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## Community-Acquired MRSA: A Virulent Pathogen

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### History

*Staphylococcus aureus* is a remarkably versatile pathogen, able to adapt to changing environments while developing increasingly complex resistance and virulence mechanisms. In the pre-antibiotic era even minor infections with *S. aureus* were serious while pneumonia and bacteremia were often fatal. In 1941 penicillin was hailed as the cure-all for gram-positive pathogens. However, within one year *S. aureus* developed penicillinases able to lyse the antibiotic before it could attach to binding proteins in the cell membrane. Similarly, it was only one year after the introduction of methicillin into clinical practice in 1960 that resistant *Staphylococcus aureus* strains emerged. However, by that time Eli Lilly had developed vancomycin from soil brought back from the jungles of Borneo. Effective but toxic, it was fortunate that this "Mississippi mud" was little needed for several decades. Indeed, it was not until the mid 1980's that methicillin resistant *S. aureus* (MRSA) infection rates started to rise in the U.S. Fostered by growing antibiotic use and increasingly invasive diagnostic and therapeutic procedures, MRSA invaded surgical wounds, ventilated lungs, and punctured blood vessels, gradually replacing both methicillin-susceptible *Staphylococcus aureus* (MSSA) and gram negative bacilli as the predominate nosocomial pathogen in the United States. Though slightly ponderous as a result of its large, resistance-focused genetic burden MRSA has been difficult to control both as a commensal and as an invader.

As MRSA infection rates grew, so did the use of vancomycin. Unfortunately suboptimal response rates to vancomycin hampered clinicians. A slowly bactericidal antibiotic, vancomycin has been shown to be inferior to that of beta-lactams in the treatment of MSSA bacteremia<sup>1</sup>. In addition, rapidly increasing use of vancomycin over the past 15 years has resulted in rising rates of MRSA resistance (vancomycin intermediate *Staphylococcus aureus* (VISA), heteroresistant VISA and vancomycin resistant *Staphylococcus aureus* (VRSA)) and also vancomycin tolerance. And perhaps even more important, recently rising MRSA MICs to vancomycin appear to result in lower response rates to serious infections<sup>2</sup>. Finally,

just as clinicians have started to feel comfortable using new antibiotics such as linezolid and daptomycin reports of non-susceptible strains have appeared in the literature.

In the 1950's the first cases of necrotizing skin infections, pneumonia and sepsis due to *S. aureus* infections in children and young adults were reported, possibly as a result of the incorporation of Pantone-Valentine leukocidin (PVL) into spa type (ST) 30 MSSA to create the invasive phage type 80/81<sup>3</sup>. Fortunately this organism gradually disappeared in the 1960's.

However, in the 1990's the first cases of community diagnosed MRSA were reported. Many of these infections involved intravenous drug users, long-term care facility residents, and patients with frequent healthcare contact and were caused by hospital strains of MRSA. However, a gradually increasing group involved a new organism: community-acquired MRSA. These infections occurred among healthy persons without risk factors for MRSA acquisition and were isolated primarily from cutaneous infections. Initially children were involved (e.g. those at daycare centers) though outbreaks soon developed in prisoners, soldiers, athletes, intravenous drug users, tattoo parlors, and Native American communities. The emerging potential danger entailed with community-associated MRSA (CA-MRSA) was illustrated by the deaths of four healthy children in Minnesota and North Dakota between 1997 and 1999 due to MW2<sup>4</sup>. Accompanied by reports first from France<sup>5</sup> and more recently from the U.S.<sup>6</sup> describing CA-MRSA pneumonia following an influenza-like illness and resulting in a significant mortality, these cases have emphasized the importance of understanding this new virulent pathogen.

### Traditional Virulence Factors

*S. aureus* is an organism with many weapons contributing to disease pathogenesis. *S. aureus* is also one of the most aggressive organisms infecting man. This combination makes *S. aureus* extremely dangerous to both the healthy and ill alike. To emphasize this we prospectively evaluated 724



oral/parenteral antibiotics. This constant bombardment has resulted in a genetic adaptation focused on the development of numerous resistant mechanisms to enhance survival. As a result this organism has acquired and incorporated a significant amount of genetic material, slowing replication and perhaps limiting additional virulence factors.

On the other hand, CA-MRSA, an “outpatient” organism, has been focused primarily on competition with other strains for colonization. Growth rate and virulence are important and resistance less so. As a result, this organism has acquired small but numerous genetic islands containing toxins but few resistance genes.<sup>15</sup>

### What We Know About the Pathogenesis of These CA-MRSA Infections: Not Much

#### Is CA-MRSA is really more virulent than traditional MRSA?

The mouse model appears to show that it is. Both the MW2 (USA400) and LAC (USA300) result in a significantly higher mortality in infected mice than MRSA252 (USA200).<sup>16</sup>

#### Where did this organism come from?

One important method of transformation of *S. aureus* is the horizontal acquisition of genetic material through mobile genetic elements (pathogenicity islands, phages, etc.) containing virulence genes in variable combinations. This transfer has the potential to create particularly virulent strains. For example, the MW2 strain of CA-MRSA associated with fulminant sepsis in four children in North Dakota appears to have acquired SCCmec IV, the *S. aureus* pathogenicity island SaPI3, and the bacteriophage Sa2 in its evolution from MSSA476. MW2 contained 19 putative virulence genes not found in 5 simultaneously examined HA-MRSA strains including several superantigens such as enterotoxin B, C, and H as well as PVL. Interestingly MW2 (USA 400) is no longer the predominant clonal complex in the U.S., having been replaced by clonal complex 8 (ST 8:USA 300).<sup>17, 18</sup>

The recent evolution of *S. aureus* appears to have started with an ST8 MSSA which then acquired the SCCmec IV gene complex to become USA500, an endemic CA-MRSA. Subsequently the acquisition of the *lukS*-PV and *lukF*-PV genes along with the genes encoding *sek* and *seq* led to the creation of ST8 MRSA IV; epidemic CA-MRSA (USA300).<sup>19</sup>

Table 2. Five Types of the SCCmec Gene Complex

SCCmec Type	Size (kb)	Antibiotic resistance	Staphylococcal cassette chromosome (SCC) class
I	94	Lacks resistance genes other than <i>bla</i> <sub>TEM</sub>	III
II	83	Associated with methicillin resistance	II
III	87	Associated with methicillin resistance	II
IV	21-24	Resistance to $\beta$ -lactams, quinolones and macrolides	II
V	28	Lacks antibiotic resistance genes other than <i>bla</i> <sub>TEM</sub>	Not known

Adapted from reference 15.

Methicillin resistance results from the production of an altered low-affinity penicillin-binding protein

(PBP2a) encoded by the *mecA* gene. This gene resides within a mobile genetic island, the staphylococcal cassette chromosome (SCCmec), which may also contain genetic elements that confer resistance to other antibiotics. There are at least 5 SCCmec types (Table 2).<sup>15</sup> HA-MRSA strains are typically associated with types II-III which are large as a result of a significant genetic burden of resistance genes. CA-MRSA is almost exclusively associated with SCCmec IV, a small, mobile, easily transferable chromosomal cassette with few resistance genes, thus allowing this organism to grow as quickly as MSSA (doubling time 29 minutes versus 39 minutes for HA-MRSA). SCCmec IV appears to have been originally transferred from coagulase negative staphylococci to MSSA. This evolutionary route is supported by the analysis of Dr. Gordon Archer’s unique collection of endocarditis-associated CNS from 1973-83 in which 36% of the CNS contained the SCCmec IV gene complex.<sup>20</sup>

In addition, SCCmec IV is often associated with (but not necessarily co-transmitted with) genes encoding PVL, staphylococcal enterotoxins, especially A, B, C, H, K, Q and other virulence factors/pathogenicity islands. Indeed, CA-MRSA is one of the most promiscuous bacteria infecting man.<sup>18, 21</sup>

#### Panton-Valentine Leukocidin (PVL)

Described in 1932, PVL is a member of leukocidin group of bacterial membrane toxins known as synergohymenotrophic toxins. PVL is encoded by the *lukF*-PV and the *lukS*-PV genes and is able to kill leukocytes by pore formation in the cell membrane with cell death. Found primarily in CA-MRSA, it has now moved into the MSSA population. PVL is closely linked epidemiologically to serious skin infections and necrotizing pneumonia, though the pathological responses in these tissues is remarkably different.<sup>22-24</sup> PVL induces inflammation in the rabbit model of cutaneous infections with an aggressive neutrophilic response. Similarly, PVL-positive strains strongly adhere to the basement membrane of pulmonary alveoli due to stronger affinity for type I and IV collagens and laminin<sup>25</sup> and induces apoptosis by disrupting mitochondrial stability. Pathologic examination of respiratory tissues infected with PVL producing SCCmec IV *S. aureus* shows small clusters of gram-positive cocci on the surface of the laryngeal mucosa with a zone of mucosal necrosis and hemorrhage. The tracheal mucosa, coated by clusters of cocci, is also extensively necrotic and hemorrhagic. And the lung parenchyma is massively hemorrhagic with alveoli filled with erythrocytes and clusters of cocci. The absence of a neutrophilic response is remarkable. But most importantly patients with PVL-positive *S. aureus* pneumonia<sup>5</sup> had a 32% mortality at 48 hours compared to 6% in patients infected with a PVL-negative strain.

On the other hand, PVL gene expression during *in vivo* *S. aureus* infection has not been clearly demonstrated. It appears that PVL is differentially expressed and does not correlate with PMN lysis *in vitro*. As a result there is still much debate whether PVL itself is a human toxin or whether it is simply a marker of more virulent strains of MRSA.<sup>26, 27</sup>

## Global/Accessory Gene Regulator (*agr*): Virulence and Resistance Factor

Virulence genes are frequently under regulatory control and are often expressed in a coordinated manner. Many critical virulence pathways, including those involved with exotoxin, exoprotein, and adhesin expression are coordinately controlled by *agr*, a key global regulon. In general, *agr* upregulates production of secreted virulence factors such as hemolysins and proteases and downregulates virulence factors expressed on the staphylococcal surface. There are 4 types of *agr* and *S. aureus* subtypes tending to be grouped in the following categories: II – VISA; I/III -CAMRSA; IV - Exfoliatin-producing strains.

Importantly, resistance also appears to be affected by *agr* function. For example, prolonged exposure to vancomycin may lead to tolerance due to *agr* dysfunction. Similarly, persistent MRSA bacteremia is associated with *agr* dysfunction. Moise-Broder *et al*<sup>2</sup> demonstrated this when they showed that 31/36 patients with the *agr* group II polymorphism failed vancomycin therapy. Interestingly, suppression of *agr* function appears to end after vancomycin is stopped. Finally, when nosocomial MRSA relinquishes *agr* function there is a loss of exotoxin release; however, these organisms produce more biofilm and tend to survive the assault by vancomycin more frequently.<sup>2,28-30</sup>

## Other

Other factors that enhance the virulence of CA-MRSA include an intrinsic resistance to killing by neutrophils as well as upregulation of virulence factors after phagocytosis. Indeed, phagocytosis often results in neutrophil lysis.<sup>16, 17</sup>

Arginine catabolic mobile element (ACME) is a mobile genetic element that appears to contribute to growth/survival of CA-MRSA by helping the organism evade the host defensive response while also helping the organism colonize various tissue surfaces. ACME is found primarily in USA 300 and *S. epidermidis*. A similar element has been found to be a virulence factor in *S. pyogenes* where it inhibits mononuclear cell proliferation and nitric oxide production.<sup>17</sup>

The distribution of other virulence genes differs by continent of origin. For example, it was found that almost all European and U.S. strains carried the *lukE-lukD* gene encoding for a non PVL leukocidin as well as the gamma-haemolysin (*blg*) gene. The *blg* gene was found in strains from Oceania. And the superantigens A, B, C, H, K have been variably detected in U.S. and European strains.<sup>31</sup>

## Horizontal Transfer of Virulence Genes is Central

The fundamental question remains: Why do some patients have more severe illness than others? Are specific *S. aureus* clones associated with more severe infection? Though there has been conflicting findings it does appear that specific clones are more frequently associated with serious infection than others.<sup>32, 33</sup> For example, a recent Intensive Care Unit – based cohort investigation from the United Kingdom found that MRSA Sequence Type 239 was significantly more likely to cause bacteremia than other strain types.<sup>34</sup> Thus, it appears that “all *S. aureus* are created

equal, but some more equal than others.” (Fowler V, personal communication) In other words, any *S. aureus* genotype carried by humans can potentially be transformed into a life-threatening human pathogen. However, strains from some clonal lineages appear to make this transformation more frequently or more “completely” than others and thus are labeled more virulent, and clonal complexes 8 (ST8:USA300) and 30 (ST30:USA1100) appear to provide the “best” genetic backbone for acquisition of new virulence factors<sup>19</sup> such as the genes for PVL and the enterotoxin Q and K genes.

## Conclusions

All common strains of *S. aureus* are able to cause all forms of disease. Importantly, however, infections with selected strains appear to result in more severe disease than others. The reasons for this are twofold: fundamental clonal differences and the acquisition of new combinations of virulence/survival genes through horizontal transfer of genetic material. The genetic causes of these strain differences are multiple and variable and as of yet only partially defined.

Community acquired MRSA is an aggressive, new, acquisitive pathogen that continues to evolve. Factors such as ACME, *agr*, enterotoxin/superantigen acquisition are but a part of the virulence profile. With its ability to acquire and combine multiple genetic complexes, future *S. aureus* infections will present the clinician with many difficult challenges. Specifically, I believe we will see:

- Sporadic outbreaks with particularly virulent organisms such as MW2
- An increase in antibiotic resistance as the organism moves into the healthcare setting
- Increasing necrotizing pneumonia associated with the looming influenza pandemic infection
- Increasing CA-MRSA bloodstream infections as a result of CA-MRSA colonization of our hospitals.

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## REFERENCES:

1. Chang F, Peacock JJ, Musher D, *et al.* Staphylococcus aureus bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. *Medicine* 2003; 82:333-9.
2. Moise-Broder PA, Sakoulas G, Eliopoulos GM, Schentag JJ, Forrest A, Moellering RC, Jr. Accessory gene regulator group II polymorphism in methicillin-resistant Staphylococcus aureus is predictive of failure of vancomycin therapy. *Clin.Infect.Dis.* 2004;38:1700-05.
3. Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, *et al.* Re-emergence of early pandemic Staphylococcus aureus as a community-acquired methicillin-resistant clone. *Lancet* 2005;365:1256-58.
4. Centers for Disease Control and Prevention. Four pediatric deaths from community-associated methicillin-resistant Staphylococcus aureus--Minnesota and North Dakota, 1997-1999. *MMWR* 1999;48:707-10.
5. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, *et al.* Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002;359:753-59.
6. Hageman JC, Uyeki TM, Francis JS, Jernigan DB, Wheeler JG, Bridges CB, *et al.* Severe community-acquired pneumonia due to Staphylococcus aureus, 2003-04 influenza season. *Emerg.Infect.Dis.* 2006;12:894-99.
7. Fowler VG, Jr., Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, *et al.* Clinical identifiers of complicated Staphylococcus aureus bacteremia. *Arch.Intern.Med.* 2003;163:2066-72.
8. Lowy FD. Staphylococcus aureus infections. *N.Engl.J.Med.* 1998;339:520-32.
9. Que YA, François P, Haefliger JA, Entenza JM, Vaudaux P, Moreillon P. Reassessing the role of Staphylococcus aureus clumping factor and fibronectin-binding protein by expression in Lactococcus lactis. *Infect Immun.* 2001;69:6296-302.
10. Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. Staphylococcus aureus sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc.Natl.Acad.Sci.U.S.A* 2000;97:5510-15.
11. Kravitz GR, Dries DJ, Peterson ML, Schlievert PM. Purpura fulminans due to Staphylococcus aureus. *Clin.Infect.Dis.* 2005;40:941-47.
12. Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindberg FP. Survival of Staphylococcus aureus inside neutrophils contributes to infection. *J.Immunol.* 2000;164:3713-22.
13. Postma B, Poppelier MJ, van Galen JC, Prossnitz ER, van Strijp JA, de Haas CJ, *et al.* Chemotaxis inhibitory protein of Staphylococcus aureus binds specifically to the C5a and formylated peptide receptor. *J.Immunol.* 2004;172:6994-7001.
14. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, *et al.* Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. *J.Exp.Med.* 2004;199:687-95.
15. Kluytmans-Vandenbergh MF, Kluytmans JA. Community-acquired methicillin-resistant Staphylococcus aureus: current perspectives. *Clin.Microbiol.Infect.* 2006;12 Suppl 1:9-15.
16. Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, *et al.* Insights into mechanisms used by Staphylococcus aureus to avoid destruction by human neutrophils. *J.Immunol.* 2005;175:3907-19.
17. Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, *et al.* Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant Staphylococcus aureus. *Lancet* 2006;367:731-39.

18. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, *et al.* Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* 2002;359:1819-27.
19. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J.Infect.Dis.* 2006;193:1495-503.
20. Wisplinghoff H, Rosato AE, Enright MC, Noto M, Craig W, Archer GL. Related clones containing SCCmec type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob.Agents Chemother.* 2003;47:3574-79.
21. Mongkolrattanothai K, Boyle S, Kahana MD, Daum RS. Severe *Staphylococcus aureus* infections caused by clonally related community-acquired methicillin-susceptible and methicillin-resistant isolates. *Clin.Infect.Dis.* 2003;37:1050-58.
22. Chambers CD, Hernandez-Diaz S, Van Marter LJ, Werler MM, Louik C, Jones KL, *et al.* Selective serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. *N.Engl.J.Med.* 2006;354:579-87.
23. Konig B, Prevost G, Piemont Y, Konig W. Effects of *Staphylococcus aureus* leukocidins on inflammatory mediator release from human granulocytes. *J.Infect.Dis.* 1995;171:607-13.
24. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, *et al.* Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003;290:2976-84.
25. de Bentzmann S, Tristan A, Etienne J, Brousse N, Vandenesch F, Lina G. *Staphylococcus aureus* isolates associated with necrotizing pneumonia bind to basement membrane type I and IV collagens and laminin. *J.Infect.Dis.* 2004;190:1506-15.
26. Genestier AL, Michallet MC, Prévost G, Bellot G, Chalabreysse L, Peyrol S, *et al.* *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J. Clin. Invest.* 2005;115:3117-27.
27. Said-Salim B, Mathema B, Braughton K, Davis S, Sinsimer D, Eisner W, *et al.* Differential distribution and expression of Panton-Valentine leucocidin among community-acquired methicillin-resistant *Staphylococcus aureus* strains. *J. Clin. Microbiol.* 2005;43:3373-9.
28. Sakoulas G, Moellering RC, Jr., Eliopoulos GM. Adaptation of methicillin-resistant *Staphylococcus aureus* in the face of vancomycin therapy. *Clin.Infect.Dis.* 2006;42 Suppl 1:S40-S50.
29. Sakoulas G, Eliopoulos GM, Moellering RC, Jr., Novick RP, Venkataraman L, Wennersten C, *et al.* *Staphylococcus aureus* accessory gene regulator (*agr*) group II: is there a relationship to the development of intermediate-level glycopeptide resistance? *J.Infect.Dis.* 2003;187:929-38.
30. Sakoulas G, Eliopoulos GM, Moellering RC, Jr., Wennersten C, Venkataraman L, Novick RP, *et al.* Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. *Antimicrob. Agents Chemother.* 2002;46:1492-502.
31. Maltezou HC, Giamarellou H. Community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Int.J.Antimicrob.Agents* 2006;27:87-96.
32. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, *et al.* How clonal is *Staphylococcus aureus*? *J.Bacteriol.* 2003;185:3307-16.
33. Melles DC, Gorkink RF, Boelens HA, Snijders SV, Peeters JK, Moorhouse MJ, *et al.* Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. *J.Clin.Invest* 2004;114:1732-40.
34. Edgeworth, JD, Yadegarfar G, Pathak S, Batra R, Cockfield JD, Wyncoll D, Beale R, Lindsay JA. An outbreak in an intensive care unit of a strain of methicillin-resistant *Staphylococcus aureus* sequence type 239 associated with an increased rate of vascular access device-related bacteremia. *Clin Infect Dis.* 2007;44:493-501.

A minimum assessment score of 80% is required.

1) The mortality of *S. aureus* bacteremia is:

- a. 8%
- b. 14%
- c. 19%
- d. 24%

Answer: \_\_\_\_\_

2) The attachment protein expressed during exponential growth phase of *S. aureus* is called:

- a. TSST
- b. SSC*mec*
- c. MSCRAMMs
- d. PVL

Answer: \_\_\_\_\_

3) Which of the following are virulence factors:

- a. CHiPs
- b. ACME
- c. Protein A
- d. ClfA
- e. all of the above
- f. a, b and c

Answer: \_\_\_\_\_

4) All of the following statements about the regulon *agr* are true except:

- a. Persistent MRSA bacteremia is associated with *agr* dysfunction.
- b. *Agr* upregulates production of secreted virulence factors such as hemolysins and proteases.
- c. *Agr* upregulates production of virulence factors expressed on the staphylococcal surface.
- d. prolonged exposure to vancomycin may lead to *agr* dysfunction and subsequent vancomycin tolerance.

Answer: \_\_\_\_\_

5) CA-MRSA is:

- a. More resistant than HA-MRSA.
- b. Often found to contain PVL.
- c. Less virulent than other staphylococci in pts with post-influenza pneumonia.
- d. None of these.

Answer: \_\_\_\_\_

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