What is behind the epidemiological difference between community-acquired and health-care associated methicillin-resistant Staphylococcus aureus?

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One of most striking features of methicillin-resistant Staphylococcus aureus (MRSA) is the strong clonal structure of the bacterial population. MRSA isolates were first reported among hospitalized patients at the beginning of the 1960s, and remain major agents of hospital infections worldwide. Typical lineages (clones) of health care-associated MRSA (HA-MRSA) are ST5 (CC5), ST239 (CC8), ST22 (CC22), and ST36 (CC30). However, these rarely cause infections in healthy individuals who are not hospitalized or attended a healthcare service in the 6–12 months before infection. A biologic consequence of acquiring antimicrobial resistance is the reduction of competitive fitness. This has generally been associated with HA-MRSA failure to efficiently spread in the community.

At the end of the 1980s, MRSA isolates were reported to cause typical community-acquired (CA) infections in Oceania; this CA-MRSA clone (ST1-SCCmecIV) was named WA-1, for Western Australia-1. After that, CA-MRSA infections were reported on all continents. Generally, typical CA-MRSA strains display important differences from HA-MRSA strains including the production of Panton-Valentine leucocidin (PVL), low-level susceptibility to non-β-lactam antibiotics, and carriage of SCCmec types IV or V. While most CA-MRSA harbor lukSF genes for PVL, exceptions have already been reported. Actually, the majority of WA-1 isolates are PVL negatives.

It is also remarkable that some CA-MRSA lineages can prevail in specific geographic regions. For instance, in Europe, ST80 is the predominant CA-MRSA lineage, and it possibly originated from a PVL-positive MSSA ancestor from sub-Saharan Africa. In Australia, 3 different lineages (ST93, ST30, and ST1) are prevailing. The ST30 is also the predominant CA-MRSA in South America. In the USA, a ST8 CA-MRSA (the so-called USA300) is the dominant clone in CA infections. Nevertheless, the factors involved in the local expansion of a specific CA-MRSA lineage and the mechanisms behind the bacterial diversification in HA- or CA-MRSA remain unclear.

In the 2000s, CA-MRSA emerged in hospital settings to compete with HA-MRSA clones, representing an important epidemiological change. The PVL-positive, CA-MRSA USA300 is now challenging the previously dominant HA-MRSA clone New York/Japan (ST5) in USA hospitals. In Orange County (CA, USA), for example, 38.0% of the MRSA isolates from 30 hospitals were genotyped as USA300, while 36.8% as NY/J clone. In Rio de Janeiro, Brazil, the CA-MRSA ST30 is frequently detected causing typical HA-infections including septicemia. A legitimate concern of the scientific community is related to the possibility that once in hospitals the CA-MRSA could exchange DNA with typical HA-MRSA clones and thus acquire multiple-resistant genes. There is also the possibility that the intense CA-MRSA dissemination in health care units might facilitate the acquisition of additional adaptive mutations to increase bacterial fitness and survival despite the costs of antibiotic resistance.

Another novel situation regarding to MRSA epidemiology involves intra-lineage diversification/evolution. Here, a PVL-negative clone related to a classical CA-MRSA lineage is largely confined to hospitals. The hospital-restricted and multiresistant ST1-SCCmecIV, PVL-negative, related to the USA400 CA-MRSA—a PVL-positive strain previously detected causing CA infections in USA and Canada—emerged in Brazilian hospitals. This is a good example of this epidemiological change that can even further
challenge the distinction between CA-MRSA and HA-MRSA. In fact, while PVL has unquestionably been correlated with CA-MRSA, PVL alone cannot explain the CA-MRSA epidemiology because some CA infections are caused by PVL-negative CA-MRSA strains.

Indeed, the spread of this USA400-related clone in hospitals was associated, at least in part, with the loss of virulence-associated genes including lukSF-PV, and sea, sec, and sek genes for enterotoxins. This loss may represent a compensation for fitness costs of antibiotic resistance. It is possible that this clone have evolved—during the intra-lineage diversification—to a more appropriate balance between virulence and fitness. In fact, in hospitals—where MRSA are mainly spread by the hands of medical staff—the bacteria may not need the entire virulence arsenal needed to cause infections in healthy people. MRSA intra-lineage diversification is an exciting and open topic that might be driven by local pressures including characteristics of the host population, settings (hospital or community), and other environmental differences in addition to several factors including migration and social-geographic characteristics.

In a paper published in this issue of *Virulence*, Mekonnen and colleagues used proteomic and genomic approaches to explain the mechanisms involved in the distinction between CA- and HA-MRSA. The researchers discovered that CA- and HA-MRSA from the same lineage—related to USA300 clone (ST8-SCCmecIV)—differ in their exoproteome profiles which are also predictive of their epidemiological behavior as CA- or HA-associated pathogens. Notably, several of these extracellular proteins were cytoplasmic. In fact, others have more recently addressed the role of these non-classical exported proteins in *S. aureus* virulence. Just to give some examples, the causality of mice infections and survival in human microphages by *S. aureus* were influenced by a leucine aminopeptidase. Two glycolytic enzymes, aldolase (FbaA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), affected the virulence ability of *S. aureus*. FbaA displayed high-level cytotoxicity to both MonoMac 6 (MM6) and HaCa T cells, while GAPDH was cytotoxic only to MM6 cells. These features were also observed using an insect infection model.

Surprisingly, when Mekonnen and collaborators correlated the virulence-gene patterns and exoprotein profiles of USA300-related isolates, they found more similarities between HA-MRSA from the Dutch and German border (HA<sub>NL–DE</sub>; Spa t008) and HA-MRSA isolates collected in Denmark (HA<sub>DK</sub>; Spa t024) than between these HA-MRSA and CA-MRSA from Denmark (CA<sub>DK</sub>; Spa t008); despite the HA<sub>DK</sub>/CA<sub>DK</sub> core genome being more closely related. Interestingly, they found few differences in the expression of proteins associated with virulence including IsdA, IsdB, SCIN and Vwb. In addition, neither PVL nor enterotoxins—identified as potentially distinguishing features for CA- and HA-MRSA based on the functional genomics studies—were detectable in the extracellular proteome. However, the exoproteome of CA- and HA-MRSA showed major differences concerning cytoplasmic exported proteins represented by the high number of these products during exponential growth phase for the CA<sub>DK</sub> group relative to the HA ones. On the contrary, in the stationary growth phase, more cytoplasmic proteins were detected in the exoproteome of HA<sub>NL–DE</sub> and HA<sub>DK</sub> compared with CA<sub>DK</sub>.

In their genomic analysis, the researchers could not detect important changes in the DNA sequences of major *S. aureus* global regulators but did find important differences in the accessory genome. Nevertheless, distinct transcript levels for *fabF*, *rpoB* and *psma1–4* genes were also detected between CA- and HA-MRSA groups, validating the exoproteome data. This study does not specify the exact mechanism responsible for such timing-modulated differences in the exoproteome of CA- and HA-MRSA. One big mystery is the evolutionary advantage for this divergence. Using human airway epithelial cells (H16HBEo–), Mekonnen and his team found that the differences in the exoproteome significantly correlated with the ability of CA isolates to multiply inside of human cells while HA isolates could not. Although they do not pinpoint the cytoplasmic proteins involved in this process, this study offers a new perspective concerning the importance of non-classical exported proteins in CA-/HA-MRSA intra-lineage diversification, which may contribute to explain the fine breakup line that separates these 2 epidemiologically distinct but closely related bacteria.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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