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Whole Genome Sequencing for a carbapenem- and colistin-resistant *Klebsiella pneumoniae* hospital outbreak

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Background

Carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) strains have been continuously isolated since 2008 in Azienda Ospedaliero-Universitaria Policlinico Modena (AOUPM), Northern Italy. Colistin-resistant CR-Kp (CRCR-Kp) appeared in 2011 with a prevalence of 11% that peaked to 36.4% in 2013 and decreased to 22% in 2014 thanks to a joint program of infection control and antimicrobial stewardship. We used whole genome sequencing to analyze the relatedness among 27 CRCR-Kp strains, isolated in AOUPM during the January 2013-March 2014 outbreak, the molecular mechanisms of antimicrobial resistance and virulence.

Methods

Twenty-seven CRCR-Kp strains were isolated from as many patients admitted in different wards (ICU 29.7%, Medicine 18.5%, Infectious Diseases 14.8%, Surgery 3.7%, and others 33.3%). Clinical samples were: rectal swab 81.5%, urine 11.1%, broncho-alveolar lavage 3.7%, blood 3.7%. CRCR-Kp isolates were sequenced with a Next Generation Sequencing approach on the Illumina MiSeq platform by using the NexteraXT DNA protocol. *In silico* Multi Locus Sequence Typing (MLST) analysis was conducted on the *Klebsiella pneumoniae* Pasteur MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) while full genome SNPs-based phylogeny was performed using kSNP v3.0 software. Antimicrobial resistance genes were analyzed by the ResFinder-2.1 software (http://www.genomicepidemiology.org), the resources of Pasteur MLST *K. pneumoniae* database, and running locally the BLAST tool. The virulence genes were investigated by the resources of Pasteur *K. pneumoniae* database.

Results

MLST characterization revealed predominance of the Sequence Type (ST) 512 (25/27, 92.6%) followed by the ST258 (2/27, 7.4%). The results of core SNPs phylogeny were consistent with MLST as ST258 samples grouped together and differentiated from ST512. Core SNPs analysis distinguished between isolates belonging to the same ST (Figure 1 with comments). Analysis of antimicrobial resistance genes revealed that ST258 were *blaKPC*2 producers, while all ST512 isolates were *blaKPC*3 producers. Among these, 20 produced *blaSHV*11 and *blaTEM*1, while 5 *blaSHV*11 only. Both ST258 strains produced *blaSHV*12, one of them also *blaTEM*1. Resistance genes for aminoglycosides, fluoroquinolones, fosfomycin were found in accordance with phenotypes results (data not shown). The transferable colistin resistance gene *mcr* was not detected. Three different types of *mgrB* mutations were present in 10 isolates: a 10-bp deletion in 8, a fully *mgrB* deletion in 1, and an insertional inactivation in 1 (Table 1).

All strains showed genes encoding virulence factors involved in biofilm formation and host cell adherence (*mrk* genes). In contrast, iron acquisition systems genes were present only in two samples. Capsular characterization showed the presence of the *wzi*154 and *wzi*29 genes in ST512 and ST258 isolates respectively (data not shown).

Figure 1: Core SNPs-based phylogenetic tree

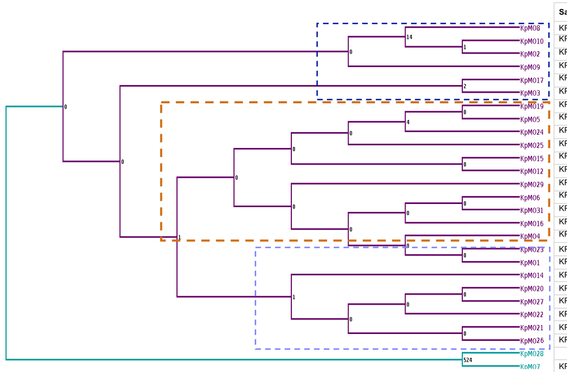

Table 1: mgrB mutations, exposure to colistin, wards movements

Sample	*mgrB*-Mutation	Col_the	Col_days	Jan13	Feb13	Mar13	Apr13	May13	Jun13	Jul13	Nov13	Dec13	Jan14	Feb14
KPMO-8	wt	no												
KPMO-10	wt	no												
KPMO-2	wt	no												
KPMO-9	insertional inactivation	no												
KPMO-17	yebO-ΔmgrB-ΔyebH	yes	12											
KPMO-3	wt	no												
KPMO-19	wt	yes	14											
KPMO-5	wt	no												
KPMO-24	wt	no												
KPMO-25	wt	no												
KPMO-15	wt	no												
KPMO-12	wt	no												
KPMO-29	wt	no												
KPMO-6	wt	no												
KPMO-31	wt	no												
KPMO-16	wt	no												
KPMO-4	wt	yes	13											
KPMO-23	Δ61-70	no												
KPMO-1	Δ61-70	no												
KPMO-14	Δ61-70	yes	30											
KPMO-20	Δ61-70	no												
KPMO-27	Δ61-70	no												
KPMO-22	Δ61-70	no												
KPMO-21	Δ61-70	yes	26											
KPMO-26	Δ61-70	no												
KPMO-28	wt	no												
KPMO-7	wt	no												

Comments

Figure 1: ST258, KpMO7 and KpMO28 (light blue) were grouped together and were well differentiated from isolates with ST512 (purple). Dashed lines show correspondance between related strains in the tree and epidemiological data in Table 1.

Table 1: Each square represent a 2 week time, different colours represent wards

ICU ● ; Medicine1 ● ; Infectious Disease ● ; Nephrology ● ; Medicine2 ● ; Pneumology ● ; Long term care ● ; Oncology ● ; Transplants ● ; # represents date of CRCR-Kp isolation.

KpMO 23, 1, 14, 20, 27, 22, 21, 26 share the same mechanism of colistin resistance and are strictly related in the tree.

Conclusions

ST512 *blaKPC*3 producers were the most prevalent isolates during the outbreak. Core SNPs analysis distinguished between related and unrelated isolates, even within the same ST. These data confirmed the epidemiological analysis of CRCR-Kp spreading during the outbreak. Two temporal distinct clusters can be noted mainly involving few wards (ICU, Infectious Disease, Nephrology). The great majority of patients did not receive colistin before CRCR-Kp isolation, suggesting a clonal dissemination of strains. CRCR-Kp endemicity in our hospital was confirmed by its presence in many different wards.

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