Complete sequence of transferable IncHI2/ST4 plasmid carrying *bla*$_{CTX-M-1}$ and *mcr-1* from an enterohaemorrhagic *Escherichia coli* (EHEC) from bovine origin

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ABSTRACT

Objective: The co-occurrence of Extended-Spectrum Beta-Lactamase (ESBL) and *mcr-1* encoding genes on plasmids have been recently demonstrated on a single cell. After finding a co-localization of these antimicrobial genes on a single plasmid here, we show the complete annotated sequence of a large plasmid encoding both *bla*$_{CTX-M-1}$ and *mcr-1* genes isolated from an enterohaemorrhagic *E. coli* (EHEC) isolate. The genomic features of this plasmid were analysed in order to provide information to better control its spread. The aim of this study is to provide complete genome of a plasmid carrying both *bla*$_{CTX-M-1}$ and *mcr-1* from EHEC.

Methods: Whole Genome Sequencing of transconjugant containing the single plasmid carrying both *bla*$_{CTX-M-1}$ and *mcr-1* isolated from EHEC.

Results: This study reports the complete sequence of a transferable *mcr-1/bla*$_{CTX-M-1}$-bearing IncHI2/ST4 plasmid harboring numerous virulence factors and resistance determinants to seven antibiotic classes, for the first time isolated from an ESBL-producing EHEC.

Conclusions: IncHI2/ST4 plasmids were already shown to disseminate *bla*$_{CTX-M-1}$ and *mcr*-1 in cattle *E. coli* in France and this data now highlights their spread among enterohaemorrhagic *E. coli* at risk for public health.

KEY WORDS: EHEC, ESBL, Colistin, Plasmid

Introduction

Since the first report of the colistin resistance *mcr-1* gene in China late 2015 [1], several studies proved its presence in phylogenetically unrelated bacteria from various geographic origins. So far, *mcr-1* has been mostly reported in *E. coli*. The co-occurrence of *mcr-1* and Extended-Spectrum Beta-Lactamase (ESBL)-encoding genes in the same isolate has also been demonstrated, including as co-localized genes on the same plasmid [2, 3]. Recently, an ESBL-producing enterohaemorrhagic *E. coli* (EHEC) of serotype O151:H16 (isolate #22593) from a French bovine in 2008 was sequenced and annotated [4]. Since the spread of *mcr-1* in *E. coli* pathotypes would constitute a further threat for public health, we looked back for the *mcr-1* sequence into the #22593 EHEC genome. Because it was not possible to extract complete plasmid sequences using only sequence analysis from this draft genome containing more than one plasmid, we transferred the plasmid pHI2-22593 from #22593 to a reference K-12 J53 rifampin resistant recipient cell and the whole genome of this TC22593D1.1 transconjugant was sequenced. We are now able to present the complete sequence of plasmid pHI2-22593, an IncHI2-type plasmid co-harboring *mcr-1* and *bla*$_{CTX-M-1}$ and conjugative. The presence of large multi-drug plasmids in pathogenic *E. coli* is a great threat for public health.

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Materials and Methods

As determined by broth microdilution, MIC to colistin was 6 mg/L for the native isolate and 2 mg/L for TC22593D1.1. Genomic DNA of TC22593D1.1 was extracted using the MasterPureTM DNA purification kit (Epicentre) and the library was prepared using the kit Ion Xpress Plus Fragment Library (Life Technologies). Size selection of adaptor ligated fragments of about 280 bp was performed by e-Gel electrophoresis (Invitrogen). The purification steps were carried out using NucleoMag NGS Clean-up and Size select magnetic beads (Macherey Nagel). Quantification of the library (155 pM) was obtained by qPCR using Ion

Library Taqman Quantitation Kit (Life Technologies). Sequencing was performed using an Ion Proton Torrent technology sequencer at the sequencing platform, Anses, France. The raw reads were trimmed by the removal of ambiguous nucleotides from read ends and when quality scores fell below 3. Reads below 36 nucleotides were also removed. To extract DNA plasmid sequences from the whole genome of the recipient cell, aligned reads with Burrows-Wheeler Aligner (BWA) against *E.coli* (Accession number U00096) were eliminated. The resultant reads were *de novo* assembled using the SPAdes assembler version 3.6.2, scaffolding was performed and paired distances were automatically detected, 314825 bases were generated in 22 contigs. The minimum contig length was set to 159 bp. The median coverage of the assemblies was 250X. The contigs belonging to Human, fungus and phages identified using BLAST (http://blast.ncbi.nlm.nih.gov/Blast. cgi) were removed from the final selection of contigs. The final 12 selected contigs corresponding to the plasmidic DNA were annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genomes Automatic Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html) and aligned with GenBank data using nucleotides BLAST. They were ordered according to the reference pS38 plasmid (Accession number KX129782.1) using Mauve version 20150226 and mapped (Figure 1).

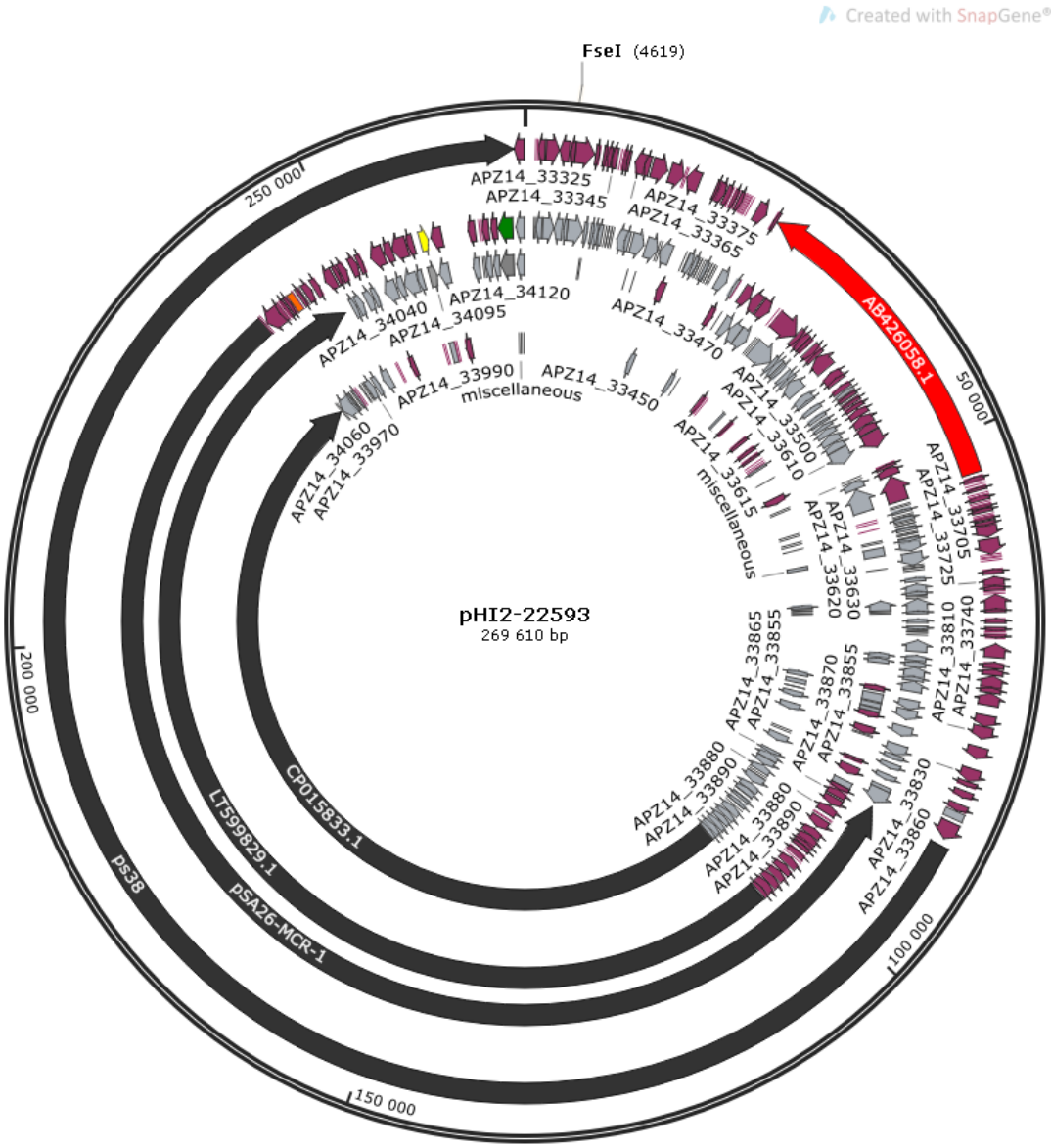
Results

pHI2-22593 was 269610 bp in length with an average G+C content of 47.1%, and harbored 320 genes including 264 protein-coding sequences (CDs) (Figure 1). The plasmid multilocus sequence typing (pMLST) analysis at (https://cge.cbs.dtu.dk/services/pMLST/) confirmed the IncHI2 replicon type and revealed a Sequence Type 4 variant. A plasmid comparison based on a full plasmid megaBLAST query (defined by ≥95% amino acid sequence identity and ≥75% aligned length coverage of a query sequence from *E. coli*) revealed that pHI2-22593 was closely related to the IncHI1 pS38 plasmid carrying also both *mcr-1* and $bla_{\text{CTX-M-1}}$ with a coverage of 83% of the query sequence (E value 0.0), the IncHI2 180-PT54 plasmid (Accession number CP015833.1) covering 83% of the query sequence (E value 0.0) and the IncHI2 p14408_M1 (Accession number LT599829.1) covering 79% of the query sequence (E value 0.0) (Table 1). A large part (53%) of the query not covered by these plasmids matched with a genomic island GEI2.43 from an enteropathogenic *E. coli* O111:H- (Accession number AB426058.1) (Figure 1).

Table 1. Characteristics of the plasmids identified in *E. coli* closely related to pHI2-22593.

Plasmid name	Replicon type	Size (pb)	Antibiotic resistance gene(s)	Native isolate, host	Geographic location (Date of isolation)	Coverage (%)	E value	Accession number
pHI2-22593	IncHI2	269610	*bla*CTX-M-1, *mcr-1*, *aadA1*, *strA*, *strB*, *sul3*, *sul1*, *tet(A)*, *dfrA1*, *mphA*,	EHEC (O151:H16) Bovine stool	France (2008)	100	0.0	LMBK01000586-LMBK01000597
pS38	IncHI1	247885	*bla*CTX-M-1, *mcr-1*, *aadA2*, *aadA1*, *estX3*, *cmlA1*, *sul3*, *tet(A)*, *dfrA1*, *mefB*	*E. coli* Poultry meat	Italy (no available date of isolation)	83	0.0	KX129782.1
180-PT54	IncHI2	240662	*aadA2*, *aadA1*, *strA*, *strB*, *cmlA1*, *sul3*, *sul1*, *tet(A)*, *dfrA1*	EHEC (O157:H7) Human	United kingdom (2012)	83	0.0	CP015833.1
pSA26-MCR-1	IncHI2	240367	*mcr-1*, *bla*TEM-1, *strA*, *strB*, *aadA2*, *aadA1*, *aph(3')-la*, *dfrA1*, *mphA*,*cml*, *floR*, *sul3*, *tet(A)*, *dfrA1*	*E. coli* Human	Kingdom of Saudi Arabia (2012)	80	0.0	KU743384
p14408_M1	IncHI2	238073	*aadA1*, *aadA2*, *strA*, *strB*, *aph(3')*, *aac(3')*, *bla*TEM-1, *mcr-1*, *cmlA1*, *sul3*, *tet(A)*	*E. coli* (no available data)	Switzerland (no available date of isolation)	79	0.0	LT599829.1

Figure 1. Map of the pHI2-22593. The regions identical to pS38, 180-PT54, pSA26-MCR-1 were noticed in black arrow and the genomic island GEI2.43 (AB426058.1) was identified in red. The *mcr-1* gene was marked in green, the *bla*$_{\text{CTX-M-1}}$ in yellow. The figure was created with the SnapGene software. ORF, open reading frame (oriV) is orange.



The plasmid pHI2-22593 encodes seven proteins associated to human pathogenicity according to PathogenFinder (https://cge.cbs.dtu.dk/services/PathogenFinder/), of which five have known functions (terminase small subunit, tail protein prophage, plasmid partition protein, YahA protein). In addition to *bla*$_{\text{CTX-M-1}}$ and *mcr-1*, pHI2-22593 harbors eight additional genes for resistances to macrolides (*mphA*), sulphonamides (*sul3*, *sul1*), tetracyclines (*tetA*), trimethoprim (*dfrA1*) and aminoglycosides (*strA*, *strB*, *aadA1*). In pHI2-22593, the *mcr-1* gene was flanked by two IS*Apl*1 elements as identified in pS38 [5].

Nucleotide sequence accession numbers

The Whole Genome Shotgun (WGS) project has been deposited at DDBJ/EMBL/GenBank under the accession number LMBK00000000. In July 2016, plasmid sequences (LMBK01000586-LMBK01000597) were added to the 01 version of LMBK00000000.

Discussion

This study reports the complete sequence of a transferable *mcr-1*/*bla*$_{\text{CTX-M-1}}$-bearing IncHI2/ST4 plasmid harboring numerous virulence factors and resistance determinants to

seven antibiotic classes, for the first time isolated from an ESBL-producing EHEC. Of note, IncHI2/ST4 plasmids were already shown to disseminate $bla_{CTX\text{-}M\text{-}1}$ and *mcr*-1 in cattle *E. coli* in France and these data now highlight their spread among enterohaemorrhagic *E. coli* at risk for public health.

Conflict of Interest

We declare that we have no conflict of interest.

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