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Inner membrane gene mgrB involved in colistin resistance is an integration hotspot for insertion sequence ISL3 carried by pKpQIL plasmids in KPC-producing K. pneumoniae.

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Background: *mcr-1* has been reported as first plasmid gene to confer colistin resistance. In KPC-producing *K. pneumoniae* (KPC-KP) isolates, however, colistin resistance is rapidly emerging by other mechanisms. This is frequently the result of inactivation of *mgrB* by insertion sequences, for instance ISL3. The original localization of this sequence is unknown. The aim of this study is to identify localization and targets of ISL3 sequences by next generation sequencing of successful KPC-KP lineages.

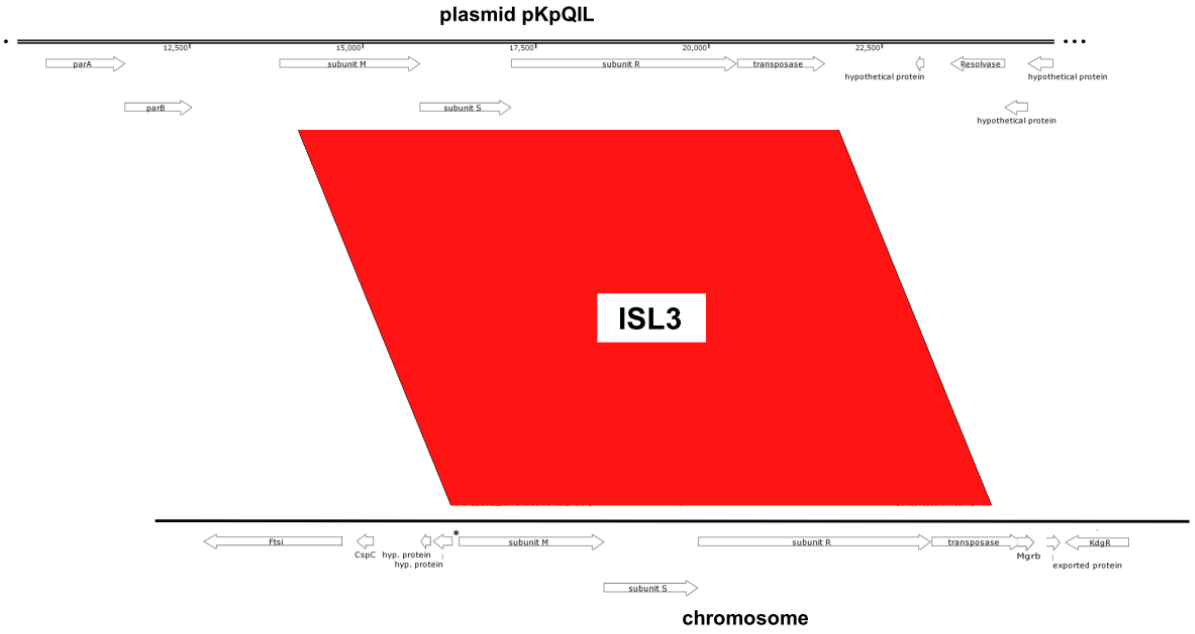
Methods: Clinical *K. pneumoniae* isolates demonstrating carbapenem and colistin resistance by AST of 10 unique patients hospitalised in the Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy, were randomly selected. Whole genome sequences (WGS) were analysed for acquired resistance genes by Resfinder, plasmids by Plasmidfinder, insertion sequences by ISfinder, and mutations in *mgrB* sequence (GI:695277517). ISL3 (GI:NC_009650) sequences were blasted against the WGS of all

isolates. Additionally, a previously characterised collection of 34 KPC-KP isolates from Crete was assessed (PRJEB10561). Plasmid sequences detected by Plasmidfinder were manually reconstructed based on complete reference plasmids.

Results: The KPC-KPs from Pisa included 8 ST512/KPC-3, and 2 ST307/KPC-3 isolates. Complete ISL3 was detected in 9 isolates: 7 ST512/KPC-3, and 2 ST307/KPC-3. Complete ISL3 was located in 9 out of 10 isolates on pKpQIL plasmids, which is inferred to as a type I restriction-modification system with a transposase. In the ISL3-negative isolate, the pKpQIL plasmid was not present. In one ST512/KPC3 isolate, with colistin MIC>8, a copy of the plasmid ISL3 was inserted in *mgrB* nucleotide position 13, marked by the asterisk (figure 1). In one ST512/KPC-3 isolate, ISL3 was inserted in inner membrane protein gene *marC*.

All 34 Crete *K. pneumoniae* isolates were ST258/KPC2, except one ST147/KPC2. Complete ISL3 was detected in 31 isolates. In the 3 ISL3-negative isolates the pKpQIL plasmid was not present. In 9 isolates, ISL3 was also inserted in *mgrB* nucleotide position 13. Chromosomal integration of ISL3 in other positions than *mgrB* was identified in 3 isolates. One isolate showed 2 ISL3-insertions: in uncharacterized gene *yibL*, and in inner membrane protein gene *yfdC*, respectively. In one isolate, ISL3 inserts in membrane protein gene *igaA*, and in another isolate ISL3 inserts in the intergenic region between the two-component system genes *ycgF* and *ycgZ*.

Conclusion: ISL3 seems to target mainly inner membrane protein genes in the chromosome, of which *mgrB* was the most common target. We showed that ISL3 is always localized on plasmid pKpQIL, and truncation of *mgrB* by ISL3 was detected in 2 different KPC-KP lineages. Thus, presence of pKpQIL may predispose for colistin resistance in general. The dissemination of ISL3 on pKpQIL plasmids may partly explain the rapid emergence of colistin resistance in KPC-KP.


plasmid pKpQIL
12,500
15,000
17,500
20,000
22,500
parA
subunit M
subunit R
transposase
hypothetical protein
Resolvase
hypothetical protein
parB
subunit S
hypothetical protein
ISL3
FtsI
CspC
hyp. protein
hyp. protein
subunit M
subunit R
transposase
MgrB
KdgR
exported protein
subunit S
chromosome