

# Third generation cephalosporin resistance in clinical non-typhoidal *Salmonella enterica* in Germany and emergence of *bla*<sub>CTX-M</sub>-harbouring pESI plasmids

Michael Pietsch<sup>1</sup>, Sandra Simon<sup>1</sup>, Anika Meinen<sup>2</sup>, Eva Trost<sup>1</sup>, Sangeeta Banerji<sup>1</sup>, Yvonne Pfeifer<sup>3</sup> and Antje Flieger<sup>1,\*</sup>

## Abstract

Non-typhoidal *Salmonella enterica* is an important gastrointestinal pathogen causing a considerable burden of disease. Resistance to third generation cephalosporins poses a serious threat for treatment of severe infections. In this study occurrence, phylogenetic relationship, and mechanisms of third generation cephalosporin resistance were investigated for clinical non-typhoidal *S. enterica* isolates in Germany. From 2017 to 2019, we detected 168 unique clinical *S. enterica* isolates with phenotypic resistance to third generation cephalosporins in a nation-wide surveillance. Compared to previous years, we observed a significant ( $P=0.0002$ ) and consistent increase in resistant isolates from 0.41% in 2005 to 1.71% in 2019. In total, 34 different serovars were identified, most often *S. Infantis* ( $n=41$ ; 24.4%), *S. Typhimurium* ( $n=27$ ; 16.1%), *S. Kentucky* ( $n=21$ ; 12.5%), and *S. Derby* ( $n=17$ ; 10.1%). Whole genome analyses revealed extended-spectrum  $\beta$ -lactamase (ESBL) genes as main cause for third generation cephalosporin resistance, and most prevalent were *bla*<sub>CTX-M-1</sub> ( $n=55$ ), *bla*<sub>CTX-M-14</sub> ( $n=25$ ), and *bla*<sub>CTX-M-65</sub> ( $n=23$ ). There was no strict correlation between serovar, phylogenetic lineage, and ESBL type but some serovar/ESBL gene combinations were detected frequently, such as *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-65</sub> in *S. Infantis* or *bla*<sub>CTX-M-14b</sub> in *S. Kentucky*. The ESBL genes were mainly located on plasmids, including IncI, IncA/C variants, emerging pESI variants, and a novel *bla*<sub>CTX-M-1</sub> harbouring plasmid. We conclude that third generation cephalosporin resistance is on the rise among clinical *S. enterica* isolates in Germany, and occurrence in various *S. enterica* serovars is most probably due to multiple acquisition events of plasmids.

## DATA SUMMARY

The authors confirm that all supporting data, code and protocols have been provided within the article. Illumina raw sequence reads were submitted to the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena/>) and can be accessed under the bioproject PRJEB41165.

## INTRODUCTION

Non-typhoidal *Salmonella* (*S.*) *enterica* (NTS) is an important agent of foodborne infections [1]. In Europe and Germany, salmonellosis, after campylobacteriosis, represents the second most reported gastrointestinal infection caused by bacteria,

encompassing 87 923 cases and 13 693 cases, respectively, in 2019 [2, 3]. *S. Typhimurium* and *S. Enteritidis* are the predominant serovars causing human infections in Germany and account for ~60% of all reported cases (<https://survstat.rki.de/>). Further prevalent serovars like *S. Infantis* and *S. Derby* represented <5% of the reported cases [3]. Although in most cases salmonellosis is self-limiting, antibiotic treatment may be required for risk group patients and more severe disease, like bloodstream infections [4]. Antibiotics for treatment of salmonellosis comprise  $\beta$ -lactams, fluoroquinolones, macrolides, and trimethoprim-sulfamethoxazole [4]. Challengingly, rising numbers of multidrug-resistant (MDR - resistant to three or more antimicrobials) *S. enterica* have

Received 09 June 2021; Accepted 27 September 2021; Published 25 October 2021

**Author affiliations:** <sup>1</sup>Unit for Enteropathogenic Bacteria and Legionella and National Reference Centre for Salmonella and other Bacterial Enterics, Robert Koch Institute, Wernigerode, Germany; <sup>2</sup>Unit for Gastrointestinal Infections, Zoonoses and Tropical Infections, Robert Koch Institute, Berlin, Germany; <sup>3</sup>Unit for Nosocomial Pathogens and Antibiotic Resistances, Robert Koch Institute, Wernigerode, Germany.

\*Correspondence: Antje Flieger, fliegera@rki.de

**Keywords:** *Salmonella enterica*; antibiotic resistance; extended-spectrum  $\beta$ -lactamase (ESBL); plasmid; pESI.

**Abbreviations:** cgMLST, Core genome multilocus sequence typing; ENA, European Nucleotide Archive; ESBL, extended-spectrum  $\beta$ -lactamase; HGT, horizontal gene transfer; MDR, multidrug-resistant; MLST, Multilocus sequence typing; NRC, National Reference Centre (for Salmonella); NTS, Non-typhoidal *Salmonella enterica*; WGS, whole genome sequencing.

**Data statement:** All supporting data, code and protocols have been provided within the article or through supplementary data files. Two supplementary tables and one supplementary figure are available with the online version of this article.

000698 © 2021 The Authors



This is an open-access article distributed under the terms of the Creative Commons Attribution License.

been observed in recent years [5]. Including a slight increase in resistance to third generation cephalosporins, which has been reported in antimicrobial resistance surveillance studies for clinical isolates, and isolates from food (including retail meat) and animals [5–9].

Resistance to third generation cephalosporins in *S. enterica* is mainly due to the production of extended-spectrum  $\beta$ -lactamases (ESBLs) or AmpC  $\beta$ -lactamases [9, 10], with CTX-M-1 as the most prevalent ESBL type identified in previous studies [5, 11]. ESBL and AmpC genes are often located on plasmids, which facilitate their spread via horizontal gene transfer (HGT) between enterobacterial species and genera [12, 13]. Interestingly, ESBL production correlates with certain serovars in *S. enterica*. Among serovars found in human infections, ESBL genes have been more frequently detected in *S. Typhimurium*, *S. Infantis* and *S. Kentucky* than in *S. Enteritidis* [5, 14]. Furthermore, recent analyses on ESBL-producing *S. Infantis* from broiler chickens, broiler meat, and humans revealed a clonal lineage harbouring an especially successful conjugative pESI-like plasmid [15]. This raises the concern for transmission of ESBL genes in *S. enterica* along the food chain from the primary production systems to clinical cases.

In the present study, we assessed the resistance of clinical NTS in Germany and further analysed whole genomes of third generation cephalosporin resistant *S. enterica* for determination of phylogeny, resistance determinants, and genetic backbones to gain knowledge about the spread of this resistance mechanism in Germany.

## METHODS

### Bacterial strains, serotyping, and antibiotic susceptibility testing

In the years 2017 – 2019, the National Reference Centre (NRC) for *Salmonella* and other Enteric bacterial Pathogens in Germany analysed 11 730 *S. enterica* isolates from human infections for pathogen surveillance. In the same time period 48083 salmonellosis cases were notified to the German surveillance system. All *S. enterica* isolates were serotyped according to the White-Kauffmann-Le Minor scheme [16]. Further, antibiotic susceptibilities to 17 antibiotics (ampicillin, cefotaxime, ceftazidime, cefoxitin, meropenem, nalidixic acid, ciprofloxacin, gentamicin, amikacin, streptomycin, chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole) were investigated by broth microdilution according to EUCAST criteria (v10.0, [https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_tables/v\\_10.0\\_Breakpoint\\_Tables.pdf](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf)). NTS isolates resistant to cefotaxime and/or ceftazidime according to EUCAST criteria (v10.0) were chosen for further analysis, however only one unique isolate per patient or infection cluster was considered. Among all NTS isolates from 2017 - 2019, 264 isolates showed phenotypic resistance to cefotaxime and/or ceftazidime and 166 unique isolates were selected for further analyses.

### Impact Statement

Non-typhoidal *Salmonella enterica* (NTS) is one of the most important agents of foodborne infections worldwide. Although in most cases salmonellosis is self-limiting, antibiotic treatment may be required. However, antibiotic resistance in NTS has been a globally emerging problem, limiting treatment options of NTS infections and further presenting a considerable threat to the public health and food safety. Knowledge about the distribution of antibiotic resistance patterns and the genetic factors in *S. enterica* is therefore essential for appropriate treatment of these infections and for early intervention to prevent the spread of antibiotic resistance, starting at the level of food production. In a nation-wide surveillance of third generation cephalosporin resistant *S. enterica* in Germany, we found that third generation cephalosporin resistance is on the rise among clinical NTS isolates. Its occurrence in various *S. enterica* serovars is likely due to multiple acquisition events of plasmids highlighting its facilitated spread.

### Bacterial cultivation and whole genome sequencing

Müller Hinton broth was used for an overnight cultivation. DNA was subsequently extracted using the GenElute Bacterial Genomic DNA Kit (Sigma, St. Louis, Missouri, U.S.). DNA quantification was performed using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.) according to manufacturer's instructions. Sequencing libraries were generated using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, United States) and paired-end sequencing was performed using a MiSeq instrument with 2×300 MiSeq v3 reagent kit (Illumina, San Diego, CA, United States), HiSeq 2500 instrument with 2×150 with 2×300 HiSeq v2 RR reagent kit or NextSeq 550 instrument with 2×300 NextSeq v2.5 high reagent kit. Whole genome sequencing (WGS) was performed for 166 NTS isolates (Table S1, available with the online version of this article) and sequences were submitted to ENA (<https://www.ebi.ac.uk/ena/>) and can be accessed under the bioproject PRJEB41165.

### Bioinformatics analyses

Quality of the raw sequence data was assessed using FastQC v0.11.5 (<https://github.com/s-andrews/FastQC>). To verify taxonomic read classification, raw data was analysed with Kraken v0.10.6 [17]. The assembly pipeline of the analysis platform SeqSphere+ v6.0.7 (Ridom, Münster, Germany) was applied for *de novo* assembly using SPAdes v3.12.0 with default parameter [18], including an additional FastQC and read trimming step [19].

Multilocus sequence typing (MLST) and core genome MLST (cgMLST) were performed using the *de novo* assembled contigs and Ridom SeqSphere+ v6.0.7 (Ridom; Münster, Germany). The Enterobase *S. enterica* cgMLST v2 scheme

template was applied. *In silico* serotyping, based on the *de novo* assembly genomes, was conducted, using the serotype prediction tool SISTR [20].

*De novo* assembled contigs were used for resistance gene and plasmid replicon type identification. For the detection of acquired antimicrobial resistance genes, the tool Abricate in combination with the NCBIres Database was used (<https://github.com/tseemann/abricate>, [21]). *In silico* replicon typing was performed to assign resistance gene contigs to plasmid replicon sequences with Abricate using the PlasmidFinder v2.0 database [22]. Identified contigs with *bla* gene and plasmid replicon sequence were further investigated by means of Geneious Prime v2020.0.5 (Biomatters, Ltd., Auckland, New Zealand). In case of presence of a plasmid replicon sequence marker and a *bla* gene on an identical contig, these contigs were additionally checked for a possible chromosomal integration via *blastn* (database nucleotide collection (*nr/nt*)) and a subsequent Mauve alignment using *S. Typhimurium* str. LT2 as reference (NC\_003197.2). If a chromosomal integration could be ruled out, *bla* gene carrying contigs were assigned to the respective identified plasmid replicon and considered as the *bla* resistance gene carrying contig. For phylogenetically closely related isolates with identical replicon and *bla* resistance gene patterns but exhibiting different *de novo* assembled contigs carrying *bla* gene and replicon sequence, a reconstruction strategy as described in Weber *et al.* was performed [23]. If multiple replicon sequences were identified in the genome sequence of an isolate and no replicon marker sequence could be addressed as genetic backbone for the *bla* gene or the *de novo* assembled *bla* gene carrying contigs exhibited not sufficient surrounding genetic environment, the *bla* gene carrying replicon was marked as ‘not determined’.

Plasmids carrying *bla*<sub>CTX-M-1</sub> ( $n=22$ ) and *bla*<sub>CTX-M-65</sub> ( $n=15$ ) in *S. Infantis* isolates were compared to variants of the emerging pESI plasmid [15]. In brief, contigs of these isolates were aligned and ordered according to the pESI plasmid backbone of the *S. Infantis* strain 119944 (accession no. NZ\_CP047882) using the MCM algorithm of Mauve [24]. These reordered plasmid drafts were subsequently compared to the pESI backbone and to a *bla*<sub>CTX-M-65</sub> carrying pESI variant (pN17S0535, accession no. CP052810). Further *bla*<sub>CTX-M-1</sub> harbouring plasmids of other serovars putatively belonging to the IncII replicon were compared to an IncII reference plasmid backbone (accession no. MK181566) using Mauve. Plasmids that carried AmpC gene *bla*<sub>CMY-2</sub> were compared to IncA/C and IncII reference plasmids (accession no. CP012929, KT186369, CP015835, CP060509) using Mauve.

### Resistance transfer experiments and molecular typing

Transfer of third generation cephalosporin resistance was tested by broth mating for three selected isolates that harboured *bla*<sub>CTX-M-3</sub> (18-04833), *bla*<sub>CTX-M-65</sub> (19-02625) and *bla*<sub>CTX-M-1</sub> (17-01817) using the sodium azide-resistant recipient strain *E. coli* J53 Azi<sup>r</sup>. Transconjugants were cultivated on LB agar with sodium azide (200 mg l<sup>-1</sup>) and ampicillin (30 mg l<sup>-1</sup>)

and screened for resistance genes as previously described [11]. The plasmid sizes of *bla* gene positive transconjugants were determined by S1-nuclease restriction and pulsed-field gel electrophoresis [25]. Transconjugants and the isolates 18-00018 and 18-00680 were screened for resistance genes as previously described [11].

## RESULTS

### Increasing proportion of third generation cephalosporin resistant *S. enterica* in Germany

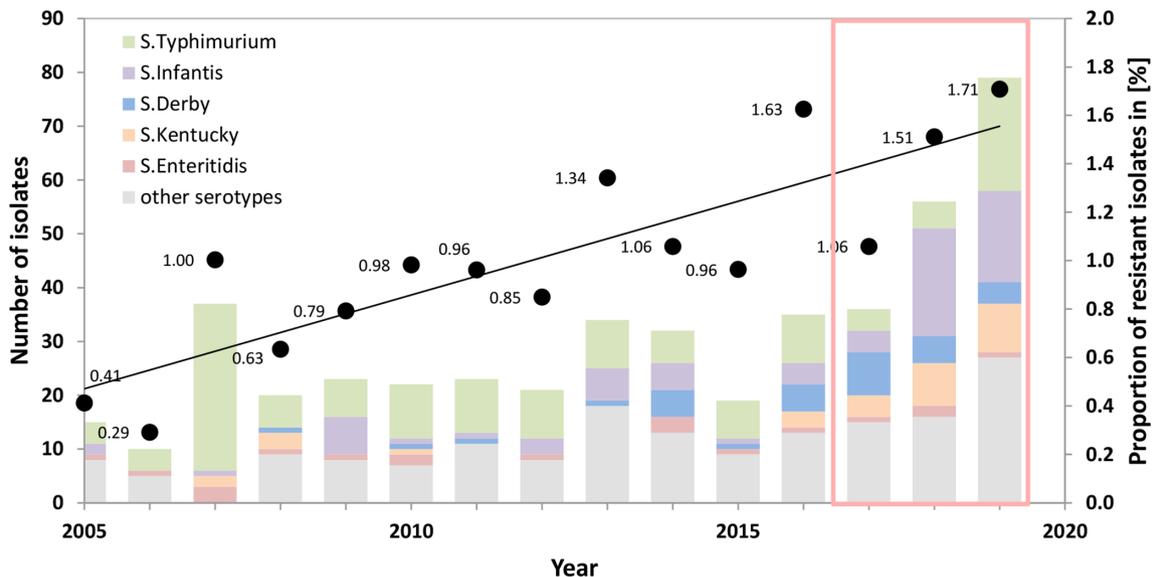
In the years 2017 – 2019, the German National Reference Centre for *Salmonella* and other Enteric Bacterial Pathogens (NRC) analysed 11 730 NTS isolates from human infections (2017=3 399, 2018=3 706, 2019=4 625 isolates). Resistance to third generation cephalosporins was detected in 264 *S. enterica* isolates, of which most were collected in 2019 (2017:  $n=36$ , 2018:  $n=56$ , 2019:  $n=172$ ). A substantial part of the isolates ( $n=100$ ) from 2019 was attributed to a single infection cluster of *S. Typhimurium* in a regional setting. Four unique/representative isolates of this cluster which harboured certain changes (see details below) were included into the subsequent analyses. Consequently, the number of third generation cephalosporin resistant isolates considered for further analysis in 2019 was 76, resulting in a total of 168 study isolates for the years 2017–2019 (Table S1).

In 2019, a proportion of 1.71% of all analysed *S. enterica* were resistant to third generation cephalosporins, which was an increase compared to 1.06% in 2017. To analyse whether the increasing trend is seen in a larger time frame, NRC data from the years 2005 to 2011 [11] and 2012 to 2016 were integrated into this analysis, using identical selection criteria. Indeed, the rising trend of third generation cephalosporin resistance was corroborated for the entire time frame from 2005 to 2019. The proportion of third generation cephalosporin resistance consistently rose from 0.41% in 2005 to 1.71% in 2019 (Fig. 1) ( $P=0.0002$ ; correlation coefficient  $r=0.8318$ ; Spearman rank correlation). In addition to resistance to third generation cephalosporins, 44 of the 168 isolates (26.2%) also showed resistance to ciprofloxacin (Table S1) and 21 isolates (12.5%) exhibited a combined resistance to ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole (Table S1). No isolate was resistant to meropenem.

The 168 third generation cephalosporin resistant isolates were assigned to 34 different serovars, of which *S. Infantis* ( $n=41$ ), *S. Typhimurium* ( $n=27$ ), *S. Kentucky* ( $n=21$ ), *S. Derby* ( $n=17$ ) and *S. Anatum* ( $n=9$ ) were most prevalent (Table S1 and S2).

### ESBL types CTX-M-1, CTX-M-14, and CTX-M-65 are the most abundant third generation cephalosporin resistance determinants

Whole genome sequence analyses including *in silico* detection of the resistance determinants revealed that for most of the isolates ( $n=163/166$ ) the third generation cephalosporin resistance was mediated by ESBLs ( $n=147$ ) or plasmid-borne AmpC  $\beta$ -lactamases ( $n=16$ ) (Table S1). For three isolates



**Fig. 1.** Increasing proportion of German clinical *S. enterica* isolates showing third generation cephalosporin resistance (cefotaxime and/or ceftazidime) from 2005 to 2019. The left axis indicates the absolute numbers of third generation cephalosporin-resistant isolates (coloured bars), the right axis indicates the proportion of third generation cephalosporin-resistant isolates in percent of all analysed isolates at the NRC (black dots). Isolates from 2017 to 2019 investigated in detail in this study are highlighted by the red box. The resistant *S. Typhimurium* isolates from 2019 included four representative isolates of an infection cluster.

no  $\beta$ -lactamase genes were detected and analyses of porin genes revealed wild-type variants without a sign of porin loss. Therefore, the underlying mechanism of resistance of these three isolates remained unclear (Table S1). The most prevalent ESBL genes in the isolate collection were *bla*<sub>CTX-M-1</sub> ( $n=55$ ), followed by *bla*<sub>CTX-M-14</sub> ( $n=25$ , different variants) and *bla*<sub>CTX-M-65</sub> ( $n=23$ ) (Table 1). The most commonly detected AmpC  $\beta$ -lactamase gene was *bla*<sub>CMY-2</sub> ( $n=13$ ), only one isolate harboured *bla*<sub>CMY-4</sub>, one isolate *bla*<sub>ACC-1</sub>, and one isolate *bla*<sub>DHA-1</sub>. Further identified  $\beta$ -lactamase genes were: *bla*<sub>OXA-1</sub> ( $n=1$ ), *bla*<sub>CARB-2</sub> ( $n=2$ ) and 39 *bla*<sub>TEM</sub> variants. Additionally, 38 isolates harboured plasmid-mediated quinolone resistance (PMQR) genes, mainly gene *qnrS1* ( $n=25$ ). Interestingly, four isolates were positive for variants of the mobile colistin resistance genes *mcr-1.1* (*S. Typhimurium*, 17-02112), *mcr-3.1* (*S. Typhimurium*, 19-03137) and *mcr-3.11* (*S. Choleraesuis* and *S. Bovismorbificans*, 17-00186 and 17-05354) (Table 1).

Although a strict correlation of serovar and ESBL/AmpC type was not detected, some ESBL types were observed in high proportions in specific serovars. For example, *bla*<sub>CTX-M-65</sub> ( $n=22/23$ ) was mainly identified in *S. Infantis*. Additionally, *bla*<sub>CTX-M-14</sub> was differentiated into three subgroups (*bla*<sub>CTX-M-14a</sub>,  $n=6$ , *bla*<sub>CTX-M-14b</sub>,  $n=16$  and *bla*<sub>CTX-M-14b2</sub>,  $n=3$ ): While *bla*<sub>CTX-M-14b</sub> was solely detected in *S. Kentucky* (Fig. 2, Table 1), the remaining *bla*<sub>CTX-M-14</sub> variants were present in various serovars.

### Plasmids as the main carriers of genes encoding ESBLs and AmpC $\beta$ -lactamases

For 137 of 163 of the ESBL/AmpC-positive isolates an attribution of the resistance gene to a respective genetic backbone / replicon was possible. The majority of the resistance genes

was located on mobile genetic elements ( $n=105$ , 65.2 %), particularly on plasmids, irrespective of their hosting *S. enterica* serovar. However, a substantial number of isolates ( $n=32$ , 18.8 %) revealed chromosomal integration of the ESBL genes. The chromosomal integration of *bla*<sub>CTX-M-14</sub> in all *S. Kentucky* isolates was identical as described by Coipan *et al.*: *bla*<sub>CTX-M-14</sub> was flanked by an upstream *ISEcp1* element, downstream of the *hcp1* gene in the type VI secretion system region of the genome [14]. In all *S. Derby* isolates the integration of *bla*<sub>CTX-M-1</sub> with an upstream *ISEcp1*, as presumable mobilizing element, took place in a different genetic region than in *S. Kentucky*. *Bla*<sub>CTX-M-1</sub> was integrated into the *kduI* gene, which was inferred from homology, causing a truncation of this pectin degradation pathway gene (accession no. A0A379QY07).

For 26 isolates it was only possible to recover the direct genetic environment but not to attribute the respective replicon type.

While for some of the ESBL genes a correlation of resistance gene and the gene carrying replicon was observed (*bla*<sub>CTX-M-65</sub> and *IncFIB(K)* (22/23), *bla*<sub>CTX-M-14b</sub> and chromosome (16/19), *bla*<sub>CMY-2</sub> and *IncI1* (6/13), *bla*<sub>CMY-2</sub> and *IncA/C* (6/13)), other genes showed a more diverse genetic background. For example, *bla*<sub>CTX-M-1</sub> was found on *IncI1*, *IncHI2*, *IncN* and *IncQ* plasmids.

### Predominance of emerging pESI plasmids in *S. Infantis* harbouring *bla*<sub>CTX-M</sub>

Detailed comparative analyses of *bla*<sub>CTX-M-65</sub> carrying plasmids revealed a high sequence identity with the previously described pESI-like plasmid identified in *S. Infantis*. Specifically, 22 of

**Table 1.** ESBL and AmpC  $\beta$ -lactamase genes identified in the third generation cephalosporin resistant clinical *Salmonella* isolates from Germany, 2017 – 2019. Information on the co-occurrence of plasmid-mediated quinolone resistance (PMQR) genes and colistin resistance-mediating genes in the respective *Salmonella* isolates is given.

ESBL/AmpC gene	n	PMQR genes (n)	Mcr genes (n)	Majorly found in (n)
<i>bla</i> <sub>CTX-M-1</sub> *	55	<i>qnrS1</i> (4)		<i>S. Infantis</i> (15) <i>S. Derby</i> (17)
<i>bla</i> <sub>CTX-M-2</sub>	1			
<i>bla</i> <sub>CTX-M-3</sub>	3			
<i>bla</i> <sub>CTX-M-9</sub>	3			
<i>bla</i> <sub>CTX-M-14</sub>	25	<i>qnrS1</i> (3)	<i>mcr-1.1</i> (1), <i>mcr-3.11</i> (2)	<i>S. Kentucky</i> (18)
<i>bla</i> <sub>CTX-M-15</sub>	7	<i>qnrS1</i> (1)		
<i>bla</i> <sub>CTX-M-32</sub>	1			
<i>bla</i> <sub>CTX-M-55</sub>	14	<i>qnrS1</i> (6)	<i>mcr-3.1</i> (1)	
<i>bla</i> <sub>CTX-M-65</sub>	23			<i>S. Infantis</i> (22)
<i>bla</i> <sub>TEM-52</sub>	2	<i>qnrB19</i> (1)		
<i>bla</i> <sub>SHV-12</sub>	13	<i>qnrS1</i> (10), <i>qnrB2</i> (1), <i>qnrA1</i> (1)		<i>S. Anatum</i> (7)
<i>bla</i> <sub>CMY-2</sub>	13	<i>qnrS1</i> (1), <i>qnrB19</i> (4), <i>qnrB6</i> (1)		
<i>bla</i> <sub>CMY-4</sub>	1	<i>qnrA1</i> (1)		
<i>bla</i> <sub>DHA-1</sub>	1	<i>qnrB4</i> (1)		
<i>bla</i> <sub>ACC-1</sub>	1			

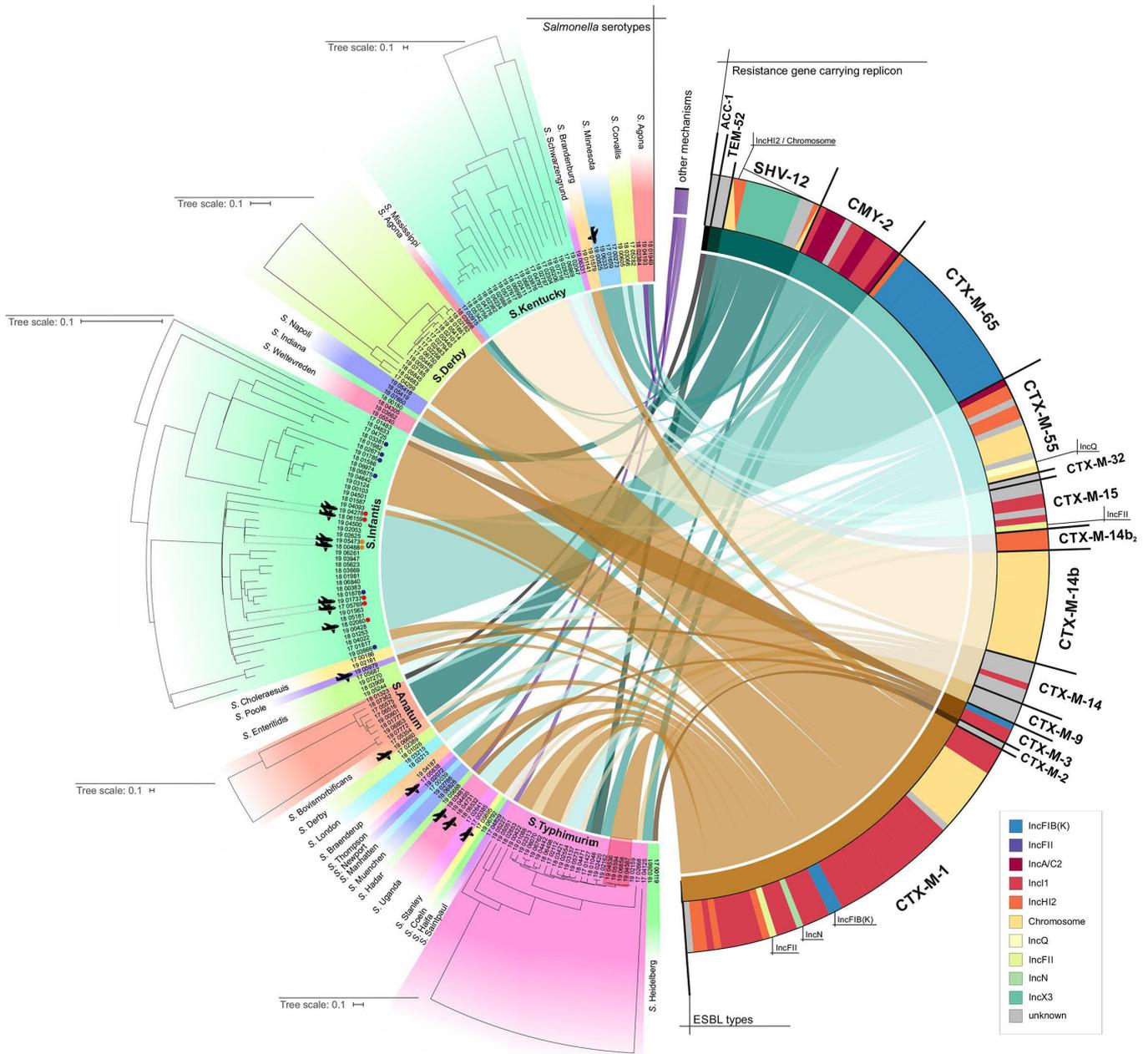
\*Includes four representative isolates originating from the 2019 *S. Typhimurium* infection cluster.

23 *bla*<sub>CTX-M-65</sub> positive isolates showed high resemblance with the chimeric backbone of the pESI-plasmid p119944 (accession no. NZ\_CP047882) and all belonged to the serovar *S. Infantis*. The non-matching isolate belonged to *S. Indiana* (18-00180) and the respective plasmid revealed no similarities to the pESI plasmid backbone. The integration of *bla*<sub>CTX-M-65</sub> usually takes place upstream of a previously described drug and heavy metal resistance gene carrying region and this was also observed for the *S. Infantis* isolates in this study [26]. Further analyses showed that the presence of *bla*<sub>CTX-M-1</sub> on a pESI-like plasmid backbone was observed only in two out of 15 *S. Infantis* isolates (17-01817 and 18-04022). We found that the integration of *bla*<sub>CTX-M-1</sub> took place in the conserved IncI1 backbone structure of pESI. The remaining 13 *S. Infantis* isolates however exhibited a high sequence identity (>95 %) with the IncI1 plasmid p15078279 (accession no. MK181566), which was previously identified in *E. coli* [27]. Additionally, in one isolate (18-04833) *bla*<sub>CTX-M-3</sub> was suspected to be located on a pESI-like plasmid. While confirmation of the insertion of *bla*<sub>CTX-M-3</sub> into the pESI based on short read sequencing data was not possible, the sequence data however suggested the presence of the full pESI-like backbone. Additional mating experiments and subsequent S1-nuclease restriction and pulsed-field gel electrophoresis revealed transferability of the *bla*<sub>CTX-M-3</sub> gene and its presence on an approx. 290 kb sized plasmid for the *bla*<sub>CTX-M-3</sub> positive transconjugant of 18-04833. In comparison, the size of the transferred *bla* gene carrying

plasmids of *bla* positive transconjugants of other pESI-like plasmid carrying strains, such as *S. Infantis* 17-01817 (*bla*<sub>CTX-M-1</sub>) and 19-02625 (*bla*<sub>CTX-M-65</sub>), was approx. 290 kb and approx. 315 kb, respectively.

### ***bla*<sub>CMY-2</sub> harbouring plasmids share high sequence similarity with IncI1 and IncA/C plasmids from Europe**

AmpC  $\beta$ -lactamase CMY-2 was the main determinant of resistance to third generation cephalosporins present in 13 isolates (mainly serovars *S. Minnesota*  $n=4$  and *S. Typhimurium*  $n=3$ ). The *bla*<sub>CMY-2</sub> gene was found to be located on plasmids of the replicon type IncI1 ( $n=6$ ) and IncA/C replicon type ( $n=6$ ) and for one isolate (19-00825) was not assignable. The plasmids of IncI1 replicon type showed a high sequence identity to two previously described variants: IncI1 belonging to the plasmid sequence type pST12 (accession no. CP012929) ( $n=4$ ) and IncI1 belonging to pST2 (accession no. KT186369) ( $n=2$ ) and shared >98%, respective >99% sequence identity with these reference plasmids. Isolates 17-05838 and 19-02072 resembled the backbone of IncA/C<sub>2</sub> plasmids of type 1 (accession no. CP015835) [28], while the other four isolates (17-00273, 17-01659, 19-02661, 19-06333) showed a high similarity with a IncA/C reference plasmid (accession no. CP060509), which was identified in NTS isolates from Russia.

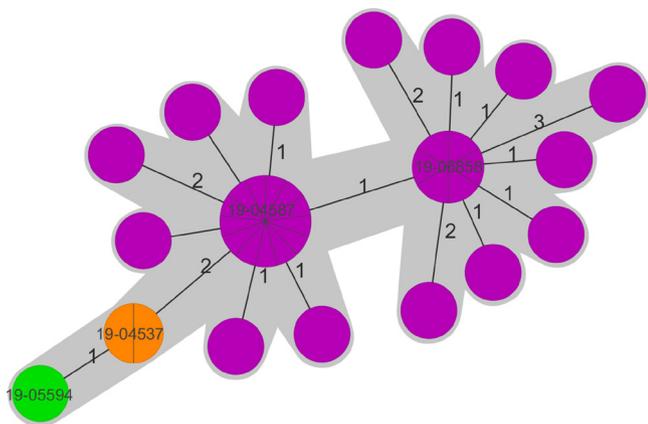


**Fig. 2.** Attribution of the *Salmonella* serovar to the respective ESBL/AmpC gene of 166 third generation resistant clinical *Salmonella* isolates from Germany 2017 – 2019. The attributed resistance gene of each isolate is indicated via coloured links. The resistance gene carrying replicon is additionally indicated in the coloured bars next to the resistance gene (see legend). The respective phylogenetic relationships of *S. Anatum*, *S. Derby*, *S. Infantis*, *S. Kentucky* and *S. Typhimurium* are highlighted by independent phylogenetic trees. Four representative isolates belonging to the *S. Typhimurium* infection cluster are shown with a red background. Isolates with possible travel history are highlighted by airplanes and coloured dots, indicating the origin of the isolates (orange: South-East Asia, red: South America, blue: Germany). The figure was created with Circos [47].

**The *S. Typhimurium* infection cluster isolates from 2019 harboured a novel plasmid carrying *bla*<sub>CTX-M-1</sub>**

As depicted above, a substantial part of the *S. Typhimurium* isolates (*n*=100) of the year 2019 originated from a single infection cluster in a regional setting. Genome analyses of 36

of these isolates were performed and confirmed their close genetic relationship. The maximum distance in the cgMLST analysis were five alleles; the majority of the genomes depicted a distance of zero or one alleles (Fig. 3). The resistance gene content and plasmid replicon analyses showed almost identical



**Fig. 3.** cgMLST-based Minimum Spanning Tree of 36 *S. Typhimurium* isolates of the 2019 infection cluster. Isolates with an identical IncHI2 plasmid structure are shown in purple, IncHI2 plasmid variants with the additional IS element in orange and the isolate with the unique IncI1 plasmid in green. Only the four representative isolates are highlighted with strain numbers.

patterns ( $bla_{CTX-M-1}$ :36/36,  $qnrS1$ :35/36, IncHI2 replicon type: 35/36). The identified resistance gene carrying plasmid showed no sequence similarity to previously published  $bla$  gene carrying plasmids. Interestingly, one isolate (19-05594) showed absence of the  $qnrS1$  gene and revealed a unique plasmid replicon background (IncI1), indicating a potential independent plasmid acquisition event. Two further isolates (19-04536 and 19-04537) exhibited a slight alteration of the  $bla_{CTX-M-1}$  carrying plasmid, varying in IS element integration sites. One representative isolate from each group (19-04587, 19-06858, 19-04536: IncHI2 plasmid alteration; 19-05594: IncI1 plasmid) was included in all here presented analyses (Figs. 2 and 3).

**No strict correlation of ESBL genes with certain serovars was observed, but some serovar/resistance gene combinations, such as *S. Infantis* with  $bla_{CTX-M-1}$  or  $bla_{CTX-M-65}$  or *S. Kentucky* with  $bla_{CTX-M-14b}$ , were detected most frequently**

cgMLST-based analysis revealed that the majority of the isolates showed no close phylogenetic relationship but clustered according to the serovar. However, among the serovars various (distinctly related) subclades were observed (Fig. 2, subclades shown for *S. Anatum*, *S. Derby*, *S. Infantis*, *S. Kentucky*, and *S. Typhimurium*). As expected, the four representative *S. Typhimurium* isolates from the infection cluster formed one cluster (sequence type ST19) (Figs 2 and 3), while the other *S. Typhimurium* isolates belonged to the ST34. Linking data on  $bla$  gene and plasmid content to phylogenetic information, a tendency regarding the presence of specific ESBL genes in certain serovars was observed. The most apparent correlations were seen for *S. Infantis* with  $bla_{CTX-M-1}$  and  $bla_{CTX-M-65}$ , *S. Typhimurium* with  $bla_{CTX-M-1}$ , *S. Derby* with  $bla_{CTX-M-1}$  and *S. Kentucky* with  $bla_{CTX-M-14b}$  (Fig. 2).

In depth cgMLST analyses of the *S. Infantis* clade showed that these strains can be differentiated into at least two different subclades: subclade one exhibited the IncI1  $bla_{CTX-M-1}$  carrying plasmid while subclade two is associated with  $bla_{CTX-M-65}$  and  $bla_{CTX-M-1}$  carrying pESI-like plasmids (Fig. S1). Additionally, one isolate (18-04833) exhibited  $bla_{CTX-M-3}$  presumably integrated into the pESI backbone. Little information on travel background of patients was available. However, for thirteen of the 41 *S. Infantis* isolates a definite exposition region was determined. Interestingly, especially isolates from patients from the subclade carrying the  $bla_{CTX-M-65}$  pESI-like plasmid exhibited travel history to South East Asia or South America (Figs 2 and S1).

## DISCUSSION

The analyses of clinical NTS from Germany showed a trend towards increasing resistance proportions for third generation cephalosporins (Fig. 1). This observation is in accordance with the worldwide emergence of antibiotic resistance in NTS. European data for clinical NTS isolates (period 2018 – 2019) by EFSA and ECDC [5] uncovered high resistance rates for different classes of antibiotics, especially ampicillin, sulfonamides, and tetracyclines. Increasing resistance was further reported for fluoroquinolones, while resistance to third generation cephalosporins was still on low levels but with rising proportions in certain serovars [5]. In Germany, five serovars (*S. Typhimurium*, *S. Derby*, *S. Kentucky*, *S. Infantis* and *S. Anatum*) accounted for over 65% of the resistant isolates in this study [5, 29, 30]. *S. Enteritidis*, while being one of the most prevalent *S. enterica* serovars, remains mostly susceptible to third generation cephalosporins [31].

In the present study we identified ESBL and AmpC  $\beta$ -lactamase genes as most important determinants of third generation resistance in clinical NTS. These observations are in concordance to reports from the EU, where in 2018/19 ESBLs were reported in 16 different serovars (among those *S. Infantis*, *S. Kentucky*, *S. Typhimurium*) and AmpC  $\beta$ -lactamases in ten different serovars, mostly in *S. Anatum*, *S. Bredeney* and *S. Thompson* [5]. The majority of isolates in this study harboured ESBL genes encoding CTX-M enzymes ( $n=132$ ; 80.0%) with CTX-M-1, CTX-M-14 and CTX-M-65 as the most prevalent types. Similar results were found in studies on *S. enterica* and *E. coli* isolated from animals, especially poultry [11, 32, 33] and in reports from the EU, where, if tested by member states, the ESBL variants CTX-M-1, CTX-M-14/14b and CTX-M-9 were frequently reported [5]. In the present study  $bla_{CTX-M-1}$  was detected in various serovars, including *S. Typhimurium*, *S. Infantis* and *S. Derby*. However,  $bla_{CTX-M-14b}$  and  $bla_{CTX-M-65}$  were almost exclusively found in *S. Kentucky* and *S. Infantis*, respectively. Detailed sequence analyses of the 23  $bla_{CTX-M-65}$  carrying plasmids of the study isolates revealed a high sequence identity among them: out of the 23 isolates, 22 belonged to the serovar *S. Infantis* and showed an identical plasmid structure, which additionally was highly similar to the chimeric backbone of the pESI-plasmid p119944 (accession no. NZ\_CP047882).

In recent studies the spread of third generation cephalosporin resistant *S. Infantis* in food-producing animals associated with the presence of a specific resistance plasmid type, the pESI-like megaplasmid, was reported [15, 34, 35]. This plasmid type/backbone was associated with the carriage of ESBL genes ( $bla_{CTX-M-65}$  and  $bla_{CTX-M-1}$ ), several other resistance genes ( $tet(A)$ ,  $sul1$ ,  $dfrA1$ ,  $dfrA14$ ,  $aadA1$ ) and heavy metal resistance genes [26]. The pESI-like megaplasmid was first identified by Aviv *et al.* in Israel [34], however, not yet harbouring third generation cephalosporin resistance genes. Subsequently findings of pESI harbouring third generation cephalosporin resistance genes were reported from Italy [15], the U.S. [28], Denmark, Netherlands and the UK [15, 34–36]. In 2018, Brown *et al.* observed the linkage of *S. Infantis* pESI-like plasmids carrying  $bla_{CTX-M-65}$  in the USA with travel activity to Latin America [37]. Despite too little information on the travel history of the patients in our study for statistical analyses, the present data partly indicate a correlation between travel activity (Asia and South America) and  $bla_{CTX-M-65}$  harbouring *S. Infantis*.

While the pESI plasmid with  $bla_{CTX-M-65}$  was highly prevalent in *S. Infantis* strains from Germany, only two of 15 *S. Infantis* strains exhibiting  $bla_{CTX-M-1}$  (17-01817 and 18-04022) harboured the resistance gene on the pESI plasmid. In Germany the occurrence of pESI was described in *S. Infantis* isolates from broiler farms since the 2010s [38]. However, these plasmids did not carry ESBL genes and were identified in *S. Infantis* strains of another lineage (ST2283) than the  $bla_{CTX-M-1}$  carrying isolates from this study (ST32). In our study one additional isolate of *S. Infantis* exhibited  $bla_{CTX-M-3}$  on a pESI-like plasmid, highlighting the potential of this particular plasmid backbone to acquire further resistance genes. Phylogenetic analysis revealed that isolates harbouring pESI with  $bla_{CTX-M-3}$ ,  $bla_{CTX-M-65}$  and  $bla_{CTX-M-1}$  are more related among each other than to the clade of the non-pESI  $bla_{CTX-M-1}$  carrying *S. Infantis* isolates. It can be speculated, that certain genetic factors enable the plasmid to establish in this specific *S. Infantis*  $bla_{CTX-M-1/65}$  clade. The presence of multiple post-segregational killing determinants on the plasmid may be of advantage for this process [34].

The majority of *S. Infantis* isolates with  $bla_{CTX-M-1}$  (13/15) exhibited a highly similar plasmid background to an IncI1 plasmid backbone. Identical or highly similar  $bla_{CTX-M-1}$  carrying plasmids were identified in the present study also in *S. Derby*, *S. Enteritidis*, *S. Typhimurium*, *S. Stanley* and *S. Brandenburg*. In contrast to the pESI plasmid, which appears to be more adapted to the *S. Infantis* host - but still can be transferred into other *S. enterica* serovars and *E. coli* [15, 39] - these non-pESI IncI1 plasmids exhibited a wider host range and were also reported in previous studies in *E. coli* [27].

Further resistance genes detected in this study were  $bla_{CTX-M-15}$  and  $bla_{CMY-2}$ . ESBL type CTX-M-15, which is highly prevalent in *E. coli* from outpatient and hospitalized patients in Germany [40], was identified only in seven clinical *S. enterica* isolates of different serovars. AmpC  $\beta$ -lactamase CMY-2, which was detected in <1% of clinical *E. coli* isolates

with third generation cephalosporin resistance in Germany [41] were detected in 12 *S. enterica* isolates in the present study (7.45 %). The observed higher proportions of CTX-M-1 and CMY-2 genes with comparable lower proportions of CTX-M-15 in our clinical *S. enterica* isolates may on the one hand indicate a possible transmission route between livestock and humans via the food chain. The active acquisition of plasmids via horizontal gene transfer along the food production chain seems rational, as identical resistance gene carrying plasmids were observed in *S. enterica* of different serovars. This is strengthened by the observation of identical resistance gene carrying plasmids in *E. coli*, *Klebsiella pneumoniae* and other Enterobacterales species in Germany, Europe, and worldwide [42–44]. Furthermore, it has been reported that approx. 6% of the healthy population of Germany carries ESBL positive *E. coli* [45]. Ingestion of other ESBL-positive Enterobacterales and transfer of resistance genes from commensal bacteria therefore might have an impact on the treatment options of severe salmonellosis, as the resistance genes may be acquired during treatment [46]. However, since CTX-M-15 is the most common ESBL type in *E. coli*/*K. pneumoniae* in the human population, these events might be limited in occurrence rather than being the main route for ESBL gene transmission.

We conclude that third generation cephalosporin resistance, is on the rise among clinical *S. enterica* isolates in Germany and that its occurrence in various *S. enterica* serovars is likely due to multiple acquisition events of plasmids. However, specific adaption processes, as observed for *S. Infantis* with pESI plasmids harbouring different  $bla_{CTX-M}$  genes, could facilitate the spread of resistant *S. enterica* isolates.

#### Funding information

The study was supported by a grant from the German Ministry of Health for the project 'Integrated Genome-Based Surveillance of Salmonella' (GenoSalmSurv, decision ZMV11-2518FSB709 of 26 November 2018).

#### Acknowledgements

We thank Monique Duwe, Susanne Kulbe, Marita Wahnfried, and Sibylle Müller-Bertling for excellent technical assistance. We also thank Andrea Thürmer and Aleksander Radonic on behalf of the core facility for genome sequencing MF2 at the Robert Koch Institute. We are also thankful to all laboratories that sent strains to the NRC for further analysis.

#### Author contributions

We describe author contributions to the paper using the CRediT taxonomy. Conceptualization: Conceptualization: M.P. and A.F. Data curation: M.P., S.S. and A.M. Formal Analysis: M.P. Funding acquisition: S.S. and A.F. Investigation: M.P., Y.P. and S.S. Methodology: M.P. Project administration: A.F. Resources: S.S. E.T. and A.F. Software: M.P. and E.T. Supervision: A.F. Validation: M.P., S.S. and A.F. Visualization: M.P. Writing – original draft: M.P. and A.F. Writing – review and editing: all authors.

#### Conflicts of interest

The authors have no conflicts of interest.

#### Ethical statement

The study does not include human subjects, human material, or human data. Solely, bacterial isolates were studied and were obtained as part of routine submissions to the National Reference Centre for Salmonella and other Enteric Bacterial Pathogens.

## References

- Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, et al. The global burden of nontyphoidal *Salmonella gastroenteritis*. *Clin Infect Dis* 2010;50:882–889.
- European food safety A, European Centre for Disease P, control. The European Union one Health 2019 zoonoses report. *EFSA J* 2021;19:e06406.
- Koch-Institut R. *Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2019*. Robert Koch-Institut, 2020.
- Hagel S, Epple HJ, Feurle GE, Kern WV, Lynen Jansen P, et al. S2k-guideline gastrointestinal infectious diseases and Whipple's disease. *Z Gastroenterol* 2015;53:418–459.
- European Food Safety Authority, and European Centre for Disease Prevention and Control. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. *EFSA J* 2021;19:e06490.
- Afema JA, Mather AE, Sisco WM. Antimicrobial resistance profiles and diversity in salmonella from humans and cattle, 2004–2011. *Zoonoses Public Health* 2015;62:506–517.
- Pardos de la Gandara M, Seral C, Castillo Garcia J, Rubio Calvo C, Weill FX. Prevalence and characterization of extended-spectrum beta-lactamases-producing *Salmonella enterica* isolates in Saragossa, Spain (2001–2008). *Microb Drug Resist* 2011;17:207–213.
- EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum  $\beta$ -lactamases and/or AmpC  $\beta$ -lactamases in food and food-producing animals. *EFSA Journal* 2011;9:2322.
- Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother* 2005;56:115–121.
- Livermore DM. Beta-lactamase-mediated resistance and opportunities for its control. *J Antimicrob Chemother* 1998;41:25–41.
- Eller C, Simon S, Miller T, Frick JS, Prager R, et al. Presence of beta-lactamases in extended-spectrum-cephalosporin-resistant *Salmonella enterica* of 30 different serovars in Germany 2005–11. *J Antimicrob Chemother* 2013;68:1978–1981.
- Carattoli A. Plasmids and the spread of resistance. *Int J Med Microbiol* 2013;303:298–304.
- Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, et al. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. *J Antimicrob Chemother* 2018;73:1121–1137.
- Coipan CE, Westrell T, van Hoek A, Alm E, Kotila S, et al. Genomic epidemiology of emerging ESBL-producing *Salmonella* Kentucky bla CTX-M-14b in Europe. *Emerg Microbes Infect* 2020;9:2124–2135.
- Franco A, Leekitcharoenphon P, Feltrin F, Alba P, Cordaro G, et al. Emergence of a clonal lineage of multidrug-resistant ESBL-producing *Salmonella* infantis transmitted from broilers and broiler meat to humans in Italy between 2011 and 2014. *PLoS One* 2015;10.
- Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemuhl J, et al. Supplement 2003–2007 (no.47) to the White-Kauffmann-Le Minor scheme. *Res Microbiol* 2010;161:26–29.
- Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol* 2014;15:R46.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
- Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
- Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VPJ, et al. The *Salmonella* In Silico Typing Resource (SISTR): an open web-accessible tool for rapidly typing and subtyping draft *Salmonella* genome assemblies. *PLoS ONE* 2016;11:e0147101.
- Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, et al. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. *Antimicrob Agents Chemother* 2019;63:11.
- Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, et al. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014;58:3895–3903.
- Weber RE, Pietsch M, Fruhauf A, Pfeifer Y, Martin M, et al. IS26-Mediated Transfer of bla NDM-1 as the main route of resistance transmission during a polyclonal, multispecies outbreak in a German Hospital. *Front Microbiol* 2019;10:2817.
- Darling AC, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 2004;14:1394–1403.
- Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem* 1995;226:235–240.
- Cohen E, Rahav G, Gal-Mor O. Genome sequence of an emerging *Salmonella enterica* serovar infantis and genomic comparison with other *S. Genome Biol Evol* 2020;12:151–159.
- Valcek A, Roer L, Overballe-Petersen S, Hansen F, Bortolaia V, et al. Incl1 ST3 and Incl1 ST7 plasmids from CTX-M-1-producing *Escherichia coli* obtained from patients with bloodstream infections are closely related to plasmids from *E. coli* of animal origin. *J Antimicrob Chemother* 2019;74:2171–2175.
- Hancock SJ, Phan MD, Peters KM, Forde BM, Chong TM, et al. Identification of IncA/C plasmid replication and maintenance genes and development of a plasmid multilocus sequence typing scheme. *Antimicrob Agents Chemother* 2017;61:e01740-16.
- European Food Safety Authority, and European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. *EFSA J* 2020;18:e06007.
- European Food Safety Authority, and European Centre for Disease Prevention and Control. The European Union One Health 2018 Zoonoses Report. *EFSA J* 2019;17:12.
- Su LH, Chiu CH, Chu C, Ou JT. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. *Clin Infect Dis* 2004;39:546–551.
- Kola A, Kohler C, Pfeifer Y, Schwab F, Kuhn K, et al. High prevalence of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in organic and conventional retail chicken meat. *J Antimicrob Chemother* 2012;67:2631–2634.
- Rodriguez I, Barownick W, Helmuth R, Mendoza MC, Rodicio MR, et al. Extended-spectrum (beta)-lactamases and AmpC (beta)-lactamases in ceftiofur-resistant *Salmonella enterica* isolates from food and livestock obtained in Germany during 2003–07. *J Antimicrob Chemother* 2009;64:301–309.
- Aviv G, Tsyba K, Steck N, Salmon-Divon M, Cornelius A, et al. A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent *Salmonella enterica* serovar Infantis strain. *Environ Microbiol* 2014;16:977–994.
- Tate H, Folster JP, Hsu CH, Chen J, Hoffmann M, et al. Comparative analysis of extended-spectrum-beta-lactamase CTX-M-65-producing *Salmonella enterica* serovar infantis isolates from humans, food animals, and retail chickens in the United States. *Antimicrob Agents Chemother* 2017;61:e00488-17.
- Gymoese P, Kiil K, Torpdahl M, Osterlund MT, Sorensen G, et al. WGS based study of the population structure of *Salmonella enterica* serovar Infantis. *BMC Genomics* 2019;20:870.
- Brown AC, Chen JC, Watkins LKF, Campbell D, Folster JP, et al. CTX-M-65 extended-spectrum beta-lactamase-producing *Salmonella enterica* serotype infantis, United States(1). *Emerg Infect Dis* 2018;24:2284–2291.
- Garcia-Soto S, Abdel-Gil MY, Tomaso H, Linde J, Methner U. Emergence of multidrug-resistant *Salmonella enterica* subspecies enterica serovar infantis of multilocus sequence type 2283 in German broiler farms. *Front Microbiol* 2020;11:1741.

39. Aviv G, Rahav G, Gal-Mor O. Horizontal transfer of the *Salmonella enterica* serovar infantis resistance and virulence plasmid pESI to the gut microbiota of warm-blooded hosts. *mBio* 2016;7:e01395-16.
40. Pietsch M, Eller C, Wendt C, Holfelder M, Falgenhauer L, et al. Molecular characterisation of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolates from hospital and ambulatory patients in Germany. *Vet Microbiol* 2017;200:130–137.
41. Pietsch M, Irrgang A, Roschanski N, Brenner Michael G, Hamprecht A, et al. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. *BMC Genomics* 2018;19:601.
42. Hansen F, Olsen SS, Heltberg O, Justesen US, Fuglsang-Damgaard D, et al. Characterization of third-generation cephalosporin-resistant *Escherichia coli* from bloodstream infections in Denmark. *Microb Drug Resist* 2014;20:316–324.
43. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, et al. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. *PLoS Genet* 2014;10:12.
44. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, et al. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. *J Antimicrob Chemother* 2018;73:1121–1137.
45. Valenza G, Nickel S, Pfeifer Y, Eller C, Krupa E, et al. Extended-spectrum-beta-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. *Antimicrob Agents Chemother* 2014;58:1228–1230.
46. Bakkeren E, Huisman JS, Fattinger SA, Hausmann A, Furter M, et al. Salmonella persists promote the spread of antibiotic resistance plasmids in the gut. *Nature* 2019;573:276–280.
47. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, et al. Circos: an information aesthetic for comparative genomics. *Genome Res* 2009;19:1639–1645.

### Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at [microbiologyresearch.org](https://microbiologyresearch.org).