Community-Associated Methicillin-Resistant *Staphylococcus aureus* and Its Emerging Virulence

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Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections are an emerging problem in the United States and many parts of the world. These infections originate in communities as opposed to hospital-acquired MRSA (HA-MRSA) infections. If adequate measures are not taken to thoroughly understand and control its changing epidemiology and clinical presentation, it may become a significant public health problem in the near future.

Methicillin-resistant *Staphylococcus aureus* (MRSA) by definition harbors a gene, *mecA*, for methicillin resistance. The *mecA* gene codes for penicillin binding protein (PBP) 2a which is different from the indigenous PBPs of *S. aureus*. PBP 2a allows MRSA to continually synthesize its cell wall in the presence of β -lactam antibiotics. Unlike HA-MRSA, CA-MRSA is susceptible to multiple classes of antibiotics, except β -lactams and occasionally to erythromycin. Its rate of prevalence in people without risk factors who live in communities is on the rise. In San Francisco General Hospital and its associated clinics, the percentage of CA-MRSA related cases had increased from 7% in 1993 to 29% in 1999 and continues to increase.¹ Based on a population-based surveillance during 2001 and 2002 from the Atlanta, Baltimore and Minnesota areas, CA-MRSA's rate of prevalence among all MRSA isolates has been reported to be between 8% and 20%.²

What is concerning about this pathogen is that we are beginning to see clinical syndromes caused by CA-MRSA that were typically not associated with staphylococci. One of these syndromes is necrotizing fasciitis, a rapidly progressive, life-threatening infection that involves skin, soft tissue, and deep fascia. Necrotizing fasciitis is a disease that could be caused by more than one pathogen, but is caused mainly by group A hemolytic streptococcus, and species of *Bacteroides*, *Clostridium*, *Peptostreptococcus*, *Klebsiella*, and members of Enterobacteriaceae. Miller et al.³ reported 14 cases of *S. aureus* causing necrotizing fasciitis in a Los Angeles county hospital, 9 of which were due to strains of CA-MRSA. Five isolates that were genotyped showed they belonged to a newer CA-MRSA clone designated as USA300 by the Centers for Disease Control and Prevention.⁴ The USA300 clone had not been seen before the year 2000. Several of these necrotizing fasciitis patients had other

co-existing illnesses or risk factors, such as previous MRSA infection, diabetes, injection drug use, hepatitis C, and human immunodeficiency virus infection.

Kravitz et al.⁵ recently reported five cases (three fatal) of purpura fulminans, a haemorrhagic condition associated with sepsis and extensive tissue necrosis, in the Minneapolis-St. Paul, Minnesota area that were due to toxin producing strains of *S. aureus*. One of these fatal cases was caused by a Pantone-Valentine leukocidin (PVL) and staphylococcal enterotoxin C (SEC) producing CA-MRSA strain of clone USA400. The four remaining cases were caused by other toxin-producing strains of community-associated methicillin-sensitive *S. aureus*, specifically SEB, SEC, PVL, or toxic shock syndrome toxin-1. Despite the numbers being relatively small, these two diseases could be viewed as a sign of CA-MRSA-associated emerging virulence. These cases are also significant in light of what we already know about other recent CA-MRSA outbreaks in communities.

Several outbreaks of CA-MRSA in people who live in communities have been reported during the last 6 to 8 years. Some of these outbreaks were reported in inmates of correctional facilities,⁶⁻⁸ professional football players,⁹ men who have sex with men,⁷ Native American populations,¹⁰⁻¹² and Alaskan natives.¹³ One of these outbreaks occurred in 1992 in a Native American population in Wisconsin.¹¹ In all these outbreak studies, the affected population lacked established risk factors for acquiring MRSA (e.g., frequent hospitalization, surgery, dialysis, living in a long-term care facility during the previous 12 months, or prior MRSA carriage), thus confirming that MRSA, once a scourge of modern hospitals, has successfully evolved and transitioned into a community-associated pathogen.

The ability of CA-MRSA to cause fatal diseases was first appreciated between 1997 and 1999 when a Midwestern hypervirulent clone of CA-MRSA (prototype strain, MW2) caused deaths of four pediatric patients in North Dakota and Minnesota.¹⁴ These patients did not have any risk factors for acquiring MRSA. Since then, many studies including data from the complete sequence of the MW2 genome have been conducted in an attempt to determine the genetic traits that make CA-MRSA virulent pathogens. Even though the genome sequence revealed at least 19 unique toxin and virulence genes in the MW2 strain,¹⁵ so far only PVL genes have been found to be consistently associated with the different clones of CA-MRSA.^{16,17} PVL genes consist of two co-transcribing genes, *lukS-PV* and *lukF-PV*.¹⁸ PVL, a two-component, pore forming cytolytic toxin has a high specificity against human polymorphonuclear cells and macrophages. It is a tissue necrosis factor and its presence in CA-MRSA has been associated with lethal necrotizing pneumonia,¹⁹ cellulitis, abscesses,²⁰ and furuncles.²¹ Besides *lukS-PV* and *lukF-PV*, the strains of CA-MRSA harbor some other staphylococcal enterotoxin genes, such as *seb*, *sec*, *seh*, and *sek*, although at a lesser frequency than PVL genes²²

(Shukla et al., unpublished results). Some of these exotoxins (e.g., SEB and SEC) function like superantigens by generating excessive immunostimulatory responses in hosts. These superantigens act by crosslinking major histocompatibility complex (MHC) class II molecules with T cell receptors.²³ The crosslinking of MHC class II molecules and T cell receptors triggers a massive release of cytokines such as IL2, IFN- γ , TNF- β from T cells, and IL-1 β and TNF- α from macrophages that manifest a toxic shock like syndrome.²³ Indeed, all *S. aureus* strains that caused deaths following purpura fulminans cases produced at least one superantigen or PVL.⁵ Even though not enough studies have been published on the precise role of superantigens in CA-MRSA associated infections, it is believed that, when present, several of these toxins may work in synergy to increase virulence.

A relevant question that remains to be understood is why we are seeing an increase in the number of cases of CA-MRSA in unrelated geographical locations but in somewhat similar populations (e.g., jail inmates in different states, ethnic populations). A simple answer could be that there might have been a bias in the selection of the population surveyed for CA-MRSA related infections. It is also likely that CA-MRSA has a better fitness in these community-like settings. CA-MRSA's genomic background, which is different than the HA-MRSA's, probably provides additional fitness factors. Certainly, multidrug susceptible CA-MRSA could thrive better in community settings where there is less antibiotic pressure than in hospitals. We may be noticing more cases of CA-MRSA in the community due to the availability of two reliable genetic markers: the type IV staphylococcal chromosomal cassette (SCCmec)²⁴ and the PVL genes. The SCCmec is a *mecA* encompassing mobile genetic element that also carries *ccr* genes²⁵ for precise integration into and excision from a *S. aureus* genome. Of the five SCCmec types (SCCmec I to V, size range 21 to 67 kbp) known thus far, type IV is the smallest (21-24 kbp) and, therefore, has the ability to be packaged into a phage and integrate into the *S. aureus* genome via transduction. Therefore, type IV SCCmec could be moving rapidly into the diverse genetic background of the methicillin-sensitive *S. aureus* strains circulating in the community.

Indeed, *S. aureus* acquires virulence genes from the species-specific temperate phages. The *lukS-PV* and *lukF-PV* genes have been identified on the genomes of at least three staphylococcal temperate phages: ϕ PVL, a defective phage; ϕ Sa2mw, a MW2 lysogenized phage; and ϕ SLT (Staphylococcal Leukocytolytic Toxin), a functional phage.^{18,26} However, only prophage ϕ SLT has been able to convert a PVL negative *S. aureus* strain into a PVL producing strain by infection.²⁶ The 42.9 kbp genome of ϕ SLT contains 62 open reading frames including PVL genes, lysis cassette, integrase, and sequences for attachment (*attL*, *attR*),¹⁸ however, the function of many of the genes on ϕ SLT is yet to be known. Since PVL genes are found in approximately 2% of

clinical *S. aureus* isolates, but at very high percentages in CA-MRSA isolates,^{17,22} it is possible that additional genes present on the ϕ SLT or other *lukS-PV* and *lukF-PV* harboring phages may add to the fitness of CA-MRSA in low-risk hosts. Acquisition of several other virulence genes, such as *sea*, *sep*, genes for staphylokinase, lipase, and enterotoxin A and B in *S. aureus*, are also phage mediated.²⁷ It is obvious that lateral transfer of virulence genes continues to play an important role in the evolution of virulence in *S. aureus*.

Why are only a few clones of CA-MRSA successful in spreading throughout the world? We do not know the precise reasons, but certainly some clones of CA-MRSA seem to establish better than others. Using multilocus sequence typing and the SCCmec typing scheme, Enright et al.²⁸ proposed the presence of only five CA-MRSA candidate clones in the world. However, molecular analysis of CA-MRSA strains from a Native American community in Nebraska by Fey et al.²⁹ suggests the inclusion of the midwestern United States CA-MRSA clone as a sixth clone.³⁰ Certainly, multilocus sequence typing allelic profile data from the CA-MRSA strain from Native Americans from Minnesota,¹⁰ North Dakota,²² and our data from Wisconsin^{11,17} strengthen this view.

The clonal spread of strain-like MW2 (USA400) in Native American populations of Minnesota, North Dakota,¹⁰ Wisconsin,¹¹ and Nebraska,¹² and now of the USA300 clonal group in correctional facilities in Georgia, California, Texas,^{6,7} and Mississippi⁸, and in football players⁹ is quite intriguing. Does a particular ecological niche or certain environmental factors favor the spread of one CA-MRSA clone over the other? Are the risk factors for acquiring different clones of CA-MRSA different? At present, it appears that USA300 and USA400 clones have preferences for different ecological niches. The USA300 clone has been reported in football players, and jail-inmates, whereas USA400 has been reported in several ethnic populations. Poor personal and group hygiene practices during and after football games and increased use of antibiotics have been attributed towards the spread of MRSA in football players.⁹ Genome sequence data from a strain of the USA300 clone and its comparison with that of the USA400 prototype strain MW2 genome will help in identifying the genetic differences between the two clones.

Certainly, there is a need for epidemiological studies to determine the risk factors for acquiring CA-MRSA in different community-like settings, including host genetic susceptibility that predisposes people to being colonized by this pathogen. Active surveillance of all community-associated *S. aureus* infections and molecular analysis of virulence genes in CA-MRSA will help us in understanding the trend of these emerging clones. Meanwhile, treatment of all *S. aureus*-related infections originating in communities should be guided by susceptibility testing of these strains.

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