Poster #
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Genomic Diversity of Multi-Drug Resistant *Klebsiella pneumoniae* ST383 Characterized by Whole Genome Mapping

J. Sabirova[1], J. Coppens[1], O. Zarkotou[2], C. Lammens[1], H. Goossens[1], S. Malhotra-Kumar[1]

[1]University of Antwerp, Antwerp, Belgium, [2]Tzaneio General Hospital, Piraeus, Greece

University of Antwerp
Laboratory of Medical Microbiology, VAXINFECTIO
Universiteitsplein 1, 2610 Wilrijk, Belgium
Tel: 32 3 820 2550
E-mail: jasmine.coppens@uantwerpen.be

Abstract

Background: In 2010, *Klebsiella pneumoniae* (KPN) harboring the metallo beta-lactamase VIM-19 and co-producing KPC-2 carbapenemase, CMY-2 cephalosporinase and CTX-M-15 extended spectrum beta-lactamase were first detected in a Greek hospital. In 2012, VIM-19 harboring KPN caused an outbreak at this hospital. We studied the genomic diversity of VIM-19 harboring KPN outbreak and non-outbreak strains collected during 2010-2013.

Methods: Non-outbreak (NO1-NO9, n=9) and outbreak (O1-O5, n=6) KPN strains were typed by multi-locus sequence typing and mapped by Whole Genome Mapping™ (WGM, Argus® system, Opgen Inc). Maps were edited and compared with *in silico* maps generated from previously sequenced KPN (NCBI) (MapSolverTM, Opgen Inc). Antimicrobial resistance (AMR) profiles were determined by disk diffusion.

Results: All typed KPN belonged to ST383. Non-outbreak strains exhibited nearly identical whole genome maps (map distance (MD) of ≤ 1.5%) (Fig. A). Outbreak strains formed two clusters: C1 (O1,O4, and O5) was similar to non-outbreak strains (MD of ≤ 0.5%), whereas C2 (O2, O3, and O6) was genotypically distinct (MD of 17.7%) (Fig. B). The two representative outbreak strains O1 and O2 differed in AMR (Fig. C), and O2 showed three genetic insertions potentially related to AMR: 16kb fragment comprising putative bleomycin resistance; 7kb fragment comprising gyrB and an outer membrane protein; 25kb fragment comprising permease and transport proteins (Fig. C).

Conclusions: WGM demonstrated the genomic diversity within non-outbreak and outbreak KPN ST383 and the emergence of an outbreak-related subclone that had acquired genes and a different AMR profile.

Background

The extensive dissemination of multi-drug resistant *Klebsiella pneumoniae* clinical strains constitutes a pressing global public health problem. Infections due to carbapenemase-producing *K. pneumoniae* are especially notorious for being difficult to treat as therapeutic options are often limited, and the potential for serious hospital epidemics, if not promptly detected and contained, is well documented. In 2010, *K. pneumoniae* belonging to a novel sequence type ST383 were detected for the first time in Greece [1]. ST383 *K. pneumoniae* isolated at the Tzaneio General Hospital harbored the metallo-beta-lactamase VIM-19 and co-produced KPC-2 carbapenemase, CMY-2 cepha-losporinase and CTX-M-15 extended spectrum beta-lactamase. In 2013, VIM-19 harboring ST383 *K. pneumoniae* strains caused an outbreak at this hospital. We studied and compared the genomic content of VIM-19 harboring ST383 *K. pneumoniae* isolated during 2010 to those that caused an outbreak during 2013.

Methods

Fifteen *K. pneumoniae* strains, isolated from patients admitted to the intensive care unit and surgical ward at the Tzaneio General Hospital were studied. These had been previously assigned to a sequence type ST383 by multi-locus sequence typing[2][3]. Of these, 9 were isolated in 2010 (non-outbreak strains, NO1-NO9) and 6 during the recorded 2013 outbreak (outbreak strains, O1-O5) (Table).

Resistance to a panel of antibiotics was determined by disk diffusion using CLSI guidelines and cut-offs (Table). The entire genomes of all 15 strains were mapped on the Argus™ Whole Genome Mapping (Opgen Inc, Gaithersburg, USA). DNA extraction, quality control, restriction using *AflII*, and loading on a mapcard were done according to manufacturer's protocols. Briefly, *K. pneumoniae* colonies grown overnight at 37°C on MH agar plates were used for isolation of high molecular weight (HMW) DNA using Argus® HMW DNA isolation kit (Opgen, Inc). Extracted DNA preps were checked for the presence of HMW DNA molecules by using Argus® QCard kit (Opgen, Inc) and subsequently used for mapping using Argus® MapCard II kit (Opgen, Inc). The assembly of restricted DNA molecules and identification of novel *AflII* restriction sites was performed using MapManager software (Opgen Inc). Visualization, editing (adjusting starting point) and analysis of maps were performed by excluding fragment size smaller than 3 kb from the analysis and a relative tolerance of 25%, combined with an absolute tolerance of 2500, were used along with UPGMA from Bionumerics v7.1 (Applied Maths, Sint-Martens-Latem, Belgium).

Results

The whole genome maps showed that non-outbreak strains were generally a homogenous population. NO1-NO7 exhibited nearly identical whole genome maps with ~99% similarity, however, NO8 had an insertion of ~24 kb fragment (**Fig. A**). Outbreak strains were clustered into two clades: C1 (O1,O4, and O5) and C2 (O2, O3, and O6) that were genotypically distinct with ~50% (**Fig. B**). The two representative outbreak strains of C1 and C2 (O1 and O2, respectively) also differed in their antibiotic resistance profiles (**Fig. C and Table**).

Table: Clinical and antibiotic susceptibility data

Strain	Dep	Source	Antibiotic Susceptibility Profile															
			AML	AMC	TZP	FEP	CTX	FOX	CAZ	ATM	ETP	MEM	CIP	FOS	F/M	MEC	SXT	GM
NO1	ICU	BLOOD	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
NO2	S	UNK	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S
NO3	ICU	CATH	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
NO4	S	URINE	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S
NO5	ICU	BLOOD	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S
NO6	ICU	CVC	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
NO7	S	URINE	R	R	S	R	R	I	R	R	S	S	R	S	R	S	R	R
NO8	ICU	CVC	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
NO9	ICU	BLOOD	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
O1	ICU	UNK	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
O2	ICU	REC. SWAB	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S
O3	ICU	REC. SWAB	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	S
O4	ICU	REC. SWAB	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
O5	S	BLOOD	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
O6	UNK	UNK	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R

Non-outbreak (NO), Outbreak (O), Surgical ward (S), Rectal swab (Rec. swab), Unknown (UNK), Amoxicillin (AML), Amoxicillin/ clavulanic acid (AMC), Piperacillin/ Tazobactam(TZP), Cefepime (FEP), Cefotaxime (CTX), Cefotaxime/ clavulanic acid (CTX-CLA), Cefoxitin (FOX), Ceftazidime (CAZ), ceftazidime/ clavulanic acid (CAZ-CLA), Aztreonam (ATM), Ertapenem (ETP), Meropenem (MEM), Ciprofloxacin (CIP), Fosfomycin (FOS), Nitrofurantoin (F/M), Mecillinam (MEC), Trimethoprim-Sulfamethoxazole (SXT), Gentamicin (GM). Resistent (R), Intermediate (I), Sensitive (S)

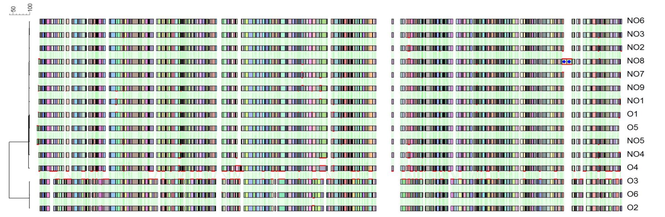

Fig A. UPGMA cluster analysis of NO strains WGMs together with O strains and clade (C2) of O strains placed as separate node.

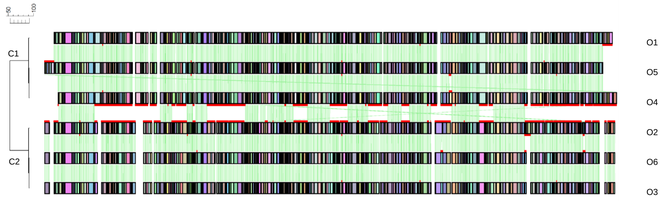

Fig B. Comparative analysis of WGMs of outbreak strains grouped in to two clades.

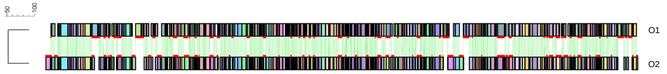

Fig C. The representatives of two clades from outbreak strains were compared to identify differences between them.

Conclusions

We demonstrate that WGM is a rapid, high-resolution technique to understand genomic diversity that can exist within strains belonging to different MLST types and especially in an outbreak situation. While genome content of non-outbreak strains was rather homologous, our results indicate that outbreak strains belonging to different ST's clustered separately through WGM.

References

[1] Papagiannitsis et al., Int J Antimicrob Agents, 2010 Dec;36(6):573-4 ;[2] Diancourt et al., J Clin Microbiol 2005, 43:4178-82;[3] http://www.pasteur.fr/recherche/genopole/PF8/mlst/*K.pneumoniae*.html

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