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MINI-REVIEW

Molecular characteristics of community-associated methicillin-resistant *Staphylococcus aureus* strains for clinical medicine

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Abstract Infections caused by methicillin-resistant *S. aureus* strains are mainly associated with a hospital setting. However, nowadays, the MRSA infections of non-hospitalized patients are observed more frequently. In order to distinguish them from hospital-associated methicillin-resistant *S. aureus* (HA-MRSA) strains, given them the name of community-associated methicillin-resistant *S. aureus* (CA-MRSA). CA-MRSA strains most commonly cause skin infections, but may lead to more severe diseases, and consequently the patient's death. The molecular markers of CA-MRSA strains are the presence of accessory gene regulator (*agr*) of group I or III, staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, V or VII and genes encoding for Panton–Valentine leukocidin (PVL). In addition, CA-MRSA strains show resistance to β-lactam antibiotics. Studies on the genetic elements of CA-MRSA strains have a key role in the unambiguous identification of strains, monitoring of infections, improving the treatment, work on new antimicrobial agents and understanding the evolution of these pathogens.

Keywords Accessory gene regulator · CA-MRSA · Epidemiology · Panton–Valentine leukocidin · Staphylococcal cassette chromosome *mec* · Virulence

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Introduction

Staphylococcus aureus is one of the most ubiquitous bacteria among human. It is resistant to many unfavorable environmental conditions such as drying or high concentration of sodium chloride, which enables it to temporary or permanent skin and nasal mucosa colonization as well as makes it one of the major human pathogen (Matouskova and Janout 2008). The rate of *S. aureus* colonization in the population is about 37.2%. Probably 1% of these are methicillin-resistant *S. aureus* colonized (Kuehnert et al. 2006).

The first antibiotic, introduced to medical use in 1940, was penicillin. In short time after its introduction, the first penicillin-resistant *S. aureus* strains were observed. In 1961, 2 years after introduction of methicillin, a penicillinase-resistant penicillin, the first methicillin-resistant *S. aureus* (MRSA) strain was reported in Great Britain (Jevons 1961). In the second half of the 1960s, multi-drug-resistant MRSA strains were described. Over the next years, *S. aureus* strains resistant to streptomycin, tetracycline, erythromycin and vancomycin were observed (Schwalbe et al. 1987; Grundmann et al. 2006). For over 40 years, MRSA strains are considered to be the main cause of the majority nosocomial infections worldwide, causing over 50% of nosocomial infections in the United States. (NNIS 2002). However, MRSA infections among people who previously did not have prolonged contact with health services appear to increase (Fridkin et al. 2005).

First, cases of community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections in drug-addicted patients, who had not been hospitalized earlier, were reported in 1982 in the United States (Saravolatz et al. 1982). Then in 1993, the presence of CA-MRSA strains in populations living in Western Australia was described

(Udo et al. 1993; Riley and Rouse 1995). Afterward, in 1999, the death of four children has been reported, caused by CA-MRSA infection (CDC 1999). Recently, more and more cases of CA-MRSA infections have been reported from all over the world (Kowalski et al. 2005).

Community-associated MRSA

With the year 1993, when new CA-MRSA clone was observed, the species were provided to enable differentiation between CA-MRSA and other MRSA strains (Udo et al. 1993). There are different clinical and epidemiological features as well as molecular markers of MRSA types. According to the definition proposed by Center for Disease Control and Prevention, CA-MRSA are strains isolated in an outpatient setting or from patient within 48 h after admission to the hospital. Furthermore, the patient's medical history cannot contain MRSA infection or colonization, hospitalization or residence in a long-term care facility (nursing home, hospice etc.), dialysis and surgery. Moreover, there cannot be risk factors for acquisition of MRSA such as indwelling catheters and medical devices that pass through the skin into the body. In majority of cases, clinical manifestation presents with skin and soft tissue infections. However, certain cases can progress to invasive tissue infections, bacteremia and death (http://www.cdc.gov).

The main molecular markers of CA-MRSA are presence of *lukF-lukS* genes, staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, V or VII (Tristan et al. 2007; Takano et al. 2008) and accessory gene regulator genotype I or III (Naimi et al. 2003; Tenover et al. 2006). In addition, CA-MRSA strains are resistant to many β-lactam agents (Kowalski et al. 2005). The differences between health-care-associated MRSA and community-associated MRSA strains are specified. They refer to clinical manifestation, epidemiology, antibiotic resistance and molecular markers (Fey et al. 2003; Huang et al. 2006; Robinson et al. 2008; Chavez and Decker 2008). The comparison of HA-MRSA and CA-MRSA is presented in Table 1.

Resistance to antimicrobial agents

CA-MRSA, in comparison with HA-MRSA strains, apart from their resistance to β-lactams, are characterized by high sensitivity toward the remaining antibiotics, mostly above 90%, whereas their resistance toward lincosamides and macrolides is observed at a various degree (Dufour et al. 2002; Naimi et al. 2003; Ellis et al. 2004; Kowalski et al. 2005; Lorber 2006). According to different authors, the sensitivity toward erythromycin and clindamycin was

Table 1 The comparison of CA- and HA-MRSA

Characteristic	CA-MRSA	HA-MRSA
Clinical manifestation	Skin and soft tissue infections, "spider bites", necrotizing pneumonia, sepsis (Robinson et al. 2008; Chavez and Decker 2008)	Pneumonia, urinary tract, bloodstream, surgical site (Robinson et al. 2008; Chavez and Decker 2008)
Risk groups	Young, drug users, prisoners, athletes, soldiers, men who had sex with men, selected ethnic populations (Robinson et al. 2008; Chavez and Decker 2008)	Elderly people, healthcare workers, preterm neonate, long-term hospitalized patients (Robinson et al. 2008; Chavez and Decker 2008)
Risk factors	Close physical contact, abrasion injuries, poor communal hygiene, hard skin contacts, school children (Robinson et al. 2008; Chavez and Decker 2008)	Indwelling devices, catheters, lines, hemodialysis, prolonged hospitalization, long-term antibiotic use, close proximity to a patient in the hospital colonized with MRSA (Robinson et al. 2008; Chavez and Decker 2008)
Transmission	Person-to-person spread: shared facilities (towels, pools etc.)	Person-to-person spread: healthcare staff (nurses, doctors, surgeons etc.)
	Environment-to-person spread (shared sports equipment etc.) (Robinson et al. 2008; Chavez and Decker 2008)	Environment-to-patient spread (hospital equipment etc.) (Robinson et al. 2008; Chavez and Decker 2008)
Resistance to antimicrobial agents	Susceptible to multiple antibiotics, β-lactam resistant (Fey et al. 2003; Huang et al. 2006)	Resistant to multiple antibiotics (Fey et al. 2003; Huang et al. 2006)
SCC*mec* type	IV, V, VII (Fey et al. 2003; Huang et al. 2006; Deurenberg and Stobberingh 2008)	I, II, III (Fey et al. 2003; Huang et al. 2006; Deurenberg and Stobberingh 2008)
PVL toxin	Usually present (Fey et al. 2003; Huang et al. 2006; Deurenberg and Stobberingh 2008)	Absent (Fey et al. 2003; Huang et al. 2006; Deurenberg and Stobberingh 2008)

44 and 83% (Kowalski et al. 2005), 18 and 87% (Lorber 2006), 44 and 83% (Naimi et al. 2003) or 20 and 95.6%, respectively. As reported by Fey et al. among CA-MRSA type IV isolates, 81% were resistant only to β-lactams (Fey et al. 2003). In other study, drug resistance was clone dependent, where clone ST30-IV(USA 1100) displayed high sensitivity toward antibiotics and clone ST8-IVa (USA 300) displayed the highest resistance and 89.4% were resistant to erythromycin, 11.7% toward clindamycin and 62.8 and 24.5% toward ciprofloxacin and tetracycline, respectively (Diep et al. 2006a, b). However, apart from the more frequent presence of the PVL gene, no differences, comparing with HA-MRSA in the distribution of genes encoding virulence factors such as adhesins or toxins, were found (Fey et al. 2003; Graham et al. 2006; Diep et al. 2006a, b; Huang et al. 2007).

Evolution

According to the most probable CA-MRSA evolution hypothesis, staphylococcal cassette chromosome *mec* was independently introduced to genomes of many different methicillin-susceptible *S. aureus* (MSSA) strains in various geographical regions. Two theories have been suggested to describe the origin of SCC*mec* in CA-MRSA strains. According to the first theory, the SCC*mec* element has been incorporated into the genome of MSSA in an outpatient setting. While the second theory describes the origin of CA-MRSA from HA-MRSA (Deurenberg et al. 2007; Deurenberg and Stobberingh 2008).

The first theory, proposed by Okuma et al. (2002), assumes that IV type of SCC*mec* was incorporated into the genome of PVL-producing MSSA strains. The Japanese studies confirmed the presence of PVL-producing MSSA strains in the 1960 s, which become then ST30-MRSA-IV strain (Ma et al. 2006; Taneike et al. 2006). Moreover, this hypothesis is confirmed by studies of Monecke et al. (2007), who suggested that CA-MRSA emerged from the various *S. aureus* isolates by integrating SCC*mec* into the genome of PVL-producing *S. aureus* (Deurenberg et al. 2007).

According to the second theory, the evolution of CA-MRSA occurred in a hospital setting; therefore, both CA-MRSA and HA-MRSA have a common ancestor (Aires de Sousa and de Lencastre 2003). Perhaps it was a penicillin-resistant strain, which appeared worldwide both in outpatient and in hospital setting in the 1950 s. As a result of the introduction of β-lactam antibiotics in the 1960 s, the strain disappeared, to reappear after its incorporation of genes encoding PVL and type IV or V SCC*mec* through horizontal gene transfer by phiSLT phage, giving a rise of CA-MRSA strain, ST30-MRSA-IV (Robinson et al. 2005; Deurenberg and Stobberingh 2008).

Epidemiology

Multiple international investigations have reported the presence of CA-MRSA in many countries and in different populations.

United States

In last few years, the number of infections caused by CA-MRSA in United States has increased. Salgado et al. (2003) reported that CA-MRSA strains are approximately 0.24% of all isolated MRSA strains. However, the research carried out in three American hospitals by Fridkin et al. (2005) showed that the incidence of CA-MRSA infections varied between 18.0 and 25.7 per 100,000 population, while 8–20% of all MRSA isolates were classified as CA-MRSA strains.

Europe

The examination of the data collected from European hospitals participating in European Antimicrobial Resistance Surveillance System (EARSS) from January 1999 through December 2002 showed the prevalence of MRSA and CA-MRSA. MRSA isolates varied between 0.5 and 44.4%. Furthermore, CA-MRSA rate remained low with a prevalence of 0.03–1.5% of all isolates (Tiemersma et al. 2004).

Isolated populations

An interesting phenomenon is the high level of colonization of CA-MRSA strains in isolated ethnic populations. Among the indigenous inhabitants of Australia, Aborigines, this level is 76% (Maguire et al. 1996), while the populations of American Indians 62% (Groom et al. 2001).

The problem of hospital infections caused by methicillin-resistant *S. aureus* (MRSA) is known and associated with hospital treatment from the 1960s (Hryniewicz 1999). The frequency of bacteremia of this etiology, according to research carried out in the years 1999–2000 in Europe, depending on the country ranged from 44.4% (Greece) to 0.6% (Denmark and Holland) (Tiemersma et al. 2004). According to the research of SENTRY carried out in the years 1997–1999 in USA, Canada, Latin America and Western Pacific, the percentage of infection in these areas was 25.3; 19.2; 20.6; and 21.6, respectively (Diekema et al. 2001). To determine strain differentiation, the method previously used was PFGE (*Pulsed Field Gel Electrophoresis*). During PFGE, bacterial DNA is immobilized in agarose blocks, in which it is digested with restriction enzymes. Next during electrophoresis, DNA is separated to fragments of different length. Electrophoresis is run in an

alternating electrical fields, which allows the separation of DNA fragments in the range of 10–800 Kb (Olive and Bean 1999). The whole genetic material in the cell is analyzed. The additional enzyme digestion of the genome with enzymes, such as *Sma*I, results in the formation of DNA fragments of different length and number (Murchan et al. 2003). Another technique used in epidemiological studies is RFLP (*Restriction Fragments Length Polymorphism*) in which differentiation is based on the analysis of restriction patterns obtained through the digestion of the genetic material with restriction enzymes. In the case of *S. aureus*, the amplified product of the coagulase gene (*coa*) or the polymorphic X region of the A protein (*spa*) is digested with restriction enzymes *Alu*I or *Rsa*I, respectively (Frenay et al. 1996; Hookey et al. 1998; Kurlenda et al. 2009). Currently, the *spa*-typing is thought to be one of the most useful and powerful typing methods (Karynski et al. 2008). One of the most simple PCR-based methods used for differentiation is RAPD (*Randomly Amplified Polymorphic DNA*). This technique is based on the use of primers consisting of 9–11 nucleotides, which begin the amplification of DNA fragments in many regions of the genome simultaneously. The products are separated in an agarose gel. This method shows sufficient level of differentiation for epidemic investigations (Kurlenda et al. 2007).

Another described and used typing method is multiplex PCR called originally multiple-locus variable-number of tandem repeats analysis (MLVA) and presently multiple-locus VNTR fingerprinting (MLVF). It analzse the variation in number of repeats in seven individual genes (*sspA*, *spa*, *clfA*, *clfB*, *sdrC*, *sdrD* and *sdrE*). The discriminatory power of MLVF method is comparable with that obtained with PFGE analysis (Sabat et al. 2006; Karynski et al. 2008). Another method used for strain typing is MLST (*multi-locus sequence typing*). In the year 2000, Enright et al. (2000) described the use of the MLST technique in epidemiological studies regarding both MRSA and methicillin-sensitive MSSA strains for the first time. MLST is the genotyping version of MLEE (*multi-locus enzyme electrophoresis*), an electrophoretic analysis of sequenced variable region, fragments of 0.5 kb in size and of seven housekeeping genes of *S. aureus: arcC, aroE, glif, gmk, pta, tpi* and *yqiL* encoding bacterial reference enzymes (Deurenberg et al. 2007). The accepted nomenclature is based on defining clones with the letters ST combined with the numbers of the SCC*mec* cassettes, for e.g. the Brazilian/Hungarian clone that was present in countries of Europe, in the USA and South America is defined as ST239-III (Deurenberg et al. 2007). At present, PFGE and MLST are regarded as the most sensitive methods for strain typing (Oliveira et al. 2002); however, as they are technically demanding and laborious, for routine purposes, regarding limited territorial ranges, more simple and quick techniques such as RAPD are used (Kurlenda et al. 2007). Currently, the most widely used method for differentiation is clone determination with the use of MLST. It allows to determine the spreading of strains, maintenance on a particular region and the appearance of new strains (Gosbell 2005; Deurenberg et al. 2007). Introducing universal nomenclature for the description of clones allows to compare centers and also to evaluate maintenance of clones in time and their exchange. Conceicao et al. (2007) presented the results of a 10-year observation where during the years 1994–1998 in Hungary the type ST239-III clone dominated and then totally vanished in the year 2003 replaced by a Southern German clone ST228-I which was present only in Europe (Deurenberg et al. 2007). In USA, in the years 1996–2004, in the region of San Francisco, the clone ST5-II was present among hospital strains. This clone was present also in Europe, Australia, Japan and South America (Diep et al. 2006a, b). In 2005, in the USA, among dialyzed patients, 92% of the isolated MRSA strains from infections belonged to the clone ST5-II (Collins 2007). Two of the biggest clones, Iberian ST247-I and Brazilian/Hungarian ST-239-III, have spread into countries of Europe and the USA, Australia, Asia and South America (Gosbell 2005; Deurenberg et al. 2007).

From the 1990s in many countries (Fey et al. 2003; Faria et al. 2005), in the outpatients infections caused by MRSA strains defined as CA-MRSA (*community-associated* MRSA) has been observed. According to different authors, it can reach 75% (Naimi et al. 2003; Wannet et al. 2004), 77% (Lorber 2006) and 84% (Faria et al. 2005).

CA-MRSA quickly spread in the outpatient population with the distinct domination of some clones in different periods, countries or continents. The most frequently determined clone ST8-IV(USA 300) was identified both in European countries and in the USA, South Africa and Asia (Pan et al. 2005; Maree et al. 2007; Manzur et al. 2007), whereas in Europe the ST80-IV clone was mainly present (Dufour et al. 2002; Witte et al. 2004). As some authors point out, in recent years, a polyclonal tendency has appeared and so have new clones (Tristan et al. 2007; Hedin and Fang 2007). These strains have widespread so much that they are more frequently isolated from hospitalized patients (Saiman et al. 2003; Maree et al. 2007). This has caused an increase in MRSA infections in hospitals, in Scandinavian countries and Holland (Kaiser et al. 2004; Skov and Westh 2005), where in the 1980 s strict MRSA monitoring was introduced, which allowed to maintain infections on a low level <1%. Since 2001, the situation has changed due to two factors, either the enrollment of patients with CA-MRSA infections (Kaiser et al. 2004; Faria et al. 2005; Lorber 2006) or the infections that developed in a hospital have been introduced by

previously enrolled carriers of CA-MRSA (Saiman et al. 2003; Collins and Hampton 2005). A new source of CA-MRSA widespreading can in the future be pig farms and people employed there. During an international conference in Denmark, 272 participants associated with the agriculture industry, among which 12.5% were CA-MRSA carriers from different countries, were analyzed (Wagenaar et al. 2007). Twenty-four cases of MRSA infections in horses hospitalized in the years 2003–2005 in veterinary clinics were also reported where clone CA-MRSA ST254-IV was found in five animals (Cuny et al. 2006).

An important issue, from the therapeutic and epidemiologic point of view, is the identification of infection and carriers of HA-MRSA and CA-MRSA. Identifying carriers is critical for the control of the widespread of multi-resistant strains and reducing the risk of infection development because *S. aureus* nasal carriage occurs quite frequently, approximately in 20–60% of a normal population (Toshkova et al. 2001; Graham et al. 2006). Kampf et al. (2003) reported that *S. aureus* carriage among hospital staff was approximately 33.8% of which 1.6% were MRSA strains (Kampf et al. 2003). The example of Holland and Scandinavian countries shows the importance of carriage monitoring where after introducing strict monitoring of enrolled patients the percentage of MRSA incidents was low and maintained on a level <1% (Kaiser et al. 2004; Skov and Westh 2005). Quick evaluation of the etiology of infection is also important in order to apply the proper treatment, as in the case of MRSA it is critical in empiric treatment not to begin treatment with β-lactam antibiotics. Introducing molecular biology methods into diagnostics reduces the time required to obtain information regarding the etiology of the infection and improves the identification of carriers (Nilsson et al. 2005; Holfelder et al. 2006; Ornskov et al. 2008).

Risk factors

Although the epidemiology of CA-MRSA has not been fully explained, several important risk groups have been described. The higher prevalence of CA-MRSA has been observed in various population groups such as military personnel, soldiers (Ellis et al. 2004), intravenous and intranasal drug users, people belonging to a lower socio-economic status, homeless persons (Charlebois et al. 2002), athletic and competitive sport participants (CDC 2003), a homosexual mans (Sztramko et al. 2007), newborns (Saunders et al. 2007), children, young people (Herold et al. 1998), prison inmates (CDC 2001), ethically closed or semi-closed communities such as Pacific Islanders (CDC 2004), rural American Indian and rural southwestern Alaska population (Groom et al. 2001) remote Aboriginal Community (Maguire et al. 1996).

Clinical manifestation

Clinical manifestation of CA-MRSA infections manifest most commonly as skin and soft tissue infection (STTI) such as furuncles, boils and abscess (Eady and Cove 2003). However, it may cause more severe symptoms as folliculitis, cellulitis, impetigo, septic arthritis, sepsis, wound infections, pneumonia (Fridkin et al. 2005), necrotizing pneumonia (Roberts et al. 2002), meningitis (von Specht et al. 2006) or endocarditis (Millar et al. 2008).

Genetic characterization of CA-MRSA strains

Accessory gene regulator (*agr*)

The accessory gene regulator, (*agr*), also termed *agrBCDA*, is the *S. aureus* global regulatory system (Cotter and Miller 1998). It consists of five genes (*hld*, *agrB*, *agrD*, *agrC* and *agrA*), whose products establish a quorum-sensing regulatory mechanism and modulate the expression of virulence factors. Moreover, it serves a crucial role in *S. aureus* virulence and reduced susceptibility to glycopeptides antibiotics.

The agr polymorphism

Polymorphism at the locus across two genes, *agrD* and *agrC*, defines four *S. aureus agr* groups (I–IV). The distribution of *S. aureus agr* groups varies with geographical region (Novick 2000; Gomes et al. 2005). The differences between *agr* groups concern the production of biofilms (Boles and Horswill 2008), expression of virulence factors and resistance to antibiotics (Campoccia et al. 2008). CA-MRSA strains correlate to *agr* group I and III (Naimi et al. 2003; Tenover et al. 2006). There is a strong correlation between *agr* group and the pathogenesis of the infection (Jarraud et al. 2002). Seventy-one percent of all MRSA strains correlate to *agr* group I what makes it the most prevalent genotype. *Agr* II group genotype is characteristic to isolates from patients with endocarditis, and it is related to reduced susceptibility to vancomycin. Furthermore, toxic shock syndrome toxin-1 (TSST)-producing isolates belong to *agr* group III, while most exfoliatin-producing strains responsible for staphylococcal scaled-skin syndrome (SSSS) correlate to *agr* group IV (Jarraud et al. 2000). Campoccia et al. (2008) compared the prevalence of antibiotic resistance in different *agr* polymorphism groups. Isolates correlated to *agr* I and II group were resistant to many antimicrobial agents including oxacillin, penicillin, gentamicin, erythromycin, clindamycin, chloramphenicol and ciprofloxacin. However, the *agr* type III strains showed the lowest prevalence of antibiotic resistance with high

susceptibility to oxacillin, clindamycin, chloramphenicol and sulfamethoxazole with trimethoprim.

Biofilm production

The activity of the *agr* system and its polymorphism influence different biofilm production in *S. aureus*. Among these bacteria, there are four types of *agr* quorum-sensing systems. Each of *agr* types (*agr* I through *agr* IV) recognizes a unique auto-inducing peptide (AIP) structure (AIP-I through AIP-IV). AIP-I and AIP-IV differ by only one amino acid for that reason there are three cross-inhibitory groups: *agr* I/IV, *agr* II and *agr* III. The addition of indicated AIP (type I *agr* strain was treated with AIP-I, type II *agr* strain was treated with AIP-II, and type III *agr* strain was treated with AIP-III) to each biofilm, resulted in its detachment over a period of 2 days (Boles and Horswill 2008). In addition, mutations in *agr* quorum-sensing system resulted in increased biofilm production (Beenken et al. 2003).

Geographic dominance

The observation of Gomes et al. (2005) showed that the isolates with different *agr* groups are most frequently recovered in distinct geographic regions. Strains of *agr* I group are mainly isolated in Europe and South America. Type II *agr* is characteristic to isolates from Japan and North America. Furthermore, strains of *agr* III group are predominant in European countries. Different geographic distribution of strains of distinct *agr* groups can be an evidence of bacterial interference mediated by *agr* operon.

The mechanism of agr regulation

The *agr* system consists of 3-kb locus and contains divergent transcription units regulated by promoters P2 and P3. The 120-bp region between promoters P2 and P3 contains sites of activation of these two promoters (Novick 2003). When bacteria reach a critical density, a 7–9-amino acid peptide (auto-inducing peptide, AIP) derived from *agrD* is secreted into the culture supernatant. For *agr* I/IV group strains, it is octapeptide, *agr* II type – nonapeptide and agr III type – heptapeptide. The structure of all auto-inducing peptides is rather similar with conserved structural feature as a gradient of increasing hydrophobicity from N- to C-termi ending with two hydrophobic residues (phenylalanine, leucine, valine, tyrosine, occasionally methionine). In addition, AIPs form a thiolactone ring with the conserved essential cysteine in central position (Ji et al. 1997; Novick 2003). *AgrD*-encoded AIP is produced in form of propeptide. Then, it is processed and secreted by *agrB*-encoded transmembrane protein AgrB (Zhang et al. 2002). Mature AIP binds to AgrC histidine protein kinase what results in phosphorylation of AgrA transcription factor. Consequently, it leads to transcriptional activation of two promoters, P2 and P3. The *agr* system is regulated via autoinduction mechanism where P2 promoter transcribes *agr* operon itself. The P3 promoter regulates expression of RNAIII transcript (Cotter and Miller 1998). RNAIII is a 0.5-kb effector of *agr* regulon. (Novick et al. 1993). Its intracellular concentration is high as well as it has a long half-time of $\approx$ 15 min (Janzon and Ardvidson 1990). In the complex secondary structure of RNAIII can be distinguished the sequence complementary to the Shine–Dalgarno sequence, by which RNAIII can block translation and non-overlapping 5′ and 3′ regions, which are active in the regulation of hemolysin-encoding gene (*hla*) transcription (Novick 2003). RNAIII regulates transcription of many genes. By binding to individual transcription factors, it can cause their allosteric modifications and change their ability to bind to target sequences. Furthermore, RNAIII could influence stability of gene transcripts (Arvidson and Tegmark 2001). RNA III inhibits the expression of genes encoding for protein A, fibronectin-binding proteins (FBP), coagulase and other surface proteins that can be important in the early stages of infection and expressed during exponential growth phase. In contrast, RNAIII serves as a transcription factor turning on expression of secreted virulence factors as hemolysins, toxic shock syndrome toxins and exotoxins (Cotter and Miller 1998).

Bacterial interference

S. aureus strains of different *agr* groups produce structurally different auto-inducing peptides. AIPs secreted by one strain can inhibit virulence gene expression in another. For that reason, colonization of competing strains can be repressed. This phenomenon is termed bacterial interference (Cotter and Miller 1998). The results obtained by Boles and Horswill (2008), who examined AIP-mediated biofilm detachment, proved the existence of this *S. aureus* phenomenon. In this research, AIP-I addition to 2-day-old biofilm of agr I type strain resulted in its detachment. In contrast, addition of interfering AIP-II did not cause biofilm dispersal.

The agr operon as a virulence factor

The *agr* operon is an important virulence factor of CA-MRSA strains. McDougal et al. (2003) obtained that *agr* system plays a crucial role in pathogenesis of staphylococcal soft tissue infections. Moreover, the virulence of *agr*-mutated stains was suppressed. Wright et al. (2005) reported a rapid growth of bacteria within first 3 h of

infection to activate *agr* system. During this time, *agr*-negative strains were eradicated and abscess formation continued in the presence of *agr*-positive bacteria. In this study, inhibition of *agr* system in early stage of infection was sufficient to block abscess formation. Furthermore, *agr* operon increases expression of Panton–Valentine leukocidin and δ-hemolysins, a virulence factors associated with CA-MRSA strains. Ninety-seven percent of CA-MRSA strains produce δ-hemolysin, while PVL is considered to be the major virulence factor of these strains (Said-Salim et al. 2003; Sakoulas 2006).

The agr operon and resistance to glycopeptide antibiotics

The *agr* system may serve a crucial role in reduced susceptibility to glycopeptide antibiotics in *S. aureus*. The analysis of glycopeptide intermediate-resistant *S. aureus* (GISA) strains showed the loss of δ-hemolysin, α-hemolysin and RNAIII production, what is associated to loss of *agr* system expression. Sakoulas et al. (2002) reported that exposition of bacteria to glycopeptide antibiotics such as vancomycin and teicoplanin resulted in decreased RNAIII production. Furthermore, the exposition to vancomycin of *agr* knockout strain resulted in increased heteroresistance compared with the wild type. The electron microscopy analysis of cell wall of *agr*-mutated strain showed its thickening in comparison with non-mutated strain (Sakoulas 2006).

Staphylococcal cassette chromosome *mec* (SCC*mec*)

In *S. aureus*, staphylococcal cassette chromosome *mec* (SCC*mec*) causes resistance to methicillin and other β-lactam antibiotics. It consists of three main regions.

The mec gene complex

The *mec* gene complex consists of following elements: insertion sequence IS431mec, *mecA* and regulatory genes - *mecR1* and *mecI*. The *mecA* gene, which is 2.1 kb in length, encodes for 78-kDa penicillin-binding protein (PBP2′ or PBP2a). It is responsible for resistance to methicillin and other β-lactam antibiotics (Ito et al. 2003).

The cassette chromosome recombinases (ccr) gene complex

The *ccr* genes, which are of invertase/resolvase class, are responsible for the mobility of SCC*mec* element. Integration and excision occurs at the specific site that is SCC*mec* attachment site (*attBscc*) at the 3′ of the open reading frame (*orfX*) of unknown function (Ito et al. 1999).

The J regions

The J (junkyard) regions contain different genes or pseudogenes whose function does not appear essential for the bacteria. The exceptions are J regions encoding for non-β-lactam antibiotic and heavy metal-resistance genes (Ito et al. 2003).

SCCmec polymorphism

At the moment, eight main types of SCC*mec* (I to VIII) are distinguished. The differences concern the presence of non-β-lactam antibiotic-resistance genes, integrated plasmids, transposons as well as insertion sequences (Ito et al. 2003; Takano et al. 2008). While all SCC*mec* types contain *mecA* gene, which causes resistance to methicillin and other β-lactam agents, SCC*mec* type II and III consist of additional integrated plasmids and transposons causing multi-drug and heavy metals resistance. Integrated plasmid pUB110 harbors the *antt (4′)* gene encoding for aminoglycosides resistance. The resistance to penicillin is determined by the presence of integrated plasmid pI258, while pT181a harbors tetracycline-resistance genes. The constitutive as well as inducible resistance to macrolide, linkosamide and streptogramin is encoded by Tn554 transposon, which harbors the *ermA* gene. In addition, the presence of ΨTn554 transposon causes resistance to cadmium, while integrated plasmid pI258 encodes for resistance to mercury (Ito et al. 2003; Oliveira et al. 2006; Takano et al. 2008). The comparison of different SCC*mec* types is showed in the Table 2. CA-MRSA strains harbor SCC*mec* type IV, V or VII, which do not contain additional integrated plasmids and transposons. Consequently, they are not resistant to multiple classes of antibiotics as well as heavy metals (Deurenberg and Stobberingh 2008).

The mechanism of resistance to β-lactam agents

β-lactam antibiotics inhibit the final stages of peptidoglycan biosynthesis, which are the components of bacterial cell wall (Giesbrecht et al. 1998). The cell wall of methicillin-sensitive *S. aureus* (MSSA) contains native penicillin-binding protein (PBP). β-lactams bind to transpeptidase domain of PBP, disrupting the synthesis of peptidoglycan layer. Whereas methicillin-resistant *S. aureus* strains acquired the *mecA* gene, which codes for low-affinity penicillin-binding protein (PBP2′ or PBP2a). Consequently, β-lactams do not inhibit cell wall biosynthesis, resulting in the growth of MRSA (Berger-Bachi and Rohrer 2002). The transcription of *mecA* gene is regulated by two regulatory elements—the MecI repressor and the transmembrane receptor MecR1, which contains a β-lactam-

Table 2 The characteristic of distinct staphylococcal cassette chromosome *mec* types

Characteristic	Staphylococcal cassette chromosome *mec* type						
	I	II	III	IV	V	VI	VII
Size (kb)	34.3	53	66.9	20.9–24.3	28	20.9	35.9
Integrated plasmids	–	pUB110	pI258, pT181	–	–	–	–
Transposons	–	Tn554	Tn554, ΨTn554	–	–	–	–
Insertion sequences	IS431, ΨIS1272	IS431	IS431	IS431, ΨIS1272	IS431	IS431, ΨIS1272	IS431
Resistance to β-lactam antibiotics	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Resistance to other antibiotics	–	Aminoglycosides, macrolide, lincosamide, streptogramin	Penicillin, tetracycline, macrolide, lincosamide, streptogramin	–	–	–	–
Resistance to heavy metals	–	–	Mercury, cadmium	–	–	–	–

induced extracellular sensor domain. The two proteins are transcribed separately. In the absence of β-lactam agents, MecI represses both transcription of the *mecA* and *mecR1-mecI*. On the other hand, in the presence of β-lactams, MecR1 is cleaved, activating its metalloprotease domain, which in turn cleaves the MecI repressor. In consequence, the transcription of *mecA* gene and PBP2 production occur (Berger-Bachi and Rohrer 2002). CA-MRSA strains, due to the presence of *mecA* gene, are resistant to β-lactam agents. However, they are susceptible to many antibiotics, comparing to HA-MRSA strains (Kilic et al. 2006; Kim et al. 2006). Kilic et al. (2006) compared antibiotic susceptibility patterns of strains harboring SCC*mec* type II (HA-MRSA) and IV (CA-MRSA). The results showed that MRSA strains of SCC*mec* type II were greater resistant that SCC*mec*-IV MRSA strains to clindamycin, erythromycin, levofloxacin, gentamicin, rifampicin, minocycline and sulfamethoxazole-trimethoprim.

The association between SCCmec type and virulence factors production

Kim et al. (2006) reported an association between SCC*mec* type of MRSA strains and toxin gene profiles. The toxin gene carriage differed in different SCC*mec* types (95.9% in SCC*mec* II, 74.4% in SCC*mec* III and 68.8% in SCC*mec* IV). Furthermore, Kilic et al. (2006) obtained that the strains of SCC*mec* type IV more frequently harbored *lukS-PV* and *lukF-PV* genes, coding for PVL than isolates of SCC*mec* type II. The PVL genes were detected in 93.6% of SCC*mec*-IV isolates, comparing to 0.2% of SCC*mec*-II isolates. In addition, higher occurrence of PVL was notified in isolates of SCC*mec*-IV in children (98.1%) than in adults (89.1%).

Panton–Valentine leukocidin

Panton–Valentine leukocidin was discovered by Van deVelde in 1894 due to its ability to lyse leukocytes. In 1932, Panton and Valentine first associated the leukotoxin with skin and soft tissue infections (Panton and Valentine 1932; Boyle-Vavra and Daum 2007). PVL shows cytolytic activity with high specificity to leukocytes. Furthermore, it is one of the molecular markers of CA-MRSA strains (Tomita and Kamio 1997; Tristan et al. 2007; Takano et al. 2008).

The lukS-PV and lukF-PV genes

In the *S. aureus* genome, the cotranscribed *lukS-PV* and *lukF-PV* genes are separated by only one thymine. The ORF of lukS-PV is 939 nucleotides long, whereas the lukF-PV ORF is 978 nucleotides long. The G + C contents of lukS-PV and lukF-PV are 31.5 and 30.3%, respectively. Two identical putative ribosome-binding sites (GAAAGGA) are located 10 bp upstream from the ATG codons of the two genes. The ribosome-binding site for the lukF-PV is included within the ORF of lukS-PV. Two hypothetical transcription signals are located 80 bp upstream from lukS-PV, whereas the putative transcription terminator is supposed to be located 67 bp downstream from lukF-PV. The unique mRNA of cotranscribed lukS-PV and lukF-PV is 2.1 kbp long. The first transcribed gene of the operon, coding for 312-amino acid protein, is lukS-PV. However, the cleavage between 28 and 29 position of amino acid results in 284-amino acid mature protein. The second transcribed gene is lukF-PV, which encodes a protein of 325 amino acids. The N-terminal end of the mature protein starts at 25 amino acid position. Consequently, the mature protein is of 301 amino acids (Prevost et al. 1995).

PVL is bicomponent, pore-forming leukotoxin, composed of two components—LukS and LukF. LukS and LukF consist of three domains—β-sandwich, rim and stem.

The structure of LukS-PV and LukF-PV

The three-dimensional structure of LukS (33 kDa) is composed of 19 strands that participate to four antiparallel β-sheets and three shorts segments of either 3_{10} or α helices (Guillet et al. 2004). In contrast, the three-dimensional structure of LukF compound (34 kDa) consists of 22 β-strands and three short segments of either 3_{10} or α helices. β-sandwich domain is composed of two antiparallel, six-stranded β-sheet domains, facing with an angle of 30°. The rim domain forms an antiparallel four-stranded structure of open-face sandwich. The stem domain consists of three antiparallel β-strands linked by one β-turn (Pedeacq et al. 1999). The two components, LukF-PV and LukS-PV, have a similar conformation. The structure of β-sandwich and stem domain of LukS-PV is more similar to their counterparts in LukF-PV, comparing to rim domain. The four peptide segments in rim domain are less conserved (Guillet et al. 2004). The two components of PVL leukocidin, the LukS-PV and LukF-PV, are secreted by *S. aureus* to the environment before they form a heptamer on polymorphonuclear leukocytes membranes. First, the LukS-PV binds to an unidentified receptor on PMN leukocyte where it dimerizes with LukF-PV. The two components bind together until the heptamer is assembled (Kaneko and Kamio 2004). Depending on the concentration of PVL, it can cause either lysis (necrosis) of the leukocyte or apoptosis. High concentrations of PVL cause the lysis of the leukocyte due to the assembly of many octameric pores on the PMNs membrane, leading to an influx of extracellular substances as ethidium ions. Furthermore, LukS-PV by binding to PMNs membrane may be phosphorylated by A or C kinase, leading to the stimulation of calcium channels of PMNs, causing influx of extracellular ions. This contributes to the production of interleukins and inflammatory mediators (Finck-Barbançon et al. 1993; Staali et al. 1998; Genestier et al. 2005). On the other hand, the low PVL concentration causes PMNs apoptosis via pathway that involves PVL-mediated pore assembly on the mitochondrial membrane. Consequently, cytochrome c is released, inducting apoptotic proteins—caspases 3 and 9 that lead to DNA fragmentation and PMNs apoptosis (Genestier et al. 2005).

The contribution of PVL in CA-MRSA pathogenesis

The role of PVL in CA-MRSA pathogenesis is controversial. On the one hand, mouse infection models used by Voyich et al. (2006) showed that PVL is not the major virulence determinant of CA-MRSA. On the other hand, the epidemiological studies as well as rabbit bacteremia model developed by Diep et al. (2008a, b) and studies carried out on neutrophils isolated from different species by Loeffler et al. (2010) confirm its major role for the pathogenesis of CA-MRSA infections.

In a PubMed database, there are more than 300 articles, published in 2002–2007, suggesting an association between PVL and CA-MRSA infections. The most frequently cited conditions are furuncles, ulcers, severe necrotizing skin infections, leucopenia and hemoptysis. Moreover, the respiratory tract infections, extensive necrosis of respiratory epithelium and necrotizing pneumonia are often mentioned (Gillet et al. 2002). Only 2% of strains isolated from patients with necrotizing pneumonia contain genes coding for PVL leukotoxin. This is small percentage; however, the infections caused by PVL-positive strains are more severe as well as the lethality rate in such infections is increased (Lopez-Aguilar et al. 2007).

Studies on animal infections models provide contradictory conclusions about the contribution of PVL in CA-MRSA pathogenesis. On the one hand, Voyich et al. (2006), using mouse bacteremia model to compare the virulence of PVL-positive and PVL-negative strains, showed that PVL is not a major virulence determinant of CA-MRSA. Strains that did not produce leukotoxin were as virulent as those containing PVL. The clinical manifestation of infections caused by PVL-positive as well as PVL-negative strains was similar and manifested as sepsis, abscess and skin disease. Furthermore, lysis of human PMN leukocytes was the same with PVL-positive and PVL-negative strains. On the other hand, Diep et al. (2008a, b) reported a modest and transient contribution of PVL in CA-MRSA infection. In contrast to Voyich et al. (2006), Diep et al. (2008a, b) developed a rabbit bacteremia model because rabbit's granulocytes are more sensitive to the leukolytic activity of PVL, compared with mouse (Szmigielski et al. 1999). At 24 and 48 h after infection, PVL plays a modest, but measurable role in pathogenesis. It is important in early stages of bacteremic seeding of the kidney. However, after 72 h postinfection, the PVL activity is lost. This is consistent with clinical manifestation of rapid and acute onset of CA-MRSA infections (Gonzalez et al. 2005). It is still unclear why PVL shows a transient effect. Perhaps the leukotoxin production in the end stages of the infection could lead to priming the innate immune response, resulting in limited survival of bacteria. Another reason may be the changes in bacterial metabolism to produce other virulence factors, important in maintaining persistent infection (Diep et al. (2008a, b). The most recent studies by Loeffler et al. (2010) showed the effect of PVL on polymorphonuclear cells from different species including humans,

mice, rabbits and monkeys. These studies demonstrated that PVL induces rapid activation and cell death in human and rabbit PMN leukocytes, but not in murine or simian cells. The reason for the differential sensitivity of cells isolated from various species is unknown. It has been suggested that animal models using mice and non-human primates do not correctly replicate S. aureus diseases in human. Taken together, PVL is a strong cytotoxic factor for human PMN leukocytes and their premature death may be extremely relevant in the virulence of CA-MRSA.

Arginine catabolic mobile element

A 30.9-kb genetic island—the arginine catabolic mobile element (ACME), present in the genome of CA-MRSA strain USA 300 which contain the SCCmec type IVa element, has been associated with emerging dominance of its background. It contains loci that encode at least 33 genes. One of these loci, arcABCD, encodes a complete arginine deiminase pathway that converts L-arginine to carbon dioxide, ATP and ammonia (Ito et al. 2001; Mongkolrattanothai et al. 2004; Diep et al. 2006a, b, 2008a, b). In addition, ACME contains an operon, *opp-3*, which comprises five genes that, together, encode a putative oligopeptide permease (Opp). It could be involved in the regulation of virulence factor expression (Wang et al. 2005).

The linkage between ACME and SCCmec

ACME could enhance growth, survival and dissemination of USA300, thus allowing for the genetic "hitchhiking" of SCC*mec*. SCC*mec* protects against exposure to β-lactams, further enhancing rapid dissemination of USA300 with antibiotic use (Diep et al. 2008a, b). Furthermore, ACME integrates into *orfX* on the USA300 chromosome, using the same attachment site as SCC*mec* element, and is flanked by repeat sequences characteristic of SCC*mec* elements. SCC*mec* provides the *ccrAB* recombinases that could catalyze mobilization and integration of ACME into the chromosome (Katayama et al. 2000).

The contribution of ACME in virulence of CA-MRSA strains

The studies of Diep et al. (2008a, b) consisting of simultaneous inoculation of rabbits with a wild-type USA300 parent strain and an isogenic ACME deletion mutant. The parent strain was recovered in greater numbers than the deletion mutant from the lungs, spleen and blood. They found that the deletion of ACME significantly attenuated the pathogenicity or fitness of USA300. The most recent studies by Montgomery et al. (2009) compared USA300 CA-MRSA clinical isolates differing in the presence or absence of ACME and a USA300 wild-type/ACME deletion mutant isogenic pair in two animal models of *S. aureus* infection. They showed that presence of ACME was not associated with increased virulence in a rat model of necrotizing pneumonia, as assessed by mortality, in vivo bacterial survival and severity of lung pathology. Furthermore, the presence of ACME was not associated with increased dermonecrosis in a model of skin infection. They concluded that ACME is not necessary for virulence in rodent models of CA-MRSA USA300 pneumonia or skin infection.

Conclusion

The studies on the genetic characterization of CA-MRSA play a crucial role in the identification of CA-MRSA strains, the monitoring of infections, improvement of treatment, working on new antimicrobial agents as well as understanding the evolution of these pathogens. First of all, the classical microbiological methods such as microscopy, culturing and biochemical identification are not sufficient for precise identification of the strain. The molecular methods, in contrast, that use genetic analysis of the nucleic acids are much more precise and are used for rapid identification, what plays an important role in monitoring of the pathogen spread and rapid identification of the bacteremia (Matouskova and Janout 2008). Then, the information about genetic elements of the strain is important in the treatment of CA-MRSA infection. It plays a significant role in clinical decision making. For instance, PVL-positive strains seem to be dangerous for pulmonary patients, so when the presence of PVL-encoding genes is confirmed, the reinforced antibiotic treatment and patient's isolation are recommended (Lopez-Aguilar et al. 2007). Another treatment of PVL-positive strains infections uses anti-PVL leukotoxin antibodies (Gauduchon et al. 2004). Furthermore, analysis of the genetic elements responsible for virulence and antibiotic resistance as well as understanding their mechanisms of action enables the development of new anti-CA-MRSA antimicrobial agents (Deurenberg and Stobberingh 2008). For example, bacterial interference, the phenomenon of producing structurally different auto-inducing peptides by *S. aureus* strains of different *agr* groups, may have potential application in the treatment of infections associated with biofilm production (Boles and Horswill 2008). In addition, genetic analysis of different CA-MRSA strains enables to study the evolution of these pathogens, both in hospital setting, as well as in outpatient. This contributes to a better understanding of the mechanisms

of spread and acquisition of virulence factors (Deurenberg and Stobberingh 2008).

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References

Aires de Sousa M, de Lencastre H (2003) Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J Clin Microbiol 41:3806–3815

Arvidson S, Tegmark K (2001) Regulation of virulence determinants in *Staphylococcus aureus*. Int J Med Microbiol 291:159–170

Beenken KE, Blevins JS, Smeltzer MS (2003) Mutation of sarA in *Staphylococcus aureus* limits biofilm formation. Infect Immun 71:4206–4211

Berger-Bachi B, Rohrer S (2002) Factors influencing methicillin resistance in Staphylococci. Arch Microbiol 178:165–171

Boles BR, Horswill AR (2008) *agr*-mediated dispersal of *Staphylococcus aureus* Biofilms. PLoS Pathog 25:4(4)

Boyle-Vavra S, Daum RS (2007) Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton–Valentine leukocidin. Lab Invest 87:3–9

Campoccia D, Baldassarri L, Pirini V, Ravaioli S, Montanaro L, Arciola CR (2008) Molecular epidemiology of *Staphylococcus aureus* from implant orthopaedic infections: ribotypes, agr polymorphism, leukocidal toxins and antibiotic resistance. Biomaterials 29:4108–4116

CDC (Centers for Disease Control, Prevention) (1999) Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*-Minnesota and North Dakota, 1997–1999. Morb Mortal Wkly Rep 48:707–710

CDC (Centers for Disease Control, Prevention) (2001) Methicillin-resistant *Staphylococcus aureus* skin or soft tissue infections in a state prison–Mississippi, 2000. Morb Mortal Wkly Rep 50:919–922

CDC (Centers for Disease Control, Prevention) (2003) Methicillin-resistant *Staphyloccocus aureus* infections among competitive sports participants-Colorado, Indiana, Pennsylvania, and Los Angeles County, 2000–2003. Morb Mortal Wkly Rep 52:793–795

CDC (Centers for Disease Control, Prevention) (2004) Community-associated methicillin-resistant *Staphylococus aureus* infections in Pacific Islanders-Hawaii, 2001–2003. Morb Mortal Wkly Rep 53:767–770

Charlebois E, Bangsberg D, Moss N (2002) Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. Clin Infect Dis 34:425–433

Chavez TT, Decker CF (2008) Health care-associated MRSA versus community-associated MRSA. Dis Mon 54:763–768

Collins RJ (2007) Community-acquired methicillin-resistant *Staphylococcus aureus* in a group home setting. Consult Pharm 22:763–767

Collins F, Hampton S (2005) Hand-washing and methicillin-resistant *Staphylococcus aureus*. Br J Nurs 14:703–707

Conceicao T, ires-de-Sousa M, Fuzi M, Toth A, Paszti J, Ungvari E, van Leeuwen WB, van Belkum A, Grundmann H, de Lencastre H (2007) Replacement of methicillin-resistant *Staphylococcus aureus* clones in Hungary over time: a 10-year surveillance study. Clin Microbiol Infect 13:971–979

Cotter PA, Miller JF (1998) In vivo and ex vivo regulation of bacterial virulence gene expression. Curr Opin Microbiol 1:19

Cuny C, Kuemmerle J, Stanek C, Willey B, Strommenger B, Witte W (2006) Emergence of MRSA infections in horses in a veterinary hospital: strain characterisation and comparison with MRSA from humans. Euro Surveill 11:44–47

Deurenberg RH, Stobberingh EE (2008) The molecular evolution of methicillin-resistant *Staphylococcus aureus*. Infect Gen Evol 8:747–763

Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE (2007) The molecular evolution of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 13:222–235

Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M (2001) Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis 32(Suppl 2):S114–S132

Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F (2006a) Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 193:1495–1503

Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF, Perdreau-Remington F (2006b) Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet 367:731–739

Diep BA, Palazzolo-Ballance AM, Tattevin P, Basuino L, Braughton KR, Whitney AR, Chen L, Kreiswirth BN, Otto M, DeLeo FR, Chambers HF (2008) Contribution of Panton–Valentine leukocidin in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. PloS ONE 3(9)

Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, Jones A, Palazzolo-Balance AM, Perdreau-Remington F, Sensabaugh GF, DeLeo FR, Chambers HF (2008b) The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin resistant *Staphylococcus aureus*. J Infect Dis 197:1523–1530

Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D, Etienne J, Richet H (2002) Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton–Valentine leukocidin. Clin Infect Dis 35:819–824

Eady EA, Cove JH (2003) Staphylococcal resistance revisited: community-acquired methicillin resistant *Staphylococcus aureus*—an emerging problem for the management of skin and soft tissue infections. Curr Opin Infect Dis 16:103–124

Ellis MW, Hospenthal DR, Dooley DP, Gray PJ, Murray CK (2004) Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. Clin Infect Dis 39:971–979

Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38:1008–1015

Faria NA, Oliveira DC, Westh H, Monnet DL, Larsen AR, Skov R, de Lencastre H (2005) Epidemiology of emerging methicillin-

resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. J Clin Microbiol 43:1836–1842

Fey PD, Said-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC, Kreiswirth BN, Schlievert PM (2003) Comparative molecular analysis of community- or hospital-acquired methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 47:196–203

Finck-Barbançon V, Duportail G, Meunier O, Colin DA (1993) Pore formation by a two-component leukocidin from *Staphylococcus aureus* within the membrane of human polymorphonuclear leukocytes. Biochim Biophys Acta 1182:275–282

Frenay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, Mooi FR (1996) Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect Dis 15:60–64

Fridkin SK, Hageman JC, Morrison M, Thomson Sanza L, Como-Sabetti K, Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM (2005) Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med 352:1436–1444

Gauduchon V, Cozon G, Vandenesch F, Genestier AL, Eyssade N, Peyrol S, Etienne J, Lina G (2004) Neutralization of *Staphylococcus aureus* Panton Valentine leukocidin by intravenous immunoglobulin in vitro. J Infect Dis 189:346–353

Genestier AL, Michallet MC, Prevost G, Bellot G, Chalabreysse L, Peyrol S, Thivolet F, Etienne J, Lina G, Vallette FM, Vandenesch F, Genestier L (2005) *Staphylococcus aureus* Panton–Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J Clin Invest 115:3117–3127

Giesbrecht P, Kersten T, Maidhof H, Wecke J (1998) Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. Microbiol Mol Biol Rev 62:1371–1414

Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piemont Y, Brousse N, Floret D, Etienne J (2002) Association between *Staphylococcus aureus* strains carrying gene for Panton–Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 359:753–759

Gomes AR, Vinga S, Zavolan M, de Lencastre H (2005) Analysis of the genetic variability of virulence-related loci in epidemic clones of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 49:366–379

Gonzalez BE, Hulten KG, Dishop MK, Lamberth LB, Hammerman WA, Mason EO Jr, Kaplan SL (2005) Pulmonary manifestations in children with invasive community-acquired *Staphylococcus aureus* infection. Clin Infect Dis 41:583–590

Gosbell IB (2005) Epidemiology, clinical features and management of infections due to community methicillin-resistant *Staphylococcus aureus* (cMRSA). Intern Med J 35(Suppl 2):S120–S135

Graham PL III, Lin SX, Larson EL (2006) A U.S. population-based survey of *Staphylococcus aureus* colonization. Ann Intern Med 144:318–325

Groom A, Wolsey D, Naimi T, Smith K, Johnson S, Boxrud D, Moore KA, Cheek JE (2001) Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. JAMA 286:1201–1205

Grundmann H, Aires-de-Sousa M, Boyce J, Tiermersma E (2006) Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet 368:874–885

Guillet V, Roblin P, Werner S, Coraiola M, Menestrina G, Monteil H, Prevost G, Mourey L (2004) Crystal structure of leucotoxin S component. J Biolog Chem 279:41028–41037

Hedin G, Fang H (2007) Epidemiology of methicillin-resistant *Staphylococcus aureus* in Southern Stockholm, 2000–2003. Microb Drug Resist 13:241–250

Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, Leitch CD, Daum RS (1998) Community-acquired methicillin-resistant *Staphylococus aureus* in children with no identified predisposing risk. JAMA 279:593–598

Holfelder M, Eigner U, Turnwald AM, Witte W, Weizenegger M, Fahr A (2006) Direct detection of methicillin-resistant *Staphylococcus aureus* in clinical specimens by a nucleic acid-based hybridisation assay. Clin Microbiol Infect 12:1163–1167

Hookey JV, Richardson JF, Cookson BD (1998) Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. J Clin Microbiol 36:1083–1089

Hryniewicz W (1999) Epidemiology of MRSA. Infection 27(Suppl 2):S13–S16

Huang H, Flynn NM, King JH, Monchaud C, Morita M, Cohen SH (2006) Comparisons of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and hospital-associated MSRA infections in Sacramento, California. J Clin Microbiol 44:2423–2427

Huang YH, Tseng SP, Hu JM, Tsai JC, Hsueh PR, Teng LJ (2007) Clonal spread of SCCmec type IV methicillin-resistant *Staphylococcus aureus* between community and hospital. Clin Microbiol Infect 13:717–724

Ito T, Katayama Y, Hiramatsu K (1999) Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob Agents Chemother 43:1449–1458

Ito T, Katayama Y, Asada K (2001) Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 45:1323–1336

Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K (2003) Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist Updat 6:41–52

Janzon L, Ardvidson S (1990) The role of delta-lysine gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. EMBO J 9:1391–1399

Jarraud S, Lyon GJ, Figueiredo AMS, Ge'rard L, Vandenesch F, Etienne J, Muir TW, Novick RP (2000) Exfoliatin-producing strains define a fourth *agr* specificity group in *Staphylococcus aureus*. J Bacteriol 182:6517–6522

Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J, Vandenesch F (2002) Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 70:631–641

Jevons MP (1961) "Celbenin"—resistant Staphylococci. BMJ 1:124–125

Ji G, Beavis R, Novick RP (1997) Bacterial interference caused by autoinducing peptide variants. Science 276:2027–2030

Kaiser AM, Schultsz C, Kruithof GJ, bets-Ossenkopp Y, Vandenbroucke-Grauls C (2004) Carriage of resistant microorganisms in repatriates from foreign hospitals to The Netherlands. Clin Microbiol Infect 10:972–979

Kampf G, Meyer B, Goroncy-Bermes P (2003) Comparison of two test methods for the determination of sufficient antimicrobial activity of three commonly used alcohol-based hand rubs for hygienic hand disinfection. J Hosp Infect 55:220–225

Kaneko J, Kamio Y (2004) Bacterial two-component and heteroheptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Biosci Biotechnol Biochem 68:981–1003

Karynski M, Sabat AJ, Empel J, Hryniewicz W (2008) Molecular surveillance of methicillin-resistant *Staphylococcus aureus* by multiple-locus variable number tandem repeat fingerprinting (formerly multiple-locus variable number tandem repeat analysis) and spa typing in a hierarchic approach. Diagn Microbiol Infect Dis 62:255–262

Katayama Y, Ito T, Hiramatsu K (2000) A new class of genetic element, Staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 44:1549–1555

Kilic A, Li H, Stratton CW, Tang YW (2006) Antimicrobial susceptibility patterns and staphylococcal cassette chromosome *mec* types of, as Well as Panton–Valentine leukocidin occurrence among, methicillin-resistant *Staphylococcus aureus* isolates from children and adults in Middle Tennessee. J Clin Microbiol 44:4436–4440

Kim JS, Song W, Kim HS, Choa CH, Leea KM, Choib MS, Kimc EC (2006) Association between the methicillin resistance of clinical isolates of *Staphylococcus aureus*, their staphylococcal cassette chromosome *mec* (SCC*mec*) subtype classification, and their toxin gene profiles. Diagn Microbiol Infect Dis 56:289–295

Kowalski TJ, Berbari EF, Osmon DR (2005) Epidemiology, treatment, and prevention of community-acquired methicillin-resistant *Staphylococcus aureus* infections. Mayo Clin Proc 80:1201–1207

Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister K, Fosheim G, McDougal LK, Chaitram J, Jensen B, Fridkin SK, Killgore G, Tenover FC (2006) Prevalence of *Staphylococcus aureus* nasal colonization in the United States 2001–2002. J Infect Dis 193:172–179

Kurlenda J, Grinholc M, Jasek K, Wegrzyn G (2007) RAPD typing of methicillin-resistant *Staphylococcus aureus*: a 7-year experience in a Polish hospital. Med Sci Monit 13:MT13–MT18

Kurlenda J, Grinholc M, Krzyszton-Russjan J, Wisniewska K (2009) Epidemiological investigation of nosocomial outbreak of staphylococcal skin diseases in neonatal ward. Antonie Van Leeuwenhoek 95:387–394

Loeffler B, Hussain M, Grundmeier M, Brueck M, Holzinger M, Varga G, Roth J, Kahl BC, Proctor RA, Peters G (2010) *Staphylococcus aureus* Panton–Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 6(1)

Lopez-Aguilar C, Perez-Roth E, Moreno A, Duran MC, Casanova C, Aguirre-Jaime A, Mendez-Alvarez S (2007) Association between the presence of the Panton–Valentine leukocidin-encoding gene and a lower rate of survival among hospitalized pulmonary patients with Staphylococcal disease. J Clin Microbiol 45:274–276

Lorber B (2006) Update in infectious diseases. Ann Intern Med 145:354–360

Ma XX, Ito T, Chongtrakool P, Hiramatsu K (2006) Predominance of clones carrying Panton–Valentine leukocidin genes among methicillin-resistant *Staphylococcus aureus* strains isolated in Japanese hospitals from 1979 to 1985. J Clin Microbiol 44:4515–4527

Maguire G, Arthur A, Boustead P, Dwyer B, Currie B (1996) Emerging epidemic of community-acquired methicillin-resistant *Staphylococcus aureus* infection in the Northern Territory. Med J Aust 164:721–723

Manzur A, Vidal M, Pujol M, Cisnal M, Hornero A, Masuet C, Pena C, Gudiol F, Ariza J (2007) Predictive factors of meticillin resistance among patients with *Staphylococcus aureus* bloodstream infections at hospital admission. J Hosp Infect 66:135–141

Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG (2007) Community-associated methicillin-resistant *Staphylococcus aureus* isolates causing healthcare-associated infections. Emerg Infect Dis 13:236–242

Matouskova I, Janout V (2008) Current knowledge of methicillin-resistant *Staphylococcus aureus* and community-associated methicillin-resistant *Staphylococcus aureus*. Biomed Pap Med Fac Univ Palacky Olomuc Czech Repub 152:191–202

McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC (2003) Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol 41:5113–5120

Millar BC, Prendergast BD, Moore JE (2008) Community-associated MRSA (CA-MRSA): an emerging pathogen in infective endocarditis. J Antimicrob Chemother 61:1–7

Monecke S, Slickers P, Ellington MJ, Kearns AM, Ehricht R (2007) High diversity of Panton–Valentine leukocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated methicillin-resistant *S. aureus*. Clin Microbiol Infect 13:1157–1164

Mongkolrattanothai K, Boyle S, Murphy TV, Daum RS (2004) Novel nonmecA-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagF* genes in a commensal Staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*. Antimicrob Agents Chemother 48:1823–1836

Montgomery CP, Boyle-Vavra S, Daum RS (2009) The arginine catabolic mobile element is not associated with enhanced virulence in experimental invasive disease caused by the community-associated methicillin-resistant *Staphylococcus aureus* USA300 genetic background. Infect Immun 77:2650–2656

Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE, Fussing V, Salmenlinna S, Vuopio-Varkila J, El Solh N, Cuny C, Witte W, Tassios PT, Legakis N, van Leeuwen W, van Belkum A, Vindel A, Laconcha I, Garaizar J, Haeggman S, Olsson-Liljequist B, Ransjo U, Coombes G, Cookson B (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J Clin Microbiol 41:1574–1585

Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, Lynfield R (2003) Comparison of community- and health-care associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290:2976–2984

Nilsson P, Alexandersson H, Ripa T (2005) Use of broth enrichment and real-time PCR to exclude the presence of methicillin-resistant *Staphylococcus aureus* in clinical samples: a sensitive screening approach. Clin Microbiol Infect 11:1027–1034

NNIS (National Nosocomial Infections Surveillance System) (2002) System report, data summary from January 1992 to June 2002, issued August 2002. Am J Infect Control 30:458–475

Novick RP (2000) Pathogenicity factors and their regulation. In: Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Rood JI (eds) Gram-positive pathogens. American Society for Microbiology, Washington, pp 392–407

Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449

Novick RP, Ross HF, Projan SJ, Kornblum J, Kreiswirth B, Moghazeh S (1993) Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J 12:3967–3975

Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, Coombs GW, Pearman JW, Tenover FC, Kapi M, Tiensasitorn C, Ito T, Hiramatsu K (2002) Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol 40:4289–4294

Olive DM, Bean P (1999) Principles and applications of methods for DNA-based typing of microbial organisms. J Clin Microbiol 37:1661–1669

Oliveira DC, Tomasz A, de Lencastre H (2002) Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. Lancet Infect Dis 2:180–189

Oliveira DC, Milheirico C, de Lencastre H (2006) Redefining a structural variant of staphylococcal cassette chromosome *mec*, SCC*mec* yype VI. Antimicrob Agents Chemother 50:3457–3459

Ornskov D, Kolmos B, Bendix HP, Nederby NJ, Brandslund I, Schouenborg P (2008) Screening for methicillin-resistant *Staphylococcus aureus* in clinical swabs using a high-throughput real-time PCR-based method. Clin Microbiol Infect 14:22–28

Pan ES, Diep BA, Charlebois ED, Auerswald C, Carleton HA, Sensabaugh GF, Perdreau-Remington F (2005) Population dynamics of nasal strains of methicillin-resistant *Staphylococcus aureus*—and their relation to community-associated disease activity. J Infect Dis 192:811–818

Panton PN, Valentine FC (1932) Staphylococcal toxin. Lancet 222:506–508

Pedeacq JD, Maveyraud L, Prevost G, Baba-Moussa L, Gonzalez A, Courcelle E, Shepard W, Monteil H, Samana JP, Mourey L (1999) The structure of a Staphylococcus leucocidin component (LukF-PV) reveals the fold of the water-soluble species of a family of transmembrane pore-forming toxins. Struct Fold Des 7:277–287

Prevost G, Couppie P, Prevost P, Gayet S, Petiau P, Cribier B, Monteil H, Piemont Y (1995) Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. J Med Microbiol 42:237–245

Riley TV, Rouse IL (1995) Methicillin-resistant *Staphylococcus aureus* in Western Australia, 1983–1992. J Hosp Infect 29:177–188

Roberts JC, Gulino SP, Peak KK, Luna VA, Sanderson R (2002) Fatal necrotizing pneumonia due to a Panton–Valentine leukocidin positive community-associated methicillin-sensitive *Staphylococcus aureus* and Influenza co-infection: a case report. Ann Clin Microbiol Antimicrob 7:5

Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, Enright MC (2005) Reemergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. Lancet 365:1256–1258

Robinson JO, Pearson JC, Christiansen KJ, Coombs GW, Murray RJ (2008) Community-associated versus healthcare-associated methicillin-resistant *Staphylococcus aureus* bacteraemia: a 10-year retrospective review. Eur J Clin Microbiol Infect Dis 28:353–361

Sabat A, Malachowa N, Miedzobrodzki J, Hryniewicz W (2006) Comparison of PCR-based methods for typing *Staphylococcus aureus* isolates. J Clin Microbiol 44:3804–3807

Said-Salim B, Mathema B, Kreiswirth BN (2003) Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. Infect Control Hosp Epidemiol 24:451–455

Saiman L, Cronquist A, Wu F, Zhou J, Rubenstein D, Eisner W, Kreiswirth BN, la-Latta P (2003) An outbreak of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. Infect Control Hosp Epidemiol 24:317–321

Sakoulas G (2006) The accessory gene regulator (*agr*) in methicillin-resistant *Staphylococcus aureus*: role in virulence and reduced susceptibility to glycopeptide antibiotics. Drug Discov Today 3:287–294

Sakoulas G, Eliopoulus GM, Moellering RC Jr, Wennersten C, Venkataraman L, Novick RP, Gold HS (2002) Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. Antimicrob Agents Chemother 46:1492–1502

Salgado CD, Farr BM, Calfee DP (2003) Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. Clin Infect Dis 36:131–139

Saravolatz LD, Markowitz N, Arking L, Pohlod D, Fisher E (1982) Methicillin-resistant *Staphylococcus aureus*: epidemiologic observations during a community-acquired outbreak. Ann Intern Med 96:11–16

Saunders A, Panaro L, McGeer A, Rosenthal A, White D, Willey BM, Gravel D, Bontovics E, Yaffe B, Katz K (2007) A nosocomial outbreak of community-associated methicillin-resistant *Staphylococcus aureus* among healthy newborns and postpartum mothers. Can J Infect Dis Med Microbiol 18:128–132

Schwalbe RS, Stapleton JT, Gilligan PH (1987) Emergence of vancomycin resistance in coagulase-negative staphylococci. N Engl J Med 316:927–931

Skov R, Westh H (2005) Epidemic increase of methicillin resistant *Staphylococcus aureus* (MRSA) in Denmark–interventions required now. Ugeskr Laeger 167:1396

Staali L, Monteil H, Colin DA (1998) The Staphylococcal pore-forming leukotoxins open Ca channels in the membrane of human polymorphonuclear neutophils. J Membr Biol 162:209–216

Szmigielski S, Prevost G, Monteil H, Colin DA, Jeljaszewicz J (1999) Leukocidal toxins of staphylococci. Zentralbl Bakteriol 289:185–201

Sztramko R, Katz K, Antoniou T, Mulvey MR, Brunetta J, Crouzat F, Kovacs C, Merkley B, Tilley D, Loutfy MR (2007) Community-associated methicillin-resistant *Staphylococcus aureus* infections in men who have sex with men: a case series. Can J Infect Dis Med Microbiol 18:257–261

Takano T, Higuchi W, Otsuka T, Baranovich T, Enany S, Saito K, Isobe H, Dohmae S, Ozaki K, Takano M, Iwao Y, Shibuya M, Okubo T, Yabe S, Shi D, Reva I, Teng LJ, Yamamoto T (2008) Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* belonging to multilocus sequence type 59 in Taiwan. Antimicrob Agents Chemother 52:837–845

Taneike I, Otsuka T, Dohmae S, Saito K, Ozaki K, Takano M, Higuchi W, Takano T, Yamamoto T (2006) Molecular nature of methicillin-resistant *Staphylococcus aureus* derived from explosive nosocomial outbreaks of the 1980 s in Japan. FEBS Lett 580:2323–2334

Tenover FC, McDougal LK, Goering VR, Killgore G, Projan SJ, Patel JB, Dunman PM (2006) Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol 44:108–118

Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundman H (2004) Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. Emerg Infect Dis 10:1627–1634

Tomita T, Kamio Y (1997) Molecular biology of the pore-forming cytolysin from *Staphylococcus aureus*, alpha- and gamma-hemolysins and leukocidin. Biosci Botechnol Biochem 61:565–572

Toshkova K, Annemuller C, Akineden O, Lammler C (2001) The significance of nasal carriage of *Staphylococcus aureus* as risk factor for human skin infection. FEMS Microbiol Lett 202:17–24

Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, Reverdy ME, Enright MC, Vandenesch F, Etienne J (2007) Global distribution of Panton–Valentine leukocidin–positive methicillin-resistant *Staphylococcus aureus*, 2006. Emerg Infect Dis 13:594–600

Udo EE, Pearman JW, Grubb WB (1993) Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J Hosp Infect 25:97–108

von Specht M, Gardella N, Tagliaferri P, Gutkind G, Mollerach M (2006) Methicillin-resistant *Staphylococcus aureus* in community-acquired meningitis. Eur J Clin Microbiol Infect Dis 25:267–269

Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, Long RD, Dorward DW, Gardner DJ, Lina G, Kreiswirth BN, Deleo FR (2006) Is Panton–Valentine leukocidin the major virulence determinant in community associated methicillin-resistant *Staphylococcus aureus* disease? J Infect Dis 194:1761–1770

Wagenaar J, van Duijkeren E, Troelstra A, van de Giessen A, Kluytmans J, Mevius D, Tiemersma E, van der Wolf P (2007) Questions and answers about MRSA in farm animals. Tijdschr Diergeneeskd 132:558–560

Wang CH, Lin CY, Luo YH, Tsai PJ, Lin YS, Lin MT, Chuang WJ, Liu CC, Wu JJ (2005) Effects of oligopeptide permease in group a streptococcal infection. Infect Immun 73:2881–2890

Wannet WJ, Spalburg E, Heck ME, Pluister GN, Willems RJ, de Neeling AJ (2004) Widespread dissemination in The Netherlands of the epidemic Berlin methicillin-resistant *Staphylococcus aureus* clone with low-level resistance to oxacillin. J Clin Microbiol 42:3077–3082

Witte W, Cuny C, Strommenger B, Braulke C, Heuck D (2004) Emergence of a new community acquired MRSA strain in Germany. Euro Surveill 9:16–18

Wright JS, Jin R, Novick RP (2005) Transient interference with staphylococcal quorum sensing blocks abscess formation. Proc Natl Acad Sci USA 102:1691–1696

Zhang L, Gray L, Novick RP, Ji G (2002) Transmembrane topology of AgrB, the protein involved in the post-translational modification of *agrD* in *Staphylococcus aureus*. J Biol Chem 277:34736–34742