



Use of Nanopore sequencing to characterise the genome architecture of mobile genetic elements encoding *bla*_{CTX-M-15} in *Escherichia coli* causing travellers' diarrhoea

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Introduction

A total of **eight isolates** of Extended Spectrum Beta Lactamase (ESBL)-producing **diarrhoeagenic *Escherichia coli* (DEC) (*bla*_{CTX-M-15} positive)** were identified from **four patients** (two isolates per one patient) who all presented symptoms of **travellers' diarrhoea**.

All patients were **returning to the UK from Pakistan**.

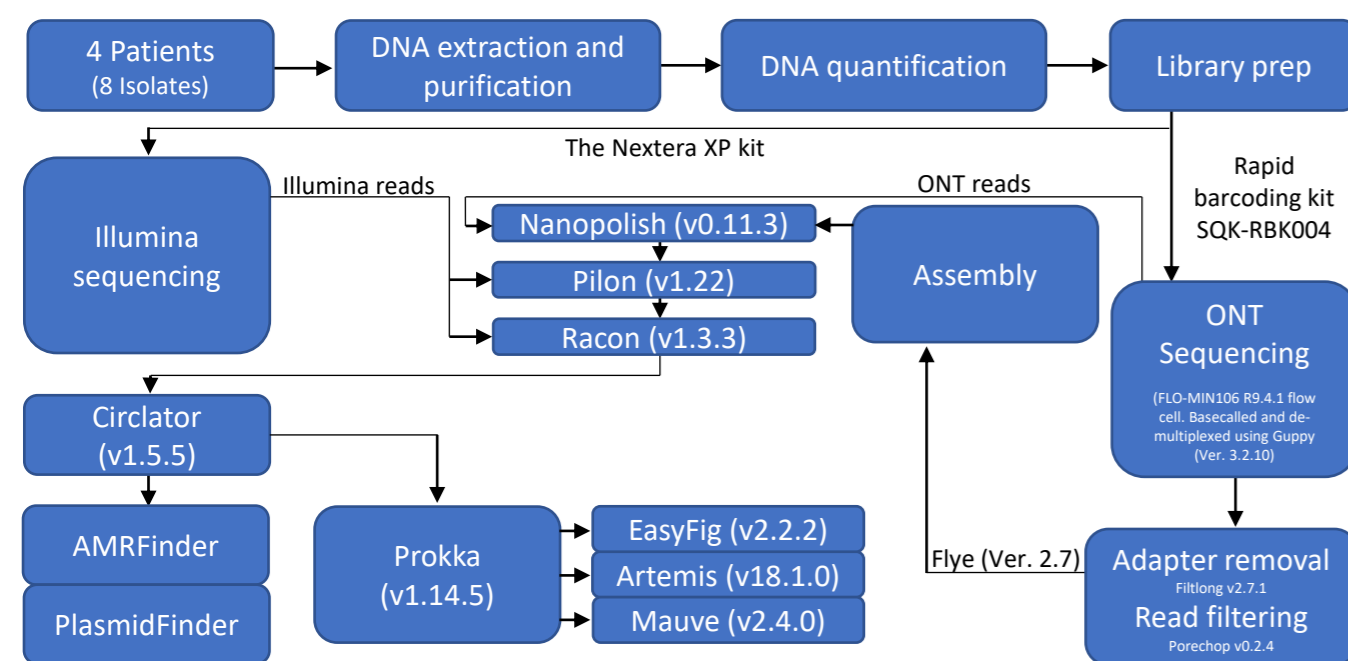
Increasing levels of antibiotics resistance to 3rd generation cephalosporins have been documented in *E. coli* and gastrointestinal bacteria can act as a **reservoir of AMR determinants** in the human gut.

The aim of this project was to determine whether *bla*_{CTX-M-15} in these isolates was **chromosomally or plasmid-encoded** to better understand the **mechanisms of transmission of AMR determinants**

Table 1: Summary of patient data including Pathotype, Serotype and Sequence type. EIEC: Enteroinvasive *E. coli*; ETEC: Enterotoxigenic *E. coli*; STEC: Shiga toxin-producing *E. coli*; EAEC: Enteraggregative *E. coli*; EPEC: Enteropathogenic *E. coli*. Position row dictates whether *bla*_{CTX-M-15} was encoded on the Chrom: Chromosome or Plas: Plasmid.

Patient	A		B		C		D	
Isolate	899091	899037	786605	788309	623214	623213	542093	542099
Pathotype	EIEC	ETEC	STEC	EAEC	EAEC	ETEC	EAEC	EPEC
Serotype	O96:H19	O167:H5	O117:H7	O:H31	O51:H30	O167:H41	O:H21	O142:H6
Sequence type	ST99	ST443	ST504	ST3032	ST38	ST182	ST227	ST1283
Position	Chrom	Plas	Plas	Plas	Chrom	Plas	Chrom	Chrom
Inc Type	N/A	IncFIB	IncI1	IncFIB	N/A	IncX1	N/A	N/A

Methods



Results

Four of the DEC isolates encoded *bla*_{CTX-M-15} on a plasmid whilst the other four encoded it on the chromosome (Table 1). *bla*_{CTX-M-15} is commonly flanked by ISEc9/ISEc1 and another IS/tnp, and may be translocated onto plasmids of the same (Fig. 1A, B) or different (Fig. 1C, D) Incompatibility group or even to different regions in the chromosome (Fig. 2, 3, 4).

Plasmids from isolates 788309 and 899037 showed a 96.98% sequence similarity (Fig. 1A, B) despite being from different patients infected with different DEC serotypes, pathotypes and sequence types (Table 1). BRIG plots for plasmids in isolates 786605 (IncI1) and 623213 (IncX1) shared significant sequence similarity to pNMBU (accession number: CP042886) and pRCS50 (accession number: LT985261), respectively (Fig. 1C, 1D).

*bla*_{CTX-M-15} was encoded on the chromosome in isolates of DEC from patients A, C and D. In Isolate 542093, *bla*_{CTX-M-15} was integrated into the chromosome at a site with plasmid/phage remanence (Fig 2). The chromosomal integration site in isolates 899091 and 542099 were the same (Fig. 3). In isolate 623214 *bla*_{CTX-M-15} integrated into a highly mosaic region with multiple drug resistance, previously described by Greig *et al.* 2018 (CP026723) (Fig. 4).

Plasmid comparisons

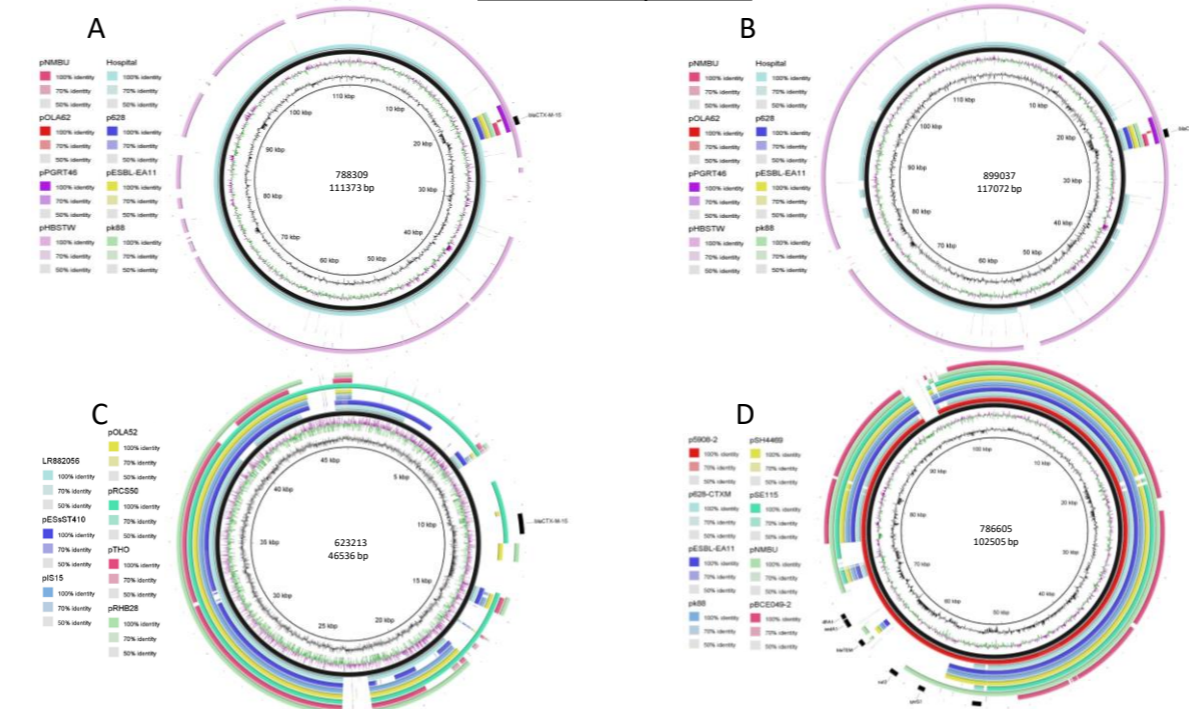


Figure 1: BRIG comparisons for plasmids encoding *bla*_{CTX-M-15} to publicly available plasmids identified using BLAST. Query plasmid identifiable by black inner ring. A: 788309; B: 899037; C: 623213 and D: 786605.

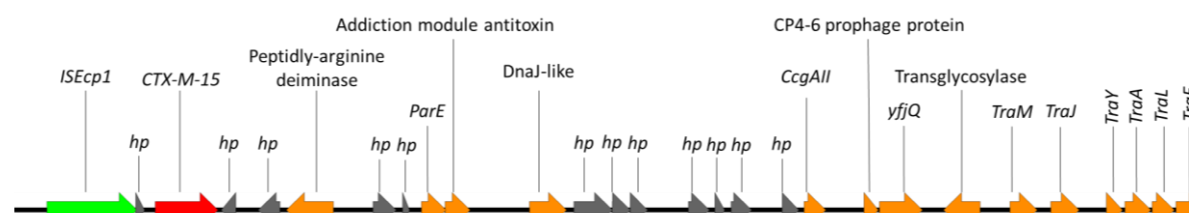


Figure 2: Chromosomal integration of *bla*_{CTX-M-15} in 542093. Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in green and other genes are shown in orange.

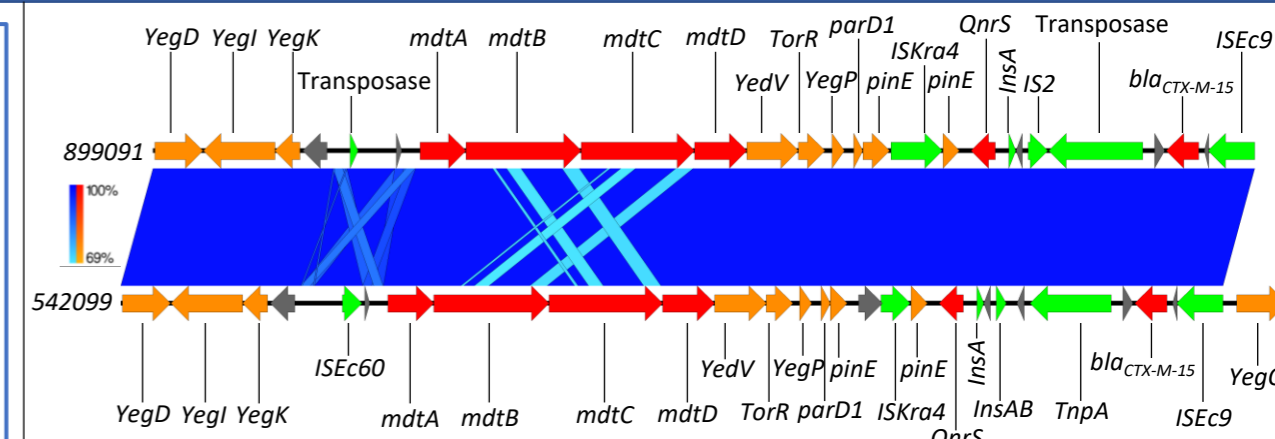


Figure 3: Chromosomal integration of *bla*_{CTX-M-15} comparison between isolates 899091 and 542099. Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in green and other genes are shown in orange. Scale bars indicate level of sequence similarity for forward (blue).

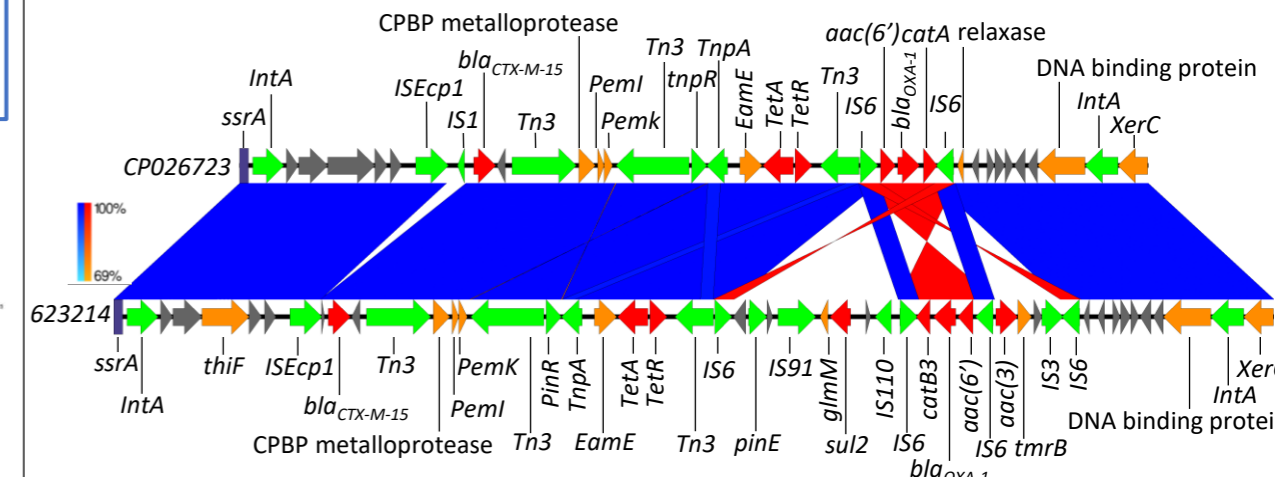


Figure 4: Chromosomal integration of *bla*_{CTX-M-15} comparison between isolates 623214 and CP026723. Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in green and other genes are shown in orange. Scale bars indicate level of sequence similarity for forward (blue) and reverse (red) sequences.

Discussion

The transmission of AMR determinants (e.g. *bla*_{CTX-M-15}) is complex and can occur in various ways and may involve different mechanisms.

In this small dataset, *bla*_{CTX-M-15} was found to be located on plasmids exhibiting three different replicon types in three different DEC pathotypes. Where *bla*_{CTX-M-15} is located on a plasmid, there is risk of transmission of the plasmid to other bacteria in the gut.

We also found *bla*_{CTX-M-15} integrated into the chromosome in three different DEC pathotypes at different locations. Integration into the chromosome may reduce the fitness cost of the maintenance of *bla*_{CTX-M-15} and facilitate persistence.

Acknowledgements

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