Applying a New Technology to an Old Question: Whole-Genome Sequencing and *Staphylococcus aureus* Acquisition in an Intensive Care Unit

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(See the Major Article by Price et al on pages 609–18.)

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Phenotypic and genotypic characteristics of *Staphylococcus aureus* have long been used to determine the relatedness of isolates obtained from patients with epidemiologic evidence of a prior common risk factor for infection. In the 1940s, at the dawn of the era of the use of antimicrobial agents, the antibiogram of 2 isolates was compared to suggest isolate relatedness. In the 1950s, staphylococcal bacteriophage typing systems [1, 2] often enabled infection control practitioners in outbreak investigations to relate strains to one another. For example, with phage typing in hospitals, a virulent and rapidly disseminated *S. aureus* strain type known as the phage 80/81 type was recognized and tracked [3].

In the early 1960s, methicillin resistance in *S. aureus* (MRSA) was recognized, a genetic trait with the phenotype of resistance to β-lactam antibiotics. The MRSA phenotype provided epidemiologists in the healthcare setting a means of studying outbreaks and monitoring epidemiologic trends.

Restriction endonuclease–based typing of strains, used increasingly in the early 1990s, changed practices for outbreak investigations. In particular, pulsed-field gel electrophoresis (PFGE), with improvements in analytical software, led to the development of standardized *S. aureus* national typing classification schemes, such as the US Centers for Disease Control and Prevention’s USA100-USA1100 scheme [4]. As novel MRSA backgrounds emerged in community settings in the 1980s in Australia and in the 1990s elsewhere, PFGE contributed to an improved understanding of the epidemiology of MRSA, both inside and outside healthcare settings.

Since 1999, with increasing use of polymerase chain reaction (PCR) and a decrease in the cost of Sanger sequencing, DNA sequence–based genotyping methods were introduced for *S. aureus*. These provide a reproducible method for defining and identifying strain types of *S. aureus* isolates. Multilocus sequence typing, which requires sequencing of a single PCR amplicon in a hypervariable region of the *spa* gene, are sequence-based classification systems that use Web-based algorithms to determine the likely relatedness of isolates. These techniques have transformed epidemiologic research by providing a common language for *S. aureus* genotyping, whether researchers work in New York, Copenhagen, Nairobi, Perth, or Hong Kong. With this common genotyping “language,” researchers have a framework for more detailed genetic, proteomic, and other studies on the determinants of virulence and fitness in circulating *S. aureus* isolates.

All of the genotyping schemes described above share a common weakness: There is an assumption that the isolates having the same strain type also share many other, unmeasured, genetic characteristics. However, mobile genetic elements may or may not be present, making up the so-called accessory genome of a given *S. aureus* isolate. There is also a continuous evolutionary process of point mutation in the core and accessory genome of *S. aureus*. Either source of variation can undermine the assumption of close isolate relatedness.

Consequences of this critical limitation in sequence-based genotyping schemes were demonstrated in the article by Price
et al [5] in the current issue of Clinical Infectious Diseases. The authors tested a cohort of patients in an intensive care unit (ICU) for S. aureus nasal and perineal colonization (with other sites tested in some patients) on admission and then weekly during the ICU stay in a 14-month study. Staphylococcus aureus isolates underwent spa typing and whole-genome sequencing (WGS). The authors defined the following standard criteria for determining that S. aureus transmission occurred from one ICU patient to another: (1) if a patient was found to be colonized with a S. aureus isolate having a spa type not previously recovered from him or her and (2) if that isolate shared a common spa type with one obtained from the body of another patient who overlapped in the period of ICU stay. These criteria rely on the assumption that other ICU patients are the primary reservoir of S. aureus that may be transmitted to ICU patients and that transmission of S. aureus occurs from patient to patient, presumably via a healthcare worker who transiently carries the bacteria on the hands. The basis of many infection control programs is to prevent just this kind of transmission, including hand hygiene and the use of gowns and gloves for contact precautions. The authors identified 44 new ICU patient acquisitions of S. aureus.

Using WGS data, however, the authors determined that spa typing could not be relied upon to determine strain relatedness. Three patients suspected of acquiring S. aureus from another patient in the ICU using the standard spa typing criteria actually carried S. aureus isolates that were genotypically quite different when WGS data were used to compare single-nucleotide variants (SNVs) differentiating them. In addition, 5 patients acquired S. aureus isolates that were either indistinguishable or had very few SNVs differentiating them from S. aureus isolates previously cultured from the body of another patient when the ICU stays of the 2 carriers did not overlap in time. This suggests that the bacterial strain type had existed in a long-term reservoir in the ICU, and that it was not transmitted from one patient to another by short-term carriage on the hands of healthcare workers.

In the study by Price et al, WGS demonstrated that only 18.9% (7/37) of newly acquired S. aureus isolates were closely related to isolates colonizing other patients in the ICU with an overlapping ICU stay. The results are limited in that the authors studied only adults and tested only the nares and the perineum in many patients. The authors found that only 16.7% of tested patients were carriers of S. aureus, lower than the prevalence found in most studies, suggesting that many carrier isolates may not have been detected.

Nevertheless, the results call into question the idea that other patients are the primary source of newly acquired nosocomial pathogens. Price et al found that S. aureus was newly acquired by an ICU patient from a nonpatient source >80% of the time. These other sources may have included visitors, healthcare workers, fomites, or undetected previous S. aureus carriage in a patient suspected of new acquisition. Assessment of these possibilities will require additional study and confirmation, and may lead to novel approaches to the control of S. aureus transmission in the ICU.

Importantly, the spectrum of common pathogenic S. aureus strain types on each continent differs, as do infection control practices. For example, the predominant US MRSA strain types, USA100 and USA300, are rarely identified in the United Kingdom; it is possible that these strain types have different transmission dynamics than strain types common in the United Kingdom. The DNA sequence of an entire S. aureus genome was first reported in 2001, and the increasing availability of WGS has enabled its application to difficult problems in epidemiology. For example, WGS of 63 ST239 MRSA isolates delineated the likely temporal and geographic dissemination of this common European, Asian, and South American healthcare-associated MRSA strain type around the world from the 1960s to 2003 [6]. WGS was also applied to a collection of 89 ST398 isolates from people and animals to demonstrate that human ST398 S. aureus was transmitted into animal populations, where it evolved, losing phage-encoded genes believed to be human innate immune modulators. Animals then likely spread ST398 clones back to human populations that were MRSA and often were resistant to tetracycline [7]. WGS was used in 2 recent studies in the healthcare setting to delineate the spread of single S. aureus clones in neonatal ICUs, in one case demonstrating that the source of an ST2371 outbreak was likely a healthcare worker [8] and in the other, focusing on the dissemination of an ST22 clone, identifying a previously undetected transmission event [9].

WGS, by revealing genetic relatedness of S. aureus isolates in detail, has been established as the new gold standard in studies of transmission dynamics and strain relatedness. However, the potential of WGS as a means of understanding the human or environmental forces that drive evolutionary change in S. aureus, and in particular, the evolution of its extensive accessory genome, has not yet been realized.

**Note**

Potential conflicts of interest. Both authors: No reported conflicts. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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