

Resistome analysis of a carbapenemase (OXA-48)-producing and colistin-resistant *Klebsiella pneumoniae* strain

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MAIN TEXT

Carbapenemase-producing *Klebsiella pneumoniae* are increasingly reported worldwide (1, 2). Therefore, polymyxins (colistin, polymyxin B) often constitute last resort antibiotics to treat infections due to those multidrug-resistant carbapenemase producers. Here the genetic basis of the antibiotic resistance determinants of a carbapenem- and colistin-resistant *K. pneumoniae* isolate was investigated.

A patient was hospitalized at the Bicêtre Hospital (Paris, France) for multiple bone fractures that occurred following a 6 floors fall in Bucarest. *K. pneumoniae* FR-1 was recovered from routine rectal screening. This first isolate was resistant to carbapenems, fluoroquinolones, rifampin, trimethoprim-sulfamethoxazole, and fosfomycin. FR-1 produced the carbapenemase OXA-48 and the extended-spectrum β -lactamase CTX-M-15, as determined by two specific PCRs. Then, the patient developed high grade fever. Since an infection due to resistant Gram negative bacteria was suspected, he received an empirical antibiotic treatment made of colistin (5 mg/kg/day) and amikacin (15mg/kg/day) for two days. Eight days later, he developed a wound infection from which *K. pneumoniae* strain FR-2 (with the same resistance profile as FR-1) was recovered. The treatment was therefore switched to tigecycline (2x100mg/day), colistin (5mg/kg/day) and amikacin (15mg/kg/day). Then, another *K. pneumoniae* isolate (FR-3) was recovered from the same wound and exhibited additional resistance to colistin. The antibiotherapy was modified for doripenem (4x1gr/day), fosfomycin (3gr/day) and tigecycline (2x100 mg/day) that cured the infection.

Genome sequencing (supplementary Methods) of FR-3 shows that it belongs to sequence type ST15, a widely distributed multidrug resistant clone (4, 5). Genomic investigations of two outbreaks involving ST15 clones in Nepal and in the Netherlands in 2012 subdivided this clade in two main lineages harbouring distinct capsule synthesis locus (*cps*) (6). FR-3 exhibits a *cps* locus serotype K24, the same serotype as the CTX-M-15-producing outbreak strains from the Netherlands

(6). FR-3 and the six ST15 strains from China, Nepal, Netherlands, and Taiwan compared here harbour the *Yersinia* high-pathogenicity island and the ferric uptake operon *kfuABC* (17, Figure 1). Eleven antimicrobial resistance genes were identified (8), in accordance with the phenotypic resistance pattern (Table 1, Table S1). Detailed analysis of the genome neither identified genes encoding ADP-ribosylation enzymes (Arr) nor RpoB polymorphism(s) that could explain the observed resistance to rifampin. PlasmidFinder (9) identified five putative distinct plasmid replicons, namely ColRNAI, IncL, ColpVC and IncFIB and two distinct incFII replicons with 95.9% identical RNAI-FII sequences. One of the IncFII replicon was identified on the same contig as a resistance gene (*tetA*). The *bla*_{OXA-48} gene was identified onto an IncL backbone. The latter plasmid sequence was 99% identical to the previously reported 62-kb pOXA-48a, known to be self-conjugative and conjugating at high frequency (10).

All contigs carrying resistance genes exhibited much lower median sequencing depth than the rest of the assembly (between 57-83%, Table 1), similarly to most regions presenting a high number of hits against the RefSeq plasmid database (Figure 1). This suggests that these genes are located on plasmids. Homologs of type IV secretion system (type F) proteins were identified on several small contigs, indicating that FR-3 may carry a second conjugative plasmid.

Two non-synonymous mutations previously reported in colistin-resistant strains (12) were identified in the PhoQ/PhoP regulator *mgrB* gene (N42Y, K43I) likely explaining the acquired resistance to colistin. Indeed, substitutions or deletions in the *mgrB* gene of *K. pneumoniae* are the most frequent molecular mechanisms of acquired resistance to colistin (11). Non-synonymous mutations were also identified in the *gyrA* (S83F, D87A) and *parC* (S80I) genes, and are likely responsible for the acquired resistance to fluoroquinolones (Table 1) (13). Efflux pumps such as *oqxA/B* might also be involved in the observed resistances.

This analysis characterizes the resistance determinants that have accumulated over time in FR strain, leading to an almost pan-resistant strain.

The sequences were submitted to ENA under the accession PRJEB20782.

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Disclosure statement

The authors did not report any potential conflict of interest.

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FIGURES

Figure 1: Genome map of the *K. pneumoniae* FR-3 isolate. Circles: **1)** Contig boundaries **2)** Open reading frames encoded on the leading and lagging strands. rRNA and tRNA are coloured in red **3)** Whole genome alignments with the following *K. pneumoniae* strains: two ST15 strains from the Netherland outbreak (GCF_001596925.1, GCF_001597245.1), two ST15 strains from Nepal (GCF_000764615.1, GCF_000943095.1), two additional ST15 from Taiwan and China (GCF_001750805.1, GCF_001663195.1) and three unrelated ST258, ST11 and ST147 (GCF_000598005.1, GCF_000240185.1, GCF_001746535.1). **4)** GC skew **5)** GC content **6)** Histogram of the sequencing depth. Regions presenting more than 3 times higher depth as the median of the assembly are highlighted in green. Regions presenting a sequencing depth lower than half of the median depth are coloured in red. **7)** Histogram of the count of significant blastp versus the RefSeq plasmid database (limited to maximum 50) **8)** Histogram of the number of significant blastp hits in the PHAST database. Outer labels highlight relevant genes or operons. **In red:** antibiotic resistance genes. **In blue:** virulence genes. **In black:** probes of known plasmids identified using PlasmidFinder.

TABLES

Table 1: List of coding sequences associated with drug resistance from the colistin-resistant *K. pneumoniae* FR-3

Gene	Best hit identity (%)	Best Hit Accession	Antibiotic Resistance	Contig size (bp)	Depth ratio gene ¹	Depth ratio contig ²
<i>aac(3)-IIa</i>	99.77	X51534	Aminoglycosides	2915	0.56	0.63
<i>aph(3')-Ia</i>	100	V00359	Aminoglycosides	1334	0.8	0.68
<i>bla_{SHV-28}</i> ³	100	HM751101	Narrow-spectrum β -lactamase	85380	0.56	0.8
<i>bla_{OXA-48}</i>	100	AY236073	Carbapenems	2231	0.51	0.57
<i>bla_{CTX-M-15}</i>	100	DQ302097	Extended-spectrum Cephalosporins	10416	0.61	0.63
<i>oqx^A</i> ³	99.23	EU370913	Quinolones	194368	0.78	1.02
<i>oqx^B</i> ³	98.86	EU370913	Quinolones	194368	1.02	1.02
<i>fos^A</i> ³	97.62	NZ_AFB001000747	Fosfomycin	362468	0.73	1.09
<i>catA1</i>	99.85	V00622	Phenicol	1204	0.95	0.83
<i>tet(A)</i>	100	AJ517790	Tetracycline	13075	0.64	0.61
<i>dfrA30</i>	99.58	AM997279	Trimethoprim	10416	0.71	0.63
Point mutations	Amino acid change					
<i>GyrA</i> ³	S83F, D87A	-	Quinolones	579439	1.12	0.99
<i>ParC</i> ³	S80I	-	Quinolones	103616	1.14	1.07
<i>MgrB</i> ³	N42Y, K43I	-	Colistin	227842	1.04	0.98

¹ ratio of the median sequencing depth of the gene as compared to the median of the whole assembly (259)

² ratio of the median sequencing depth of the contig encoding the gene as compared to the median of the whole assembly (259)

³ encoded on the chromosome

