Acuitas® Whole Genome Sequence Analysis (WGS) is a high-resolution tool to identify a full spectrum of antibiotic resistance genes and strain type in multidrug-resistant (MDRO) pathogens. WGS-based Resistome analysis is an essential component of a comprehensive infection control plan to effectively manage and control multidrug-resistant organisms (MDROs).

### Methods and Materials

**Clinical Samples**

Total of seventeen clinical microbe isolates (eleven K. pneumoniae and six E. coli) from two institutes were sequenced with MiSeq (Illumina). Institution names were blinded and sample names were coded to protect identity. In addition, twenty isolates from the National Institutes of Health (NIH) were also used to validate OpGen Acuitas Whole Genome Sequence Analysis pipeline.

**Analysis Work Flow**

Assembly: The whole genome sequences of clinical isolates were assembled from Illumina MiSeq 2x250bp sequencing data using assembler Velver.

**Determination of the resistance genes variants**

To determine the variants of antibiotic resistance genes, the whole genome sequences were used to query antibiotic resistance gene databases created at OpGen (Gaithersburg, MD, USA).

**MLST+ Schema and strain typing**

The MLST+ targets of each species were defined at OpGen in conjunction with Ridom. Strain typing of isolates was conducted with Ridom SeqSphere+ software using assembled whole genome sequences.

### Results

1. **Acuitas whole genome sequence methods detected more resistance genes from clinical isolates than traditional methods (Table 1).** Resistance gene profiles have previously been established for seven isolates (six E.coli and one K. pneumoniae) with traditional methods by our collaborator. Using whole genome sequencing, we have been able to confirm most of the resistance genes. We can also detect more antibiotic resistance genes: CMY-6 in Sample 586301, CMY-7 in sample 4492611, TEM-1 in sample 6295541 and TEM-1 in sample 8491211.

![Figure 1. High resolution strain typing with Acuitas Whole Genome Sequence Analysis.](image)

#### Table 1. Acuitas Whole Genome Sequence Analysis detected more antibiotics resistance genes than traditional methods based on PCR-Sanger DNA sequencing.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acuitas WGS</th>
<th>MLST+</th>
<th>PCR-Sanger</th>
<th>Sanger</th>
<th>SeqSphere+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td>Acuitas WGS</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sample2</td>
<td>Acuitas WGS</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

2. **Acuitas whole Genome Sequence Analysis can resolve closely related resistance gene variants (Table 2, 3).** Acuitas Resistome Test (OpGen) detect the resistance gene families in clinical isolates. Acuitas Whole Genome Sequence Analysis not only confirmed all resistance gene families detected by Acuitas Resistome Test, but also provided variant information of these resistance genes in 10 clinical isolates (Table 2) and 19 clinical samples obtained from NIH (Table 3).

![Figure 2. Comparison of Acuitas Whole Genome Sequence Analysis strain typing and strain typing based on Whole Genome Mapping.](image)

#### Table 2. Acuitas Whole Genome Sequence Analysis resolved closely related antibiotic resistance genes in ten K. pneumoniae isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Acuitas WGS</th>
<th>MLST+</th>
<th>PCR-Sanger</th>
<th>Sanger</th>
<th>SeqSphere+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate1</td>
<td>Acuitas WGS</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Isolate2</td>
<td>Acuitas WGS</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

3. **Acuitas Whole Genome Sequence Analysis efficiently strain types clinical isolates.**

Using whole genome sequences of 10 K. pneumoniae isolates from NIH dataset, Ridom SeqSphere+ correctly placed two outbreak strains together (Figure 1 (A), KPNH1 and KPNH10) with the K.pneumoniae MLST+ targets defined. It also placed two strains sharing genetic similarity in the same group (Figure 1(A): KPR0928 and KPNH301). (2) Using whole genome sequences from 8 E.coli isolates from Table 1 and three biological replicates of an additional E.coli strain, the MLST+ correctly identified three biological replicates as the same (Figure 1(B)).

![Figure 3. Comparison of Acuitas Whole Genome Sequence Analysis strain typing and strain typing based on Whole Genome Mapping.](image)

#### Table 3. Acuitas Whole Genome Sequence Analysis resolved closely related antibiotic resistance genes in 19 clinical isolates (Biological materials associated with these results were contributed by Dr. Karen Frank, National Institutes of Health, Sci. Transl. Med. 6, 254ra126 (2014). Note: * These genes were detected as two parts in two contigs. + These genes are new variants.}

![Table 3](image)

### Conclusion

1. Whole genome sequencing is a high resolution tool to identify full spectrum of antibiotic resistance genotypes and resolve closely related variants of antibiotic resistance genes.
2. Acuitas Resistome Test accurately detected the same antibiotic resistance gene families as the Acuitas Whole Genome Sequence Analysis; the whole genome sequencing test provided additional resolution by identifying the specific variants of the same genes.
3. Acuitas Whole Genome Sequence Analysis can strain type closely related clinical bacterial isolates using thousands of MLST+ targets. The results will help prevention and control of hospital acquired infections (HAIs) along with improved antibiotic stewardship toward reduction of infection rates and patient length of stay as well as better patient outcomes.