2 Ogawa T, Terao Y, Sakata H *et al*. Epidemiological characterization of *Streptococcus pyogenes* isolated from patients with multiple onsets of pharyngitis. *FEMS Microbiol Lett* 2011; **318**: 143–51.

3 Capoor MR, Nair D, Deb M *et al*. Resistance to erythromycin and rising penicillin MIC in *Streptococcus pyogenes* in India. *Jpn J Infect Dis* 2006; **59**: 334–6.

4 Kimura K, Suzuki S, Wachino J *et al*. First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* 2008; **52**: 2890–7.

5 Nagano N, Nagano Y, Kimura K *et al*. Genetic heterogeneity in *pbp* genes among clinically isolated group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* 2008; **52**: 4258–67.

6 Dahesh S, Hensler ME, Van Sorge NM *et al*. Point mutation in the group B streptococcal *pbp2x* gene conferring decreased susceptibility to β-lactam antibiotics. *Antimicrob Agents Chemother* 2008; **52**: 2915–8.

7 Gaudreau C, Lecours R, Ismaïl J *et al*. Prosthetic hip joint infection with a *Streptococcus agalactiae* isolate not susceptible to penicillin G and ceftriaxone. *J Antimicrob Chemother* 2010; **65**: 594–5.

8 Longtin J, Vermeiren C, Shahinas D *et al*. Novel mutations in a patient isolate of *Streptococcus agalactiae* with reduced penicillin susceptibility emerging after long-term oral suppressive therapy. *Antimicrob Agents Chemother* 2011; **55**: 2983–5.

9 Kimura K, Wachino J, Kurokawa H *et al*. Practical disk diffusion test for detecting group B streptococcus with reduced penicillin susceptibility. *J Clin Microbiol* 2009; **47**: 4154–7.

10 Nagano N, Nagano Y, Toyama M *et al*. Penicillin-susceptible group B streptococcal clinical isolates with reduced cephalosporin susceptibility. *J Clin Microbiol* 2014; **52**: 3406–10.

J Antimicrob Chemother 2015
doi:10.1093/jac/dku486
Advance Access publication 27 November 2014

Carbapenem and amikacin resistance on a large conjugative *Acinetobacter baumannii* plasmid

Steven J. Nigro[1]*, Kathryn E. Holt[2], Derek Pickard[3] and Ruth M. Hall[1]

[1]School of Molecular Bioscience, The University of Sydney, Sydney, New South Wales, Australia; [2]Department of Biochemistry and Molecular Biology and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia; [3]Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

*Corresponding author. Tel: +61-2-9351-3809. Fax: +61-2-9351-5858; E-mail: steven.nigro@sydney.edu.au

Keywords: *A. baumannii*, resistance plasmids, *aphA6*, bla_{OXA-23}

Sir,

Worldwide, most multiply antibiotic-resistant (MAR), and particularly carbapenem-resistant, *Acinetobacter baumannii* causing infections belong to global clone 1 (GC1) or global clone 2 (GC2). Though there have been incidences where a different clonal group was predominantly responsible for an epidemic outbreak, MAR isolates that are not members of these clones have been observed to cause infections in hospitals but are not as common.[1]

Isolate D46, collected at Royal North Shore Hospital, Sydney in 2010, was shown to be resistant to ampicillin, imipenem, meropenem, ticarcillin/clavulanate, ceftazidime, cefotaxime, streptomycin, spectinomycin, sulfamethoxazole, tetracycline, trimethoprim, chloramphenicol, florfenicol, kanamycin, neomycin, gentamicin, amikacin, tobramycin, nalidixic acid and ciprofloxacin, making it extensively antibiotic resistant. D46 was previously shown to be ST110 (Oxford MLST) and to harbour the *aphA6* amikacin resistance gene within Tn*aphA6* and an *aadB* gene cassette (gentamicin, kanamycin and tobramycin resistance) in the small plasmid pRAY.[2] An ISAba1 upstream of the chromosomal *ampC* confers resistance to third-generation cephalosporins, ceftazidime and cefotaxime.[3]

Here, we have further examined the causes of resistance. Using PCR as described previously,[4] D46 was shown to contain the *strA-strB*, *sul2* and *tetA*(B) genes, responsible for streptomycin, sulfamethoxazole and tetracycline resistance, respectively. These genes were in the same configuration as in AbGRI1-2 (Tn*6167*),[4] but D46 does not have an island in *comM*. Carbapenem and ticarcillin/clavulanate resistance was due to the bla_{OXA-23} carbapenemase gene, which was within Tn*2006*.

To better understand how Tn*2006* was acquired, conjugation was performed as described previously,[5,6] using D46 as a donor and a rifampicin-resistant mutant of *A. baumannii* ATCC 17978 as a recipient. Transconjugants resistant to meropenem, imipenem and ticarcillin/clavulanate, indicative of bla_{OXA-23}, and resistant to kanamycin, neomycin and amikacin, indicative of *aphA6*, were recovered. Hence, both Tn*2006* and Tn*aphA6* were located on a conjugative plasmid.

The whole genome sequence of D46 was determined using Illumina HiSeq and assembled as described previously.[5] The draft genome comprised 115 contigs with an average read depth of 88.7× coverage and D46 was determined to be ST25 according to the Pasteur MLST scheme. Three plasmids were detected. The sequence of the smallest plasmid, pD46-1 (6078 bp), contained *aadB* and was almost identical to pRAY* (a single base difference).[2] pD46-2 is an 8731 bp cryptic plasmid that was identical to p1ABTCDC0715 (GenBank accession number CP002523) from a GC2 isolate.

To determine whether D46 harboured Tn*2006* together with Tn*aphA6* on a plasmid related to pAb-G7-2[5] and pD72-2,[7] contigs that matched them were retrieved from the draft genome. Seven contigs were recovered and assembled using PCR.[5,7] The amplicons were sequenced, finalizing the assembly. Plasmid pD46-3 has a 67027 bp backbone sharing 99.99% identity (five single-base changes) with that of pD72-2 (Figure 1a). pD46-3 also harboured Tn*aphA6* in the same position as pD72-2 (Figure 1a). One end of two backbone contigs had inversely oriented fragments of ISAba1 and they were linked with bla_{OXA-23} in Tn*2006* using PCR (Figure 1a). The complete 74916 bp sequence of

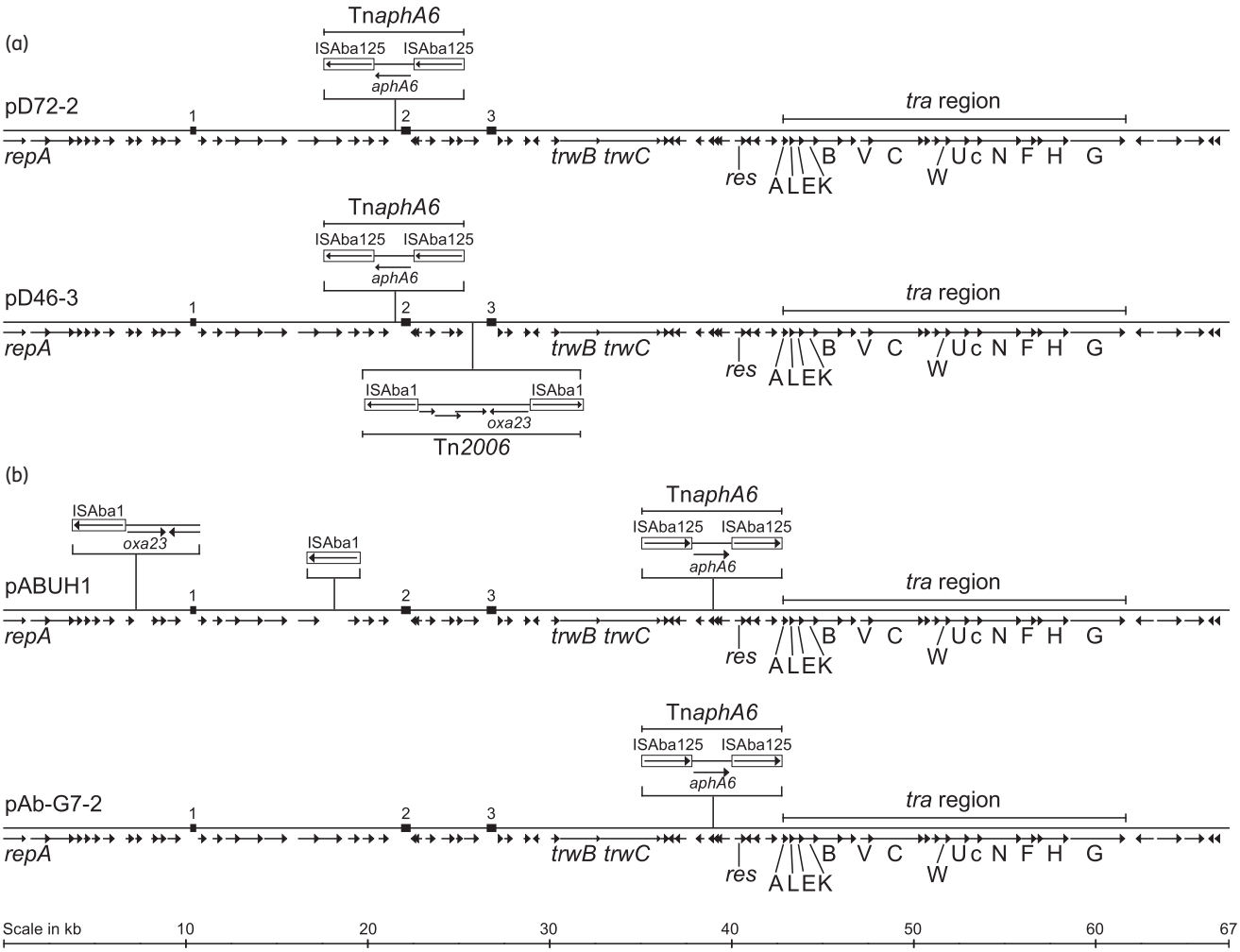

Figure 1. Comparison of *repAci6* plasmids pD72-2, pD46-3 (a), pABUH1 and pAb-G7-2 (b). The plasmid backbones, linearized and opened at *repA*, are represented by horizontal lines. The extent of ORFs and genes are shown as arrows beneath the lines, with names given where a function is known. The transfer region is indicated above each line, the *tra* genes are indicated with capital letters and *trbC* is shown as c. The three repeat regions are indicated by the numbered boxes. The structures of Tn*aphA6*, Tn*2006* and Tn*2008*-like are shown above or below the backbone, with IS represented as open boxes containing an arrow showing their orientation.

pD46-3 was deposited in GenBank under accession number KM977710. As the backbone of pD46-3 was almost identical to that of pD72-2 and pAb-G7-2, pD46-3 also contained the complete set of transfer genes (Figure 1). This is the first report of a completely sequenced *A. baumannii* plasmid that has been demonstrated to simultaneously transfer resistance to carbapenems and aminoglycosides.

A recent genomic study of a GC2 *A. baumannii* collection from a US hospital reported the sequence of pABUH1 (GenBank accession number AYOH01000010), which the authors suggested could be transferable.[8] It was described as a plasmid related to pACICU2, harbouring ISAba1 upstream of *aphA6* and having a copy of bla_{OXA-23} flanked by two copies of ISAba125 that is in the same position as ISAba125 in pACICU2.[8] However, it was recently shown that pACICU2 is likely identical to pAb-G7-2 and in fact harbours Tn*aphA6* at this location.[9] Our analysis of the sequence of pABUH1 revealed that it in fact carries Tn*aphA6* in this position (Figure 1b). Furthermore, ISAba1 is actually upstream of bla_{OXA-23}, in a structure similar to Tn*2008* (GenBank accession number GQ861438) that is flanked by a 9 bp direct repeat. However, in pABUH1 the sequence adjacent to the ISAba1 was 1613 bp whereas it is only 1351 bp in Tn*2008*.

The backbones of the *repAci6* plasmids, pAb-G7-2, pD72-2, pD46-3, pABUH1 and pACICU2, are all very closely related, but they can be separated into two lineages based on the position of Tn*aphA6* (Figure 1). Representatives of the two lineages, pD46-3 and pABUH1, have each acquired the bla_{OXA-23} gene on separate occasions and a sixth *repAci6* plasmid, pA85-3, has also gained bla_{OXA-23} within AbaR4.[6] The acquisition of bla_{OXA-23} in different structures and in separate events indicates that this group of related plasmids plays a vital role in the dissemination of this carbapenemase gene and could be one of the factors responsible for making it a worldwide problem. Hence, surveillance of this group of *A. baumannii* plasmids will be vital in curtailing the spread of carbapenem resistance in *A. baumannii*.

Funding

This study was supported by grants from the School of Molecular Bioscience and the Wellcome Trust Sanger Institute. S. N. was supported by an Australian Postgraduate Award. K. E. H. was supported by NHMRC fellowship 628930.

Transparency declarations

None to declare.

References

1 Visca P, Seifert H, Towner KJ. *Acinetobacter* infection—an emerging threat to human health. *IUBMB Life* 2011; **63**: 1048–54.

2 Hamidian M, Nigro SJ, Hall RM. Variants of the gentamicin and tobramycin resistance plasmid pRAY are widely distributed in *Acinetobacter*. *J Antimicrob Chemother* 2012; **67**: 2833–6.

3 Hamidian M, Hall RM. ISAba1 targets a specific position upstream of the intrinsic *ampC* gene of *Acinetobacter baumannii* leading to cephalosporin resistance. *J Antimicrob Chemother* 2013; **68**: 2682–3.

4 Nigro SJ, Hall RM. Tn*6167*, an antibiotic resistance island in an Australian carbapenem-resistant *Acinetobacter baumannii* GC2, ST92 isolate. *J Antimicrob Chemother* 2012; **67**: 1342–6.

5 Hamidian M, Holt KE, Pickard D *et al*. A GC1 *Acinetobacter baumannii* isolate carrying AbaR3 and the aminoglycoside resistance transposon Tn*aphA6* in a conjugative plasmid. *J Antimicrob Chemother* 2014; **69**: 955–8.

6 Hamidian M, Kenyon JJ, Holt KE *et al*. A conjugative plasmid carrying the carbapenem resistance gene *bla*OXA-23 in AbaR4 in an extensively resistant GC1 *Acinetobacter baumannii* isolate. *J Antimicrob Chemother* 2014; **69**: 2625–8.

7 Nigro SJ, Hall RM. Amikacin resistance plasmids in extensively antibiotic-resistant GC2 *Acinetobacter baumannii* from two Australian hospitals. *J Antimicrob Chemother* 2014; **69**: 3435–7.

8 Wright MS, Haft DH, Harkins DM *et al*. New insights into dissemination and variation of the health care-associated pathogen *Acinetobacter baumannii* from genomic analysis. *MBio* 2014; **5**: e00963-13.

9 Hamidian M, Hall RM. pACICU2 is a conjugative plasmid of *Acinetobacter* carrying the aminoglycoside resistance transposon Tn*aphA6*. *J Antimicrob Chemother* 2014; **69**: 1146–8.

J Antimicrob Chemother 2015
doi:10.1093/jac/dku488
Advance Access publication 27 November 2014

Identification of a novel *fosX*^{CC} gene conferring fosfomycin resistance in *Campylobacter*

Yang Wang[1]†, Hong Yao[1]†, Fengru Deng[1], Dejun Liu[1], Yi Zhang[2] and Zhangqi Shen[3]*

[1]*National Centre for Veterinary Drug Safety Evaluation, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China;* [2]*The First School of Clinical Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100700, China;* [3]*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA*

*Corresponding author. Tel: +1-515-294-2381; Fax: +1-515-294-8500; E-mail: szq@iastate.edu
†These authors contributed equally to this work.

Keywords: foodborne pathogens, multidrug resistance, food-producing animal, food safety

Sir,

Fosfomycin [(2*R*,3*S*)-3-methyloxiran-2-yl]phosphonic acid is a broad-spectrum antibiotic with bactericidal activity against both Gram-positive and Gram-negative bacteria.[1] Fosfomycin inhibits bacterial cell wall synthesis by inactivating the enzyme UDP-*N*-acetylglucosamine-3-enolpyruvyl transferase (MurA), which is essential for the catalysis of bacterial peptidoglycan biosynthesis. Thus, modification or overexpression of MurA can contribute to the development of fosfomycin resistance in bacteria.[2] Other common mechanisms of resistance to fosfomycin include decreased drug uptake and inactivation of the antibiotic by addition of various groups.[3] To date, several fosfomycin resistance genes have been functionally characterized in bacteria. FomA and FomB enzymes can inactivate fosfomycin by phosphorylation.[4] FosA, FosB and FosX can inactivate fosfomycin by catalysing the addition of glutathione, L-cysteine and H_2O, respectively, to its epoxide ring.[2]

Campylobacter species are the leading foodborne pathogens worldwide, accounting for 400–500 million cases of diarrhoea each year.[5] Over the past few decades, *Campylobacter* species have become increasingly resistant to several clinically important antimicrobial agents, compromising the effectiveness of clinical therapy.[6] Various mechanisms of resistance to macrolides, fluoroquinolones, tetracyclines, aminoglycosides and β-lactams in *Campylobacter* have been extensively described.[7] In addition, clinical trials have indicated that fosfomycin can effectively treat *Campylobacter* enteritis in humans.[8] To date, no mechanism of fosfomycin resistance has been characterized in *Campylobacter* species. Here, we identified a novel *fosX*-like gene from *Campylobacter coli* (designated *fosX*^{CC}), located in the multidrug resistance genomic island (MDRGI), which confers fosfomycin resistance to *Campylobacter* species.

C. coli DZB4 was isolated from swine faeces in China in 2012. Our recent study indicated that DZB4 contains a type II MDRGI consisting of 10 ORFs that is inserted between *cadF* and *CCO1582* on the chromosome (GenBank accession number KC876749) and this MDRGI can mediate antibiotic resistance, at least to macrolides, aminoglycosides and tetracycline.[9] Further analysis revealed that the fifth ORF, immediately upstream of *erm*(B), in type II MDRGI encodes a 136 amino acid protein that exhibits 26.9%, 34.2% and 63.9% identity to fosfomycin resistance determinants FosA, FosB and FosX found in *Serratia marcescens* (M85195),[10] *Staphylococcus haemolyticus* (X89875)[11] and *Listeria monocytogenes* (NP_465227.1),[2] respectively. Protein structure prediction indicated that this putative protein has identical quaternary structure to FosX and contains the conserved active site residue E44, which plays a key role in catalysis in FosX but not in FosA or FosB.[2,12,13] Considering that the fosfomycin MIC for isolate DZB4 was >512 mg/L, which is much higher than that for reference strains