Characterization of an IncFIB/IncHI1B Plasmid Encoding Efflux Pump TMexCD1-TOprJ1 in a Clinical Tigecycline- and Carbapenem-Resistant *Klebsiella pneumoniae* Strain

Xuemei Yang,^a Lianwei Ye,^a Edward Wai-Chi Chan,^b Rong Zhang,^c Sheng Chen^a

^aDepartment of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong

^bState Key Lab of Chemical Biology and Drug Discovery, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Hong Kong

^cDepartment of Clinical Laboratory, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

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A novel plasmid-encoded resistance-nodulation-division (RND) efflux pump, TMexCD1-TOprJ1, conferring resistance to multiple drugs, including tigecycline, has recently been identified in *Klebsiella pneumoniae* strains (1, 2). However, the gene cluster *tmexCD1-toprJ1* was rarely identified in clinical *Enterobacteriaceae* isolates (1). Here, we report a novel plasmid from a clinical *K. pneumoniae* strain isolated in Hong Kong that harbored this gene cluster and exhibited resistance to tigecycline.

A *K. pneumoniae* strain HKU57 isolate was recovered from the blood culture of a clinical patient in a hospital in Hong Kong in 2017. Antimicrobial susceptibility testing showed that it was resistant to tetracycline and tigecycline; the β -lactam antibiotics aztreonam, cefotaxime, ceftazidime, meropenem, imipenem, and ertapenem; the aminoglycoside antibiotics gentamicin and amikacin; ciprofloxacin; chloramphenicol; and azithromycin but remained susceptible to colistin (see Table 1).

Strain HKU57 was subsequently characterized by whole-genome sequencing and *in silico* data analysis. The complete genome of strain HKU57 was obtained by hybrid assembly of both Illumina and Nanopore reads using Unicycler (3). The isolate harbored a 5.11-Mb chromosome and three plasmids, i.e., pHKU57_1 (273,789-bp), pHKU57_2 (111,290-bp), and pHKU57_3 (59,223-bp). This strain was found to belong to ST15 by Kleborate based on multilocus sequence typing (MLST) (<https://github.com/katholt/Kleborate>) (4) and KL48 and O1v1 serotype by Kaptive based on capsule and lipopolysaccharide synthesis loci (5). BLASTn against the virulence genes database (<http://bigsdbs.pasteur.fr/klebsiella/klebsiella.html>) showed that strain HKU57 harbored virulence genes *mrkABCDHFIIJ* (type 3 fimbriae) and *kfuABC* (ferric uptake system). This strain did not harbor virulence plasmid-bearing virulence genes, such as *iro*, *iuc*, *rmpA*/ *rmpA2*. The newly reported RND efflux pump gene cluster *tnfxB1-tmexCD1-toprJ1* was located on plasmid pHKU57_1 (Fig. 1a). The MIC of tigecycline testing strain HKU57 was 8 μ g/ml (Table 1), which was consistent with the previously reported *tnfxB1-tmexCD1-toprJ1* harboring strains (1, 2). Plasmid pHKU57_1 was an IncFIB/IncHI1B plasmid containing multiple resistance genes, including *strAB*, *armA*, *aph(3')-Ia*, *qnrB4*, *sul1*, *mphA*, *mphE*, *msrE*, and *bla*_{DHA-1}. In BLAST against the NCBI database, this plasmid was found to show the highest similarity (82% coverage, 99% identity) to plasmid pHNWH61-1, a 256,606-bp IncFIB/IncHI1B plasmid recovered from a *K. pneumoniae* strain, which also harbored the *tnfxB1-tmexCD1-toprJ1* gene cluster (Fig. 1a). Another similar plasmid was pF10AN_1, with 73% coverage and 99% identity, from a *K. pneumoniae* strain, which did not harbor this gene cluster. Plasmid pHKU57_1 shared a

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Address correspondence to Sheng Chen, shechen@cityu.edu.hk.

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TABLE 1 MIC profiles of strain HKU57

Strain	Species	MIC ($\mu\text{g ml}^{-1}$) ^a													
		TIG	TET	MEM	IMP	ETP	CAZ	CTX	ATM	AZI	AMK	CIP	GEN	CHL	CLS
HKU57	<i>K. pneumoniae</i>	8	>128	8	16	16	>128	>128	>128	>128	>128	>128	>128	64	1
25922	<i>E. coli</i>	<0.25	0.5	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	2	2	<0.25	1	4	0.5

^aTIG, tigecycline; TET, tetracycline; MEM, meropenem; IMP, imipenem; ETP, ertapenem; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; AZI, azithromycin; AMK, amikacin; CIP, ciprofloxacin; GEN, gentamicin; CHL, chloramphenicol; CLS, colistin. All tests were performed in duplicate, and each test included three biological replicates.

similar backbone with plasmid pGH27_175, a 175,909-bp plasmid recovered in Hong Kong, which lacked the mosaic resistance sequences in pHKU57_1 (6). Strain HKU57 was found to harbor the *tet(A)* gene, which was located on plasmid pHKU57_3. The *tet(A)* gene was identified as *tet(A)*_AJ517790 with one mutation (p.T42A) and conferred resistance to tetracycline (7). Other genes conferring glycylicycline resistance were further analyzed, and a mutation in RamR (p.A19V) was identified. This mutant was frequently identified in *K. pneumoniae* clinical isolates and had no significant effect on tigecycline resistance (8).

The *tnfxB1-tmexCD1-toprJ1* cluster was located within transposon Tn5393 as part of an insertion that disrupted the transposase in plasmids pHNAH81-1 and pKA9-4 (1, 2, 9). In plasmid pHKU57_1, the *hp2* gene located upstream of this cluster was truncated by insertion sequence IS26 with other parts remaining the same, indicating that the *tnfxB1-tmexCD1-toprJ1* cluster in plasmid pHKU57_1 may have evolved from plasmids such as pHNAH81-1 and pKA9-4 by genetic recombination (Fig. 1b). Similar to pHKU57_1, IS26 truncated the *hp2* gene in plasmid p18-29-MDR, which was also recovered from a *K. pneumoniae* strain. The downstream sequences of Δ Tn5393 in these two plasmids were different, indicating that multiple genetic recombination processes happened during evolution (Fig. 1b).

Strain HKU57 was also resistant to the carbapenem antibiotics meropenem, imipenem, and ertapenem, with MICs of 8, 16, and 16 $\mu\text{g/ml}$, respectively. Whereas no carbapenemase was identified in this strain, further analysis indicated that the outer membrane porin-encoding gene *ompK35* was intact, while *ompK36* was disrupted by ISVsa5 in the chromosome of strain HKU57. Thus, together with the extended-spectrum beta-lactamase DHA-1 it produces, this strain exhibited resistance to carbapenem antibiotics (10–12).

Our data indicate that the *tnfxB1-tmexCD1-toprJ1* gene cluster has disseminated on various plasmids in clinical *K. pneumoniae* strains. Dissemination of such a gene cluster in carbapenem-resistant *K. pneumoniae* strains may pose a great threat to clinical infection control because the treatment choices for this multidrug-resistant pathogen are already very limited.

Data availability. Complete sequences of the chromosome of strain HKU57 and plasmids pHKU57_1, pHKU57_2, and pHKU57_p3 have been deposited in the GenBank database under accession no. CP063215 to CP063218.

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