Community-associated MRSA: what makes them special?

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Summary

While infections with methicillin-resistant *Staphylococcus aureus* (MRSA) were traditionally restricted to the hospital setting, novel MRSA strains emerged over the last two decades that have the capacity to infect otherwise healthy people outside of the hospital setting. These communityassociated (CA-) MRSA strains combine methicillin resistance with enhanced virulence and fitness. Interestingly, CA-MRSA strains emerged globally and from different backgrounds, indicating that the "trade-off" between maintaining sufficient levels of methicillin resistance and obtaining enhanced virulence at a low fitness cost was achieved on several occasions in convergent evolution. However, frequently this process comprised similar changes. First and foremost, all CA-MRSA strains typically carry a novel type of methicillin resistance locus that appears to cause less of a fitness burden. Additionally, acquisition of specific toxin genes, most notably that encoding Panton-Valentine leukocidin (PVL), and adaptation of gene expression of genome-encoded toxins, such as alpha-toxin and phenol-soluble modulins (PSMs), further contributed to the evolution of CA-MRSA. Finally, the exceptional epidemiological success of the USA300 CA-MRSA clone in particular may have been due to yet another gene acquisition, namely that of the *speG* gene, which is located on the arginine catabolic mobile element (ACME) and involved in detoxifying harmful host-derived polyamines.

Keywords

Introduction

Antibiotic resistance is a big problem during the treatment of bacterial infections. While antibiotics are an effective means to control such infections, antibiotic overuse triggers the spread of resistant strains in the population. As a result, many strains of dangerous bacterial pathogens nowadays are resistant to antibiotics, with some strains combining even multiple resistances to different antibiotics.

Possibly the most infamous example of an antibiotic-resistant pathogen is *Staphylococcus aureus* (Lowy, 2003). This bacterium asymptomatically colonizes about a third of the population and may cause moderately severe to severe and occasionally life-threatening

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infections(Lowy, 1998). Notably, it is the most common cause of nosocomial infections and a leading cause of death in hospitalized patients. This extreme morbidity and mortality is due largely to the fact that many *S. aureus* strains carry genes that provide resistance to a variety of antibiotics, including the most efficient and widely used anti-staphylococcal drugs.

Penicillin and its derivatives are very effective against staphylococci. However, soon after the introduction of penicillin intro clinical use, penicillin-resistant, penicillinase-containing *S. aureus* strains spread all over the world(Barber and Rozwadowska-Dowzenko, 1948; Kirby, 1944). As a response to the fact that penicillin became ineffective against many infectious *S. aureus* strains, the penicillinase-resistant penicillin derivative methicillin was introduced in 1959. However, methicillin-resistant *S. aureus* (MRSA) was found within a year (Barber, 1961). Beginning in the 1980s, MRSA spread globally to such an extent that many countries now report MRSA rates of 50% or higher among infective *S. aureus* isolates in hospitals(Diekema et al., 2001). Only some countries, such as the Netherlands and the Scandinavian countries, which have effective search-and-destroy policies and/or control antibiotic overuse, have so far succeeded in keeping MRSA rates at a low level.

Until the mid 1990s, MRSA infections were limited to hospitals, infecting primarily the elderly, very young, and patients with weakened immune systems or undergoing surgery. However, within the last ~ 15 years, MRSA outbreaks were reported in healthy individuals without connection to health care institutions, such as sports teams, army recruits, or prisoners (Chambers, 2001). It soon became clear that these infections were due to the rise of new, distinct strains of MRSA, now called CA-MRSA strains. In the present review I will address the question what makes these strains different from hospital-associated (HA-) MRSA, enabling them to spread sustainably in the population and cause disease in otherwise healthy people.

Epidemiology and CA-MRSA disease manifestations

The first well documented CA-MRSA cases appeared in the upper midwestern United States between 1997 and 1999in children (CDC, 1999). These infections, which were fatal cases of sepsis and severe pneumonia, were caused by strain MW2 (pulsed-field type USA400). In the meantime, closely related strains belonging to pulsed-field type strain USA300 have replaced USA400 strains in the U.S. (Moran et al., 2006), but USA400 CA-MRSA infections are still observed in Alaska (David et al., 2008). While the U.S. has experienced the most pronounced CA-MRSA epidemic, CA-MRSA is also a global problem. Global strains of CA-MRSA belong to a series of different lineages; and specific strains are predominant in different countries (Mediavilla et al., 2012). For example, infections with CA-MRSA strains belonging to sequence type (ST) 80 are common in Europe and ST30 CA-MRSA infections occur predominantly in Australasia.

By far the most frequent disease manifestation associated with CA-MRSA is infection of the skin and soft tissues(Fridkin et al., 2005). Skin and soft tissue infections (SSTI) account for at least 90% of CA-MRSA infections. CA-MRSA SSTI are usually moderately severe to severe, often very painful, and are treated by simple incision and drainage. However, rare cases of very dramatic skin infections, such as necrotizing fasciitis, have been reported with CA- in contrast to HA-MRSA(Miller et al., 2005).CA-MRSA strains also cause infections of the bones and joints, such as osteomyelitis, and respiratory infections, such as pneumonia, sepsis, and urinary tract infections. Depending on the survey, these types of infections each account for <1 and 4% of all CA-MRSA infections.

Antibiotic resistance

With respect to antibiotic resistance, CA-MRSA strains are distinguished from HA-MRSA strains by three characteristics: First, CA-MRSA harbor different types of SCC*mec* elements, the mobile genetic elements (MGEs) encoding methicillin resistance genes. Traditional SCC*mec* elements found in HA-MRSA are most frequently of types I, II, and III; in contrast, CA-MRSA have SCC*mec* elements of types IV and V, which are shorter and thus believed to cause less of a fitness burden(Daum et al., 2002; Hiramatsu et al., 2001; Lee et al., 2007).

Second, CA-MRSA isolates are usually sensitive to most antibiotics other than methicillin and beta-lactams, while multi-resistance is common in HA-MRSA isolates. There have been reports on multi-resistant CA-MRSA; for example, a specific USA300 clone caused an outbreak of multi-resistant CA-MRSA in San Francisco (Diep et al., 2008a). This outbreak clone had accumulated resistance to mupirocin and clindamycin, and most isolates were also resistant to tetracycline. Non-multi-resistant USA300 isolates commonly only are resistant to erythromycin and ciprofloxacin, in addition to methicillin and beta-lactams. Fortunately, multi-resistant CA-MRSA isolates are still an exception.

Third, MIC (minimal inhibitory concentration) values of HA-MRSA clones are usually higher than those of CA-MRSA clones. For example, strain MRSA252 (USA200, EMRSA-16), a typical HA-MRSA clone from the United Kingdom, has an MIC that is > 50 times higher than that of strain LAC, a USA300 CA-MRSA clone isolated from prisoners in Los Angeles that is often used in laboratory research(Cheung et al., 2011).

Virulence characteristics

The observation that CA-MRSA strains have the capacity to infect otherwise healthy people indicates enhanced virulence. Accordingly, Voyich et al. showed in 2005 that the CA-MRSA strains MW2 and LAC are more virulent in a mouse infection model than common HA-MRSA strains such as COL and MRSA252(Voyich et al., 2005). Furthermore, these authors found that the enhanced virulence of CA-MRSA strains was accompanied by enhanced survival in human neutrophils, suggesting that the interaction of the pathogen with neutrophils, a critical step in the establishment of *S. aureus* infection, determines the increased virulence of CA-MRSA compared to HA-MRSA (Voyich et al., 2005).

It is important to stress that CA-MRSA strains are not more virulent than many MSSA strains. For example, MSSA strains of the ST8 lineage, such as those of the NCTC8325 family that are frequently used for laboratory experiments, are at least as virulent in animal infection models as CA-MRSA strains (Cheung et al., 2011). What distinguishes CA-MRSA strains is the combination of methicillin resistance with virulence characteristics not previously seen with HA-MRSA strains.

Toxins

The species *S. aureus* is infamous for producing a plethora of toxins, some of which are found virtually in all *S. aureus*, while others are linked to MGEs and restricted to a subset of strains. Therefore, a specific toxin repertoire or an enhanced production of toxins appeared as a likely basis for the enhanced virulence characteristics of CA-MRSA. As evasion of neutrophil killing was at least one of the predominant factors assumed to be associated with that enhanced virulence, staphylococcal leukocidins were in the center of investigations that were undertaken to delineate the molecular basis of CA-MRSA virulence.

Panton-Valentine leukocidin

Panton-Valentine leukocidin (PVL) is a two-component leukocidin that belongs to a beta-barrel forming family of cytolytic toxins, also comprising several other leukocidins, gamma-toxin, and alpha-toxin (Szmigielski et al., 1999). PVL is encoded by the prophage-encoded adjacent *lukS* and *lukF* genes,which produce the two toxin parts, LukS and LukF. Both are needed for the cytolytic activity of the toxin. Already in 1932, Panton and Valentine noted an association between PVL production and abscess formation(Panton and Valentine, 1932), but interest in PVL increased enormously when an epidemiological association between presence of the *lukSF* genes and CA-MRSA was detected (Vandenesch et al., 2003). Most CA-MRSA strains have *lukSF* genes, while their frequency in MSSA is much lower, and they are absent from predominant HA-MRSA clones.

PVL is lytic to human neutrophils at concentrations between 0.3 and 2 μg/ml (Genestier et al., 2005). Concentrations of PVL reaching or exceeding that range were demonstrated in human skin abscesses and in some clinical specimens from different infection types (Badiou et al., 2008; Badiou et al., 2010). In laboratory experiments, the contribution of PVL to the lytic activity toward human neutrophils when assayed with CA-MRSA culture filtrates proved strongly dependent on the type of growth media used(Graves et al., 2010). For that reason, such studies are barely conclusive when judging the role of PVL in CA-MRSA virulence. When assayed with live bacteria, PVL did not have a significant contribution to neutrophil killing by strains LAC and MW2 (Voyich et al., 2006). Quite unexpectedly, these laboratory experiments thus indicated that the contribution of PVL to the neutrophil killing capacity of important CA-MRSA strains is minor.

Accordingly, several skin and lung infection studies in mice with isogenic deletion mutants of the *lukSF* genes in strains LAC (USA300) and MW2 (USA400) did not reveal differences in virulence (Bubeck Wardenburg et al., 2007; Bubeck Wardenburg et al., 2008; Voyich et al., 2006). However, some other mouse studies showed differences (Brown et al., 2008; Labandeira-Rey et al., 2007), although in part these results may have been caused by unintended secondary mutations (Villaruz et al., 2009). Notably, it was found later that mouse neutrophils are much less sensitive to the cytolytic activity of PVL than those of humans (Hongo et al., 2009). Mechanistically, this difference was recently explained by the discovery of species-specific interaction of PVL with C5a receptors (Spaan et al., 2012), explaining the negative results of the earlier mouse infection studies regarding the contribution of PVL to CA-MRSA virulence. These findings lay to rest the intense scientific discussion about the initial controversial findings of the mouse infection studies, which now have no more than historical importance.

The sensitivity of rabbit neutrophils to PVL is in the range of those of humans (Loffler et al., 2010; Szmigielski et al., 1999); and therefore, more recent infection studies were performed in rabbits. In a model of severe pneumonia, rabbits showed increased morbidity and mortality when infected with PVL-containing USA300 as compared to an isogenic *lukSF* mutant, indicating a significant role of PVL in the development of severe CA-MRSA pneumonia (Diep et al., 2010). Notably, in this study the contribution of PVL to pathogenesis could be linked entirely to its interaction with neutrophils. Furthermore, PVL had a significant effect in experimental CA-MRSA (USA300) osteomyelitis when assayed in rabbits (Cremieux et al., 2009). However, results in rabbit skin infection models with USA300 were controversial. Lipinska et al. demonstrated a moderate yet significant impact of PVL on rabbit skin infection (Lipinska et al., 2011), whereas Kobayashi et al. failed to detect such an effect (Kobayashi et al., 2011). Notably, in the Kobayashi study, *lukSF* mutants were also compared to mutants in *hla* (alpha-toxin) and *psma* (phenol-soluble modulinα operon), both of which showed a strong impact on the development of skin infection. Furthermore, Li et al. compared the virulence of different globally occurring PVL-

positive and -negative CA-MRSA clones and did not find a correlation between presence or expression of the *lukSF* genes and virulence, while they found such a correlation for expression of the *psma* and *hla* genes (Li et al., 2010). The reason why divergent results were obtained in these rabbit skin infection studies is unclear. Possibly, intradermal injection as used in the Lipinska et al. and Li et al. studies may better reflect the natural infection scenario leading to the types of skin infection that are typical for PVL-positive strains, such as furunculosis or folliculitis (Del Giudice et al., 2011). However, while using the same mode of injection, the Lipinska et al. and Li et al. studies reached contrasting conclusions on the role of PVL in skin infection.

In addition to causing lysis of neutrophils, PVL has substantial pro-inflammatory effects that can be observed at concentrations as low as 5 ng/ml (Konig et al., 1995). As with many toxins, it is not clear whether such pro-inflammatory effects are of benefit to the bacteria, as they may cause excessive phagocyte infiltration and tissue damage, or whether they serve the human innate immune system to recognize infiltrating bacteria and launch a defensive response. Most likely, both mechanisms contribute to infection outcome and it depends on the specific scenario, which has a stronger impact. Diep et al. provided evidence in a rabbit pneumonia infection model suggesting that the pro-inflammatory effects of PVL contribute to increased infectivity (Diep et al., 2010). In contrast, Yoong et al. showed that presence of PVL stimulates the immune system, leading to better clearance of the bacteria (Yoong and Pier, 2010).

Finally, it needs to be noted that an increasing number of CA-MRSA clones are found that do not contain *lukSF* genes (Otto, 2010), for example in Korea (Lee et al., 2010) and the United Kingdom (Otter and French, 2008). Li et al. showed in rabbit skin infection studies that these clones are in average not less virulent than *lukSF*-containing CA-MRSA clones (Li et al., 2010). Furthermore, there was no difference in virulence between USA500, the PVL-negative progenitor of USA300, and USA300 itself. Altogether, the intensive recent research on PVL and its role in CA-MRSA infection has shown that the importance of PVL as a determinant of CA-MRSA virulence was initially overestimated. PVL certainly is a virulence factor, but only in specific infection types and scenarios, and only in some strains, does it appear to significantly contribute to the severity of disease caused by CA-MRSA.

Phenol-soluble modulins

Phenol-soluble modulins (PSMs) are a family of amphipathic, α-helical peptide produced by staphylococci (Wang et al., 2007). Many members of the PSM family have pronounced cytolytic activity toward a variety of human cells, including neutrophils and erythrocytes (Cheung et al., 2012; Cheung et al., 2010; Wang et al., 2007). In addition, PSMs trigger inflammatory responses by interaction with the formyl peptide receptor 2 (FPR2) (Kretschmer et al., 2010). PSMs also contribute to staphylococcal biofilm formation by forming fibril-like structures (Schwartz et al., 2012), structure biofilms, and cause biofilm detachment, resulting in the dissemination of biofilm-associated infection (Periasamy et al., 2012; Wang et al., 2011).

While the ~ 40–45 amino acid long β-type PSMs are barely cytolytic, PSMs of the α-type, ~ 20–25 amino acids in length, may have strong cytolytic activity (Cheung et al., 2012; Cheung et al., 2010; Wang et al., 2007). In particular, the PSMα peptides of *S. aureus*, PSMα1 – PSMα4, which are encoded in the *psma* operon, contain the potent cytolysin PSMα3. *S. aureus* delta-toxin, a member of the α-type PSMs, is a moderately potent yet often strongly expressed cytolysin.

PSMs are produced in high amounts in CA-MRSA strains, whereas production is in average lower in typical HA-MRSA strains such as USA100 and USA200 strains (Li et al., 2009;

Wang et al., 2007). This may at least in part be due to the facts that (i) the accessory gene regulator (Agr) virulence regulator exerts an exceptionally strict control over PSM expression (Queck et al., 2008), and (ii) HA-MRSA strains often show low, while CA-MRSA strains commonly have high Agr activity (Li et al., 2009).

The PSMα peptides have a significant impact on CA-MRSA virulence in experimental skin infection using mice or rabbits and bacteremia in mice (Kobayashi et al., 2011; Wang et al., 2007). Notably, PSMα peptides, above all PSMα3, are responsible for the increased neutrophil killing capacity that distinguishes CA- from HA-MRSA strains (Wang et al., 2007). Recent findings suggest that PSMs are expressed after neutrophil ingestion of the bacteria in the neutrophil phagosome as a result of Agr induction, identifying PSMs as the main mediator of quorum- (or diffusion-) sensing-induced neutrophil killing after *S. aureus* ingestion (Surewaard et al., 2012).

Alpha-toxin

Alpha-toxin is a cytolysin that is produced by most *S. aureus* strains (Bhakdi and Tranum-Jensen, 1991). While it is not lytic to human neutrophils (Valeva et al., 1997), it lyses a series of other cell types including macrophages and erythrocytes. The cytolytic activity of alpha-toxin is dependent on the interaction with the ADAM10 receptor (Wilke and Bubeck Wardenburg, 2010); and ADAM10-deficient mice are protected from lethal pneumonia and severe *S. aureus* skin infection (Inoshima et al., 2011; Inoshima et al., 2012). Alpha-toxin also leads to neutrophil chemotaxis (Bartlett et al., 2008)and has pro-inflammatory effects, including induction of the inflammasome (Craven et al., 2009) and generation of highly pro-inflammatory cytokines such as IL-1β and IL-18 (Craven et al., 2009). Finally, alpha-toxin has been shown to contribute to the penetration of the epithelial barrier during skin infection with USA300 (Soong et al., 2012).

Alpha-toxin has a demonstrated impact on virulence in many infection models. In CA-MRSA (USA300) pneumonia, alpha-toxin has a significant effect on morbidity and mortality(Bubeck Wardenburg et al., 2007). Immunization of mice with the mutant, non-toxic H35L derivative of alpha-toxin or passive immunization with anti-alpha-toxin antibodies protects from lethal pulmonary challenge(Bubeck Wardenburg and Schneewind, 2008). Furthermore, alpha-toxin has a strong impact on the development of USA300 skin infection (Kobayashi et al., 2011), which is also prevented by passive or active immunization (Kennedy et al., 2010).

Gene expression versus gene acquisition

The evolution of virulence in *S. aureus* clones and lineages is frequently only analyzed in terms of acquisition or loss of virulence-associated genes. However, significant changes in virulence may arise from minute changes in the genome, for example in regulatory loci such as Agr (DeLeo et al., 2011; Kennedy et al., 2008),which may remain unrecognized in such analyses. PSMs and alpha-toxin are expressed at high levels in CA- compared to HA-MRSA strains (Li et al., 2009), in accordance with the notion that gene expression changes contributed to the evolution of highly virulent CA-MRSA. This is likely at least in part due to the high activity in CA-MRSA of Agr, which controls expression of most *S. aureus* toxins and has a strong impact on CA-MRSA virulence(Cheung et al., 2011). Interestingly, it was reported that Agr controls genes in CA-MRSA strains in a fashion that is somewhat different from other strains (Cheung et al., 2011). However, the specific genetic events that led to these gene expression changes are still unknown.

Notably, explaining the evolution of virulence of CA-MRSA based on the core genomeencoded alpha-toxin and PSMs or the MGE-encoded PVL is thus categorically

different in terms of recognizing the contribution of gene expression versus only that of gene acquisition or presence. However, these two mechanisms are not mutually exclusive and it is likely that they both contributed to the evolution of CA-MRSA virulence. Furthermore, because PVL is also under control of Agr (Queck et al., 2008), both gene acquisition and enhanced expression mechanisms may have contributed to the evolution of the part of CA-MRSA virulence that is based on PVL.

Fitness

The epidemiological success of pathogenic *S. aureus* is not solely due to virulence factors in the stricter sense, such as toxins. It is also dependent on factors enhancing what can be called "fitness", i.e. the capacity to grow and persist in the human host. *S. aureus* is a colonizer at least in parts of the human population (Wertheim et al., 2005)and infections commonly originate from colonizing strains(von Eiff et al., 2001). Whether this is always true for CA-MRSA infection is not entirely clear, as direct infection from patients with *S. aureus* skin infection or from infected fomites appears possible. Nevertheless, factors that enhance fitness of CA-MRSA during colonization likely contribute to the spread of CA-MRSA in the population, by increasing the reservoir for subsequent infection and thus the epidemiological success of CA-MRSA as pathogens. Furthermore, genes that promote growth and persistence during infection also ultimately increase CA-MRSA virulence. The investigation of such factors has lacked behind the analysis of CA-MRSA toxins. However, as mentioned above, certain SCC*mec* elements are characteristic for CA-MRSA. In-vitro results indicate that SCC*mec* type IV has a role in enhancing CA-MRSA fitness(Lee et al., 2007), but in an in-vivo model of rabbit bacteremia, no impact on competitive fitness was detected (Diep et al., 2008b). Nevertheless, the fact that all CA-MRSA strains detected so far harbor one type of the novel, short SCC*mec* types IV or V indicates that they played a crucial role in the evolution of CA-MRSA.

Enhanced success of the USA300 CA-MRSA strain in particular has recently been linked to genes present in the arginine catabolic mobile element (ACME), which among CA-MRSA clones is limited to strain USA300, and was most likely acquired from *S. epidermidis* (Diep et al., 2006; Diep et al., 2008b). The most predominant features of ACME are an arginine deiminase and an oligopeptide transport gene cluster. These were frequently speculated to contribute to the fitness of strain USA300, such as by increasing survival in an acidic environment as found on the human skin or enhancing nutrient uptake, respectively (Diep et al., 2006; Diep and Otto, 2008). However, while ACME overall appeared to increase pathogen survival, no particular role could be attributed to either of these two clusters. Recently, Joshi et al. demonstrated that a decisive function in fitness might be due to another gene present on ACME, namely a spermidin Nacetyltransferase encoded by the *speG* gene (Joshi et al., 2011). While *S. aureus* has a unique sensitivity among bacteria to polyamines such as spermidin, SpeG detoxifies polyamines and may thus enhance survival of USA300 on the human skin.

Conclusions

Recent research revealed that CA-MRSA strains have increased virulence and fitness properties compared to traditional HA-MRSA strains. Mechanistically, this is likely due to a combination of (i) the acquisition of novel genes on MGEs, such as novel SCC*mec* elements, the PVL-encoding phage or, in case of USA300, ACME, and (ii) adaptations in gene expression, most importantly resulting in enhanced toxin expression (Fig. 1).

The development of high virulence and the acquisition of methicillin resistance cannot be separated when analyzing the evolution of CA-MRSA. The acquisition of the low fitness-

cost SCC*mec* elements appears to have been a necessary step in that evolution. In addition, results obtained so far indicate that there is a trade-off between the expression of methicillin resistance and that of important virulence determinants in MRSA. In support of that idea, CA-MRSA have lower methicillin resistance than HA-MRSA and HA-MRSA express toxins and other virulence genes at lower levels. The effective combination of methicillin resistance with high toxin expression likely required a specific strain background in which the acquisition of SCC*mec* did not lead to significant fitness and toxin expression decreases, or genetic adaptations subsequent to the acquisition of methicillin resistance. The precise nature of these genetic changes is largely unknown. Interestingly, recent findings indicate that there are regulatory components on *SCCmec* elements, including the methicillin resistance genes themselves, which control expression of genome-encoded genes (Pozzi et al., 2012). Furthermore, the genome-encoded virulence regulator Agr regulates expression of *mecA* in USA300 (Cheung et al., 2011). The "trade-off" between methicillin resistance and virulence gene expression may thus happen in a fashion that is more direct than previously thought.

The contribution of toxins to CA-MRSA virulence has received most attention, in part because these may represent promising targets for therapeutic intervention. For such endeavors, it is of utmost importance to analyze and rank their relative importance for CA-MRSA pathogenesis. The key players that have been linked to CA-MRSA virulence, alpha-toxin, PSMα peptides, and PVL, were analyzed in a comparative fashion in rabbit pneumonia and skin infection models. Alpha-toxin and PSMα turned out to strongly impact skin infection, but PVL had no effect in that study (Kobayashi et al., 2011). All three toxins significantly affected the progression of severe pneumonia, with PVL showing the greatest impact (Diep, 2010). These findings reflect that different types of CA-MRSA disease may require different toxins and thus, different therapeutic approaches. Notably, these studies were performed with USA300, while we do not yet understand which factors mainly contribute to disease progression in CA-MRSA other than USA300, particularly in PVL-negative CA-MRSA.

In conclusion, there are certain factors that CA-MRSA have in common and distinguish them from other MRSA (Table 1). These comprise the presence of low-fitness cost *SCCmec* elements and increased virulence characteristics. However, different CA-MRSA clones appear to have achieved the goal of simultaneous expression of sufficiently high methicillin resistance and aggressive virulence characteristics using different approaches in convergent evolution (Fig. 1). Most appear to have up-regulated toxin expression. Some, but not all, relied on acquisition of additional toxins such as PVL. Specific ones, such as USA300, relied on yet other factors such as ACME to increase resistance to host-derived toxic agents and thus survival in the human host.

Acknowledgments

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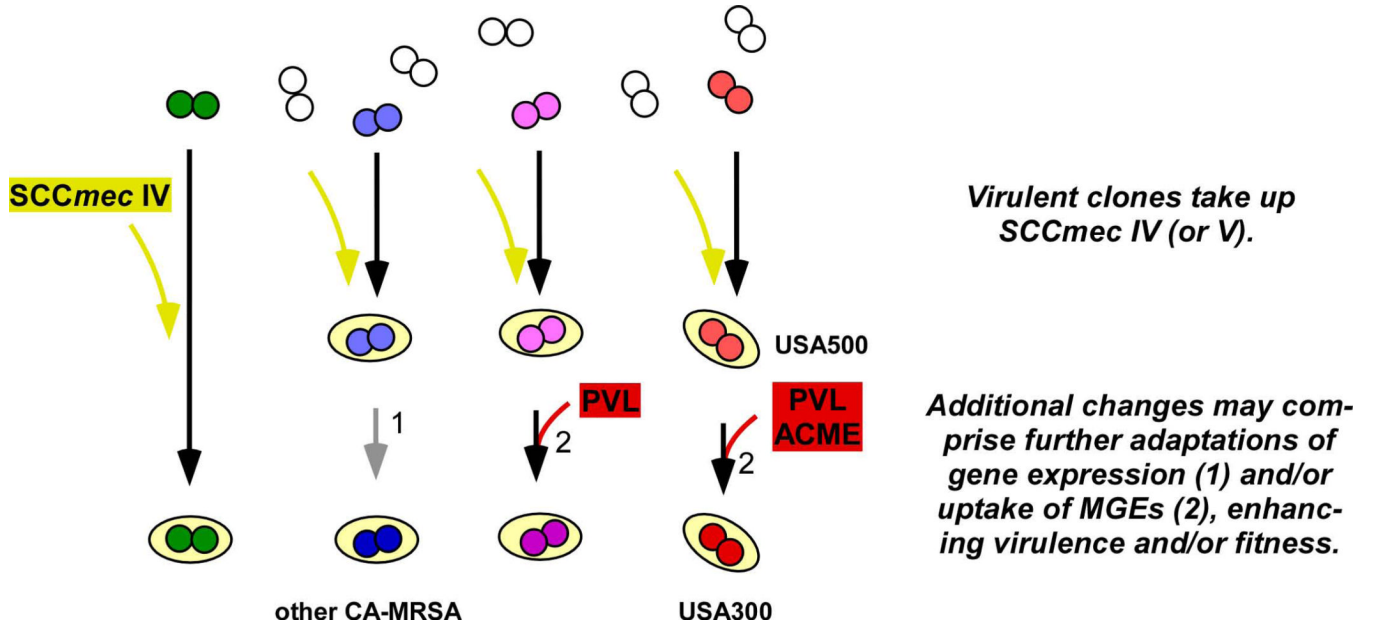
Fig. 1. Evolution of CA-MRSA
The acquisition of SCC*mec* type IV (or V) by virulent strains appears to have been a
common first step in the evolution of CA-MRSA strains. In some strains, as shown on the
left, this combination may have been sufficient to produce highly virulent, methicillin-
resistant strains with the capacity to infect healthy people, while in others, additional steps
were required. These additional steps may have involved adaptations of gene expression,
such as to enhance toxin production, and/or the uptake of MGEs, including or not including
the acquisition of PVL-encoding genes. On the right, the assumed scenario for the evolution
of USA300 is shown. Uptake of SCC*mec* type IV by a virulent ST8 strain resulted in a
virulent MRSA strain (USA500), whose fitness was further improved by uptake of ACME.
Acquisition of PVL-encoding genes appears to have increased virulence at least in some
infection types.

Table 1

Characteristic virulence- and fitness-associated features of CA-MRSA.

Factor	Role	Presence	Remarks
Virulence			
Alpha-toxin	Cytolysin, pro-Inflammatory	All CA-MRSA Increased Expression compared to HA-MRSA	Affects outcome of CA-MRSA pneumonia, skin Infection
PSMα peptides (PSMα1 - PSMα4)	Cytolysins, leukocidins, pro-Inflammatory	All CA-MRSA Increased Expression compared to HA-MRSA	Affects outcome of CA-MRSA pneumonia, skin Infection
Panton-Valentine leukocidin (PVL)	Leukocidin, pro-Inflammatory	Some CA-MRSA, including USA300 Located on prophage, absent from HA-MRSA	Affects outcome of CA-MRSA pneumonia, controversial reports on skin infection
Agr	Virulence regulator	All CA-MRSA Increased Expression compared to HA-MRSA	Controls expression of alpha-toxin, PSMα, PVL and PSMα, PVL and other toxins
Fitness			
SCC*mec* types IV or V	Carry methicillin resistance genes	All CA-MRSA Absent from HA-MRSA	Less fitness cost compared to other SCC*mec* types?
SpeG	N-spermidin-acetyltransferase	On ACME (only in USA300)	Abrogates *S. aureus* sensitivity to host-derived toxic polyamines