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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.

Material/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

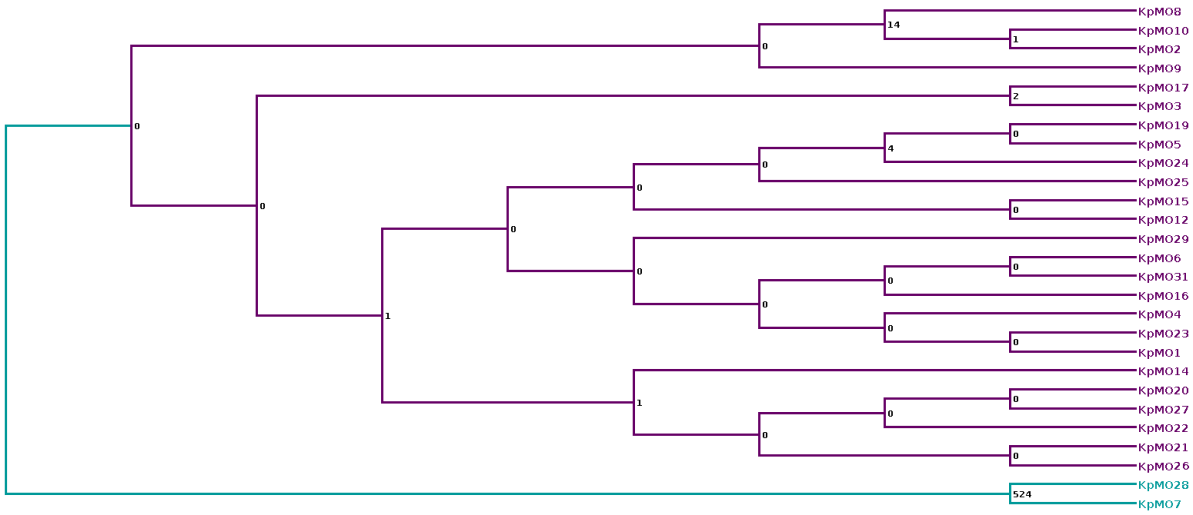
33.3%). Clinical samples were: rectal swab 81.5%, urine 11.1%, broncho-alveolar lavage 3.7%, blood 3.7%. CRCR-Kp isolates were sequenced with a Next Generation Sequencing approach on the Illumina MiSeq platform by using the NexteraXT DNA protocol. *In silico* Multi Locus Sequence Typing (MLST) analysis was conducted on the *Klebsiella pneumoniae* Pub MLST database (http://bigsdb.pasteur.fr/perl/ bigsdb/bigsdb.pl? db=pubmlst _ klebsiella _seqdef_public &page=sequenceQuery) 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). Analysis of antimicrobial resistance genes revealed that all ST512 isolates were bla_{KPC3} producers. Among these, 20 produced bla_{SHV11} and bla_{TEM1}, while 5 bla_{SHV11} only. Both ST258 strains produced bla_{KPC2} and bla_{SHV12}, one of them also bla_{TEM1}. The transferable colistin resistance genes *mcr1/mcr1.2* and *mcr2* were not detected. Three different type of *mgr*B mutations were present in 9 isolates: a 10-bp deletion in 7, a fully *mgr*B deletion in 1, and an insertional inactivation in 1. All strains showed genes encoding virulence factors involved in biofilm formation and host cell adherence. In contrast, iron acquisition systems genes were present only in two samples. Capsular characterization showed the presence of the *wzi*154 and *wzi*29 in ST512 and ST258 isolates respectively.

Conclusions: ST512 bla_{KPC3} producers were the most prevalent isolates during the outbreak. Core SNPs analysis distinguished between related and unrelated isolates, even within the same ST. We could correlate colistin resistance only to mutations associated with *mgr*B gene. The study of other potential mechanisms for colistin resistance is ongoing.

Figure 1

Phylogenetic tree core SNPs-based


KpMO8
KpMO10
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KpMO7
14
1
2
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4
524