DIAGNOSTICS

Rapid diagnosis of lung infections

Nanopore sequencing of the sputum metagenome identifies respiratory pathogens in six hours.

Andrei Prodan, Morten O. A. Sommer and Max Nieuwdorp

Respiratory infections are the fourth largest cause of mortality worldwide and account for more than half the volume of antibiotics prescribed in human medicine. Identifying the causative agents of respiratory infections using traditional culture methods can take several days, so patients are often treated with broad-spectrum antibiotics, a practice that accelerates the emergence of antibiotic resistance. In this issue, Charalampous et al.[1] present a metagenomics protocol that achieves untargeted diagnosis of respiratory infections in just six hours (Fig. 1). Sputum samples are depleted of human DNA using saponin, thereby increasing the relative abundance of microbial DNA, and then analyzed by nanopore sequencing. This study is the first sequencing-based diagnostics protocol applicable to a respiratory infection sample that can provide rapid, untargeted diagnostics matching the accuracy of culture- and PCR-based techniques.

Speed is crucial in infectious disease diagnostics. Physicians need to rapidly identify the causative pathogen as well as its drug sensitivity profile to maximize the chances of therapeutic success. Broad-spectrum antibiotics have two major unwanted side effects. First, they can severely disrupt the patient's gut microbiome, leading to short-term gastrointestinal discomfort or long-term metabolic and inflammatory effects[2,3]. Second, they contribute to the selection and spread of antibiotic resistance genes, exacerbating global concerns regarding multidrug-resistant pathogens[4,5]. Rapid and reliable identification of respiratory pathogens is therefore crucial both for successful treatment and for antibiotic stewardship.

Molecular methods are already being applied in infection diagnostics. PCR-based assays require just a few hours, but they are targeted: finding each individual pathogen requires the use of a specific, carefully designed set of primers. Sequencing-based techniques are untargeted, yet come with their own set of limitations. Nanopore, the fastest and most compact sequencing technology available, produces long reads but with high error rates and comparatively low output. Its speed and portability have led to its use for Ebola surveillance[6] and urine infection diagnostics[7] where either the pathogen load is high[6] or the complexity of the sample is low[7].

One of the main challenges in respiratory infection diagnostics is that human DNA is several orders of magnitude more abundant in sputum than is microbial DNA. To get rid of human DNA, Charalampous et al.[1] turned to saponin-based depletion[8]. This method takes advantage of differences between human and bacterial cell surfaces. In specific conditions, human cells incubated with saponin become susceptible to osmotic lysis whereas bacterial cells remain largely intact. Protocol optimization enabled the authors to obtain a detection sensitivity of 96.6% and reduced the sample preparation time to under four hours. Using nanopore sequencing, the authors could generate enough sequencing data to identify the pathogens in a sample in two hours, bringing the total time from sample receipt to pathogen identification down to six hours.

Charalampous et al.[1] used 41 respiratory samples to benchmark the optimized metagenomic pipeline against culture-based methods. The specificity of metagenomics was initially only around 50%. Some pathogens detected by metagenomics could not be detected by culture, yet quantitative PCR assays confirmed the presence of DNA derived from these pathogens. Additionally, amendment of the computational mapping analysis to include species-specific genes for use as markers to differentiate pathogens from closely related bacteria increased sensitivity and specificity to 100% compared to the gold-standard methods. Future improvements in the metagenomic classification algorithms may enable low false-positive rates without the need for manually selected marker genes, thus expanding the method's applicability.

Metagenomic diagnostics could enable detection of antibiotic resistance genes in clinical samples. Nanopore sequencing has previously been used to identify antibiotic resistance genes in this setting[9]. However, sequencing-based identification of antibiotic resistance is still hampered by three main issues. First, our understanding of the entire set of genes and mutations that can cause resistance is incomplete, potentially leading to false negatives. Second, homology-based annotation often relies on incomplete sequence identity to a validated reference gene, potentially leading to false positives. Third, resistance-gene databases frequently include regulatory genes or housekeeping genes that may not directly cause antibiotic resistance, another source of false positives. Finally, even the presence of a confirmed resistance gene does not guarantee that the gene is expressed or confers antibiotic resistance on its host. These limitations were demonstrated by Charalampous et al.[1], who identified both resistance phenotypes without annotated resistance genes and resistance genes without a resistance phenotype. Accordingly, the metagenomics diagnostic method reported by these authors cannot yet fully replace culture-based

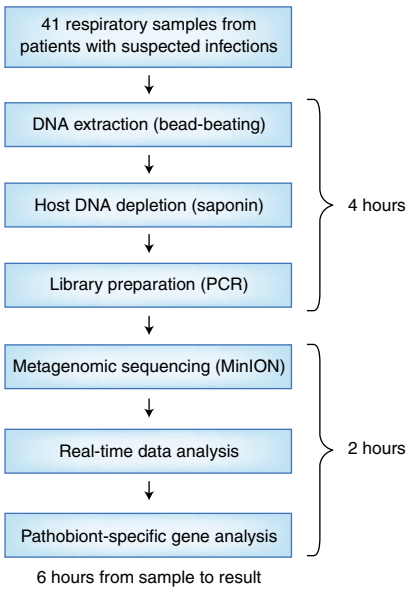

Fig. 1 | Workflow for respiratory sample metagenomics[1].

techniques if the goal is to confidently assess antibiotic resistance.

In the future, clinical application of metagenomics diagnostics could enable a comprehensive assessment of the genomic epidemiology of antimicrobial resistance, which would inform efforts to manage antibiotic resistance. Routine application of metagenomic diagnostics in chronic lung infections could enable the identification of pathogen vulnerabilities, such as collateral sensitivity recently identified in *Pseudomonas aeruginosa* from patients with cystic fibrosis[10]. Finally, data generated by metagenomic diagnostics could be fed to machine learning algorithms in order to identify biomarkers to guide treatment.

To assess its real world applicability, the nanopore sequencing-based diagnostic developed by Charalampous et al.[1] is being tested in the INHALE clinical trial (https://www.ucl.ac.uk/inhale-project). Further improvements are needed to enable implementation in routine clinical use, but the speed and sensitivity compared with culture-based techniques show great promise. ❐

Andrei Prodan[1], Morten O. A. Sommer[2*] and Max Nieuwdorp[1*]

[1]*Department of Vascular Medicine, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, the Netherlands.* [2]*DTU Biosustain, Technical University of Denmark, Kongens Lyngby, Denmark.*

**e-mail: msom@bio.dtu.dk; m.nieuwdorp@amsterdamumc.nl*

Published online: 24 June 2019
https://doi.org/10.1038/s41587-019-0174-3

References

1. Charalampous, T. et al. *Nat. Biotechnol.* https://doi.org/s41587-019-0156-5 (2019).
2. Thaiss, C.A., Zmora, N., Levy, M. & Elinav, E. *Nature* **535**, 65–74 (2016).
3. Komaroff, A. L. *J. Am. Med. Assoc.* **317**, 355–356 (2017).
4. Berendonk, T. U. et al. *Nat. Rev. Microbiol.* **13**, 310–317 (2015).
5. Sommer, M. O. A., Munck, C., Toft-Kehler, R. V. & Andersson, D. I. *Nat. Rev. Microbiol.* **15**, 689–696 (2017).
6. Quick, J. et al. *Nature* **530**, 228–232 (2016).
7. Schmidt, K. et al. *J. Antimicrob. Chemother.* **72**, 104–114 (2017).
8. Hasan, M. R. et al. *J. Clin. Microbiol.* **54**, 919–927 (2016).
9. van der Helm, E. et al. *Nucleic Acids Res.* **45**, e61 (2017).
10. Imamovic, L. et al. *Cell* **172**, 121–134.e14 (2018).

Competing interests
The authors declare no competing interests.

RESEARCH HIGHLIGHTS

Papers from the literature

Selected by the *Nature Biotechnology* editors.

Rational engineering of XCaMPs, a multicolor GECI suite for in vivo imaging of complex brain circuit dynamics
Inoue, M. et al. *Cell* https://doi.org/10.1016/j.cell.2019.04.007 (2019).

A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment
Van Hove, H. et al. *Nat. Neurosci.* https://doi.org/10.1038/s41593-019-0393-4 (2019).

Complex signal processing in synthetic gene circuits using cooperative regulatory assemblies
Bashor, C. J. et al. *Science* https://doi.org/10.1126/science.aau8287 (2019).

A white-box machine learning approach for revealing antibiotic mechanisms of action
Yang, J. H. et al. *Cell* https://doi.org/10.1016/j.cell.2019.04.016 (2019).

Learning the signatures of the human grasp using a scalable tactile glove
Sundaram, S. et al. *Nature* https://doi.org/10.1038/s41586-019-1234-z (2019).

Follow us on Twitter @NatureBiotech #nbtHighlight