Whole Genome Sequencing Analysis of *Klebsiella pneumoniae* Isolates Reveals Diversity in Genetic Antibiotic Resistance Patterns

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CLUSTERS

Hospital 1	Unit A		CG-258					
	Unit B						CC-20	
	Unit C	CG-15	CG-15	CG-258	CC-20	CC-20	CC-20	CG-258
	Unit D	CG-15		CG-258	CC-20		CG-15	
	Unit E	CG-258		CG-258	CC-20			
				CG-258				
	Unit F	CG-258	CG-15		CC-20	CC-20		
			CG-15					
			CG-15					
Hospital 2	Unit G					CC-20	CG-15	
	Unit H	CG-258	CG-258	CC-20	CC-20			
		CG-258		CC-20	CC-20			
				CC-20	CC-20			
	Unit I	CG-258					CG-258	
	Unit J	CG-258						
	Unit K		CG-15					
	Unit L	CG-258	CG-15	CC-20		CG-258	CG-258	
			CG-15					
			CG-15					

Table 1. Clusters arranged by hospital and by unit. No predominant or localized cluster identified. Arranged for visual clarity; does not reflect actual geographic room locations.

STRAINS

Hospital 1	Unit A		ST-258					
	Unit B						ST-1243	
	Unit C	ST-14	ST-15	ST-348	ST-29	ST-280	ST-584	N/A
	Unit D	ST-14		ST-348	ST-29		ST-551	
	Unit E	ST-11		ST-348	ST-29			
				ST-348				
	Unit F	ST-11	ST-15		ST-29	ST-20		
			ST-15					
			ST-15					
Hospital 2	Unit G					ST-20	ST-107	
	Unit H	ST-11	ST-258	ST-16	ST-1694			
		ST-11		ST-16	ST-1694			
				ST-16	ST-1694			
	Unit I	ST-11					ST-2670	
	Unit J	ST-11						
	Unit K		ST-15					
	Unit L	ST-11	ST-15	ST-16		ST-834	ST-54	
			ST-15					
			ST-15					

Table 2. Strains arranged by hospital and by unit. Four hospital-specific and one unit-specific strain identified by MST analysis. Arranged for visual clarity ; does not reflect actual geographic room locations.

BACKGROUND

Klebsiella pneumoniae is among the leading causes of healthcare-associated infections (HAI). Multidrug-resistant (MDR) *Klebsiella* variants are difficult to treat and have been reported with increasing frequency in hospitals. Under the hypothesis that nosocomial strains should bear genetic similarities if they are breeding and spreading throughout a hospital or unit, we sought to compare genetic similarities/differences of hospital-acquired *Klebsiella pneumoniae* isolates using whole genome multi-locus sequence typing (wg-MLST) analysis, and then compare the antibiotic resistance genetic patterns against their epidemiologic typing.

METHODS

- We attempted to find hospital and/or unit-specific nosocomial *K. pneumoniae* strains by sourcing from two geographically disparate tertiary care hospitals in the Detroit, Michigan area. Samples were collected after 48 hours of admission to select for HAI etiologies not present on arrival. A total of 46 clinical bacterial HAI isolates were collected between 2017—2019 for analysis.
- We extracted genomic DNA using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). We used the Nextera® DNA Flex Library Prep Kit (Illumina, San Diego, CA) to build libraries from 200 ng of DNA and generated paired-end reads (2 × 151 bp) using the Illumina NextSeq reagent kit and instrument (Illumina, San Diego, CA).
- We *de novo* assembled the genome using the SPAdes (version 3.7.1) assembler on the Bionumerics calculation engine, and unique MLST Pasteur sequence type (ST) was then assigned for each isolate.
- We then compared each isolate the international *ResFinder* Database (version 3.2) to search for known chromosomal mutations and resistance genes for each isolate.
- We then analyzed for patterns against geographic hospital and unit, as well as within each ST.

RESULTS

There was significant genetic diversity among the isolates, and no predominant strain or gene pattern was identified.

CLUSTER & STRAIN ANALYSIS

- Isolates belonged to one of three distinct phylogenetic clusters: CG-258 | CG-15 | CG-20.
- However, no cluster was hospital or unit specific, and each cluster contained multiple strains.
- MLST analysis revealed 17 unique strains. Seven strains had genetically unique resistance genes detected in more than one isolate, but only five of these seven were hospital-specific.
  - **ST-14** did not have any unique or specific ABX resistance genes
  - **ST-16** was unique for several *bla-SHV* (beta-lactam) genes (26, 78, 98, 145, 179, 194, 199). It represents 4 of the 22 samples from Hospital 2 and was found across two different units.
  - **ST-29** did not have any unique or specific ABX resistance genes
  - **ST-348** was unique for the *bla-SHV-81* gene
  - **ST-1694**, the only unit-specific strain, lacked any unique or strain-specific ABX resistance genes.

GENE ANALYSIS

- 75 unique resistance genes detected among all isolates.
- Only 8 hospital-specific genes detected in more than one isolate, and most with very low prevalence:

  Hospital 1: *qnrB1* (43%), *blaSHV-110* (17%), *blaSHV-1* (9%)

  Hospital 2: *blaCTX-M-3* (23%), *ac(6')-Ib3* (18%), *blaSHV-36* (9%), *blaSHV-80* (9%), *blaSHV-178* (9%)

- Most prominent hospital-1-specific gene (*qnrB1*) non-specifically found in all 3 clusters, 4 of 6 units, and 5 of 12 strains.
- No colistin resistance genes were detected.
- Carbapenemase resistance genes were detected in two isolates.
- Comparisons of phenotypic expression against gene detection show significant differences when trying to establish nosocomial patterns.

CONCLUSION

Genetic analysis of antibiotic resistance patterns and wg-MLST serotypes revealed **significant heterogeneity** among HAI *K. pneumoniae* isolates, much more than was initially expected. The low prevalence of specific genes and absence of geographic clustering indicates **no common source of transmission** for either hospital. Although *K. pneumoniae* is a very common nosocomial pathogen, etiologic analysis suggests **diverse community strains** (*e.g.* gut colonization) may actually be responsible for previously-designated HAI.

ANTIBIOTIC RESISTANCE

Phenotypic Prevalence			Genotypic Prevalence		
Hospital 1 (N=23)	Hospital 2 (N=22)	Drug Class	Resistance Gene	Hospital 1 (N=23)	Hospital 2 (N=22)
100%	100%	FOSFOMYCIN	*fosA*	100%	77%
			fosA5	0%	23%
100%	100%	QUINOLONE	*oqxA*	100%	100%
			oqxB	100%	100%
			qnrB1	43%	0%
		AG / FQ	*aac(6')-Ib-cr*	74%	50%
96%	86%	AMINOGLYCOSIDE	*aadA2*	22%	55%
			aac(3)-IIa	52%	14%
			aph(3'')-Ib	52%	14%
			aph(6)-Id	52%	14%
			aph(3')-Ia	22%	32%
74%	36%	PHENICOL	*catB3*	74%	32%
78%	77%	TRIMETHOPRIM	*dfrA12*	22%	55%
			dfrA14	48%	27%
78%	64%	SULPHONAMIDE	*sul1*	26%	64%
			sul2	48%	14%
			sul3	9%	18%
100%	100%	β-LACTAM	*blaCTX-M-15* [ESBL]	87%	64%
			blaOXA-1 [ESBL]	74%	32%
			blaTEM-1B [ESBL]	57%	50%
			blaSHV-28	17%	18%
			blaSHV-106	17%	18%
			blaSHV-182	13%	23%
			blaCTX-M-3 [ESBL]	0%	23%
			blaFOX-5 [Amp-C]	4%	0%
			blaKPC-2	0%	5%
			blaKPC-3	0%	5%
35%	64%	MACROLIDE	*mph(A)*	30%	64%
43%	9%	TETRACYCLINE	*tet(A)*	30%	5%
13%	18%	RIFAMPICIN	*ARR-3*	13%	18%
0%	0%	COLISTIN	--	--	--
0%	0%	FUSIDIC ACID	--	--	--
0%	0%	GLYCOPEPTIDE	--	--	--
0%	0%	NITROIMIDAZOLE	--	--	--
0%	0%	OXAZOLIDINONE	--	--	--

Table 3. Hospital-specific prevalence for specific ABX resistance genes as well as expected phenotypic drug class expression on traditional susceptibility testing. Low-prevalence genes < 18% are omitted for visual clarity (except for KPC and Amp-C for clinical significance).

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