

# Surveillance of Extended Spectrum Beta-Lactamase Resistance in *Salmonella* in England, 2014-2022

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## INTRODUCTION

*Salmonella* are Gram negative bacilli belonging to the Enterobacteriales family

- Prevalent in animals such as poultry, pigs, and cattle which can cause transmission via the food chain<sup>[1]</sup>
- Salmonellosis is generally transmitted via consumption of contaminated food of animal origin<sup>[1]</sup>
- Salmonella* is responsible for 180 million (~9%) cases of diarrhoeal illness that occurs globally each year<sup>[2]</sup>
- In England, between 7,000 and 12,000 cases of salmonellosis are detected annually<sup>[3,4]</sup>

Symptoms of salmonellosis are relatively mild, but invasive infections can occur

- Fever, abdominal pain, diarrhoea and nausea, sometimes vomiting<sup>[1]</sup>
- Infection is usually self-limiting, but antibiotics are needed in more severe cases<sup>[1]</sup>

Antimicrobial resistance is a global public health concern<sup>[5]</sup>

- Extended Spectrum Beta Lactamases (ESBLs) were first discovered in the 1980s, have spread worldwide and are now endemic in the Enterobacteriales.
- ESBLs are enzymes which confer resistance to most beta-lactam antibiotics (penicillins, cephalosporins)
- Previously, *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> were the most prevalent ESBL genes. Currently, *bla*<sub>CTX-M</sub> genes are the predominant cause of ESBL resistance → *bla*<sub>CTX-M-15</sub> is the dominant variant worldwide
- Other prevalent ESBL genes include *bla*<sub>OXA</sub>, *bla*<sub>CMY</sub> and *bla*<sub>DHA</sub>
- ESBL genes are frequently found on plasmids, transposons and insertion sequences, facilitating spread
- The presence of ESBL's in *Salmonella* has been studied and characterised in other countries, however it has not been widely characterised within England

The aim of this study was to analyse Whole Genome Sequencing (WGS) data to infer and describe the prevalence of ESBL resistance in *Salmonella* in England.

## METHODS

*Salmonella* isolates (n=77,258) received by the Gastrointestinal Bacteria Reference Unit (GBRU) between January 2014 and December 2022 in England were selected for the dataset

- These were sequenced via paired-end sequencing on an Illumina HiSeq platform
- This was part of routine surveillance<sup>[10]</sup>

Bioinformatic pipelines are used for the analysis of the raw sequencing data to obtain:

- Identification (Kmer, ST, eBG, Seqsero)
- Typing for strain discrimination (SNP address)
- Detection of antimicrobial resistance genes<sup>[10]</sup>

The data was analysed retrospectively for surveillance and presence of ESBL genes

- Isolates included in this study were selected based on the presence of six predominant ESBL-encoding genes; *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CMY</sub> and *bla*<sub>DHA</sub> (n=1873)
- Note: Non-ESBL variants of these genes, present in the dataset that are known not to confer phenotypic resistance to extended spectrum beta lactams, were excluded, e.g. *bla*<sub>TEM-1</sub>, *bla*<sub>TEM-57</sub>, *bla*<sub>TEM-135</sub>, *bla*<sub>OXA-9</sub>, *bla*<sub>OXA-10</sub>, *bla*<sub>OXA-50</sub>

cgMLST using Enterobase was used to explore the distribution of ESBL genes within the *Salmonella* population structure.

- The top 10 eBURST Groups (eBG)/serotypes (accounting for 81% of *Salmonella* harbouring ESBL genes) were then explored

## RESULTS

GBRU received 77,258 *Salmonella* isolates between 2014 and 2022 (Figure 1b). Of these, 1873 isolates (2.4%) harboured one or more of 6 predominant ESBL-encoding genes:

*bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CMY</sub>, *bla*<sub>DHA</sub>.

- Overall, ESBL resistance in *Salmonella* increased by 88% between 2014 to 2022 (150 isolates in 2014 to 283 isolates in 2022) with a decline between 2020-2021 (Figure 1a)
- Sample numbers received also declined between 2020-2021 due to COVID pandemic (Figure 1b)
- In terms of each gene, *bla*<sub>CTX-M</sub> increased by 336% (39 isolates in 2014 to 131 isolates in 2022), *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> and *bla*<sub>OXA</sub> remained stable, *bla*<sub>CMY</sub> increased by 318% (27 isolates in 2014 to 86 isolates in 2022) and *bla*<sub>DHA</sub> decreased by 290% (26 isolates in 2014 to 10 isolates in 2022)

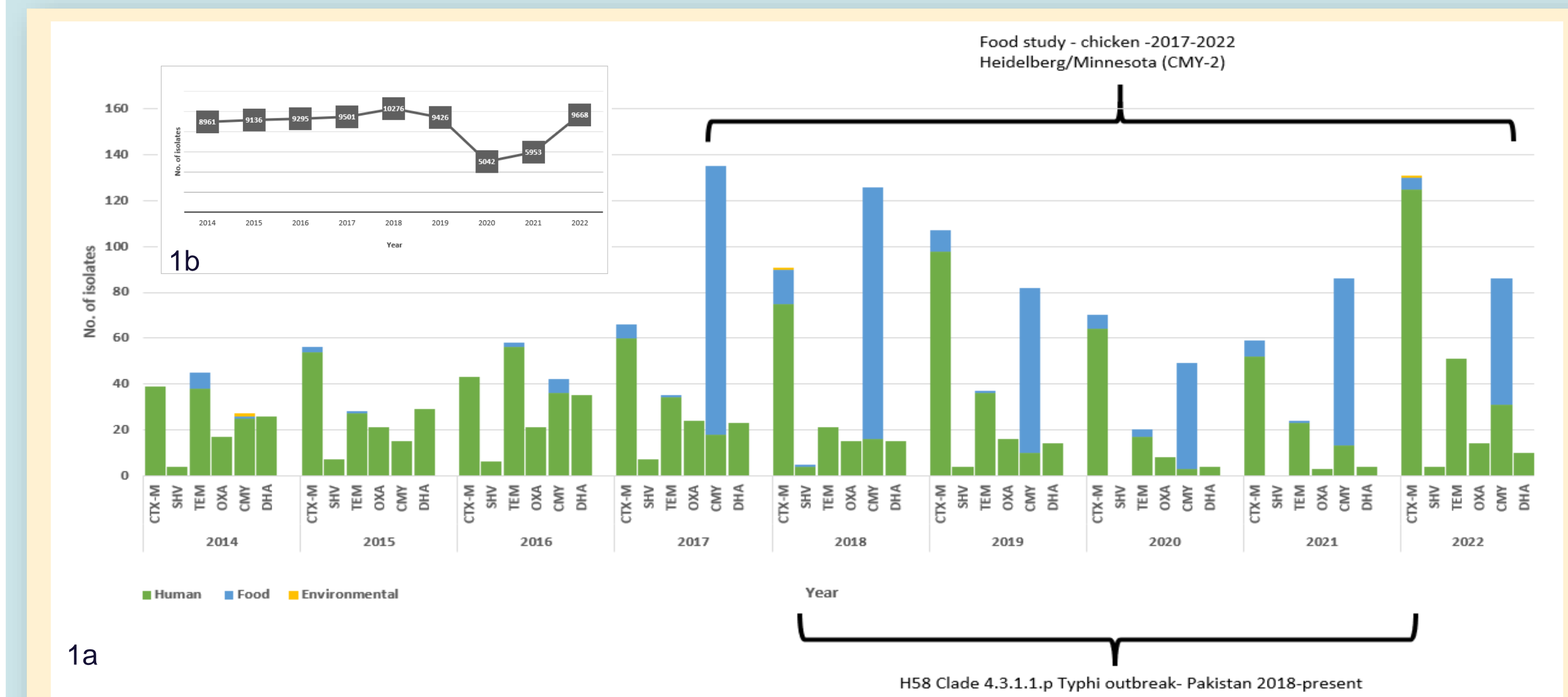


Figure 1a - Graph showing the number of *Salmonella* isolates between 2014 and 2022 harbouring the six predominant ESBL genes, and the distribution per year and per gene

Figure 1b (inset) - Total number of *Salmonella* isolates received per year

Of 1872 isolates, there were 667 *bla*<sub>CTX-M</sub>, 37 *bla*<sub>SHV</sub>, 141 *bla*<sub>OXA</sub>, 319 *bla*<sub>TEM</sub>, 649 *bla*<sub>CMY</sub> and 166 *bla*<sub>DHA</sub> genes, including variants of these.

- The overall most prevalent ESBL gene in the dataset was *bla*<sub>CTX-M</sub>, and least prevalent was *bla*<sub>SHV</sub>
- The most prevalent variants of the six predominant genes were *bla*<sub>CTX-M-15</sub> (n=276), *bla*<sub>SHV-12</sub> (n=34), *bla*<sub>TEM-117-p</sub> (n=97), *bla*<sub>OXA-1</sub> (n=126), *bla*<sub>CMY-2</sub> (n=614) and *bla*<sub>DHA-1</sub> (n=77)

90 different serotypes harboured ESBL genes – 88 non-typhoidal (n=1,672) and 2 typhoidal (Typhi and Paratyphi A) (n=201).

- The top 10 eBGs/serotypes in terms of prevalence of ESBL genes were: eBG26 - Heidelberg (n=392), eBG1/138 - Typhimurium (n=226), eBG13 - Typhi (n=200), eBG77 - Minnesota (n=152), eBG1 - monophasic Typhimurium (n=130), eBG4 - Enteritidis (n=119), eBG31 - Infantis (n=113), eBG56 - Kentucky (n=96), eBG54 - Agona (n=52), eBG14 - Saintpaul (n=31) (Figure 2).
- These 10 eBGs (serotypes) account for 81% of all *Salmonella* isolates harbouring ESBL genes

cgMLST was used to determine the distribution of ESBL genes in the *Salmonella* population of the top 10 eBG/serotypes harbouring ESBL genes (Figure 2).

- Some ESBL genes were specific to a particular eBG (serotype) and were rarely found in other eBGs (serotypes); e.g. *bla*<sub>CTX-M-65</sub> in eBG31 (*S. Infantis*)
- Some ESBL genes distributed throughout the *Salmonella* population e.g. *bla*<sub>CTX-M-15</sub>, *bla*<sub>CMY-2</sub>
- Each eBG (serotype) had one or more predominant ESBL genes present, as well as multiple other sporadic ESBL genes in lower numbers – suggesting both clonal and non-clonal spread of ESBL determinants within these *Salmonella* eBGs (serotypes). e.g:
  - eBG13 (*S. Typhi*) – Predominant gene: *bla*<sub>CTX-M-15</sub>, other genes: *bla*<sub>CTX-M-55</sub>, *bla*<sub>SHV-12</sub>, *bla*<sub>TEM-10</sub>, *bla*<sub>TEM-190</sub>
  - eBG31 (*S. Infantis*) – Predominant genes: *bla*<sub>CTX-M-65</sub> and *bla*<sub>CTX-M-1</sub>, other genes: *bla*<sub>SHV-11</sub>, *bla*<sub>SHV-12</sub>, *bla*<sub>DHA-1</sub>, *bla*<sub>DHA-5</sub>, *bla*<sub>DHA-9</sub>, *bla*<sub>DHA-12</sub>, *bla*<sub>DHA-13</sub>, *bla*<sub>DHA-14</sub>, *bla*<sub>DHA-17</sub>, *bla*<sub>TEM-52</sub>, *bla*<sub>TEM-117-p</sub>, *bla*<sub>TEM-159</sub>, *bla*<sub>TEM-191-p</sub>

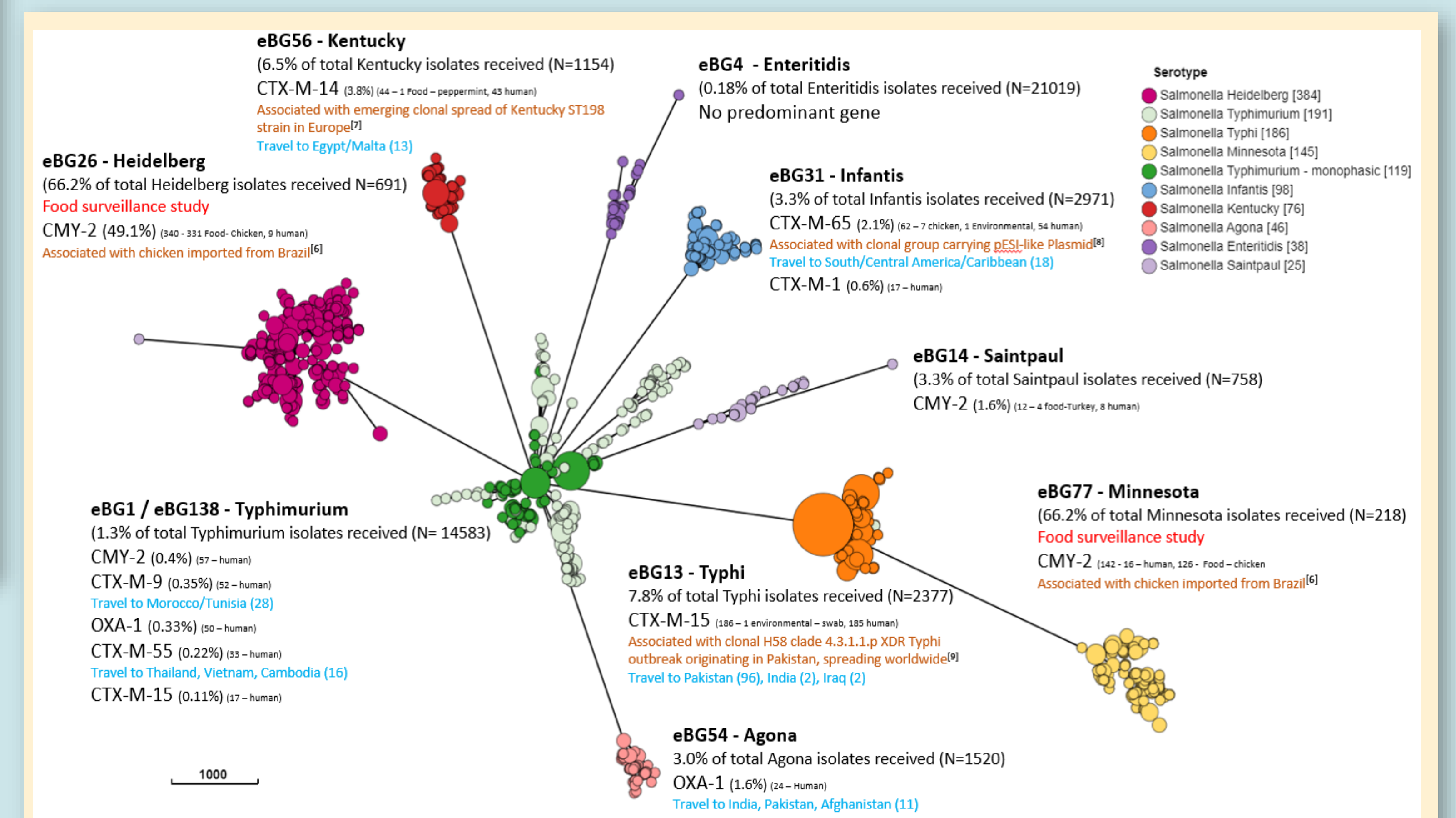


Figure 2 - Grapetree showing the population structure of the top 10 eBGs (serotypes) of *Salmonella* harbouring ESBL genes 2014-2022. Predominant ESBL gene(s) in each eBG (serotype) are listed, including travel association, food importation and published studies. (Proportion of isolates with ESBL genes out of total number of isolates received (N=), is given as a percentage per eBG (serotype).)

## DISCUSSION

- Our surveillance data shows an 88% increase of ESBL resistance in *Salmonella* between 2014 and 2022
- The 6 predominant ESBL encoding genes in this dataset were: *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CMY</sub> and *bla*<sub>DHA</sub>
- ESBL encoding genes are seen in multiple *Salmonella* eBGs (serotypes) with the top 4 being: eBG26 (Heidelberg), eBG1 (Typhimurium), eBG13 (Typhi), and eBG77 (Minnesota)
- The large increase in *bla*<sub>CMY</sub> between 2014 and 2022 in *S. Heidelberg* and *S. Minnesota* is due to poultry surveillance studies from 2017-2022. This was traced back to chicken imported from Brazil<sup>[6]</sup>
- Excluding food isolates, *bla*<sub>CMY</sub> levels increased by 24% in all the serotypes in this study
- Food surveillance is important in detection of AST, as it highlights the potential spread and transmission of AMR genes in the food chain
- The significant rise in *bla*<sub>CTX-M</sub> was predominantly associated with a global spread of the H58 (clade 4.3.1.1.p) XDR Typhi harbouring *bla*<sub>CTX-M-15</sub> from an outbreak originating from Pakistan since 2017<sup>[9]</sup>
- This study confirmed a further 3 eBG/serotypes (eBG1/eBG138 - Typhimurium, eBG54 - Agona, eBG14 - Saintpaul) with emerging ESBL resistance that have not been previously described

## CONCLUSIONS

- This study highlights the importance of continued WGS surveillance data to monitor the increase and spread of antimicrobial resistance in the *Salmonella* population
- Surveillance of antimicrobial resistance allows detection of trends over time; it is important to detect an increase of ESBLs, since third generation cephalosporins such as cefotaxime are recommended for the treatment of invasive or severe Salmonellosis<sup>[11]</sup>
- This will allow changes and updates to treatment guidelines where required, if specific antibiotics are no longer deemed appropriate

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