Detection and characterisation of *Salmonella enterica* serovar Infantis (eBG31) harbouring *bla*$_{CTX-M-1}$ causing clinical disease in humans in England

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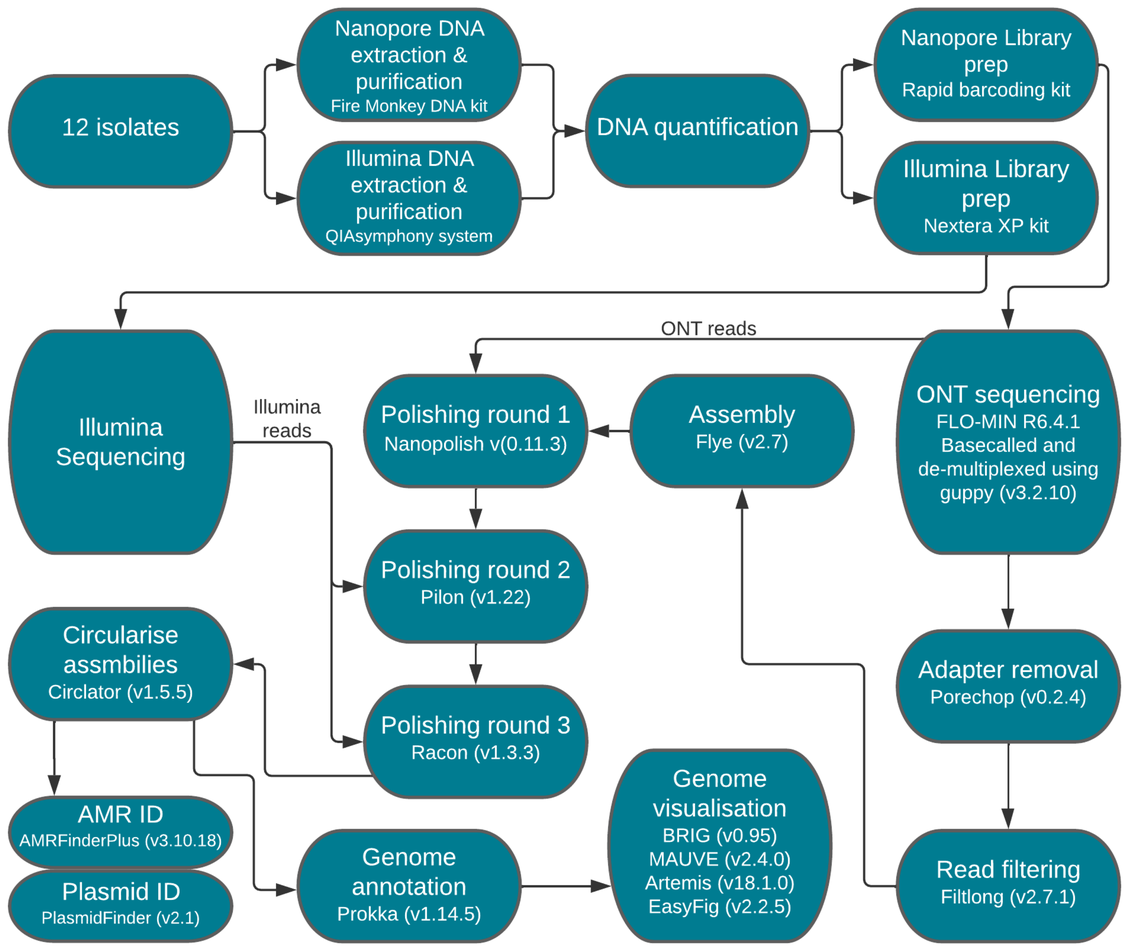
INTRODUCTION

- *Salmonella enterica* serovar Infantis, comprising eBurst groups (eBGs), eBG31 and eBG297, is the fourth most common serovar in humans across Europe. eBGs31 causes the highest burden of human disease and is the predominant group in animal reservoirs, especially in poultry.
- There is an increasing number of reports of S. Infantis harbouring plasmids carrying multiple resistance determinants, termed 'plasmid of emerging S. enterica Infantis' (pESI). pESI plasmids often carry genes conferring resistance to tetracycline, trimethoprim, sulfamethoxazole, antiseptics, heavy metals and genes associated with virulence.
- UKHSA undertake routine genomic surveillance of critically important serovars for purposes of One Health, especially in those serovars/eBGs with know animal reservoir. Such routine surveillance has recently identified an isolate of S. Infantis eBG31 harbouring *bla*$_{CTX-M-1}$.
- Of the 2509 clinical isolates of *S*. Infantis in the UKHSA archives between 2012 and 2022, 21 (0.8%) had *bla*$_{CTX-M-1}$. 12 of these isolates were chosen for re-sequencing using Oxford Nanopore Technologies (ONT) and were the focus of this study (Table 1).
- The aim of this study was to interrogate the UKHSA archive of *Salmonella* isolates for the present of *S*. Infantis eBG31 harbouring *bla*$_{CTX-M-1}$ to characterise the AMR determinants using a combination of short and long-read sequencing technologies.

NGS ID	Receipt Date	Sex	Foreign Travel	Travel Destination	EBG	SNP Address
1059469	24/12/2020	F	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
1048341	07/12/2020	F	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
1045028	03/12/2020	F	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
1042326	01/12/2020	F	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
1038175	25/11/2020	F	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
557658	15/06/2018	F	Y	Unknown	31	1.1.36.107.107.1618.1759
1551502	10/09/2015	F	Y	Italy	31	1.1.36.107.107.2115.2728
1375619	18/08/2021	M	Unknown	Unknown	31	1.1.36.107.1912.2081.2561
1153217	31/03/2021	M	Unknown	Unknown	31	1.1.841.1323.1831.1955.2398
1048349	07/12/2020	M	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
1044958	02/12/2020	M	Unknown	Unknown	31	1.1.841.1323.1831.1955.2288
152369	25/08/2015	M	N	Unknown	31	1.1.36.107.107.107.107

METHODS



RESULTS

- SNP Typing identified 7 of the 12 isolates studied fell within the same 5 SNP single linkage cluster, indicating close genetic similarity and likely to be from a common source (Table 1). Although no source could be identified the cases were geographically dispersed indicating that a nationally distributed, imported or domestically produced product was a possible vehicle.
- Short read sequencing technologies was able to identify other resistant determinants (in addition to *bla*$_{CTX-M-1}$) including *gyrA*[87:D-G], *tetA*-1, *sul*-1, *aph3'-la* and *dfrA*-1/*dfrA*-14 or *dfrA-1*, known to confer resistance to fluoroquinolones, tetracyclines, sulphonamides, aminoglycosides and trimethoprim, respectively.
- ONT sequencing was then able to confirm that these resistant determinants (including *bla*$_{CTX-M-1}$) were located on a pESI-like plasmid (on average 286kbp in size) and fell into one of two resistant sites, previously described by Tate et al. (2017) (Fig. 1). However, unlike Tate et al. (2017) who had identified *bla*$_{CTX-M-65}$ in site 1 (Fig. 2) we identified *bla*$_{CTX-M-1}$ in site 2 (Fig. 3).
- ONT analysis and BRIG visualisation also highlighted a 5kbp deletion (containing *bla*$_{CTX-M-1}$) in one of the outbreak strains – 1045028. When sequenced previously on the illumina platform, *bla*$_{CTX-M-1}$ was detected (Fig. 1). Comparative long-read investigations also found one of the sporadic cases (1375619) exhibited loss of two co-located AMR determinants *aph(3')-la* and *drfA14* (Fig. 1). The short read sequencing data for this isolate (1375619) indicated that *aph(3')-la* was present at least in variants in the original culture, but *drfA14* was not detected in either the Illumina or ONT data.
- Resistance site 1 (Fig. 1) and 2 (Fig. 3) comparisons conducted between the UKHSA isolate (1153217); p119944 isolated from Israel by Cohen et al., (2020) & pFSIS1502169 isolated from the USA discussed by Tate et al., (2017) highlights the dynamic evolution of the drug regions and that *bla*$_{CTX-M}$ variants can insert into 6 hotspot regions in the megaplasmid as mentioned by Cohen et al., (2020) .

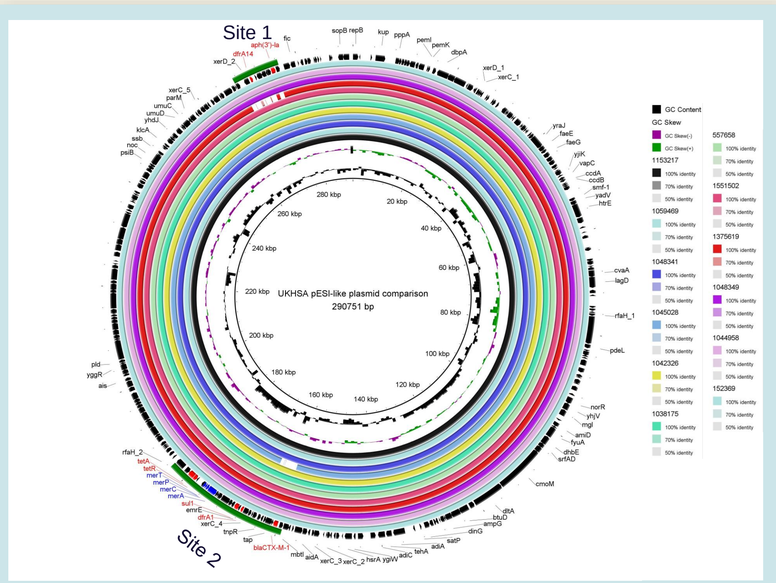


Figure. 1: BRIG comparison of UKHSA pESI-like plasmids.

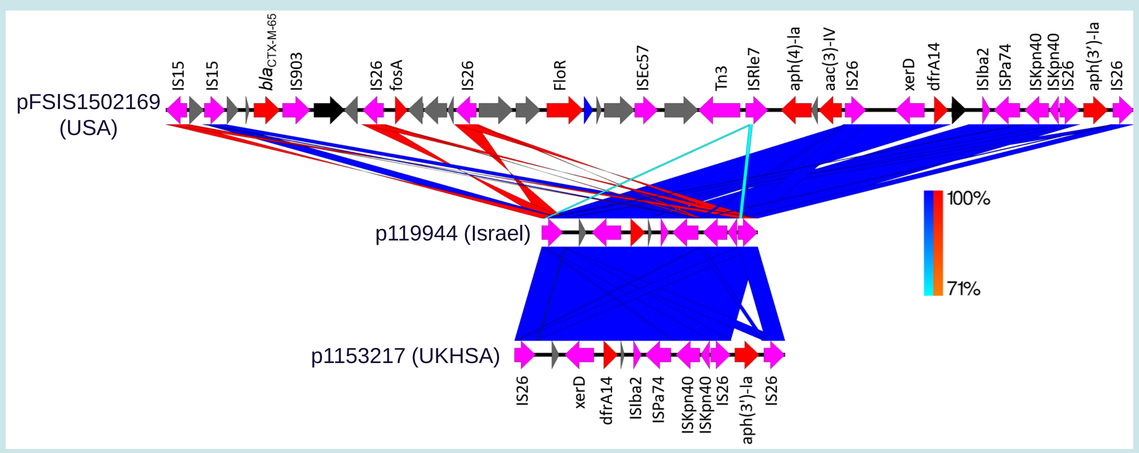


Figure. 2: Resistance site 1 comparison. Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants in red; Mobile elements in pink; Regulatory genes in blue & unannotated genes in black. Scale bars indicate level of sequence similarity for forward (blue) and reverse (red) sequences.

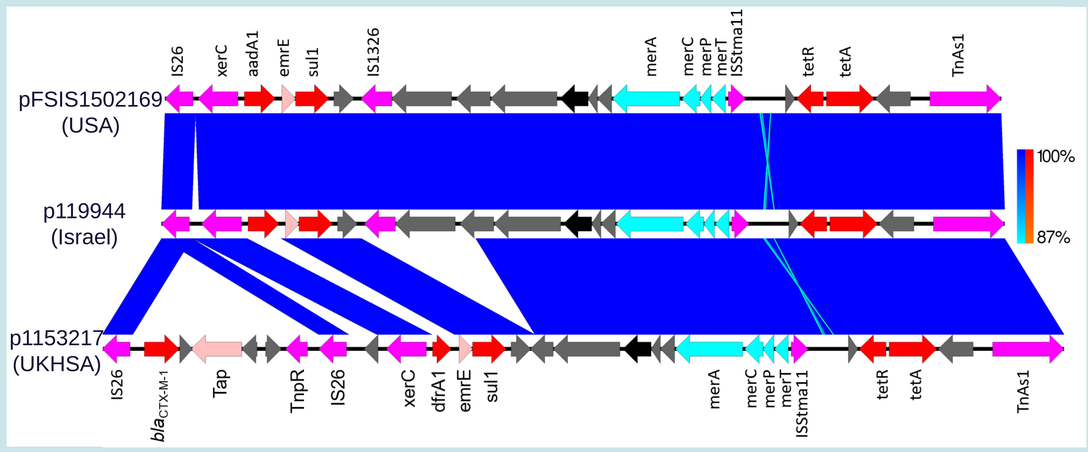


Figure. 3: Resistance site 2 comparison. Arrows indicate gene direction while colours indicate gene function. Hypothetical proteins are shown in grey; AMR determinants are shown in red; Mobile elements are shown in pink; Heavy metal genes are shown in cyan; Multidrug efflux pumps are shown in light pink & unannotated genes are shown in black

DISCUSSION & CONCLUSIONS

- Twenty-one human cases infected with S. Infantis eBG31 harboring *bla*$_{CTX-M-1}$ was detected from the total amount of diagnosis of S. Infantis eBG31 (2486 isolates). Within the UKHSA archive there were two other S. Infantis eBG31 *bla*$_{CTX-M}$ variants – *bla*$_{CTX-M-65}$ (65 isolates) and *bla*$_{CTX-M-14}$ (1 isolate). Indicating the presence of ESBL resistance within the UK S. Infantis population. Our data indicates that, despite an overall reduction in Salmonellosis since 2019, most likely due to the impact of reduction in testing and access to healthcare due to the COVID-19 pandemic, the majority of S. Infantis with *bla*$_{CTX-M-1}$ cases (n=15/21, 71%) have occurred over the last 3 years.
- The first detection of S. Infantis eBG31 harbouring *bla*$_{CTX-M}$ variants occurred in Italy, and two of our earlier cases (1551502 & 557658) reported travel in the days prior to onset of symptoms. No travel history was recorded for any of the subsequent cases, and so a domestic or non-domestic source could not be confirmed. Although, the detection of a *bla*$_{CTX-M-1}$ variant in the animal reservoir in the UK indicates that a domestic source is possible, either via the food chain, animal contact, or exposure to a contaminated environment.
- ONT analysis also identified the loss of a 5kbp regions containing *bla*$_{CTX-M-1}$ in isolate 105028. A possible explanation for this observation is that the colony that was selected for Nanopore sequencing had lost its *bla*$_{CTX-M-1}$ during storage. Maintenance of the plasmid but loss of the *bla*$_{CTX-M}$ determinant located on the plasmid, on subculture has been observed previously (Lock et al. 2021).
- Whilst Short-read WGS can identify the presence or absence of AMR determinants it cannot interrogate their genomic architecture. Long-read analysis of WGS data however enables the characterisation of mobile genetic elements on which the key AMR determinants are located and identifies the combination of different AMR determinants co-located on the same mobile element. This approach enables us to monitor the emergence and spread of *bla*$_{CTX-M}$ variants in all *Salmonella* species at both the local and global level.

The research was part funded by the National Institute for Health Research Protection Research Unit (NIHR HPRU) in Gastrointestinal Infections at University of Liverpool in partnership with the UK Health Security Agency (UKHSA), in collaboration with University of Warwick. Health Protection Research Unit (HPRU) in Genomics and Enabling Data is a collaboration funded by the National Institute for Health Research (NIHR) between UK Health Security Agency (UKHSA), the University of Warwick, the Centre for Genomic Pathogen Surveillance and the University of Cambridge. The views expressed are those of the author(s) and not necessarily the NIHR, the Department of Health and Social Care or the UK Health Security Agency.

FUNDED BY NIHR | National Institute for Health Research

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