Methicillin-resistant Staphylococcus aureus (MRSA) is an important human pathogen that causes serious infectious diseases and was endemic in hospitals by the late 1960s. Beginning with its first report in the late 1990s, the rapid emergence of community-associated MRSA (CA-MRSA) worldwide responsible for a wide spectrum of diseases ranging from minor skin infections to fatal necrotizing pneumonia has been found in previously healthy individuals without established risk factors for MRSA acquisition. Recently, various virulence determinants unique to CA-MRSA have been uncovered, which explain how the pathogen spreads easily and causes severe CA-MRSA infections among humans. However, the role of Panton-Valentine leukocidin (PVL) in the pathogenesis of CA-MRSA infection is currently a matter of much debate because of conflicting data from epidemiologic studies of CA-MRSA infections and various murine disease models. Identifying specialized pathogenic traits of CA-MRSA and the concerted regulation of these factors remains a challenge that will foster development of vaccines and therapies designed to control CA-MRSA infections. This review focuses on the current status of molecular epidemiology associated with CA-MRSA in Taiwan and progresses toward understanding the enhanced virulence properties of CA-MRSA, with an emphasis on the role of Panton-Valentine leukocidin.

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1. Background

*Staphylococcus aureus* is a ubiquitous human pathogen and a leading cause of bacterial infections involving the bloodstream, lower respiratory tract, and skin and soft tissue.\(^1\) The high morbidity and mortality resulting from *S. aureus* were abated by penicillin in the 1940s, but this was short-lived, as penicillin-resistant *S. aureus* (PRSA) producing β-lactamase quickly emerged in the mid-1940s. The prevalence of PRSA then increased dramatically within a few years.\(^2\)\(^-\)\(^4^\) Methicillin, a β-lactamase-insensitive β-lactam, provided new treatment options for PRSA infections in the late 1950s. However, after first being reported in 1961, methicillin-resistant *S. aureus* (MRSA) that was cross-resistant to the entire β-lactam class of antibiotics, rapidly increased and spread during the 1980s, primarily in health care environments.\(^5\)\(^-\)\(^6^\)\(^-\)\(^8^\) Since then, MRSA has become endemic in hospital settings in many developed countries and accounts for most nosocomial *S. aureus* infections.\(^9\)\(^-\)\(^10^\) In Taiwan, MRSA was first documented in the early 1980s and rapidly increased in the 1990s.\(^10^\) In 2000, methicillin resistance was identified in 53%–83% of all *S. aureus* isolates at the 12 major hospitals in Taiwan.\(^11\)

2. Emergence of Community-associated MRSA Clones

Historically, MRSA infections represent a considerable burden on health care; infected patients require prolonged hospital stays, entail high hospital costs, and have increased in-hospital mortality.\(^12\)\(^-\)\(^13^\) With few exceptions, MRSA was a problem in most patients who had traditional risk factors associated with treatment in nosocomial settings.\(^13\)\(^-\)\(^14^\) However, the traditional notion of MRSA as a pathogen that is seemingly confined to the nosocomial arena was recently challenged with the recognition of community-associated MRSA (CA-MRSA) among children and adults lacking traditional risk factors.\(^15\)\(^-\)\(^18^\)

CA-MRSA was first reported in Western Australia in the 1990s and, by the end of the 1990s, reports of serious and rapidly progressive fatal disease in children in Minnesota and North Dakota in the USA because of virulent CA-MRSA were the focus of attention.\(^19\)\(^-\)\(^20^\) Since these early reports, CA-MRSA isolates have become globally pervasive and epidemiologically unassociated outbreaks of CA-MRSA have been reported throughout the world, including in Taiwan.\(^5\)\(^-\)\(^21\) Two categories of MRSA are now recognized: health care–associated (HA) MRSA, the leading cause of HA infections worldwide, particularly in intensive care units, and CA-MRSA.\(^43^\) The isolates that cause CA-MRSA infection are identified by using a definition advocated by the Centers for Disease Control and Prevention in 2000.\(^24\) This definition states that a case of CA-MRSA infection is diagnosed in an outpatient or within 48 hours of hospitalization if the patient lacks the following traditional risk factors for MRSA infection such as receipt of hemodialysis, surgery, residence in a long-term care facility, or hospitalization during the previous year; the presence of an indwelling catheter or a percutaneous device at the time culture samples were obtained; or previous isolation of MRSA.\(^44\)\(^,\)\(^45^\)

CA-MRSA strains have unique microbiologic characteristics such as limited antibiotic resistance (except to β-lactam antimicrobial agents), different exotoxin gene profiles [e.g., Panton-Valentine leukocidin (PVL)], and smaller staphylococcal cassette chromosome mec (SCCmec) variants: either SCCmec type IV or less frequently, type V.\(^32\) They have common pulsed gel electrophoresis patterns that are distinct from those of the major pandemic clones of HA isolates.\(^46\)

Nowadays, the vast majority of CA-MRSA diseases worldwide result from strains belonging to at least five clonal lineages such as sequence types (ST)1, ST8, ST30, ST59, and ST80. Evidently, CA-MRSA clones appear to be epidemiologically successful, fit, and well adapted for dissemination in the community, and predominate over methicillin-susceptible *S. aureus* in certain populations.\(^5\)\(^-\)\(^7\)\(^-\)\(^9\)\(^,\)\(^47\)\(^-\)\(^51^\) Furthermore, the virulence of CA-MRSA seems to parallel its epidemiologic success.

3. CA-MRSA Epidemiology and Clinical Manifestations in Taiwan

CA-MRSA causes a wide range of diseases, ranging from infection of skin or soft tissue to severe invasive diseases, such as severe sepsis, necrotizing pneumonia, necrotizing fasciitis, and disseminated invasive osteomyelitis.\(^34\)\(^,\)\(^52\)\(^-\)\(^54^\) In Taiwan, CA-MRSA infections have been increasingly reported in pediatric patients since 2002.\(^25\)\(^,\)\(^55^\) After pooling the data from different retrospective studies with similar designs between 1997 and 2001, the MRSA rate is estimated to be 44% in pediatric cases of community-associated *S. aureus* infections.\(^57\)\(^-\)\(^59^\) However, a substantial proportion (41%–65%) of the pediatric cases with CA-MRSA infection had identified risk factors, although the definition of health care–associated risks varied among different studies.\(^60^\)

Currently, the characteristics of CA-MRSA infecting strains in Taiwan can be demonstrated in three different ways. First, they are characterized by multiple antibiotic resistance [including clindamycin (93%–100%), erythromycin (94%–100%), and chloramphenicol (57%–65%)], which differs from that in the United States. Additionally, they are less resistant to gentamicin (11%–34%), ciprofloxacin (0%), fusidic acid (0%), trimethoprim-sulfamethoxazole (0%–9%), and minocycline (7%) than are health care–associated MRSA.\(^27\)\(^,\)\(^57\)\(^,\)\(^58^\) Second, although skin and superficial soft tissue infections are the major clinical manifestations, significant morbidity and mortality because of CA-MRSA infections in pediatric cases are increasingly reported.\(^56\)\(^,\)\(^61\)\(^-\)\(^64^\) Third, they have a common genotype (e.g., ST59/USA1000) that differ from those of the major pandemic clones of nosocomial MRSA isolates, possess PVL, staphylococcal enterotoxin B, and smaller SCCmec variants: either SCCmec type IV (now designated type VII), or less frequently, type IV.\(^27\)\(^,\)\(^29\)\(^,\)\(^60\)\(^,\)\(^65\)\(^,\)\(^66^\)

In addition to the hospital-based studies, several community-based colonization studies and the latest island-wide survey were conducted to estimate the extent of MRSA in community children.\(^29\)\(^,\)\(^61\)\(^,\)\(^65\)\(^,\)\(^67\)\(^-\)\(^70^\) These studies indicated that the prevalence of the nasal MRSA colonization in Taiwan increased significantly during the period of 2005–2006 compared with those during the period of 2001–2002 (from 1.9% to 9.5% for northern Taiwan and from...


4. PVL in CA-MRSA Virulence and Pathogenesis

In 1894, van de Velde identified a substance named “substance leukocide” or leukocidin, which is a molecule produced by S. aureus that lyses leukocytes.72 Panton and Valentine then discovered a leukocidin clearly distinct from a hemolytic toxin that was nonlethal for rabbits.73 However, they showed a correlation between presence of this nonlethal leukocidin (later named PVL) and severe skin infections, especially carbuncles (large abscesses), some of which were ultimately fatal in the absence of antibiotics.73

PVL is a two-component cytotoxin that targets human and rabbit polymorphonuclear neutrophils, and monocytes or macrophages, or both.74 The products of pvl genes (LukS-PV and LukF-PV), which are encoded by contiguous located cotranscribed genes (lukS-PV and lukF-PV) within specific bacteriophages assemble as heterooligomers and synergistically exert cytolytic pore-forming activity on the surface of susceptible host cells, although the plasma membrane binding site and/or receptor is unknown.74–76 Notably, in vitro studies with recombinant PVL demonstrated a concentration-dependent effect leading to either apoptosis or necrosis in neutrophils exposed to different toxin concentrations, with Bax-independent apoptosis occurring by means of a novel pathway that presumably involved PVL-mediated pore formation in the mitochondria membranes.1,77,78 Sublytic concentrations of PVL trigger apoptosis of human neutrophils within 6 hours of treatment, whereas higher toxin concentrations cause cell lysis within 1 hour.1,78 In addition to its ability to form pores in membranes of target leukocytes, PVL is also a polymorphonuclear neutrophil priming agent.77,79–81 More recently, the signal peptide of PVL has been implicated as a S. aureus adherence molecule; these findings are consistent with earlier studies by de Bentzmann et al. and Tristan et al.,82,83 who reported that PVL-positive S. aureus isolates associated with necrotizing pneumonia have strong affinity for host extracellular matrix proteins.

Both epidemiologic and clinical data provide compelling evidence that the high virulence potential of CA-MRSA originates from PVL.32,84,85 For example, Lina et al.85 and Gillet et al.84 reported a link between PVL-positive S. aureus and severe skin infections and necrotizing pneumonia. Moreover, despite the large repertoire of transferrable toxin genes among S. aureus strains, pvl is the most consistently present transferrable toxin locus among CA-MRSA strains and is considered a stable marker of CA-MRSA strains worldwide (e.g., 100% for the US clone, the Southwest Pacific clone, the Europe clone, and 73.3%–100% for the Taiwan clone).84,85,92,96 Although compelling, epidemiologic data alone are not sufficient to establish a direct role for PVL in S. aureus pathogenesis or whether it directly contributes to widespread dissemination of CA-MRSA clones.88,89

To test directly the role of PVL in the pathogenesis of CA-MRSA infection, Voyich et al. compared USA300 and USA400 wild-type and isogenic PVL-deletion strains in murine abscess and sepsis models of infection.90 However, regardless of strains used, they found that the course of infection was virtually identical in both models and PVL had no effect on neutrophil phagocytosis or lysis after uptake. Conversely, Varshney et al.91 reported that methicillin-susceptible S. aureus and MRSA strains that produced high levels of PVL caused larger skin abscesses, higher bacterial burdens, and more tissue inflammation than did low-PVL producing strains in a mouse model of skin infection. Furthermore, on the basis of the results reported by Labanderia-Rey et al.,92 they suggested a potential role of PVL in a murine model of staphylococcal pneumonia by use of laboratory strains transduced to express the leukocidin and proposed a model in which PVL functioned as a global regulator of gene expression. Furthermore, as shown in a recent study on gene expression directly in human tissue, PVL, together with other secreted toxins, had a high level of expression during superficial and invasive CA-MRSA infections.93 However, in contrast to the observation of Labanderia-Rey et al., subsequent microarray-based and proteomics-based studies demonstrated that PVL has no impact on gene or protein expression in either USA300 or USA400 strains in vitro or in vivo.94,95 Thus, although the involvement of PVL in CA-MRSA virulence is a topic of high interest and is under intense investigation, conflicting results have been reported in the potential role of PVL using diverse animal models for CA-MRSA disease.96–100

The reason for the discrepancy between studies remains unclear, but collectively, most mouse models of CA-MRSA pathogenesis support either no role or a limited transient role for PVL in promoting disease.94 It is possible that contribution of PVL to CA-MRSA disease is limited to a specific host genetic background or susceptibility factor (e.g., antecedent influenza), or is either too subtle to detect in current models of pathogenesis or the models do not accurately reflect human disease, as suggested by Kobayashi and DeLeo.77 Very recently, a study by Löffler et al.101 provided a definite explanation for these conflicting results. They demonstrated that PVL induces rapid activation and cell death in human and rabbit neutrophils, but not in murine or simian cells. Therefore, they questioned the value of infection models in mice and nonhuman primates to elucidate the impact of PVL and suggested that PVL has an important cytotoxic role in human neutrophils, which has major implications for the pathogenesis of CA-MRSA infections. In further support of this notion, series of studies indicate that the clinical sequelae of pvl-positive S. aureus infections tend to be more severe than infections with pvl-negative S. aureus.54,84,102 Currently, the state of knowledge regarding pathogenesis of CA-MRSA infection is also focusing on additional factors beside PVL, such as the type I arginine catabolic mobile element and α-type phenol-soluble modulins, in the prototypical strains MW2 and USA300.103,104

5. Conclusion

CA-MRSA virulence and pathogenesis seems complex, and there are likely multiple factors involved in transmission
and establishment of CA-MRSA disease. Further understanding will be gained by continued identification of specific pathogenic traits of prevalent CA-MRSA strains, including ST59, to yield new diagnostic tools and therapeutic targets for further drug and vaccine development.

References


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