Genome wide analysis of colistin-resistant *Klebsiella pneumoniae* ST383

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Introduction

Colistin is one of the last-line antibiotics against infections caused by multi-drug resistant Gram-negative bacteria and its extensive use has inevitably led to emergence of colistin resistance. KPC-2 producing *K. pneumoniae* (KPN) belonging to ST383 were first detected in 2010 in Greece[1]. Since then, this clone has not only spread rapidly to other parts of Europe including Scandinavia, but has also caused outbreaks in Greek hospitals. We carried out a genome-wide analysis of sequential isolates of colistin-resistant (110-CR), sensitive (116-CS) and resistant (117-CR) sequential isolates of ST383 KPN recovered from a Greek patient who received colistin therapy.

Methods

The sequentially isolated strains (110-CR, 116-CS and 117-CR) were screened for antibiotic susceptibility and resistance genes, and by MLST, PFGE (*XbaI*) and whole genome mapping (WGM). For WGM, DNA extraction, quality control, restriction using *AflII*, according to manufacturer's protocols (Argus® system, Opgen,Inc), and analysed using BioNumerics (Applied Maths, Belgium). Whole genome sequencing of both isolates was performed via 2×150b paired end sequencing (Nextera XT) **(Figure 1).** Prophages were identified using http://phast.wishartlab.com/.

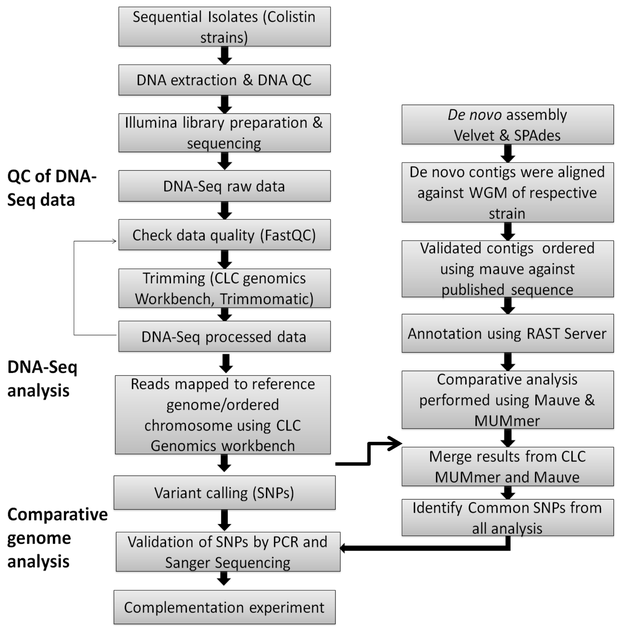

Figure 1: Workflow

Results

Sequential isolates were belonged to same MLST type, similar PFGE and antibiogram patterns, except for colistin resistance **(Table).** eBURST (http://pubmlst.org) analysis showed that the international hybrid KPN clone ST258 (allelic profile 3–3-1–1-1–1-79) and ST383 (allelic profile 2–6-1–3-8–1-18) were five-locus variants. These results were also in line with our PFGE profiles. WGMs of the sequential isolates showed complete identity (only ~2% differences) (**Figure 2**). Genome assemblies of 110-CR, 116-CS and 117-CR generated a chromosome size of ~5.4 Mb assembled into ~118 scaffolds. Draft scaffolds were annotated (RAST) http://rast.nmpdr.org/rast.cgi, and ordered as a pseudo chromosome and compared **(Figure 3).** 23 non-synonymous SNPs were identified. Genes or biosynthetic pathways with a potential association with colistin resistance development included the colanic acid biosynthesis UDP-glucose lipid carrier transferase (*WcaJ*), stress regulator protein, ferric uptake regulation protein, sensory histidine kinase, phospholipid phosphatase (*PgpB*).

The promoter region of the PhoP/PhoQ regulator gene, *mgrB,* was also interrupted by a short insertion element. Comparative genomic analysis also showed loss of prophages in the colistin resistant strain.

Conclusions

We report here the first genome-wide analysis of the novel ST383 KPN clone. Importantly, we identified a novel set of SNPs in genes involved in lipopolysaccharide synthesis and in the capsular polysaccharide cluster that might be potentially involved in mediating colistin resistance in KPN.

Strain	Colistin E test (µg/ml)	MIC Broth Dilution (µg/ml)	Source	MLST (ST) type	PFGE type	*bla*$_{CTX-M}$	*bla*$_{SHV}$	*bla*$_{TEM-37}$	aac6‘ Ib + In26
110R	32	32	BLOOD	ST-383	2a	-	**SHV-12**	**TEM-37**	**aac6’-Ib**
116S	0,125	2	CVC	ST-383	2b	-	**SHV-1**	**TEM-37**	**aac6’-Ib**
117R	24	32	BLOOD	ST-383	2c	-	**SHV-1**	**TEM-37**	**aac6’-Ib**

Table: Strain characteristics: clinical data, MLST, PFGE types and colistin MICs (CVC= central venous catheter)

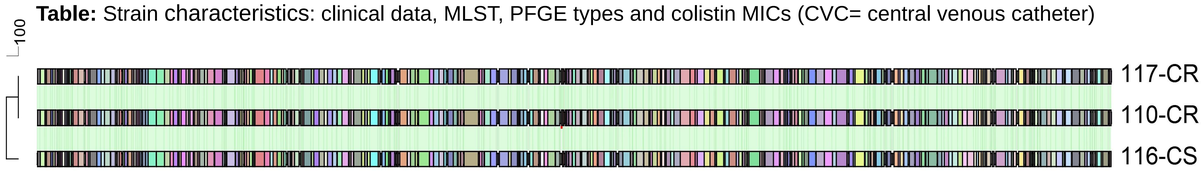

Figure 2: Whole genome maps of sequential colistin-resistant, sensitive and resistant ST383 KPN isolates. Green lines indicate identity of restriction pattern among the maps and red horizontal marks represent the variations.

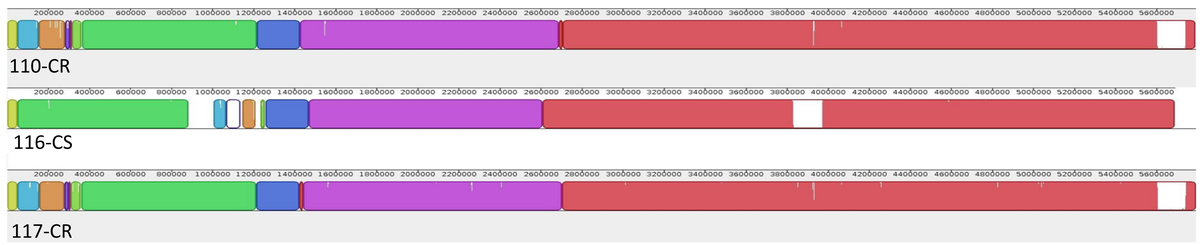

Figure 3: Chromosomes synteny were derived from whole genome sequencing of sequential colistin-resistant, sensitive and resistant ST383 KPN isolates. Identical regions are shown as similar color blocks and few variations as shown as colorless blocks.

Bibliography

1. Papagiannitsis, CC., et al., Emergence of *Klebsiella pneumoniae* of a novel sequence type (ST383) producing VIM- 4, KPC-2 and CMY-4 β-lactamases. Int J Antimicrob Agents. 2010;36(6):573-4.

Funding

This work and B.B.X. are supported by University of Antwerp Research funds (BOF-DOCPRO 2012-27450).

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