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The role of virulence determinants in community-associated MRSA pathogenesis

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Abstract

The recent emergence of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) marked a quantum step change in the biology and epidemiology of a major human pathogen. Various virulence determinants unique to CA-MRSA have been recently uncovered, shedding light on how these strains spread easily and sustainably among humans and frequently cause severe disease. The role of the Panton Valentine leukocidin (PVL) in CA-MRSA pathogenesis is a matter of much debate. While epidemiological data have suggested a role of PVL in the CA-MRSA disease process, recent data from relevant animal models suggest that PVL does not impact virulence of prevalent CA-MRSA strains. Identifying specialized pathogenic traits of CA-MRSA remains a challenge that will yield new diagnostic tools and therapeutic targets for drug and vaccine development.

Emergence of Community-associated Clones

Staphylococcus aureus is a major human pathogen and the leading cause of hospital-associated infections. *S. aureus* strains have developed resistance to almost all antibiotics, thereby complicating disease management. After first being reported in 1961, methicillin resistant *Staphylococcus aureus* (MRSA) has become endemic in hospitals worldwide, rendering the entire β -lactam class of antibiotics ineffective. Until recently, feral descendants of hospital-associated MRSA were the most common cause of sporadic disease in the community, frequently infecting persons with a history of recent hospitalization, or close contact with a person who had been hospitalized [3]. In contrast, community-associated MRSA (CA-MRSA) was first described in the late 1990s to cause severe and fatal infections in children without antecedent health care exposure [1,2]. Since then, epidemiologically unassociated outbreaks of CA-MRSA have been reported throughout the world [6-24] (Box 1). The vast majority of CA-MRSA infections worldwide are caused by strains belonging to only 5 clonal lineages (Figure 1). CA-MRSA lineages differ markedly in genotypic and phenotypic characters from traditional hospital-associated MRSA lineages. Evidently, these five CA-MRSA strains are fit and well-adapted for dissemination in the community (Box 1).

Here, we discuss recent advances in our understanding of CA-MRSA pathogenesis, focusing on the specialized pathogenic traits of CA-MRSA that are not typically found in traditional hospital-associated MRSA strains and methicillin susceptible *S. aureus*. Although numerous adhesins, toxins, and other important components of the staphylococcal background genome

contribute to the disease process, a broad discussion of staphylococcal pathogenesis is beyond the scope of this review.

Unique Genomic Contents

Based on clonal analysis (Figure 1), two prominent CA-MRSA strains from the United States were selected for further characterization to elucidate the molecular basis for inter-strain variations in epidemiologic characters, disease frequencies and disease severity. Genome sequencing of two CA-MRSA strains, the Midwest Clone MW2 (USA400) and the pandemic clone USA300 [7,9-11,25,26], revealed that ~20% of the unique genomic contents of CA-MRSA strains are due to the horizontal acquisition of multiple mobile genetic elements, including prophages and pathogenicity islands, which are absent from the traditional hospital-associated MRSA strains COL, N315 and MRSA252 (Figure 2). The prophages and pathogenicity islands contained numerous specialized pathogenicity factors, such as enterotoxins and exoproteins that could allow CA-MRSA to evade or subvert host defenses [25,26]. The MW2 strain, for example, contained 18 toxins not typically found in traditional hospital-associated MRSA strains [25]. Of these, superantigenic enterotoxin H, which is reported to have the highest binding affinity to major histocompatibility complex class II molecules ever measured for a staphylococcal enterotoxins [27], is uniquely found in MW2 and not other CA-MRSA strains or hospital-associated MRSA strains [6,14].

MW2 and USA300 have two mobile genetic elements in common: (1) the prophage ϕ SA2 pvl encoding the cytolytic toxin, Panton-Valentine leukocidin (PVL), and (2) the type IV staphylococcal chromosome cassette *mec* (SCC*mec*) element conferring resistance to the entire class of β -lactam antibiotics. Although PVL is found in sporadic CA-MRSA and methicillin susceptible *S. aureus* strains, it is epidemiologically linked only to the 5 predominant CA-MRSA clonal lineages widely associated with large disease outbreaks (see Figure 1). In comparison, the type IV SCC*mec* is present not only in the 5 predominant CA-MRSA clonal lineages but also almost all CA-MRSA strains causing sporadic disease in the community (Figure 1) [28,30,31]. The type IV SCC*mec* found in CA-MRSA strains is smaller in size than type I-III SCC*mec* elements found in traditional nosocomial strains (Figure 2), which could explain its extensive horizontal transfer among *S. aureus* lineages to generate the broad diversity of CA-MRSA strains (Figure 1). In addition, the genome sequence of the pandemic clone USA300 revealed the horizontal acquisition of the type I arginine catabolic mobile element (ACME) from the ubiquitous skin commensal *S. epidermidis* [26]. Type I ACME has been postulated to contribute to growth and survival of USA300 within the host, and promote colonization of the human skin [26]. ACME is integrated downstream of type IV SCC*mec* (Figure 2), utilizing the same cassette chromosome recombinases A and B (*ccrAB*) contained with SCC*mec* for mobilization and transfer [15-24,34]. Type I ACME is a distinctive genetic feature of the USA300 pandemic clone [26,34,35], although ACME-like elements have been detected in sporadic MRSA [32-34].

Is PVL the major virulence determinant of CA-MRSA?

The epidemiological association between genetically diverse *S. aureus* strains carrying the *pvl* genes and fatal necrotizing pneumonia has renewed and intensified interest in understanding the biological role of PVL [36]. A PubMed search for articles on PVL published in 2002-2007 identified more than 250 articles noting an association between PVL and CA-MRSA disease outbreaks. Although compelling, epidemiological data alone is insufficient to establish whether PVL directly contributes to widespread dissemination of CA-MRSA clones [37,38]. Of note, PVL-deficient variants of the predominant CA-MRSA strains also cause endemic community-associated disease [39]. For example, PVL-positive ST1 strains (MW Clone) are prevalent in the United States, but PVL-negative ST1 was earlier frequent in

communities in Western Australia [4]. Similarly, whereas PVL-positive ST59 are endemic in Taiwan and Vietnam, the vast majority of ST59 strains in the United States do not carry the PVL-harboring prophage [11-13,29]. Despite the prevalence of PVL-deficient CA-MRSA strains, PVL is widely regarded as the primary virulence determinant driving the epidemic spread of the major CA-MRSA clones worldwide [14,40,41]. We summarize here our current understanding of the biochemistry, molecular biology, and general function of PVL in CA-MRSA pathogenesis.

PVL and its effects as purified toxin

PVL belongs to a family of similar bi-component leukocidal toxins (synergohymenotropic toxins) produced by staphylococci, with one component belonging to the F and one to the S class [42]. PVL is encoded by the *lukPV* operon encoding the LukF-PV and LukS-PV components on the ϕ SLT, ϕ PVL, ϕ SA2MW, or ϕ SA2usa phages [26,42]. These β -barrel structured toxins form pores almost exclusively in leukocytes, via a mechanism similar to that used by α -hemolysin (α -toxin) [43]. Notably, α -toxin does not lyse neutrophils and the molecular underpinnings of the target cell specificity of these structurally very similar toxins are not completely understood [44]. Interestingly, early reports have concluded that PVL alone is not a very toxic substance [45], as intravenous injection of PVL in rabbits resulted in granulocytopenia followed by a marked granulocytosis, but was not lethal. Purified PVL also caused dermonecrotic lesions in rabbits [46], contributing to the hypothesis that this toxin plays a role in skin and soft tissue infections [42]. Recently, PVL has been shown to cause lethal necrotic lesions in the lungs of mice via nasal instillation of purified toxin [47].

Isogenic PVL⁺/PVL⁻ CA-MRSA strains in abscess and sepsis models

The effects of deleting the *lukPV* operon in MW2 and USA300 genetic backgrounds were tested in a mouse abscess model, to reproduce the most common clinical disease associated with PVL-positive CA-MRSA disease [18]. No significant difference in weight loss, abscess size, or bacteria colony forming units per abscess were detected when using Crl:SKH1-hrBR hairless and BALB/c mice to compare the following isogenic strain pairs: 1) MW2 Δ *pvl* mutant, in which *lukPV* operon was deleted by allelic replacement with a spectinomycin resistance cassette, and the parental strain MW2; and 2) LAC Δ *pvl* mutant and the parental strain LAC (Los Angeles County), which is a prototypical USA300 strain [48,49]. As bacteremia accounted for 65% of invasive CA-MRSA disease [24], the same isogenic strain pairs were compared in a CD1 Swiss mouse bacteremia model but no differences in lethality were observed, indicating that PVL is dispensable for CA-MRSA pathogenesis [48]. As rabbit and human PMNs are 10-100 times more sensitive than murine PMNs to the leukolytic effects of purified PVL [50,51], a rabbit model of bacteremia was used to test the potential effects of PVL. Using both co-infection and single-strain infection designs in 117 outbred rabbits, no difference in lung, spleen or kidney infectivity were detected using the same MW2 and LAC isogenic strain pairs [52]. As the same mouse and rabbit models have been used successfully to demonstrate the role of other CA-MRSA virulence factors [53,54], the null effect of PVL in these models strongly indicates that this toxin is not a major virulence factor of MW2 and USA300 strains.

PVL in experimental pneumonia: an exception?

Pneumonia is a rare disease caused by CA-MRSA [17,24]. Using purified toxin or a laboratory strain of *S. aureus* that overproduced PVL via a multicopy plasmid, PVL was shown to impact mouse survival in a model of pneumonia using BALB/c mice [47]. It is of interest that when comparing isogenic *S. aureus* strains lysogenized with either wild-type ϕ SLT or mutated ϕ SLT in which the *lukPV* operon was deleted, no difference in mouse survival was found [47],

indicating that PVL does not exhibit a lethal effect when expressed from a single transgene copy. In contrast, using isogenic Δpvl mutants in the MW2 and USA300 backgrounds, and when over-expressing PVL in *S. aureus* strain Newman, no significant contribution of PVL to lethal pneumonia was found using C57/black and BALB/c mice [49,55]. Passive immunization with anti-PVL immune sera also failed to protect mice against challenge with USA300 in the murine pneumonia model [56], indicating that PVL is not necessary for the pathogenesis of pulmonary disease.

Isogenic PVL+/PVL– CA-MRSA strains in the interaction with human neutrophils

Neutrophils are the main human leukocyte type to eliminate invading bacteria and the main cellular target of PVL [42]. As animal models can only partially reproduce how pathogens cause disease in humans, the null effect of PVL in the mouse and rabbit models may be due to a specific interaction of PVL with human neutrophils. Surprisingly, culture supernatants prepared from MW2 and USA300 clinical strains and their Δpvl isogenic mutants did not exhibit differences in capacity for pore formation or lysis of human neutrophils, even though PVL was secreted in abundance into the supernatants of the parental strains [48]. Thus, despite a detailed understanding of the molecular mechanistic basis of the leukolytic activities of purified PVL [57], the relevance of this toxin in the context of host-pathogen interactions remains unclear, particularly because CA-MRSA strains also secrete other exotoxins with much more potent leukolytic activities than PVL (see the section on PSMs below).

Does PVL have a gene regulatory effect?

Recently, a pronounced global gene regulatory effect has been ascribed to PVL [47], with the regulatory changes reminiscent of disrupting the accessory gene regulator *agr* [58]. For example, expression of PVL in a laboratory *S. aureus* strain background lysogenized with ϕ SLT appeared to result in a very strong up-regulation of protein A compared to the isogenic strain lysogenized with a mutated ϕ SLT in which the *lukPV* operon was deleted, suggesting that PVL regulates the expression of protein A via an unknown mechanism [47]. Protein A not only plays a classical role in immune evasion by binding to the Fc part of human IgG but also causes inflammatory effects in mouse lungs by interacting with TNFR1 on airway epithelial cells [59]. The interrelated effects of PVL and protein A were thought to be central in causing the overwhelming inflammation and necrosis of the mouse lungs [47]. However, misinterpretation of the microarray data due to the apparent lack of confirmatory experiments might have led to the model in which PVL plays a role in global gene regulation [47]. The possibility of spontaneous mutations or polar effects could have been ruled out by genetic complementation analysis, which however was not done for these microarray experiments [47]. Further transcriptional analysis using the SH1000 strain (a laboratory strain deficient in the *rsbU* component of the alternative sigma factor *sigB*, complemented with a functional *rsbU* copy) did not provide appropriate external validation, as it was compared to a strain that was not isogenic, containing both the *lukPV* operon and the rest of the phage ϕ SLT [47]. In contrast, no effects of PVL on global gene regulation and protein expression were detected in three independent CA-MRSA strains belonging to MW2 and USA300 backgrounds and their Δpvl isogenic mutants (Diep *et al.*, unpublished). While the reasons underlying these extreme differences will need to be investigated further, the failure to replicate the regulatory effects of PVL in clinically relevant CA-MRSA strains suggests that unintended genetic perturbations—not ruled out by appropriate controls—may have caused the dramatic gene regulatory differences reported in that study [47].

Taken together, it is clear that PVL has no impact on CA-MRSA pathogenesis when the *lukPV* operon is expressed via its own promoter at single gene copy. Only purified PVL at high

concentrations or over-expression of the *lukPV* operon via a multicopy plasmid may cause lung inflammation and necrosis. Notwithstanding the compelling epidemiological linkage between PVL and CA-MRSA strains, is there anything overlooked when analyzing the contribution of PVL to the pathogenesis of CA-MRSA? For example, PVL may be produced in abundance in the human host, perhaps in the context of antibiotic-induced bacterial SOS response that could result in prophage induction and enhanced expression of phage-encoded PVL [60,61]. Also, PVL may harm human cells other than leukocytes in a yet undiscovered interaction, which in this case would have to be human-specific, as mouse and rabbit infection models clearly do not show a PVL effect. Future research on the biological relevance of PVL is cautiously warranted. Nonetheless, attributing enhanced CA-MRSA pathogenicity to PVL alone ignores the possible contributions of numerous other genetic determinants unique to these epidemic strains (Figure 2) [25,26,53,62].

The linkage of *SCCmec* and ACME

MRSA strains contain distinct allotypes of the *SCCmec* element. In the absence of antibiotic selection pressure, *SCCmec* is thought to reduce the biological fitness of MRSA. The type IV *SCCmec* element found in CA-MRSA strains is smaller in size than the type I-III *SCCmec* elements found in hospital-associated MRSA strains. Thus, it may impose only a slight cost to fitness, as it does not encode resistance determinants other than *mecA*. When comparing isogenic strains with different allotypes of the *SCCmec* element, type I *SCCmec* but not type IV *SCCmec* was found to impose a fitness cost to the bacterial host in terms of decreased *in vitro* growth rate and cell yield per mole of ATP consumed [63]. A highly discriminating rabbit co-infection model was used to further demonstrate that a USA300 clinical strain and its isogenic mutant with precise deletion of the type IV *SCCmec* exhibited no difference in infectivity of vital rabbit organs [34]. Taken together, these data strongly indicate that type IV *SCCmec* does not engender a biological fitness cost. Accordingly, stable populations of CA-MRSA strains have emerged worldwide by transfer of type IV *SCCmec* into genetically diverse *S. aureus* strains (Figure 1) [5,64]. In contrast, the burden that other *SCCmec* allotypes (type I-III) impose on bacterial fitness (i.e. decreased *in vitro* growth rate [63]) has likely restricted traditional hospital-associated strains bearing these elements from spreading into the community. Hospital-associated MRSA seems to depend critically on high rates of antibiotic use in hospitals to overcome the fitness burden associated with the presence of antibiotic resistance elements.

Type IV *SCCmec* is physically linked to type I ACME in the pandemic clone USA300 (Figure 2), suggesting that selection for antibiotic resistance and pathogenicity may be interconnected [34]. Precise deletion of ACME in a USA300 clinical isolate resulted in attenuated pathogenicity or fitness when compared to the parental strain in the rabbit co-infection model, thereby providing evidence that ACME contributes to pathogenesis [34]. Two gene clusters identified in ACME, *arc* and *opp-3*, could confer the phenotypes of interest. As L-arginine is a substrate for nitric oxide production, depletion of L-arginine by arginine deiminase system (*arc*) could inhibit nitric oxide production, a molecule used in both the innate and adaptive immune responses against bacterial infections [65]. L-arginine catabolism could also be important for ATP production and pH homeostasis on the acidic human skin (pH 4.2–5.9) [66]. *opp-3* belongs to the ABC transporter family, members of which have a wide variety of functions, including peptide nutrient uptake, quorum sensing, pheromone transport, chemotaxis, eukaryotic cell adhesion, binding of serum components, and resistance to antimicrobial peptides. Thus, in community settings largely devoid of antibiotic selection pressure, ACME may enhance the growth, survival and dissemination of USA300, thus allowing for the genetic “hitchhiking” of *SCCmec*. In turn, *SCCmec* protects against exposure to β -lactam antibiotics, further enhancing rapid dissemination of USA300 into health care settings with high rates of antibiotic use.

The SCCmec-ACME linkage alone, however, is likely not sufficient to bestow the epidemic properties of USA300. USA300 variants lacking the SCCmec-ACME composite island are also prevalent among methicillin susceptible *S. aureus* causing sporadic disease in the United States [18,67], suggesting a substantial contribution of the genomic background to the epidemic properties of USA300 [26,29]. It is also of interest that ACME-like elements have been found in MRSA isolates in association with other SCCmec elements, including type IV SCCmec in ST1, type II SCCmec in ST5 and ST59, and type V SCCmec in ST97 strains [32-34]. The associated SCCmec allotypes II, IV, and V, contained genes encoding CcrAB recombinases essential for mobilization and integration of ACME into a chromosomal attachment site found at the terminus of SCCmec [34,68]. ACME-like elements found in ST5 and ST59 strains differed significantly in gene content from the type I ACME found in USA300, including the lack of the *opp-3* operon that could contribute to virulence [34]. Although it appears that these ACME-like elements are found in MRSA strains causing sporadic disease, it remains to be determined whether they contribute to bacterial pathogenesis or fitness.

Phenol-soluble modulins and other toxins

While *S. aureus* produces many molecules with the capacity to lyse human leukocytes, it has remained obscure which molecules are mainly responsible for neutrophil lysis in vivo and the pronounced lytic activity seen in CA-MRSA. Recently, novel cytolytic peptides have been found in *S. aureus*, the α -type phenol-soluble modulins (PSMs), which are encoded in an operon on the genomes of all sequenced *S. aureus* strains (Figure 3a). The α -helical and amphipathic α -type PSMs are secreted in a yet undiscovered fashion as the primary translation products carrying an N-terminal N-formyl methionine (Figure 3b). They have pronounced in vitro and in vivo leukocidal (Figure 3c), in addition to pro-inflammatory and chemotactic activities [53]. However, whereas hospital-associated MRSA often lack PSM production or produce PSMs only at reduced levels, PSMs are expressed at considerable levels in CA-MRSA. Notably, over-expression of α -type PSMs in a hospital-associated MRSA strain caused leukocidal activity equal to that observed in CA-MRSA strains, indicating that expression of these peptides is the main cause for the extreme difference in lytic activity between CA-MRSA and hospital-associated MRSA and a possible major contributor to the pronounced pathogenic potential of CA-MRSA strains. In fact, a dramatic influence of the α -type PSMs on the infectivity of CA-MRSA was demonstrated using the bacteremia and abscess mouse infection models (Figure 3d) [53].

The results obtained with the PSMs indicate that we will have to consider differential gene expression between CA-MRSA and HA-MRSA strains to explain differences in virulence. For example, it is known that α -hemolysin, a strong pro-inflammatory and pore-forming toxin, may differ considerably in expression among different strains. Although α -hemolysin does not lyse neutrophils, it has recently been shown to have a key impact on the outcome of necrotizing pneumonia by CA-MRSA [56,69]. Finally, as these toxins and the PSMs are under control of the global regulator *agr* and there is strong expression of the *agr* regulatory molecule RNAIII in CA-MRSA strains, whereas hospital-associated MRSA strains are often *agr*-negative, there may be a key role of this virulence regulator in CA-MRSA disease that remains to be investigated [53].

Concluding Remarks

In recent years perhaps no infectious disease problem has generated as much interest as the epidemic spread of CA-MRSA strains. In 2005, 18,650 deaths were estimated in patients with invasive MRSA disease in the United States, which was comparable to the number of deaths attributed to HIV/AIDS [24,70]. The pandemic clone USA300 has contributed more than any other strain to the dramatic increase in MRSA disease because it is unusually virulent and

especially well-adapted for transmission in the community. A frequently overlooked attribute of USA300 pathogenicity is its enhanced transmission between susceptible hosts. The precise mechanism involved in transmission is poorly understood, although specific bacterial factors, such as ACME, may facilitate colonization of new niches within the host [26]. Recent studies further indicate that the molecular basis of CA-MRSA pathogenicity resides not solely in the horizontal acquisition of novel pathogenicity determinants but also in the differential regulation of effector molecules encoded by all *S. aureus* genomes. Specifically, the α -type PSMs exemplify the importance of understanding the regulatory mechanisms underlying increased expression of virulence determinants in CA-MRSA strains [53]. The current state of knowledge regarding CA-MRSA pathogenesis is summarized in Figure 4. It should be noted that the prototypical strains MW2 and USA300 have been used exclusively for studies of CA-MRSA pathogenesis, although the proposed virulence mechanisms may not be present or active in other CA-MRSA strains. The specific pathogenic traits of other prevalent CA-MRSA strains, including ST30, ST59 and ST80, will need to be identified and their relative contributions to the disease process elucidated in order to prioritize targets for drug and vaccine development.

Box 1

Community-associated Clones of MRSA

Five CA-MRSA strains account for the vast majority of CA-MRSA disease worldwide.

- The first widely recognized CA-MRSA strain is commonly known as the Midwest Clone, coined after the region in the United States where it emerged [1,2]. This clonal lineage, however, was prevalent in communities in Western Australia earlier [4]. According to multi-locus sequence typing (MLST) analysis, the Midwest Clone belongs to the sequence type ST1 clonal lineage (Figure 1) [5].
- The second CA-MRSA strain—ST30 clonal lineage—is known as the Southwest Pacific/Oceania Clone, as it was implicated in localized community outbreaks in Australia, Greece, Mexico and United States [7-11].
- The third CA-MRSA strain—ST80 clonal lineage—is known as the European Clone for causing endemic disease in many European communities [8,14].
- The fourth CA-MRSA strain—ST59 clonal lineage—is known as the Pacific Clone as it is endemic in the United States, Taiwan, and Vietnam [11-13].
- The fifth CA-MRSA strain—ST8 clonal lineage—is better known as USA300, after the name of its unique pulsed-field gel electrophoresis profile [28]. Not seen before the year 2000, USA300 is now pandemic in communities across 38 U.S. states, Canada, and 9 European countries [11]. The pandemic clone USA300 has been implicated in unusually severe human diseases, including endocarditis, pneumonia, sepsis, and necrotizing fasciitis [16,17,29]. Remarkably, the introduction of USA300 into a new geographic area has often been associated with the displacement of locally endemic CA-MRSA strains belonging to ST1, ST30, ST59 and ST80 clonal lineages [22,23,32,33]. In many locales, USA300 alone accounts for more than 50% of all disease caused by the entire *S. aureus* species [18], which points to the unique capacity of this clone to spread easily and sustainably among humans.

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Figure 1. Genetic diversity among *S. aureus* strains that have independently acquired type IV SCCmec (blue). The five predominant CA-MRSA clones (red) all have the *lukPV* gene operon encoding Pantone Valentine leukocidin. The graph was constructed with Splitstree (version 4.8) using concatenated sequences of seven housekeeping gene fragments used in MLST for *S. aureus*. The scale bar represents the evolutionary distance (number of substitutions per nucleotide position).

Figure 2.

Interstrain differences in gene content among methicillin resistant *Staphylococcus aureus*. Concentric circles represent the circular chromosomes of USA300, COL, N315, MW2, and MRSA252. The core chromosome, consisting of genes shared by all strains of *S. aureus*, is shown in gray. The accessory chromosome, consisting of genes acquired through horizontal acquisition of mobile genetic elements, includes the different allotypes of *SCCmec* (blue), ACME (light blue), pathogenicity islands (red or green), and prophages (black).

Figure 3.

The α -type phenol-soluble modulins (PSMs). (a), arrangement of the α -type PSM genes in the *S. aureus* genome (shown for strain USA300). (b), amino acid sequences of α -type PSMs. (c), α -type PSMs cause neutrophil lysis. Representative images of neutrophils incubated with PSM α 3 (10 μ g) or buffer after 60 min of incubation. (d), α -type PSMs are crucial for CA-MRSA skin and soft tissue infection. Representative images of dermonecrosis formed by USA300 wild-type and isogenic PSM α operon deletion strains.

Figure 4.

Molecular basis of CA-MRSA infection. The exceptional success of CA-MRSA strains in the community is likely due to enhanced virulence and colonization. In addition to an enhanced capacity for nasal carriage [11], CA-MRSA strains are also commonly carried on the skin, thereby facilitating skin-to-skin spread and even sexual transmission [71-75]. Using isogenic mutant strains in CA-MRSA, 3 factors have a proven impact on CA-MRSA pathogenesis, as determined in animal models. PSMs (α -type) are crucial for abscess formation and bacteremia, the most widely found manifestations of CA-MRSA disease. α -toxin has a dramatic impact on the development of necrotizing pneumonia, one of the rarer and more dramatic diseases CA-MRSA may cause. Whether PSMs have a role in necrotizing pneumonia, or α -hemolysin in abscess formation and bacteremia of CA-MRSA, has not been tested yet. Both factors are widely found in *S. aureus* strains. However, CA-MRSA strains may differ from HA-MRSA strains in the expression of these factors, as shown for PSMs. The mobile genetic element ACME is thought to enhance colonization and survival on the skin and is only found in the USA300 background. USA300 CA-MRSA strains with ACME outcompete isogenic strains without ACME in a rabbit infection model.