Genotypic and Phenotypic Diversity of Contemporaneous Carbapenem Resistant *Klebsiella pneumoniae* from Blood Cultures of Individual Patients.

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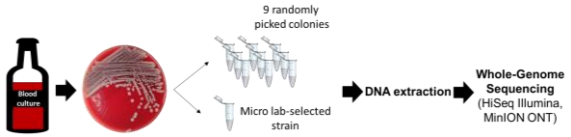
Background

The longstanding paradigm is that almost all bloodstream infections (BSIs) stem from a single, clonal organism. We hypothesized that carbapenem resistant *K. pneumoniae* (CRKP) from individual patients (pts) with BSIs are genetically diverse and manifest phenotypic differences that are not typically recognized by the clinical microbiology laboratory at time of diagnosis.

Methods



Results

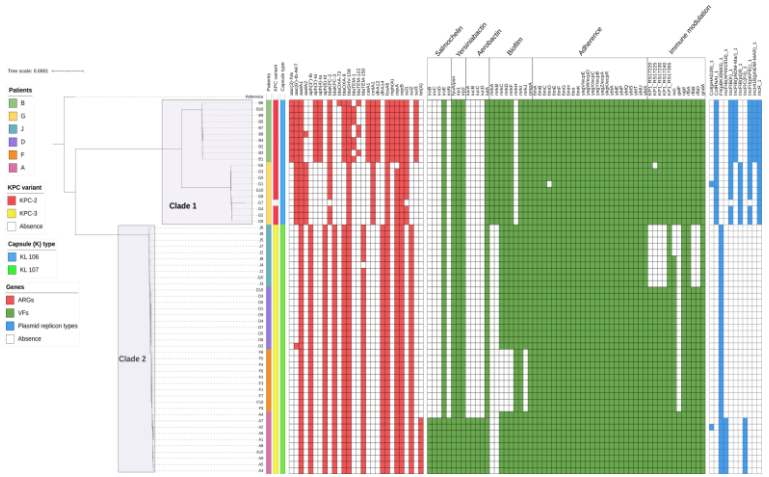


Figure 2. Comparisons of CRKP strains by core genome single nucleotide polymorphism (SNP) phylogeny, and presence/absence of specific genes.

- In each pt strains differed from each other by presence/absence of specific antibiotic resistance genes, mutations of capsular genes and at other loci involved in host interactions, and/or loss of plasmids.

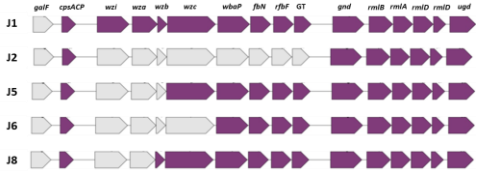


Figure 3. Comparison of capsular gene composition in patient J CRKP strains having a KL107 (wzi154) capsule type (grey indicates absence, purple indicates presence).

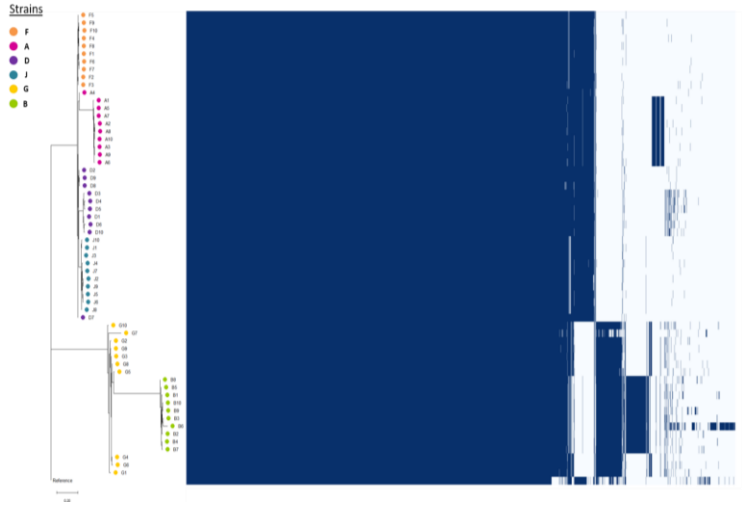


Figure 4. Phylogenetic tree based on accessory gene composition and pangenome analysis (gene presence/absence matrix, blue indicates presence, white indicates absence).

- Pangenome showed accessory gene composition diversity among strains from all pts (**Fig. 4**)
- Intra-pt genetically diverse strains exhibited phenotypic differences in viscosity and mucosity, capsular content, and resistance to serum killing (**Table 1**) and antibiotic resistance (**Table 2**).

Phenotype	Patient A				Patient G			Patient J					
	A1	A4	A8	p-values	G1	G7	p-values	J1	J2	J5	J6	J7	p-values
CPS (uronic acid, nmole/mL)	140.8±4.8	159.6±10.9	134.6±15.4	NS (0.07)	109.2±4.1	114.4±7.8	NS (0.10)	76.2±5.8	28.4±3.0	39.3±5.9	29.6±3.8	35.9±1.3	0.0001
Hypermucosity (OD_{600}, mean ± S.D.)	0.67±0.03	0.72±0.01	0.68±0.04	NS (0.2)	0.68±0.01	0.61±0.03	NS (0.10)	0.69±0.01	0.41±0.001	0.48±0.03	0.44±0.01	0.47±0.01	<0.0001
% serum kill (mean ± SD)	87.0±3.4	52.2±1.27	86.6±0.8	NS (0.07)	56.2±4.4	52.9±4.5	NS (0.86)	85.7±2.3	100%±0	100±0	100±0	100±0	0.01

Table 1. in vitro phenotypes of CRKP strains from three patients (A, G, J). CPS (capsule polysaccharide; OD (optical density); SD (standard deviation); NS (non significant)

	MEM	MVB	CAZ	CZA	TET	GEN
A	>16 (100%)	2 (100%)	>256 (90%) >128 (10%)	8 (40%) 4 (40%) 2 (20%)	256 (90%) 4 (10%)	4 (40%) 2 (60%)
G	>16 (90%) 0.5 (10%)	2 (10%) 0.25 (10%) 0.06 (80%)	126 (30%) 64 (60%) 0.5 (10%)	4 (10%) 2 (20%) 1 (40%) 0.5 (30%)	8 (10%) 4 (90%)	4 (50%) 2 (50%)
J	>16 (100%)	0.06 (100%)	>128 (100%)	16 (30%) 8 (70%)	0.25 (100%)	4 (80%) 1 (20%)

Table 2. Antimicrobial MICs (mg/L) of selected agents for strains from pts A, G and J. MEM (Meropenem), MVB (Meropenem Varbobactam), CAZ (Ceftazidime), CZA (Ceftazidime Avibactam), TET (Tetracycline), GEN (Gentamicin).

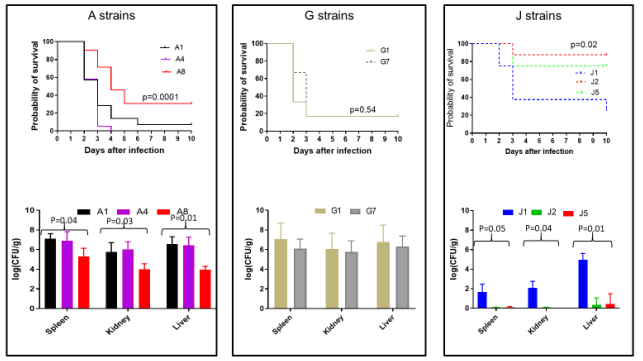


Figure 5. Tissue burdens and mortality in a mouse model of disseminated infection induce by IV infection. Strains from pt A, G and J were tested. Survival studies are represented on top panel and tissue burden studies in spleen, kidney and liver are presented on the bottom panel.

- Various strains from pts A and J differed in ability to cause target organ infections or mortality in a mouse model of intravenous disseminated infection **(Fig. 5)**.

WGSs of CRKP from seeded blood cultures. To assess if CRKP genetic diversity might arise during growth within a blood culture, we seeded blood culture bottles with index strains A1, G1 or J1, and performed short-read WGS on strains recovered following incubation at 37°C.

- In each patient, resultant colonies were morphologically similar
- Single strains were isolated from ten randomly selected colonies and were indistinguishable from respective index strains in core genome SNP, gene and plasmid content as well as in growth rates *in vitro* and antibiotic MICs

Conclusion

- We identified genotypic and phenotypic variant ST258 *K. pneumoniae* strains from blood cultures of individual patients, which were not detected by the clinical laboratory or in seeded blood culture bottles
- Our data suggest a new, population-based paradigm for BSIs by CRKP, with potentially profound implications for medical, microbiology laboratory and infection prevention practices.

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