A novel PhoP substitution in *Klebsiella pneumoniae* with decreased susceptibility to colistin

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Introduction

The increasing prevalence of multidrug-resistant Gram negative bacteria pose a serious threat to human health worldwide, as treatment options become increasingly limited.(1,2,3) Polymyxin antibiotics were dismissed in the 1980s due to concerns of nephrotoxicity and neurotoxicity but are now coming back into clinical use as antibiotics of last resort to treat these infections.(4) *Klebsiella pneumoniae* is a Gram negative bacterium which causes community and hospital acquired infections in humans but particularly pneumonia. Hospital based colistin treatment has been shown to result in the culture of *K.pneumoniae* with reduced susceptibility polymyxins.(5) Previous work demonstrated that substitutions in the PhoP/PhoQ two component system can confer resistance to colistin.(6) Three isolates of *Klebsiella pneumoniae* (AJ1, AJ2 and AJ3) were isolated from a hospitalized patient. AJ1 and AJ2 were resistant to colistin, with no known mechanism found to confer colistin resistance. We aimed to compare the colistin resistant isolates to the susceptible isolate and identify mechanisms of colistin resistance, using whole genome sequencing and bioinformatic tools.

Methods

The isolates were whole genome sequenced using Illumina paired end sequencing. Assembly and annotation were achieved using tools available on *Galaxy Orione* (https://orione.crs4.it/). The reads were assembled by *de novo* assembly using Velvet and annotated using Prokka. Multi Locus Sequence Typing (MLST) and plasmid identification was achieved by using tools from the Centre of Genetic Epidemiology (CGE, https://cge.cbs.dtu.dk/). Known resistance genes were identified using the Comprehensive Antibiotic Resistance Gene Database (CARD, http://arpcard.mcmaster.ca) and a CGE tool. The reads were mapped against the *K.pneumoniae* reference genome NTUH-K2044 (7)and analysed for Single Nucleotide Polymorphisms (SNPs), using Burrows Wheeler Aligner (BWA).

Results

Table 1. MIC of colistin for the three *K. pneumoniae* isolates and control organism using different recommended methods.

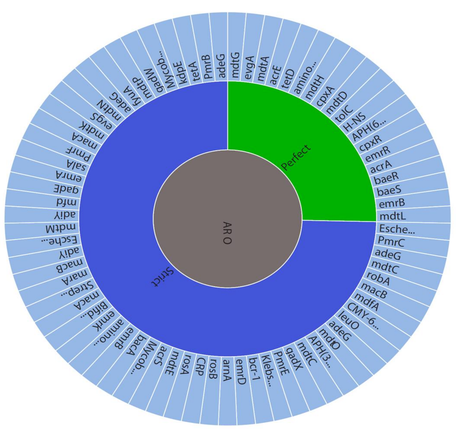
Strain	Method used			
	Agar dilution EUCAST	Broth dilution EUCAST	Broth dilution CLSI	Broth dilution +Tween 80 CLSI
	MIC (µg/mL)			
Control (*K. pneumoniae* NCTC 9633)	4	8	8	2
AJ1	**16**	**32**	**32**	**16**
AJ2	**16**	**32**	**32**	**16**
AJ3	**0.5**	**2**	**4**	**4**

Plasmid mediated genetic factors contributing to an antibiotic resistance phenotype

- Four plasmids; IncFII(K), IncL/M(pOXA-48), IncFIB(K) and IncR were identified in all three isolates.
- AJ1 and AJ2 carried a fifth plasmid, IncFIA(HI1), not present in AJ3.
- The transmissible colistin resistance genes, mcr-1, mcr-2 and mcr-3 were not present in the isolates.

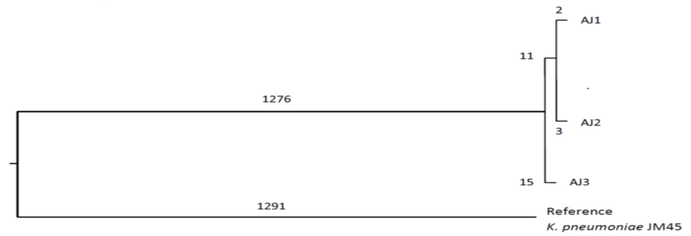
Chromosomal mediated factors conferring antibiotic resistance

Figure 1. Output from the Comprehensive Antibiotic Resistance Gene Database (CARD).



- All three isolates contained substitutions Cys30Phe, Val42Leu and Glu322Arg in PmrC, and a Ser30Thr substitution in PmrF.
- Colistin resistant isolates AJ1 and AJ2 also contained mutations in *pmrL and pmrE* respectively
- A PhoP substitution, Glu36Lys, was identified in the colistin resistant isolates, AJ1 and AJ2, but not in susceptible isolate AJ3.

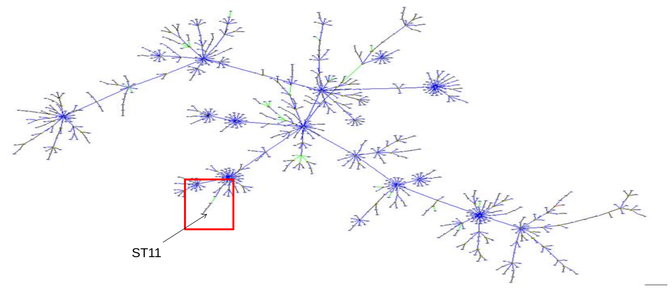
Figure 2. Relatedness of the three *K. pneumoniae* isolates using an identical MLST type *K. pneumoniae* JM45 as the reference genome.



The dendrogram was created using SNP calling data. It demonstrates that the three isolates are closely related and evolved from a common ancestor. AJ1 and AJ2 are divergent to AJ3 and probably evolved in parallel.

- Multi locus Sequence Typing showed that AJ1, AJ2 and AJ3 are all sequence type 11
- The eBURST diagram shows that ST11 is a founder of a clonal complex with descendants including ST258
- ST258 is multi-drug resistant strain, thought to be the main cause of the spread of *K.pneumoniae* carbapenemases (6)
- AJ1, AJ2 and AJ3 are closely related to other strains of multi-drug resistant *K.pneumoniae*

Figure 3. eBURST diagram of the *K.pneumoniae* MLST database



Conclusions

- AJ1, AJ2 and AJ3 are very closely related and colistin resistance is likely to have evolved in the patient.
- A novel substitution in PhoP (Glu36Lys) was found in both colistin resistant isolates (AJ1 and AJ2) but not in the colistin susceptible isolate (AJ3).
- We hypothesize that this PhoP substitution is responsible for the colistin resistance phenotype of AJ1 and AJ2.
- Further work is in progress to determine the role of this mutation as a mechanism of polymyxin resistance.

References

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