

Community-acquired methicillin-resistant *Staphylococcus aureus*: community transmission, pathogenesis, and drug resistance

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Abstract Methicillin-resistant *Staphylococcus aureus* (MRSA) is able to persist not only in hospitals (with a high level of antimicrobial agent use) but also in the community (with a low level of antimicrobial agent use). The former is called hospital-acquired MRSA (HA-MRSA) and the latter community-acquired MRSA (CA-MRSA). It is believed MRSA clones are generated from *S. aureus* through insertion of the staphylococcal cassette chromosome *mec* (SCC*mec*), and outbreaks occur as they spread. Several worldwide and regional clones have been identified, and their epidemiological, clinical, and genetic characteristics have been described. CA-MRSA is likely able to survive in the community because of suitable SCC*mec* types (type IV or V), a clone-specific colonization/infection nature, toxin profiles (including Pantone-Valentine leucocidin, PVL), and narrow drug resistance patterns. CA-MRSA infections are generally seen in healthy children or young athletes, with unexpected cases of diseases, and also in elderly inpatients, occasionally surprising clinicians used to HA-MRSA infections. CA-MRSA spreads within families and close-contact groups or even through public transport, demonstrating transmission cores. Re-infection (including multifocal infection) frequently occurs, if the cores are not sought out and properly eradicated. Recently, attention has been given to CA-MRSA (USA300), which originated in the US, and is growing as HA-MRSA and also as a worldwide clone. CA-MRSA infection in influenza season has increasingly been noted as well. MRSA is also found in

farm and companion animals, and has occasionally transferred to humans. As such, the epidemiological, clinical, and genetic behavior of CA-MRSA, a growing threat, is focused on in this study.

Keywords Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) · Community transmission · Pathogenesis · Drug resistance · Animals

Introduction

Staphylococcus aureus colonizes the nasal mucosa of approximately 30% of individuals. It is associated with a wide range of infections including skin and soft tissue infections (SSTIs, such as bullous impetigo, abscesses, furunculosis, and staphylococcal scalded skin syndrome), systemic or fatal infections [such as blood stream infection (BSI), endocarditis, and pneumonia], and toxin-associated diseases (such as toxic shock syndrome and food poisoning) [1–4]. Historically, several classes of drug-resistant *S. aureus* have emerged [5–7]. They include penicillin G-resistant *S. aureus*, which produced penicillinase (PCase, penicillin-hydrolyzing enzyme) in the mid 1940s, and multidrug-resistant *S. aureus* (which was resistant to penicillin G, chloramphenicol, tetracycline, and erythromycin) in the late 1950s. Methicillin-resistant *S. aureus* (MRSA), isolated in 1961, continues to be a life-threatening multidrug-resistant bacterium, changing its resistance patterns by acquiring resistance to each new antimicrobial agent. Such MRSA has been a major cause of nosocomial infections, and thus is alternatively called hospital-acquired MRSA (HA-MRSA). In addition, since the period from 1997 to 1999, another class of MRSA has become a major concern worldwide as an emerging pathogen in the

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community [8–11]. This new class of MRSA is called community-acquired MRSA (CA-MRSA).

Ideally, MRSA in the community should impose no biological cost, i.e., it carries a certain type of staphylococcal cassette chromosome *mec* (SCC*mec*) and exhibits resistance to only limited numbers of antimicrobial agents. In turn, it can be selected by low levels of antimicrobial agent use in the community [12–14]. By contrast, MRSA in hospitals carries some other SCC*mec* types or exhibits multiple drug resistance, i.e., it imposes a high biological cost. Therefore, it has to be selected by high levels of antimicrobial agent use in hospitals to overcome the biological cost (in turn, transfer to the community is restricted) [12, 15, 16]. Moreover, CA-MRSA must have high colonization and infection abilities to distribute among healthy individuals in the community.

Current MRSA typing

MRSA is typed based on genetic characteristics such as multilocus sequence type (ST) [17], protein A gene (*spa*) type [18–20], *agr* type [21, 22], coagulase type [23, 24], and SCC*mec* type [25–28]. Generally, ST typing [and analysis of the clonal complex (CC) to which each ST type belongs] is a standard for genotyping (Fig. 1). Typing is based on the seven housekeeping gene sequences, with the allelic profile (allele no. representing the ST type) and CC obtained from the MLST website (<http://www.mlst.net/>) and eBURST software (<http://eburst.mlst.net/>), respectively. In contrast, *spa* typing targets a single gene (*spa*), and the *spa* type is given through a public *spa* type database (<http://tools.eugenomics.com/>) and the Ridom Spa Server (<http://spaserver.ridom.de/>), but with different assignment (e.g., *spa1* for the former and *spat008* for the

latter). Each ST type clone contains some *spa* variants. The *agr* type is determined by PCR; two methods are reported, demonstrating types I–IV. There are subtypes in *agr*I. Coagulase typing is conducted using an antiserum kit (Denka Seiken, Tokyo, Japan), determining types I–VIII. Attempts have been made for coagulase gene (*coa*) typing by PCR, but with incomplete results. Coagulase types well correlate with ST types. SCC*mec* typing is performed by multiplex PCR targeting the *mec* and *ccr* (recombinase gene) complexes or targeting even the junk regions, determining types I–VIII. There are subtypes in SCC*mec*II, III, and IV. Generally speaking, SCC*mec* I, II, and III are of HA-MRSA, while SCC*mec*IV and V are of CA-MRSA [9, 12, 15, 16, 29–32].

For molecular epidemiology of MRSA epidemics due to certain clones, SmaI-digestion of MRSA DNA is used in pulsed-field gel electrophoresis (PFGE), aiming at PFGE typing (i.e., identification of epidemic strains and analysis of their divergence) [33, 34]. Indeed, in the US, MRSA clones have been classified according to SmaI PFGE types, such as USA100 (for ST5 HA-MRSA), USA300 (ST8 CA-MRSA), USA400 (ST1 CA-MRSA), and USA1000 (ST59 CA-MRSA) [33, 34]. Moreover, when an epidemic MRSA clone possesses a unique virulence gene or a combination of unique genes and unique DNA sequences, it can serve as a target for rapid and less-laborious screening of epidemic clones in PCR, multiplex PCR [35–38], or real-time PCR assay.

HA-MRSA

HA-MRSA is typically defined as MRSA isolated from inpatients that had been MRSA-negative at the beginning of hospitalization or MRSA isolated from inpatients 48 h or more after hospitalization [9, 39–41]. There are several types in HA-MRSA [29, 30, 42]. It is believed that MRSA is generated from *S. aureus* (methicillin-susceptible *S. aureus*, MSSA) by acquisition of SCC*mec* and that this has occurred only a limited number of times. Yet such limited numbers of MRSA clones, emerged, have disseminated worldwide, resulting in the current epidemics in hospital settings [42, 43]. Invasive MRSA epidemics were especially noted in the US and Japan in the late 1970s to early 1990s [5–7], when some second-generation cepheims (such as cefoxitin and latamoxef) were widely used. These pandemics were followed by MRSA episodes in Europe. Some are still continuing.

Well-known MRSA clones [11, 42, 44–48] include the archaic clone (ST250/SCC*mec*I; reported in the UK from the 1960s), Iberian clone (ST247/SCC*mec*IA; reported in Spain from 1989), New York/Japan clone (ST5/SCC*mec*II; reported in the US from 1998), EMRSA-16 clone (ST36/SCC*mec*II; reported in the UK from 1993), Brazilian clone

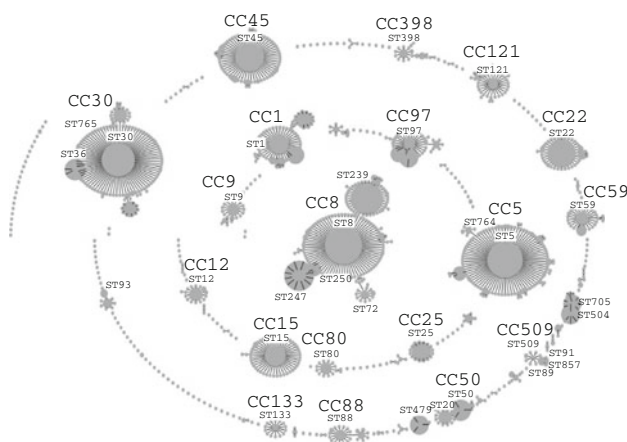


Fig. 1 eBURST analysis of *S. aureus*. Data from the MLST website (<http://www.mlst.net/>) and eBURST software (<http://eburst.mlst.net/>), 23 December 2009. Major CC and ST types are indicated

(ST239/SCCmecIIIa; reported in Brazil from 1992), Hungarian clone (ST239/SCCmecIII; reported in Hungary from 1993), EMRSA-15 clone (ST22/SCCmecIV; reported in the UK from 1993), pediatric clone (ST5/SCCmecIV or IVa; reported in Portugal from 1992), and Berlin clone (ST45/SCCmecIVa; reported in Germany from 1998) (Fig. 2) [11, 42, 44–57]. The Russian clone (ST239/SCCmecIII_R) can also be noted [57]. They are all Pantone-Valentine leucocidin (PVL)-negative. Among them, six clones (Iberian, Brazilian, Hungarian, EMRSA-15, New York/Japan, and pediatric) have disseminated more internationally, and are referred to as pandemic clones [42].

Due to the replacement nature of MRSA clones, distribution patterns in each country or area may change. In Hungary, southern Germany (ST228-I) and New York/Japan (ST5) clones have appeared recently, instead of the Hungarian clone (ST239) [47]. In Hong Kong, three major clones, the Hungarian clone (ST239), New York/Japan clone (ST5), and ST45 clone, have appeared recently [58]. The TW clone, a variant of ST239 MRSA, was recently noted as the cause of an outbreak in an intensive care unit (ICU) in London [59, 60]. The association with bacteremia of TW was four times higher than that of other common MRSA clones (such as EMRSA-15 or EMRSA-16). Therefore, it was described as highly transmissible MRSA

[59, 60]. ST239 HA-MRSA is a growing MRSA group, which includes at least nine members (EMRSA-1, EMRSA-4, EMRSA-7, EMRSA-11, and the Brazilian, Portuguese, Hungarian, Viennese, and Russian clones), and is widely spreading in Asia, South America, and Eastern Europe [44, 61–66].

MRSA incidence among *S. aureus* varies country by country, with a low frequency (around 1%) in some countries in Europe (e.g., the Netherlands and Sweden) and a high frequency (>60%) in the US and Japan (Fig. 2) [51, 55, 67]. In the US, invasive MRSA infections have become a serious problem, with an estimated patient number of 94,360 and 18,650 deaths in 2005. MRSA infections generally occur most frequently among inpatients who undergo invasive medical procedures, who have weakened immune systems (or are immunocompromised hosts) or whose age is 50 or 65 years and older [67–70].

Criteria and risk factors for CA-MRSA

Epidemiologically, CA-MRSA is defined as MRSA isolated from outpatients with no history of hospitalization within the past 1 year, and who presented no other established risk factors for MRSA infection, such as surgery, residence in a

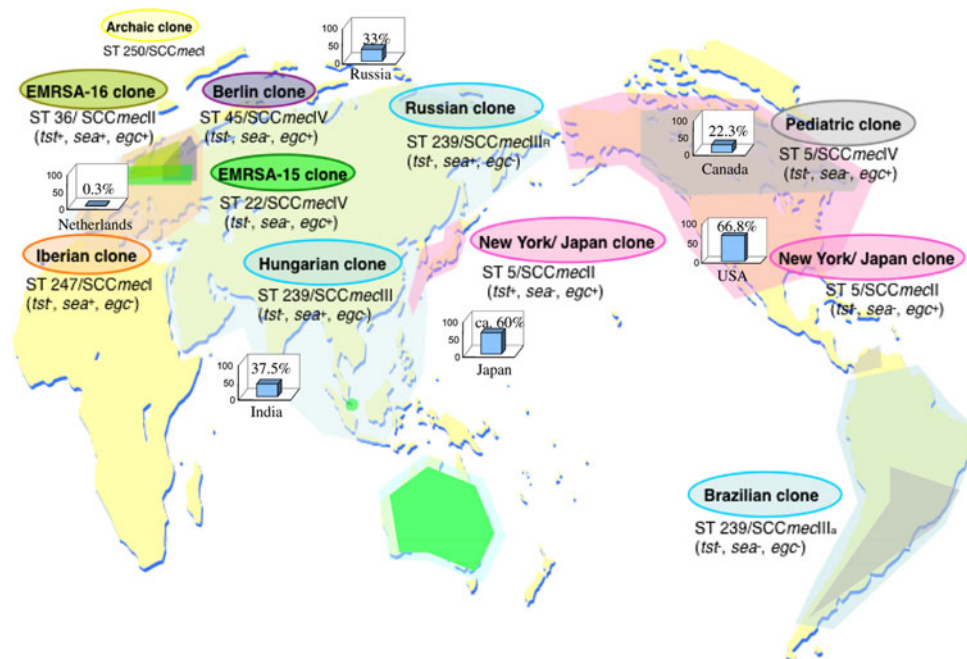


Fig. 2 Worldwide distribution of major HA-MRSA clones. Data from Ref. [11, 42, 44–57]. The colored areas indicate the areas of each spreading clone. Yellow represents the region where the archaic clone was isolated. Orange represents the region where the Iberian clone was isolated. Pink represents the region where the New York/Japan clone was isolated. Lime green represents the region where the EMRSA-16 clone was isolated. Blue represents the region where the

ST239 clone, including the Hungarian clone, Brazilian clone, and Russian clone, was isolated. Purple represents the region where the Berlin clone was isolated. Green represents the region where EMRSA-15 was isolated. Gray represents the region where the pediatric clone was isolated. Graphs indicate the rate of MRSA in all *S. aureus* isolates from the hospitals in each country

long-term care facility, dialysis, or indwelling percutaneous medical devices or catheters [9, 39–41].

Since some MRSA clones have been well established as CA-MRSA, and CA-MRSA has spread into hospital settings, CA-MRSA may also be defined bacteriologically [9, 32–34, 71, 72]. This is probably the case when it is genetically identified (based on, e.g., ST type, *spa* type, and *SCCmec* type), when it is identified by PFGE typing, when it produces PVL, when it possesses *SCCmecIV*, or when it is resistant only to β -lactam antimicrobial agents (in some regions or countries). Low MIC values for oxacillin (MICs, around 32 $\mu\text{g/ml}$ or less) or imipenem (MICs, around 1 $\mu\text{g/ml}$ or less) may also serve as a marker of CA-MRSA; HA-MRSA exhibits higher MIC values for oxacillin (MICs, ≥ 128 $\mu\text{g/ml}$) or imipenem (MICs, 32 $\mu\text{g/ml}$) in many cases [73]. Such a bacteriological definition would be needed to investigate CA-MRSA spread within hospital settings.

CA-MRSA infection occurs in healthy individuals, especially children and adolescents, through skin-to-skin contact. Reflecting this, risk factors for CA-MRSA infection includes (e.g.) university and school students, adolescent athletes, military trainees, jail inmates, men who have sex with men (MSM), members of an infected family, hurricane evacuees, and people with tattoos [10, 33, 74–76].

Clinical aspects of CA-MRSA infections

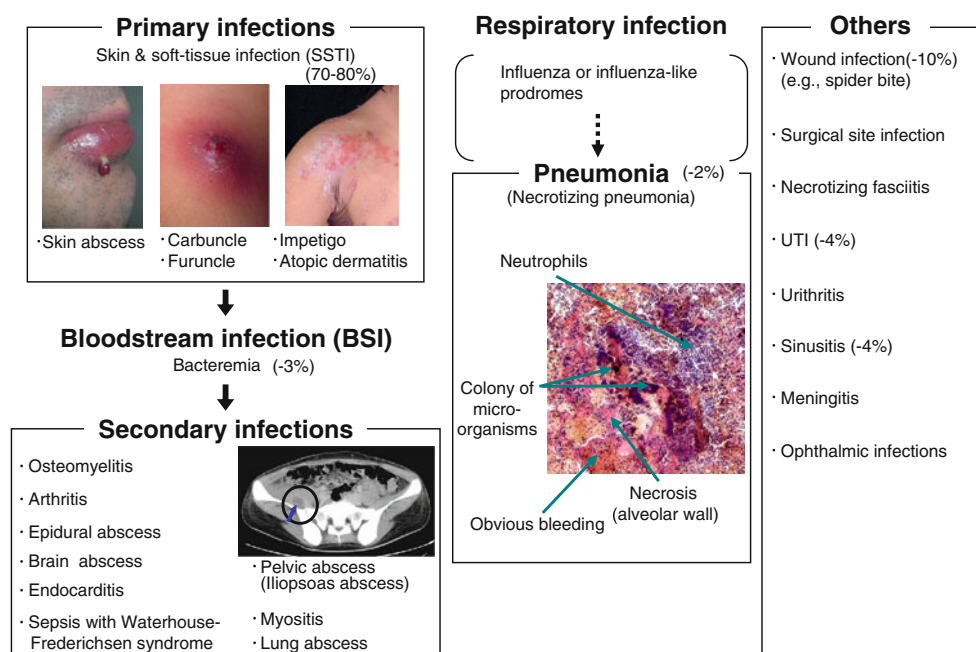
Clinical aspects of CA-MRSA infection are summarized in Fig. 3 [4, 40, 75, 77–95]. CA-MRSA has emerged as a cause of serious infections. The vast majority (70–80%) of

infections with CA-MRSA manifest as SSTI including pyogenic skin infection [40]. The incidence of SSTI caused by CA-MRSA has been increasing (e.g., 24 cases per 100,000 persons in 2000 to 164.2 cases per 100,000 persons in 2005 in the US) [96]. Most people who have developed CA-MRSA skin infection have been healthy individuals (such as children, prisoners, and athletes) without risk factors such as hospitalization [97].

Some CA-MRSA strains produce PVL, which lyses white blood cells. PVL-positive CA-MRSA has been especially isolated from “deep” skin infection and SSTI, such as furuncle, carbuncle, and cellulitis [4]. These patients complain of a severe pain due to unusually large abscesses. On the other hand, impetigo, the most common SSTI disease, which occurs in superficial skin, is related to PVL-negative CA-MRSA. A study of nasal MRSA carriers (53% positive for USA300) among American soldiers has shown that 29% of nasal MRSA carriers developed SSTI 1 year later. Of the nasal MRSA carriers who developed SSTI, 97% were positive for USA300, indicating that more than 50% of nasal USA300 carriers developed SSTI [98]. This strongly suggests that nasal carriage of MRSA is a risk factor for SSTI; however, the significance of nasal carriage may be varied among the clones. Especially, nasal USA300 carriers may be the high-risk group for SSTI.

CA-MRSA can also cause wound infections. Bacterial chronic wounds (diabetic ulcer, venous stasis ulcer) have increased over the past years, and the majority of these are due to CA-MRSA [80]. CA-MRSA has also been detected from surgical site infection, urinary tract infections (UTI) [82], infections of the eye and orbit [83], meningitis [85], and sinusitis.

Fig. 3 Diseases related to CA-MRSA infection. Data from Refs. [4, 40, 75, 78–84, 86–95]. Arrow in computer-assisted tomography image points to pelvic abscess. Numbers in parentheses represent incidence of the disease



Severe necrotizing fasciitis cases caused by CA-MRSA have been described as well. Necrotizing fasciitis is a rapidly progressive infection of adjacent subcutaneous tissue and superficial fascia. A 36-year-old Hispanic male with human immunodeficiency virus (HIV) infection suffered necrotizing fasciitis caused by CA-MRSA. In that case, the strain had the USA300 genotype, which is the most common CA-MRSA clone in the US and now spreading worldwide [99]. In another report, five cases of necrotizing fasciitis caused by USA300 were also described [87].

Nasal carriage and SSTI caused by CA-MRSA are also the risk factors for bloodstream infections (BSIs). USA300-associated BSIs have been described [100, 101]. Nasal carriage of *S. aureus* associates with higher incidence of BSIs, compared to non-carriers. Previous studies have reported that in approximately 80% of the patients who were initially positive for nasal *S. aureus* at admission and then developed BSIs, the PFGE genotypes of isolates from blood cultures were identical to those of the isolates from nasal swabs taken at admission, indicating endogenous infection [79, 81].

Management of SSTI caused by CA-MRSA includes incision and drainage of infected tissue and appropriate antimicrobial therapy. Most people with CA-MRSA primary infections can be cured with appropriate treatment. On the contrary, some patients with CA-MRSA primary infection will unfortunately develop severe life-threatening infections, so-called CA-MRSA-related secondary infection, probably through BSIs.

As has been reported, the mortality rates of community-acquired and hospital-acquired BSIs were 10 and 15%, respectively [78]. Malani et al. [102] have reported that advanced age, chronic renal insufficiency, catheter-related infection, Charlson weighted index of comorbidity score, and presence of MRSA were associated with in-hospital and 6-month mortality of *S. aureus* BSI. A case of BSI with CA-MRSA in four drug users was described in the UK [103].

Secondary infection with CA-MRSA includes pelvic abscess [75], lung abscess [84], osteomyelitis [86], arthritis [88], brain abscess [89], myositis [90], and endocarditis [91]. The prevalence of *S. aureus* secondary infection is very rare, but severe. Severe systemic infection due to CA-MRSA represents as an initial cutaneous abscess progressing to bacteremia, osteomyelitis, and simultaneous iliopsoas and piriformis abscesses [75]. Lung abscesses due to CA-MRSA are also commonly associated with SSTI [104]. Septic arthritis caused by USA300 isolates has been described. The arthritis was associated with longer duration of fever [104]. Although rare, CA-MRSA causes purpura fulminans and Waterhouse-Friderichsen syndrome, which are associated with a very high mortality [93].

CA-MRSA infections of the respiratory tract are less common in comparison with HA-MRSA, but can be severe. Typical symptoms of CA-MRSA-associated community-acquired pneumonia (MRSA-CAP) include high temperature, arterial hypotension, hemoptysis, rapid aggravation to the point of septic shock, and the need for ventilatory support [105]. In the US, two pediatric fatal cases (ages of 13 years and 12 months) due to necrotizing pneumonia of CA-MRSA (USA400) were reported in 1997 and 1998, respectively [8]. Fatal MRSA-CAP caused by PVL-positive ST30 CA-MRSA in a 1-year-old boy has been reported in Japan [92]. Most importantly, co-infection of influenza patients with CA-MRSA is associated with severe pneumonia with high fatality. In the US during the 2003–2004 influenza season, USA300- and USA400-caused pneumonia among patients with seasonal influenza or influenza-like disease (mean age 21 years, range 3 months–62 years) showed approximately 30% fatality [94]. In Consensus Guidelines, the American Thoracic Society and Infectious Diseases Society of America emphasized that CA-MRSA pneumonia, as well as SARS and novel influenza, should be paid attention to as life-threatening CAP [95]. In 2009, outbreak of the novel swine origin influenza A (H1N1) occurred worldwide. Fatal co-infection of a 42-year-old sailor with swine origin influenza virus A (H1N1) and PVL-positive ST30 CA-MRSA has already been reported in Hong Kong [106]. Therefore, for all age groups, pneumonia due to co-infection with influenza virus (including novel swine origin influenza virus A) and CA-MRSA should be given serious attention.

Molecular characteristics and epidemiology of major PVL-positive CA-MRSA

Well-known and well-characterized CA-MRSA clones include CA-MRSA belonging to ST1 and ST8 (USA400 and USA300, respectively), which are mostly found in the US [33, 34] and Canada [107, 108]. The USA300 clone associates with SSTI, and also invasive infections such as sepsis, pneumonia, and necrotizing fasciitis. It has the ability to replace preexisting MRSA clones, and now is the predominant CA-MRSA clone in the US [33, 34]. It is also expected to become a major clone even in hospital settings.

As for the evolution of USA300 (type strain USA300-0114), based on MLST data, seven cell surface protein genes, and virulence genes including (e.g.) enterotoxins, PVL, and the arginine catabolic mobile element (ACME) [34, 109], it has been proposed that HA-MRSA USA500 (ST8/SCC*mecIV*; negative for enterotoxins, PVL, and ACME) initially emerged from ST8 MSSA through the acquisition of SCC*mecIV*; subsequently, USA300 emerged

from USA500 through mutation in two cell surface protein genes, and subsequent acquisition of staphylococcal pathogenicity island 5 (SaPI5, carrying *sek* and *seq*), PVL prophage, and ACME.

Drug resistance of USA300 (USA300-0114) has been diverging. Analysis of early 2000s USA300 strains revealed that 90% of the strains carried a 3.1-kb cryptic plasmid and 30-kb plasmid carrying a macrolide transporter gene (*msrA*) and *blaZ* gene, resulting in macrolide resistance, as well as β -lactam resistance (“core resistance”) [33]. Some of those strains further showed additional resistance to i) tetracycline through acquisition of a 4.3-kb plasmid with a *tetK* gene; ii) clindamycin through acquisition of a 2.6-kb plasmid with an *ermC* gene; or iii) levofloxacin [33] through *gyrA* mutation. Genomic analysis of an early 2000s strain (TCH1516) also revealed another β -lactamase plasmid (accession no. CP000731; 27 kb in size) with an aminoglycoside-resistant gene (*aphA-3*) and cadmium-resistant gene (*cadD*), in addition to *msrA* and *blaZ* [110].

Since the mid 2000s, clindamycin resistance and mupirocin resistance have increasingly been noted [111, 112]. Particularly, mupirocin resistance has been highly associated with multiple drug-resistant strains that frequently show resistance to fluoroquinolone, tetracycline, macrolide, lincosamide, and mupirocin. Genomic analysis of a multiple drug-resistant strain of USA300 [USA300-0114 (strain FPR3757), accession no. NC_007793] revealed carriage of a 37-kb plasmid with an *ermC* and mupirocin resistant gene (*mupA*), as well as *gyrA* mutation and a 4.4-kb *tetK* plasmid, but not a β -lactamase plasmid [72].

More recently, vancomycin-intermediate strains of USA300 (designated as VISA) have been noted [113, 114]. Although mutations associated with vancomycin-intermediate phenotypes have been proposed [115], the exact responsible genes for the vancomycin-intermediate phenotypes of USA300 are still unknown.

In the US, there has been recent clonal expansion of a subset of USA300 isolates, originating from the common ancestor [116]. Those USA300 isolates are identified primarily based on i) PFGE patterns, but also based on other genetic markers such as ii) ST type (ST8), iii) *spa* types [major type: *spa1* (t008)], iv) PVL genes, v) ACME, vi) SCCmecIVa, vii) the 40-bp direct repeat unit (*dru*) region, which is located adjacent to IS431 within SCCmecIVa, and viii) a signature AT repeat sequence within the hypothetical SACOL0058 gene, which is located 1.4-kb downstream of the 3' end of SCCmecIVa [33, 34, 37, 72, 117]. A subset of USA300 isolates with modest genetic changes, however, may differ in their virulence potentials. Major types in the US are USA300-0114 (*spa1*) and USA300-0247 (*spa1*), and minor types include (e.g.) USA300-0114 (*spa168*). In addition, USA300-0114 (*spa168*) exhibited low virulence in a mouse sepsis/bacteremia model [116].

The USA300 clone has already spread across the Atlantic and Pacific oceans [108, 111, 112, 116, 118–130]. It has been isolated in, e.g., Canada (North America), the UK, Germany, Denmark, Switzerland, Austria, and Italy (Europe), Colombia (South America), Australia (Oceania), and Japan, Korea, and Taiwan (Asia), as well as the US. Among USA300 clones, clones with *spa1* (t008) have been widely identified in North America and Europe (Table 1). However, hospital-associated USA300 with *spa363* (t024), as well as a community-associated clone with *spa1* (t008), has been isolated in Denmark. Other variants with *spa163* and *spat063* have also been isolated in the US and Australia, respectively. In Japan, USA300 clones with *spa363* (t024) and *spa985* (t711) have been isolated.

In the US, multidrug-resistant USA300 clones, which were resistant to levofloxacin, tetracycline, erythromycin, clindamycin, and mupirocin, have been isolated [72, 111, 112]. On the other hand, USA300 clones isolated in the other countries have shown resistance to limited drugs (Table 1).

ST80 CA-MRSA is mostly found in Europe [9], though also in North Africa (Algeria) [131] and the Middle East [132]. Fusidic acid resistance, mediated by *fusA*, *fusB*, or *fusC* genes, is one of the genetic and phenotypic characteristics [9, 133, 134]. Currently, high-level mupirocin-resistant strains, whose MIC levels were >1,024 $\mu\text{g}/\text{ml}$, emerged in Kuwait, suggesting the difficulty of eradication of ST80 CA-MRSA [135]. This was mediated by different sizes (21 and 26 kb) of plasmids with *mupA* (high-level mupirocin resistance gene).

ST59 CA-MRSA is spreading in Taiwan [32] and has also been isolated from Hong Kong [136], the US (USA1000) [33] and Japan. ST59 CA-MRSA from Taiwan carries a unique SCCmec structure (initially designated as SCCmec-VII and currently reclassified as SCCmecV) that involves two distinct *ccrC* genes [32, 137]. In contrast to the initial common understanding that CA-MRSA is generally resistant to only β -lactam agents, it was multiple drug-resistant to tetracycline, kanamycin, streptomycin, erythromycin, clindamycin, and chloramphenicol. ST59 CA-MRSA carries a PCase plasmid with a tetracycline resistance gene (*tetK*), composite transposon-bearing streptomycin resistance gene (*aadE*), kanamycin resistance gene [*aph(3')*-III], and erythromycin/clindamycin resistance gene (*ermB*) [32]. In contrast to many other cases [in which the chloramphenicol resistance gene (*cat*) is carried by a plasmid], the *cat* gene in the Taiwanese clone is located in the chromosome. PVL-negative ST59/SCCmecIVx strains with the same multiple drug resistance pattern are present in Taiwanese hospital settings [32]. Recently, a very similar PVL-positive ST59/SCCmecVII strain was found in Japan [138].

PVL-positive ST30 CA-MRSA [9, 33, 89, 139–141] is a global clone, which is spreading worldwide including

Table 1 Characteristics of USA300 isolates in each country

Country	<i>spa</i> type ^a / PFGE type	Major (non- β lactam) drug resistance	Reference
North America			
USA	1 (t008) /USA300-0114	EM, LVFX	[111, 112, 116]
	1 (t008)/USA300-0114	EM, LVFX, TC, CLDM, MUP	
	1 (t008)/USA300-0247	EM, LVFX, TC, CLDM, MUP	
	168	ND	
Canada	1 (t008)	EM, CPFEX	[108]
Europe			
UK	1 (t008)	EM	[118]
Germany	1 (t008)	EM, CPFEX, etc.	[119, 120]
Denmark	1 (t008) ^b	KM, EM	[121]
	363 (t024) ^c	EM, CLDM	
Switzerland	1 (t008)	ND	[122]
Austria	1 (t008)	EM, CPFEX	[123]
Italy	1 (t008)	EM, CPFEX	[124]
Asia			
Japan	363 (t024), 985 (t711)	EM	[125, 126]
Korea ^d	ND	ND	[127]
Taiwan	ND	ND	[128]
Oceania			
Australia	ND	EM (<i>msrA</i>), KM (<i>aphA3</i>)	[129]
South America			
Colombia	ND	ND	[130]

EM ycerythromycin, LVFX levofloxacin, TC tetracycline, CLDM clindamycin, MUP mupirocin, CPFEX ciprofloxacin, KM kanamycin, ND no data available

^a *spa* types from the Ridom Spa Server are shown in parentheses

^b CA-MRSA associated

^c HA-MRSA associated

^d USA300-like strain

Japan. Nosocomial outbreak-derived MRSA in Japan in the 1980s was largely PVL-positive ST30 MRSA with *spa43* and *SCCmecIVa*, *IVc*, or *IVx* [142], while current PVL-positive ST30 strains exhibit *spa19* and *SCCmecIVa* or *IVc*. Some PVL-positive ST30 strains acquired a plasmid (pGKT1) conferring resistance to gentamicin, kanamycin, and tetracycline [141].

In the 1950s and 1960s, PVL-positive and -negative penicillin-resistant *S. aureus* (ST30/*spa43*) spread in hospitals and the community worldwide (e.g., Australia, Denmark, the UK, US, and Cuba) [143]. One of the possibilities is that PVL-positive ST30 CA-MRSA evolved from this worldwide ST30 MSSA. PVL-positive ST30/*spa19*/*SCCmecIVc* with the same PFGE pattern was isolated from Far Eastern Russia, while PVL-positive ST30/*SCCmecV* with the same PFGE pattern from Egypt exhibited *spa251*. PVL-positive CA-MRSA clones also include other ST types such as emerging ST22, spreading to Europe and Japan, [144, 145] and ST93 [146].

CA-MRSA factors associated with colonization and spread in the community

Some major CA-MRSA clones have unique colonization and infection ability, which may play a role in community

transmission. The USA300 clone (ST8) possesses the ACME-*SCCmecIV* linkage as a merit of colonization and spread [72]. ACME-*SCCmecIV* linkage, which was first found in USA300, is considered to play a key role in the persistence of USA300 in the community. ACME (type I) in USA300 appears to originate in *S. epidermidis*, a representative skin-colonizing bacterium [12]. ACME contains two operons associated with pathogenesis, *arc* (encoding for the arginine deaminase pathway), and *opp3* (encoding for the oligopeptide permease system) [12, 72]. Arginine deaminase is a virulence factor of *Streptococcus pyogenes*, and plays a role in (e.g.) survival at low pH, inhibition of human peripheral blood mononuclear cell proliferation, or invasion [147, 148]. Oligopeptide permease is associated with (e.g.) MRSA growth and infection [149]. ACME is also found in certain strains of ST5 (USA100) and ST59 (USA1000), but such ACME lacks one possible virulence component (the *opp3* operon) found in USA300 [12].

The presence of *SCCmecIV* is an advantage for CA-MRSA, because low levels of antimicrobial agents use in the community could be sufficient to drive the spread of MRSA with *SCCmecIV* (imposing no biological cost) [12–14]. In contrast, *SCCmecI*, *SCCmecII*, or *SCCmecIII* (all of which impose biological cost) would need high levels of antimicrobial agent use (in hospitals) to overcome the

biological cost, and may restrict such HA-MRSA from spreading into the community [12, 15, 16].

The PVL-positive ST30 clone carries the collagen adhesin gene (*cna*) and bone sialoprotein adhesin gene (*bbp*) [140], and the combination of PVL, *cna* and *bbp* would play a role in colonization to matrix within the body. The presence of the PVL gene and the *cna* gene correlates with pulmonary manifestations, probably due to viral infection-caused epithelial damage permitting binding of PVL-positive *S. aureus* to exposed collagens and laminin [150, 151]. In addition, presence of the *bbp* gene allows *S. aureus* adherence to bone sialoprotein, leading to hematogenously spread osteomyelitis, arthritis, and iliopsoas abscess [75, 152–154].

The PVL-positive ST22 clone exhibits heavy biofilm formation (Fig. 4), which results in heavy colonization among family members and their relatives [of the 12 members in 4 families, 9 (75%) were colonized with the same MRSA, Fig. 4] [144]. In these families, cutaneous abscess occurred every 2 months.

CA-MRSA is also spreading among healthy (asymptomatic) people in the community (including families or public transport). CA-MRSA colonizes the nares or cheek

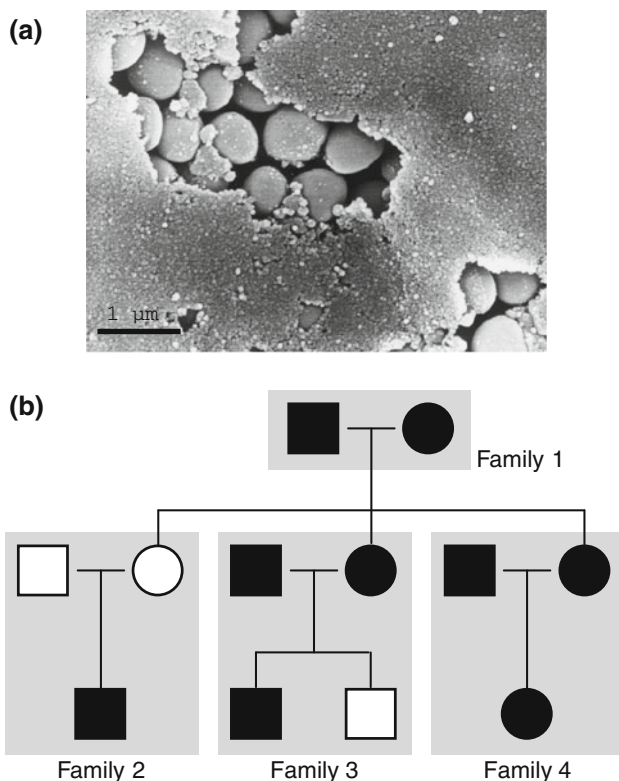


Fig. 4 Scanning electron micrograph showing ST22 MRSA and its biofilm (a) and family pedigree with ST22 MRSA infection (b). Data are from Ref. [144]. a Strains were grown on trypticase soy agarII with 5% sheep blood at 37°C overnight. Bar is 1 μm. b Square and circle represent male and female, respectively. Closed mark represents the person who was colonized on infected with MRSA

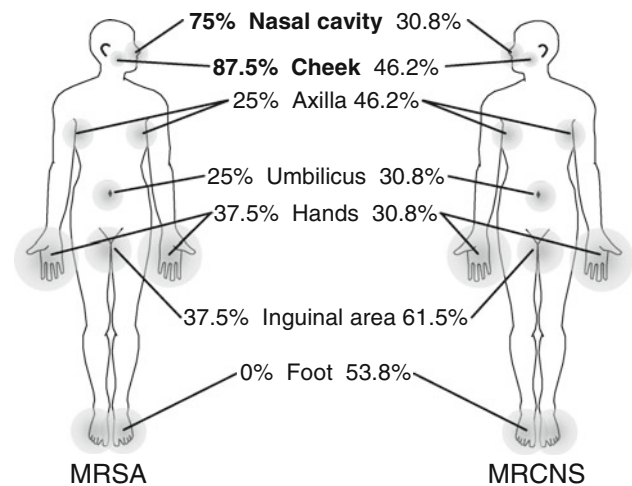


Fig. 5 Distribution of MRSA and MRCNS in the body. Data from Refs. [144, 155]. Percentages show MRSA incidence isolated from patients and family members or MRCNS incidence isolated from healthy volunteers

in many cases, but also axilla, umbilicus, and groin (Fig. 5) [144]. Although methicillin-resistant strains (MR-CNS) of coagulase-negative staphylococci, a major pathogen of nosocomial BSI, colonize even the feet [155], no MRSA is isolated from the feet [144]. Cheeks and the nasal cavity can be highly colonized by CA-MRSA in comparison with other parts of possible colonization. So it is highly possible that CA-MRSA can be transmitted through close body contact (hugs, kisses, etc).

Virulence factors

Adhesins common to MRSA

Adhesins involve not only microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [156, 157], which are spike-like protein structures anchored to the cell wall, but also biofilms [158], and signal peptides of PVL [159]. MSCRAMMs commonly found in MRSA are (e.g.) adhesins for laminin, fibronectin, elastin, fibrinogen, and protein A (Spa). Some adhesins play a role not only in cell adherence but also in cell invasion [160].

Immuno-modification factors at the initial stage of infection

Spa (protein A) commonly presents on the cell surface of most *S. aureus* clinical strains, and, in cases, is released in culture supernatants [161–163]. The N-terminus of Spa neutralizes immunoglobulins through direct binding to Fc and Fab fragments, indicating immunoresistant protein (which prevents efficient elimination of bacteria by immune

cells). The C-terminus is the transmembrane domain. Short sequence repeats (SSRs), consisting of eight amino acids, are located between the Fc binding domain and transmembrane domain and are used for *spa* typing [18–20]. Structures of SSRs could vary the virulence of Spa by changing the distance of the Fc binding domain from the cell surface. It is also known that Spa activates tumor necrosis factor receptor 1 signaling of human airway epithelial cells through the Fc binding domain, resulting in release of IL-8 [164]. PVL and Spa synergistically induce severe damage of lung tissue and high mortality in murine necrotizing pneumonia models using PVL-positive *S. aureus* [163].

Toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxin B (SEB) could suppress the motility of polymorphonuclear neutrophils (PMNs) through the inhibition of MRSA exoproteins expression [165, 166]. SEG, SEI, SEM, SEN, and SEO [all located on the enterotoxin gene cluster (*egc*)] [167] modify polyclonal T cell proliferation and induce immune anergy by T cell suppressor activity, B cell depletion, and inhibition of antibody responses [167–170]. And also, they may reduce *S. aureus* pathogenicity, and may contribute to bacterial colonization [171, 172].

PVL targets PMNs and induces cell death, resulting in bacterial evasion from bactericidal functions of PMNs [173]. ACME inhibits human peripheral blood mononuclear cell proliferation, and also inhibits nitric oxide production of the immune cells, which is important in both innate and adaptive immune responses [72]. Fibronectin-binding proteins play a role in adherence to extracellular matrix and invasion of host cells including epithelial cells and fibroblasts [174]. Therefore, possession of those factors could be an advantage in colonization and/or invasion of MRSA.

Cytolysins

Hemolysins include α -hemolysin (α -toxin; gene, *hla*), β -hemolysin (β -toxin; gene, *hlyB*), γ -hemolysin (γ -toxin; genes, *hlgA*, *hlgB*, *hlgC*), and δ -hemolysin (δ -toxin; gene, *hld*) [174, 175]. Of those, at least α -, γ -, and δ -hemolysin exhibit cytolytic activity even for non-erythrocytes. α -Hemolysin is a strong cytolytic toxin in murine models. γ -Hemolysin consists of three component proteins, HlgA, HlgB, and HlgC. Combination of HlgA and HlgB acts as hemolysin, and combination of HlgB and HlgC acts as a leucocidin [173]. δ -Hemolysin is a phenol-soluble modulin that may be classified as a cytolytic peptide [176]. The δ -hemolysin gene is located within the RNAPIII region in the *agr* locus.

Leucocidins are a family consisting of LukED, PVL, γ -hemolysin (HlgB and HlgC), and LukM [173, 175, 177]. The first three leucocidins are virulent for humans [173], while LukM is virulent for bovines (not for humans) [177]. PVL is unique to CA-MRSA. The PVL and LukM genes are carried by a phage.

Cytolytic peptides

Cytolytic peptides are 22–25 amino acids or 40 amino acids long [178]. *S. aureus* (including MRSA) harbors three distinct cytolytic peptide genes on three different loci on the chromosome. The three genes are the δ -hemolysin gene (as described above), the PSM α gene (4 tandemly-repeated genes, PSM α 1 to PSM α 4), and the PSM β gene (2 tandemly-repeated genes, PSM β 1 and PSM β 2) [178]. They are expressed to a higher extent in CA-MRSA [such as USA300 (ST8), USA400 (ST1), ST30, and ST59], than in HA-MRSA [such as New York/Japan (ST5)] [178, 179]. In addition, HA-MRSA carries additional cytolytic peptide genes on SCCmecII and SCCmecIII [180]. The PSM-mec gene is not present in SCCmecIV, which is common in CA-MRSA.

Superantigens

Superantigens bind to major histocompatibility complex class II (MHC II) molecules and the variable part of the β -chain of certain T-cell receptors (V β -TCRs), resulting in unusual activation of T-cells [181–185]. Staphylococcal superantigens are also called SE, and include TSST-1 (previously SEF), SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, SES, SET, SEU, and SEV [185, 186]. SEA (and a combination of multiple superantigens) is associated with severe diseases [171, 187]. 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 [165, 166].

Superantigen genes are located on a pathogenicity island [165, 188], such as SaPI5 carrying *seq2* and *sek2*, Φ Sa3mw carrying *sek*, *sea*, *sek2*, and *seq*, SaPI3 carrying *seb*, *sek*, *seq*, or *seb*, *sel*, and *sek*, SaPI1 carrying *tst*, and *sek*, and SaPI_{m1/n1} carrying *tst*, *sek*, and *sel*. At least SaPI1 and likely SaPI3 are mobile pathogenicity islands, and can be transferred from bacterial cell to cell by the action of a phage in such a way that SaPI is excised from the chromosome, and packed into phage particles to be transferred to other cells [189].

Exfoliative toxins

Exfoliative toxins are proteinase from *S. aureus*, which digest desmoglein 1, resulting in exfoliation of the epidermis cells [190]. Exfoliative toxin involves ETA, ETB, and ETD. Of those, ETA and ETB are responsible for staphylococcal scalded skin syndrome (SSSS) in infants and bullous impetigo in children, while ETD is responsible for not only bullous impetigo but also deep pyoderma in adults [191]. ETA is expressed in ST88 CA-MRSA, and ETB in ST89 or ST91 CA-MRSA. ST89 CA-MRSA is

rarely positive for both ETA and ETB [141]. ETD is found in ST80 CA-MRSA [35, 71]. The ETA gene is carried by a phage [192], while the ETB gene is located on a plasmid [193]. The ETD gene is located on a pathogenicity island on the chromosome [194].

Structure, activity, and clinical importance of PVL

PVL is considered as an immunoresistant factor due to its cytotoxicity against human PMNs and monocytes [195]. Possible association of PVL with necrotizing lesion development has been described. PVL, a member of

staphylococcal two-component pore-forming toxins, consists of PVL-S (284 amino acids, 32 kDa) and PVL-F (301 amino acids, 34 kDa) (Fig. 6a) [196–199], which can be purified from bacterial culture supernatants as a monomeric form [173]. Crystal structures of PVL-S and PVL-F water-soluble monomers were reported in 2004 [199] and 1999 [196], respectively. Sequences of those crystal structures were identical to those of the PVL gene core sequence (in ST30 CA-MRSA and others) in Fig. 7 [32, 200, 201]. PVL-S monomer, but not PVL-F monomer, can bind to PMN membrane with high affinity in vitro, indicating that PVL-S mediates receptor binding

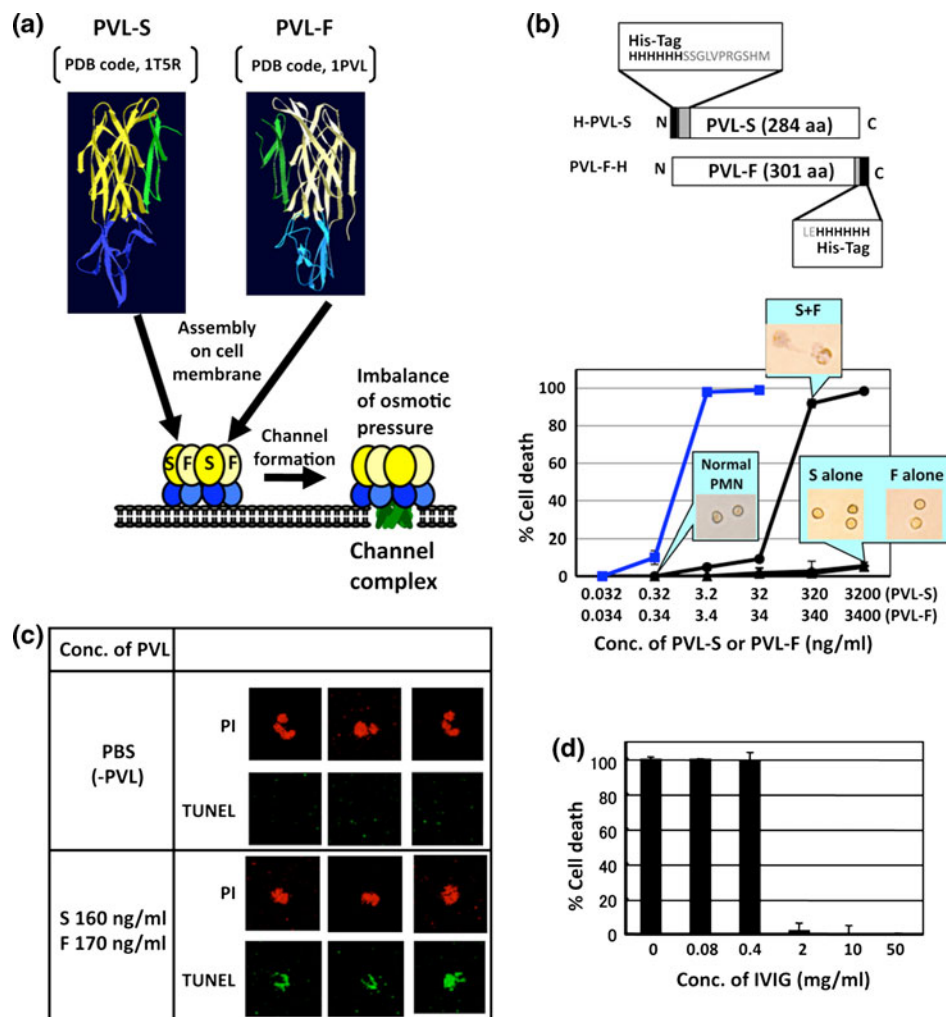


Fig. 6 Structures and functions of PVL. **a** Data from Refs. [173, 196–204]. Protein Data Base (PDB) codes of PVL-F monomer (PDB code, 1PVL) [196] and PVL-S monomer (PDB code, 1T5R) [197] were downloaded from the RCSB Protein Data Bank (<http://www.pdb.org/>) [198], and their crystal structures were reconstructed using SWISS-MODEL and the Swiss-Pdb Viewer (<http://www.expasy.org/spdbv/>) [199]. **b** Schematic representation of primary structures of recombinant histidine (His)-tagged PVL-S (H-PVL-S) and PVL-F (PVL-F-H) and their cell lysis activities are shown. Necrosis of human PMNs and monocytes was determined by

morphological change and trypan blue staining. Percents of cell lysis (mean \pm SD) are shown (black lines, human PMNs; blue line, human monocytes). In the boxes, light micrographs of PMNs (incubated with the indicated concentrations of PVL) are also shown. **c** PVL-induced apoptosis of human PMNs was detected by TUNEL staining (green) under a fluorescent microscope. Nuclei were also stained with propidium iodide (PI). **d** Neutralization of PVL activity with intravenous immunoglobulin G (IVIG) was examined. PVL proteins were pre-incubated with the different concentrations of IVIG for 15 min at 37°C and then incubated with human PMNs

of PVL in CA-MRSA (USA300)-induced muscle lesions and necrotizing pneumonia in the murine model and protective effects of PVL immunization [206]. These data suggest the role of PVL in lesion development. In contrast, Voyich et al. [207] and Bubeck-Wardenburg et al. [208, 209] provided counterevidence. They showed no significant difference in virulence (e.g., abscess volume, lesion size, mortality) between PVL-positive CA-MRSA and its PVL-knocked down mutants in the murine models (skin abscess, dermonecrosis, pneumonia, and bacteremia). Actually, necrotizing fasciitis caused by PVL-negative MRSA has also been reported clinically [210].

Currently, the role of PVL in bacterial survival at the early stage of infection has also been described elsewhere [211]. Other than cytotoxicity, the possible role of the PVL-S signal peptide that attaches to the cell wall after cleavage in bacterial adhesion to collagens (components of tissue extracellular matrix) has also been described [159]. Although the exact role of PVL as a cytotoxin is still controversial, possession of PVL may be an advantage for CA-MRSA at the early stage of infection. Since patients with PVL-positive CA-MRSA infection complain of severe pain and much pus, it is also possible that PVL may induce higher cytotoxicity in human than that in other animal species.

Subinhibitory concentrations of clindamycin, rifampicin, and linezolid inhibit PVL production, while β -lactam agents enhanced PVL production [212, 213]. Intravenous immunoglobulin G (IVIG), a product of Japan, neutralizes PVL activity against human PMNs at the levels of normal blood IgG (8.7–17 mg/ml) or less (Fig. 6d). This was the case even for human monocytes. Therefore, these agents may have therapeutic potential for infection with PVL-positive CA-MRSA.

PVL gene

The PVL region is a polycistronic operon, which contains the S component gene (*luk_{PV}S*, 939 bp) upstream and the F component gene (*luk_{PV}F*, 978 bp) downstream, with a single-base intercistronic region [214, 215].

Gene mutations and amino acid changes of the PVL gene that have been identified are summarized in Fig. 7a [32, 200, 201]. All gene mutations were substitutions: 11 substitutions in the S gene and 5 substitutions in the F gene. In the S gene, 6 of 11 were non-synonymous substitutions, which change amino acids; all of the amino acid changes occurred in the mature S component. In contrast, in the F gene, of five synonymous substitutions (which do not change amino acids), only one caused amino acid change in the mature F component. The facts that amino acid changes accumulate in the S component and the F component is highly conserved strongly suggest that the S

component plays a role in receptor bindings in tissues and the F component plays a role as a toxic component. This result is consistent with a previous notion, obtained by purified component analysis, that the S component acts as a receptor binding, while the F component acts as a toxic component.

The PVL gene is found in various MSSA and MRSA clones. Phylogenetic analysis of the PVL gene is summarized in Fig. 7b [32, 200, 201]. The PVL gene has been identified in 12 ST types. Genes encoding the PVL component with different amino acid sequences are distinguished by different colors. Based on phylogenetic tree analysis, the core sequences could be those of the ST30/ST1335 (single locus variant of ST30)/ST50 group—ST30 includes CA-MRSA and MRSA in the 1980s and 1990s, and ST50 includes CA-MSSA associated with SSTIs; the ST30/ST1335/ST50 group could be an origin of various PVL gene sequences. The same PVL amino acid sequences as those of the ST30/ST1335/ST50 group can be found in ST1, ST22, ST25, ST59, ST80, ST121, and ST1010 (although the gene nucleotide sequences were divergent from those of the ST30/ST1337/ST50 group). However, it should be noted that there existed distinct PVL amino acid sequences in ST30, ST1, and ST8. Distinct PVL amino acid sequences are also found in five other minor cases (Fig. 7b). Based on the previous data of PVL and its homologues [32, 173], and amino acid changes in PVL crystal structures [196–204] and deduced mode of action of PVL are shown in Fig. 7c. In PVL-S, a change at His68 is located at the bottom surface of rim domain (blue, Fig. 7c), suggesting the effect on receptor binding. Of four amino acid changes located in β -sandwich domain (yellow, Fig. 7c), Val27, His148, and Asn233 are located on the surface contacting adjacent PVL-F. These three, as well as one more change at Phe129 located in pre-stem (membrane spanning) domain (green, Fig. 7c), may alter channel formation. In contrast to PVL-S, Val 11, only one amino acid change in PVL-F, is located at the loop-out region of β -sandwich domain (Fig. 7c). Therefore, PVL-F is functionally conserved, suggesting that PVL-F may play a critical role in PVL activity in addition to channel formation.

Genome and SCCmec

Genome

The entire genome structure of USA300 [USA300-0114 (strain FPR3757)] [72] is shown in Fig. 8a. The PVL genes are located in the prophage called Φ SA2USA. At the opposite position on genome USA300, SCCmec(IVa) is present. USA300 SCCmecIVa is linked to ACME. The

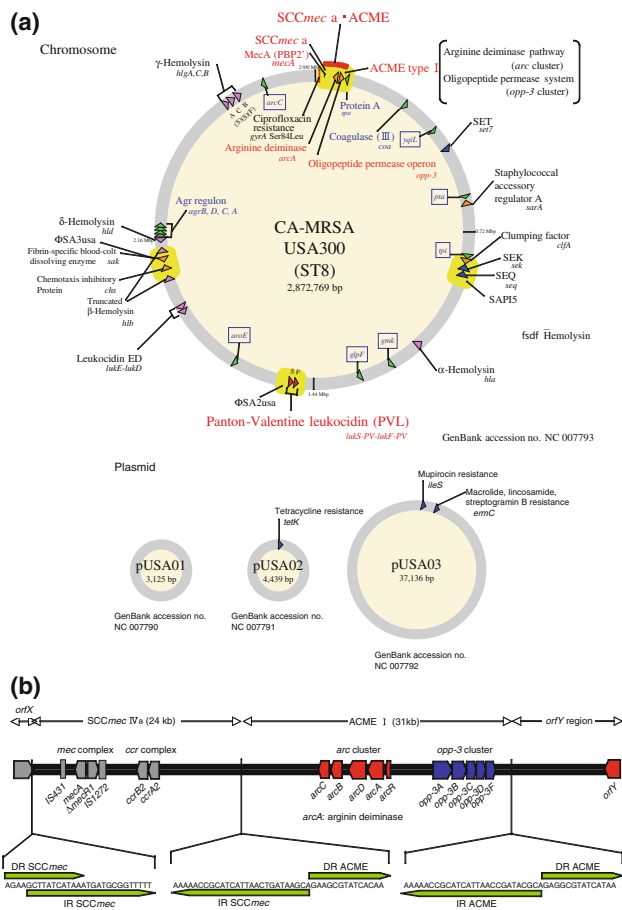


Fig. 8 Structures of the USA300 genome (a) and ACME-SCCmecIVa linkage structure (b). Data from Ref. [72]. **a** Genomic structures of the USA300 chromosome (accession no. NC 007793) and plasmids (accession nos. NC 007790, NC 007791, and NC 007792) are shown. Positions of the ACME-SCCmecIVa linkage, *spa*, coagulase gene (*coa*), seven housekeeping genes for MLST, drug resistance genes, and virulence genes including SaPI structures are also indicated. **b** The ACME-SCCmecIVa linkage structure (*orfX*–*orfY* region [137]) is shown. Positions of the *mec* complex, *ccr* complex (in SCCmecIVa), *arc* cluster, *opp-3* cluster (in ACME), *orfX*, and *orfY* are indicated. Sequences at the 5'- and 3'- ends of SCCmecIVa and ACME are also shown below the scheme. The positions of direct and inverted repeats are indicated by green arrows

connection of SCCmec to ACME is most probably a recombination process at invert repeat (IR) sequences (Fig. 8b). Moreover, SCCmecIVa and ACME could be excised by the *ccr* recombinase function at the repeat sequences, and the circular DNA generated could be transferred to other bacterial cells [12, 43].

USA300 (USA300-0114) is resistant to fluoroquinolones (e.g., ciprofloxacin) macrolides (erythromycin), lincosamides (clindamycin), and tetracycline. Of those resistances, fluoroquinolone resistance is due to the gyraseA (*gyrA*) mutation on the chromosome, while the other resistance genes are on the plasmid (Fig. 8a).

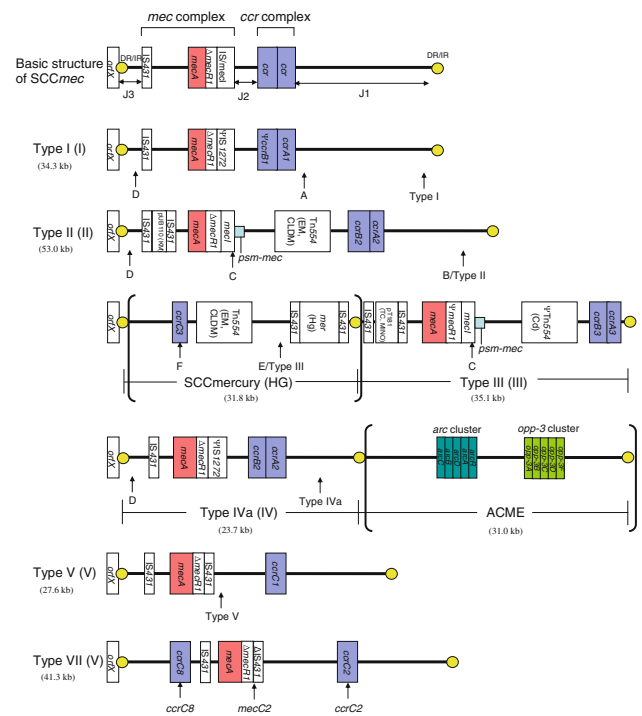
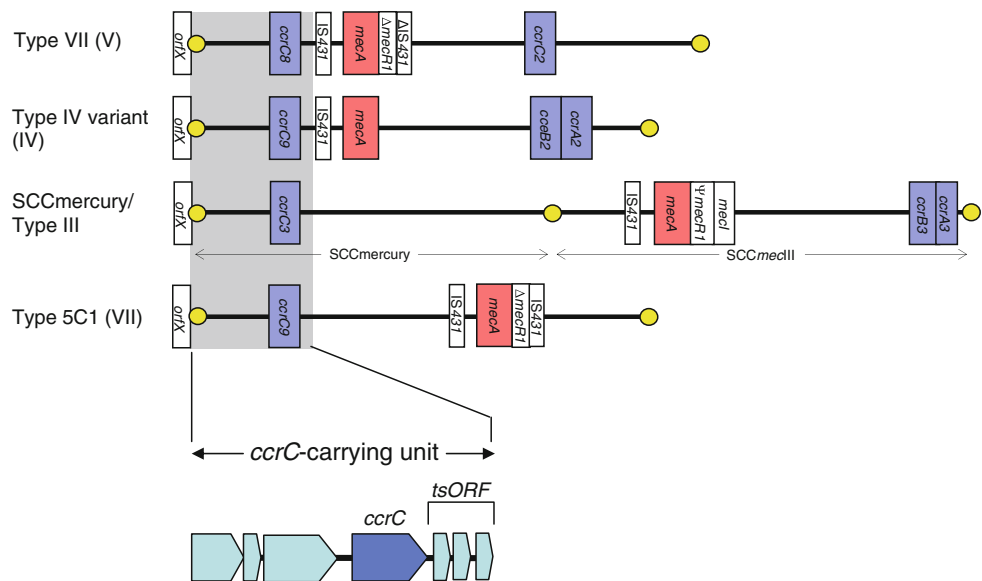


Fig. 9 Basic structures of SCCmec. Data from Refs. [25–28, 32, 137]. Roman numerals in parentheses represent a new type of SCCmec proposed by the SCCmec Working Committee (IWG-SCC). Arrows indicate the region of detection by PCR method to decide the SCCmec type. ACME is located downstream of SCCmecIVa in USA300

SCCmec

SCCmec types were reported previously [25–27]; however, SCCmec typing has recently been repropoed by the SCCmec Working Committee (IWS-SCC) [28]. SCCmec types, including those repropoed by the committee (shown in parenthesis) [25–28, 32, 137], are shown in Fig. 9. Since SCCmec is inserted at the attachment site (*att*), which is located at the right end of *orfX*, all SCCmec structures contain the *att* sequence at both ends. SCCmec contains core structures consisting of a *mec* complex representing methicillin resistance and a *ccr* complex. The locations of primers used for previous typing are shown in Fig. 9 (arrows). On the other hand, retying recommended by the committee is determined by the *mec* complex and *ccr* complex, located to the right side of the *mec* complex. In some cases, SCCmec type III is linked to SCCmercury (HG). SCCmec type IVa (of USA300) is linked to ACME. SCCmec types II and III carry the peptide toxin gene (PSM-mec) [180]. Besides methicillin resistance, SCCmec-II confers kanamycin resistance and erythromycin/clindamycin resistance. SCCmercury/type III carries the erythromycin/clindamycin resistance gene, tetracycline resistance gene, and cadmium resistance gene. SCCmec types II and III are seen in pandemic HA-MRSA clones.

Fig. 10 SCCmec structures containing the *ccrC*-carrying unit. Data of SCCmecVII (current from SCCmecV) from Ref. [137]. The structure of the *ccrC*-carrying unit of SCCmecVII [137] is indicated below. The position of the *ccrC*-carrying unit in each SCCmec structure is indicated as gray



SCCmecVII (currently SCCmec type V) contains the *ccrC*-carrying unit at the *orfX* end side and adjacent to *IS431* [137]. This core unit is also seen in other SCCmec structures, suggesting that it is used to construct SCCmec mosaic structures through recombination (Fig. 10).

Rapid detection of PVL-positive CA-MRSA

Multiplex PCR is developed for the rapid detection of major PVL-positive CA-MRSA clones. The targets for an assay are a combination of the gene or DNA sequence unique to each clone. Targets include the PVL gene, the MW1409 and MW0756 loci, and the *seh* gene for ST1 (USA400) [35, 36]; the PVL gene and the *arC* gene (on ACME) for ST8 (USA300) [35–37]; the PVL gene and the *etd* gene for ST80 [35]; and the PVL gene and the *cna* and *bbp* genes for ST30 [38]. The *mecA* gene and the *nuc* gene (encoding for *S. aureus*-specific thermostable nuclease) may also be included in an assay to differentiate MRSA from MSSA and *S. aureus* from CNS, respectively [36].

Drug resistance

Drug resistance genes and their resistance mechanisms in MRSA are summarized in Table 2. Some of them are found in CA-MRSA. Breakpoints for drug resistance have been defined by the CLSI.

β-lactam agents [32, 35, 71, 73, 216–219]

Ampicillin resistance is encoded for by the *blaZ* gene. Methicillin resistance is encoded by the *mecA* gene, whose product is methicillin-resistant penicillin binding protein 2

(peptidoglycan-associated transpeptidase, PBP2), known as PBP2a or PBP2'. The expression level of the *mecA* gene is under the control of chromosomal genes such as *fem*. Some MRSA exhibits a low oxacillin MIC level (MIC, <4 μg/ml), and thus are susceptible to oxacillin according to the CLSI's breakpoints; such MRSA is called borderline MRSA. To improve controversial phenomena, and to diagnose borderline MRSA, ceftioxin is used to judge MRSA.

CA-MRSA exhibits low oxacillin and carbapenem (especially imipenem) MIC levels compared to HA-MRSA. For instance, oxacillin MIC_{90s} are 32 μg/ml for CA-MRSA and ≥256 μg/ml for HA-MRSA, and imipenem MIC_{90s} are 1 μg/ml for CA-MRSA and 64 μg/ml for HA-MRSA. This phenomenon is even more remarkably observed: imipenem MIC_{90s} 0.12 μg/ml for PVL-positive CA-MRSA and 2 μg/ml for PVL-negative CA-MRSA. It is possible that HA-MRSA strains that have been selected in more cases over longer periods by *β*-lactam agents in hospitals have resulted in higher resistance. For example, in Japan, MRSA isolated from hospitals from 1976 to 1989 was susceptible, or only slightly resistant, to imipenem (MIC₉₀, 12.5 μg/ml), while that isolated in 1990 and 1991 was highly resistant to imipenem (MIC₉₀, ≥100 μg/ml). If this is the case, in the future CA-MRSA could also manifest high oxacillin and imipenem resistance, just as HA-MRSA has been selected.

As for distribution in the community in Japan, MRSA is isolated from children at a few percent, no MRSA is isolated from university students, and it is isolated in public transport (trains and subways) at 2.5%.

Vancomycin [220–223]

Vancomycin is a first-line antimicrobial agent for the treatment of MRSA infections. Vancomycin-intermediate *S. aureus* (VISA, MIC; 4–8 μg/ml) is characterized by cell

Table 2 Summary of drug resistance in MRSA

Antimicrobial agent group	Presence of resistance gene in CA-MRSA	Resistance gene	Product of resistance gene	Mechanism of resistance	Resistance pattern	Reference
β-Lactam	Yes	<i>mecA</i>	Penicillin binding protein 2' (PBP2')	Acquisition of resistant PBP	PCG, MIPIC	[32, 35, 71, 216–219]
	Yes	<i>blaZ</i> [PCase plasmid]		Decomposition	ABPC	
Quinolone	Yes	<i>gylA</i> mutation	Mutated DNA topoisomerase IV	Target mutation	CPFX or LVFX	[32, 72, 234, 235]
	Yes	<i>gyrA</i> mutation	Mutated DNA gyrase	Target mutation	CPFX or LVFX	
Macrolide-lincosamide-streptogramin B (MLS _B)	Yes	<i>ermA</i>	23S rRNA adenine N-6-methyltransferase (ErmA)	Target modification	EM, CLDM	[32, 35, 71, 72, 111, 145, 219, 239–243]
	Yes	<i>ermB</i> [pTPC1]	23S rRNA adenine N-6-methyltransferase (ErmB)	Target modification	EM, CLDM	
	Yes	<i>ermC</i> [pUSA03]	23S rRNA adenine N-6-methyltransferase (ErmC)	Target modification	EM, CLDM	
Macrolide	Yes	<i>ereB</i>	Erythromycin esterase	Lactone ring hydrolyse	EM	
	Yes	<i>mphB</i>	Macrolide 2'-phosphotransferase	Phosphorylation	EM	
Phenicol	Yes	<i>msrA</i>	Efflux pump (MsrA)	Efflux	EM	
	Yes	<i>cat</i>	Chloramphenicol acetyltransferase (CAT)	Acetylation	CP	
Tetracycline	Yes	<i>tetK</i> [PCase plasmid, pUSA02, pGKT1]	Tetracycline efflux protein (TetK)	Drug-specific efflux pump	TC, OTC, DOXY	
	Yes	<i>tetM</i>	Tetracycline resistance protein (TetM)	Ribosomal protection	TC, OTC, DOXY	
Aminoglycoside	Yes	<i>aac(6')-aph(2'')</i> [pGKT1]	6'-Aminoglycoside N-acetyltransferase-2''-aminoglycoside O-phosphotransferase (AAC(6')-APH(2''))	Acetylation and phosphorylation	GM, KM, ABK, TOB	[32, 35, 71, 142, 145, 219, 226–232]
	Yes	<i>ant(4')-I</i>	4',4''-Aminoglycoside-O-phosphoryltransferase I (ANT(4',4''))	Adenylylation	KM, FRM, TOB	
	Yes	<i>ant(6')</i> [pTPC1] (<i>aadE</i>)	6'-Aminoglycoside O-adenyltransferase (AAD(6'))	Adenylylation	SM	
Glycopeptide	Yes	<i>aph(3')-III</i> [pTPC1]	3'-Aminoglycoside O-phosphoryltransferase III (APH(3'))	Phosphorylation	KM, lividomycin, FRM	
	Yes	<i>sat</i>	Streptothricin acetyltransferase	Acetylation	Nourseothricin	
	Yes	<i>spc</i>	Streptomycin 3''-adenyltransferase	Adenylylation	SM	
Fusidic acid	Yes	<i>vana</i>	D-Ala-D-Lac ligase (VanA)	Target modification	VCM, TEIC	[220–223]
	Yes	–	–	Cell wall thickness	VCM ^a	
Fusidic acid	Yes	<i>fusA</i> mutation	Mutated EF-G	Target mutation	FA	[145, 247–249]
	Yes	<i>fusB</i> [pUB101]	EF-G binding protein (FusB)	Translation protection	FA	

Table 2 continued

Antimicrobial agent group	Presence of resistance gene in CA-MRSA	Resistance gene	Product of resistance gene	Mechanism of resistance	Resistance pattern	Reference
Streptogramin A	Yes	<i>fusC</i>	EF-G binding protein	Translation protection	FA	
Mupirocin		<i>vatB</i> <i>ileS</i> mutation <i>mupA (ileS-2)</i> [pUSA03]	Acetyltransferase Mutated isoleucyl tRNA synthetase	Acetylation Target mutation	QPR/ DPR MUP	[72, 111, 245, 246]
Ansamycin	Yes	<i>mupA (ileS-2)</i> [pUSA03] <i>rpoB</i> mutation	Resistant isoleucyl tRNA synthetase Mutated RNA polymerase beta subunit	Acquisition of resistant enzyme Target modification	MUP RFP	[236–238]
Oxazolidinone		23S rRNA mutation <i>cfr</i>	Mutated 23S rRNA 23S rRNA methyltransferase	Target mutation Target modification	LZD LZD	[224, 225]
Trimethoprim		<i>dfrA</i>	Resistant dihydrofolate reductase	Acquisition of resistant enzyme	TMP	[244]

PCG penicillin, *MPIPC* oxacillin, *ABPC* ampicillin, *CPFX* ciprofloxacin, *LVFX* levofloxacin, *EM* erythromycin, *CLDM* clindamycin, *CP* chloramphenicol, *TC* tetracycline, *OTC* oxytetracycline, *DOXY* doxycycline, *GM* gentamicin, *KM* kanamycin, *TOB* tobramycin, *FRM* framidomycin, *SM* streptomycin, *VCM* vancomycin, *FA* fusidic acid, *QRP/ DPR* quinupristin/dalfopristin, *MUP* mupirocin, *RFP* rifampicin, *LZD* linezolid, *TMP* trimethoprim

^a Intermediate for vancomycin

wall thickness (although the responsible gene has not been identified yet). VISA is MRSA in many cases. Heterogeneous VISA (hVISA) has also been noted. Vancomycin-resistant *S. aureus* (VRSA, MIC; ≥ 16 $\mu\text{g/ml}$), which is always MRSA, carries the *vanA* gene (on the Tn1546-like structure in a plasmid). The *vanA* gene is transmissible. The *vanA* plasmid pLW1043 confers multiple-drug resistance, including ampicillin (*blaZ*), gentamicin (*aacA/aphD*), trimethoprim (*dfrA/thyA*), and quaternary ammonium compound resistance (*qacC*). VRSA has been isolated not only from the US, but also from India and Iran.

VRSA is highly associated with USA100 (New York/Japan clone) and USA800 (pediatric clone) in the US. VISA has also been found in the most remarkable CA-MRSA clone in the US, USA300.

Linezolid [224, 225]

Linezolid is an alternative to vancomycin for the treatment of MRSA infections. Linezolid is able to penetrate into tissues (such as lung tissues) better than vancomycin. One of the resistance mechanisms is G to U substitution at position 2576 in the peptidyl transferase center of 23S rRNA, exhibiting MIC levels ≥ 8 $\mu\text{g/ml}$. The G2576U mutation-mediated resistance levels are affected by the number of copies of mutated 23S rRNA genes on the chromosome (present in five copies per cell). The other mechanism is methylation of adenine at position 2503 in the peptidyl transferase center of 23S rRNA, exhibiting 8 $\mu\text{g/ml}$. The responsible methyltransferase is encoded by the *cfr* gene; *cfr* also confers on host bacterial cells multiple resistance to chloramphenicol, clindamycin, tiamulin, and quinupristin/dalfopristin, in addition to oxazolidinones.

Aminoglycosides [32, 35, 71, 142, 145, 219, 226–233]

Gentamicin has widely been used for the treatment of *S. aureus* infections in the community, such as bullous impetigo, in Japan. Arbekacin has been used as an anti-MRSA agent in Japan. Resistance to gentamicin is mainly due to gentamicin modification (inactivation) by 6'-aminoglycoside *N*-acetyltransferase-2''-aminoglycoside *O*-phosphotransferase [AAC(6')-APH(2'')] (Fig. 11). Higher production of AAC(6')-APH(2'') results in resistance to arbekacin as well; a breakpoint for arbekacin resistance is 2 $\mu\text{g/ml}$ (Japanese Society for Chemotherapy).

Fluoroquinolones [32, 72, 234, 235]

In *S. aureus*, fluoroquinolone resistance is caused by a mutation in the DNA gyrase gene (*gyr*) and/or topoisomerase IV gene (*gri*). The quinolone resistance-determining region (QRDR) is located in *gyrA* and *griA*.

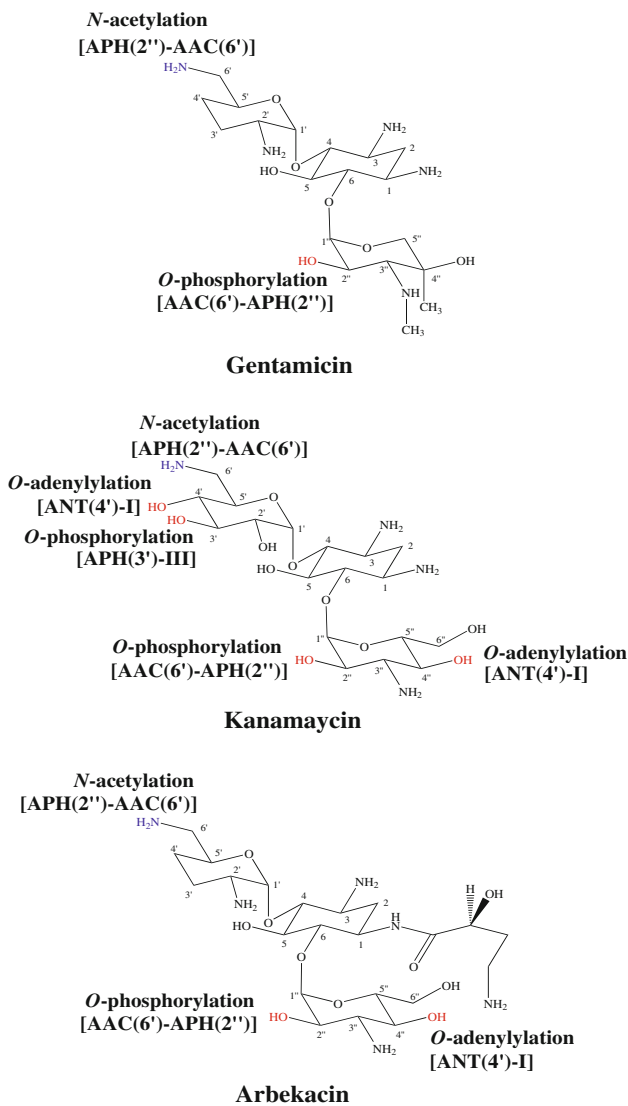


Fig. 11 Chemical structures of aminoglycosides and target residues of enzymatic modification. Chemical structures of gentamicin, kanamycin, and arbekacin are shown. Hydroxyl and amino residues shown in red and blue, respectively, are the targets of the enzymes. The inactivating enzymes are from Table 2

Fluoroquinolone resistance is at a severe level for HA-MRSA. Ciprofloxacin and levofloxacin resistance has been noted. Some CA-MRSA clones have also become fluoroquinolone resistant. For example, the USA300 clone is now multiple-drug resistant, and ciprofloxacin resistance (one of such multiple resistance) is due to a Ser84Leu chromosomal mutation of gyrase A encoded by the *gyrA* gene.

Rifampicin [236–238]

Rifampicin exhibits extremely high in vitro activity against *S. aureus* including MRSA. However, single use of rifampicin results in the rapid emergence of resistance.

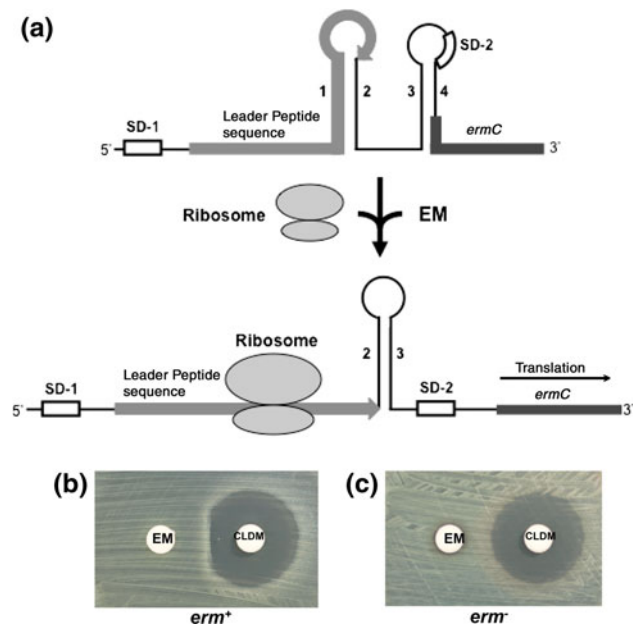


Fig. 12 Model for translational regulation of *ermC* expression (a) and D test (b, c). Data from Refs. [298, 299]. a Secondary structures upstream of *ermC* mRNA in the absence and presence of erythromycin are shown. The leader peptide sequence (19 amino acids) and *ermC* are indicated as gray and dark gray lines, respectively. SD-1 Shine-Dalgarno sequence for leader peptide translation, SD-2 Shine-Dalgarno sequence for *ermC* translation. In the absence of EM, SD-2 is masked by stem-loop (constructed by sequences 3 and 4). In the presence of EM, a complex of ribosome and EM tightly binds to the leader peptide sequence. This induces disruption of the two stem-loops, resulting in initiation of *ermC* translation from unmasked SD-2. b, c Results of D test of *ermC*-positive strain (b) and *ermC*-negative erythromycin-resistant strain (c) are shown. EM erythromycin, CLDM clindamycin

Rifampicin resistance is caused by a mutation in the RFP resistance-determining clusters I and II located in the β -subunit gene (*rpoB*) of bacterial RNA polymerase.

To prevent rifampicin-resistance, combination therapy with appropriate drugs (such as linezolid) has been continuously investigated. Moreover, rifampicin-resistant MRSA has been isolated in tuberculosis wards.

Macrolide-lincosamide-streptogramin B (MLS_B) [32, 35, 71, 72, 111, 145, 219, 239–243]

Clindamycin has been, in part, used for the treatment of *S. aureus* infections including MRSA infections. However, failure of treatment by clindamycin has already been noted due to the presence of inducible clindamycin resistance. Inducible-clindamycin-resistant strains are always resistant to erythromycin, and inducible clindamycin resistance can be induced by erythromycin. Inducible clindamycin resistance assay is called the D test (Fig. 12b, c). Clindamycin cannot be an effective inducer. The mechanism of clindamycin resistance is considered to be that in the absence of

an erythromycin inducer, a ribosome-binding (SD) sequence of clindamycin resistance mRNA is hidden due to the mRNA secondary structure, and thus clindamycin resistance mRNA cannot be translated, while with the presence of the inducer, this secondary structure of mRNA is disrupted resulting in translation of clindamycin resistance mRNA (Fig. 12a).

Trimethoprim–sulfamethoxazole (TMP–SMX) [244]

Mild SSTIs may be treated with TMP–SMX. Occasionally, the *dfrA* gene is associated with *SCCmec-n1*.

Mupirocin [72, 111, 245, 246]

Mupirocin has been used in the eradication of naris-colonizing MRSA. As for resistance, *ileS* mutation is low-level resistance (MICs; 8–256 µg/ml), while the *mupA* (*ileS-2*) gene confers high-level resistance (MICs; ≥512 µg/ml). The multiple-drug-resistant USA300 clone (ST8) carries the *mupA* gene on a plasmid. Also, the multiple-drug-resistant ST80 clone carries the *mupA* gene.

Fusidic acid [145, 247–249]

High-level fusidic acid-resistance (MICs; >12 µg/ml) involves mutation of the *fusA* gene. ST80 CA-MRSA, a worldwide clone, carries the *fusA* gene, and thus the *fusA* gene has been a marker of ST80 CA-MRSA spread in Europe. Occasionally, the *fusB1* gene is found on *SCCmec* (*SCCmec-n1* carrying *dfrA*).

MRSA in the community in Japan

Young school age children have nasal MRSA at higher levels with a peak at 5–9 years (isolation rate 2.2%), compared with other age groups, e.g., university students (isolation rate <0.25%) [250]. In other reports, isolation rates were 3.8 or 4.3% for children in kindergartens and day-care centers [251, 252]. All MRSA strains are PVL-negative. In their families, MRSA transmits among family members [250]. Examples include ST8/*SCCmecIVx* MRSA or ST764/*SCCmecII* MRSA. Moreover, children have ST88/*SCCmecIVx* MRSA (associated with bullous impetigo), ST857/*SCCmecX* (IIb) MRSA (new type), or ST380/*SCCmecIVc* and ST22/*SCCmecI* MRSA (rare isolates). The HA-MRSA New York/Japan clone (ST5/*SCCmecII*) is also detected from children; in one study, it was only from pediatric outpatients [250].

MRSA is also distributed in the environment. Although no MRSA was isolated from play settings such as karaoke, pachinko, amusement arcades, and gyms, MRSA was

isolated from public transport (trains, subways, or buses) at 2.5% [155]. They were all PVL-negative. Such MRSA falls into three groups: i) bullous impetigo-related CA-MRSA, such as ST8/*SCCmecIVx* or I MRSA and ST88/*SCCmecIVx* MRSA, ii) the HA-MRSA New York/Japan clone, and iii) uncategorized MRSA [155]. It is conceivable that such MRSA is transferred from the hands of passengers in trains, subways, or buses.

The New York/Japan clone, a typical HA-MRSA clone in Japan, is occasionally isolated from the community, as described above. It could be due to (e.g.) frequent pediatric visits to hospitals (which could be a high risk factor for pediatric MRSA colonization); for instance, some children visit hospitals for treatment several times a month. More importantly, ST764/*SCCmecII* MRSA is spreading not only in hospitals but also widely in the community [155, 253]. The ST764 clone is a derivative of the New York/Japan clone and is multiple-drug-resistant just like the New York/Japan clone; however, it seems to persist more in the community. The ST764 MRSA clone was first isolated from bacteremia in an inpatient in Sado (Niigata) in 2005 [253], and then from the hands of a medical student (undergoing clinical practice) in 2007 [155]. ST764 was also isolated from an intrafamilial transmission case in 2008 [250], from necrotizing fasciitis in the community in 2008 (unpublished data), and in enteritis in the community in 2009 (unpublished data), suggesting that the ST764 clone is an emerging hybrid-type clone with a higher ability to adapt to the community. The ST764 clone lacks SaPI_{m1/n1} (carrying three superantigen genes, *tst*, *sec*, and *sel*) present in the New York/Japan clone, but instead has a superantigen gene *seb2* [a variant of *seb* (*seb1*)] [138]. The *seb1* gene is detected in the major PVL-positive CA-MRSA [ST59/*SCCmecVII* (or V)] in Taiwan [32]. The *seb* gene may contribute to adaptation to the community.

It has also turned out recently that ST8/*SCCmecIVx* is a major CA-MRSA clone in Japan. It exhibits mainly *spa606* (with some minor *spa* types) and is PVL- and ACME-negative and distinct from USA300. It has been associated with a wide range of community infections, including bullous impetigo [141], atopic dermatitis (unpublished data), slight wound infection in a sumo-wrestling team [254], bacteremia and subsequent epidural abscesses [255], and fatal pneumonia [256]. A very similar MRSA was also isolated from cutaneous abscesses (in the community) of Japanese family members who had moved to Hong Kong [137].

Bullous impetigo is a common disease among children, and CA-MRSA is frequently isolated from these infected children (at 19–51%) [141, 194]. Such bullous impetigo-associated CA-MRSA (biCA-MRSA) belongs to ST8, ST88, ST89, and ST91 [141]. Those isolates are positive for the *cna* gene in many cases (at 86.4%) [257]. We

speculate that biCA-MRSA infects skin soft tissues through binding to collagens, exposed by scratching, to cause bullous impetigo. biCA-MRSA is highly resistant to gentamicin (at 80–90%) [141, 258], reflecting frequent use of gentamicin ointment for the treatment of bullous impetigo.

Recently, a variety of PVL-positive MRSA clones have been accumulating in Japan (albeit still at a low frequency), including ST30/SCC*mecIVa* [92], ST30/SCC*mecIVc* [75, 141], and their ST single locus variants, ST765/SCC*mecIVx* (or I) [71] and ST1335/SCC*mecIVc* (unpublished data), ST59/SCC*mecVII* (or V) [138], ST8/SCC*mecIVa* (USA300 clone) [125], and ST22/SCC*mecIVa* [144]. Of those, ST59 may originate in Taiwan (although the patient and his family members had never been to Taiwan). As for USA300, the first isolate was from a visitor (Indian girl) from the US in 2007 in Tokyo. This was followed by a Japanese child infection case in Saitama in 2008 (this patient and her family members had never been abroad), indicating the spread of USA300 in Japan, although the two isolates in 2007 and 2008 are divergent [126].

CA-MRSA infections in other areas of Asia

Taiwan

In Taiwan, the ST59/SCC*mecVII* (or alternatively assigned as V) clone has been noted as a major CA-MRSA clone [32, 259, 260]. They are mostly PVL-positive [32, 259]. On the other hand, ST59/SCC*mecIV* is mostly PVL-negative [32, 259, 260]. Nasal colonization of healthy adults (age of >18 years) with MRSA has also been reported (isolation rate 3.8%) [260]. Of those, PVL-positive ST59 and PVL-negative ST59 accounted for approximately 38 and 46%, respectively. The PVL-positive and -negative ST59/SCC*mecIV/V* clones were relatively multidrug-resistant [(e.g.) tetracycline, kanamycin, MLS_B, chloramphenicol] [32]. Although the most frequent clone in the hospital in Taiwan is ST239/SCC*mecIII* (Hungarian clone), PVL-positive and -negative ST59 clones are spreading not only in the community but also in the hospital [32, 259]. Currently, a fatal case of a 12-year-old boy who was infected by USA300 was reported [128].

China

Recent studies have shown the appearance of a worldwide clone, ST30 CA-MRSA, in China [106, 261, 262]. Yu et al. [261] investigated community- and hospital-acquired *S. aureus* isolates from the patients of a teaching hospital in Wenzhou and found two CA-MRSA isolates. One was a worldwide clone, PVL-positive ST30/

SCC*mecIV*, and mostly susceptible to non- β -lactam antimicrobial agents; the other was PVL-positive ST398/SCC*mecIV*, resistant to erythromycin and gentamicin. In the same study, ST398/SCC*mecIV* was also found as HA-MRSA.

More information about the molecular characteristics of CA-MRSA is available in Hong Kong [136, 262, 263]. Ho and his colleagues collected CA-MRSA strains from patients and their household members from 2004 to 2007 [136, 263]. Most of the CA-MRSA isolates (78% of all CA-MRSA isolates) were associated with SSTI. Among the CA-MRSA isolates, 72% were positive for PVL. Sixty-seven percent of the PVL-positive CA-MRSA isolates showed ST30/SCC*mecIV*, typically characteristic of worldwide clones, and were mostly susceptible to non- β lactam antimicrobial agents. The other set of PVL-positive CA-MRSA isolates in Hong Kong was ST59. ST59 clones showed multidrug resistance (MLS_B, tetracycline, or chloramphenicol), indicating similar characteristics to PVL-positive ST59 seen in Taiwan [32]. PVL-negative CA-MRSA isolates were also isolated and showed ST8/SCC*mecIVx* [262], which has typical characteristics of PVL-negative CA-MRSA in Japan [141]. Recently, after PVL-negative ST88/SCC*mecIV* CA-MRSA was reported in Japan [250], a similar PVL-negative ST88/SCC*mecIVa* CA-MRSA has also been isolated in Guangzhou, a city adjacent to Hong Kong [264]. A PVL-negative ST59/SCC*mecIVa* CA-MRSA was isolated in this study as well.

In hospitals in Hong Kong, ST239/SCC*mecIII/IIIA* is a major clone, and ST5/SCC*mecII* and ST45/SCC*mecIV* (and its variant) are found as well [58]. Currently, the ST45/SCC*mecIV* clone is increasing.

These data indicate that CA-MRSA strains in China have divergent genotypes closely related to clones isolated worldwide and in areas around mainland China including Taiwan and Japan.

Russia

There are many distinct MRSA clones in the territory of the Russian Federation. Among them, the ST239 Hungarian clone could be a major clone in Russian hospitals [265]. In addition, another unique ST239 MRSA clone (so-called Russian clone) was found in the far eastern part of Russia [57]. It can transfer multidrug resistance to other *S. aureus* or MRSA strains. In addition, one strain of PVL-positive worldwide CA-MRSA was isolated. Its genotype was ST30/*spa19*/SCC*mecIVc* [139]. A multidrug-resistant ST239 clone, described above, has been isolated from outpatients, in addition to inpatients, in Russia, indicating that the multidrug-resistant ST239 clone is spreading even in the community. This may be, in part, because the

Table 3 Cases of MRSA transmission between humans and animals

Country	Animal	Transmission to humans (numbers of infected persons)	Genotype and resistance						References
			MLST	<i>spa</i> type	<i>SCCmec</i> type	PVL gene	PFGE type (SmaI type)	Drug resistance	
Europe									
Hungary	Cows	1	1	t127	IVa	–	ND	TC, EM	[267]
The Netherlands	Pigs	7	ND	t108	ND	ND	Non-typeable	ND	[268]
The Netherlands	Pigs	3	398	t108	V	–	Non-typeable	ND	[269]
The Netherlands	Pigs	4	398	t011	IV	ND	Non-typeable	GM, KM, TC, EM, TS, LCM	[270]
The Netherlands	Dogs	3	80 ^a	ND	IV ^a	+	RIVM cluster 28	TC, FA	[271]
The Netherlands	Dogs	65	ND	ND	NT	ND	RIVM cluster 35	OFLX, ST, FA	[272]
Germany	Dogs	1	398	t034	V	–	Non-typeable	TC, EM, CLDM	[273]
Germany	Dogs	1	225	t014	II	–	ND	EM, CLDM, ERFX	[273]
Germany	Cats	4	80	t131	IV	+	ND	FA	[274]
North America									
USA	Dogs	7	ND	ND	II	–	Canadian epidemic MRSA-2	EM, CLDM, CPFEX, MUP (5/10)	[275]
USA	Cats	4	ND	ND	II	–	Canadian epidemic MRSA-2	EM, CLDM, CPFEX	[275]
USA	Cats	1	8	ND	IVa	+	USA300	EM, ERFX	[276]
Canada	Dogs	2	ND	ND	II	–	Canadian epidemic MRSA-2	EM, CLDM, CPFEX, MUP (2/4)	[275]
Canada	Cats	1	ND	ND	II	–	Canadian epidemic MRSA-2	EM, CLDM, CPFEX, MUP	[275]
Canada	Horses ^b	13	ND	ND	IV	–	Canadian epidemic MRSA-5 ^c	GM, TC, DOXY, EM	[277]

TC tetracycline, EM erythromycin, GM gentamicin, KM kanamycin, TS tylosin, LCM lincomycin, FA fusidic acid, OFLX ofloxacin, ST sulfamethoxazole–trimethoprim, CLDM clindamycin; ERFX enrofloxacin, CPFEX ciprofloxacin, MUP mupirocin, DOXY doxycycline, ND no data available, NT non-typeable

^a In other references, RIVM cluster 28 has been reported to be ST80 and *SCCmec*IV [278]

^b Infection in veterinary hospital of neonatal intensive care unit of horse

^c Canadian epidemic MRSA-5 is related to USA500 [277]

patients in Russia can obtain antibiotics without doctor's prescription in the community. Therefore, selection pressure by antibiotics in the community is slightly higher than that in other countries like Japan or the US, suggesting that different criteria for CA-MRSA should be considered. However, further study is needed.

Korea

In Korea, MRSA is spreading in hospitals and in the community like in other countries [266]. But the major ST type is very unique. ST72, which is *SCCmec*IV and PVL negative, is the major CA-MRSA clone in Korea, but does not spread in other areas. The ST5/*SCCmec*II and ST239/*SCCmec*IIIa clone are spreading both in hospitals and in

the community [266]. Only one case of a USA300-like clone has been reported [127].

MRSA in animals

MRSA has also been isolated from companion and livestock animals. Those MRSA strains have currently been noted as a source of infection in humans, and transmission cases are summarized in Table 3 [267–278]. For example, in case of companion animals, the EMRSA-15 clone (ST22/*SCCmec*IV/PVL-negative) has been isolated from dogs and cats in Europe, including the UK, Ireland, and Germany [279–284]; the Canadian epidemic MRSA-2 clone (ST5/*SCCmec*II/PVL-negative) from dogs and cats

[275]; and the Canadian epidemic MRSA-5 clone (ST8/SCC*mecIV*/PVL-negative) from horses in North America [285]. In addition, human PVL-positive CA-MRSA clones have been isolated from companion animals [274, 276]. Sing et al. [274] have reported a recurrent infection case of PVL-positive ST80 CA-MRSA through intrafamilial transmission among households and cat. To prevent recurrent infections, the eradication of MRSA from companion animals, as well as patients and their households, is important. Other than the ST80 clone, transmission of the USA300 strain from a cat has also been reported [276].

On the other hand, MRSA strains have also been isolated from livestock (e.g., pigs, cattle, and chickens) in (e.g.) the Netherlands, Belgium, Germany, Hungary, the US, Australia, and Korea [267, 269, 286–290]. van Rijen et al. [291] concluded in their report that the infection risk for pig and cow farmers (32%) is three times higher than for people who do not have direct contact with those animals. ST398 clones with SCC*mecIV* or V, as animal-associated CA-MRSA clones, have been widely spreading among livestock [269, 286–290, 292] and companion animals (e.g., horses) [293]. ST398 clones were originally known as non-typeable MRSA in PFGE due to methylation of C-residues in the SmaI recognition site [294]. Currently, transmission of those animal clones to farmers and their households has been noted (Table 3). Furthermore, isolation of the ST398 clone from a patient, family member (carrier), and nurse (carrier) who was taking care of that patient has also been observed, indicating possible transmission from human to human [268]. Those ST398 clones were PVL-negative. However, PVL-positive ST398 CA-MRSA has also been isolated from a human patient with abscesses in China although there had been no description of contact with animals [261].

Other than ST398, possible transmission of ST9 clones with SCC*mecIII* or V among pigs and farmers has also been reported in Asia. In those cases, no ST398 clones were observed in contrast to Europe, North America, and Australia, suggesting a spread of different clones in Asia [295, 296].

In Japan, MRSA with SCC*mecIV* has been isolated from chicken meat, although MLST was unknown [297]. On the other hand, we have examined the carriage of MRSA among healthy companion animals (mainly dogs and cats) in Japan. However, no MRSA has been isolated from healthy companion animals, although other methicillin-resistant *Staphylococcus* spp., such as methicillin-resistant *S. intermedius*, have been isolated.

Companion and livestock animals, which are carriers of MRSA, are potential risk factors for MRSA infection in humans. In cases of recurrent MRSA infection among family members, tests should be conducted on MRSA carriage in animals in the house or farm. If positive, MRSA eradication should be carefully considered.

Closing remarks

Historically, several waves of MRSA infections have attacked humans, including those due to penicillin G-resistant *S. aureus* in the mid 1940s, multidrug-resistant *S. aureus* in the late 1950s, and MRSA since 1961 (but with worldwide outbreaks in the 1970s–1980s). They all seem to have been driven by high levels of antimicrobial agent use in hospitals. The concept of MRSA infection has drastically changed since the late 1990s, when CA-MRSA was noted worldwide. CA-MRSA is well adapted to the community and triggers even unexpected cases of diseases for clinicians. Apparently, new countermeasures against the emerging threat (such as active surveillance of CA-MRSA in high risk groups, such as children and their families, and its eradication) must be considered and constructed.

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