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A Comparative Analysis of Highly-Multiplexed Real-time PCR and Whole Genome Sequence Analysis for Outbreak Investigation

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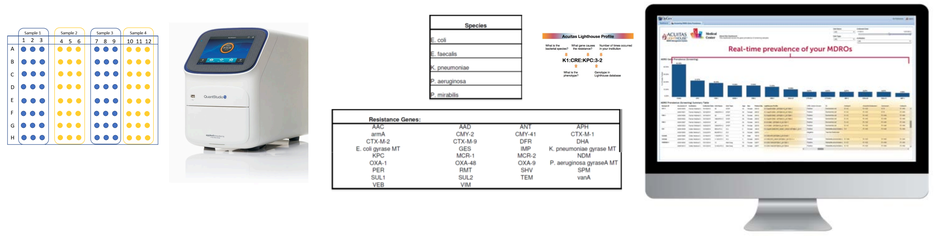
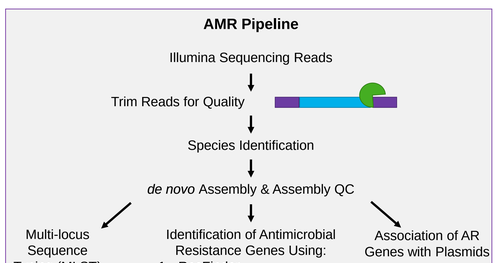
Abstract

Carbapenemase-producing carbapenem-resistant organisms (CPO) are an emerging public health threat in the United States. In October 2018, the Wadsworth Center (WC) began collaborating with OpGen, Inc. as part of the New York State Life Sciences Initiative, which addresses public health issues through laboratory analysis, research, and education. As part of this collaboration, a sample set of 80 clinical isolates was selected from the WC culture collection. The strains chosen included isolates received over two outbreak investigations, as well as 10 unrelated strains representing a wide range of antibiotic resistance genes. Each strain selected was identified using MALDI-TOF mass spectrometry and characterized with carbapenemase production, molecular testing for carbapenemase resistance genes, and drug susceptibility testing. In addition whole genome sequencing (WGS) was performed, and analysis of all 80 isolates was 100% concordant with WC developed real-time PCR assays. The WC developed WGS analysis pipeline includes assessment of isolate relatedness through single nucleotide polymorphism (SNP) analysis, multi-locus sequence typing (MLST), and mobile genetic element identification. As further evaluation, we used the Acuitas Lighthouse ® Software (RUO) and Core Genome MLST (cgMLST) to perform *in silico* analysis using assembled genomes from the WC pipeline. This software is used to assess results from the Acuitas ® AMR Gene Panel (RUO), a rapid PCR test for identification of 5 bacterial species and 47 resistance genes. The WC WGS sequence analysis and the *in silico* evaluation produced concordant clustering dendrograms for the 80 strains assessed with slight discrepancies. Both WGS analysis methods accurately detected the two distinct outbreaks and classified the other strains as unrelated. However, the *in silico* analyses clustered additional isolates that most likely share a common ancestor but were unrelated to the same recent common exposure. The WC pipeline has increased resolution when determining strain relatedness, however it has a longer turn-around time. This study suggests that the Acuitas® test in conjunction with the Acuitas Lighthouse ® Software (RUO) can be used as a front-line tool in clinical settings for transmission and outbreak detection with follow-up confirmation of isolate relatedness by WGS . This study has improved our understanding of the detection of healthcare associated outbreaks and implementation of infection control using rapid molecular testing of resistance genes followed by detailed confirmation by WGS.

Method Comparison



The Acuitas® AMR Gene Panel is a multiplexed real-time PCR assay for the detection of 47 antibiotic resistance genes and species-specific genes for *E. coli, K. pneumoniae/K. variicola, P. mirabilis, P. aeruginosa and E. faecalis*. It is important to note that antibiotic resistance genes can be detected from other bacterial species as well. Bacterial suspensions from pure culture isolates are extracted using the Qiagen EZ1 Advanced XL instrument. DNA is detected using real-time PCR performed on the ABI QuantStudio 5 real-time PCR System. The results are uploaded into the Acuitas Lighthouse® Software where a unique profile is generated, compared, and potentially matched to all samples run previously.



Bacterial suspensions from pure culture isolates are extracted using the QIAcube automated extractor. DNA is quantitated, library prepped and sequenced with the Illumina MiSeq. DNA assembly, QC, and analysis are performed using a lab-developed pipeline to assess isolate relatedness through single nucleotide polymorphism (SNP) analysis, multi-locus sequence typing (MLST), and mobile genetic element identification.

Results Comparison

80 clinical isolates were selected, including isolates from two outbreak investigations, as well as unrelated strains representing a wide range of antibiotic resistance genes. The WC developed WGS analysis pipeline results were directly compared to the Acuitas Lighthouse ® Profile results using *in silico* analysis.

Wadsworth Whole Genome Sequencing

Outbreak 1: *Klebsiella pneumoniae*/*bla*$_{KPC}$

Cluster #1	Ref: # 7	8	7	9	10	3	11	12	13	14	16	4	17	18	5	40	1	2
	8	0	8	6	8	8	6	7	6	6	9	8	10	10	9	11	4234	5127
	7	8	0	1	10	47	4	5	4	4	51	6	8	8	43	32	4352	5312
	9	6	1	0	4	4	2	3	2	2	5	4	6	6	5	7	4202	5087
	10	8	10	4	0	6	2	3	2	2	5	4	6	6	6	8	4270	5172
	3	8	47	4	6	0	2	3	2	2	13	4	6	6	5	7	1611	2207
	11	6	4	2	2	2	0	1	0	0	3	2	4	4	3	5	4237	5129
	12	7	5	3	3	3	1	0	1	1	4	3	5	5	4	6	4209	5104
	13	6	4	2	2	2	0	1	0	0	3	2	4	4	3	5	4206	5110
	14	6	4	2	2	2	0	1	0	0	3	2	4	4	3	5	4247	5148
	16	9	51	5	5	13	3	4	3	3	0	1	3	3	5	7	4309	5213
	4	8	6	4	4	4	2	3	2	2	1	0	2	2	3	5	4271	5171
	17	10	8	6	6	6	4	5	4	4	3	2	0	2	5	7	4283	5185
	18	10	8	6	6	6	4	5	4	4	3	2	2	0	5	7	4254	5159
	5	9	43	5	6	5	3	4	3	3	5	3	5	5	0	2	1673	2276
	40	11	32	7	8	7	5	6	5	5	7	5	7	7	2	0	1633	2235
	1	4234	4352	4202	4270	1611	4237	4209	4206	4247	4309	4271	4283	4254	1673	1633	0	144
	2	5127	5312	5087	5172	2207	5129	5104	5110	5148	5213	5171	5185	5159	2276	2235	144	0

Cluster # 2	Ref: # 21	21	22	23	19	25	26	24	20
	21	0	0	9	9	10	11	15	1016
	22	0	0	9	9	10	9	15	982
	23	9	9	0	0	1	1	7	942
	19	9	9	0	0	1	1	8	973
	25	10	10	1	1	0	2	9	964
	26	11	9	1	1	2	0	9	116
	24	15	15	7	8	9	9	0	753
	20	1016	982	942	973	964	116	753	0

Cluster #4	Ref: # 41	41	42	43	44
	41	0	1	17	17
	42	1	0	17	17
	43	17	17	0	1
	44	17	17	1	0

Isolates from this outbreak were categorized into 4 distinct clusters using SNV analysis:

- Cluster 1: WGS analyses show that isolates 1 and 2 are not related to the other isolates in this cluster, and are also not related to one another.
- Cluster 2: WGS analyses show that isolate 20 is not related to the other isolates in this cluster. All other isolates in this cluster are likely to be related.
- Cluster 3: WGS analyses show that all isolates in this cluster are related
- Cluster 4: WGS analyses show that all isolates in this cluster are related.

Cluster #3	Ref: # 34	34	35	31	32	28	29	37	39	30	38	36
	34	0	3	4	4	4	4	5	5	4	3	6
	35	3	0	3	2	3	3	2	2	1	2	5
	31	4	3	0	0	0	0	1	3	2	3	6
	32	4	2	0	0	0	0	0	2	1	3	6
	28	4	3	0	0	0	0	1	3	2	3	6
	29	4	3	0	0	0	0	1	3	2	3	6
	37	5	2	1	0	1	1	0	2	1	4	7
	39	5	2	3	2	3	3	2	0	1	4	7
	30	4	1	2	1	2	2	1	1	0	3	6
	38	3	2	3	3	3	3	4	4	3	0	5
	36	6	5	6	6	6	6	7	7	6	5	0

Outbreak 2: *Pseudomonas aeruginosa*/*bla*$_{VIM}$

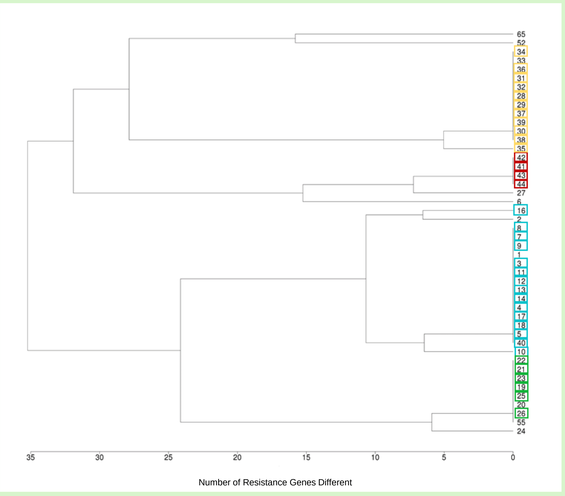
	46	75	47	56	72	74	62	79	66	67	69	81	80	78	76	73	61	53
46	0	3499	2339	4104	2028	2401	2048	2006	2054	2070	2276	1910	2340	3175	2615	2467	33999	58480
75	3499	0	876	878	633	722	643	557	551	638	574	620	867	2136	1309	1222	34683	58286
47	2339	876	0	19	13	5	10	9	8	11	10	11	15	11	687	845	35522	58136
56	4104	878	19	0	17	14	15	14	14	16	15	16	17	1274	696	852	35970	59417
72	2028	633	13	17	0	9	8	7	7	9	8	9	13	15	14	8	35514	57993
74	2401	722	5	14	9	0	5	4	4	6	5	6	10	6	593	752	35839	58222
62	2048	643	10	15	8	5	0	1	1	3	2	3	7	11	11	16	35489	57917
79	2006	557	9	14	7	4	1	0	0	1	0	0	6	10	9	14	35361	57758
66	2054	551	8	14	7	4	1	0	0	2	1	2	6	31	10	14	35088	57391
67	2070	638	11	16	9	6	3	1	2	0	1	0	8	13	11	17	35751	58330
69	2276	574	10	15	8	5	2	0	1	1	0	1	7	11	571	605	35912	58420
81	1910	620	11	16	9	6	3	0	2	0	1	0	8	12	9	15	34864	57542
80	2340	867	15	17	13	10	7	6	6	8	7	8	0	16	686	840	35000	57027
78	3175	2136	11	1274	15	6	11	10	31	13	11	12	16	0	681	838	35709	57814
76	2615	1309	687	696	14	593	11	9	10	11	571	9	686	681	0	162	35827	58209
73	2467	1222	845	852	8	752	16	14	14	17	605	15	840	838	162	0	35967	58298
61	33999	34683	35522	35970	35514	35839	35489	35361	35088	35751	35912	34864	35000	35709	35827	35967	0	55897
53	58480	58286	58136	59417	57993	58222	57917	57758	57391	58330	58420	57542	57027	57814	58209	58298	55897	0

Isolates from this outbreak are shown above

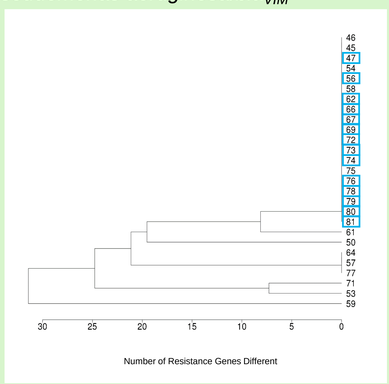
- Isolates 46, 75, 61, and 53 are unrelated to this outbreak.
- All other isolates in this outbreak are considered to be related, although some more closely related than others.

Acuitas Lighthouse®

Outbreak 1: *Klebsiella pneumoniae*/*bla*$_{KPC}$



Outbreak 2: *Pseudomonas aeruginosa*/*bla*$_{VIM}$



Isolates from both of these outbreaks were assessed based upon the Acuitas Lighthouse ® Profile using *in silico* analysis. The number of resistance genes different between isolates is represented above. Isolates are color coded to match the SNV analysis clustering. Overall isolates cluster similarly to that seen with SNV analysis, however a few additional isolates that were determined to be unrelated with SNV analysis cluster as well.

- The WC WGS sequence analysis and the Acuitas Lighthouse® evaluation produced concordant clustering dendrograms for the strains assessed with slight discrepancies.
- Both methods accurately detected the two distinct outbreaks and classified the other strains as unrelated (data not shown for *Escherichia coli*, *Enterobacter cloacae*, and *Serratia marcescens* isolates included in this study)
- The Acuitas Lighthouse ® analyses clustered additional isolates that were likely not from the same recent exposure
- The WC pipeline has increased resolution when determining strain relatedness and can identify additional antimicrobial resistance genes, however it has a longer turn-around time.

Conclusions

- Acuitas ® AMR Gene Panel and Acuitas Lighthouse ® Software (RUO) can be used as a front-line tool in clinical settings for transmission and outbreak detection
- Follow-up confirmation of isolate relatedness should be performed using WGS
- This study highlights the importance of detection of healthcare associated outbreaks and implementation of infection control which can be done using rapid molecular testing of resistance genes followed by detailed confirmation by WGS