Performance Of Two Methods For Detection Of Carbapenem Resistance Mechanisms Among Enterobacteriaceae

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Background

Multi-drug resistance organisms (MDROs), especially MDR gram-negative bacteria, are of clinical and epidemiological importance, causing both therapeutic and diagnostic challenges worldwide. Of particular concern are carbapenem-resistant Enterobacteriaceae (CRE), whose resistance is conferred by the presence of extended spectrum β-lactamases (ESBL), AmpC enzymes and/or carbapenemases, often in combination with a permeability defect. Knowledge of the resistance mechanism is critical to epidemiological studies, but few laboratories have the capacity to routinely evaluate for mechanisms of resistance among carbapenem-resistant Enterobacteriaceae (CRE). From Jan 2011 thru Jan 2014, UCLA isolated 233 CRE from clinical specimens (non-duplicate), 57.5% of which contained a carbapenemase when tested by a laboratory-developed real-time PCR (RT-PCR), and 42.5% negative for any of the targeted genes (Figure 1). The purpose of this study was to evaluate the accuracy of a laboratory-developed CRE PCR in comparison to Acuitas® Resistome Test, a rapid and comprehensive molecular genotyping assay for MDRO isolates.

Methods

Bacterial isolates

- Select clinical isolates (N=20) of Enterobacteriaceae non-susceptible to imipenem or meropenem (i.e. MIC >1 μg/ml) by reference broth microdilution were included in the study.
- Isolates were subcultured onto sheep’s blood agar plates from frozen glycerol stocks prior to molecular analysis.

UCLA lab-developed real-time PCR (RT-PCR)

- DNA was extracted using the NucliSENS easyMAG (bioMérieux).
- Three multiplexed Taqman-based real-time PCR reactions (Table 1) were performed on each isolate, as previously described by Pollet et al. (1).
- A previously published alternate multiplex PCR assay targeting OXA-23-like and OXA-51-like genes (2) was used for discordant analysis of 1 isolate.

OpGen Acuitas® Resistome Test (OpGen Inc. Gaithersburg, MD)

- Testing of clinical isolates was performed by OpGen Clinical Services Laboratory using the Acuitas Resistome Test. The OpGen Acuitas Resistome Test is a microfluidic PCR array that analyzes culture isolates from Gram-negative bacilli for approximately 50 antibiotic resistance genes families across several hundred variants associated with MDROs. The targets include genes that encode carbapenemases, ESBLs, and AmpC β-lactamase.
- In brief, 100 μl of total nucleic acid was extracted from each culture isolate. PCR amplifications specific to the Acuitas Resistome Test
- Targets were performed using the template from each sample and primers and fluorescent reporter probe for each target.
- Results were analyzed on a BioMark HD System (Fluidigm).

Results

- In total, 10 Klebsiella pneumoniae, 4 Escherichia coli, 2 Citrobacter freundii, 2 Enterobacter aerogenes, 1 E. cloacae, and 1 Providencia rettgeri were tested.
- 16/20 isolates yielded initial concordant carbapenemase results by the Acuitas Resistome Test and RT-PCR (Table 2).
- These included 1 KPC, 2 IMP, 2 NDM and 1 OXA-48-like enzymes, and 10 isolates negative for carbapenemase genes targeted in the RT-PCR.

Conclusions

- The Acuitas Resistome Test performed comparably to RT-PCR for the detection of carbapenemases.
- Furthermore, this test provides data on resistance mechanisms outside the presence of carbapenemases, helping to resolve some UCLA CRE that were negative by the lab-developed RT-PCR.
- Laboratories should be aware that discordant results might be observed in CRE, one possibility being due to plasmid loss.

References