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# Novel approach obtains protein signatures from host and pathogen with one small sample

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A novel study from researchers at Nationwide Children's Hospital has shown that it is possible to obtain both host and bacteria protein signatures in a sample smaller than the average human biopsy. The technique described in the paper—unbiased two-dimensional liquid chromatography-tandem mass spectrometry—could change how researchers study infectious diseases.

"This technology can be applied to any disease state," says Kevin Mason, PhD, principal investigator in the Center for Microbial Pathogenesis in The Research Institute at Nationwide Children's. "With infectious diseases, it's particularly effective, because you can obtain results for the pathogen and the host from just one sample."

According to the researchers, this is the first comprehensive study of host protein and metabolite profiles in vivo in response to infection. Additionally, the study, published in the March issue of *Molecular and Cellular Proteomics*, shows the feasibility of extensive characterization of host protein profiles during disease.

"The potential impact of the technology is not limited to research," says Sheryl Justice, PhD, principal investigator in the Center for Microbial Pathogenesis. "We can now think differently about how we might analyze samples for personalized medicine."

The disease state studied, otitis media caused by nontypeable Haemophilis influenzae (NTHI), continues to vex the medical community. With the ever-present threat of antibiotic resistance and the burden of recurrent illness in children, otitis media continues to be an area of directed innovation in the scientific community.

Through this study, researchers gained new ground by obtaining a snapshot of the proteomic and metabolomic landscape of ear infections.

The research team conducted two-dimensional liquid chromatography-tandem mass spectrometry using unlabeled proteins to identify peptides from in vivo samples. The samples were collected after 48 hours of infection with NTHI. While the size of a typical human biopsy is 100 ug, the tiny samples required for the proteomics study were only 3 ug.

"In the chinchilla model we used, there is a lot of genetic diversity because they are not an inbred animal model," says Dr. Justice, who is also associate professor of Pediatrics and Urology at The Ohio State University College of Medicine. "So we were concerned that there might be too much diversity to get a consistent proteomic picture that would be attributable to the disease state. However, even with our small sample size in an outbred model, we were able to identify dynamic changes in 105 chinchilla proteins and 66 metabolites, thus defining the early proteomic and metabolomics signature of otitis media."

Ultimately, knowing which metabolic pathways are activated in the disease state compared to a healthy state could provide targets for new therapies. Those therapies could replace or support current antibiotic treatments for ear infections. And based on the proteins identified in the initial study, one such therapy is being developed.

The team experimentally demonstrated the role of the protein Apr2/3 protein complex in NTHI invasion. By using an inhibitor specific to the

protein, the team was able to alter bacterial invasion and provide evidence for a potential adjunct therapy for otitis media infections.

"The potential to develop new therapeutic approaches to NTHI based on a deeper understanding of the metabolic activity is one way that we can make a big impact on this disease and ultimately on other diseases," says Dr. Mason, who is also assistant professor of Pediatrics at The Ohio State University School of Medicine. "We can make the pathogen cells more susceptible to current and future treatments through adjunct therapies aimed at the active metabolic pathways. We can improve the way infections are treated."

According to Dr. Mason, the collaboration among the authors on the paper was essential to the success of the project. The multi-institutional team included Joseph Kerschner, MD, dean and executive vice president of the Medical College of Wisconsin, who provided the chinchilla genome, and a team from Duke University's Proteomics and Metabolomics Core Facility, who conducted the two-dimensional liquid chromatography-tandem mass spectrometry.

Now, the team is taking more snapshots of otitis media infection by collecting samples at different time points in the disease process. "This will help us build a proteomic timeline, providing a level of understanding that is unprecedented," says Dr. Mason.

**More information:** Comprehensive proteomic and metabolomic signatures of nontypeable Haemophilus influenzae-induced acute otitis media reveal bacterial aerobic respiration in an immunosuppressed environment. *Mol Cell Proteomics*. 2015 Dec 28. pii: mcp.M115.052498. [Epub ahead of print]