Comprehensive Analysis of Antibiotic Resistance in Multidrug-Resistant Organisms (MDROs) by Whole Genome Sequencing using Acuitas® Whole Genome Sequence Analysis

Weizhong Chang, Rossio Kersey, Alex Saeed, Vadim Sapiro, Terry Walker

OpGen, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878, USA.

Abstract

The timely and efficient determination of the antibiotic resistance genes in clinical isolates is crucial for the prevention of outbreaks and the treatment of patients. In this study, we developed pipelines to comprehensively analyze antibiotic resistance genes in carbapenem-resistant Enterobacteriaceae (CREs) and extended spectrum beta-lactamase (ESBL) producers using Acuitas® Whole Genome Sequence Analysis with next generation sequencing (NGS) technology.

To be able to comprehensively determine the resistance genes in clinical isolates of MDROs, we built a database consisting of all beta-lactamase variants with NCBI accession numbers from Lahey Clinic (http://www.lahey.org/Studies/). All genes of beta-lactamases are manually curated for the coding sequences with start codon and stop codon if those exist.

The database was tested using whole genome sequence (WGS) data assembled from Illumina MiSeq data generated on eight species of clinical isolates: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumanii, Enterobacter cloacae, Citrobacter freundii, Klebsiella oxytoca, and Serratia marcescens;* all such isolates were reported to harbor CRE and ESBL antibiotic resistance genes based on results of Sanger sequencing technology and the Acuitas® Resistome Test. Using Acuitas Whole Genome Sequence Analysis, we resolved closely related gene variants across the antibiotic resistance gene families KPC, NDM, OXA, CTX-M, CMY, TEM, SHV, ACT, IMP, VIM, DHA, PER, and VEB in these clinical isolates. For example, WGS resolved single nucleotide differences between gene variants KPC-2 and KPC-3, or single nucleotide differences between NDM-1 and NDM-4. Similarly, WGS resolved closely related gene variants 2, 3, 14, 15, and 79 of CTX-M.

The depth of our database facilitated our discovery of antibiotic resistance genes which were previously reported for these clinical isolates. Furthermore, our variant determination isn't limited to the contents of the database we developed. If the homologous sequence of a resistance gene is identified but identical to the gene variant in the database, the coding sequence will be retrieved and searched against NCBI database to find the identical gene. If the identical variant still can't be found, the gene is reported as a new variant of that family of beta-lactamases and then dynamically added to our database.

In conclusion, we have created a database consisting of all beta-lactamase genes from Lahey Clinic website. Using the database with the Acuitas Whole Genome Sequence Analysis pipeline, we can comprehensively determine antibiotic resistance genes in multidrug-resistant organisms (MDROs), providing tools to help the prevention of outbreaks and the treatment of patients.

Objectives

To develop pipelines to comprehensively analyze antibiotic resistance genes in carbapenem-resistant Enterobacteriaceae (CREs) and extended spectrum beta-lactamase (ESBL) producers using Acuitas Whole Genome Sequence Analysis with next generation sequencing (NGS) technology.

Methods and Materials

Creation of Databases

A database was created at OpGen (Gaithersburg, MD, USA) based on the gene list from Lahey Clinic. All genes of beta-lactamases are manually curated for the coding sequences with start and stop codons if those exist.

Clinical Samples

Total of 69 clinical isolates of eight species: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumanii, Enterobacter cloacae, Citrobacter freundii, Klebsiella oxytoca, and Serratia marcescens* have been chosen for the validating the analysis workflow of Acuitas Whole Genome Sequence Analysis.

Analysis Work Flow

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

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.

Validation of Analysis of Antibiotic Resistance using Acuitas Whole Genome Sequence Analysis: 69clinical isolates were tested for antibiotic resistance using both Acuitas Resistome Test and Acuitas Whole Genome Sequence Analysis. The results were compared to assess the accuracy and sensitivity of the Acuitas Whole Genome Sequence Analysis. The reproducibility of Acuitas Whole Genome Sequence Analysis workflow has been further validated by five replicated culture of one isolate for each species.

Results

1. **Acuitas Whole Genome Sequence Analysis can resolve closely related gene variants across the antibiotic resistance gene families:** We have been able to resolve closed related variants of all species we tested. In Table 1, we presented the test results for CREs and ESBLs in seven *E.coli,* four *C. freundii* and four *E. cloacae* isolates.

Table 1. Determination of antibiotic resistance genes *in E.coli*, C. *freundii* and *E.cloacae* isolates. Resistance genes with colored background aren't present in Lahey resistance gene list: blue - identical to the resistance gene in NCBI databases; red – new variant of the resistance gene.

E. coli

	CTX-M	CMY	KPC	NDM	OXA	SHV	TEM
4492611		CMY-7		NDM-1		SHV-12	TEM_AIT37459.1
5860301	CTX-M-15	CMY-6		NDM-4			
6295541			KPC-2				TEM-1
8464341	CTX-M-15				OXA-1		
8491211			KPC-3				TEM_AIE31043.1
8714631	CTX-M-55						
8728551	CTX-M-14				OXA-48		TEM-1

C. freundii

Sample	OXA	VIM	CTX-M	TEM	CMY	SHV	IMP	FOX
871537	OXA-1		CTX-M-3	TEM-1	CMY-34			
1083058				TEM-1	CMY-New			FOX-3
924198		VIM-1			CMY-48	SHV-AKJ19228 KP975077		
931525	OXA-142				CMY-110		IMP-1	

E. cloacae

Sample	ACT	VEB	OXA	VIM	CTX-M	TEM	NDM
584208	ACT-KJ949095						
586750	ACT-DQ478710	VEB-1	OXA-2, 10	VIM-5			
686828	ACT-KJ949098		OXA-2		CTX-M-2	TEM-1	
874361	ACT-new			VIM-4	CTX-M-3, 14	TEM-1	NDM-1

2. **Validation of the reproducibility of Acuitas Whole Genome Sequence Analysis:** We have selected one isolate from each species. These isolates were cultured in five replicated cultures and the DNA from these cultures were sequenced and the antibiotic resistance genes profiles were determined by Acuitas Whole Genome Sequence Analysis. As shown in Table 2, Five technical replicates of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* have the same antibiotic resistance profile.

Table 2 The antibiotic resistance profile of five technical replicates of one *P. aeruginosa* and one *A. baumanii* (100X coverage). The same antibiotic resistance gene profiles for all five replicates of each species demonstrated the reproducibility of the Acuitas Whole Genome Sequence Analysis.

P. aeruginosa

Samples	OXA		VEB	IMP
TR1	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR2	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR3	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR4	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR5	OXA-10	OXA-50g	VEB-1a	IMP-14a

A. baumanii

Sample	OXA		TEM
TR1	OXA-23	OXA-66	TEM-1(AGW28875)
TR2	OXA-23	OXA-66	TEM-1(AGW28875)
TR3	OXA-23	OXA-66	TEM-1(AGW28875)
TR4	OXA-23	OXA-66	TEM-1(AGW28875)
TR5	OXA-23	OXA-66	TEM-1(AGW28875)

3. **Determination of Coverage Threshold for Acuitas Whole Genome Sequence Analysis:** To determine the coverage threshold for the analysis, we have down-sampled the reads from the technical replicates described in section 2. for all 8 species we are currently studying in this project. For most species, all resistance genes keep being intact from 100X coverage down sampled to 70X. For some species, if the coverage is reduced to 60X, some antibiotic resistance genes were broken into pieces and become impossible to assign into a specific variant and we cannot determine if isolate has the complete resistance gene with high confidence. Table 3 shows coverage threshold analysis of the *Pseudomonas aeruginosa.*

Table 3. Coverage threshold analysis of the *Pseudomonas aeruginosa.* At 60X VEB-1a will be split into two pieces and the existence of the complete gene can't be determined with high confidence.

70X Coverage

Samples	OXA		VEB	IMP
TR1	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR2	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR3	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR4	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR5	OXA-10	OXA-50g	VEB-1a	IMP-14a

60X Coverage

Samples	OXA		VEB	IMP
TR1	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR2	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR3	OXA-10	OXA-50g	VEB-1a	IMP-14a
TR4	OXA-10	OXA-50g	VEB-1a (385, 601)	IMP-14a
TR5	OXA-10	OXA-50g	VEB-1a	IMP-14a

4. **Validation of Sensitivity and Specificity of Acuitas Whole Genome Sequence Analysis:** To prove the sensitivity and specificity of Acuitas Whole Genome Analysis, we performed Acuitas Resistome Test and Acuitas Whole Genome Analysis of 48 clinical isolates. Table 4 lists validation results of 14 clinical isolates from seven species. As shown in the table, All the results from Acuitas Whole Genome Sequence Analysis are consistent with the Acuitas Resistome Test. Some name differences come from Acuitas Resistome Test naming convention which reflect the detection of several variants with the same pair of the probes.

Table 4 Validation of Sensitivity and Specificity of Acuitas Whole Genome Sequence Analysis. Fourteen clinical isolates were analyzed by Acuitas Resistome and Acuitas Whole Genome Sequence Analysis.

Samples	Organism	Acuitas Resistome	Whole Genome Sequencing
920964	*A. baumannii*	OXA-51	Oxa-58, Oxa-69
936844	*A. baumannii*	OXA-24; OXA-51; TEM-G238/E240; TEM-R164; TEM-E104	OXA-66; OXA-72; TEM-1
948753	*C. freundii*	CMY-2; OXA-2; TEM-G238/E240; TEM-R164; TEM-E104	CMY-AJ746169; OXA-2; TEM-1
931525	*C. freundii*	CMY-2; IMP-1; OXA-10	OXA-142; CMY-2; IMP-1
847268	*E. cloacae*	CTX-M-2; ACT-1/MIR-1; OXA-2; TEM-G238/E240; TEM-R164; TEM-E104	OXA-2; TEM-1; ACT-25; ; CTX-M-2
924384	*E. cloacae*	CTX-M-1; ACT-1/MIR-1; OXA-10	CTX-M-KJ802507; ACT-33; OXA-101
600277	*E. coli*	CTX-M-9; OXA-10	CTX-M-65; OXA-10
686817	*E. coli*	CTX-M-9; CTX-M-1	CTX-M-15;24;
N/A	*K. pneumoniae*	KPC-2; SHV-G238S/E240K; SHV-G156	KPC-2; SHV-12
921030	*K. pneumoniae*	KPC-2; OXA-10; VEB-1; SHV-G238/E240; SHV-G156; TEM-G238/E240;TEM-R164; TEM-E104	OXA-9;10; TEM-1; KPC-2; SHV-11; VEB-1
926467	*P. aeruginosa*	IMP-2; OXA-10; OXA-50	OXA-10; new2; IMP-48
959963	*P. aeruginosa*	IMP-2; OXA-10; OXA-50; VEB-1	OXA-10; OXA-50; VEB-9; IMP-14
874608	*S. marcescens*	Negative results for all reported genes.	All negative
450082	*S. marcescens*	CTX-M-2; OXA-2; TEM-G238/E240; TEM-R164; TEM-E104	CTX-M-2; OXA-2; TEM-1

Conclusion

1. We have created a database consisting of all beta-lactamase genes from the Lahey Clinic website.
2. Using the database with the Acuitas Whole Genome Sequence Analysis pipeline, we can comprehensively determine antibiotic resistance in multidrug-resistant organisms (MDROs).

Acuitas Whole Genome Sequence Analysis is for research use only and not for use in diagnostic procedures.