expressed in colony-forming units/ml. However, CMg is only able to quantify microorganisms in a relative fashion, expressed as a percentage. Hence, a proxy is required to translate percentages into colony-forming units/ml. Recently, a spike-in-based calibration to total microbial load protocol (6), which consists of adding controlled amounts of whole cells, was tested as an appealing approach to estimate absolute bacterial concentrations. Yet, the choice of the spike-in organism regarding its susceptibility to cell lysis and the form in which it will be used (fresh, stabilized, or lyophilized) remains to be defined to allow, as accurately as possible, quantification of various bacteria from different communities.

Lastly, the authors reported a short turnaround time (9 h for the first case), which could allow a rapid optimization of the antibiotic treatment under three conditions: (1) the sequencer would be available 24 hours a day and the results rapidly sent to the physician, (2) the output of the sequencer would be actionable for the physician, and (3) the resistance pattern of the pathogen(s) would be inferred. Indeed, a simple identification of the microorganisms involved (which currently can be achieved overnight by mass spectrometry) is not enough to finely adjust the antibiotic regimen. As metagenomic sequencing is able to recover antibiotic resistance determinants, efforts should be made to infer resistance patterns from metagenomic data.

In conclusion, we acknowledge that the article by Pendleton and colleagues is an important contribution that supports the use of CMg in the context of hospital-acquired pneumonia but note that several limitations need to be addressed before CMg can eventually be used in the routine setting. ■

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

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Reply

From the Authors:

We appreciate the thoughtful letter from Dr. Emonet and colleagues in response to ours (1) [this issue, pp. 1610–1612]. Our proof-of-principle report is the first demonstration that pathogens in pneumonia can be identified using real-time metagenomics. To our knowledge, no one has advocated for routine clinical use of this emerging technology, which, however promising, presents numerous challenges. We appreciate the opportunity to address and expand upon current hurdles to bringing clinical metagenomics to the bedside.

As stated in our letter, the high host/microbe ratio of genetic material in respiratory specimens presents a challenge for identifying pathogens via metagenomics. We wish to highlight recent advances in strategies for selective enrichment of microbial DNA that are relevant to this issue (2). These techniques, performed before sequencing, selectively enrich the microbial fraction of specimen DNA using either differences in CpG methylation density (3) or chaotropic lysis of eukaryotic cells followed by degradation of eukaryotic DNA (4). Additionally, a novel “read until” protocol for nanopore sequencers was recently shown to be capable of selectively sequencing DNA of interest while rejecting unwanted sequences (5). This “selective sequencing” approach, currently in the proof-of-concept stage, may prove to be a powerful tool for metagenomic analysis of mixed-origin specimens.

Contamination of sequencing results by environmentally and procedurally introduced DNA is an important consideration whenever highly sensitive sequencing platforms are used. One advantage of nanopore sequencing is the relatively long read length, which provides superior taxonomic resolution compared with commonly used 16S amplicon-based approaches. As an illustration, the long read length of the microbial DNA detected in one of our patient specimens permitted species-level identification of a known respiratory pathogen, *Pseudomonas aeruginosa*. Yet, 16S amplicon-based sequencing of the same specimen could only detect the presence of members of the *Pseudomonas* genus, which contains numerous nonpathogenic environmental and procedural contaminants. It is also worth noting that our metagenomics strategy did not employ DNA amplification, thus removing a major source of contamination in low-biomass sequencing studies. In our reported cases, results from direct specimen sequencing were subsequently validated both by culture and by alignment with whole-genome sequencing of cultured isolates. That said, we agree with the endorsement of negative extraction controls, regardless of the sequencing platform used.

Mere detection of microbiota in respiratory specimens does not discriminate between colonization and infection. Although quantitative culture is commonly used for this purpose, the distinction

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between colonization and infection remains ultimately an integrated clinical one, informed by clinical context, microbial identification, microbial burden, and host response. As we suggested, microbial burden may potentially be determined with the use of complementary quantification tools, such as amplification of “universal” microbial primers (e.g., 16S and internal transcribed spacer) (6) by novel rapid, ultrasensitive polymerase chain reaction techniques (7). Characterization of the host response, which is currently restricted to coarse indices such as the alveolar cell count and differential, may soon be richly and rapidly characterized using real-time host transcriptomics, now possible with the same nanopore sequencer we used for metagenomics in our study (MinION; Oxford Nanopore Technologies, Oxford, UK).

Finally, to incorporate rapid metagenomic results into antimicrobial selection, clinicians will need to overcome numerous temporal barriers. Although we believe that our ultimate goal should be rapid, direct detection of antimicrobial resistance genes, we observe that pathogen speciation alone is often sufficient to support narrowing empiric antimicrobial coverage (e.g., discontinuing vancomycin or linezolid in the absence of methicillin-resistant *Staphylococcus aureus*). Recently, the manufacturer of the sequencing device used in our study (Oxford Nanopore Technologies) released a platform capable of sequencing up to 48 specimens simultaneously. The ability to perform parallel sequencing of multiple aliquots from the same respiratory specimen would both dramatically increase the speed of sequencing and permit greater sequencing depth. This approach, especially if combined with the microbial DNA enrichment strategies discussed above, should make the goal of direct sequencing of antimicrobial resistance genes more attainable.

We again thank Dr. Emonet and colleagues and the *Journal* for the opportunity to discuss the future directions of this promising and rapidly evolving technology and its application to pneumonia diagnostics. Our report demonstrates the potential of real-time metagenomics to identify pathogens in pneumonia, and we believe immediate experimental optimization and clinical studies are warranted. ■

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Erratum: FIFTY YEARS OF RESEARCH IN ARDS. Gas Exchange in Acute Respiratory Distress Syndrome

Because of a typesetting error, there were mistakes in two equations in the final published version of the October 15, 2017, State of the Art (1). Equation 3 in the DEAD SPACE section on page 966 should read:

$$\dot{V}_D/\dot{V}_E = V_D/V_T = (P_{aCO_2} - P_{ECO_2})/P_{aCO_2}.$$

[The bold “P” incorrectly appeared as “F”.]

In the ALVEOLAR GAS EQUATION section on page 967, Equation 6 should read:

$$P_{AO_2} = P_{IO_2} - P_{aCO_2} \cdot [F_{IO_2} + (1 - F_{IO_2})/RER].$$

[The bold “F” incorrectly appeared as “P”; the equals sign incorrectly appeared as a plus sign.] ■

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