Complete Sequence of the FII Plasmid p42-2, encoding *bla*$_{CTX-M-55}$, *oqxAB*, *fosA3* and *floR* from *Escherichia coli*

Qiu E Yang[a,b†], Timothy Rutland Walsh[c], Bao Tao Liu [a,b], Meng Ting Zou [a,b], Hui Deng[a,b], Liang Xing Fang[a,b], Xiao Ping Liao [a,b], Jian Sun[a,b*] and Ya Hong Liu[a,b, d*]

[a] National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, South China Agricultural University, Guangzhou, P. R. China.

[b] College of Veterinary Medicine, South China Agricultural University, Guangzhou, P. R. China.

[c] Department of Medical Microbiology, Institute of Infection & immunity, School of Medicine, Cardiff University, Cardiff, UK.

[d] Jiangsu Co-Innovation Centre for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, Jiangsu, P. R. China.

Running title: Complete Sequence of the FII Plasmid p42-2

† Present address: Department of Medical Microbiology, Institute of Infection & immunity, School of Medicine, Cardiff University, Cardiff, UK.

*Corresponding author: Ya-Hong Liu, PH.D, National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, College of Veterinary Medicine, South China Agricultural University, Guangzhou, P. R. China. E-mail: gale@scau.edu.cn. Tel: +86-13602706880; Fax: +86-020-85284896

Jian Sun, PH.D, National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, College of Veterinary Medicine, South China Agricultural University, Guangzhou, P. R. China. E-mail: jiansun@scau.edu.cn. Tel: +86-020-85285507; Fax: +86-020-85285507

Abstract

We sequenced a novel conjugative multidrug resistance IncF plasmid, p42-2, isolated from an *Escherichia coli* strain 42-2 previously identified in China. p42-2 is 106,886 bp in size, composed of a typical IncFII-type backbone (~54 kb) and one distinct acquired DNA region spanning ~53 kb, harboring 12 antibiotic resistance genes ($bla_{CTX-M-55}$, *oqxA*, *oqxB*, *fosA3*, *floR*, *tetA*(A), *tetA* (R), *strA*, *strB*, *sul2*, *aph(3')-II* and Δbla_{TEM-1}). The spread of these multi-drug-resistance determinants on the same plasmid is of great concern and because of co-resistance to antibiotics from different classes is therapeutically challenging.

Keywords: FII Plasmid, multidrug resistance, complete sequencing, mobile genetic elements (MGEs), *Escherichia coli*

Widespread antibiotic resistance poses an enormous threat for human and animal health worldwide (1). This problem has been exacerbated in recent years with the emergence of multidrug-resistant (MDR) plasmids conferring resistance to most classes of antimicrobials (2). Plasmids of the incompatibility F group (IncF), representing one of the most frequently encountered plasmid types (3), have frequently been associated with MDR phenotypes including extended-spectrum β-lactamases (ESBLs) and plasmid-mediated quinolone (PMQR) genes (4, 5). In our previous study, IncF plasmids recovered from *Escherichia coli* of food-producing and companion animals were investigated and most of them carried numerous resistance determinants, such as $bla_{\text{CTX-M}}$, *rmtB*, *oqxAB*, and *floR* (4, 6). The spread of these multi-resistance plasmids has prompted worldwide concern because of co-resistance to multiple antimicrobial agents that will facilitate the survival of bacteria under the selective pressure of antibiotics. Herein, we analysed a common subtype IncF plasmid, F33: A-: B- (FII, FIA, FIB; FAB formula), this plasmid, designated p42-2, contains 12 different resistance genes, was fully sequenced and the data compared with other IncF plasmids.

E. coli 42-2 was recovered from the faeces of a healthy duck in Guangzhou, China. Conjugation was performed by mixing *E. coli* 42-2 and *E. coli* C600 in a liquid medium and isolating for *E. coli* C600 (p42-2) by selecting on MacConkey agar containing streptomycin (1000 mg/L) and cefotaxime (2 mg/L) as previously described (4, 6). p42-2 was extracted from the *E. coli* C600 transconjugant using a commercial kit (Qiagen Midi kit, Qiagen, Germany). Sequencing of p42-2 was carried out on an Illumina genome analyzer IIx with a 500bp paired-end library (approximately 100M available reads, 935-fold genome coverage) and 2000bp paired-end library (approximately 337M available reads, 3150-fold genome coverage), respectively. These raw data were assembled by SOAPdenovo (7). Gene prediction and annotation were performed using RAST tools (8). The sequence comparison

and map generation were performed using BLAST (http://blast.ncbi.nlm.nih.gov) and Easyfig version 2.1 (9).

Plasmid p42-2 confers resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, sulfonamides/trimethoprim, tetracycline, olaquindox, fosfomycin, cephalosporin and florenicol. Because of its broad resistance spectrum, p42-2 was sequenced and fully annotated. p42-2 contains 106,886 bp with 141 predicted open reading frames (ORFs) and is composed of a typical IncFII-type backbone (~54 kb), encoding functions for IncF plasmid replication, horizontal transfer, maintenance and stability functions. The remaining ~53 kb is an acquisition region carrying various resistance genes and mobile genetic elements (MGEs). The whole sequence of p42-2 is organized similarly to other IncF plasmids, including pHNFP460-1 (GeneBank access ion number KJ020575, mainland China) (Fig.1), and pHN7A8 (GeneBank accession number JN232517, mainland China) (10), with a 61%~63% coverage and an overall nucleotide identity of 99%. Sequencing analysis confirm that p42-2 is a chimera plasmid made up of basic an IncF plasmid backbone with an additional ~53 kb mobile region.

The p42-2 backbone (replication and transfer regions) is highly conserved with other known IncF plasmids, such as pHNF460-1, pHN7A8 and pHK23a (GeneBank accession number JQ432559, Hongkong) (Fig. S1). p42-2 contains one replication region (*copB*, *repA3*, *repA1* and *repA4*), belonging to Inc group F, with plasmid replicon type, F33: A-: B-, according to FAB (FII, FIA, FIB) criteria (11). The p42-2 replication region was flanked by plasmid functional modules (*pemK/pemI* and *snrB*), which contributes to plasmid maintenance in the bacterial population (12). This region is indentical to that in other IncFII plasmids pHNFP460-1 and pHN7A8 (Fig.S1). The transfer region of p42-2 comprises of 26 *tra* genes

and 6 *trb* genes (*traM*, *traJ*, *traY*, *traA*, *traL*, *traE*, *traK*, *traB*, *traP*, *traD*, *traG*, *traV*, *traR*, *traC*, *trbI*, *traW*, *traU*, *trbC*, *traN*, *trbE*, *traF*, *traQ*, *trbB*, *trbJ*, *trbF*, *traH*, *traG*, *traS*, *traT*, *traD*, *traI*, *traX*). The gene order of this region is totally conserved in pHNFP460-1 and pHN7A8 (Fig.S1).

The ~53 kb variable region (position 3996 to 56766) comprises 12 resistance determinants, complete or truncated insertion sequences and transposons, as well as many genes of unknown function and was located downstream to the replication region. In comparison with pHNFP460-1 and pHN7A8, p42-2 is composed of a larger multi-resistance region (MRR), which is flanked by seven different oriented IS*26* elements into five succinct regions harboring antibiotic resistance genes (Fig.1): The first region comprises six antibiotic resistance genes (*floR*, *tetA*(A), *tetA*(R), *strA*, *strB* and *sul2*), conferring resistance to florenicol, tetracycline, streptomycin and sulfonomide. This region has highest identity (99.9%) with part of pCFSA007428.01 (GeneBank accession number CP009414, USA). p42-2 possesses an additional sequence (~20 kb) in the second region, with many genes of putative functions, such as plasmid maintenance and stability determinants (*stbD/stbE*) (13), and genes associated with virulence factors (*vagC/vagD*). Interestingly, partial replication genes (*bis-repX-pir*) of IncX plasmid also have been found in this region, suggesting that only part of an IncX plasmid has been captured or acquired of IncX followed be a partial deletion event. The third region possesses two multi-drug efflux pumps *oqxA* and *oqxB*, which are active against fluoroquinlones. IS*26*-like sequences flank this locus (IS*26*-*oqxA*-*oqxB*-IS*26*) which is closely related to pACN001-A (GeneBank accession number KC853434, China) and pOLA52 (GenBank accession number EU370913, Denmark) (14). There are an additional four resistance determinants, *tetA*(R), *fosA3*, $\Delta bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M-55}}$, combined by three IS*26* elements. This region, IS*26*-*tetA*(R)-*fosA3*-IS*26*-$\Delta bla_{\text{TEM-1}}$-*orf477*-

113 *bla*$_{CTX-M-55}$-*Δ*IS*Ecp1*-IS*26*, was identical to plasmids pHNFP460-1 and pHP588 (GenBank
114 accession number AB778291, Japan). p42-2 MRR is flanked by fragments of IS*1* with the
115 same IS*26*/IS*1* backbone as seen in pHN7A8 (Fig.1) and was shown to be combined with a
116 diverse range of MGEs (IS*5075*, IS*50*, IS*CR2*, IS*26*, IS*1294*, IS*Ecp1* and Tn*21*). These
117 MGEs are important in horizontal gene transfer and may provide a favorable genetic
118 environment for plasmid plasticity. For instance, a total of seven IS*26* copies are located on
119 p42-2 (Fig.1), each aasociated with different antibitoic resistance genes such as *bla*$_{CTX-M-55}$,
120 *oqxAB* and *fosA3*. This observation proves further evidence that IS*26* plays an important role
121 in the dissemination and evolution of IncF antibiotic resistance plasmids by creating regions
122 containing multiple antibiotic resistance genes through stepwise integration and/or
123 recombination events mediated by IS*26* (15,16). Thus, the variable region of p42-2, encoding
124 12 antibiotic resistance determinants, may have arisen from MRR acquisition by the actions
125 of MGEs from multiple plasmid sources, with an IncF plasmid backbone.
126
127 In conclusion, p42-2 is a chimera plasmid made up of basic an IncF plasmid backbone with a
128 large multi-resistance region. This region appears to have evolved through the integration of
129 multiple resistance determinants from different sources by the action of MGEs and
130 recombination. The association of these important antibiotic resistance genes (such as *bla*$_{CTX-M-55}$, *oqxAB*, *fosA3* and *floR*) on the same plasmid is therapeutically challenging due to co-
132 selection by various drugs and may confer a selection advantage antibiotic resistant *E. coli*
133 clones.
134
135 **Nucleotide sequence accession number**
136 The annotated sequence of plasmid p42-2 from strain 42-2 has been submitted to GenBank
137 accession number KT990220.

Acknowledgements

This work was supported by the Program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (Grant No. IRT13063), the National Natural Science Foundation and Natural Science Foundation of Guangdong Province, China (Grant No. U1201214), the Natural Science Foundation of Guangdong Province (Grant No. S2012030006590) and the National Natural Science Funds of China (Grant No. 31402247).

Reference

1. **Aminov RI.** 2009. The role of antibiotics and antibiotic resistance in nature. Environ Microbiol **11:**2970-2988.
2. **DeNap JC, Hergenrother PJ.** 2005. Bacterial death comes full circle: targeting plasmid replication in drug-resistant bacteria. Org Biomol Chem **3:**959-966.
3. **Carattoli A.** 2013. Plasmids and the spread of resistance. Int J Med Microbiol **303:**298-304.
4. **Liu BT, Yang QE, Li L, Sun J, Liao XP, Fang LX, Yang SS, Deng H, Liu YH.** 2013. Dissemination and Characterization of Plasmids Carrying *oqxAB-bla*(CTX-M) Genes in *Escherichia coli* Isolates from Food-Producing Animals. Plos One **8:**e73947.
5. **Karisik E, Ellington M, Pike R, Warren R, Livermore D, Woodford N.** 2006. Molecular characterization of plasmids encoding CTX-M-15 β-lactamases from *Escherichia coli* strains in the United Kingdom. J Antimicro Chemother **58:**665-668.
6. **Yang QE, Sun J, Li L, Deng H, Liu BT, Fang LX, Liao XP, Liu YH.** 2015. IncF plasmid diversity in multi-drug resistant *Escherichia coli* strains from animals in China. Front Microbiol **6:**964.

7. **Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J.** 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience **1:**18.

8. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M.** 2008. The RAST Server: rapid annotations using subsystems technology. BMC genomics **9:**75.

9. **Sullivan MJ, Petty NK, Beatson SA.** 2011. Easyfig: a genome comparison visualizer. Bioinformatics **27:**1009-1010.

10. **He L, Partridge SR, Yang X, Hou J, Deng Y, Yao Q, Zeng Z, Chen Z, Liu JH.** 2013. Complete nucleotide sequence of pHN7A8, an F33:A-:B- type epidemic plasmid carrying $bla_{CTX\text{-}M\text{-}65}$, *fosA3* and *rmtB* from China. J Antimicrob Chemother **68:**46-50.

11. **Villa L, García-Fernández A, Fortini D, Carattoli A.** 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicro Chemother **65:**2518-2529.

12. **Gerdes K, Christensen SK, Lobner-Olesen A. 2005.** Prokaryotic toxin-antitoxin stress response loci. Nat Rev Microbiol **3:**371-382.

13. **Hayes F.** 1998. A family of stability determinants in pathogenic bacteria. J Bacteriol **180:**6415-6418.

14. **Norman A, Hansen LH, She, QS, Søren J.** 2008. Nucleotide sequence of pOLA52: a conjugative IncX1 plasmid from Escherichia coli which enables biofilm formation and multidrug efflux. Plasmid **60:**59-74.

15. **He S, Hickman AB, Varani AM, Siguier P, Chandler M, Dekker JP, Dyda F.** 2015. Insertion sequence IS*26* reorganizes plasmids in clinically isolated multidrug-resistant bacteria by replicative transposition. mBio **6:**e00762-00715.

16. **Harmer CJ, Moran RA, Hall RM.** 2014. Movement of IS26-associated antibiotic resistance genes occurs via a translocatable unit that includes a single IS26 and preferentially inserts

adjacent to another IS26. MBio **5**:e01801-01814.

Figure legend

Fig.1 Comparison of p42-2 MRR with other plasmids containing the same resistance modules. The extents and directions of antibiotic resistance (dark arrows) and other selected genes (grey arrows) are indicated by labelled arrows (HPs, hypothetical proteins, are indicated by black boxes). ISs are shown as white boxes labelled with their number/name and *Δ* represents a truncated gene. The plasmid backbone is indicated by dark lines. Light-grey shading and oblique dotted lines between two regions indicate these regions with homology. Diagrams are drawn from sequences available under the following GenBank accession numbers: p42-2, KT990220; pCFSAN007428_01; CP009414, pACN001-A, KC853434; pHNFP460-1, KJ020575 and pHN7A8, JN232517.