

Tet(X4) in *A. caviae*, a potential reservoir for the dissemination of tigecycline resistance into different environmental niches. The *tet(X4)*-carrying element analysis suggests that the *catD-tet(X4)*-ISCR2 gene cassette is highly active and may further spread into different bacterial species. Continuous monitoring of *tet(X4)* in humans, animals and environments should be considered, to improve understanding and address the spread of resistance to tetracyclines, including tigecycline.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as [Supplementary data](#) at JAC Online.

References

- 1 He T, Wang R, Liu D *et al.* Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. *Nat Microbiol* 2019; **4**: 1450–6.
- 2 Sun J, Chen C, Cui C-Y *et al.* Plasmid-encoded *tet(X)* genes that confer high-level tigecycline resistance in *Escherichia coli*. *Nat Microbiol* 2019; **4**: 1457–64.
- 3 Batra P, Mathur P, Misra MC. *Aeromonas* spp.: an emerging nosocomial pathogen. *J Lab Physicians* 2016; **8**: 1–4.
- 4 Walsh TR, Weeks J, Livermore DM *et al.* Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011; **11**: 355–62.
- 5 Wang X, Zhai W, Li J *et al.* Presence of an *mcr-3* variant in *Aeromonas caviae*, *Proteus mirabilis*, and *Escherichia coli* from one domestic duck. *Antimicrob Agents Chemother* 2018; **62**: e02106–17.
- 6 Ling Z, Yin W, Li H *et al.* Chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat. *Antimicrob Agents Chemother* 2017; **61**: e01272–17.
- 7 Clinical and Laboratory Standards Institute. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria—Second Edition: M45*. CLSI, Wayne, PA, USA, 2010.

8 Wick RR, Judd LM, Gorrie CL *et al.* Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: e1005595.

9 Poirel L, Mugnier PD, Toleman MA *et al.* ISCR2, another vehicle for *bla_{VEB}* gene acquisition. *Antimicrob Agents Chemother* 2009; **53**: 4940–3.

10 He YZ, Li XP, Miao YY *et al.* The ISAp1₂ dimer circular intermediate participates in *mcr-1* transposition. *Front Microbiol* 2019; **10**: 15.

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In vivo acquisition of fosfomycin resistance in *Escherichia coli* by *fosA* transmission from commensal flora

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Sir,
Fosfomycin is increasingly used to treat infections caused by MDR bacteria.¹ Fosfomycin acts by inhibiting UDP-N-acetylglucosamine enolpyruvyl transferase (*murA*), which prevents the formation of N-acetylmuramic acid, an essential component of peptidoglycan.¹ Although resistance to fosfomycin is still low in *Escherichia coli*, the acquisition of *fosA* may reduce future activity of fosfomycin to treat infections caused by *E. coli*.² FosA is a glutathione transferase that inactivates fosfomycin through catalysing the addition of glutathione. *fosA* genes are often present in the chromosome of *Klebsiella pneumoniae*, but not in the chromosome of *E. coli*.^{2,3} *Klebsiella variicola* is closely related and often misidentified as *K. pneumoniae*.⁴ While horizontal spread of *fosA* has been demonstrated *in vitro*,⁵ we here provide evidence for *in vivo fosA* transmission from *K. variicola* to *E. coli*, resulting in development of fosfomycin resistance.

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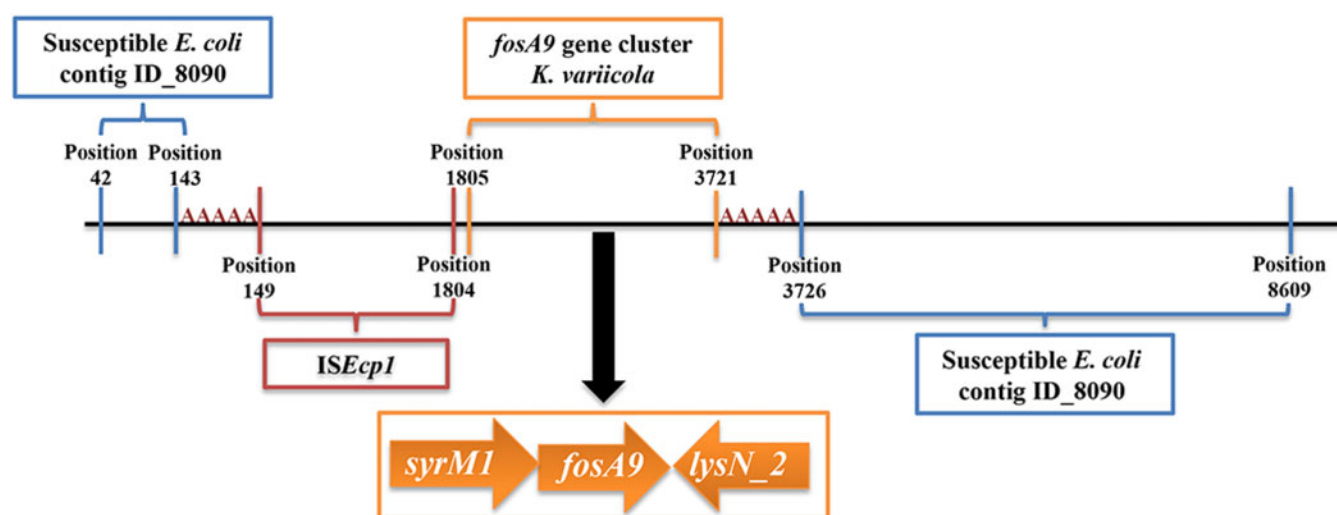


Figure 1. Schematic representation of the contig (ECO-BAB-IMI-103297_P-ACH-BAB-IMI-103242_1528359160_131_length_8653_cov_18.1163_ID_8928, 8653 bp) in the fosfomycin-resistant *E. coli* isolate containing a *fosA9* gene cluster originating from a *K. variicola* isolate. The *ISEcp1*-*syrM1*-*fosA9*-*lysN2* region is flanked by 5 bp DRs (AAAAA), suggesting mobilization from *K. variicola* by *ISEcp1*. Upstream and downstream sequences of the insertion region align to contig ECO-BAB-IMI-103298_P-ACH-BAB-IMI-103242_1528359160_92_length_16411_cov_29.2905_ID_8090 from the first susceptible *E. coli* isolate. Sequence information of complete genomes of all isolates and separate sequences of the relevant contigs (containing *fosA9* in *E. coli* and *K. variicola*, and ECO-BAB-IMI-103298_P-ACH-BAB-IMI-103242_1528359160_92_length_16411_cov_29.2905_ID_8090 from the susceptible *E. coli*) have been deposited in the ENA under project number PRJEB32329. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

The Medical Research Ethics Committee of the University Medical Center Utrecht confirmed that the Medical Research Involving Human Subjects Act does not apply to this study (reference number WAG/mb/18/027282). We were not able to obtain informed consent because the patient died a few years ago. All information including gender, age, dates and medical history that was not directly clinically relevant has been omitted to protect the privacy of the patient.

An aged patient had a suspicion of chronic endovascular infection of their aortic bifurcation graft, which the patient received after an acute aortic aneurysm 22 years earlier. The patient had suffered from recurrent episodes of sepsis, with blood cultures yielding *Propionibacterium* spp., *K. variicola*, *Citrobacter koseri* and *Pseudomonas aeruginosa*, as determined by MALDI-TOF MS. Positron emission tomography (PET)-CT findings were compatible with prosthetic graft infection. The patient subsequently developed septic shock with *E. coli* bacteraemia without a clear source of infection that was treated successfully with intravenous ceftriaxone. The isolate was resistant to amoxicillin/clavulanic acid and ciprofloxacin that had been used to suppress chronic infection, prompting the addition of oral fosfomycin at 3 g every 48 h. Seven months later, while still using fosfomycin, the patient developed spondylodiscitis. Blood cultures drawn at the time isolated *E. coli* with an identical resistance pattern, except being resistant to fosfomycin. Fosfomycin was discontinued and the patient received a prolonged course of ceftriaxone.

Fosfomycin susceptibility, determined by agar dilution according to CSLI guidelines,⁶ demonstrated a rise in the MIC from 2 mg/L for the initial *E. coli* isolate to >1024 mg/L for the second *E. coli* isolate. WGS revealed five SNP differences between *E. coli* isolates in the core genome, based on core genome MLST (cgMLST) analysis.⁷ Yet, the second *E. coli* isolate has a 3573 bp insertion consisting of *ISEcp1*, a *fosA* gene we named *fosA9* as the next available number

according to NCBI, *syrM1* and *lysN2*. The insertion is flanked by 5 bp DRs (AAAAA) suggesting mobilization of this *fosA9* gene cluster by *ISEcp1* (Figure 1).⁸ Genes other than *fosA9* responsible for fosfomycin resistance were not found. At the time of the first *E. coli* sepsis episode, six *K. variicola* had been isolated from rectum swabs and blood cultures over a period of 20 months (Table S1, available as [Supplementary data](#) at JAC Online). cgMLST analysis revealed a maximum of 16 SNP differences between *K. variicola* isolates.⁷ The same cluster as above containing *fosA9*, without the mobile genetic element *ISEcp1*, was identified in the *K. variicola* isolates, suggesting *K. variicola* to be the source of *fosA9* acquired by *E. coli* (Figure 1). *fosA* genes were not identified in other clinical isolates from this patient. Sequence information of all isolates has been deposited in the European Nucleotide Archive (ENA) under project number PRJEB32329.

fosA transfer from *Klebsiella* spp. to *E. coli*, leading to fosfomycin resistance, has been demonstrated *in vitro*.³ Based on publicly available genomes, *fosA* and adjacent genes are well conserved in *K. variicola* (minimum 98% identity to *fosA9*) and *K. pneumoniae* (minimum 94% identity to *fosA9*) isolates. According to mPlasmids, PlasmidFinder and contig coverage, *fosA9* was predicted to be located in the chromosome of the second *E. coli* and all *K. variicola* isolates.^{9,10} However, based on BLASTn, the contig containing *fosA9* aligns to plasmid sequences. The localization of *fosA9* in *E. coli* can thus only be confirmed by completely assembling its genome using long-read sequencing, as the mobilization of the *fosA9* gene cluster by an IS element might switch its genomic background. We postulate that *fosA9* transfer from *K. variicola* to *E. coli* occurred in the gastrointestinal tract, as *K. variicola* was not co-cultured in the blood at the time of *E. coli* bacteraemia. We hypothesize that fosfomycin pressure played a role in this transfer; however, this

has to be confirmed with further experiments *in vitro*. Acquisition of *fosA9* was associated with an 8-fold increase in the MIC for *E. coli* (from 2 to 1024 mg/L) while, despite the presence of *fosA9* in the chromosome of the *K. variicola* isolates, the fosfomycin MICs were below the EUCAST susceptibility breakpoint of ≤ 32 mg/L (Table S1).⁶ This could suggest either higher dependency of *E. coli* growth on glutathione or a difference in *fosA9* expression or metabolism, i.e. higher expression by the *ISEcp1* promoter present upstream of the *fosA9* gene cluster.⁸

In conclusion, our case illustrates the potential of long-term use of oral fosfomycin to promote horizontal gene transfer of *fosA9* from commensal gut flora to potential pathogenic microorganisms, such as *E. coli*.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as [Supplementary data](#) at JAC Online.

References

- 1 Karageorgopoulos DE, Wang R, Yu XH et al. Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-negative pathogens. *J Antimicrob Chemother* 2012; **67**: 255–68.
- 2 Ito R, Mustapha MM, Tomich AD et al. Widespread fosfomycin resistance in Gram-negative bacteria attributable to the chromosomal *fosA* gene. *MBio* 2017; **8**: e00749–17.
- 3 Guo Q, Tomich AD, McElheny CL et al. Glutathione-S-transferase FosA6 of *Klebsiella pneumoniae* origin conferring fosfomycin resistance in ESBL-producing *Escherichia coli*. *J Antimicrob Chemother* 2016; **71**: 2460–5.
- 4 Linson SE, Long SW, Ojeda Saavedra M et al. Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*. *mSphere* 2017; **2**: e00290–17.
- 5 Klontz EH, Tomich AD, Günther S et al. Structure and dynamics of FosA-mediated fosfomycin resistance in *Klebsiella pneumoniae* and *Escherichia coli*. *Antimicrob Agents Chemother* 2017; **61**: e01572–17.
- 6 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Eleventh Edition: M07*. CLSI, Wayne, PA, USA, 2018.

7 De Been M, Pinholt M, Top J et al. Core genome multilocus sequence typing scheme for high-resolution typing of *Enterococcus faecium*. *J Clin Microbiol* 2015; **53**: 3788–97.

8 Poirel L, Decousser JW, Nordmann P. Insertion sequence *ISEcp1B* is involved in expression and mobilization of a *bla*_{CTX-M} β -lactamase gene. *Antimicrob Agents Chemother* 2003; **47**: 2938–45.

9 Arredondo-Alonso S, Rogers MRC, Braat JC et al. mPlasmids: a user-friendly tool to predict plasmid- and chromosome-derived sequences for single species. *Microb Genom* 2018; **4**: e000224.

10 Carattoli A, Zankari E, Garcia FA et al. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895–903.

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Emergence of *Klebsiella pneumoniae* and *Enterobacter cloacae* producing OXA-48 carbapenemases from retail meats in China, 2018

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Sir,
Carbapenem-resistant Enterobacteriaceae (CRE) have been globally reported, not only in hospitals, but also in the community, animals (including livestock, companion animals and wildlife), the environment and food,^{1,2} and they are recognized as a serious threat to human health. Recently, an increased prevalence of *Escherichia coli* strains carrying *bla*_{NDM} from food in China from 2015 to 2018 has been reported, highlighting the risk of human exposure to food polluted by strains producing NDM carbapenemase.^{3,4} OXA-48-producing CRE have been frequently reported in Europe and have been identified in many ecosystems. However, Enterobacteriaceae producing OXA-48 had so far