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-acetylmuramiduronic acid 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.

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/18/002782). 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 prostatic 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 and 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, symM1 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 the 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). fosA4 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.

FosA9 transfer from Klebsiella spp. to E. coli, leading to fosfomycin resistance, has been demonstrated in vitro.1 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 plasmids, 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
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.

References

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Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online.