INTRODUCTION

- Shiga toxin-producing Escherichia coli (STEC) are a group of zoonotic, foodborne pathogens defined by the presence of phage-encoded Shiga toxin genes (strX) [1]. STEC causes gastrointestinal disease in humans and symptoms include severe bloody diarrhoea, abdominal pain and nausea. In 5-15% of cases infection leads to Haemolytic Uremic Syndrome (HUS), characterised by kidney failure and/or cardiovascular and neurological complications [2].

- STEC genome size ranges from 4.9 to 5.6 Mbp in size, and a high proportion (8-12%) is comprised of mobile elements and prophage [3].

- There are two primary subtypes of Shiga toxin, strA and strB of which can be further subdivided into three strX subtypes (1a, 1c and 1d) and seven strY subtypes (2a – 2j) [4]. StrX2a and StrX2d have a higher association with disease progression to HUS, particularly so in children and the elderly.

- In May 2017, a one-year-old male was admitted to hospital presenting with bloody diarrhoea and was subsequently diagnosed with HUS. STEC O63:H6 carrying strX2 was isolated from his faecal sample.

- STEC encoding strX2 are rarely associated with causing HUS.

- We used single-molecule real-time (SMRT) Oxford Nanopore sequencing to characterise the accessory genome, including prophage content.

RESULTS

Phylogenetic analysis:

- STEC O63:H6 belongs to Clonal Complex 122 (CC122).

- CC122 is comprised of two sequence types, ST122 and ST583 and three serotypes, O71:H6, O125ac:H6 and of O63:H6 (Figure 1).

- E. coli O71:H6 is stx negative. E. coli O125ac:H6 is strX2 positive and E. coli O63:H6 is located on two separate branches, one group harbors the strX2f and the other does not.

- There appears to be two separate events involving the co-acquisition of strX2 encoding prophage and IncFIB plasmid.

Genome features:

- Sample 377323 assembled into two contigs, one chromosome (4,905,296 bp) and one plasmid (85,191 bp).

- The locus of enterocyte effacement was adjacent to a RNA gene (selC).

- 377323 contained 11 prophages comprising 8.96% of the chromosome (Figure 2B).

- The prophages did not significantly match (>50% sequence similarity and coverage) any known prophages from the Sakai (STEC) reference genome (NC_002695).1.

- Prophage 10 encoded the strX2 gene and was inserted adjacent to the strX2 gene encoding a transfer-messenger RNA (Figure 2C).

- The cell-cycler-inhibiting factor (cif) was located on prophage 9 and is inserted next to the yedD gene.

- The plasmid identified (p377323) contained an astA gene which codes for arginine succinyltransferase and a BpaA cassette encoding for a type IV pilin and Hha-like haemolysin modulators. Over 30% of the plasmid comprised a tetr conjugation cassette (Figure 2D).

DISCUSSION and CONCLUSIONS

- In this study we show the emergence of STEC in a previously non-STEC lineage of Escherichia coli.

- We show the acquisition of the strX2f encoding prophage across two different clades within CC122.

- We have also noted the co-acquisition of an IncFIB plasmid which further contributes to disease severity by carrying multiple virulence factors such as the bfp cassette.

- The acquisition of the strX2f encoding prophage corresponds with the acquisition of additional mobile genetic elements such as prophages and plasmids that encode for multiple virulence factors including cell cycle-inhibiting factor (cif), the bfp cassette and an array of non-LEE effectors (nel).

- Pathogenic potential of STEC is multifactorial. STEC encoding strX2f may cause severe symptoms including HUS when present in combination with other virulence factors.

- The emergence of strX in a STEC Non-O157 lineage is a major public health concern.

REFERENCES:


AMMENAGEMENTS

I would also like to thank the NIHR Health Protection Research Unit (NIHR HPRU) for gastrointestinal infections for funding this project.

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