**Next-generation sequencing** holds potential for improving clinical and public health microbiology. In addition to identifying pathogens more rapidly and precisely than traditional methods, high-throughput technologies and bioinformatics can provide new insights into disease transmission, virulence, and antimicrobial resistance. The US public health system is integrating pathogen genome sequencing into infectious disease surveillance with support from the Advanced Molecular Detection (AMD) program, established by Congress at the Centers for Disease Control and Prevention (CDC) in 2014. Population-level data on pathogen genomes in turn support the development of more precise and efficient clinical diagnostics. In time, laboratories may be able to replace many traditional microbiology processes with a single workflow that accommodates a wide array of pathogens.

**How Next-Generation Sequencing of Pathogens Works**

Next-generation sequencing is a versatile technology, broadly applicable to viruses, bacteria, fungi, parasites, animal vectors, and human hosts. Choosing among available methods depends on sequencing objectives and involves tradeoffs in accuracy, efficiency, and cost. For routine sequencing, most US clinical and public health microbiology laboratories use short-read sequencing platforms, which produce sequence fragments up to 1000 base-pairs long. Although microbial genomes are generally smaller and less complex than human genomes, long-read sequencing technologies (such as single-molecule real-time sequencing) are useful for constructing complete, highly accurate genomes and sorting out plasmids, repeats, and other complex regions.

A different approach, nanopore sequencing, relies on threading individual DNA or RNA molecules through engineered protein nanopores and monitoring the electric current across each pore. The first such commercially available instrument offers relatively long sequence reads and allows data analysis to begin while sequencing is still in progress. Early limitations in throughput and accuracy have been mitigated by continued improvements in hardware and reagents. Because of device portability, fast sample preparation, flexibility, and relatively low cost, nanopore sequencing is becoming a feasible first-line strategy for pathogen sequencing in clinical and public health settings.

The transformation of raw sequence data into actionable information is complex and computationally intensive (Figure). The first step is typically to assemble shorter fragments into a complete sequence, either by mapping against a known reference genome or by assembling the sequence de novo using overlapping reads. Comparing the assembled genome with reference strains facilitates many different inferences, such as pathogen identification, high-resolution strain typing, and prediction of important phenotypic characteristics (eg, virulence, antimicrobial resistance). Well-curated and up-to-date reference databases are crucially important because microbial pathogens evolve rapidly and bacteria can exchange plasmids—often encoding virulence and antimicrobial resistance traits—across strains and species. Assembled genomes can be compared with others to look for phylogenetic clustering as evidence of transmission. Each step—assembly, strain typing, phylotyping, and clustering—requires different bioinformatics tools that must be harmonized into a consistent workflow.

**Important Practice Considerations**

In public health, next-generation sequencing offers crucial advantages for surveillance and outbreak investigation in terms of speed and resolution of sequence differences. For example, the transition to next-generation sequencing from an older molecular method (pulsed-field gel electrophoresis) is well underway in PulseNet, the foodborne disease surveillance system maintained by CDC and its public health partners. PulseNet is now able to detect outbreaks earlier, to distinguish clusters of related cases more accurately, and to link illnesses to potential contaminated food sources more quickly.

Integrating pathogen genomics with epidemiology is enhancing public health efforts to prevent transmission of communicable diseases, such as tuberculosis. Genotyping tuberculosis isolates can corroborate transmission inferred from contact investigations or suggest connections among apparently unrelated cases, helping health departments to better focus their resources. Next-generation sequencing has the potential to yield information on...
Next-generation sequencing data are amenable to standardization and sharing, important advantages for global health partnerships like the World Health Organization’s influenza surveillance system. An open, “sequencing first” approach can help produce timely and accurate data for selection of candidate influenza vaccines, quickly identifying prevalent variants while monitoring the dynamics of co-circulating viral populations.1

Next-generation sequencing also offers advantages for challenging field investigations. For example, a research team from the United Kingdom packed a nanopore sequencing laboratory into standard luggage for transport to Guinea during the 2015 Ebola outbreak.2 During an 8-month period, they sequenced 142 Ebola virus genomes on site, usually within 1 working day; data were transmitted to the cloud for analysis and results returned the next day. Despite significant logistical challenges, including unreliable electrical power and internet service, the team provided actionable information for epidemic response without exporting samples from the country.

US public health laboratories, with support from the AMD program, are rapidly adopting next-generation sequencing for surveillance and investigation of foodborne disease, tuberculosis, hepatitis C, Legionella, and other pathogens.2 Nevertheless, the transition from research to routine public health and clinical use will have to overcome substantial challenges.3 At the laboratory level, these include infrastructure, workforce development, efficiency, and cost. At a broader, systemic level, substantial efforts are needed to develop standard protocols, proficiency-testing programs, professional guidelines, and regulatory requirements.3

Value
Compared with conventional methods, next-generation sequencing increases speed, accuracy, and detail but also increases cost. For example, a CDC analysis estimated that next-generation sequencing cost approximately $150 to $250 per bacterial isolate, compared with $94 for pulsed-field gel electrophoresis. Consolidating workflows for multiple pathogens may improve laboratory efficiency and help offset this cost; however, the transition to next-generation sequencing also entails significant up-front investment in laboratory equipment, computer resources, and training. Much more information will be needed to evaluate the value of next-generation sequencing technologies for microbiology at patient, programmatic, and societal levels.

Evidence
Evidence-based guidelines exist for only a few specific, clinical uses of pathogen sequence data, for example, in selecting antiretroviral treatment for HIV infection. Informative sequences from bacterial, viral, fungal, and parasite genomes are the basis for many new, nucleic acid-based diagnostic tests, including “point-of-care” tests that bypass the microbiology laboratory completely. As multiplex panels for syndromic diagnosis (eg, for diarrhea) become more widely used, systematic efforts will be needed to assess their clinical validity and utility, as well as their effect on laboratory-based public health surveillance.

Bottom Line
Next-generation sequencing is transforming the public health approach to infectious diseases, as well as the treatment of individual patients. Better coordination in establishing quality standards, reporting, and interpretation of next-generation sequencing data could make these efforts synergistic.

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REFERENCES

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