The *mgrB* gene involved in colistin resistance is an integration hotspot for insertion sequence ISL3 carried by pKpQIL-like plasmids in KPC-KP

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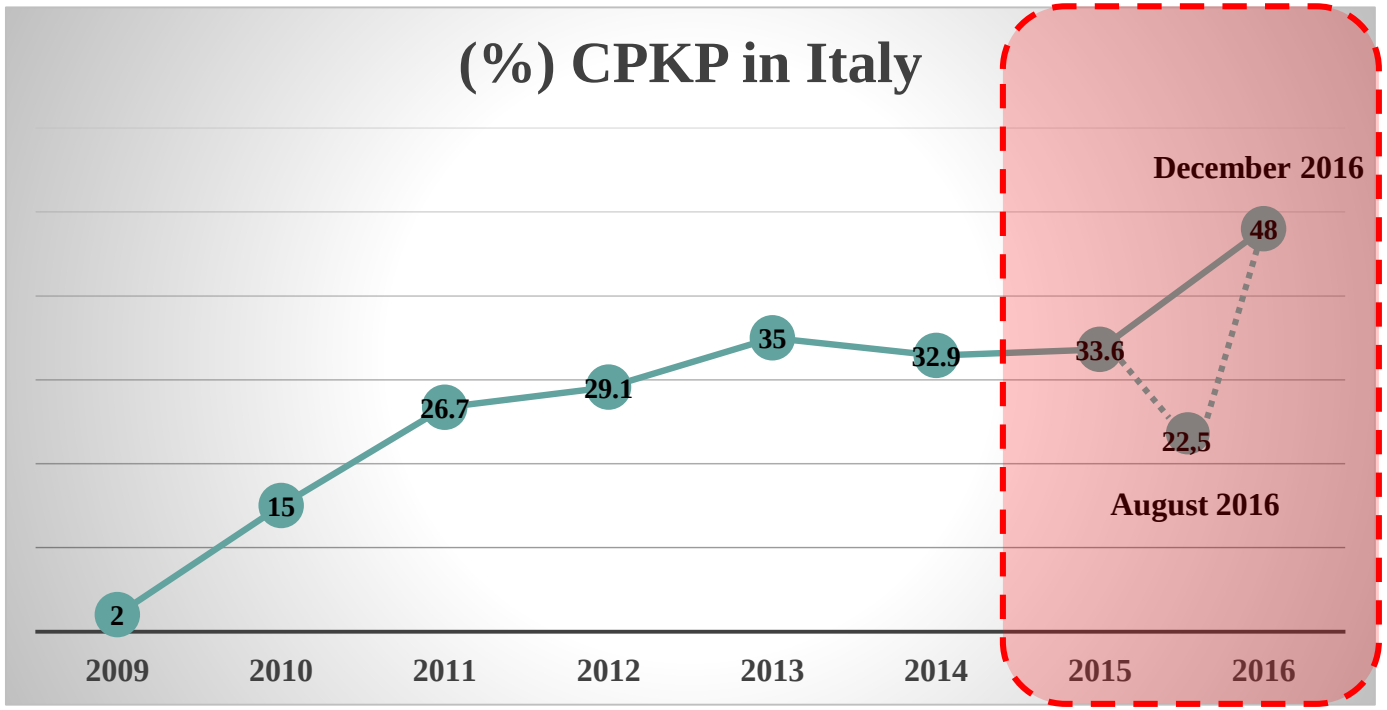
Nothing to disclose

Introduction

- In Italy the first isolation of a KPC-positive *K. pneumoniae* was reported in **2009**.
- The percentage of invasive isolates of CPKP, that was until 2009 lower than one to 2%, increased to 15% in **2010**, 26.7 in **2011**, 29.1% in **2012** to reach 35% in **2013** (EARS-Net).
- This trend apparently reversed in **2014**, when 33% CPKP were reported. (Giani *et al.* 2015)



Pisa

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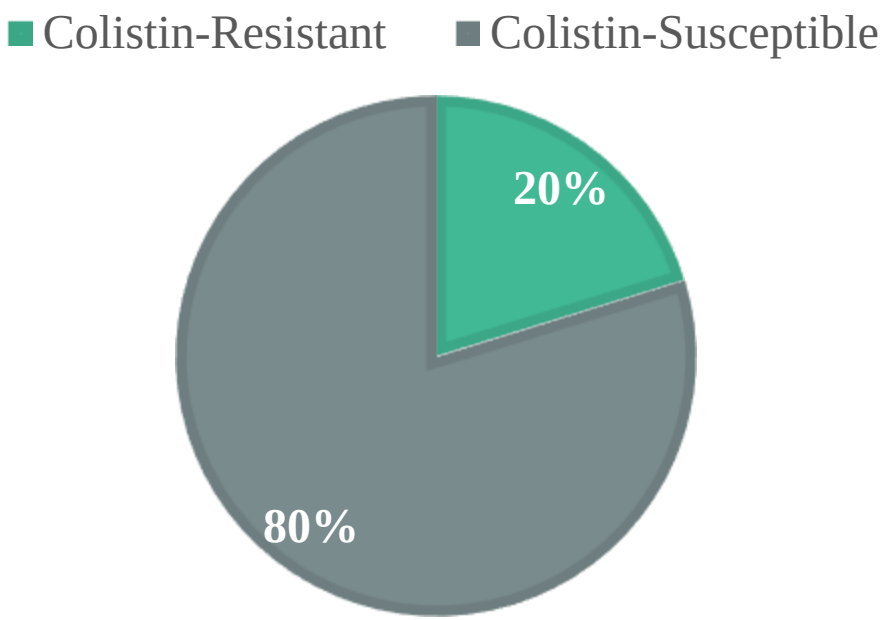
Colistin resistant *Klebsiella pneumoniae*

Resistance to colistin among CPKP is 40,1% in Italy, 19% in Greece, and 10% in Spain. (EuSCAPE, Nov-2016)

Resistance to colistin among CPKP during 2016 in Pisa



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Aim of the study

The aim of the study was to investigate the molecular bases of colistin-resistance

Methods

- Thirty clinical isolates of *K. pneumoniae* were collected from unique patients in Pisa University Hospital during 2015-2016 and the whole genome sequences were analyzed on Illumina MiSeq platform (study PRJEB19808).

- Additionally, a previously characterized collection of 34 CPKP isolates collected between 2010-2014 in Crete, Greece, deposited at the European Nucleotide Archive (study PRJEB10561) was assessed.

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Results (1a)

Characteristics of the Italian CPKP isolates

Isolate	Isolation date	MLST	MIC COL (mg/L)	Mutations in *mgrB*
1084	07/2015	512	>8 R	-
1091	07/2015	512	>8 R	-
1122	08/2015	512	>8 R	delta 109/119
1123	02/2015	512	4 R	IS5 75
1129	09/2015	307	4 R	-
1145	10/2015	512	>8 R	delta 109/119
1147a	02/2015	512	>8 R	-
1196	04/2015	307	>8 R	-
1201b	04/2015	512	4 R	-
1206	12/2015	512	>8 R	ISL3 133
1043	05/2015	512	>8 R	delta 109/119
1059	06/2015	512	>8 R	delta 109/119
1076	06/2015	512	>8 R	delta 109/119
1079	06/2015	512	>8 R	delta 109/119
1088	07/2015	512	>8 R	delta 109/119

Isolate	Isolation date	MLST	MIC COL (mg/L)	Mutations in mgrB
1126	09/2015	512	>8 R	delta 109/119
1133	02/2015	512	>8 R	delta 109/119
1136	09/2015	512	>8 R	delta 109/119
1152	11/2015	512	>8 R	delta 109/119
1168	07/2015	37	>8 R	A7T Stop codon
1187	03/2015	512	>8 R	delta 109/119
1195	04/2015	512	>8 R	delta 109/119
1203	04/2015	512	>8 R	delta 109/119
1140	09/2015	512	>8 R	- **Mcr 1.2**
1147b	10/2015	512	>8 R	delta 109/119
1208	01/2016	512	>8 R	ISL3 133
1235	02/2016	512	>8 R	IS5 75
1236	02/2016	37	>8 R	A7T Stop codon
1307	06/2016	512	>8 R	delta 109/119

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Results (1b)

CPKP isolates from Greece

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Journal of Antimicrobial Chemotherapy

Emergence of pan-resistance in KPC-2 carbapenemase-producing *Klebsiella pneumoniae* in Crete, Greece: a close call

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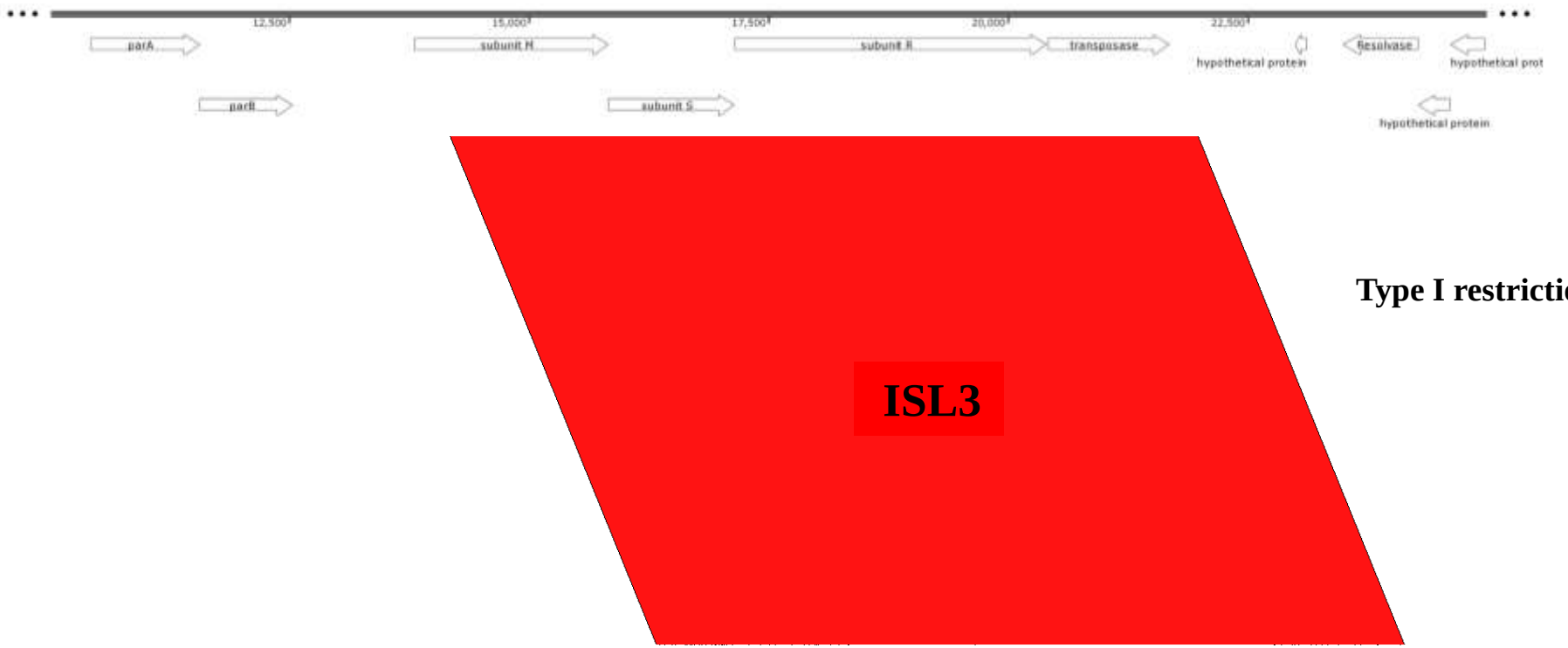
Isolate	Hospital	Isolation date	MIC COL (mg/L)	Mutations in mgrB
510	A	17-02-10	4 R	IS5 126
506	A	18-02-10	24 R	**ISL3 3**
523	A	25-02-10	3 R	IS5 126
545	C	31-08-13	16 R	IS5 75
529	C	17-09-13	96 R	-
542	C	21-09-13	12 R	**ISL3 133**
551	C	17-03-14	12 R	**ISL3 134**
550	C	23-03-14	12 R	G110C point mutation
538	A	09-04-14	256 R	IS5 75
535	A	11-04-14	12 R	**ISL3 133**
541	A	14-04-14	6 R	**ISL3 133**
543	A	14-04-14	4 R	**ISL3 133**
531	A	16-04-14	32 R	**ISL3 133**
528	A	17-04-14	8 R	**ISL3 133**
536	C	06-05-14	8 R	G110C point mutation
540	C	08-05-14	8 R	G110C point mutation
555	B	18-06-14	8 R	**ISL3 133**
549	B	19-06-14	16 R	Gene deletion
552	B	03-07-14	12 R	**ISL3 133**

Results (2a)

pKpQIL plasmids



Type I restriction-modification system + transposase

R restriction endonuclease subunit
S DNA sequence specificity subunit
M DNA methyl transferase subunit

chromosome

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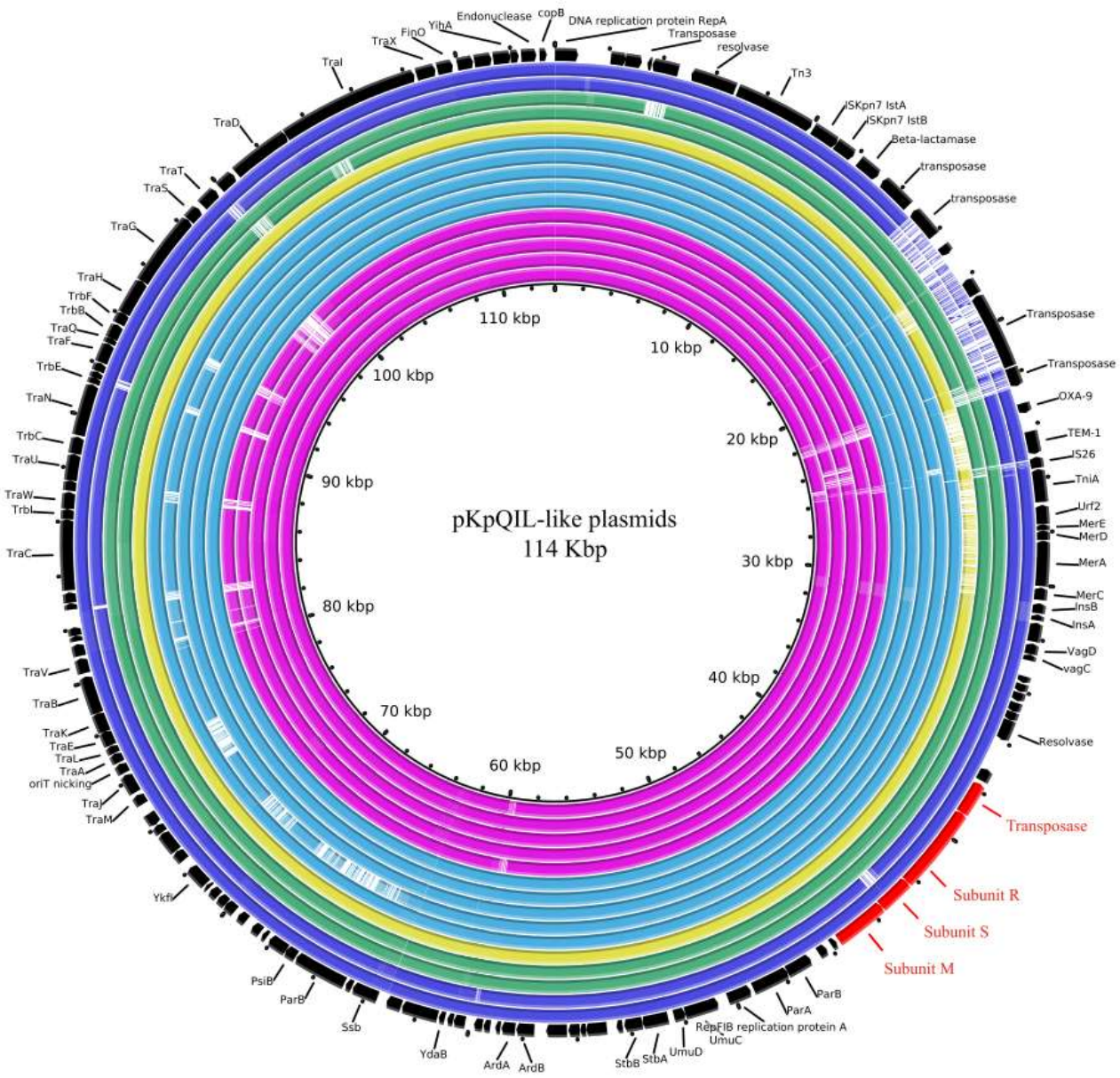
Genome comparison of ISL3-carrying pKpQIL-like plasmids in Italian and Greek isolates.



Results (2b)

ISL3 location

Complete ISL3 sequence was localized on pKpQIL-like plasmids, which were detected in all five MLST types

ST512 KPC-3, Italy

ST307 KPC-3, Italy

ST37 KPC-3, Italy

ST258 KPC-2, Greece

ST147 KPC-2, Greece

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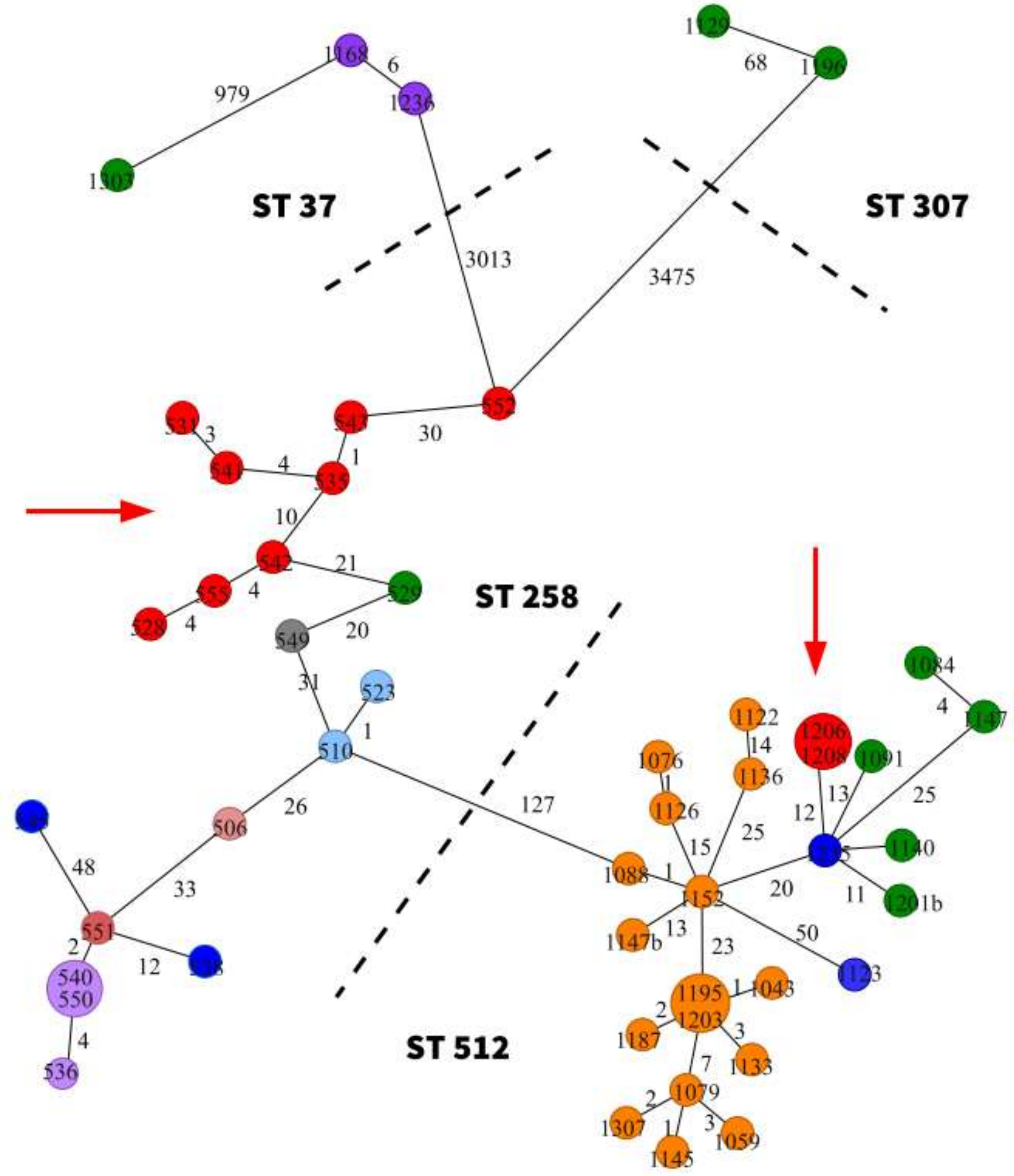

Results (3)

Minimum spanning tree

Minimum spanning tree (4891 alleles) based on allelic mismatch between Italian isolates and Greek isolates with mutations in *mgrB.*

The **red arrows** point out clusters of isolates with identical insertions of ISL3 in nucleotide position 133 of mgrB.

Results (4)

ISL3 insertion targets in the chromosome

ISL3 not only targets *mgrB*, but also other inner membrane protein coding sequences such as *marC*, *yfdC*, *igaA*, and *sbmA*.

Isolate	Country	Insertion	Gene Function
1084	Italy	marC	Inner-membrane protein
1235	Italy	sbmA	Inner-membrane protein
192914	Greece	yidL	AraC family putative transcriptional regulator
	Greece	yfdC	Inner-membrane protein
192903	Greece	igaA	Inner-membrane protein
192875	Greece	ycgF/ycgZ	Two-component system genes for regulation of biofilm and acid-resistance
2 isolates	Italy	mgrB	Inner-membrane protein
10 isolates	Greece	mgrB	Inner-membrane protein

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Concluding remarks

1. Colistin-resistance in CPKP was sporadically reported in the early days of the CPKP outbreak from 2007 to 2010. Nowadays, is very common among CPKP in countries like Greece and Italy.
2. We found that mgrB was disrupted at identical nucleotide positions in different lineages, which shows that ISL3 recombines at specific target sequence sites.
3. The frequent insertion of ISL3 in *mgrB*, and in other inner membrane protein sequences suggest that these targets are not random, but are hotspots for integration of ISL3.
4. The ISL3-carrying plasmid is omnipresent in CPKP, in both KPC-2-KP lineages isolated in Greece, as well as KPC-3-KPs originating from Italy.
5. We showed both horizontal transmission by plasmids, and vertical clonal expansion of colistin resistance by the same molecular mechanism. Both represent important epidemiological explanations for the emergence of colistin resistance in CPKP.

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John W. Rossen
Alexander W. Friedrich

Erik Bathoorn