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Insights into the evolution of gene organization and multidrug resistance from *Klebsiella pneumoniae* plasmid pKF3-140

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ABSTRACT

Plasmid-mediated transfer of drug-resistance genes among various bacterial species is considered one of the most important mechanisms for the spread of multidrug resistance. To gain insights into the evolution of gene organization and antimicrobial resistance in clinical bacterial samples, a complete plasmid genome of *Klebsiella pneumoniae* pKF3-140 is determined, which has a circular chromosome of 147,416 bp in length. Among the 203 predicted genes, 142 have function assignment and about 50 appear to be involved in plasmid replication, maintenance, conjugative transfer, iron acquisition and transport, and drug resistance. Extensive comparative genomic analyses revealed that pKF3-140 exhibits a rather low sequence similarity and structural conservation with other reported *K. pneumoniae* plasmids. In contrast, the overall organization of pKF3-140 is highly similar to *Escherichia coli* plasmids p1ESCUM and pUTI89, which indicates the possibility that *K. pneumoniae* pKF3-140 may have a potential origin in *E. coli*. Meanwhile, interestingly, several drug resistant genes show high similarity to the plasmid pU302L in *Salmonella enterica* serovar Typhimurium U302 strain G8430 and the plasmid pK245 in *K. pneumoniae*. This mosaic pattern of sequence similarities suggests that pKF3-140 might have arisen from *E. coli* and acquired the resistance genes from a variety of enteric bacteria and underscores the importance of a further understanding of horizontal gene transfer among enteric bacteria.

1. Introduction

Klebsiella pneumoniae, a gram-negative bacteria, which causes pneumonia, liver abscess, meningitis, urinary tract infections and other infectious diseases, is considered to be the most important iatrogenic pathogen to humans (Podschun and Ullmann, 1998). This bacterium has been reported to be increasingly antibiotic-resistant, posing a great challenge to the successful control and treatment of patients with severe underlying diseases or nosocomial infections worldwide (Jurczak et al., 2007; Lederman and Crum, 2005). Although complete mechanisms of *K. pneumoniae* virulence are still lacking, the molecular underpinnings of pathogenicity in multiple-resistance for such pathogenic bacteria are generally regarded as plasmid mediated (Hastings, 2004; Shen et al., 2008). Plasmids have been considered the causative agents because of their ability to acquire antibiotic resistance in different species and can lead to rapid spread of antibiotic resistance (Levy and Marshall, 2004).

Currently, more than 30 plasmids ranging from 3 kb to 270 kb have been identified in *K. pneumoniae* in the past decade (http://www.ncbi.nlm.nih.gov/genomes/genlist.cgi?taxid=2&type=2&name=Bacteria%20Plasmids). Studies on plasmid pJHCMW1 (accession number AF479774, 11,354 bp, harbored by a clinical *K. pneumoniae* strain) characterized its antibiotic resistance due to the transposon Tn*1331*, which contains *aac(6′)-Ib*, *aadA1*, bla_{OXA-9}, and bla_{TEM-1} genes (Sarno et al., 2002). The complete nucleotide sequence of plasmid pKP96 (accession number NC_011617), from *K. pneumoniae* isolated in China, exhibited 91% identity with the IncN plasmid R46 (accession number NC_003292) and encoded quinolone resistance genes determined by *qnrA1*, *aac(6′)-Ib-cr* and $bla_{CTX-M-24}$ (Shen et al., 2008). The plasmid

Abbreviations: bp, base pair(s); C, cytidine; CLSI, Clinical and Laboratory Standards Institute; DHPS, dihydropteroate synthetase; G, guanosine; MIC, minimal inhibitory concentration; NICPBP, National Institute for the Control of Pharmaceutical and Biological Products; Nt, nucleotide; ORF, open reading frame; P, plasmid; PCR, polymerase chain reaction; RM, restriction modification; μg, microgram.

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pK245 (accession number NC_010886), a 98,264 bp in length harboring *qnrS* and bla_{SHV-2} genes, was found to be responsible for quinolone resistance and extended spectrum β-lactam resistance in a clinical *K. pneumoniae* (Chen et al., 2006b). It was shown that the *qnrS*-containing region of pK245 is nearly identical (>99% identity) to the other three *qnrS*-carrying plasmids (pVQ01 from *Salmonella enterica* subsp. *enterica* serovar Virchow, accession number JQ609357, pINF5 from *S. enterica* subsp. *enterica* serovar Infantis, accession number AM234722 and pAH0376 from *Shigella flexneri*, accession number AB187515). Moreover, complete sequencing and comparative analysis revealed that the 41,723 bp multiple-resistance plasmid pMET1 from *K. pneumoniae* (accession number EU383016), was closely related to *Yersinia pestis* plasmid pCRY (accession number AE017044) and most possibly can be mobilized between *Yersinia* and other Enterobacteriaceae (Soler Bistue et al., 2008).

In order to relieve growing pressures on public health and biodefense threat, considerable efforts should be made to completely sequence increasing number of drug resistance plasmids to understand the mechanisms of the emergence and spread of antimicrobial resistance in clinical bacterial strains. Previously, we have reported that a 70 kb plasmid named pKF3-70 from a *K. pneumoniae* strain (accession number FJ494913) isolated by the laboratory of the first affiliated hospital of Wenzhou Medical College, Wenzhou, China (Yi et al., 2010). This plasmid is closely related to some *E. coli* plasmids in its backbone structure and carries an extended-spectrum beta-lactamase gene, $bla_{CTX-M-14}$, which favors the transfer via conjugation with *K. pneumoniae* to recipient cells. In this study, we performed extensively comparative and evolutionary analyses on the previously reported multiple-resistance plasmid pKF3-140 isolated from the identical host strain as pKF3-70. Similar to pKF3-70, pKF3-140 is also quite different from other known *K. pneumoniae* plasmids, and shares a certain degree of sequence and structural similarity to the plasmids from *E. coli*.

2. Materials and methods

The host strain (*K. pneumoniae* KF3) of pKF3-140 was collected from the sputum samples of a patient hospitalized in 2006 at First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China. Isolation, sequencing and sequence assembly of pKF3-140 were carried out as described previously (Yi et al., 2010; Zhao et al., 2010).

Potential open reading frames (ORFs) were predicted with the Glimmer3 software (http://www.cbcb.umd.edu/software/glimmer) and ORFs larger than 90 bp were retained as functional genes (Guo et al., 2003). Database searches were performed against SWISS-PROT/TrEMBL, NCBI/CDD database, and RefSeq using BLAST programs with cutoff values of 10^{-3} and 25% identity. Domain characterization was performed using the Interproscan program with the default settings (http://www.ebi.ac.uk/interpro). The plasmids used for extensive comparative analysis were downloaded from NCBI (http://www.ncbi.nlm.nih.gov). Orthologous groups of genes from pKF3-140, pUTI89 and p1ESCUM were identified using the OrthoMCL program with the default parameters (http://www.orthomcl.org/cgi-bin/OrthoMclWeb.cgi), which has been proven to be a very powerful tool in identifying orthologous families from multiple genomes, and it has been widely

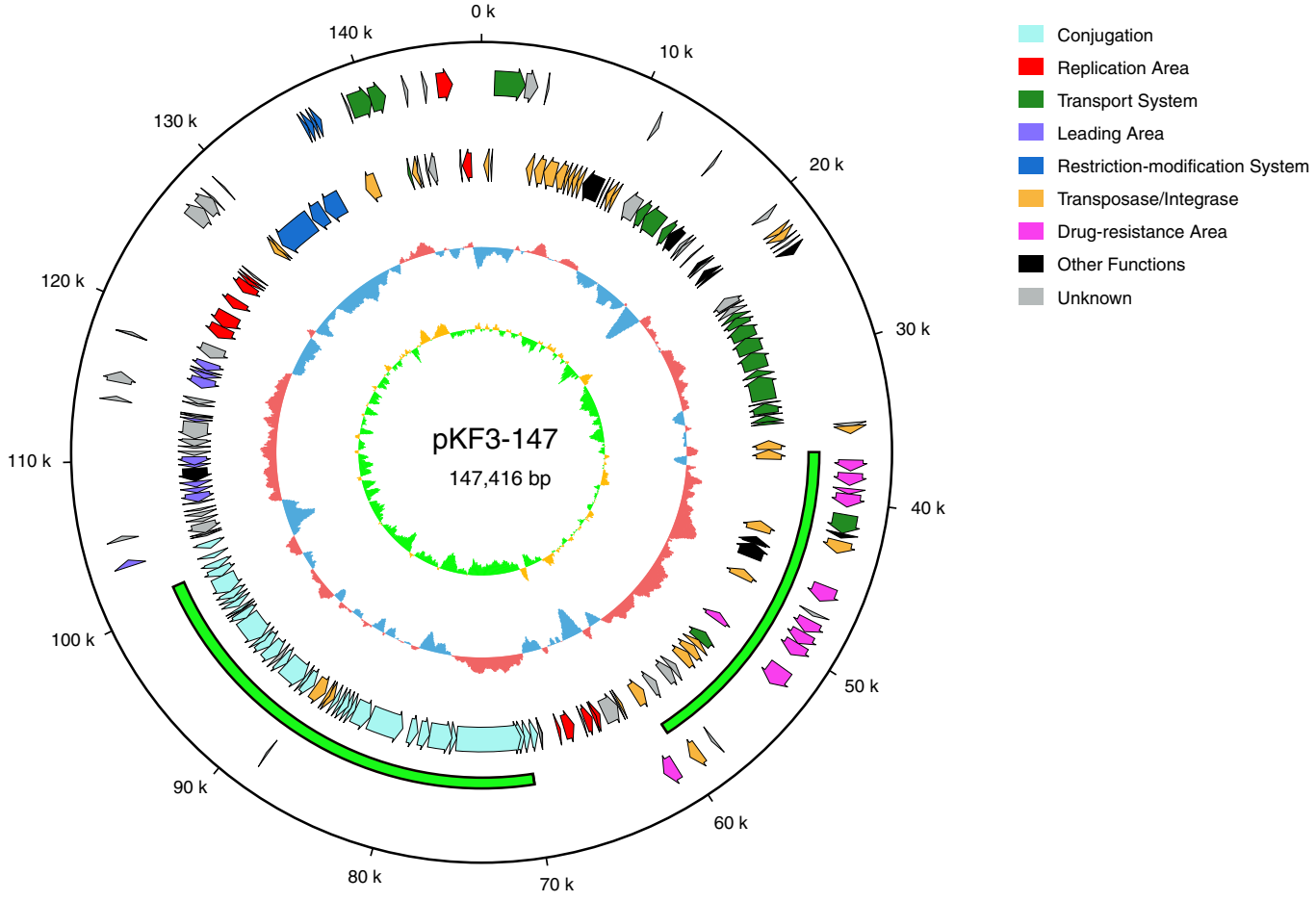

Fig. 1. The circular organization of pKF3-140. The outer circle indicates the genomic location. The second and third circles indicate genes in the forward and the reverse orientations, respectively. The fourth circle shows GC skew (G−C/G+C) with red being areas of positive GC skew and blue being areas of negative GC skew. The fifth circle shows GC content, where a G+C content of >50% is shown in yellow and a G+C content of <50% is shown in green. The MDR-encoding region (between 37 and 60 kbp) and the tra-encoding genes (between 70 and 101 kbp) were marked by green arc area.