

# Strain Typing and High Resolution Analysis of Antibiotic Resistance in Multidrug-Resistant Organisms (MDROs) by Whole Genome Sequencing

Weizhong Chang, Alex Saeed, Vadim Sapiro, Terry Walker

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



## Abstract

Better methods are required to detect antibiotic resistance genes and strain type MDROs such as carbapenem-resistant Enterobacteriaceae (CRE) and extended spectrum beta-lactamase (ESBL) producers. We developed an analysis pipeline to resolve closely related antibiotic resistance genes and strain type CRE and ESBL producers using whole genome sequencing.

Using data generated with Illumina MiSeq<sup>®</sup>, Acuitas<sup>®</sup> Whole Genome Sequence Analysis was performed on clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* reported to harbor CRE and ESBL antibiotic resistance genes based on Sanger sequencing technology. Acuitas Whole Genome Sequence Analysis resolved closely related gene variants across the antibiotic resistance gene families KPC, NDM, OXA-48, CTX-M, CMY, TEM and SHV in these clinical isolates. For example, single nucleotide differences were resolved between gene subtypes KPC-2 and KPC-3 and single nucleotide differences between NDM-1 and NDM-4. Similarly, closely related gene subtypes 14, 15, 55 and 79 were resolved for CTX-M. An analysis pipeline was built to interrogate clinical isolates for additional antibiotic resistance gene variants associated with carbapenem resistance, ESBL producers and AmpC beta lactamases. The analysis pipeline identified previously unreported antibiotic resistance genes for these clinical isolates.

Additionally, Ridom<sup>®</sup> SeqSphere+ software was used for MLST+ analyses of thousands genes to resolve closely related clinical strains of *E. coli* and *K. pneumoniae*. We compared dendrograms of strain types based on MLST+ sequence analysis with dendrograms based OpGen's Whole Genome Mapping<sup>™</sup> System. Results demonstrated the high resolution of Acuitas Whole Genome Sequencing Analysis and the complementary nature of the two genome technologies in terms of genome resolution, cost, and time-to-results for fast and effective strain typing to track clinical isolates from hospital outbreaks or epidemiology studies.

Acuitas Whole Genome Sequence Analysis is a high resolution tool to identify a full spectrum of antibiotic resistance genotypes and strain type closely related clinical bacterial isolates for 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.

## Objectives

To develop methods to detect and resolve closely related antibiotic resistance genes, and strain type MDROs such as CRE and ESBL producers using whole genome sequences with next generation sequencing technology. The goal is to provide high resolution component of MDRO surveillance system provided by OpGen, Inc.

## Methods and Materials

### Clinical Samples

Total of seventeen clinical microbe isolates (eleven *K. pneumoniae* and six *E.coli*) from two institutes have been 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 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 (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.

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

## Results

**1. Acuitas whole genome sequence methods detected more resistance genes from clinical isolates than traditional methods** (Table1). 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 5860301, CMY-7 in sample 4492611, TEM-1 in sample 6295541 and TEM AIE31043.1 in sample 8491211.

Sample	Traditional Methods							Acuitas Whole Genome Sequence Analysis							Organism	
	CTX-M	CMY	KPC	NDM	OXA	SHV	TEM	CTX-M	CMY	KPC	NDM	OXA	SHV	TEM		
611		CMY-2		NDM-1		SHV-12	TEM								AIT37459.1	<i>E.coli</i>
301	CTX-M-15	CMY-2		NDM-4				CTX-M-15	CMY-2,6		NDM-4		SHV-12			<i>E.coli</i>
541			KPC-2							KPC-2				TEM-1		<i>K.pneumoniae</i>
341	CTX-M-15				OXA-163			CTX-M-15				OXA-1				<i>E.coli</i>
211			KPC-3							KPC-3				AIE31043.1		<i>E.coli</i>
631	CTX-M-55,79							CTX-M-55								<i>E.coli</i>
551	CTX-M-14				OXA-48		TEM-1	CTX-M-14				OXA-48		TEM-1		<i>E.coli</i>

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

**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 Sequencing 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).

Sample	Acuitas Resistome Test				Acuitas Whole Genome Sequence Analysis				Orgnism
	Antibiotic Resistance Gene Family				Antibiotic Resistance Genes				
F11	KPC	OXA-2	SHV	TEM	KPC-3	OXA-2	SHV-11	TEM-1	<i>K.pneumoniae</i>
F12	KPC	OXA-2	SHV	TEM	KPC-3	OXA-2	SHV-11	TEM-1	<i>K.pneumoniae</i>
F25	KPC	OXA-2	SHV	TEM	KPC-3	OXA-2	SHV-11	TEM-1	<i>K.pneumoniae</i>
F28	KPC	OXA-2	SHV	TEM	KPC-3	OXA-2	SHV-11	TEM-1	<i>K.pneumoniae</i>
F13	KPC	OXA-2	SHV	TEM	KPC-3	OXA-2	SHV-11	TEM-1	<i>K.pneumoniae</i>
F29	KPC	OXA-2	SHV	TEM	KPC-2	OXA-2	SHV-11	TEM-1	<i>K.pneumoniae</i>
F19	KPC		SHV-ESBL	TEM	KPC-2		SHV-12	TEM-1	<i>K.pneumoniae</i>
F30	KPC		SHV-ESBL	TEM	KPC-2		SHV-12	TEM-1	<i>K.pneumoniae</i>
F34	KPC		SHV	TEM	KPC-2		SHV-11	TEM-1	<i>K.pneumoniae</i>
F31	KPC CTX-M	OXA-50	SHV	TEM	KPC-2 CTX-M-15	OXA-50	SHV-28	TEM-1	<i>K.pneumoniae</i>

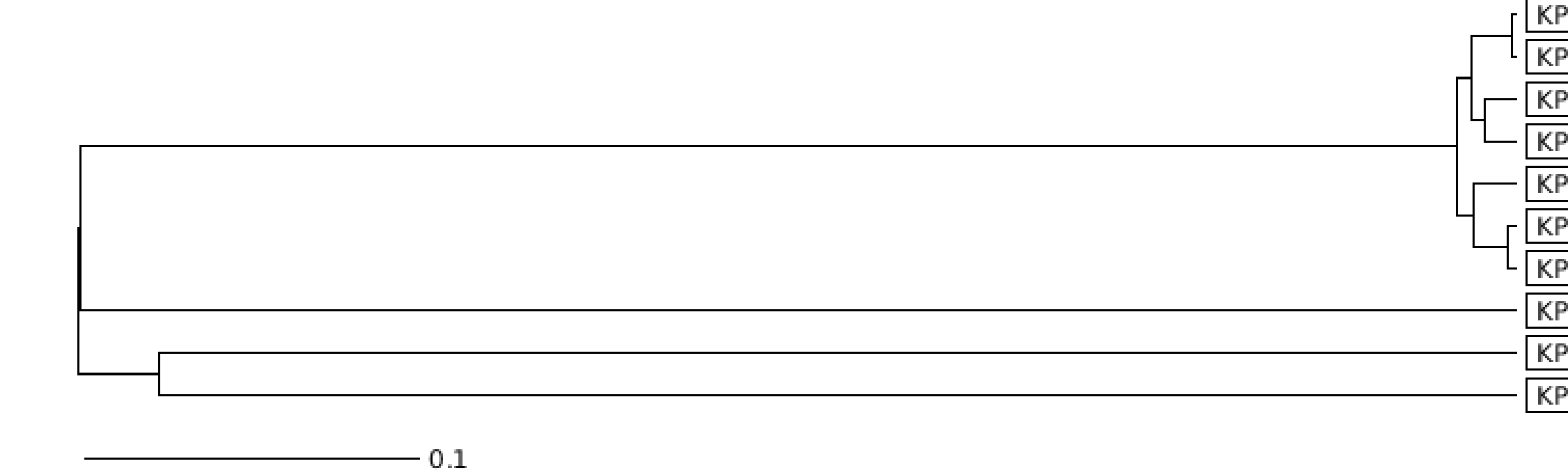
**Table 2.** Acuitas Whole Genome Sequence Analysis resolved closely related antibiotic resistance genes in ten *K. pneumoniae* isolates.

**3. Acuitas Whole Genome Sequence Analysis efficiently strain types clinical isolates.** (1) Using whole genome sequences of 10 *K. pneumoniae* isolates from the NIH dataset, Ridom SeqSphere+ correctly placed two outbreak strains together (Figure 1 (A), KPNIH1 and KPNIH10) with the *K.pneumoniae* MLST+ targets we defined. It also placed two strains sharing genetic similarity in the same group (Figure 1(A): KPR0928 and KPNIH30). (2) Using whole genome sequences from 6 *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)).

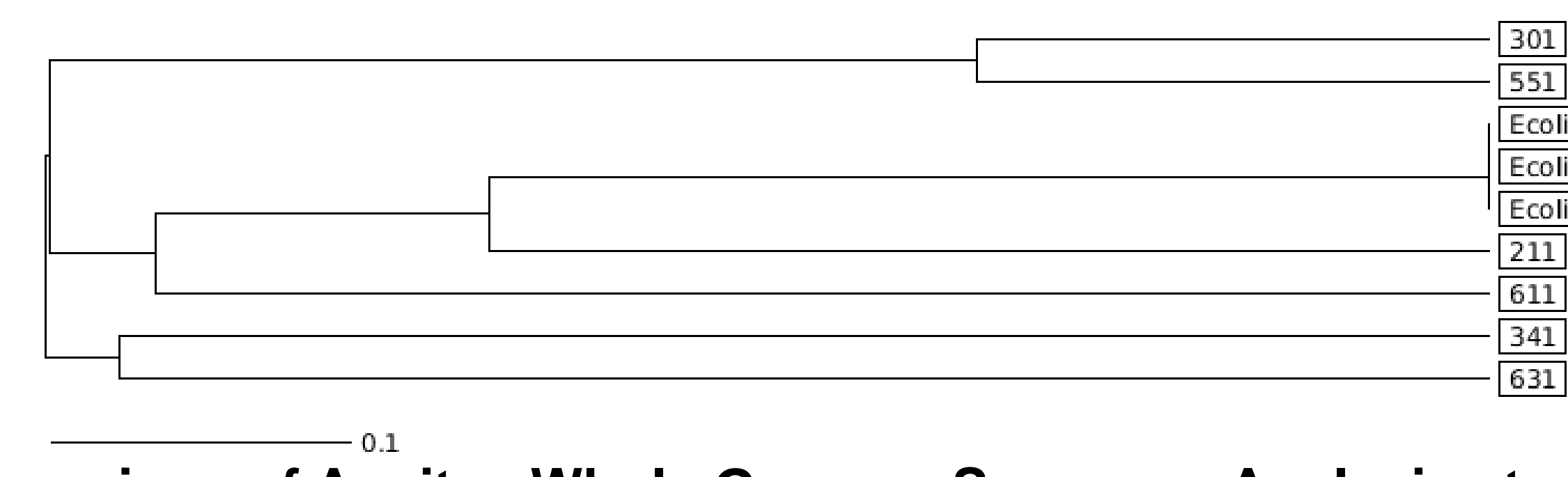
Sample	Acuitas Resistome Test				Acuitas Whole Genome Sequence Analysis				Organism				
	Antibiotic Resistance Gene Family				Antibiotic Resistance Gene								
ECONIH1	KPC		TEM	CTX-M	KPC-2		TEM-1		CTX-M-15/15	<i>E. coli</i>			
KPNIH31	KPC	SHV		CTX-M	KPC-2	SHV*			CTX-M-15*	<i>K. pneumoniae</i>			
KPNIH27	KPC	SHV	ESBL	FOX	KPC-2	SHV-5/12/26	TEM-1	FOX-5		<i>K. pneumoniae</i>			
KONIH1	KPC		TEM	FOX	KPC-2/2		TEM-1*	FOX-5		<i>K. oxytoca</i>			
CFNIH1	KPC	SHV	ESBL	FOX	KPC-2	SHV-5		FOX-5		<i>C. freundii</i>			
PSNIH2	KPC	SHV	ESBL	FOX	KPC-2	SHV-5		FOX-5		<i>Pantoea</i>			
ECNIH2	KPC	SHV	ESBL	TEM	FOX	ACT		KPC-3/3/2	SHV-5/12/26	TEM-1/1	FOX-5	ACT-DQ478712	<i>E. cloacae</i>
ECNIH5	KPC	SHV	ESBL	TEM	ACT			KPC-2	SHV-12	TEM-1		ACT-KJ949120	<i>E. cloacae</i>
ECNIH3	KPC	SHV	ESBL		ACT			KPC-2	SHV-12			ACT-KJ949120	<i>E. cloacae</i>
PSNIH1	KPC	SHV	ESBL					KPC-2	SHV-5				<i>E. cloacae</i>
KPNIH29	KPC	SHV	TEM					KPC-3	SHV-11	TEM*			<i>K. pneumoniae</i>
KPNIH1	KPC	SHV	TEM					KPC-3	SHV-11	TEM-1			<i>K. pneumoniae</i>
KPNIH10	KPC	SHV	TEM					KPC-3	SHV-11	TEM-1			<i>K. pneumoniae</i>
KPNIH33	KPC	SHV	TEM					KPC-3/3/3	SHV-11	TEM-1			<i>K. pneumoniae</i>
KPNIH30	KPC	SHV	TEM					KPC-2	SHV-11	TEM-1			<i>K. pneumoniae</i>
KPR0928	KPC	SHV	TEM					KPC-2	SHV-11/12	TEM-1			<i>K. pneumoniae</i>
KPNIH24	KPC	SHV	ESBL	TEM				KPC-2	SHV-11/12	TEM-1			<i>K. pneumoniae</i>
KPNIH32	KPC	SHV	ESBL	TEM				KPC-3	SHV-11/12	TEM-1			<i>K. pneumoniae</i>
ECNIH4	KPC	SHV	ESBL	TEM				KPC-2	SHV-12	TEM-1			<i>E. cloacae</i>

**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 gene variants.

A



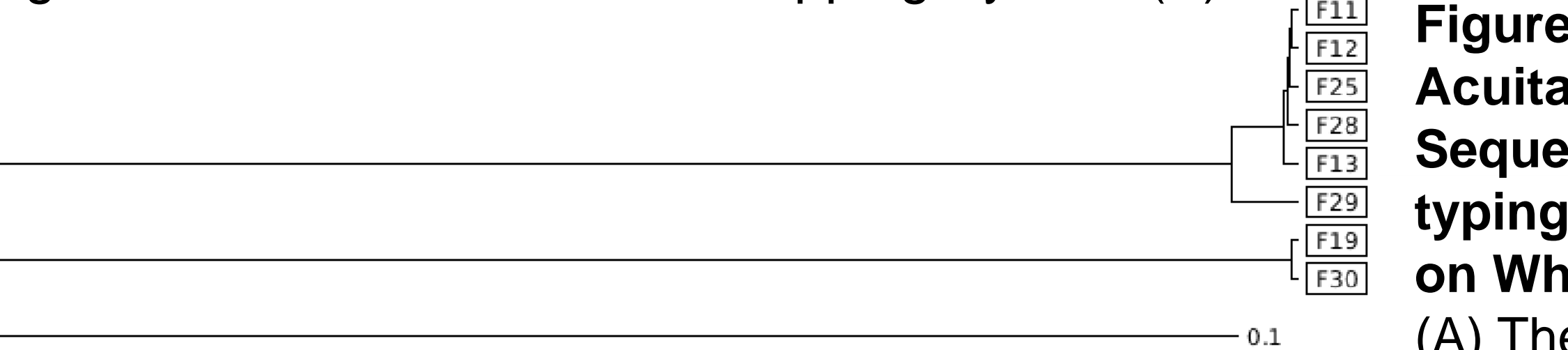
B



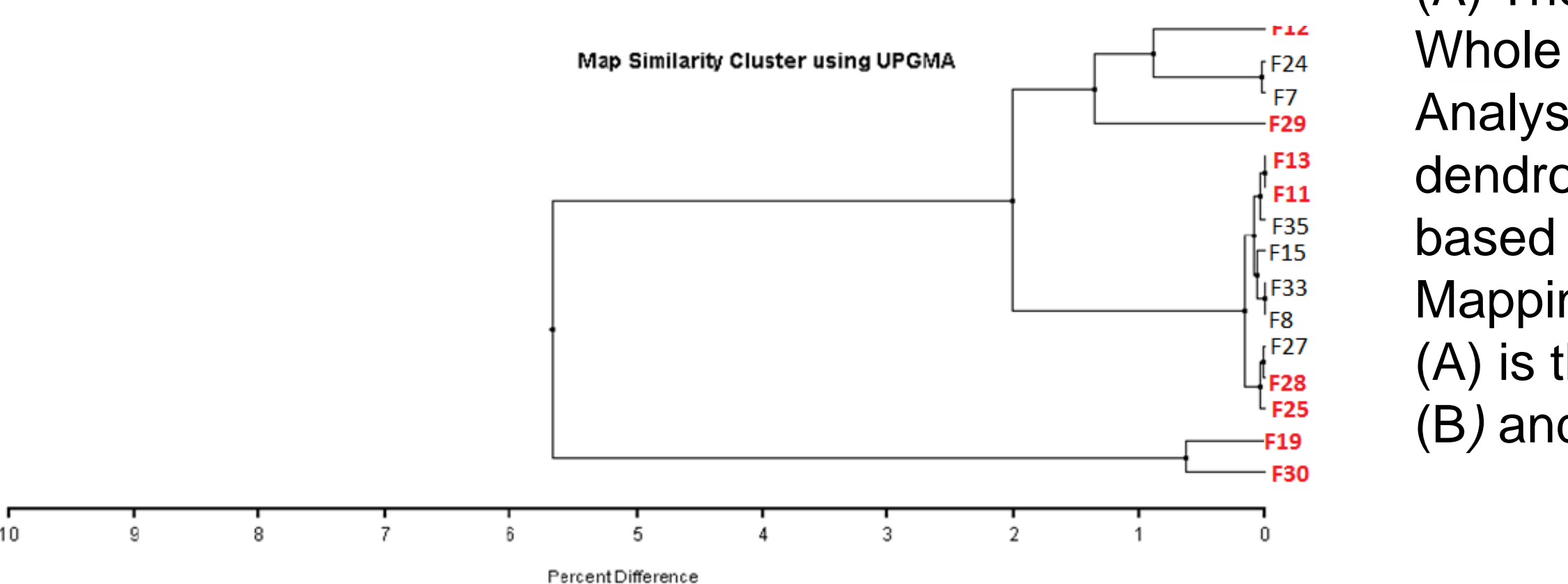
**Figure 1. High resolution strain typing with Acuitas Sequence Analysis.** (A) Strain typing of 10 *K.pneumoniae* isolates from NIH dataset in Table 3. (B) Strain typing of seven *E.coli* isolates with Acuitas Whole Genome Sequence Analysis. Ecoli\_BR1, 2, 3 are biological replicates of the same isolates.

**4. Comparison of Acuitas Whole Genome Sequence Analysis strain typing and strain typing based on the Whole Genome Mapping** (Figure 2). We have strain typed eight *K.pneumoniae* in Table 2 with Acuitas Whole Genome Sequence Analysis. The dendrogram (A) is complementary to the dendrogram previously generated by strain typing based on Whole Genome Mapping System (B).

A



B



**Figure 2. Comparison of Acuitas Whole Genome Sequence Analysis strain typing and strain typing based on Whole Genome Mapping.** (A) The dendrogram of Acuitas Whole Genome Sequence Analysis strain typing. (B) The dendrogram of strain typing based on Whole Genome Mapping. Eight *K.pneumoniae* in (A) is the subset of 15 isolates in (B) and they are labelled in Red.

## Conclusion

- 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.
- 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 these genes.
- 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.