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

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# In the Wrong Place at the Wrong Time: Microbial Misplacement and Acute Respiratory Distress Syndrome

Patients with acute respiratory distress syndrome (ARDS) are gravely ill, so any knowledge of actionable factors at play in their lungs will be of great clinical value. It is well recognized that pulmonary bacterial compositions are abnormal in patients with ARDS and that the presence of gut microbiota are a bad prognostic factor. In this issue of the *Journal*, Dickson and his colleagues (pp. 555–563) build on previous work that found gut-specific bacteria were common and abundant in the airways of patients with ARDS and that the presence of enteric organisms correlated with the intensity of inflammation (1–3).

The authors now report a prospective observational study of 91 mechanically ventilated critically ill patients, showing that outcomes were predictable at the time of admission to ICU by measurements in BAL fluid. None of their patients had received antibiotics prior to ICU admission. The authors assessed bacterial burden by quantitative PCR (qPCR), and they estimated the relative abundance of specific gut-associated bacteria by amplification and sequencing of the 16S ribosomal RNA gene. The principal finding was that sequences identifying bowel bacteria predicted the length of time on a ventilator.

The authors are careful and honest about the limitations of the study. The study materials came from the lower respiratory tract, which normally has a relatively low microbial biomass. A significant technical problem arose from the use of dilute miniature BAL specimens that had been previously centrifuged to separate eukaryotic cells. The consequent depletion of bacterial numbers in the studied supernatant magnifies a risk that background contamination may bias the results (4). The risk is addressed carefully in the paper, and a significant message may be that improved protocols are likely to yield even more conclusive results in future investigations.

DNA sequencing of highly variable regions of bacterial genomes (such as the universally present 16S ribosomal RNA gene) allows quantification of almost all the bacteria present in biological samples. Bacteria are identified as operational taxonomic units (OTUs) through database matches. A limitation of 16S sequencing is that OTUs can usually define phyla and genera but rarely identify species. Intraspecific strains of the same species can differ by 20% of their genes, so although 16S shows us the ballpark it tells nothing about individual players.

Bacterial communities can be described with strategies that derive historically from microbial ecology. Measures such as the Shannon diversity index, richness, and evenness capture community structures that are of value in environmental surveys or epidemiological studies. In human microbiology, these parameters may be used to predict microbial community resilience to infection. Their value in the catastrophic events leading to ARDS is uncertain, and it may not be surprising that diversity of lung bacteria did not significantly predict ICU outcomes in this study.

These community parameters are derived from relative abundances (i.e., percent of each sample) after random pruning of sequence reads to match the lowest sample count. This rarefaction can bias the interpretation of disease-associated bacteria, and it is recommended that unrarefied data be used in analysis of differences in taxa between disease states (5).



Originally Published in Press as DOI: 10.1164/rccm.202001-0004ED on January 24, 2020

Much more information can be gleaned by asking which bacterial species differ between disease and normal, or which taxa differ with varying disease outcomes. It is fortuitous that 16S sequencing yields data that are similar in complexity and distribution to the results of human gene expression studies, meaning that a sophisticated toolkit is available for the interpretation of OTU abundance tables. In their investigation, Dickson and his colleagues used random forest analysis to show that Lachnospiraceae and Enterobacteriaceae families were most strongly predictive of days on a ventilator.

These findings confirm previous studies that found gut-associated bacteria in the lung microbiota in patients with ARDS, primarily *Enterobacteriaceae* spp (2, 3). Importantly, the most significant taxon found in the present study (OTU0005: *Enterobacteriaceae*) (1) was nearly identical to that of the ARDS-associated bacterial taxon 342 found in an earlier study by Panzer and colleagues (3).

Dickson and colleagues succinctly state, "The crucial and unanswered question is whether lung microbiota are a modifiable risk factor" (1). The implication of a specific Enterobacteriaceae OTU in premorbid ARDS is a very material finding that may indicate that particular bacterial taxa, rather than a general dislocation of bowel flora, underlies disease progression. In that case, therapeutic activities may be directed at the organism and the immune responses it initiates.

The study also highlights the general significance of bacterial identification in ARDS. Failure to identify causal organisms occurs in half of ICU patients admitted with pneumonia (6), and similarly low diagnostic rates are found in patients with pneumonia after immunosuppressive therapy or bone marrow transplants (7, 8). qPCR assays for (non-16S) sequences specific to common pathogens have the potential to deliver results by the bedside. As exemplified by Dickson and colleagues, qPCR with general primers can measure the overall bacterial burden, allowing critical assessment of the relative abundance of a pathogen among other organisms (1, 9).

Metagenomic shotgun sequencing is a more advanced methodology that can identify all of the genes present in a complex microbial mixture. Many examples from bowel metagenomics show insights into disease can be gleaned without direct knowledge of the bacterial species contributing the genes. Unfortunately, pulmonary samples are currently problematic for microbial metagenomics because very high human-to-microbial DNA ratios may require a 100-fold increase in sequence depth (and 100 times the cost of sequencing).

Since the time of Robert Koch in 1880, medical bacteriology has depended on the ability to culture pathogens. Sequence analysis does not replace this technology but instead improves its direction. The patients in the investigation of Dickson and colleagues were not studied using clinical culture-based techniques, restricting the authors' ability to match sequence and bacterial burden to pneumonia assessments. Should particular Enterobacteriaceae OTUs be observed consistently in ARDS patients, there is a strong case to isolate the organisms and to study their antimicrobial sensitivities and their effects on pathogenesis in model systems. It might also be considered that the ICU environment is very selective for multidrug-resistant pathogens, which can persist and spread within hospitals (10).

Dickson and colleagues cautiously but correctly conclude that the lung microbiome is an important and understudied source of variation in outcomes among critically ill patients. Their intriguing results indicate a high priority for ambitious investigations with samples optimally gathered for microbial testing. Novel therapeutic targets for the prevention and treatment of lung injury may be found through accompanying sequence studies with robust efforts to culture and characterize the Enterobacteria associated with ARDS. ■

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

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