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SHORT COMMUNICATION

Characterization of an IncL/M plasmid carrying bla_{OXA-48} in a *Klebsiella pneumoniae* strain from Italy

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SUMMARY

Here we report the complete nucleotide sequence of a 49.257-bp IncL/M conjugative plasmid (pRAY) carrying the bla_{OXA-48} gene collected from a *Klebsiella pneumoniae* clinical strain isolated in Italy. The genetic environment of pRAY plasmid revealed that the bla_{OXA-48} gene was located within a *Tn1999.2* transposon. The pRAY plasmid differed from bla_{OXA-48}-harboring IncL/M plasmids by genetic context and size. Comparative analysis demonstrated that pRAY plasmid lacked a region of ~15 kb carrying genes encoding proteins involved in pilus assembly and plasmid conjugative apparatus.

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Recent years have seen a rapid spread of carbapenem-resistant *Enterobacteriaceae* (CRE) worldwide (Nordmann *et al.*, 2011). The carbapenem resistance in *Enterobacteriaceae* is mainly due to the production of ß-lactamases belonging to the Ambler Class A, B and D with carbapenemase activity. The circulation of the different types of carbapenemase varies geographically, showing a different epidemiology for each country. In particular, KPC is the most common carbapenemase in Israel, China, Italy, Greece, the USA and South America, NDM is endemic in Indian subcontinent, while OXA-48 is widely disseminated in Turkey, North Africa and in different European countries (Nordmann and Poirel, 2014).

It was demonstrated that bla_{OXA-48} gene is located within a transposon designated Tn*1999* and molecular studies revealed that this transposon is commonly associated with IncL/M-type conjugative plasmids (Poirel *et al.*, 2012). Currently, four different transposons, designated Tn*1999* to Tn*1999.4,* have been found to be associated to the bla_{OXA-48} gene (Poirel *et al.*, 2012; Patron *et al.*, 2013).

In this study, we characterized the complete sequence of an bla_{OXA-48}-carrying IncL/M-type plasmid that was recovered from a clinical *K. pneumoniae* strain isolated in Italy.

In November 2013, a multidrug-resistant *K. pneumoniae* strain was isolated from urine sample of a 23-year-old male patient at the University-Hospital St. Orsola-Malpighi, Bologna, Italy. The isolate was identified by MALDI-TOF MS assay and antimicrobial susceptibility testing was performed by Vitek2 and confirmed by E-test assay. MIC results were interpreted according to EUCAST clinical breakpoint v7.1 (EUCAST). PCR and sequencing were performed to identify the carbapenemase gene.

Whole-genome DNA was extracted from transformant *E. coli* and treated with Plasmid-Safe ATP-Dependent DNase (Epicentre) to remove contaminant chromosomal DNA. Sequencing was performed using the 454 GS Junior (Roche) and MySeq (Illumina) systems. Newbler and Mira assemblies were merged using CISA (Lin and Lia, 2013) and scaffolded with SSPACE (Boetzer *et al.*, 2011) software and gap closure was performed using PCR followed by Sanger sequencing. Genome annotation was

Table 1 - *Antimicrobial susceptibility of the K. pneumoniae isolate and E. coli DH10B transformant carrying the* bla_{OXA-48} *plasmid pRAY.*

	MIC (mg/liter)	
Antimicrobial agent	*K. pneumoniae (pRAY)*	*E. coli DH10B (pRAY)*
Ampicillin	≥256	≥256
Amoxicillin-clavulanic ac.	>256	≥256
Temocillin	≥1024	≥1024
Piperacillin-tazobactam	≥256	≥256
Cefotaxime	2	2
Ceftazidime	1	1
Imipenem	8	4
Meropenem	4	2
Ciprofloxacin	8	0.06
Gentamicin	32	0.25
Amikacin	4	2
Tigecycline	1	0.25
Colistin	0.125	0.125

Key words:
Klebsiella pneumoniae, Plasmid, OXA-48.

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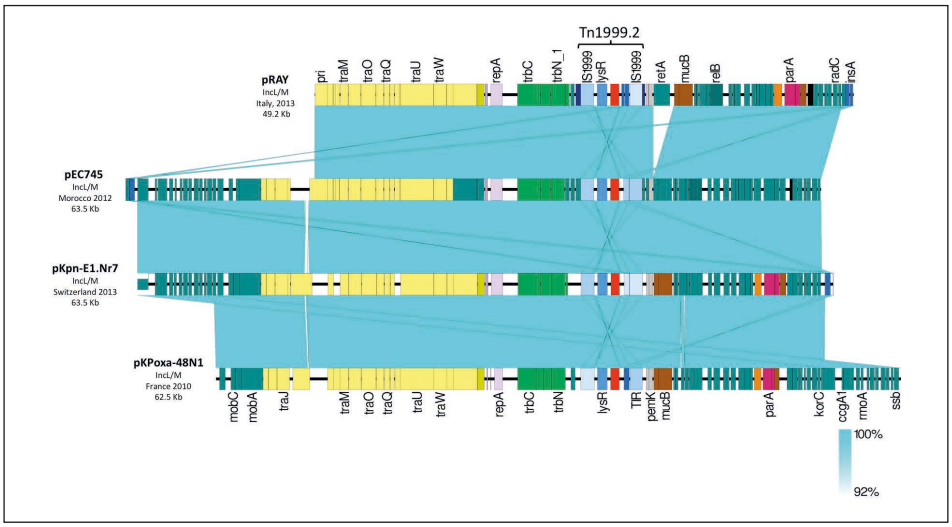

Figure 1 - *Comparative analysis of plasmid pRAY (Accession no. KX524525.1) with others bla*$_{OXA-48}$*-harboring IncL/M plasmids, pEC745 (CP015075.2) pKpn-E1.Nr7 (KM406491.1) and pKPoxa-48N1 (KC757416.2). Yellow boxes indicate conjugal transfer genes. Red boxe indicates bla*$_{OXA-48}$ *gene. Blue boxes indicate mobile elements. Light-blue shading indicates regions with identity higher than 90%.*

performed with a modified version of Prokka (Seemann, 2014) and features were manually curated with Artemis (Sanger Institute).

The *K. pneumoniae* strain was resistant to penicillins, imipenem, ciprofloxacin, gentamicin, while it exhibited intermediate susceptibility to meropenem and susceptibility to cephalosporins, amikacin, tigecycline and colistin (*Table 1*). PCR amplification and sequencing showed that *K. pneumoniae* strain was positive for *bla*$_{OXA-48}$ carbapenemase gene. Molecular analysis demonstrated the *bla*$_{OXA-48}$ gene was identified on a plasmid of ~50 Kb. Plasmid transferibility was confirmed by electroporation into electrocompetent *E. coli* DH10B (*Table 1*).

The OXA-48-harboring plasmid, named pRAY (GenBank accession number KX524525), was 49.257 bp in size with a G+C content of 52.08%. It harbored 64 potential coding sequences (CDSs) including OXA-48 carbapenemase and a large region containing Tra conjugal transfer proteins. Genetic analysis of pRAY sequence revealed that *bla*$_{OXA-48}$ gene was found to be a part of the transposon Tn*1999.2*. Comparative analysis demonstrated that pRAY possessed a similar architecture to that of other *bla*$_{OXA-48}$-harboring IncL/M plasmids previously sequenced (*Figure 1*). In detail, BLAST analysis showed that the pRAY has high similarity with pEC745 (96% identity for 99% coverage), pKPoxa-48N1 (95% identity 99% coverage) and pKpn-E1.Nr7 (96% identity 99% coverage) plasmids. However, pRAY differed from *bla*$_{OXA-48}$-harboring IncL/M plasmids by insertion of a bacterial retroelement belonging to the group II intron (*retA* gene) upstream *muc* operon. In addition, a ~15 kb region located between insertion sequence (IS) IS1R and *tra* operon was absent in pRAY (*Figure 1*). This region carried *traHIJK* and *mobCAB* genes encoding proteins involved in pilus assembly and plasmid conjugative apparatus (Zatyka and Thomas, 1998; Smillie *et al.*, 2010) These findings indicate a lower mobility of this plasmid thus suggesting lower spreading rather than common ~62 kb *bla*$_{OXA-48}$-harboring IncL/M plasmids.

Although *bla*$_{OXA-48}$ gene is located in an active transposon, the dissemination of this carbapenemase is commonly associated with the spread of a single epidemic plasmid (Poirel *et al.*, 2012). Molecular studies have shown that *bla*$_{OXA-48}$ is located within a composite transposon in plasmids of ~62 Kb (Poirel *et al.*, 2012; Stoesser *et al.*, 2016; Zatyka and Thomas, 1998; Beyrouthy *et al.*, 2014). Here, we describe the complete sequence of a *bla*$_{OXA-48}$-carrying plasmid from a *K. pneumoniae* isolated in Italy, a country where this carbapenemase is rare. Unlike other reports, this study showed the complete sequence of a plasmid that differs from common IncL/M *bla*$_{OXA-48}$-harboring plasmids for its genetic context and size. In particular, pRAY plasmid is smaller than commonly reported *bla*$_{OXA-48}$- carrying plasmids, thus demonstrating a wider variability in the size of these plasmids (Poirel *et al.*, 2012; Stoesser *et al.*, 2016). In conclusion, our findings broaden our knowledge of the *bla*$_{OXA-48}$-carrying plasmids and provide important information that can be used to better define the molecular epidemiology of carbapenemase-producing *Enterobacteriaceae*.

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