**Global genomic dissection of antimicrobial resistance in *Salmonella* Typhimurium**

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## Abstract

### Background

*Salmonella* Typhimurium (STm) is a globally prevalent pathogen causing disease in both humans and animals. Antibiotics are required for the treatment of invasive salmonellosis and increasing resistance poses a treatment challenge. Comprehensive whole-genome sequencing based surveillance efforts, especially of USA and UK, and open access databases presented an opportunity to comprehensively analyse the genomic antimicrobial resistance (AMR) to key clinically-relevant antibiotics within this dataset.

### Methods

In this study, we identified and analysed resistance to fourteen key antibiotics using AbritAMR, and integrated the identified resistance with multilevel genome typing (MGT). AMR carriage and trends were assessed by genomic types at different MGT levels.

### Findings

In the complete dataset, 47% of the isolates were resistant to at least one drug – however resistance varied considerably by genomic types, geography, and time. When comparing data from 2019-2022, we observed USA had higher resistance to cefotaxime (AmpC) and gentamicin, whereas UK had higher resistance to multiple drugs including azithromycin and cefotaxime (ESBL). Within the 2015-2022 isolates, we identified 166 sequence types (STs) at different MGT levels with >80% resistance to at least one drug. We grouped these STs over time to reveal 20 predominant temporal patterns. We also identified STs that were expanding regionally, and those were source specific.

### Interpretation

The availability of global datasets enabled delineation of AMR trends within STm. Furthermore, integration of AMR with MGT genome typing provided sharable, standardised, and specific identification and tracking of resistant genomic types. This integrated analysis presents a unique approach for global surveillance of AMR and AMR strains.

## Introduction

*Salmonella enterica* serovar Typhimurium (STm) is one of the two most frequently isolated Non-typhoidal *Salmonellae* (NTS) serovars globally [1], and the most frequent in Australia [2]. NTS has a wide host range (such as domesticated animals, birds, pets, fruits and vegetables), and infects humans through direct contact with hosts or consumption of contaminated food [3]. NTS infects the gastrointestinal tract, and disease severity is generally mild to moderate. However, NTS infections can also result in bacteraemia, and cause endocarditis, abscesses, or meningitis [4]. Antibiotics are not recommended for mild to moderate cases of salmonellosis in healthy individuals, however, are required in severe cases. For such cases, the US CDC recommends [5] fluoroquinolones (e.g. ciprofloxacin) as first-line treatment in adult travellers. Then, ceftriaxone (a third-generation cephalosporin antibiotic) or azithromycin (macrolide) is recommended for children or adults with invasive disease. Recommendations in Australia are similar [6, 7]. Additionally, in Australia, amoxicillin, or trimethoprim-sulfamethoxazole may be prescribed after confirming an isolate's sensitivity to these drugs.

By multilocus sequence typing (MLST), the STm population is consisting of a few predominant sequence types (STs), ST19, ST34, ST313 and ST36 [8]. ST19 is the largest ST while ST34 is the monophasic STm. ST313 comprises two multidrug resistant lineages (L1 and L2) associated with invasive disease in Africa [9]. In our previous work, we developed multilevel genome typing (MGT) for characterising STm isolates at multiple levels of resolutions [10]. MGT comprises nine MLST-like schemes that increase in resolution from MGT1 to MGT9 within a single system. A set of nine MGT STs, one for each level, are assigned to every isolate – thus enabling the comparison of isolates at multiple resolutions. MGT1 is the traditional 7-gene MLST for the *Salmonella* species - hence MGT1 STs are the same as the well-known 7-gene MLST STs, such as ST19 or ST34. MGT8 comprises the species core genome MLST (cgMLST), and MGT9 is the serovar core genome MLST. MGT2 to 7 are composed of increasing numbers of core genes from MGT8. Many well studied clades and types can be described using these intermediate resolution levels. MGT3 ST11 describes phage type DT104 while two large groups composed of mainly USA avian isolates are described by MGT3 ST8 and MGT3 ST171. Monophasic STm (MLST ST34) can be identified using MGT3 ST26, MGT3 ST18 and MGT3 ST225. The invasive disease-causing lineages within ST313 (L1 and L2) are described via MGT3 ST725 and MGT3 ST3, respectively.

The emergence of antimicrobial resistance (AMR) and multi-drug resistance (MDR) in STm has been well documented [11, 12]. For example: within ST34, resistance follows the ASSuT pattern (i.e. resistance to **A**mpicillins, **S**treptomycins, **Su**lphathiazoles, and **T**etracyclines), with the corresponding genes $bla_{TEM-1}$, *strA-strB*, *sul2*, and *tet(B)* present on a chromosomal resistance island [13]. Additionally, ST34 may confer resistance to quinolones, cefotaxime, colistin, gentamicin, and chloramphenicol [14]. Pigs are an important reservoir of this ST [14]. Phage type DT104 or MGT3 ST11 has the well-known resistance pattern ACSSuT (the additional 'C' refers to **C**hloramphenicol) [15], also with the corresponding genes chromosomally encoded [16, 17]. DT104 has rarer genes such as *tet(G)*, *tetR* and *floR*, and resistance to additional drugs such as gentamicin, kanamycin, trimethoprim-sulphamethoxazole has also been observed [15]. The prevalent bloodstream-infection-associated lineage, ST313 L2, or MGT3 ST3, has well known resistance to multiple drugs such as ampicillins, trimethoprim-sulphamethoxazole and chloramphenicol – with reports also emerging of resistance to azithromycin and cephalosporins [9].

In recent years, whole genome sequencing (WGS) has been adopted for the surveillance of *Salmonella* in the UK [18] and USA [19] which have made complete WGS surveillance datasets available publicly. With the public availability of a large number of genomes from long term *Salmonella* surveillance projects, we conducted a comprehensive genomic survey of AMR in STm. We particularly focussed on combining AMR determinant detection with MGT typing to track AMR strains. We demonstrated the utility of the standardised and multi-resolution MGT approach, to identify groups of resistant strains with precision, especially to drugs of clinical importance and determined global temporal trends of AMR MGT STs in STm.

## Results

### Dataset

Our dataset comprised 65,895 genomes available within MGTdb [20] until August 31, 2023. The genomes were assembled and assigned MGT STs. Over half of the isolates had metadata (**Table 1**): 63% with country, 59% with year, and 58% with both country and year. The isolates spanned 90 countries and were sampled between 1900 to 2022. Of isolates annotated with both year and country, 73% were from USA and UK. Sampling has been increasing

steadily in recent years, especially since approximately 2013 onwards with 42% of the data sampled from 2015 to 2022 (**Figure S1, Table 1**).

As the UK adopted genomic surveillance for *Salmonella* from 2015 [18], and USA from 2019 [19], we considered the following subsets for detailed analysis, two timeframes 2015-2018 and 2019-2022, and three geographies, UK, USA and other countries (which we designate as 'Other' throughout this paper). For the 'other' data, since the temporal and geographic coverage of the isolates was very heterogenous, we presented the results but refrained from discussion of their significance as any trends observed may be the result of sampling bias.

### Resistance score

Using resistance predictions obtained for 14 drug classes using AbritAMR [21], we calculated a resistance score for each isolate defined by the number of drugs an isolate was non-susceptible to. Approximately half (53%) of the isolates were susceptible to all drugs (**Table S1**), however this varied by location. In 2015-2022 isolates, a higher percentage of isolates in USA and Other were susceptible (55% and 59%, respectively), whereas in UK the percentage was much lower (41%). Resistance to four drugs was the common resistance score in the complete dataset (16%) and in UK (23%). In USA resistance to two and four drugs was in similar frequency (18% and 14%, respectively).

Variability in resistance was also seen by time (**Table S1**). We compared the average resistance scores between the complete dataset to UK and USA over two timeframes, 2015-2018 and 2019-2022, and found that in both timeframes UK had the highest resistance, while USA had lower resistance compared to the complete dataset. When comparing the resistance within UK and USA, between two timeframes, resistance within UK was lower in the later timeframe; whereas in USA, resistance was higher in the later timeframe.

We further used the resistance score to analyse the frequency of resistance to the three key prescribed drugs (ciprofloxacin, cefotaxime, and azithromycin), in 2021-2022. We found that within UK and USA, only 9% being resistant to one drug and 1% being resistant to two drugs (**Table S2**). Resistance to all three drugs was very rare in this set, only seen in seven isolates (three from USA, and four from Other).

## Resistance to individual drugs

Resistance to any individual drug in the complete dataset varied from negligible at 0.02% for meropenem to 36% for tetracycline (**Figure 1)**. The UK in 2015-2018 had one of the highest resistance rates, especially to the four drugs tetracycline (54%), streptomycin (52%), ampicillin (49%) and sulfathiazole (46%). In the UK in 2019-2022, compared to 2015-2018, there was a significant increase in resistance to only two drugs, kanamycin (from 1% to 5%) and gentamicin (from 1.7% to 2.9%), and a significant reduction in resistance to eight drugs namely, azithromycin, cefotaxime (ESBL), cefotaxime (AmpC), ciprofloxacin, sulfathiazole, ampicillin, streptomycin and tetracycline (**Figure 1**). However, an opposite trend was seen in USA, wherein resistance significantly increased to eight drugs, and decreased to two drugs in 2019-2022, compared to 2015-2018. In particular, resistance was increased to tetracyclines (from 38% to 44%), streptomycin (from 19% to 22%), sulphathiazole (from 33% to 39%), trimethoprim (1.8% to 2.3%), trimethoprim-sulphathiazole (1.7% to 2.1%), ciprofloxacin (2.7% to 3.4%), gentamicin, (4.3% to 8.2%), and cefotaxime (ESBL) (0.3% to 0.7%), and decreased to chloramphenicol and cefotaxime (AmpC).

We also compared the resistance to individual drugs, between UK and USA in 2019-2022 (**Figure 1**). UK had significantly higher resistance to 10 drugs, namely streptomycin (higher by 22%), ampicillin (by 25%), chloramphenicol (by 8%), trimethoprim (by 8%), trimethoprim-sulphathiazole (by 7%), ciprofloxacin (by 5%), kanamycin (by 1.3%), azithromycin (by 0.5%), and meropenem (by 0.1%), and USA had higher resistance to only two drugs, namely, cefotaxime (AmpC) (higher by 6%) and gentamicin (by 7%).

## Resistance within MGT1 (MLST) STs

MGT1 STs are identical to the traditional MLST STs. Difference in AMR carriage within and between these STs are well known [12]. For the total dataset, 64% isolates within ST19 (the most frequent ST) were susceptible to all drugs, followed by resistance to four drugs within 11% of the isolates; whereas within ST34 (the second frequent ST) only 4% isolates were susceptible to all drugs with majority (55%) being resistant to four drugs (**Figure 2**, **Table S3**). The average resistance to multiple drugs followed the order: ST313 > ST34 > other STs > ST19 > ST36.

Resistance rates for each drug in each ST was compared between UK and USA for the 2019-2022 timeframe only (**Table S4**, **Figure S2**). Within ST19, UK had significantly higher

resistance to seven drugs, including ampicillin (higher by 10%), ciprofloxacin (by 7%), and cefotaxime (ESBL) (by 0.4%), whereas USA had higher resistance to five drugs, including cefotaxime (AmpC) (higher by 7%), along with kanamycin (by 1%) and gentamicin (by 8%). Differences in resistance to individual drugs between STs was also compared using the 2019-2022 data without taking location into account (**Table S4**). Between ST19 and ST34, ST34 had higher resistance to most drugs (12 of 14), with the exception of cefotaxime (AmpC) which was higher in ST19.

### MGT STs with 80% of the isolates resistant to a given drug

Analysis of AMR using MLST (MGT1) STs gave a broad overview of resistance, especially in the context of the most frequent STs, ST19 and ST34. However, to obtain a finer-grade understanding of resistance, we used higher MGT levels to break up the dataset into higher-resolution MGT STs. In particular, we computed percentage resistance for each ST at each MGT level. We then examined STs at each level from MGT1 to MGT9 to assign resistant STs (defined as containing over 80% of isolates resistant to a given drug). Resistant STs identified at different MGT levels were uniquely identified so that they contained mutually exclusive sets of isolates (i.e. for a given isolate, it was only included in one ST at any MGT level).

We used all isolates between 2015-2022 to identify resistant MGT STs for each drug and found a total of 161 MGT STs with resistance to one or more drug classes. Resistant STs were identified for 13 of the 14 drugs (**Table 2**, **Table S5**). There were no meropenem resistant MGT STs. These STs spanned all MGT levels, from MGT1 to MGT9. We additionally used a subset of data, ranging between 2021-2022 to identify the MGT STs which were resistant in recent years. This enabled the identification of MGT STs that were either present and resistance in 2021-2022, or present in 2015-2022, but only became resistant in 2021-2022. We identified 43 MGT STs using this set, spanning 11 of the 14 drugs – none were identified for cefotaxime (ESBL), azithromycin and meropenem resistance (**Table 2**, **Table S5**). Of these 43 MGT STs, five were new compared to the 2015-2022 dataset, namely MGT3 ST11 (newly resistant to tetracycline and chloramphenicol), MGT3 ST18 and MGT5 ST3341 (both newly resistant to sulfathiazole), and, MGT5 ST9 and MGT6 15776 (both newly resistant to ampicillin, and cefotaxime (AmpC)).

To identify when the 166 resistant MGT STs (161 identified using the 2015-2022 set, and 5 newly identified using the 2021-2022 set) emerged, and how their frequency changed over

time, we normalised the ST counts over time (referred to as profiles) such that each ST abundance was relative to the average yearly count for that ST. We then identified clusters of ST profiles that showed similar temporal trends (referred to as temporal patterns). We identified 20 predominant temporal patterns from the STs – which we ordered by time according to when the average abundance within a temporal pattern was highest (**Figure 3**). For example, Cluster 1 consisted of 12 STs with similar patterns of isolate counts over time – these STs contained 2-fold more isolates in 2015 relative to other years. In contrast, Cluster 2 comprised 10 STs, that were between 1- to 2-fold higher in 2015, and gradually reduced in frequency in later years. Of the 20 clusters, 9 clusters showed an increasing trend in later-years namely Clusters 3, 7, 8, 12, 14, 17, 18, 19, and 20 – with resistance to 11 of 14 drugs (which excluded the drugs cefotaxime (ESBL), azithromycin and meropenem). Finally, 16 of 20 clusters were associated with a clear geographical majority (≥50%). In particular, Clusters 1, 2, 3, 4, 7, 9, 10, 12, 13 and 19 comprised isolates predominantly from UK with resistance to 13 drugs (excluded meropenem), Clusters 8, 11, 14, and 20 comprised isolates predominantly from USA with resistance to 11 drugs (excluded cefotaxime (ESBL), azithromycin and meropenem), and Clusters 6 and 15 from Other countries with resistance to 10 drugs (excluded trimethoprim-sulphathiazole, cefotaxime (AmpC), azithromycin and meropenem). The remaining four clusters (Clusters 5, 16, 17, and 18), comprised resistance to 12 drugs (excluded azithromycin and meropenem) and were not dominated by one geographic category.

### Resistance in 2021-2022 dataset

We then focussed the analysis on the most recent data (2021-2022). This subset comprised 7,613 isolates, of which 45% were resistant to one or more drugs. We summarised the distribution of these isolates, as those that were uniquely within any resistant MGT STs (including 2015-2022 or 2021-2022 set), and those that were not (**Table 3**). Resistant isolates were grouped within 44 resistant MGT STs (comprising resistance to 11 of 14 drugs) – tetracycline had the highest proportion of resistant isolates described by these STs at 89%, while ciprofloxacin was the lowest at 24%. The 44 resistant MGT STs comprised 42 resistant MGT STs identified using the 2021-2022 dataset, and two additionally identified using the 2015-2022 dataset (namely, MGT5 ST8218 and MGT5 ST973).

To further elucidate the epidemiology of these 44 resistant MGT STs, we created a visual representation depicting the MGT hierarchy, the annotated source organism, the identified plasmids, and resistance to all drugs, as a hierarchical network (**Figures 4** and **5**). These 44 resistant MGT STs were distributed within four MGT1 STs (**Figures 4** and **5**), as follows, MGT1 ST19 comprised 29 resistant MGT STs ranging from MGT levels 2 to 6, MGT1 ST34 comprised 13 resistant MGT STs (from MGT1 to MGT6), and both MGT1 ST313 and MGT1 ST213 comprised one resistant MGT3 ST each. Of the 29 resistant MGT STs within MGT1 ST19, 17 comprised isolates from USA, eight from UK, two were global and two from South Africa. These resistant isolates were annotated as being isolated from a variety of sources, such as human, chicken, pig, cattle, sheep and turkey; and were resistant to one or more of the 10 drugs detected in these STs (excluding kanamycin, cefotaxime (ESBL), azithromycin and meropenem). Of the 13 resistant MGT STs within MGT1 ST34, 11 comprised isolates from UK, one from USA, and one was global. These STs were also annotated as being from a variety of sources, such as human, animal, pig, food, turkey and environment; and were resistant to one or more of the 10 drugs (excluding ciprofloxacin, cefotaxime (ESBL), azithromycin and meropenem). Finally, MGT3 ST3 within MGT1 ST313, comprised isolates predominantly from South Africa, which were annotated as being isolated from human, and was MDR; and MGT3 ST207 within MGT1 ST213 comprised isolates from USA and Mexico, which were sporadically annotated as being isolated from human and water sources, and was also MDR (including to ciprofloxacin).

### Resistance to prescribed drugs in 2021-2022 isolates

In the 2021-2022 isolates, resistance to azithromycin, cefotaxime and ciprofloxacin were low and mostly sporadic across different MGT STs as detailed below.

### Azithromycin

Only a small percentage (45 isolates (**Table 3**), 0.6% of total in 2021-2022) of isolates in the 2021-2022 isolates were resistant to azithromycin. Most were isolated in UK (27 of 45 (**Table 3**)), followed by USA and Other (9 each). By mechanisms, *mph(A)* was found globally, whereas *mef(B)* was only found in UK (**Figure S3**). The *mef(B)* isolates were within MGT1 ST34, whereas isolates with the other eight mechanisms were distributed within MGT1 ST34 (9 isolates), MGT1 ST19 (12 isolates), MGT1 ST36 (3 isolates) and MGT1 ST213 (2 isolates).

### Cefotaxime (ESBL mediated resistance)

In the 2021-2022 isolates, only 61 isolates (0.8% of total; **Table 3**) were resistant to third generation cephalosporins such as cefotaxime via the ESBL mechanism with nine ESBL genes identified (**Figure S4).** Within MGT1, resistant isolates were found in MGT1 ST19 (26 isolates), MGT1 ST34 (26 isolates), MGT1 ST 313 (5 isolates), MGT1 ST213 (2 isolates) and MGT1 ST36 (2 isolates).

### Cefotaxime (AmpC mediated resistance)

In the 2021-2022 isolates, 277 isolates (3.6% of total) were resistant to third generation cephalosporins via the AmpC-mediated mechanism (**Table 3**), 57% (159 isolates) of which were within nine cefotaxime (AmpC) resistant MGT STs (**Figures 4** and **5**). One of these (MGT3 ST1705) was within MGT1 ST34 and comprised isolates from UK (25 isolates), and was sparsely annotated as being from pigs; and the other eight were within MGT1 ST19, with isolates mostly from USA (107, and 4 from Other), with five MGT STs comprising isolates from chicken (MGT3 ST171, MGT5 ST7526, MGT5 ST952, MGT6 ST1515 and MGT5 ST9), two from cattle (MGT4 ST3060 and MGT6 ST15776) and one with no source metadata (MGT5 ST9858) . The isolates in all these MGT STs were resistant via the *blaCMY-2* gene. The remaining 43% (118 of 227) isolates were not within any resistant MGT STs and were predominantly from USA (112 isolates). At the MGT4 level, these isolates were distributed across 51 MGT4 STs.

### Ciprofloxacin

In the 2021-2022 isolates, 372 isolates (4.9% of total) were resistant to ciprofloxacin (**Table 3**), Only 24% (89) of these resistant isolates were within four ciprofloxacin resistant MGT STs (**Figures 4** and **5**). Three MGT STs (MGT2 ST59, MGT5 ST118, and MGT5 ST264) were within MGT1 ST19, and one (MGT3 ST207) was within MGT1 ST213. MGT2 ST59 comprised isolates mostly from UK (10 of 14, additionally 2 from USA, and 2 from India), which had the mutation D87Y in *gyrA*, and were isolated from humans. MGT5 ST118 and MGT5 ST264 comprised isolates only from UK (40 isolates), and had the mutation D87N in *gyrA*. Both of these MGT STs were only sporadically annotated as isolated from ‘human’ or ‘animal’. MGT3 ST207 comprised 12 isolates from USA, and two from Mexico. This MGT ST had a high diversity in the underlying resistance mechanism. The mutation S83Y in *gyrA*, was the most common mutation mechanism found in 11 isolates of which four had additional

genes (one also had both *oqxA* and *oqxB*, and three had *qnrB19*). Two isolates also had *qnrB19* by itself without the *gyrA* mutation.

The majority of the ciprofloxacin resistant isolates (76%, 283 isolates) were not within any resistant MGT STs and were found in all geographies: UK (122 isolates), USA (107 isolates) and other countries (54 isolates) (**Figure S5**). These resistant isolates comprised 32 different resistance mechanisms (**Figure S5**) and distributed across 88 MGT3 STs.

## Discussion

In this study, we analysed resistance to 14 drug classes (including clinically relevant classes) in 65,000 publicly available STm genomes. The public dataset included high-quality surveillance genome data from UK and USA. We integrated MGT for AMR surveillance, to identify trackable resistant STs (≥80% isolates of an ST resistant to a drug).

### Resistance varied by geography, time, and MGT1 STs

Overall, in the global dataset, nearly half of the isolates were resistant to one or more drugs. Resistance to four drugs (16%) was most frequently observed, followed by resistance to six drugs (6%) and 25% had resistance to other numbers of drugs (up to a maximum of 12 drugs). When the entire dataset was split into two timeframes, 2015-2018 and 2019-2022, and into three geographies UK, USA and Other. Resistance varied considerably across time, geography and MGT STs.

Although there is yearly variation in resistance between 2015 to 2022, resistance was generally higher in UK (overall average resistance score of 2.4; average resistance score amongst resistant isolates of 4) compared to USA (overall average resistance score of 1.5; average resistance score amongst resistant isolates of 3.4). This also holds true at the individual drug level. Upon comparing the resistance to each drug between UK and USA in the 2019-2022 set, we found USA had significantly higher resistance to cefotaxime (AmpC) and gentamicin, whereas UK had significantly higher resistance to multiple drugs, including azithromycin, ciprofloxacin, kanamycin, trimethoprim-sulphathiazole, and ampicillin.

When we considered change in resistance within the same country. Resistance in UK decreased significantly over time, while there was an increase in USA. Again, this was also observed at the individual drug level. In UK, resistance increased significantly only to

kanamycin and gentamicin, whereas in USA resistance increased for several drugs including ciprofloxacin, trimethoprim-sulphathiazole, cefotaxime (ESBL), and ampicillin. The significant decrease in resistance in UK could be attributed to various implemented antimicrobial stewardship programs [22, 23]. Although stewardship recommendations are also available in USA [24, 25], the significant increase in USA can be partly attributed to implementation of the national *Salmonella* surveillance program with unbiased and more comprehensive sampling. As surveillance was not part of the national framework in 2015-2018, we cannot ascertain if the higher resistance seen in 2019-2022 did not already exist in the previous time-frame and was simply not sampled.

We compared the average resistance in various MGT1 or MLST STs, and observed the following order of STs by decreasing resistance: ST313 > ST34 > other STs > ST19 > ST36. ST313 and ST34 are well known MDR STs [12], and the dataset here reflected previously known resistance differences in the various MLST STs. The higher resistance observed in the UK (compared to USA) could be attributed to higher presence of ST34 isolates; the decrease in isolation of ST34 in 2019-2022 compared to 2015-2018 could explain the observed decrease in overall resistance; and similarly, the increase in isolation of ST34 isolates in USA in 2019-2022, compared to 2015-2018, could partly explain the observed increase in resistance in USA. As surveillance was part of the national framework throughout the 2015-2018 timeframe in the UK, the observed decrease in UK likely represented a real reduction in resistance.

### MGT STs enable standardised identification and tracking of resistant STs

As MGT can separate a dataset into smaller STs, via higher resolution MGT-levels – we utilised this approach to identify higher-resolution STs comprising non-overlapping isolates with ≥80% of the isolates resistant to each drug class. MGT STs identified for each drug were then combined for analysis to identify mutually exclusive MGT STs with resistance to one or more drugs. This novel approach allowed precise identification and tracking of resistant strains. We had identified two sets of MGTs STs, one using all isolates in the 2015-2022 dataset, and other, using the 2021-2022 only subset. Using the 2015-2022 dataset, 161 MGT STs with resistance to 13 drugs were identified, while using the 2021-2022 dataset, 43 MGT STs with resistance to 11 drugs were identified. Of these 43 MGT STs identified using the 2021-2022 set, five were not considered resistant in earlier timeframe of 2015-2022. In particular MGT5 ST9 and MGT6 ST15776, were not resistant to third-generation

cephalosporins using the 2015-2022 dataset but were resistant in 2021-2022. These STs therefore either gained resistance or had an increasing number of resistant isolates expanding in the 2021-2022 period. Conversely, MGT STs with resistance to cefotaxime (ESBL) (namely MGT3 ST1583, MGT5 ST645, MGT5 ST5868 and MGT6 ST5443) and azithromycin (namely MGT4 ST4133) were identified using 2015-2022, but not using the 2021-2022 timeframe – indicating that these STs were not present or had possibly reduced resistance in recent years.

Temporal trend clustering of the 166 resistant MGT STs revealed 20 predominant temporal trends. Most temporal trends comprised UK isolates, followed by USA, and then Other. Multiple UK dominant trends also began earlier in time (e.g. Clusters 1 and 2), while most USA trends comprised MGT STs expanding and peaking later in time (e.g. Clusters 14 and 20). Other dominant trends had only yearly peaks (e.g. Cluster 6 and 15). These observed trends correlated with the underlying sampling of isolates from these three geographies: UK had continuous surveillance over this time period, USA from 2019 onwards, and only sporadic sampling of Other isolates.

Although the application of unsupervised clustering to multi-variate temporal data are common in other life-sciences domains (such as time series proteomic measurements [26]), to our knowledge its application to epidemiological data, by considering MGT STs as multivariate is novel. This approach provided a natural setting to understand the temporal trends of resistance within a dataset – and enabled comparison between different MGT STs with different abundances. As we were standardising and clustering the ‘number of isolates within an ST, the interpretation of an observed trend would depend on the underlying sampling – i.e. if the underlying sampling is unbiased and continuous the observed trend of an ST is a true representation of its epidemiology. As the data from UK comprises continuous surveillance data, the observed trends were likely to represent a true temporal-epidemiology of the resistant MGT STs.

### MGT enabled the identification of geographically restricted resistant STs

Within MGT1 ST19, we identified three ciprofloxacin resistant MGT STs, namely MGT2 ST59, MGT5 ST118 and MGT5 ST264. All three of these MGT STs comprised isolates annotated as predominantly from UK, however MGT2 ST59 comprised sporadic isolates from other countries. The two MGT5 STs were hierarchically within MGT3 ST11. In our

previous work we showed MGT3 ST11 was equivalent to DT104 [10], which is globally disseminated; and as expected, both MGT5 ST118 and MGT5 ST264 had the additional resistance pattern ACSSuT, commonly found in DT104. Thus, using MGT STs we can identify ciprofloxacin-resistant trackable types within DT104 – which are geographically restricted.

Both the MGT5 STs are recent STs, with MGT5 ST264 (temporal trend Cluster 18; peaking in 2021) emerging earlier than MGT5 ST118 (Cluster 19; peaking in 2022). MGT5 ST264 also had a higher diversity in resistance mechanisms compared to MGT5 ST118, suggesting that MGT5 ST118 may be a clone that expanded either from MGT5 ST264 or from the same ancestor. All three of these MGT STs had mutations in the *gyrA* gene. In particular, MGT2 ST59 contained the mutation D87Y, and both the MGT5 STs contained D87N. These mutations impart intermediate resistance (or elevate MIC) [27]. Isolates from all three STs were annotated as isolated from humans – with a few isolates in MGT5 ST118 also annotated with source as 'animal'. In UK, decreased susceptibility to ciprofloxacin via the same D87N mutation in *gyrA* has been observed in DT104 isolates from dairy cattle since at least 2000 [28]. Furthermore, certain *Salmonella* infections may persist for life within an animal [29], or may persist in herds via various farming practices [30], this suggests that the previously implicated dairy farms may be a potential source of currently observed infections. Using MGT STs these isolates can be further tracked and investigated.

### MGT STs can differentiate STs with resistance to the same drug that originated from different sources

We identified nine cefotaxime (AmpC) resistant MGT STs – all with the gene *blaCMY-2*. Of these nine, one (MGT3 ST1705) was within MGT1 ST34 and from UK, and eight (MGT3 ST171, MGT5 ST7526, MGT5 ST952, MGT5 ST9858, MGT6 ST1515, MGT5 ST9, MGT4 ST3060, MGT6 ST15776) within MGT1 ST19 and from USA. MGT3 ST1705, within ST34, along with cefotaxime (AmpC) comprised additional resistance to ASSuT and was sporadically annotated as being isolated from pigs. ASSuT is traditionally observed in ST34, and as expected was also observed in this ST.

Of the eight cefotaxime (AmpC) resistant MGT STs in USA within ST19, two were annotated as isolated from cattle (MGT4 ST3060 and MGT6 ST15776), four from poultry

(MGT5 ST7526, MGT5 ST952, MGT5 ST9858, MGT6 ST1515), and one was unannotated (MGT5 ST9858). At the MGT3 level, both MGT4 ST3060 and MGT6 ST15776 are hierarchically within MGT3 ST23, and the other five MGT STs are hierarchically within MGT3 ST8. MGT3 ST23 has been isolated predominantly from cattle and human, and MGT3 ST8 predominantly from poultry and human. All poultry-associated resistant MGT-STs were additionally resistant to tetracyclines and sulfathiazoles, and the cattle MGT STs were additionally resistant to tetracyclines, streptomycins, sulfathiazoles and chloramphenicols – further distinguishing STs from the two sources. Thus, MGT can enable identification of STs resistant to the same drug, but associated with different reservoirs.

Considering the two cattle cefotaxime (AmpC) resistant MGT STs, MGT4 ST3060 arose earlier initially in 2018 (Cluster 14), MGT6 ST15776 in 2019 (Cluster 20), both expanding in 2022. Previous studies have noted the use of third generation cephalosporins in dairy cattle (e.g. in mastitis) and found cattle and associated products such as milk and beef contain third-generation cephalosporin resistant *Salmonella* [31]. Furthermore resistance to the same antibiotic-classes seen in the two MGT STs here (tetracyclines, streptomycins, sulfathiazoles, chloramphenicols, and cefotaxime (AmpC)) have been reported in recent isolates from cattle in USA [32]. As isolates in MGT3 ST8 also have been sporadically annotated from humans – cattle may potentially be an important source of cefotaxime resistant STm infections in humans.

### MGT enables the identification of closely related STs with resistance to distinct drugs

As mentioned in the previous section, MGT3 ST8 comprises poultry isolates. Within this MGT3 ST, five MGT STs (namely, MGT5 ST7526, MGT5 ST952, MGT5 ST9858, MGT6 ST1515, and MGT5 ST9) were resistant to cefotaxime (AmpC) and two MGT STs (MGT5 ST652 and MGT5 ST973) were resistant to gentamicin. Interestingly, these five cefotaxime (AmpC) resistant MGT STs were mutually exclusive to the gentamicin resistant MGT STs. Thus, an MGT ST at a lower resolution level, can be split into higher resolution STs that can provide a clear separation of different resistance profiles.

In USA, both third generation cephalosporins and gentamicin may be prescribed to poultry under certain conditions [33]. Resistance mediated via AmpC in USA chickens has been observed from a very early time [34]. These AmpC based resistant isolates occasionally cause outbreaks, for example a multistate STm outbreak identified by the US CDC linked to

chicken salad, with isolates showing multidrug resistance, including to third generation cephalosporins and gentamicin [35]. In our work here, since we identified multiple STs carrying resistance to one drug or the other within MGT3 ST8, and, the MGT STs with resistance to different drugs were seen to expand at the same time: the gentamicin resistant STs, MGT5 ST973 (Cluster 17) and MGT4 ST652 (Cluster 14), peaked in 2021 and 2022, respectively, and the cefotaxime (AmpC) resistant STs MGT5 ST7526, MGT5 ST9858, MGT6 ST1515 (all three clustered in Cluster 19), and MGT5 ST952 (Cluster 20), also peaked in 2022. The cefotaxime (AmpC) resistant ST MGT5 ST9 (Cluster 5) peaks earlier in 2017, however, was only identified as a highly resistant ST in the 2021-2022 dataset. This suggests that different drugs were potentially used in different regions leading to independent regional singular drug selection pressure at the same time.

## Conclusion

In this study, we paint a comprehensive picture of AMR in the publicly available global STm dataset. In particular, we demonstrated the utility of integrating genomic typing to identify and track highly resistant strains. We demonstrated the utility of MGT in identifying resistant STs that expanded regionally, STs that were source specific, STs that enabled distinction between different resistant classes within the same source. Thus, MGT offers an ideal platform to track AMR at strain level with high precision. These systems can provide opportunities for early detection, data-driven decision making, targeted interventions, and measurements of effectiveness of interventions. We believe the approach presented here can be effectively applied to standardised global comprehensive surveillance of AMR within STm and should be applicable to other organisms.

## Methods

### Genome assembly and assignment of MGT STs

The MGTdb server regularly downloads newly available sequencing reads from NCBI SRA [36], and assembles them using an inhouse pipeline which uses SKESA [10, 20, 37]. All publicly available isolates until 2023, August 31 within MGTdb that were assigned an ST at atleast one MGT level were used for further analysis.

## AMR and plasmid annotation

AbritAMR [21] (version 1.0.14) was run using the default options on each assembly to identify AMR determinants grouped into drug classes. We ran the 'report' option to obtain a phenotypic interpretation of resistance based on the genotype. For *Salmonella*, AbritAMR provided a phenotypic interpretation of resistance to 14 classes (out of 16). An interpretation was not provided for colistin, and aminoglycoside resistance medicated via rRNA methyltransferases, hence we excluded these two classes from our analysis.

PlasmidFinder [38] (downloaded on 2023, June 4) using it's default database was run on each isolate to identify the presence of plasmids via their replicon sequences.

A file was generated which contained for each isolate it's MGT STs, AbritAMR outputs, and PlasmidFinder outputs, along with any available metadata annotations.

We performed AbritAMR validation using isolates from NCBI Pathogen Detection [39] on 14 December, 2022 for which both phenotypic and whole genome sequences were available for STm. We compared the AbritAMR resistance predictions with the corresponding antimicrobial susceptibility testing (AST) results as listed in NCBI Pathogen Detection. The accuracy was 98.6% which was very close to the originally reported accuracy. We considered resistance as binary only, and grouped all non-susceptible resistance (reported either by AST or by AbritAMR) as 'resistant' for simplicity.

## Resistance score

For each drug, we converted susceptibility to 0 and non-susceptibility to 1. A resistance score was then defined by as the sum of the Boolean values across the 14 drug classes.
We also used the resistance score to analyse the frequency of non-susceptibility to commonly prescribed drugs for invasive salmonellosis in 2021-2022. We found only two isolates resistant to both cefotaxime (AmpC) and cefotaxime (ESBL) – hence we combined the two classes (that were differentiated on the bases of mechanism for this drug), i.e. resistance to at least one was reported as resistance to cefotaxime.

We performed a bootstrap (9999 times) of the average resistance score at each year between 2015-2022 for UK, USA and Other subsets and calculated a 95% confidence interval of the resistance score.

To compare if the distribution of resistance scores was larger in one subset over another, we used the Mann-Whitney U test (with continuity set to False, and significant difference was defined at $p<0.05$). In particular, we compared the distributions in the complete datasets, with UK, USA and Other, in 2015-2018 and 2019-2022. We also compared the distributions in the same geography over the two timeframes, and within the three geographies in each of the two timeframes, and similarly for within different STs.

All three statistical tests were performed using the scipy.stats package (version 1.10.1) in python3 (version 3.9.6).

### Comparison of resistance within individual drugs

The Binomial test, in the scipy package (version 1.10.1) in python3 (version 3.9.6) was used to compare if in two given subsets, the Binomial distributions representing resistance to a drug were significantly different ($p<0.05$).

### Identification of highly resistant MGT STs

To identify MGT STs that were highly resistant to a drug, for any given set of isolates typed with MGT STs, we started at the lowest level and considered STs that were ≥ 80% resistant (and had a minimal count of 10). We designated these STs at highly resistant, and removed the isolates assigned this ST. The isolates were removed to ensure that an isolate is counted just once, i.e. the resulting set of resistant STs comprises a non-overlapping set of isolates. The process was then repeated at the next MGT level. This analysis was performed in python3 (version 3.9.6).

We identified highly resistant MGT STs, within three sets of data: the complete dataset, the 2015-2022 dataset, and the 2021-2022 dataset.

### Identification of temporal clusters

We combined the MGT STs identified for each drug, and both the 2015-2022 and 2021-2022 set, to identify predominant temporal patterns in the dataset. We first ensured an isolate was uniquely represented in the set of MGT STs for each drug, and then picked a profile (the

number of isolates over time) with the largest count. Each profile was standardized (to enable comparison between the temporal patterns), via the usual method, as follows:

$$(x_i - m)/s,$$

where $x_i$ are the number of isolates in an MGT ST at one timepoint, $m$ is the mean count between 2015-2022, and $s$ is the standard deviation.

We then clustered the profiles into 20 clusters. We had used silhouette index to identify the optimal number of clusters – as eight; however, we further separated the clusters, and found 20 was optimal to reveal epidemiologically useful patterns.

These analyses were performed in R (version 4.3.1), we used the package bios2mds [40] (version 1.2.3) to calculate the silhouette index, and the package Mfuzz [41] (version 2.60.0) to perform the temporal clustering. We used a custom function to plot the resulting clusters – which were manually ordered by visually inspecting when the standardized score of the cluster was highest.

### MGT hierarchy

For each MGT STs (that was level 2 or above), we displayed the lower-level MGT ST, if atleast 50% isolates were assigned the same ST at the lower MGT level, otherwise we designated an X. We displayed a plasmid if ≥50% isolates within the ST comprised it.

A heatmap of resistance to all drugs was generated and displayed in the order “tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem”. If an ST was a resistant ST (≥80%) to a drug, that drug in the heatmap was marked with a black outline. This enabled the visualisation of new resistance acquired by this ST compared to the lower-level MGT STs. MGT2 ST2 was common between all identified STs, however, to give an indication of resistance at the MGT2 level within that given MGT1 ST, we displayed MGT2 ST2 separately within each MGT1 ST.

We displayed the top five source of the isolates within an ST. However, the source was manually simplified to represent the closest associated animal. E.g. “Human, stool” was simplified to “human”, “ground turkey” to “turkey. When the isolates were only sparsely annotated with a source, i.e. <10 isolates were available with a given source, we indicated this with a “*”.

We used python (version 3.9.6) to generate the hierarchy in dot format, which was converted to scalable vector graphic format using graphviz’s (version 8.0.5) hierarchical layout engine [42].

## World map

We simplified the “country” annotations of the isolates, e.g. “United States of America”, “United States, California” and “United States”, were all harmonized to “USA”. We then used the python3 (version 3.9.6) package, geopy (version 2.3.0), to identify the latitude and longitude for each country, which was then plotted on a world map using the packages ggplot2 [43] (version 3.4.4) and scatterpie (0.2.1) in R (version 4.3.1).

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## Figure legends

**Figure 1**. Resistance to 14 drugs within the complete dataset, and various subsets. The drugs are arranged from left to right, in descending order of resistance in the complete dataset. Significantly higher resistance in 2019-2022, compared to 2015-2018, in UK and USA, is indicated via ↑, and significantly lower resistance is indicated via ↓. Significantly higher resistance between UK and USA in 2019-2022 is indicated via a pink outline. Resistance to a drug between two groups was tested using the Binomial test. Significance was assessed at $p < 0.05$.

**Figure 2**. Resistance to each drug and aggregate resistance score for MGT1 STs. The MGT1 STs are ordered in descending order of number of genomes – and only STs with a minimum

count of 20 isolates are shown. `0' indicates a new ST, empty indicates that an ST was not assigned (for example due to missing data).

**Figure 3**. 166 MGT STs grouped into 20 normalised temporal trend clusters. The number of isolates in each MGT ST, was normalised then clustered into 20 clusters using unsupervised c-mean clustering. Each of the clusters has a unique temporal pattern - revealed by the bold-black line - representing a weighted-average of all temporal profiles within the cluster. The clusters have been ordered in time according to the normalised score. Each MGT ST within a cluster is indicated by its MGT-level and the ST number, and has been uniquely coloured. The cluster header indicates the number of MGT STs within the cluster, the total number of isolates in the MGT STs within the cluster, as well as, the geographical distribution of the isolates. This is followed by counts in the order: "tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem", representing the number of MGT STs identified as resistant to the drug at that position.

**Figure 4**. MGT hierarchy of resistant MGT STs within MGT1 ST19. The MGT STs indicated by white or coloured headers are resistant to at least one drug (i.e. ≥80% of the isolates in that ST were resistant), and the MGT STs indicated in grey show the MGT hierarchy but were not identified as resistant. For a given MGT ST, if a single MGT ST could not be identified at the lower resolution level, i.e. ≥50% isolates did not have a single ST at the lower resolution MGT level, then the lower-level MGT ST was designated as ‘X’. The heatmap indicated resistance to 14 drugs in the order “tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem”. MGT STs with 80% or more of the isolates resistant to ciprofloxacin are highlighted in orange, to cefotaxime (AmpC) in yellow, and, to gentamicin in pink. The source of the isolates (manually simplified to indicate the closest organism) is indicated. When <10 isolates are annotated with a source, then an ‘*’ was placed next to the source.

**Figure 5**. MGT hierarchy of resistant MGT STs within MGT1 ST34, MGT1 ST213 and MGT1 ST313. The MGT STs indicated by white or coloured headers are resistant to at least one drug (i.e. ≥80% of the isolates in that ST were resistant), and the MGT STs indicated in

grey show the MGT hierarchy but were not identified as resistant. For a given MGT ST, if a single MGT ST could not be identified at the lower resolution level, i.e. ≥50% isolates did not have a single ST at the lower resolution MGT level, then the lower-level MGT ST was designated as 'X'. The heatmap indicated resistance to 14 drugs in the order "tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem". MGT STs with 80% or more of the isolates resistant to ciprofloxacin are highlighted in orange, to cefotaxime (AmpC) in yellow, to kanamycin in blue, and, both kanamycin and gentamicin in purple. The source of the isolates (manually simplified to indicate the closest organism) is indicated. When <10 isolates are annotated with a source, then an '*' was placed next to the source.

## Tables

Table 1. Number of isolates from top 10 countries in descending order.

| Country | With country | | With country and year | | 2015-2018 | | 2019-2022 | |
|---|---|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % | Count | % |
| USA | 15643 | 37.9 | 13519 | 35.1 | 4791 | 32.5 | 5216 | 42.2 |
| United Kingdom | 15117 | 36.6 | 14733 | 38.3 | 7127 | 48.4 | 5066 | 41 |
| Canada | 1737 | 4.2 | 1692 | 4.4 | 840 | 5.7 | 130 | 1.1 |
| Australia | 1557 | 3.8 | 1557 | 4 | 494 | 3.4 | 121 | 1 |
| Denmark | 1148 | 2.8 | 1141 | 3 | 31 | 0.2 | 4 | 0 |
| South Africa | 1049 | 2.5 | 1049 | 2.7 | 32 | 0.2 | 992 | 8 |
| Germany | 959 | 2.3 | 959 | 2.5 | 544 | 3.7 | 96 | 0.8 |
| Taiwan | 483 | 1.2 | 483 | 1.3 | 115 | 0.8 | 1 | 0 |
| Italy | 332 | 0.8 | 332 | 0.9 | 88 | 0.6 | 3 | 0 |
| Japan | 319 | 0.8 | 319 | 0.8 | 136 | 0.9 | 0 | 0 |
| **Total displayed** | 38344 | 92.9 | 35784 | 93 | 14198 | 96.4 | 11629 | 94.1 |
| **Total overall** | 41285 | | 38482 | | 14738 | | 12361 | |
| **%** | 62.7 | | 58.4 | | 22.4 | | 18.8 | |

Table 2. Number of resistant MGT STs for each drug identified using the 2015- 2022 and the 2021-2022 dataset.

| Drug | 2015-2022 | 2021-2022 | New in 2021-2022 |
|---|---|---|---|
| Tetracycline | 85 | 17 | 2 |
| Streptomycin | 59 | 16 | 1 |
| Ampicillin | 51 | 16 | 3 |
| Sulfathiazole | 98 | 24 | 3 |
| Chloramphenicol | 58 | 9 | 1 |
| Trimethoprim | 24 | 4 | 0 |
| Trim-Sulpha | 20 | 4 | 0 |
| Ciprofloxacin | 21 | 4 | 0 |
| Kanamycin | 13 | 2 | 0 |
| Cefotaxime (AmpC) | 18 | 9 | 2 |
| Gentamicin | 11 | 3 | 0 |
| Cefotaxime (ESBL) | 4 | 0 | 0 |
| Azithromycin | 1 | 0 | 0 |
| Meropenem | 0 | 0 | 0 |
| **Total** | 161 | 43 | 5 |

Table 3: Distribution of 2021-2022 isolates resistant to each drug class.

| Drug | Total R | In 80% R MGT STs | | | | Not in 80% R MGT STs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | UK | USA | Other | Shannon entropy[a] | UK | USA | Other | % of total R | Shannon entropy[b] |
| Tetracycline | 2995 | 1335 | 1382 | 242 | 0.7 | 105 | 152 | 76 | 11.1 | 1.3 |
| Streptomycin | 2327 | 1371 | 624 | 253 | 0.8 | 110 | 217 | 64 | 16.8 | 2 |
| Ampicillin | 2107 | 1279 | 617 | 149 | 0.5 | 114 | 163 | 53 | 15.7 | 1.2 |
| Sulfathiazole | 2609 | 995 | 1178 | 210 | 0.6 | 210 | 196 | 80 | 18.6 | 1.2 |
| Chloramphenicol | 886 | 436 | 238 | 50 | 0.6 | 119 | 86 | 48 | 28.6 | 1.8 |
| Trimethoprim | 475 | 191 | 9 | 25 | 0.4 | 129 | 73 | 63 | 55.8 | 1.5 |
| Trim-Sulpha | 436 | 191 | 9 | 25 | 0.5 | 112 | 63 | 56 | 53 | 2.6 |
| Ciprofloxacin | 372 | 69 | 17 | 4 | 0.7 | 122 | 107 | 54 | 76.1 | 1.9 |
| Kanamycin | 343 | 125 | 8 | 9 | 0 | 83 | 109 | 23 | 62.7 | 0.5 |
| Cefotaxime (AmpC) | 277 | 25 | 137 | 8 | 0 | 2 | 112 | 4 | 42.6 | 0.3 |
| Gentamicin | 395 | 109 | 93 | 2 | 0 | 20 | 178 | 12 | 53.2 | 0.7 |
| Cefotaxime (ESBL) | 61 | 1 | 0 | 0 | 0 | 21 | 27 | 12 | 98.4 | 1.3 |
| Azithromycin | 45 | 0 | 0 | 0 | 0 | 27 | 9 | 9 | 100 | 1.2 |
| Meropenem | 7 | 0 | 0 | 0 | 0 | 5 | 0 | 2 | 100 | 0 |

[a]Shannon entropy was calculated as the average entropy of resistance mechanisms in each MGT ST.
[b]Shannon entropy was calculated as the average entropy of resistance mechanisms in UK, USA and Other.

## Supplementary

### Supplementary tables

Supplementary tables are provided in the file supplementary_tables.pdf.

### Supplementary figures

Supplementary figures are provided in the file supplementary_figures.pdf.

### Supplementary code

**Supplementary Code –** Scripts to enable all the analysis presented in this paper will be available at https://github.com/Lanlab/AMR_analysis.

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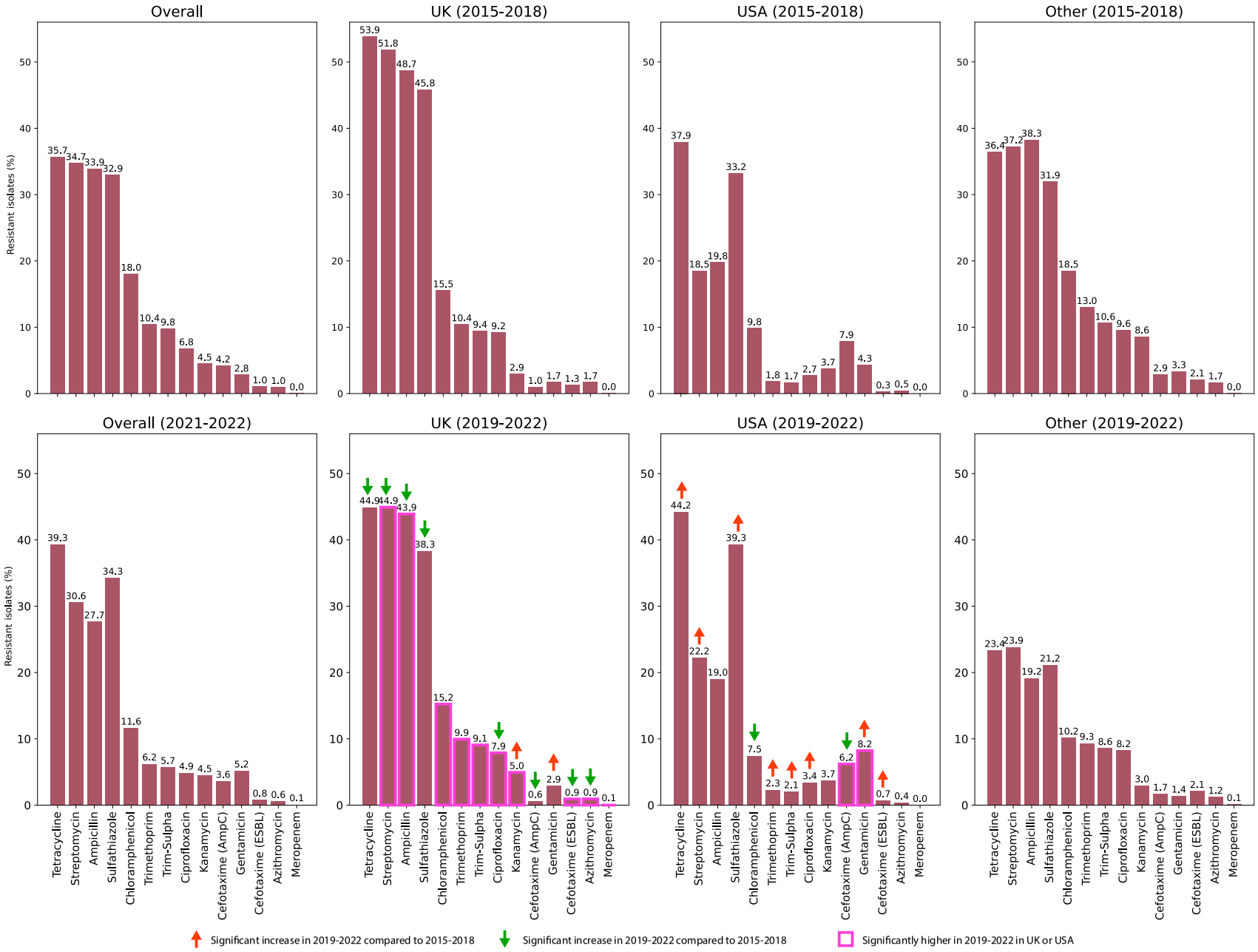


Figure 1: Resistance to 14 drugs within the complete dataset, and various subsets. The drugs are arranged from left to right, in descending order of resistance in the complete dataset. Significantly higher resistance in 2019-2022, compared to 2015-2018, in UK and USA, is indicated via ↑, and significantly lower resistance is indicated via ↓. Significantly higher resistance between UK and USA in 2019-2022 is indicated via a pink outline. Resistance to a drug between two groups was tested using the Binomial test. Significance was assessed at $p < 0.05$.

| MGT1 | Resistance to each drug (%) | | | | | | | | | | | | | | No. of genomes | Resistance score (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tetracycline | Streptomycin | Ampicillin | Sulfathiazole | Chloramphenicol | Trimethoprim | Trim-Sulpha | Ciprofloxacin | Kanamycin | Cefotaxime (AmpC) | Gentamicin | Cefotaxime (ESBL) | Azithromycin | Meropenem | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 19 | 30 | 24 | 23 | 23 | 16 | 5 | 5 | 4 | 4 | 5 | 3 | 1 | 1 | 0 | 46667 | 64 | 4 | 7 | 3 | 11 | 4 | 3 | 2 | 1 | 0 | 0 | 0 |
| 34 | 87 | 83 | 83 | 80 | 12 | 13 | 12 | 7 | 7 | 1 | 4 | 2 | 2 | 0 | 9122 | 4 | 10 | 3 | 8 | 55 | 7 | 4 | 3 | 3 | 3 | 0 | 0 |
| 313 | 9 | 78 | 79 | 77 | 70 | 77 | 76 | 7 | 1 | 0 | 4 | 5 | 2 | 0 | 3147 | 19 | 2 | 1 | 1 | 1 | 6 | 58 | 6 | 1 | 2 | 3 | 0 |
| 36 | 18 | 15 | 14 | 17 | 9 | 10 | 10 | 19 | 8 | 4 | 2 | 1 | 3 | 0 | 2053 | 73 | 6 | 2 | 3 | 4 | 2 | 1 | 2 | 3 | 2 | 1 | 1 |
| 0 | 21 | 22 | 21 | 21 | 11 | 9 | 8 | 25 | 2 | 2 | 1 | 0 | 1 | 0 | 699 | 53 | 21 | 2 | 4 | 10 | 3 | 3 | 1 | 2 | 0 | 0 | 0 |
| 102 | 11 | 26 | 35 | 37 | 26 | 28 | 28 | 99 | 0 | 0 | 0 | 0 | 6 | 0 | 472 | 1 | 60 | 3 | 0 | 9 | 2 | 1 | 21 | 4 | 0 | 0 | 0 |
| 99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 466 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 213 | 66 | 69 | 53 | 68 | 54 | 38 | 37 | 41 | 14 | 33 | 36 | 1 | 1 | 0 | 444 | 27 | 2 | 1 | 2 | 3 | 11 | 12 | 8 | 10 | 12 | 12 | 0 |
| | 68 | 74 | 67 | 35 | 53 | 10 | 9 | 13 | 7 | 2 | 0 | 0 | 2 | 0 | 441 | 17 | 4 | 8 | 7 | 41 | 14 | 3 | 2 | 2 | 1 | 0 | 0 |
| 2072 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 402 | 98 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 302 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 304 | 98 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 568 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 188 | 99 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2076 | 3 | 3 | 6 | 3 | 2 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 158 | 92 | 1 | 3 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 128 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 135 | 99 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 323 | 2 | 6 | 4 | 5 | 1 | 3 | 3 | 2 | 0 | 0 | 2 | 2 | 1 | 0 | 127 | 91 | 3 | 1 | 1 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 0 |
| 328 | 67 | 58 | 78 | 60 | 72 | 50 | 46 | 87 | 57 | 4 | 0 | 23 | 0 | 0 | 101 | 3 | 6 | 1 | 16 | 12 | 6 | 3 | 9 | 17 | 22 | 6 | 0 |
| 3631 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 96 | 97 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 44 | 53 | 53 | 57 | 57 | 57 | 57 | 85 | 0 | 0 | 0 | 1 | 0 | 0 | 81 | 4 | 40 | 0 | 0 | 0 | 0 | 11 | 7 | 38 | 0 | 0 | 0 |
| 2210 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 72 | 99 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2089 | 13 | 13 | 13 | 4 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 87 | 0 | 0 | 0 | 9 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2066 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2379 | 91 | 89 | 83 | 89 | 9 | 9 | 9 | 9 | 6 | 0 | 6 | 3 | 0 | 0 | 35 | 3 | 6 | 6 | 0 | 77 | 0 | 0 | 0 | 3 | 6 | 0 | 0 |
| 2 | 36 | 6 | 39 | 55 | 46 | 55 | 55 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 42 | 3 | 0 | 0 | 15 | 6 | 30 | 0 | 3 | 0 | 0 | 0 |
| 3438 | 60 | 30 | 10 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 70 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 513 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 97 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 11 | 11 | 25 | 11 | 21 | 4 | 4 | 32 | 18 | 0 | 0 | 21 | 0 | 0 | 28 | 64 | 11 | 0 | 4 | 0 | 11 | 0 | 4 | 7 | 0 | 0 | 0 |
| 2297 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 93 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 376 | 52 | 62 | 52 | 62 | 57 | 19 | 19 | 24 | 33 | 0 | 43 | 0 | 0 | 0 | 21 | 38 | 0 | 0 | 0 | 10 | 0 | 24 | 10 | 5 | 5 | 10 | 0 |
| 1544 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 60 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Figure 2: Resistance to each drug and aggregate resistance score for MGT1 STs. The MGT1 STs are ordered in descending order of number of genomes – and only STs with a minimum count of 20 isolates are shown. '0' indicates a new ST, empty indicates that an ST was not assigned (for example due to missing data).

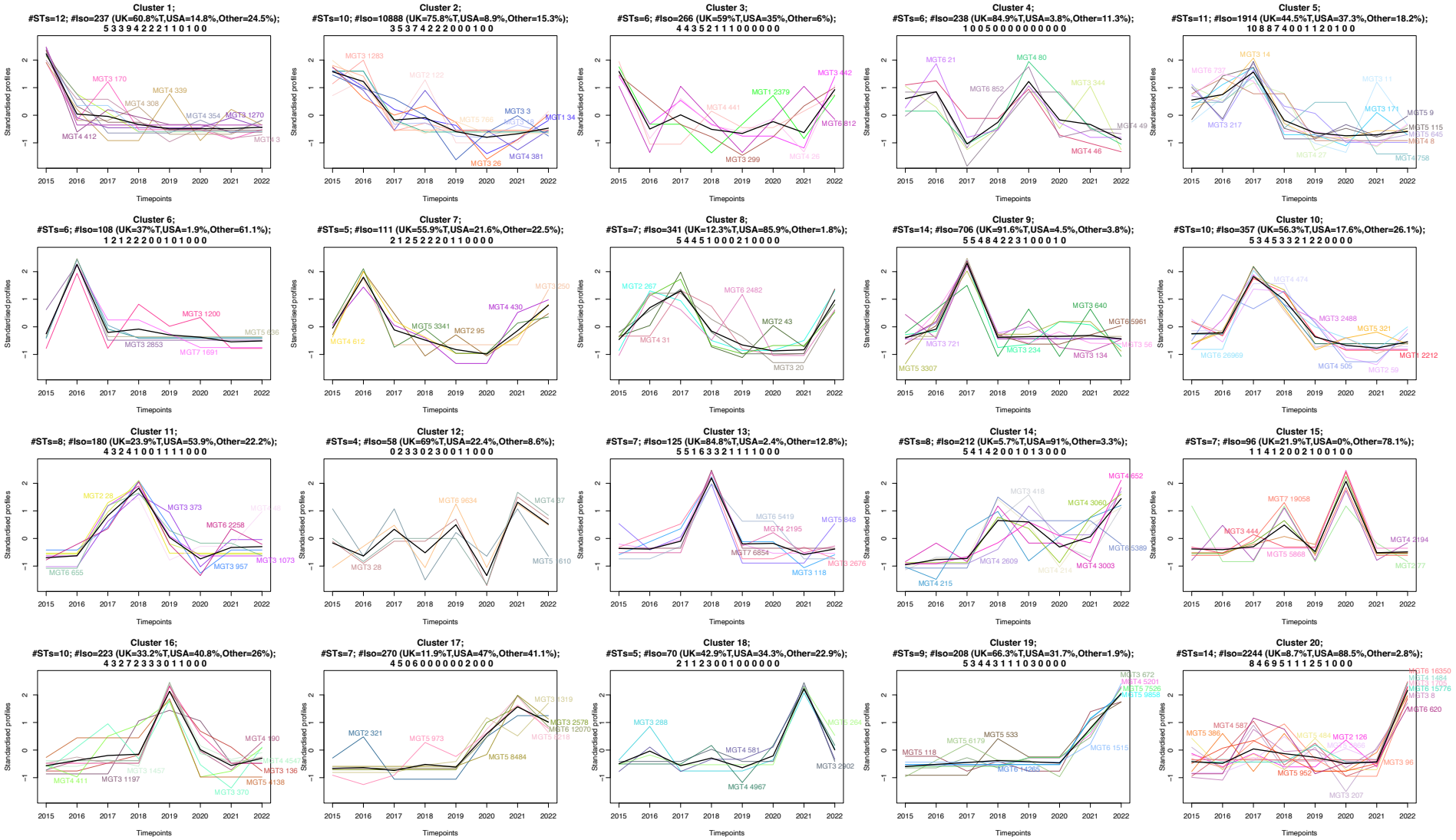


Figure 3: 166 MGT STs grouped into 20 normalised temporal trend clusters. The number of isolates in each MGT ST, was normalised then clustered into 20 clusters using unsupervised c-mean clustering. Each of the clusters has a unique temporal pattern - revealed by the bold-black line - representing a weighted-average of all temporal profiles within the cluster. The clusters have been ordered in time according to the normalised score. Each MGT ST within a cluster is indicated by its MGT-level and the ST number, and has been uniquely coloured. The cluster header indicates the number of MGT STs within the cluster, the total number of isolates in the MGT STs within the cluster, as well as, the geographical distribution of the isolates. This is followed by counts in the order: "tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem", representing the number of MGT STs identified as resistant to the drug at that position.

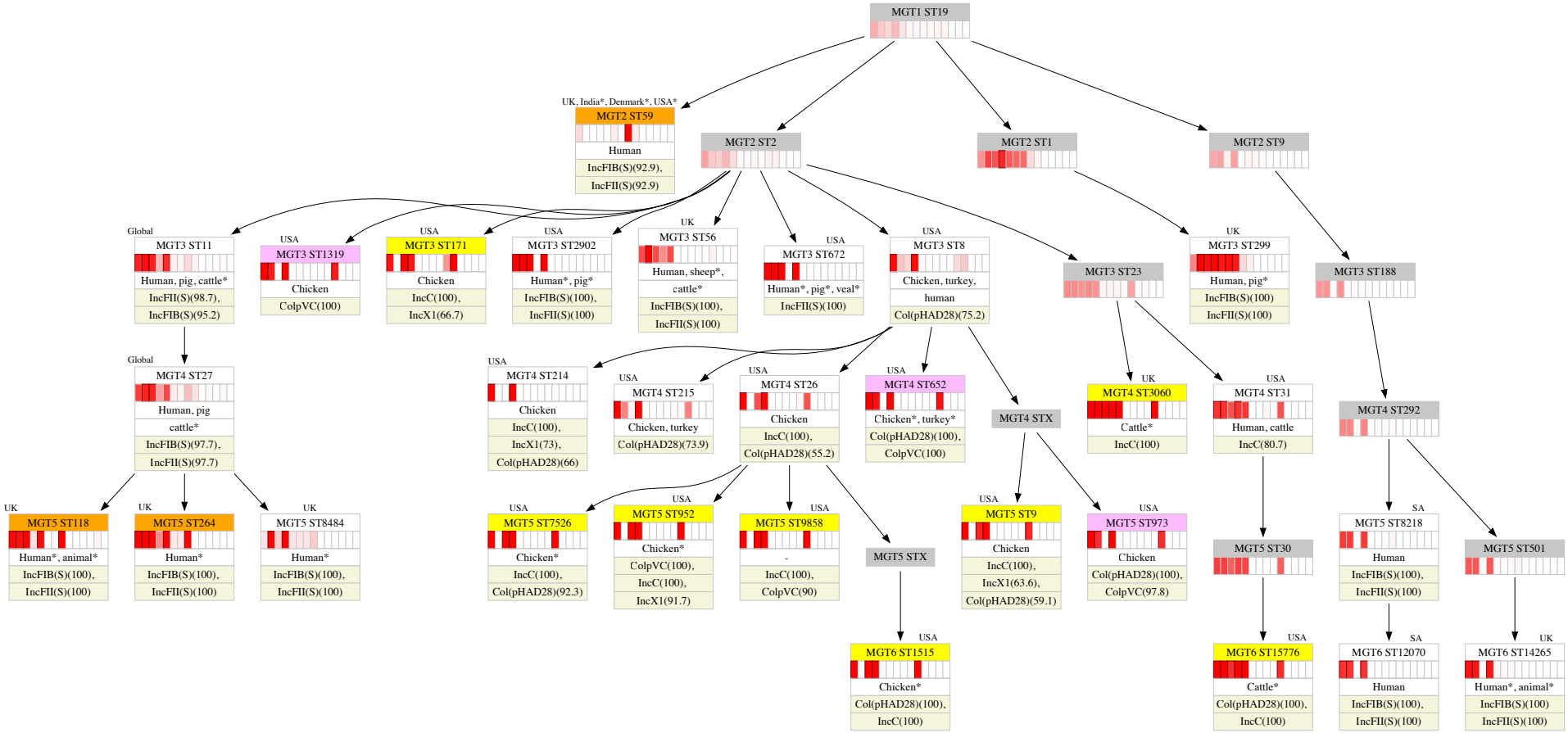


Figure 4: MGT hierarchy of resistant MGT STs within MGT1 ST19. The MGT STs indicated by white or coloured headers are resistant to at least one drug (ie. ≥80% of the isolates in that ST were resistant), and the MGT STs indicated in grey represent the MGT hierarchy, but were not identified as resistant to a drug. For a given MGT ST, if a single MGT ST could not be identified at the lower level, i.e. ≥50% isolates did not have a single ST at the lower MGT level, then the lower-level MGT ST was designated as 'X'. The heatmap indicated resistance to 14 drugs in the order "tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem". MGT STs with 80% or more of the isolates resistant to ciprofloxacin is shown in orange, to cefotaxime (AmpC) in yellow, and, to gentamicin in pink. The source of the isolates (manually simplified to indicate the closed organism) is indicated. When <10 isolates are annotated, then an '*' was placed next to the source.

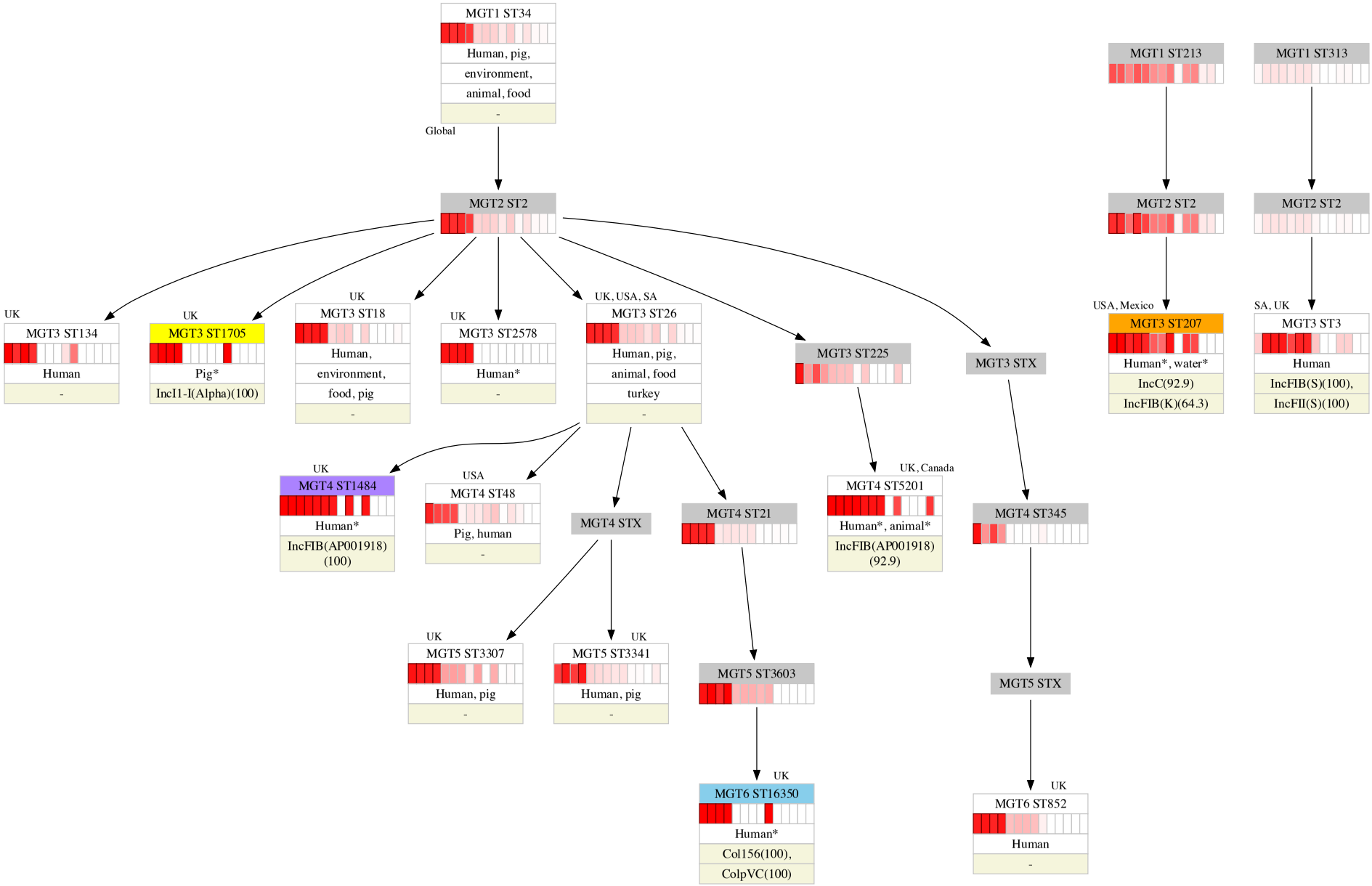


Figure 5: MGT hierarchy of resistant MGT STs within MGT1 ST34, MGT1 ST213 and MGT1 ST313. The MGT STs indicated by white or coloured headers are resistant to at least one drug (ie. ≥80% of the isolates in that ST were resistant), and the MGT STs indicated in grey represent the MGT hierarchy, but were not identified as resistant to a drug. For a given MGT ST, if a single MGT ST could not be identified at the lower level, i.e. ≥50% isolates did not have a single ST at the lower MGT level, then the lower-level MGT ST was designated as 'X'. The heatmap indicated resistance to 14 drugs in the order "tetracycline, streptomycin, ampicillin, sulphathiazole, chloramphenicol, trimethoprim, trimethoprim-sulphathiazole, ciprofloxacin, kanamycin, cefotaxime (AmpC), gentamicin, cefotaxime (ESBL), azithromycin, meropenem". MGT STs with 80% or more of the isolates resistant to ciprofloxacin is shown in orange, to cefotaxime (AmpC) in yellow, to kanamycin in blue, and, both kanamycin and gentamicin in purple. The source of the isolates (manually simplified to indicate the closed organism) is indicated. When <10 isolates are annotated, then an '*' was placed next to the source.