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Understanding the epidemic of community-associated MRSA and finding a cure: are we asking the right questions?

Michael Otto
National Institute of Allergy and Infectious Diseases, The National Institutes of Health, Bethesda, MD

Staphylococcus aureus remains a dangerous pathogen. It is notorious as the prime cause of hospital-associated (nosocomial) infections. In addition, many *S. aureus* strains are resistant to penicillin, penicillinase-resistant beta-lactams, and many other antibiotics. Methicillin-resistant *S. aureus*, or MRSA, represent an especially big threat to the public health system. Interestingly, MRSA strains were found only about 1 year after introduction of the antibiotic into the market [1]. Worldwide epidemics of MRSA developed in the following decades, soon rendering methicillin ineffective for most nosocomial *S. aureus* infections [2]. Similar scenarios are being observed or to be expected for more recently developed antibiotics, if they are widely used, underlining the urgent need for more intense efforts in antibacterial drug development.

Traditionally, MRSA infections have been limited to hospitals and predisposed individuals such as the elderly, immune-compromised, and patients undergoing surgery. More recently however, MRSA infections occurred outside of hospital settings [3]. These community-associated (CA-) MRSA were first reported in the Northern U.S., where they caused fatal infections in otherwise healthy children [4]. Soon after, CA-MRSA outbreaks occurred in sports teams, prison inmates, and other groups with often close physical contact and low hygiene. In the meantime, CA-MRSA is a global problem with the most serious epidemic seen in the U.S. [5].

While a total of 5 clonally unrelated strains are responsible for worldwide outbreaks of CA-MRSA [5], the epidemic in the U.S. is almost entirely caused by a clonal group of strains belonging to sequence type USA300 [6], which has undergone rapid diversification [7]. USA300 has almost completely replaced the clone that caused the initial fatal outbreak in children in the Northern U.S. and is now the most frequent cause of all infections reporting to emergency departments in the U.S. [6]. According to recent estimates, MRSA infections cause more deaths annually in the U.S. than HIV/AIDS [8], with CA-MRSA infections and particularly USA300 responsible for a recent surge in those numbers. USA300 has appeared in other countries and continents and appears to be spreading there [5,9], although it is not yet clear if it will cause a similar scenario in those places. Notably, while most infections with USA300 present as mild to moderately severe skin and soft tissue infections, this strain appears to cause severe and even fatal infections, such as necrotizing fasciitis or pneumonia, more frequently than other MRSA [10,11].

The CA-MRSA epidemic poses two main questions for biomedical research. First, we would like to understand which molecular determinants are responsible for the fact that these strains are more successful in causing disease and spreading sustainably in the population, compared to traditional, hospital-associated (HA-) MRSA. Second, we need to understand which determinants are the main contributors to the exceptional virulence potential seen in CA-MRSA, in order to select the best molecular targets for modern target-oriented drug development aimed at controlling virulence [12]. Unfortunately, these two separated questions

have not always been strictly distinguished, although the underlying molecular determinants are likely to be different. For example, a specific virulence determinant may not contribute to a great extent to the overall virulence potential of CA-MRSA strains, but may represent the molecular cause for the difference that renders CA-MRSA more effective in causing disease in otherwise healthy individuals. Identification of such a factor is important to understand CA-MRSA pathogenesis, but it may not necessarily represent a good drug target. Furthermore, some determinants may contribute to CA-MRSA success by increasing asymptomatic colonization and transmissibility of CA-MRSA, while not affecting the virulence potential. They might be interesting targets for decolonization approaches, but not therapeutic strategies aimed at virulence expression. Notably, factors involved in CA-MRSA transmissibility remain virtually unexplored.

With regard to the first question, the comparison of CA-MRSA with other *S. aureus* and MRSA genomes has been helpful in delineating genes with known functions, such as toxins, that were only present in CA-MRSA, but not other strains, and thus represent good candidates for determinants of the exceptional success of CA-MRSA. First and foremost, this led to the hypothesis that the Panton-Valentine leukocidin (PVL), whose genes are present in almost all CA-MRSA strains, but largely absent from HA-MRSA, was largely responsible for the pathogenic success of CA-MRSA [13]. This hypothesis was attractive because the presence of PVL, which lyses white blood cells, could very well have explained increased virulence. However, results from multiple animal infection studies using isogenic deletions of the PVL-encoding genes in CA-MRSA strains revealed that PVL has no or only a transient role in CA-MRSA pathogenesis [14–17]. Moreover, presence of PVL genes in CA-MRSA did not impact the capacity to lyse human neutrophils as the most important white blood cells eliminating invading *S. aureus* [15]. Thus, it is quite clear that the contribution of PVL to CA-MRSA virulence is much smaller than hypothesized in the beginning. The striking frequency of PVL-encoding genes in CA-MRSA strains still indicates it may have an important role in defining the exceptional CA-MRSA pathogenic potential compared with other MRSA. However, this role has yet to be revealed experimentally.

In addition, analysis of the USA300 genome revealed the presence of a mobile genetic element called ACME that is uniquely present in that *S. aureus* strain, and likely has been acquired from the coagulase-negative relative *S. epidermidis* [18]. Owing to the clonality of USA300, presence of ACME in USA300isolates does not directly indicate that genes present on ACME are involved in the pathogenesis of USA300. It was shown experimentally that an ACME-negative strain exhibited lower persistence in a rabbit infection model, but only when injected with the isogenic wild-type in a competitive fashion, indicating a significant, yet small role of ACME in pathogenesis [19]. However, elements present on ACME are being discussed to impact colonization rather than virulence [5,18] and we will need to await the urgently needed integration of colonization models into CA-MRSA research to see if ACME is involved in those processes. Nevertheless, ACME is absent from CA-MRSA other than USA300 and could thus only serve to explain the exceptional success of USA300 compared to other CA-MRSA.

Thus, mere comparisons of gene content have not been able to identify determinants with a major influence on the pathogenic success of CA-MRSA. Therefore, it appears that we need to consider differences in gene expression to a much higher extent than before to explain the exceptional features of CA-MRSA. This is supported by recent investigation and also leads us to the second question: which are the main factors determining CA-MRSA virulence?

So far, two determinants showed a dramatic impact on CA-MRSA pathogenesis in infection models: alpha-toxin and the alpha-type of phenol-soluble modulins (PSMs) [16,20]. Both are leukolytic toxins with additional pro-inflammatory effects. While alpha-toxin is very weak in lysing specifically neutrophils and may exert the pronounced effect that was found in

experimental pneumonia rather by its pro-inflammatory properties, deletion of the *psm*-alpha genes led to a very substantial reduction in neutrophil lysis in vitro and in vivo, and to a severely reduced formation of abscesses in a skin infection model. Genes for both toxins are found in virtually all *S. aureus*, which indicates, first, that these two toxins form excellent targets for target-oriented drug development not only for CA-MRSA but all *S. aureus*, and second, differential gene expression needs to be considered as a key factor contributing to the increased success of CA-MRSA. Indeed, higher expression of PSMs in CA-MRSA compared to HA-MRSA has been shown [20].

In conclusion, to understand the CA-MRSA epidemic, we will need to consider differences in gene expression in addition to continuing the analysis of specific genes uniquely present in CA-MRSA. Additionally, there will have to be a much bigger effort to investigate increased colonization capacity and transmissibility as likely reasons for CA-MRSA success. On the other hand, we will need to perform comparative analyses exploring the relative contribution of virulence determinants to pathogenesis in order to select the best targets for attacking CA-MRSA virulence. Notably, these experiments will need to be performed in CA-MRSA strains, rather than laboratory strains, to allow valid conclusions.

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