Measuring hydrolytic activity of carbapenemase-producing *Klebsiella pneumoniae* isolates

Cole Hudson, Dr. Vincent Tam
Department of Pharmacy Practice and Translational Research

UNIVERSITY of HOUSTON

Background

The overuse of antibiotics for decades has led to the emergence of antibiotic-resistant bacteria, some of which are immune to nearly all available drugs. The CDC reported over 35,000 deaths and 2.8 million infections from antibiotic-resistant bacteria in the U.S. in 2017 alone[1]. Novel therapies designed to treat resistant bacterial infections are of great importance.

Identification of the enzymes responsible for antibiotic resistance is a key step in selecting appropriate therapies. *Klebsiella pneumoniae* carbapenemases (KPCs) have been identified as resistance enzymes in highly drug-resistant gram-negative bacterial infections[2,3]. Understanding the activity of these resistance enzymes is necessary for optimizing current antibiotic treatments and generating effective new therapies.

Results

Clinical isolates KP286 and KP1575 both demonstrated significant hydrolytic activity against Imipenem as compared to the negative control. This is demonstrated by a loss of absorbance at 299 nm (**Fig. 1**). The degradation of Imipenem is quantified in **Fig. 2** and indicates that the unknown, KP286, contains significant levels of carbapenemase. KP286 demonstrated higher activity than the positive control strain even when normalized for total protein concentration suggesting that it either produces more carbapenemase or contains a more efficient enzyme (**Fig.3**) .

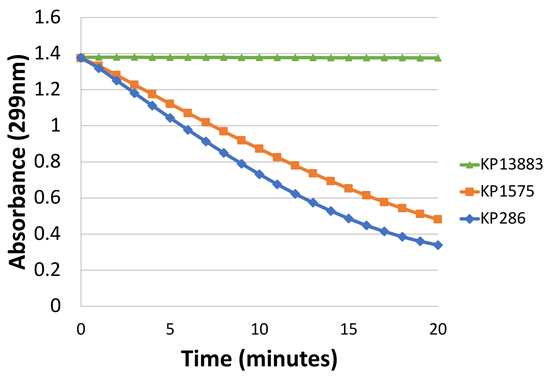

Figure 1. Absorbance at 299nm measured for 20 minutes after the addition of crude cell lysate

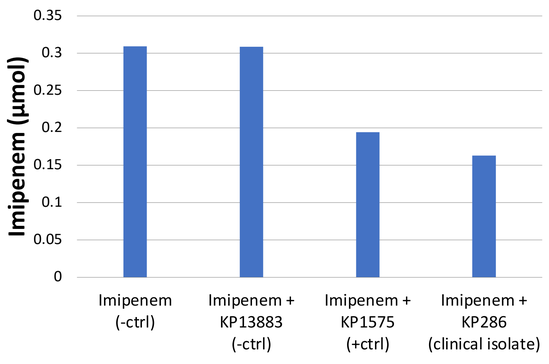

Figure 2. Levels of Imipenem measured 10 minutes after the addition of cell lysate compared to Imipenem in solution (-ctrl)

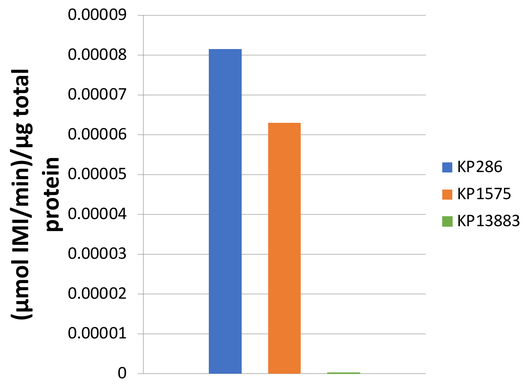

Figure 3. Enzymatic activity ((µmol IMI/min)/µg total protein) normalized for total cellular protein concentration

Conclusions

The results indicate that KP isolates vary in their rate of carbapenemase activity even when normalized for total protein concentration. This suggests that a fixed dosing regimen for antibiotic treatment may not be optimal.

Future work on this project will involve using the established assay to identify more resistant clinical isolates and quantify their enzymatic activity.

Methodology

- Bloodstream isolates of *Klebsiella pneumoniae* (KP) demonstrating resistance to carbapenem class antibiotics were identified.
- Enzymatic activity of crude cell lysate was quantified by spectrophotometric assay of Imipenem (IMI) degradation.
- KP13883 was used as a negative control, clinical isolate KP1575 served as a positive control, and clinical isolate KP286 was an unknown.
- Total protein content was measured using Pierce BCA protein assay and used to normalize enzymatic activity.

References

1. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.
2. Arnold, R. S., Thom, K. A., Sharma, S., Phillips, M., Kristie Johnson, J., & Morgan, D. J. (2011). Emergence of Klebsiella pneumoniae carbapenemase-producing bacteria. *Southern medical journal*, *104*(1), 40–45.
3. Henrietta Abodakpi, Kai-Tai Chang, Ana María Sánchez Díaz, Rafael Cantón, Todd M. Lasco, Katrina Chan, Amelia K. Sofjan & Vincent H. Tam (2018) Prevalence of extended-spectrum beta-lactamase and carbapenemase-producing bloodstream isolates of Klebsiella pneumoniae in a tertiary care hospital, Journal of Chemotherapy, 30:2, 115-119

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Contact information

Vincent Tam
VHTam@central.uh.edu