Molecular diversity and resistance mechanisms of *Klebsiella pneumoniae* bloodstream infections in Peru

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BACKGROUND

- *Klebsiella pneumoniae*, is a leading pathogen for mortality associated with antimicrobial resistance (AMR), responsible for > 250,000 deaths in 2019.
- Genomic surveillance is gaining traction as an important tool for AMR surveillance, to identify high risk clones and emerging mechanisms of AMR.
- Important gaps currently exist in publicly available genomic data
  - Limited representativity of low- and middle-income countries
  - Limited epidemiological and clinical data linked to genomic data

OBJECTIVES

- Assess the genetic diversity among *K. pneumoniae* blood isolates recovered from different regions of Peru
- Identify the AMR and virulence genetic determinants of surveilled K. pneumoniae blood isolates
- Evaluate if epidemiological characteristics (age group, origin of infection, region) and clinical characteristics (severity and outcome) are associated with specific clonal groups (CGs), AMR genes and/or virulence genes.

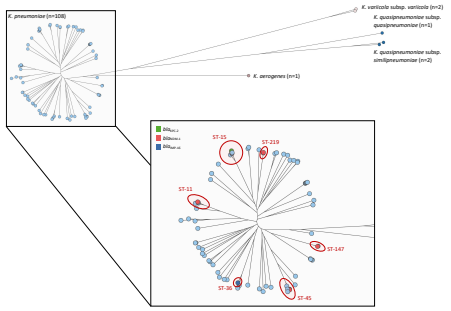
MATERIALS & METHODS

- Consecutive non-duplicate *K. pneumoniae* blood culture isolates collected during an AMR surveillance study (VIRAPERU), from Jul 2017 to Oct 2019, from 15 tertiary hospitals across Peru, were reactivated for whole genome sequencing.
- DNA extraction (GeneJET, Thermo Fisher Scientific), DNA library (Nextera XT, Illumina) and genome sequencing (MiSeq 500bp-V2, Illumina)
- De novo assembling (SPAdes v3.13.1), quality assessment and annotation (Nullarbor v2.0), and identification of species, sequence type (ST) group, K/O loci, and antimicrobial resistance genes (ARGs) and mutations (Kleborate v2.0.1)
- SNP trees were constructed using CSI Phylogeny 1.4 and visualized using Microreact.

RESULTS

1 Six *Klebsiella* species were identified by WGS. The most prevalent was *K. pneumoniae sensu stricto* (n= 108, 95%).

Fig 1. SNP based phylogenetic tree of all 114 Klebsiella genomes.



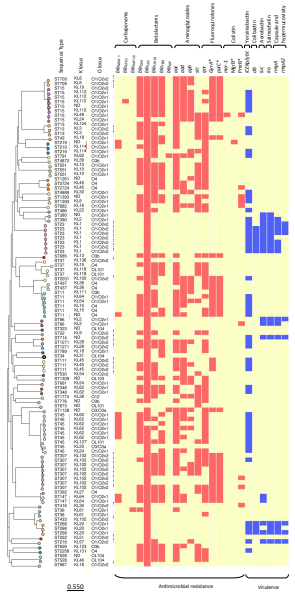
2 Wide genetic diversity was observed among *K. pneumoniae* genomes along with a vast resistome.

- 54 ST groups
- 52 distinct K-loci
- 9 distinct O-loci
- 80 distinct ARGs
- 3 carbapenemase genes

Carriage of more than one ARG was present in 75.4% (86/114) *Klebsiella* genomes, with an average of 8.25 (95%CI: 0.52-7.22) ARGs per genome.

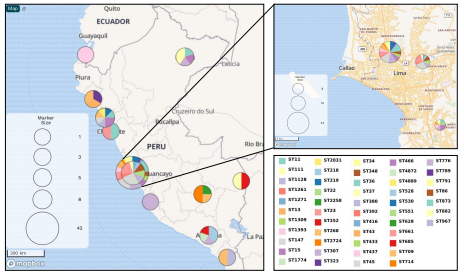
Isolates with a high number of virulence genes carried less ARGs.

Fig 2. SNP based phylogenetic tree of 108 *Klebsiella pneumoniae* genomes, along with their sequence type, K-loci and O-loci, virulence genes and ARGs and AMR mutations.



From a total of 118 isolates, 114 were recovered and sequenced (4 did not grow).

Fig 3. Geographical distribution of 108 Klebsiella pneumoniae genomes colored by sequence type (ST)



3 Carbapenem resistance was found in 13% of isolates. The most common carbapenemase gene was *bla*NDM-1

Colistin resistance conferred by *mcr-1* was found in one isolate, with co-carriage of ESBL gene *bla*CTX-M-15.

Table 1. Frequency of resistance to different antibiotics and the identified genetic determinants

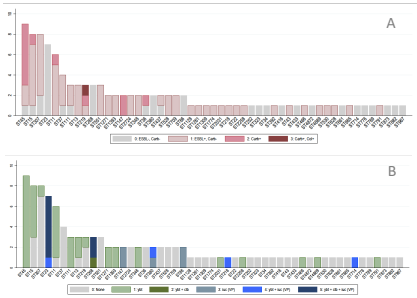
Resistance profile	Frequency of resistance	Related AGRs & alleles	Related AMR mutations	Nº of isolates with no genetic determinant identified
Third-generation cephalosporins	77 (71.3%)	**blaCTX-M-15 (74.0%)** blaTEM-26 (11.7%) blaCTX-M-55 (5.2%) blaCTX-M-14 (2.6%) blaCTX-M-27 (2.6%) blaSHV-12 (2.6%) blaSHV-2 (1.3%) blaSHV-45 (1.3%)		1 (1.3%)
Carbapenems	14 (13.0%)	**blaNDM-1 (78.6%)** blaKPC-2 (7.1%) blaIMP-16 (7.1%)		1 (7.1%)
Fluoroquinolones	70 (64.8%)	**qnrB1 (34.2%)** qnrB19 (28.6%) qnrE2 (15.7%) qnrS1 (5.7%) qnrB4 (1.4%) qnrB6 (1.4%) qnrE1 (1.4%) qepA2 (1.4%)	**GyrAB3 (48.6%)** ParC80 (44.3%) GyrA87 (15.7%)	1 (1.4%)
Aminoglycosides	55 (50.9%)	**aac(6')-Ib-cr.v2 (87.3%)** aac(3)-IIa v1 (69.1%) strA (54.5%) strB (56.4%) aadA2 (43.6%) aph3-Ia (25.5%) aac(3)-IV (12.7%) aph(4)-Ia (12.7%) aac(3)-IId (10.9%) aadA16 (10.9%) aadA5 (5.5%) aph(3)-Ia (1.8%)		0 (0%)
Colistin	12 (11.5%)	mcr-1 (8.3%)	pmrB (8.3%)	11 (91.7%)*

AGRs, antimicrobial resistance genes; AMR, antimicrobial resistance
*Only the presence of mcr-1 and PmrB or MgrB mutations was assessed

4 Eight high-risk ST groups were identified, based on their association with carbapenemase carriage (Figure 4A) or with high virulence (Figure 4B).

- Carbapenemase-carriage: ST45, ST219, ST147, ST15, ST11, ST36
- Higly-virulent: ST23, ST268

Fig 4. Distribution of ST groups based on Kleborate resistance score (A) and virulence score (B)



5 *K. pneumoniae* isolates recovered from bloodstream infections from the community had a higher virulence score and lower resistance score compared with those from hospital origin.

Hospital isolates had significantly higher number of ARGs than community isolates.

Table 2. Genomic features assessed by epidemiological characteristics and clinical outcome

	Infection origin*			Age group				Discharge status			
	Community N=13		Hospital N=42	p-value	Neonatal N=25	Pediatric N=10	Adult N=78	p-value	Survived N=50		Died N=22	p-value
	n	(%)	n (%)		n (%)	n (%)	n (%)		n	(%)	n (%)	
Genomic virulence score*												
0	3	(23.1)	24 (57.1)	0.001	16 (64.0)	4 (40.0)	39 (50.0)	0.693	22	(44.0)	13 (59.0)	0.927
1	3	(23.1)	16 (38.1)		8 (32.0)	4 (40.0)	24 (30.8)		20	(40.0)	7 (31.8)	
2	1	(7.6)	0 (0.0)		0 (0.0)	0 (0.0)	1 (1.3)		1	(2.0)	0 (0.0)	
3	2	(15.4)	1 (2.4)		0 (0.0)	0 (0.0)	5 (6.4)		3	(6.0)	1 (4.6)	
4	2	(15.4)	0 (0.0)		0 (0.0)	0 (0.0)	4 (5.1)		1	(2.0)	0 (0.0)	
5	2	(15.4)	1 (2.4)		1 (4.0)	2 (20.0)	5 (6.4)		3	(6.0)	1 (4.6)	
Genomic resistance score*												
0	9	(69.2)	11 (26.2)	0.035	8 (32.0)	5 (50.0)	27 (34.6)	0.907	16	(32.0)	6 (27.3)	0.479
1	4	(30.8)	24 (57.1)		15 (60.0)	4 (40.0)	41 (52.6)		28	(56.0)	11 (50.0)	
2	0	(0.0)	6 (14.3)		2 (8.0)	1 (10.0)	9 (11.5)		6	(12.0)	4 (18.2)	
3	0	(0.0)	1 (2.4)		0 (0.0)	0 (0.0)	1 (1.3)		0	(0.0)	1 (4.5)	
Nº of antibiotic classes with genotypic resistance	0	(0-4)	7 (5-8)	< 0.001	7 (5-9)	6 (0-8)	7 (1-8)	0.448	7	(1-8)	7 (5-9)	0.263
Nº of antimicrobial resistance genes	0	(0-8)	10 (6-12)	< 0.001	11 (7-14)	10 (0-13)	10 (0-11)	0.278	10	(0-11)	11 (6-15)	0.101

*Genomic virulence score is calculated by Kleborate, based on the following criteria: 0 = no yersiniabactin, colibactin or aerobactin; 1 = yersiniabactin only; 2 = yersiniabactin and colibactin (or colibactin only); 3 = aerobactin without yersiniabactin or colibactin; 4 =aerobactin with yersiniabactin (no colibactin); 5 =yersiniabactin, colibactin and aerobactin. †Genomic resistance score is calculated by Kleborate, based on the following criteria: 1 = ESBL; 2 = Carbapenemase; 3 = Carbapenemase plus colistin resistance; 0 otherwise. ‡Only assessed for non-neonatal bloodstream infections.

CONCLUSIONS

- Bloodstream infections in Peru are caused by a wide diversity of *K. pneumoniae* strains, carrying multiple AMR genes. Carbapenem resistance is principally a result of *bla*NDM-1 carriage, found across 6 specific ST groups.
- Genomic surveillance of *K. pneumoniae* can be conducted in Peru following published genomic surveillance frameworks and using publicly available genomic tools.
- This study constitutes a benchmark for genomic surveillance of *K. pneumoniae* in Peru and a potential roadmap for other low-resource settings.

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