 1994 [18], and the same genotype MRSA (the New York clone) was isolated in Connecticut, New Jersey, and Pennsylvania in 1998 [19]. In 2000, it was found that the Japanese major MRSA clone isolated in 1997 and 1998 in Tokyo and the New York clone shared the same genotype (I:A:A) and drug resistance pattern, including ciprofloxacin, suggesting the transcontinental spread of the same single MRSA clone from Japan to the United States [20]. In 2001 [16] and 2002 [15,21], the MRSA clone from Japan and the United States was recognized as the epidemic New York/Japan clone with genotype ST5/SCCmecII. The New York/Japan clone spread to several US states and other (at least five) countries between 1995 and 2004. The Japanese type is positive for tst (encoding TSST-1), while the US type is negative [22,23]. In Taiwan, the Japanese type appeared in the early 2000s and is currently a dominant hospital-acquired MRSA (HA-MRSA) clone [24,25]. Vancomycin-intermediate MRSA was first detected in this lineage (strain Mu50) in Tokyo in 1997 [23].

2.2.2. ST239/SCCmecIII lineage

This worldwide-disseminated lineage was first identified as the Brazilian clone (with SCCmecIII), which spread among hospitals in Brazil in 1992 [26], and then spread intercontinentally to hospitals in Portugal, possibly linked to the Brazil-to-Portugal migration of human populations since 1992-1993 [16,27]. The Hungarian clone (with SCCmecIII) emerged in hospitals in Hungary in 1993 and became predominant in hospitals until 1998. The ST239 lineage consisted of more than five MRSA clades reflecting their continental origin, such as Asia, North America, South America, Europe (with marked divergence), and Australia, with some intercontinental transmission cases [28]. For instance, the ST239 TW clone (strain TW20), which was noted as intensive care unit (ICU)-associated MRSA in London between 2002 and 2004 [29], clustered within the Thai clade, most probably suggesting transmission from south-east Asia to London [28]. Besides transmission in hospital settings, ST239 MRSA also spreads in the community as an agent of urethritis in Russia [30]. In Taiwan, the ST239 lineage (with SCCmecIII and III) appeared in the 1990s, and ST239/SCCmecIII is currently a dominant HA-MRSA clone [31]; some strains have exhibited fusidic acid resistance with fusA as a dominant gene (fusC since 2007) [32,33]. In Japan, no
detectable ST239 MRSA is spreading. ST239 includes regional variants [28] in terms of spa, SCCmec, and others. For instance, ST239 in Krasnoyarsk, Russia (ST239c1ns), carried a novel pathogenicity island (SaPI2R) with the tst gene for the first time in the ST239 lineage [34].

2.3. CA-MRSA

CA-MRSA includes divergent MRSA lineages, which are also divergent from MRSA in hospital settings [35–38]. CA-MRSA often produces Panton-Valentine leukocidin (PVL) [35–38]. Initially, CA-MRSA was synonymous with PVL-positive MRSA; with representatives such as ST30, which was a worldwide clone [37]; ST80, which was dominant in Europe [40,41]; ST1 (USA400), which was dominant in the United States [38]; ST8 (USA300), which is currently dominant in the United States [35,38]; and ST59 (Taiwan clone), which was (and is still) dominant in Taiwan [39].

The origin of CA-MRSA is drug-susceptible MSSA (in many cases) in the community, and CA-MRSA is generated by the acquisition of SCCmec types IV or V at the orfX (att) [40,41]. Essentially, CA-MRSA of community origin is resistant to β-lactam agents only or to some agents in restricted classes, although some successful PVL-positive CA-MRSA (e.g., ST8 and ST59) has become or was initially resistant to multiple antibiotics [38,42]. Moreover, for “newborn” CA-MRSA, MIC levels of (e.g.) imipenem and oxacillin are lower than those for MRSA in hospital settings, probably reflecting the exposed period of time or mass in antibiotic use [43].

2.3.1. ST8/SCCmecIV lineage (USA300)

USA300 (PVL-positive) was isolated locally from two infectious cases of college football players in Pennsylvania and prisons in Missouri in 2000 in the United States [44]; however, MRSA infection, largely USA300, then swept through the United States reaching a peak in 2005–2006 with an estimated patient number of 94,360 and 18,650 deaths (rate: 19.8%) from invasive MRSA infections [44,45]. USA300 became remarkable in both community and hospital settings in the United States [45]; it accounts for >50% of all S. aureus infection [46] or the majority (67%) of invasive community MRSA infection [45]. Moreover, USA300 has also spread globally, including Europe [44,47] and Japan [48]. In Europe, USA300-related MRSA is growing as a major clone in the community (the most common clone in some cases), together with the ST80/SCCmecIV European clone [49,50]; PVL-positive clones are the predominant MRSA in Europe [49]. USA300 is one of the most characterized CA-MRSA [41,51].

2.3.2. ST59/SCCmec IV and V lineages (Taiwan clones)

Taiwan clones have been isolated from skin and soft tissue infection (SSTI) cases (and pyomyositis cases) of children since 1997 in Taiwan [52]. ST59/SCCmecV (PVL-positive) has become the most dominant CA-MRSA in Taiwan, accounting for 69–84% in CA-MRSA isolates in 1997–2005 [39,52,53]. ST59/SCCmecIV (PVL-negative) is the second most dominant CA-MRSA (accounting for 8.8–17.6%), but it is the most dominant (accounting for 46–59%) MRSA in nasal carriage in the community in Taiwan [54–57]. ST59/SCCmecIV has also been recognized as an important nosocomial pathogen [31,58]. ST59 Taiwan clones have been isolated even from livestock [59] and from pets such as dogs and cats [60]. Moreover, ST59 Taiwan clones have spread globally, including to Europe [49] and Japan [61].

2.3.3. ST8/SCCmec IV lineages (ST8 CA-MRSA/J)

ST8 CA-MRSA/J (PVL-negative) was isolated from bullous impetigo cases of children in Niigata in 2003 in Japan [62,63] and has become a successful CA-MRSA in Japan, accounting for 16–34% in the community during 2008–2010; it has even spread to public transport [64]. ST8 CA-MRSA/J was also isolated in Hong Kong from an infected/colonized Japanese family [63].

2.4. Definition of CA-MRSA and HA-MRSA

The Centers for Disease Control and Prevention (CDC) has described the criteria for the epidemiological definition of CA- and HA-MRSA [45]; according to the definition, CA-MRSA is identified as MRSA from outpatients (patients in the community) with no history of hospitalization, surgery, dialysis, or indwelling percutaneous medical devices and catheters in the past one year or inpatients isolated within 48 hours after hospital admission; that is, MRSA from patients with no MRSA risks. The CDC CA-MRSA definition was later evaluated by David et al [65].

In addition, accumulated knowledge has established the bacteriological identification of CA- and HA-MRSA clones, as described above. For instance, for genotypes, CA-MRSA is discriminated from HA-MRSA in terms of ST types, spa types, agr types, and coagulase types [35,37,38]. For SCCmec types, CA-MRSA carries SCCmecIV or V in many cases, while HA-MRSA carries SCCmecI or III in many cases [41]. For virulence, CA-MRSA likely possesses a CA-MRSA-specific adhesin/colonization factor, such as arginine catabolic mobile element (ACME) [51], unique cell-wall-anchored bacterial surface proteins (CWASPs) [66,67], and enhanced biofilm [68]; often produces toxins, such as PVL [35,37], exfoliative toxin (ET) [62,69], or staphylococcal enterotoxin B (SEB) [24,42]; and expresses the cytolytic peptide (or phenol-soluble modulin, PSM) genes (e.g., psmA [encoding PSMa] or hld [encoding δ-hemolysin]) at higher levels than HA-MRSA [70].

3. CA-MRSA infections

3.1. Associated diseases

CA-MRSA infection occurs in healthy individuals, especially children, adolescents, and the elderly, through skin-to-skin contact, causing nasal or other body surface carriage [36,37,40,45,71–74]. Clinically noted CA-MRSA infections are summarized in Fig. 1 [38,40,63,68,72,74–78; unpublished data]. The vast majority (70–80%) of CA-MRSA infections manifest as SSTIs, including pyogenic skin infection. PVL-positive CA-MRSA has been especially isolated from “deep” skin infection, such as furuncle, carbuncle, and cellulitis; patients complain of severe pain due to unusually large abscesses. CA-MRSA (mostly PVL-negative) has also been
isolated from impetigo, the most common SSTI disease in children, which occurs in superficial skin; bullous impetigo is generally caused by ET-positive \textit{S. aureus} \cite{79}, albeit with 10-30\% cases due to CA-MRSA \cite{69,72,80}. In some ET-negative CA-MRSA cases, MRSA is isolated in combination with ET-positive \textit{S. aureus} \cite{80}. ET-positive CA-MRSA has also been isolated in a neonatal intensive care unit (NICU) from neonates with staphylococcal scalded skin syndrome (SSSS) \cite{79,81}. CA-MRSA has also been isolated from young athletes (e.g., with atopic dermatitis) \cite{63,82,83}. MRSA SSTIs may be decreasing in the United States \cite{84}.

Moreover, CA-MRSA can cause wound infections and has been detected from surgical site infection \cite{85}. Bacterial chronic wounds (diabetic ulcer and venous stasis ulcer) have increased over the past years and the majority of these are due to CA-MRSA \cite{86}.

The prevalence of CA-MRSA invasive infection is rare; however, it occasionally surprises clinicians who are used to MRSA (HA-MRSA) infections. The first surprise was fatal necrotizing pneumonia and sepsis in children in the United States in 1997 to 1999 \cite{7}; this was followed by a wide range of invasive infections worldwide, as shown in Fig. 1. CA-MRSA nasal carriage and SSTI are risk factors for bloodstream infections (BSIs) \cite{87,88}. Iliopsoas abscesses (IPAs), which could have originated in bacteremia, may be increasing \cite{89}. CA-MRSA accounts for 2.4\% of severe community-acquired pneumonia (CAP) in 2006 and 2007 \cite{90}, including influenza prodrome cases \cite{77,91}. MRSA coinfection increased the mortality from influenza (H1N1) in children by eightfold \cite{78}. In the United States, most CA-MRSA cases are from USA300 \cite{73}.

### 3.2. Transmission core of CA-MRSA in the community

CA-MRSA is likely transmitted from a core in the community, such as infected families, athlete groups, and hurricane evacuees \cite{68,72,82,92}. In the United States, the most common \textit{S. aureus} colonized from household contacts of patients with skin infections was USA300 (53\%) \cite{73}. MRSA transmission to household contacts has also been reported in Europe \cite{93}. Since long-term CA-MRSA carriage/transmission with invasive diseases in a community core has not been understood well, two family infection clusters from CA-MRSA were carefully investigated in Japan.

#### 3.2.1. PVL-positive ST22 CA-MRSA case

The series of cutaneous abscesses started with a Japanese infant, who had stayed in India with her parents \cite{68}. Most probably, MRSA transmission had occurred from a housemaid (the first person with deep cutaneous abscesses, who was in contact with the residents) to the infant. MRSA infection spread to the parents in India and then to four other related families after their return to Japan. For four related families, the targets in the first study, at least six of 12 individuals (50\%) developed deep cutaneous abscesses, including recurrent and multifocal abscesses, every 1.2 months on average. MRSA persisted for up to three years, finally causing infection/

---

**Fig. 1** – Summary of diseases related to CA-MRSA. Data are taken from Refs. \cite{36,40,63,68,74,78; unpublished data}. Numbers (%) represent the incidence of the disease. CAP = community-acquired pneumonia; PVL = Panton-Valentine leukocidin; UTI = urinary tract infection.
colonization in at least 11 of 14 members (79%) of five related families. It should be noted that the economic levels of the five related families were higher than the average in Japan, and no individuals had deep abscesses before. This super sticky MRSA exhibited strong biofilm formation.

3.2. USA300 transmission case
The series of cutaneous abscesses started with a housewife, who visited Hawaii, United States, and was treated in a hospital there [94]. After her return to Japan, she suffered from SSTIs, which spread to seven of 11 members (64%) of three related families. Nine months later, an infant, a family, distinct from the housewife in the first episode, suffered from cellulitis and sepsis, followed by osteomyelitis two months later [94,95]. After the successful treatment of the infant, no USA300 was detected in the three related families.

3.2.3. MRSA-positive pus acted as a repeated source
The families in the two cases in Japan had infants or children and frequently got together on New Year’s Day or other holidays; therefore, MRSA was likely transmitted through close body contact such as hugs and kisses. The most common colonized body surface site was the cheek (87.5%) and nares (75%), followed by the inguinal area (37.5%), hands (37.5%), axilla (25%), and umbilicus (25%) [68]. MRSA occasionally changed its location at the body surface sites; sometimes no MRSA was detected from nares. Especially for PVL-positive cases, MRSA-positive pus acted as a repeated source, and pus contact enabled long-term infection/colonization in a family cluster; household members never wore gloves.

3.3. MRSA nasal carriage at specific ages in the community
Children are at high risk for CA-MRSA carriage [96,97]. Healthy children in the community carry nasal MRSA at 1.9-11.6% in Taiwan [55,98,99] and at 3.7-4.3% in Japan [96,97]. In Japan, MRSA carriage is age-dependent which peaks in the 5-9-year age group (3.7%); more MRSA strains were isolated from healthy children in the community than from pediatric outpatients [96]. By contrast, no MRSA was isolated from healthy university students (<0.2%); however, recent preliminary data suggested markedly high MRSA carriage in the elderly (>10% in some cases), suggesting a possible important source of infection (because the elderly population has been increasing worldwide).

4. SCCmec: Classification and linked structures

4.1. SCCmec structure and classification
SCCmec basically consists of the mec gene complex (encoding β-lactam resistance) and the ccr gene complex (encoding recombinase) with three joining regions J1–J3 (Fig. 2A) [10,51,67,100–105; unpublished data]. The mec gene complex includes six types, classes A, B, C1, C2, D, and E, while the ccr gene complex includes three types, ccrA, ccrB, and ccrC. Moreover, allotypes exist in each type: five (A1–A5) for ccrA, six (B1–B6) for ccrB, and one (C1) for ccrC. Based on the combination, SCCmec is typed as I–XI according to the International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) [10], as shown in Fig. 2A. Recently, SCCmecXI carrying a novel homologue of mecA (called mecC or mecAG251) was found in the MRSA isolates [mainly ST130 clones (including other STs of clonal complex 130) and ST425 clones] from animals and humans (albeit rarely) in European countries [106,107]. The mecAG251 has approximately 70% identity (63% in amino acid level) with mecA [100]. The entire SCCmec type, including J1, J2, and J3 regions, is expressed as e.g., SCCmecIV.1.1.1, SCCmecIV.1.1.2, or SCCmecIV.new.1.1. Traditional nomenclature has also been used for subtyping SCCmecIV, such as IVa or IVc.

As shown in Fig. 2A, SCCmecIV (ZH47) and V (TSGH17, PM1) carry an additional ccr gene complex in J3 region, which was previously named as the ccrC-carrying unit [108]. This ccrC region is indicated by the type 5 ccr gene complex (5S) (description: 2B&5 and SC2&5), according to IWS-SCC [10]. By contrast, the corresponding region of SCCmecVII (JCSG082) is assigned as ccr gene complex 5 (description: 5C1). This complication (5S vs. 5) is followed by the suggestion of two groups (I and II) [109].

4.2. SCCmec-linked or -associated virulence functions

4.2.1. ACME
The ACME is a mobile genetic element with att at both ends, which was found linked to SCCmecIVa of USA300 (Fig. 2B, top figure) [51]. The ACME of USA300 (ACMEI) carries the arc gene cluster (arcC, arcB, arcD, arcA, and arcR) and the oligopeptide permease operon (opp-3 operon; opp-3A, opp-3B, opp-3C, opp-3D, and opp-3E). These gene clusters are possibly associated with, for example, growth under lower pH conditions (at the skin surface), interruption of nitric oxide production of the host, uptake of peptide nutrition, and quorum sensing [51]. USA300 ACME is considered to enhance colonization and survival on the skin [41]. Type II ACME (ACMEII), carrying only the arc gene cluster, is also found linked to ST5, ST8, ST22, ST59, ST239, and ST764 MRSA [41,101,105,110–112], linked to SCCmec as shown in Fig. 2B (middle and bottom figures). Such ACMEII is also linked with part of the J1 region of SCCmecI (called ΔJ1 SCCmecI or cJ1), which encodes a bacterial cell surface protein. The role of ACMEII and ΔJ1 SCCmecI (or cJ1) remains less understood, but may be associated with MRSA colonization.

4.2.2. IE25923
IE25923 is a mobile genetic element with att at both ends and carries a putative transposase gene [102]. IE25923 is linked to SCCmecIVc and to SCCmecI, albeit rarely (Fig. 2C) [102,103]. Its function remains unknown.

4.2.3. Bacterial cell surface proteins from the J1 region
A successful PVL-negative CA-MRSA clone in Japan, ST8 CA-MRSA/J, carries SCCmecIV.new.1.1; the J1 region of SCCmecIVJ carries the spj gene encoding a 1602-amino acid CWASP with an LPXTG motif (CWASP/J) [67]. CWASP/J may contribute to ST8 CA-MRSA/J colonization in the community.
Fig. 2 – SCCmec structures and classification (A), SCCmec-linked structures (B, C), and SCCmecI-associated virulence structures (D). Data are taken from Refs. [10, 51, 67, 100–105; unpublished data] and http://www.sccmec.org/Pages/SCC_HomeEN.html, and described in the text. ACME = arginine catabolic mobile element; SCCmec = staphylococcal cassette chromosome mec.
In addition, the J1 region of SCCmec in strain COL carries the pls gene encoding a 1548-amino acid CWASP with an LPXTG motif (Pls) [113]. The expression of Pls reduces either bacterial adherence to, for example, fibronectin and the Fc domain of immunoglobulin or cellular invasion of bacteria in vitro [114]. Accumulated knowledge, therefore, suggests that the J1 region of SCCmec is not a “junkyard” [10], but rather it may act as a “hot spot” and play an important role in MRSA colonization/infection.

4.2.4. PSM-mec from mec gene complex

PSM-mec, 22-amino acid peptide, exhibits cytolytic activity to human polymorphonuclear neutrophils (PMNs) and induces IL-8 production [115]. PSM-mec is encoded by the psm-mec gene present in class A mec gene complex, in the order of IS431-mec-mecR1-mecL-psm-mec/fudoh (psm-mec is overlapped by fudoh in the opposite direction) [116]. Class A mec gene complex is associated with, for example, SCCmecII and III, indicating that PSM-mec is a virulence factor of HA-MRSA (and maybe of HA-MRSA variants adapted to the community) [64,76,96,105].

5. Emergence of multidrug resistance in the community

Some successful CA-MRSA became multidrug resistant through multiple steps, but rarely by a single step. HA-MRSA is also spreading in the community through mutations.

5.1. Multidrug-resistant CA-MRSA

5.1.1. USA300

USA300 was initially resistant to erythromycin (gene: msrA) only, followed by the acquisition of a series of resistance, depending on strains, to mupirocin (gene: ileS-2, mupA), macrolides/clindamycin (genes: ermA and C), and tetracycline (genes: tetK, and M), mostly by plasmids; resistance to levofloxacin (gene: gyrA mutation), gentamicin (gene: aac(6’)-aph [2’]), and sulfamethoxazole-trimethoprim (gene: dfrA); reduced susceptibility to vancomycin and daptomycin; and resistance to phenolics and others (genes: cfr and fexA) [117,118].

5.1.2. ST30 and ST80 CA-MRSA

ST30 CA-MRSA (a worldwide clone) is generally susceptible to non-β-lactam agents, but a strain acquired a gentamicin/kanamycin and tetracycline resistance plasmid (pGKT1 carrying aac(6’)-aph[2’] and tetK) in Japan [62]. ST80 CA-MRSA in Europe carries a plasmid encoding fusidic acid resistance (gene: fusB) and also other resistance genes [119].

5.1.3. ST59/SCCmecV Taiwan clone

By contrast, the ST59 Taiwan clone has been unusually multidrug resistant since its initial isolation [24,42,52,54] and carries a unique mobile multidrug resistance structure (IS1216V-mediated composite transposon MESPM1, originating from enterococci), which encoded resistance to erythromycin/clindamycin (gene: ermB), kanamycin (gene: aac(6’)-Ila), streptomycin (gene: aadE), and chloramphenicol (gene: cat) [42]. ST59 CA-MRSA also carried a tetracycline resistance-encoding (tetK) penicillinase plasmid, pPM1. There is a possibility that ST59 CA-MRSA originated from “multidrug-resistant” ST59 MSSA with MESPM1 (and pPM1) by acquiring SCCmecV, or that initial “non-β-lactam-susceptible” ST59 CA-MRSA acquired MESPM1 and became multidrug resistant in a single step. The distinct resistance patterns of ST59 CA-MRSA may have resulted from IS1216V-mediated deletion within the IS1216V-rich structure [42].

5.2. Adaptation of HA-MRSA to the community

The variants of HA-MRSA (multidrug resistant), which were epidemiologically classified as CA-MRSA, have been isolated from patients or households in the community.

5.2.1. ACMEII-positive ST764 MRSA

A variant, exhibiting ST764 (single locus variant of ST5), of the ST5/SCCmecII lineage (New York/Japan) was positive for ACMEII, SEB, and the enhanced expression of cytolytic peptide genes (psm and hld), but was negative for TSST-1 (lacking SaPIm1/n1) [105]. It was isolated from the blood of a 54-year-old patient with bacteremia, who had been infected in the community, in addition to patients or students in hospitals (unpublished data). ST764 MRSA is the second most frequent isolate, following ST8 CA-MRSA/j, in household colonization in Niigata, Japan. ST764 MRSA is multidrug resistant, including levofloxacin and fosfomycin.

5.2.2. ST5/SCCmecII MRSA with a characteristic of CA-MRSA

A variant of the ST5/SCCmecII lineage (New York/Japan), which was positive for enhanced expression of psm and hld, was isolated from an 89-year-old patient with necrotizing CAP [76]. The variant (named NY/Jv) was multidrug resistant, including levofloxacin. This fatal MRSA CAP case of multidrug resistance may pose a new threat, since the elderly population has been increasing.

6. Virulence factors in CA-MRSA

Diep et al [35], based on data from animal infection models, emphasized the role of some key virulence factors in USA300 infection: ACME in colonization, α-hemolysin (Hla) in necrotizing pneumonia, and PSMs (with high expression levels) in bacteremia and abscess formation. In addition to these USA300 prevalent factors, many virulence factors have been reported for S. aureus and MRSA, depending on strains. Those virulence factors are summarized in Fig. 3 [41,67,70,120–149]; virulence factors may exhibit multiple functions and synergistic actions.

6.1. Factors for adherence

S. aureus adhesins include microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [150,151], such as spike-like proteins anchored to the cell wall (CWASPs) through a C-terminal LPXTG-motif, secretable
I. Adherence (colonization) to skin, tissues, and medical devices

Fig. 3 – Summary of *Staphylococcus aureus* virulence factors and their possible roles in the pathogenicity, proposed in vitro and in animal models. *S. aureus*, including MRSA, produce a number of virulence factors for adherence to skin, tissues, and medical devices (I), immune evasion (II), and development of lesions and symptoms (III). Some of these factors and their specific functions are summarized here. A Data are taken from Refs. [41, 67, 70, 120–149] and described in the text.

**II. Immune evasion**

**III. Development of lesions and symptoms**

expanded repertoire adhesive molecules (SERAMs) [152], biofilms [153], and signal peptides of PVL-S [154]. MSCRAMMs commonly found in MRSA are, for example, adhesins for laminin (Eno), fibronectin (FnBPA, FnBPB), elastin (EbpS), fibrinogen (SdrC, SdrD, and SdrE), and protein A (Spa). SERAMs do not possess an LPXTG motif, and are secreted into culture supernatant or associated with the bacterial cell surface by noncovalent binding [152]. SERAMs include, for example, fibrinogen-binding protein (Efb) and extracellular adherence protein (Eap; alternatively, known as major histocompatibility complex class II [MHC class II] analog protein [Map]) [152].

Therefore, *S. aureus* (and MRSA) is potentially adhesive to host skins, mucosa, the endothelial cell surface, extracellular matrix molecules, and plasma proteins. In addition, some MRSA clones or strains have unique additional adhesin or colonization factors (including candidates), such as ACME for USA300 [51], ACMEII for some ST types (ST764 and others [101, 105, 110]), CWASP/J for ST8 CA-MRSA/A clon (spj gene); Eap = extracellular adherence protein (alternatively, MHC class II analog protein [Map]); ETs = exfoliative toxins; FnBP = fibronectin-binding protein; Hla = α-hemolysin; Hlb = β-hemolysin; Hld = δ-hemolysin; HlgBC = γ-hemolysin (HlgB and HlgC); LukED = leukocidin (LukE, LukD); MSCRAMMs = microbial surface components recognizing adhesive matrix molecules; NTED = neonatal TSS-like exanthematous disease; PMNs = polymorphonuclear neutrophils; PSMx = phenol soluble modulin α; PVL = Panton-Valentine leukocidin; SAgs = superantigens; SCIN = staphylococcal complement inhibitor; SEB = staphylococcal enterotoxin B; SEC = staphylococcal enterotoxin C; SERAMs = secretable expanded repertoire adhesive molecules; Spa = staphylococcal protein A; SSLs = staphylococcal superantigen-like proteins; SSSS = staphylococcal scalded skin syndrome; TSST-1 = toxic shock syndrome toxin-1.

6.2. Factors for immune evasion

*S. aureus* (and MRSA) possesses various factors that interfere with the functions of immunoglobulins and complements, disrupt host phagocytes, and promote internalization.

Spa is an MSCRAMM with an LPXTG motif, but is partially released into culture supernatant. Spa captures Fc domains of IgG and IgM, resulting in interruption of Fc-receptor-mediated phagocytosis and the classical pathway of complement activation [130]. In addition, Spa is a proinflammatory factor through TNF receptor activation [155] and its expression level is stimulated by the expression of PVL [143].
PVL, γ-hemolysin (Hlg), and LukED are bi-component leukocidins; e.g., PVL consists of PVL-S and PVL-F. They disrupt human phagocytes, including PMNs, monocytes, and macrophages [72,127–129]; e.g., PVL induces necrosis at >10 nM and apoptosis at <5 nM against human PMNs [72,126,156].

The immune evasion cluster (IEC) includes secretory factors, staphylococcal complement inhibitor (SCIN, encoded by \( \text{scn} \)) and chemotaxis inhibitory protein of \( S. \) aureus (CHIPS, encoded by \( \text{chp} \)), and also staphylococcal enterotoxin A (SEA, encoded by \( \text{sea} \)) and staphylokinase (SAK, encoded by \( \text{sak} \)) with some exceptions [157]. The SCIN interrupts complement activation by inhibiting C3 converting enzymes [133], while CHIPS inhibits the binding of C5a and formylated peptides to their receptors, resulting in interruption of phagocyte chemotaxis [132]. The IEC is located on a phage (with att in the \( \beta \)-hemolysin gene, \( \text{hbl} \)) [157].

Staphylococcal superantigen-like proteins (SSLs; with no superantigen activity) include 14 SSL species [158] and interfere the functions of immunoglobulins and complements (for SSL7 and SSL10) [134], matrix metalloproteinase 9 function (for SSL5) [135], and adherence of PMNs to endothelial cells (for SSL11) [138].

FnBPA and FnBPB [125] and also Eap (Map) [120] mediate \( S. \) aureus (MRSA) internalization by professional and non-professional phagocytes. \( \beta \)-Hemolysin (Hlb; splingomyelinase) and \( \delta \)-hemolysin (Hld; cytolytic peptide) synergistically disrupt phagosome membrane, resulting in staphylococcal escape into cytosol (intracellular survival) [131]. Further studies are needed because hlb is disrupted by IEC-carrying phage in ST8 CA-MRSA (such as USA300 and CA-MRSA/J); in this regard, the potential virulence of ST59 Taiwanese MRSA, with intact hlb and \( \beta \) genomic island \( \text{vSAj} \beta \) with IEC [42], is noted.

6.3. Factors for specific lesions and symptoms

ET digests desmoglein 1 (Dsg-1) of desmosome, causing the disruption of stratum granulosum and subsequent blister formation and exfoliation of the epidermis [159]. ET involves ETA, ETB, and ETD. ETA and ETB are responsible for SSSS in infants and bullous impetigo in children, while ETD is responsible for bullous impetigo and deep pyoderma in adults [140]. The genetic location is phage (for ETA), plasmid (for ETB), and SaPI (for SED) [160,161]. ETA or ETB-producing MRSA is a successful CA-MRSA clone (ST88 or ST89/ST91), accounting for 10–30% of \( S. \) aureus from bullous impetigo in Japan [80]. ST80 European CA-MRSA carries the ETD gene [162,163].

Bi-component toxins (PVL, Hlg, LukED, and LukGH) and a single-component toxin (Hla) are \( \beta \)-barrel pore-forming exotoxins (BPTs) [129,164,165]. Of those, PVL has historically been noted as a causative factor of cutaneous abscesses, such as furunculosis by \( S. \) aureus [166–168], and as the first CA-MRSA-associated virulence factor for necrotizing pneumonia and sepsis [143,169–171]. Synergistic exacerbating effects of PVL and Spa or influenza virus on lung tissue damage were also reported [143,172]. For osteomyelitis, PVL was epidemiologically and experimentally associated with severe tissue damage (e.g., severe bone deformation, muscle inflammation, and abscess formation) as an exacerbating factor [145,173].

In vitro, PVL induces potent cytolytic activity in human and rabbit phagocytes by binding to lipid rafts, but minor activity to murine cells [126]. In addition, PVL at lower concentrations induces the production of inflammatory mediators (e.g., IL-18, IL-8, IL-18, and TNF-\( \alpha \)) by host phagocytes [174,175]. PVL genes are carried by a phage [176]. PVL action in animal models is controversial. In rabbit models using USA300, PVL showed virulence in necrotizing pneumonia, osteomyelitis, and skin infection, but minor effects in bacteremia [139,144,145,177]. In murine pneumonia models, Hla, which shows potent cytotoxicity in monocytes and lymphocytes [178], has been considered as a major virulence factor [141]. Hla also played a role in abscess formation in rabbit skin infection models using USA300 [139].

Hld and PSMs are peptide cytolsins: Hld has 26 amino acids; PSMz1-4, 20-22 amino acids; and PSM\( \beta \)1, 2, 44 amino acids [70]. To date, all \( S. \) aureus (and MRSA) carry genes for PSMz, PSM\( \beta \), and Hld (and also for Hla); hld is located in the RNAII region; however, for PSMs and Hld, the expression levels of the genes are greater in community isolates than in hospital isolates [63,70,76,103]. In vitro, Hld and PSM\( \alpha \) induce the lysis of human PMNs. These peptides activate the inflammatory reaction of PMNs through formylated peptide receptor [179]. In animal models using USA100 and USA300, PSMz, but not PSM\( \beta \), contributed to skin lesion development. PSM\( \alpha \) and Hld contributed to bloodstream infections in a murine model using USA100. In addition, PSM-mec is also found in SCC\( \text{mec} \) regions (class A mec gene complex), associated with SCC\( \text{mec} \)II, III, and VIII, [116]. In a murine bacteremia model, PSM-mec affected mortality, although in skin infection models, the role of PSM-mec is controversial [115].

Staphylococcal superantigens include TSST-1 (previously called SEF) and at least 24 SEs (SEA-E, SEG-V, and SEX) [146,147,180]. Superantigens cross-link MHC class II molecules of antigen-presenting cells and the variable part of the \( \beta \)-chain of certain T-cell receptors (V\( \beta \)-TCRs), resulting in the abnormal activation of T cells [147]. SEA (and a combination of multiple superantigens) is associated with severe diseases [149]. TSST-1 is responsible for TSS and neonatal TSS-like exanthematous disease (NTED) [146,148]. SEB is included in the list of Category B biological weapons by CDC. A recent study also showed the virulence of TSST-1, SEB, and SEC in the development of lethal pneumonia using a rabbit model [142]. Moreover, TSST-1 and SEB may suppress the motility of PMNs through the inhibition of exoprotein expression, and allow MRSA to invade and damage tissues [181]. Superantigen genes are located on a pathogenicity island such as SaPI5 (seq2 and sek2), USA3mw (sek, sea, sek2, and seq), SaPI3 (sek, seq, seq, or sek, sel, and sek), SaPI1 (sek and sek), SaPI2R (tek), SaPlm1/n1 (tek, sec, and sek), and SaPlm50 (tek, seq, and sel) [34,42,63,182,183]. In addition, sea is sometimes located on the IEC-carrying phage [157].

7. Human invasive infections in the community: MRSA genotypes and symptoms

The MRSA invasive infections in the community in Japan, which surprised clinicians, were investigated for MRSA genotypes, and the data are summarized in Table 1.
### Table 1 – Invasive MRSA infections in the community in Japan and the MRSA characteristics.

<table>
<thead>
<tr>
<th>Case</th>
<th>MRSA genotype</th>
<th>MRSA virulence factor</th>
<th>Relevant adherence/colonization factor</th>
<th>Relevant toxin or toxin-like factor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PVL</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td></td>
<td></td>
<td></td>
<td>Superantigen</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PSMα or Hld expression level</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hla (common)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>SSTI</td>
<td>16 months</td>
<td>ST30/SCC mec IVa</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influenza, athlete</td>
<td>15 years</td>
<td>ST8/SCC mecIVV.new 1.1 (CA-MRSA/J)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>?</td>
<td>ST5/SCC mec II (variant, NY/Jv)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cna, Bbp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CWASP/J</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidural abscess</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSTI</td>
<td>11 years</td>
<td>ST8/SCC mec IVV.new 1.1 (CA-MRSA/J)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influenza</td>
<td>25 years</td>
<td>ST8/SCC mec IVV (USA300)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CWAASP/J</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSTI</td>
<td>17 years</td>
<td>ST30/SCC mec IVc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSI</td>
<td>62 years</td>
<td>ST8/SCC mec IVV (variant, NY/Jv)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cna, Bbp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteomyelitis, Bacteremia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSTI</td>
<td>11 months</td>
<td>ST8/SCC mec IVs (USA300)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteremia</td>
<td>54 years</td>
<td>ST764/SCC mec II (variant of ST5/SCC mec II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| ACME  = arginine catabolic mobile element; Bbp = bone sialoprotein-binding protein; Cna = collagen-binding protein; CWASP/J = CWASP produced by ST8 CA-MRSA/J clone (spj gene); egc = enterotoxin gene cluster; Hla = α-hemolysin; Hld = δ-hemolysin; ND = no description; PSMα = phenol soluble modulin α; PVL = Panton-Valentine leukocidin; SCCmec = staphylococcal cassette chromosome mec; SEB, SEC, SEG, SEI, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, and SEU = staphylococcal enterotoxins B, C, G, I, K-Q, and U, respectively; SSTI = skin and soft tissue infection; TSST-1 = toxic shock syndrome toxin-1. 
a 5 SEs: SEG, SEI, SEM, SEN, SEO.  
b 2 SEs: SEC, SEL.
In this table, SSTI cases are omitted; however, invasive infections could be caused by successful MRSA clones in the community, mostly causing SSTIs (Table 1). Of nine MRSA cases, eight possessed bacterial adhesin/colonization factor (including a candidate) related to CA-MRSA, suggesting the importance of these factors not only in community spread, but also in the pathogenesis.

“Deep” pyogenic skin infection (with unusually large abscesses) was especially noted for PVL-positive USA300 [48, 95, 187] and ST22 CA-MRSA [68]; however, no severe invasive infections were observed for ST22 CA-MRSA in a previous study [68]. Instead, PVL-negative CA-MRSA, such as ST8 CA-MRSA/ [63], often caused severe invasive infections; ST8 CA-MRSA/J was also associated with SSTIs, albeit without unusually large abscesses [63].

Initially, PVL was especially emphasized as a causative toxin of necrotizing pneumonia [143] and pleural infiltrates with multiple nodular and cavity lesions have been noted, especially in necrotizing pneumonia caused by PVL-positive CA-MRSA [171]. Although cavity formation may be observed in some cases of CAP from methicillin-susceptible S. aureus and Klebsiella pneumoniae and in septic pulmonary embolism, it has not usually been observed in HA-MRSA infections (in Japan); however, such necrotizing pneumonia cases have been found even in PVL-negative MRSA, including ST8 CA-MRSA/J [63] and a variant of ST5 HA-MRSA (NY/JV) [76]. Some toxin patterns were consistent with those described by Diep et al [35].

Nine invasive cases were caused by only three genotype groups: ST30 worldwide CA-MRSA, ST8 CA-MRSA (CA-MRSA/J, its variant CA-MRSA/J, and USA300), and ST5 HA-MRSA-related variants (NY/JV and ST764). Further studies, such as close collaboration between clinical and basic aspects, are necessary for better and accurate understanding of invasive CA-MRSA infections in terms of virulence genotyping, including comparative genomics.

8. Characteristics found by CA-MRSA genome analysis

USA300 [51] carried ACME, which may have originated in Staphylococcus epidermidis and is linked with SCCmecVa. ST59/SCCmecV Taiwan clone [42] carried MESm1 originating in enterococci; and ST59/SCCmecV Taiwan clone carried an MESm1-related structure (unpublished data). ST8 CA-MRSA/J in Japan [63] carried SCCmecV.new.1.1 with spj and SaPIj50 with tst loci, originated in ST5 HA-MRSA (New York/Japan). CA-MRSA evolution, as above, includes horizontal gene transfer with an insertion sequence (IS), transposon (Tn), pathogenicity island (SaPI), SCC, genomic island (vSa), plasmid, and phage, and also includes gene salvage.

9. Conclusions

Successful CA-MRSA clones possess a unique adhesin/colonization factor to facilitate MRSA spread in the community, albeit with unknown causes. PVL, which causes secretion of MRSA as pus at the skin surface, allows more MRSA spread through touch (thus acting as a spread factor). There are infection cores in the community. Hand hygiene is strongly requested in the cores. MRSA SSTIs may be decreasing in some countries, but invasive infections (rare cases compared to SSTIs) are increasing in some cases. CA-MRSA invasive factors have been proposed, but further molecular genetic study including comparative genomics is needed, especially to clarify the role of combination (adherence-colonization-spread factor/toxin/immune evasion factor) in the pathogenesis. Some CA-MRSA acquired their virulence cassette from HA-MRSA or other staphylococci, and a resistance cassette from enterococci, and some have spread even to livestock, pets, and public transport, or originated from animals. The evolution and the resulting epidemic of MRSA with “community characteristics” are dynamic, posing a threat even to hospitals and the elderly.

R E F E R E N C E S


