Rapid Pathogen Identification in Bacterial Pneumonia Using Real-Time Metagenomics

To the Editor:

Pneumonia remains a tremendous cause of morbidity, mortality, and healthcare expense (1). Despite the recent revolution in culture-independent microbiology (2), clinical identification of respiratory pathogens still relies on the culture-based techniques used by Pasteur in the 1880s (3). Delayed identification of pathogens in pneumonia can result in increased morbidity and mortality, as well as indiscriminate use of broad empiric antibiotics, impeding antimicrobial stewardship. Although novel sequencing-based approaches to pathogen identification have shown considerable promise in research settings (4), to date, these techniques have been too slow, expensive, labor intensive, and informatically challenging for clinical use. The MinION (Oxford Nanopore Technologies, Oxford, UK) is a new-to-market palm-sized DNA sequencer that has previously been used for real-time detection of viral and bacterial pathogens in blood (5, 6) and urine (7), as well as for rapid species identification in tuberculosis (8). We here report the first use of real-time metagenomic sequencing to identify bacterial pathogens in the lungs of patients with pneumonia.

Case 1

A 41-year-old immunosuppressed woman with connective tissue disease-associated interstitial lung disease was admitted to the intensive care unit with hypoxic respiratory failure requiring mechanical ventilation. A chest X-ray revealed bilateral infiltrates (Figure 1A), and a mini-bronchoalveolar lavage (mini-BAL) was performed. Lavage fluid was aliquoted and sent both for conventional microbiologic studies and for concurrent sequencing analysis in our research laboratory. Gram stain of the lavage fluid revealed neutrophils and “oral flora.” Lavage fluid was centrifuged, and DNA was extracted (Qiagen DNeasy Blood and Tissue Kit, Qiagen, Venlo, the Netherlands) and sequenced using the MinION device. Nine hours after lavage, we identified a 3,217 base-pair sequence of DNA that was well aligned ($E \leq 10^{-199}$) with published genomes of numerous previously sequenced strains of *Pseudomonas aeruginosa* (Figure 1B). Twenty-three hours after lavage, the clinical microbiology laboratory reported the growth of more than 10^4 colony-forming units of *P. aeruginosa*

(Figure 1C). In a subsequent confirmatory analysis, we isolated DNA from a pure culture of this clinically isolated *P. aeruginosa* and, during a 48-hour period, performed whole-genome sequencing using the same MinION sequencer. The initial 3,217 base-pair sequence from the lavage fluid was well aligned with the genome of the clinically isolated *P. aeruginosa* strain ($E \leq 10^{-199}$), which was, in turn well aligned with that of a reference *P. aeruginosa* genome (SCV20265; $E \leq 10^{-199}$) (Figure 1D). The bacterial genome sequenced from the cultured *P. aeruginosa* isolate revealed the presence of numerous antibiotic resistance genes (e.g., the Mex efflux pump gene complex) that accurately predicted the phenotypic resistance pattern identified by the clinical microbiology laboratory, using conventional susceptibility testing. Using previously reported methods (9), we amplified the 16S ribosomal RNA gene from DNA isolated from the lavage fluid and performed community sequencing using the MiSeq platform (Illumina, San Diego). This revealed a low-diversity bacterial community dominated (65% relative abundance) by *P. aeruginosa* (Figure 1E), consistent with community features we have previously shown to be present in bacterial pneumonia (4).

Case 2

A 59-year-old man with abdominal sepsis developed hypoxic respiratory failure requiring mechanical ventilation. A mini-BAL was performed, and lavage fluid was aliquoted, processed, and analyzed as described earlier. Real-time sequencing using the MinION revealed six high-quality DNA sequence reads measuring between 909 and 8,288 base pairs, each well-aligned with the genomes of previously sequenced strains of *Staphylococcus aureus* ($E \leq 10^{-199}$). The following day, the clinical microbiology laboratory reported growth of greater than 10^4 colony-forming units of *S. aureus* from the same mini-BAL specimen. Subsequent community analysis of the BAL specimen revealed a low-diversity bacterial community dominated (95% relative abundance) by *Staphylococcus* sp.

Discussion

These cases, which represent the first use of real-time metagenomic sequencing to identify pathogens in pneumonia, demonstrate the enormous potential of rapid sequencing-based techniques to accelerate and improve our diagnosis and management of this common and lethal disease. We here demonstrate that in principle, a real-time metagenomic approach using currently available tools is capable of identifying pathogens faster than conventional in-use culture-based techniques. An acceleration in pathogen identification in pneumonia holds promise to improve our delivery of tailored antimicrobial therapy, improving patient outcomes and facilitating antimicrobial stewardship by minimizing the need for broad, empiric antimicrobial coverage. In addition to accelerating time to diagnosis of culturable pathogens, real-time metagenomics holds potential to identify pathogens that cannot be grown in culture. Unlike polymerase chain reaction (PCR)-based and 16S ribosomal RNA gene-based sequencing approaches, a metagenomic approach is “agnostic” regarding the taxonomy of pathogens and can identify bacteria, viruses, and fungi alike. This sequencing platform has proven capable of identifying RNA viruses in nonlung specimens (5, 6), demonstrating its potential for an impressive breadth of taxonomic detection. Thus, a real-time

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Figure 1. Rapid identification of a respiratory pathogen using real-time metagenomics. (A) A 41-year-old woman developed hypoxic respiratory failure with bilateral infiltrates on chest X-ray. A miniature bronchoalveolar lavage was performed. (B) Nine hours later, a 3,217 base pair DNA sequence was detected in the lavage fluid using a MinION sequencer. The sequence was well aligned with previously sequenced strains of *Pseudomonas aeruginosa* ($E \leq 10^{-199}$). (C) Twenty-three hours after lavage, the hospital's clinical microbiology laboratory reported the growth of >10⁴ colony forming units of *P. aeruginosa*. (D) The genomic identity of the cultured strain was subsequently (48 hours later) confirmed via whole-genome sequencing performed with the MinION sequencer. The 3,217 base-pair sequence from the bronchoalveolar lavage fluid was well aligned with a segment of the sequenced genome of the cultured *P. aeruginosa* strain, which was in turn well aligned with a reference strain of *P. aeruginosa* (SCV20265) ($E \leq 10^{-199}$ for both). (E) Subsequent analysis of the lavage fluid using community sequencing of the bacterial 16S ribosomal RNA gene confirmed the presence of a low-diversity bacterial community dominated by *P. aeruginosa*. NCBI = National Center for Biotechnology Information.

metagenomic approach may prove to be a powerful tool both in the detection of known pathogens as well as in identification of emerging diseases (10). Further, our ability to rapidly (48 hours) sequence the entire genome of a cultured isolate (Figure 1D) demonstrates the power of real-time sequencing to improve our tailoring of antimicrobial therapy by identifying the presence or absence of known resistance genes.

Our experience with real-time metagenomic pathogen identification, although promising, identifies several key areas in need of immediate study. Although our sequencing approach demonstrates extraordinary specificity in detecting the presence of pathogens, the method's sensitivity for pathogen detection is unknowable without dedicated, systematic study. Novel, rapid techniques of molecular quantification of DNA (such as droplet digital PCR and nanorod PCR) may prove useful adjuncts

in helping clinicians discriminate between states of health, contamination, colonization, and infection. Although in principle our metagenomic approach is capable of identifying nonbacterial pathogens, we have not yet established whether our current approach can detect fungal, viral, or protozoal pathogens in the lungs. Additional methodological work is needed to optimize the detection of microbial signal in host-DNA-rich respiratory specimens. Finally, further work is needed to streamline the bioinformatic analysis of metagenomic sequencing data before this approach can be scaled for testing in a clinical context.

Pneumonia is a 21st-century problem, yet its diagnosis still relies on 19th-century tools. Our results demonstrate the feasibility and promise of introducing real-time metagenomics to our diagnostic arsenal. Clinical study is warranted, as the revolution in molecular microbiology has at last reached the bedside. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Kathryn M. Pendleton, M.D.
John R. Erb-Downward, Ph.D.
Yuwei Bao
William R. Branton, B.S.
Nicole R. Falkowski, B.S.
Duane W. Newton, Ph.D.
University of Michigan Medical School
Ann Arbor, Michigan

Gary B. Huffnagle, Ph.D.
University of Michigan Medical School
Ann Arbor, Michigan
and
University of Michigan
Ann Arbor, Michigan

Robert P. Dickson, M.D.
University of Michigan Medical School
Ann Arbor, Michigan
and
Michigan Center for Integrative Research in Critical Care
Ann Arbor, Michigan

ORCID IDs: 0000-0002-9943-2449 (D.W.N.);
0000-0002-4505-9642 (G.B.H.); 0000-0002-6875-4277 (R.P.D.).

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Triplicate Sputum Cultures for Efficacy Evaluation of Novel Antituberculosis Regimens

To the Editor:

Sputum culture conversion from positive to negative remains the standard endpoint in recent studies of antituberculosis drugs (1–3). In most protocols, a single sputum sample is collected for culture at each patient visit. With ongoing efficacious treatment, however, sputum production and quality decreases (4), while the frequency of “contaminated” results increases. Such results are equivocal, do not prove the absence of viable mycobacteria, and increase the cost of the trial. Various measures at different steps of the sputum collection and culture process can reduce, but not completely eliminate, the problem of contaminated cultures (5–8).

TMC207-C208 (NCT00449644) was a 120-week, randomized, double-blind, phase 2 study that examined addition of bedaquiline or placebo to the first 24 weeks of a five-drug treatment regimen in 160 adults with multidrug-resistant tuberculosis (2). For the first time, we used a simple strategy to minimize the occurrence of microbiologically uninformative visits by collecting triplicate sputa at 30-minute intervals during each visit, separate routine processing of each sputum sample using only mycobacteria growth indicator

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