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

Matthew T. Bird[1, 2], David R. Greig[1, 3], Satheesh Nair[1], Claire Jenkins[1, 3], Saheer E. Gharbia[1, 2]

[1]National Infection Service, Public Health England; [2] Health Protection Research Unit in Genomes and Enabling Data; [3] Health Protection Research Unit in Gastrointestinal infection

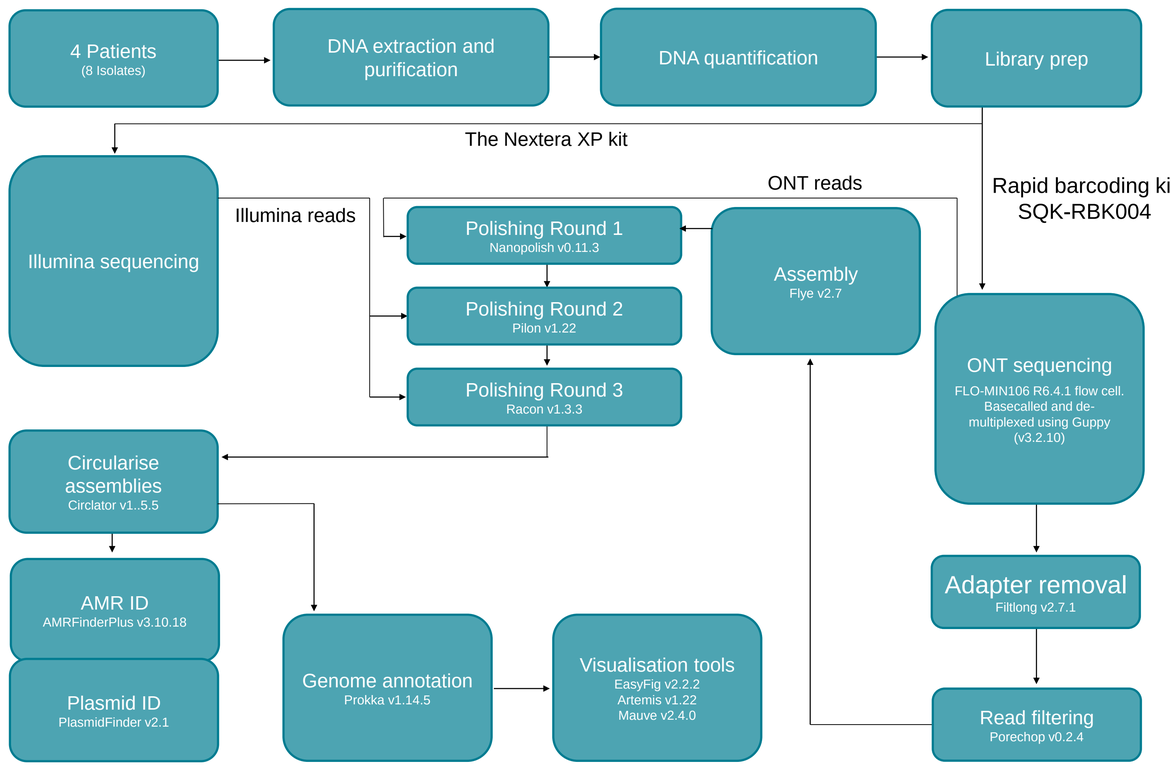
Health Protection Research Unit in Gastrointestinal Infections at University of Liverpool

## 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

| 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).

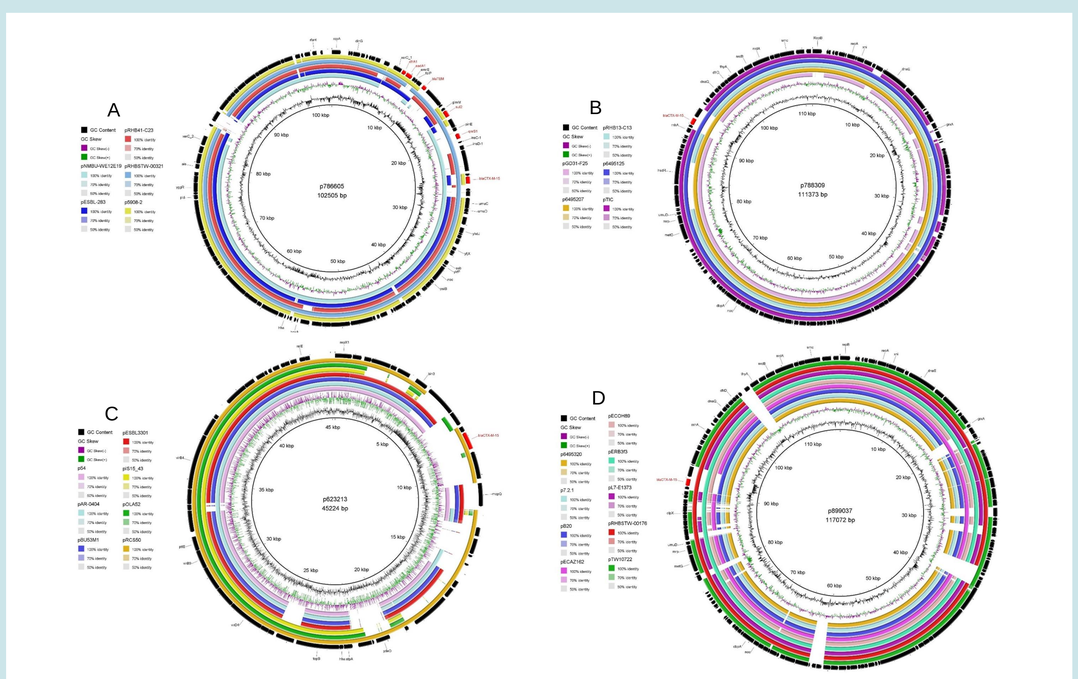



**Figure. 1: BRIG comparisons for plasmids encoding bla$_{CTX-M-15}$ to publicly available plasmids identified using BLAST**. A: p786605; B: p788309; C: p623213 and D: p899037.

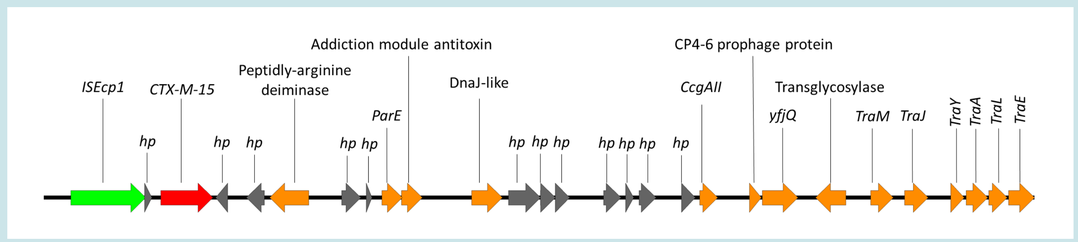



**Figure. 2: Chromosomal integration of bla$_{CTX-M-15}$ in 542093.** Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in green and other genes are shown in orange.

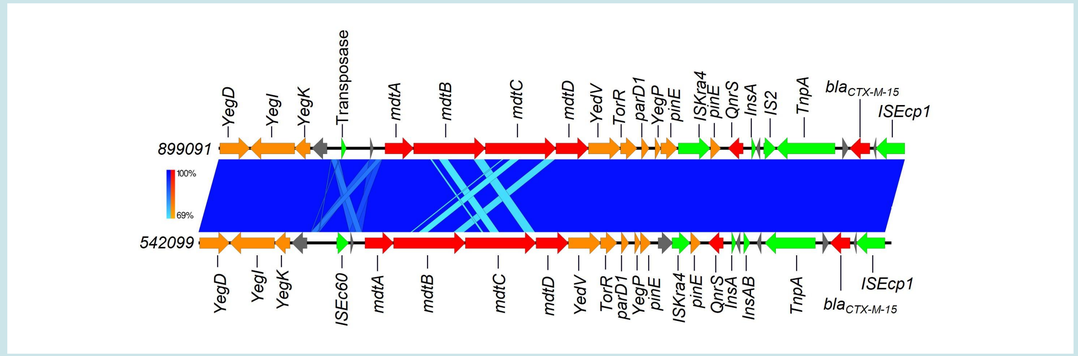



**Figure. 3: Chromosomal integration of bla$_{CTX-M-15}$ comparison between isolates 899091 and 542099.** Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in green and other genes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

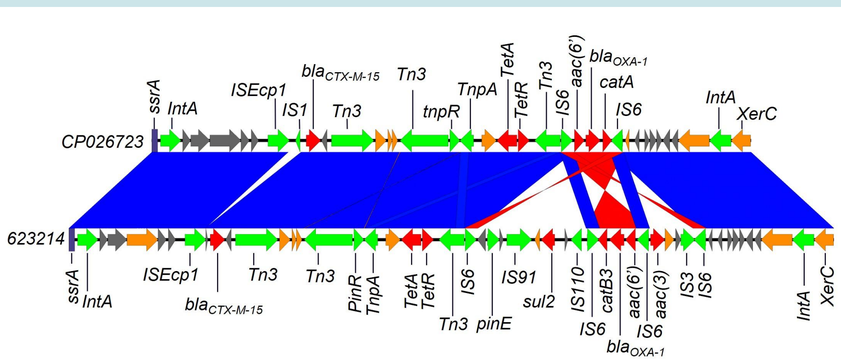



**Figure. 4: Chromosomal integration of bla$_{CTX-M-15}$ comparison between isolates 623214 and CP026723.** Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in green and other genes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

## DISCUSSION & CONCLUSION

The transmission of AMR determinants (e.g. *bla*$_{CTX-M-15}$) is complex and can occur in various ways and may involve different mechanisms. Here we present *bla*$_{CTX-M-15}$ located on plasmids exhibiting three different replicon types in three different DEC pathotypes, which carries a risk of transmission of the plasmid to other bacteria in the gut. We also identified *bla*$_{CTX-M-15}$ integrated into the chromosome in three different DEC pathotypes. Integration may reduce fitness cost of the maintenance of *bla*$_{CTX-M-15}$ and facilitate persistence.

Characterising and understanding *bla*$_{CTX-M-15}$-encoding plasmids as well as chromosomal integration events will inform strategies to ease the burden and spread of AMR. Furthermore, determining the mechanisms that contribute to the global spread of AMR will confer improvements in infection prevention and allow for the conservation of existing antibiotics, and to ultimately reduce the threat to public health.

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