Bacteriophage amplification and MALDI-TOF MS as a means of rapid *Burkholderia pseudomallei* diagnostic identification and antibiotic resistance determination

Christopher R. Cox¹, Nicholas R. Saichek², Herbert P. Schweizer², and Kent J. Voorhees¹

¹Advanced Biodetection Technology Laboratory, Colorado School of Mines, Golden CO 80401,
²Colorado State University, Department of Microbiology, Immunology and Pathology, Fort Collins, CO

## Introduction

- *Burkholderia pseudomallei* is a facultative intracellular Gram-negative pathogen and the etiologic agent of melioidosis, a debilitating disease of humans and domesticated animals.
- Disease symptoms range from acute localized septic infection resulting from exposure to contaminated soil and water, to severe pneumonia, neurologic impairment, and disseminated septicaemia. Associated mortality rates range from 20-50%.
- The Centers for Disease Control and Prevention classifies *B. pseudomallei* as a Category B priority pathogen because of its relatively widespread accessibility and ease of airborne transmission as an agent of biowarfare.
- Of greater concern with regards to potential widespread impact on human health is *B. pseudomallei*’s intrinsic resistance to many widely used antibiotics including amoxicillin, cephalosporins, macrolides, penicillin, quinolones, and rifampins. Because of this, cefazidime is the drug of choice for treatment of melioidosis.
- Reports continue to increase on the incidence of cefazidime failure during treatment of melioidosis.
- Current methods for diagnostic *B. pseudomallei* identification are time-intensive and rely on tedious culturing practices (>24 hrs) while showing insufficient capability for rapid antimicrobial resistance determination.
- Diagnostic MALDI-TOF MS has been applied to rapid clinical bacterial identification of an increasing number of human pathogens.
- However, the necessity for relatively large cell concentrations for direct bacterial protein profiling, and the requirement for exact growth conditions have somewhat delayed its widespread use. Further, such methods do not afford the capability to distinguish drug-resistant isolates.
- To address these shortcomings we employed *Burkholderia*-specific bacteriophage φK216 amplification assayed using phage-specific MALDI-TOF MS methods developed and patented (1) at the Colorado School of Mines for rapid, simultaneous identification and antibiotic resistance profiling.

## Methods

### Bacterial strains and conditions

- *Burkholderia pseudomallei* cefazidime-sensitive strain Bp82 and, cefazidime-resistant strain Bp82.3 were plated on Luria Bertani (LB) agar supplemented with 80μg/μl adenine, and incubated for 24 hrs at 37°C (2).
- A single colony was back diluted in 10ml LB broth with 80μg/μl adenine and incubated with aeration at 37°C to an optical density (OD600) of 0.15 (~4x10^5 cfu/mL) prior to phage infection.
- Antibiotic resistance determination was conducted with the addition of 15μg/mL cefazidime per reaction at the onset of infection.

### MALDI-TOF MS analysis

- Bacteria and phage samples were prepared for mass spectrometry using a ferulic acid matrix (15μg/mL) in a 17:33:50 mixture of 88% formic acid, acetonitrile, and DI water (3).
- Bacterial and phage concentrations were measured hourly by MALDI-TOF MS with a 337nm N2 laser in linear mode as well as by spot titer assay (4).

### Computational Modeling

- In silico prediction of phage infection was conducted using an algorithm (5) based on the Payne and Jansen phage therapy model (6) to approximate the point during amplification that phage concentrations would surpass MALDI-TOF MS detection threshold.
- All computations were done using Mathematica 9 (Wolfram software).

## Results

- In vitro amplification of φK216 in drug-sensitive strain Bp82 + 15μg/mL cefazidime showed no evident signal at 2hrs.
- Initial infecting phage concentration and infection reactions are allowed for simultaneous identification and differentiation of *B. pseudomallei* and *φK216* amplification readily surpassed MALDI-TOF MS detection threshold within 2 hrs of infection, whereas drug-sensitive strain Bp82 was not observed.
- Mathematical modeling of phage infection allowed accurate and reproducible pre-analysis prediction of the point in time during phage amplification when the MALDI-TOF MS detection threshold would be surpassed.

## Conclusions

- Phage amplification coupled with MALDI-TOF MS was demonstrated as a useful tool for *B. pseudomallei* identification, eliminating the need for extensive pre-analysis bacterial enrichment.
- Spectra of *B. pseudomallei* Bp82, Bp82.3 and phage φK216 were obtained by MALDI-TOF MS showing its utility for identification.
- Because of its reliance on a viable host, phage amplification readily allowed for simultaneous identification and differentiation of cefazidime-resistant *Burkholderia*.
- MALDI-TOF MS spectra clearly indicating the occurrence of phage drug resistance of *Burkholderia* were predictably observed within 2 hrs of infection, whereas drug-sensitive strain Bp82 was not observed.
- Mathematical modeling of phage infection allowed accurate and reproducible pre-analysis prediction of the point in time during phage amplification when the MALDI-TOF MS detection threshold would be surpassed.

## References


## Acknowledgements

This work was funded by:
- NIH Career Development Award USA A006357
- DTRA Award W81XWH-07-C-0061