New molecular diagnostic method to identify Hypervirulent *Klebsiella pneumoniae* O1 strains

Klebsiella pneumoniae is an encapsulated gram-negative bacillus that frequently causes outbreaks of nosocomial infections in hospitalized patients. *K. pneumoniae* can also afflict ambulatory persons and cause community-acquired invasive diseases—including pyogenic liver abscess, endophthalmitis, meningitis, empyema, lung abscess, and necrotizing fasciitis. *The* worldwide emergence of multidrug-resistant strains and hypervirulent strains of *K. pneumoniae* has become an increasing clinical challenge and public health concern. A new molecular diagnostic method based on detection of the genetic determinants of lipopolysaccharide (LPS) O-antigen has been developed by Dr. Chi-Tai Fang of National Taiwan University to rapidly and accurately identify hypervirulent *K. pneumonia*e O1 strains. This study was published in March 2016 in the *Journal of Clinical Microbiology*, a distinguished journal of the American Society for Microbiology.

The research was conducted by Dr. Chi-Tai Fang, Professor of Epidemiology and Preventive Medicine at the College of Public Health, National Taiwan University, and his two Master's students, Ms. Yun-Jui Shih and Ms. Cheng-Man Cheong.

K. pneumoniae strains can be distinguished by their capsular polysaccharide (CPS) K-antigen types (77 serotypes) and LPS O-antigen types (9 serotypes). Strains with K1 CPS are extremely virulent, causing metastatic endophthalmitis and meningitis from pyogenic liver abscess. Nevertheless, only 60% of strains causing pyogenic liver abscess are K1 strains. The remaining strains are of K2, K5, K20, K54, K57, and other K types. On the other hand, more than 90% of strains causing pyogenic liver abscess have O1 LPSs. Therefore, O1 is a better marker for virulent *K. pneumoniae* strains capable of causing pyogenic liver abscess. However, previous research has not produced a simple and reliable method for detecting O1 in clinical and epidemiological samples because *K. pneumoniae* O-serotyping is cumbersome and the reagents are not commercially available.

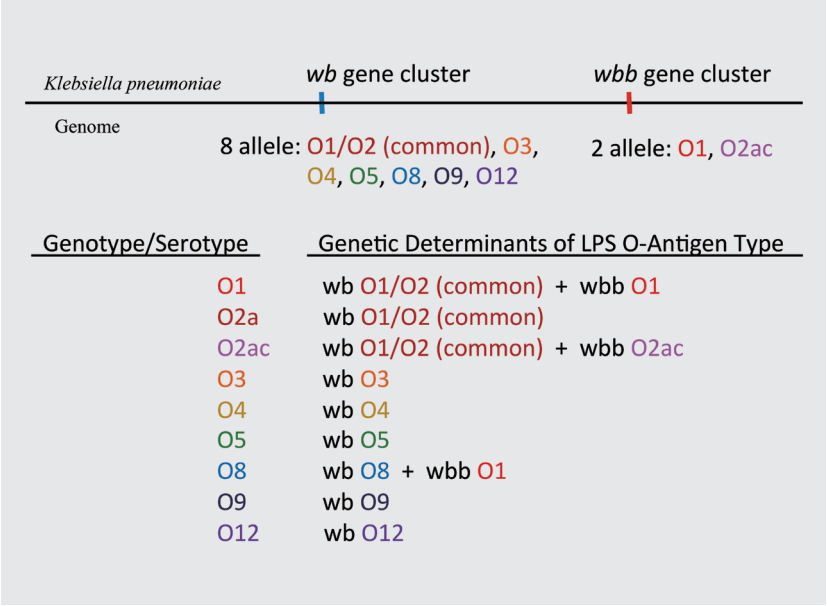

Figure 1. The two genetic determinants of lipopolysaccharide (LPS) O-antigen types in Klebsiella pneumoniae.

To develop a new molecular diagnostic method to quickly and accurately identify O1 *K. pneumoniae*, Dr. Fang and his students sequenced the genetic determinants of LPS O-antigen from O1, O2a, O2ac, O3, O4, O5, O8, O9, and O12 (Figure 1). They then successfully developed allele-specific PCR primers that detect the genes specific to O1 and other O types. His team further tested the sensitivity and specificity of the new molecular detection method against the O and K reference strains provided by the World Health Organization Collaborative Center for *Escher-*

ichia coli and *Klebsiella* at Statens Serum Institut (Copenhagen, Denmark). Both the sensitivity and specificity were found to be 100%.

Given the high accuracy and great convenience, the new PCR detection method represents an important breakthrough in the molecular diagnosis of hypervirulent *K. pneumoniae*. This new technology provides a highly useful tool for clinical and epidemiological investigations of *K. pneumoniae* and associated diseases.

Reference
Chi-Tai Fang, Yun-Jui Shih, Cheng-Man Cheong, and Wen-Ching Yi. (2016). Rapid and accurate determination of lipopolysaccharide O-antigen types in *Klebsiella pneumoniae* with a novel PCR-based O-genotyping method. *Journal of Clinical Microbiology*, 54, 666-675. DOI: 10.1128/JCM.02494-15.

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Determining the global metabolic effects of acute inhalation of nano- and fine-sized ZnO particles in the rat lung using an NMR-based metabolomic approach

Nano- and fine-sized zinc oxide (ZnO) particles are widely used for environmental and industrial applications. Previous studies revealed that inhalation of ZnO particles can induce acute occupational inhalation illnesses such as metal fume fever in humans and rats. Although studies have illustrated the association between ZnO-induced adverse effects and pulmonary inflammation, injury, and oxidative stress, the molecular mechanisms in the respiratory system are still unclear. In addition, there is debate regarding the influence of particle size on the toxicity of ZnO particles. Thus, a high-throughput approach was applied to examine the metabolic effects induced by ZnO particles.

This study was published in *Nanotoxicology* in 2016 (10(7): 924–934) and was conducted by Dr. Tsun-Jen Cheng, a professor at the Institute of Occupational Medicine and Industrial Hygiene in the College of Public Health at NTU, Dr. Ching-Yu Lin, an associate professor at the Institute of Environmental Health in the College of Public Health at NTU, and Mr. Sheng-Han Lee, a Ph. D. candidate at the Institute of Environmental Health in the College of Public Health at NTU.

A metabolomic (metabonomic) approach can record a “snapshot” of low-molecular weight metabolites to suggest plausible molecular mechanisms and develop potential biomarkers for different environmental stresses and diseases. To examine the global metabolic responses of the respiratory system of rats that inhaled ZnO particles, a nuclear magnetic resonance (NMR)-based metabolomic approach was used in rats dosed with a series of nano-sized (35 nm) or fine-sized (250 nm) ZnO particles. Bronchoalveolar lavage fluid (BALF) and lung tissues were collected for NMR instrumental analysis and subsequent multivariate statistical analyses such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA).

The results of the PCA and