Pandemic Escherichia coli ST648 isolate harbouring fosA3 and blaCTX-M-8 on an IncI1/ST113 plasmid: A new successful combination for the spread of fosfomycin resistance?

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Genome Note

Pandemic *Escherichia coli* ST648 isolate harbouring *fosA3* and $bla_{CTX\text{-}M\text{-}8}$ on an IncI1/ST113 plasmid: A new successful combination for the spread of fosfomycin resistance?

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ABSTRACT

Objectives: The emergence of Enterobacteriaceae isolates resistant to the last-resort antibiotic fosfomycin outside of Asia is a public-health issue. Here we report the draft genome of an *Escherichia coli* isolate presenting both an extended-spectrum β-lactamase (ESBL) and the *fosA3* gene in a healthy cow in Brazil.
Methods: Whole genomic DNA from *E. coli* E12 was extracted and 2 × 150-bp paired-end reads were generated using Illumina sequencing technology. De novo genome assembly was performed using SPAdes v.3.11 and the draft genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline. Further analyses were performed using the Center for Genomic Epidemiology databases.
Results: The 5 045 934-bp genome displayed several resistance genes, including the *fosA3* and $bla_{CTX\text{-}M\text{-}8}$ genes. Southern blot experiments showed that they were co-located on an IncI1/ST113 plasmid.
Conclusion: Presence of the *fosA3* gene on the same common plasmid as $bla_{CTX\text{-}M\text{-}8}$ will have to be monitored. This draft genome provides data that will help in tracing the dissemination of this gene and the evolution of its plasmidic support.

Fosfomycin is a last-resort antibiotic in human medicine for the treatment of carbapenem-resistant Enterobacteriaceae, and the emergence of resistant isolates is a public-health concern. The human and animal reservoirs of the plasmid-borne *fosA3* gene conferring fosfomycin resistance are located in China, where it is mostly found in association with extended-spectrum β-lactamase (ESBL) $bla_{CTX\text{-}M\text{-}55/\text{-}65}$ genes on IncF and IncI1 plasmids [1].

Outside of Asia, the *fosA3* gene has been reported occasionally, such as in humans in Brazil [2] and in autochthonous cases from bovines in France and poultry in Brazil [1,3]. In these latter studies, *fosA3* was associated with $bla_{CTX\text{-}M\text{-}55}$ on an IncF plasmid and in *Escherichia coli* from different sequence types (STs).

E. coli E54 was collected in 2014 from a 4-year-old cow on an intensive production farm in the Northeast region of Brazil. This isolate was detected in the framework of a study on the evaluation of faecal carriage of extended-spectrum cephalosporin-resistant *E. coli* in healthy cattle. Strain E54 was isolated from a faecal sample by selection on MacConkey agar supplemented with cefotaxime (2 μg/mL) following an enrichment step in tryptic soy broth also supplemented with cefotaxime. Identification was confirmed using an API20E gallery (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing performed by the disk diffusion method showed an ESBL phenotype (resistant to amoxicillin, piperacillin, cefuroxime, cefotaxime, ceftiofur and ceftaroline and intermediate susceptibility profile to cefepime) as well as additional resistances to streptomycin, kanamycin, sulfonamides, trimethoprim and fosfomycin. The isolate was susceptible to ceftazidime, aztreonam, cefoxitin, amoxicillin/clavulanic acid, piperacillin/tazobactam, carbapenems (meropenem, imipenem, ertapenem and doripenem), tetracycline, tigecycline, amikacin, gentamicin, tobramycin, netilmicin, nalidixic acid, ciprofloxacin, enrofloxacin, levofloxacin, nitrofurantoin and chloramphenicol. The *fosA3* and $bla_{CTX\text{-}M\text{-}8}$ genes were detected by PCR.

Whole-genome sequencing was performed on genomic DNA extracted using a Microbial DNA Extraction Kit (Macherey Nagel, Hœrdt, France). A genomic library was prepared using a Nextera XT DNA Library Preparation Kit (Illumina Inc., Cambridge, UK) according to the manufacturer's protocol, and 2 × 150-bp paired-

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end reads were generated using Illumina NextSeq sequencing technology (Illumina Inc., San Diego, CA). De novo genome assembly was performed using SPAdes v.3.11 and the draft genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). In total, 2 456 726 paired-end reads were generated with a minimum 73-fold coverage, which were assembled into 127 contigs. The genome size was calculated as 5 045 934 bp, which comprised a total of 4771 protein-coding sequences. The G + C content of this strain added up to 50.5%. Further characterisation of strain E54 was performed through the databases of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org).

According to Achtman's multilocus sequence typing (MLST) scheme, *E. coli* E54 belonged to phylogroup D and ST648 and presented an O83:H42-*fim58* profile (https://cge.cbs.dtu.dk/services/SerotypeFinder/ and https://cge.cbs.dtu.dk/services/FimTyper/). The virulence factors (https://cge.cbs.dtu.dk/services/VirulenceFinder/) *air*, *eilA* and *lpfA* were also detected, along with 499 proteins of pathogenic families (https://cge.cbs.dtu.dk/services/PathogenFinder/).

Interestingly, CTX-M-15-producing ST648 was described as a pandemic clone in pets and horses in Europe, prone to colonise diverse ecological niches. Whether the CTX-M-8-producing ST648 clone will also successfully disseminate in animals in Brazil will thus have to be monitored.

The *fosA3*, *aadA12, aadA2, aph(3')-Ic, strA, strB, sul1, sul2* and *drfA12* antimicrobial resistance genes were detected using the ResFinder 2.1 database (https://cge.cbs.dtu.dk/services/ResFinder/), which confer resistance to, respectively, fosfomycin, aminoglycosides (streptomycin and kanamycin), sulfonamides and trimethoprim. The detected genes are in total coherence with the observed phenotypes. The ESBL phenotype was due to the $bla_{\text{CTX-M-8}}$ gene, which is frequently detected in Brazil where it was first described in 1996. The IncQ1 and IncI1 incompatibility groups were found using PlasmidFinder 1.3 (https://cge.cbs.dtu.dk/services/PlasmidFinder/), and plasmid subtyping using an online database (http://pubmlst.org/plasmid/) showed that the IncI1 plasmid belonged to ST113.

Southern blot experiments on S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) gels proved that the *fosA3* and $bla_{\text{CTX-M-8}}$ genes co-localised on the IncI1/ST113 plasmid whose size was ca. 90 kbp. The co-localisation was further proved by co-transfer of the *fosA3* and $bla_{\text{CTX-M-8}}$ genes to an *E. coli* HB101 recipient strain by conjugation. Since the transconjugant did not display resistance phenotypes to streptomycin, kanamycin or sulfonamides/trimethoprim, the corresponding resistance genes are most probably not located on the same plasmid, which is corroborated by their occurrence on different contigs from the *fosA3* and $bla_{\text{CTX-M-8}}$ genes in the assembled genome.

In conclusion, here we report the draft genome of an *E. coli* ST648 isolate presenting plasmidic co-localisation of the *fosA3* and $bla_{\text{CTX-M-8}}$ genes. Until now, the *fosA3* gene has been reported with $bla_{\text{CTX-M-55/-65}}$ genes predominantly on IncF and to a lesser extent IncI1 (belonging to ST71 and ST136 subtypes) plasmids, which are typical Asian combinations. Here, *fosA3* was co-localised with $bla_{\text{CTX-M-8}}$ on an IncI1/ST113 plasmid, a plasmid subtype that is epidemic in Brazil and beyond [4,5]. And (i) CTX-M-8 enzymes are widespread in Brazil both in humans and animals and (ii) IncI1/ST113 plasmids are frequent vectors of these genes.

This Whole Genome Sequencing project has been deposited at DDBJ/EMBL/GenBank under the accession no. PVMT00000000.

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Competing interests

None declared.

Ethical approval

Not required.

References

[1] Lupo A., Saras E, Madec JY, Haenni M. Emergence of $bla_{\text{CTX-M-55}}$ associated with *fosA*, *rmtB* and *mcr* gene variants in *Escherichia coli* from various animal species in France. J Antimicrob Chemother 2018;73:867–72.

[2] Cunha MP, Lincopan N, Cerdeira L, Esposito F, Dropa M, Franco LS, et al. Coexistence of CTX-M-2, CTX-M-55, CMY-2, FosA3, and QnrB19 in extraintestinal pathogenic *Escherichia coli* from poultry in Brazil. Antimicrob Agents Chemother 2017;61:, doi:http://dx.doi.org/10.1128/AAC.02474-16 pii: e02474-16.

[3] Fernandes MR, Sellera FP, Moura Q, Souza TA, Lincopan N. Draft genome sequence of a CTX-M-8, CTX-M-55 and FosA3 co-producing *Escherichia coli* ST117/B2 isolated from an asymptomatic carrier. J Glob Antimicrob Resist 2018;12:183–4.

[4] Norizuki C, Wachino JI, Suzuki M, Kawamura K, Nagano N, Kimura K, et al. Specific $bla_{\text{CTX-M-8}}$/IncI1 plasmid transfer among genetically diverse *Escherichia coli* isolates between humans and chickens. Antimicrob Agents Chemother 2017;61:, doi:http://dx.doi.org/10.1128/AAC.00663-17 pii: e00663-17.

[5] Eller C, Leistner R, Guerra B, Fischer J, Wendt C, Rabsch W, et al. Emergence of extended-spectrum β-lactamase (ESBL) CTX-M-8 in Germany. J Antimicrob Chemother 2014;69:562–4.