Understanding the Molecular Pathogenesis of Methicillin-Resistant Staphylococcus aureus

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(See the major article by Calderwood et al on pages 571–7.)

Keywords. MRSA; genotyping; pathogenesis; virulence factors; Staphylococcus aureus.

Staphylococcus aureus has been referred to as the “persistent pathogen” [1, 2] owing to its high prevalence for decades, its cause of a significant burden of disease, and its ability to cause a wide spectrum of clinical infections at various sites, including skin and soft tissue, bone and joint, heart valves, bloodstream, and cerebrospinal fluid. These organisms possess numerous toxins and virulence factors that may account for their virulence and ability to evade host defenses [3, 4]. The acquisition by S. aureus of the staphylococcal cassette chromosome containing the mecA gene (SCCmecA), which encodes for methicillin resistance, has added to its armamentarium and increased the difficulty in its eradication in human infections [5,6]. The epidemiology of methicillin-resistant S. aureus (MRSA) is closely intertwined with that of methicillin-susceptible S. aureus (MSSA), and has become more complex with the recognition of healthcare-associated, healthcare-associated community-onset, and community-associated (CA) MRSA strains [4, 7, 8].

With the advent of numerous molecular typing methods has come an enhanced understanding of the complex evolution and epidemiology of MRSA [5, 9, 10]. There is a predominance of multilocus sequence type ST5 or clonal complex (CC) 5, with pulsed-field gel electrophoresis (PFGE) type USA100 as the most common healthcare-associated strains and ST8 or CC8 PFGE USA300 as the most common among community-associated strains [8, 9]. Many of the MRSA strains are thought to have originated from predominant MSSA strains that acquired SCCmecA [6, 11]. The genotype classifications, while critical in elucidating the evolution, transmission, and epidemiology of both MSSA and MRSA infections in populations at risk, have only recently been recognized as potential predictors of specific clinical infections.

As examples, in a large international data set of MSSA isolates, isolates causing infective endocarditis were more likely to be CC33 and to contain various adhesins and enterotoxins [12]. Furthermore, an analysis of both MSSA and MRSA isolates from one US academic medical center demonstrated that CC5 and CC30 strains were responsible for hematogenous complications of infection [13]. A predominant Asian strain of MRSA, ST239, was recently responsible for a large intensive care unit outbreak in London, and was highly associated with vascular access device-related bacteremia [14]. A related predominant Brazilian clonal complex of MRSA has been demonstrated to have enhanced ability to produce biofilm and adhere to and invade airway epithelial cells, properties that may promote its nosocomial transmission and ability to cause pneumonia [15]. In a study of patients with S. aureus bacteremia, CC15 was associated with 30-day mortality, and CC22 with osteo-articular infections [16]. These and other findings suggest that clonal typing may be a potential predictor of specific clinical infections. Additional research is needed to analyze the specific features and characteristics of individual clonal types that could be responsible for their predilection to cause or associate with specific types of clinical infections. One very important area of future research is linking the profiles of S. aureus toxins and virulence factors to specific genotypes.

Because of their physiologic functions, specific toxins and virulence factors are probably the key to and the best predictors of important infection [3]. One of the most commonly cited factors in MRSA infections has been the Panton-Valentine leukocidin (PVL), a toxin [4, 17] that is found in many strains of CA-MRSA and is cytotoxic to human and rabbit monocytes and macrophages and to human polymorphonuclear leukocytes.
It has been proposed that PVL is one element responsible for the propensity of CA-MRSA to cause significant skin and soft tissue and other necrotizing infections. This hypothesis remains controversial, and there are other studies to suggest that PVL may not be the primary determinant of skin and soft tissue infections [18]. Regardless, the occurrence of PVL in CA-MRSA has underscored the importance of investigating the roles of all toxins and virulence factors, as linked to clonal typing, in the molecular pathogenesis of S. aureus.

Furthermore, superantigens have also been implicated as critical elements in S. aureus sepsis, infective endocarditis, and acute kidney injury [19]. Among staphylococcal toxins and superantigens, the staphylococcal enterotoxin family has been implicated because enterotoxins bind to major histocompatibility complex class II molecules on antigen-presenting cells and specific Vβ regions of T-cell receptors, resulting in stimulation of the immune system [19].

In this issue of The Journal, Calderwood and colleagues [20] describe the role of one staphylococcal enterotoxin, enterotoxin P, in predicting bloodstream infections in patients colonized with MRSA. These authors performed a nested case-control study in the setting of ongoing active surveillance for MRSA at their hospital. Case patients were patients colonized with MRSA in whom a bloodstream infection developed after 2 days of hospitalization. Controls were patients with culture-confirmed MRSA colonization in whom no bloodstream infection or other evidence of invasive MRSA infection appeared.

Whole-genome sequencing was performed on clinical isolates from both case patients and controls. Several genes encoding for virulence factors that mediate host invasion and evade the immune system were included in the analysis, including PVL, other leukocidins, staphylococcal enterotoxins A–U, staphylococcal chemotaxis inhibitor protein, staphylococcal complement inhibitor, staphylokinase, toxic shock syndrome toxin, SCCmecA type, accessory gene locus group, and single-nucleotide gene polymorphisms for α-hemolysin and phenol-soluble modulin. Using univariate and multivariable Cox proportional hazard regression models, the authors determined predictors of MRSA bloodstream infections.

There were 1578 banked MRSA isolates from 492 of 8203 intensive care unit patients included in the study, with 52 patients in whom healthcare-associated MRSA blood stream infections occurred after or concurrent with MRSA colonization. Of these, 19 patients were excluded because their infection was due to secondary bacteremia. The 33 case patients with primary bacteremia and 121 controls were analyzed for host and pathogen factors. Genome sequencing was completed in both groups.

In the univariate analysis, clinical predictors of infection were cancer comorbidity, presence of a central venous catheter, and glucose level >200 mg/dL. Among all of the potential genes associated with MRSA bacteremia, only the presence of enterotoxin P was a significant predictor. In the multivariant model, the presence of enterotoxin P was the most significant predictor of MRSA bacteremia (hazard ratio, 26.74; 4.74–150.79; P < .01), with the presence of cancer comorbidity and central venous catheter also significant predictors. Although not statistically significant, the use of an anti-MRSA antimicrobial was suggested to be protective. The CC5 strain was responsible for most (88%) of the bacteremia cases and was predominant in the colonized patients (89%).

As outlined by Calderwood et al [20], enterotoxin P is part of an innate immune evasion cluster encoding for proteins that promote evasion of the host immune system through inhibition of neutrophil chemotaxis, binding, and phagocytosis [21, 22]. Enterotoxin P expresses other mechanisms of immune disruption. An earlier study [23] noted that infections in patients with S. aureus with enterotoxin P were associated with higher mortality rates. As further discussed Calderwood et al it is important to note that the presence of enterotoxin P gene may not correlate with expression of the gene and the actual presence of the enterotoxin. Studies that correlate the phenotypic expression with infection will be crucial in determining the actual role of enterotoxin P, which may also function as a superantigen, as has been the case for other enterotoxins such as staphylococcal enterotoxin C [19].

This findings reported by Calderwood et [20] underscore the value of collecting and banking isolates for future molecular testing and microbial analysis. The importance of banked isolates was clearly demonstrated in the recent description of the NAP1 BI epidemic strain of Clostridium difficile [24], where it was recognized that historic NAP1 BI isolates, which had existed for years but had not been linked to outbreaks, were fully susceptible to fluoroquinolones. The strains associated with significant outbreaks, however, had acquired resistance to these antimicrobial agents. In the setting of markedly increased use of fluoroquinolones, these resistant strains had a selective advantage with the development of subsequent outbreaks. This fact, which is key in understanding the development and epidemiology of these C. difficile outbreaks, would have remained unrecognized without historically banked isolates. In another study evaluating a banked set of MRSA isolates, a previously unrecognized cluster of MRSA ST239-III cases in Ohio was detected [25]. Given the important antimicrobial resistance, outbreak potential, and virulence associated with this strain, its identification emphasizes the need for such molecular surveillance. The continued collection and storing of isolates is critical to understand the molecular pathogenesis of microbial infections.

The study by Calderwood et al [20] also illustrates the value of linking clinical and epidemiologic data with characterization of microbial characteristics or, as coined by the investigators, looking at “combined host and pathogen factors.”
References