Contemporary IncI1 plasmids involved in the transmission and spread of antimicrobial resistance in Enterobacteriaceae

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Abstract

IncI1 has become one of the most common plasmid families in contemporary Enterobacteriaceae from both human and animal sources. In clinical epidemiology, this plasmid type ranks first as the confirmed vehicle of transmission of extended spectrum beta-lactamase and plasmid AmpC genes in isolates from food-producing animals. In this review, we describe the epidemiology and evolution of IncI1 plasmids and closely related IncIγ plasmids. We highlight the emergence of *epidemic* plasmids circulating among different bacterial hosts in geographically distant countries, and we address the phylogeny of the IncI1 and IncIγ family based on plasmid Multilocus Sequence Typing.

Highlights

- Contemporary and historical IncI1 plasmids share a conserved backbone, and evolution is attributed to acquisition of clinically relevant antimicrobial resistance determinants.
- Phylogenetic analysis performed on pMLST alleles identified two major plasmid lineages that differ in their *pil* cluster sequences.
- IncI1 and IncIγ plasmids that carry ESBL and AmpC genes spread among different Enterobacteriaceae isolated from human, animal and environmental sources.

Introduction

Plasmids have contributed significantly to adaptation and evolution of Enterobacteriaceae. They promote acquisition and horizontal transmission of antimicrobial resistance (AMR), a major threat to public health. Development of new molecular approaches has helped us recognize and describe specific plasmids involved in AMR transmission. Whole genome sequencing and complete plasmid sequencing have facilitated accurate bacterial typing which helps track the spread of AMR in bacteria from different sources and geographic origin. Tracing resistance genes on definitive plasmid types has helped clarify the routes by which AMR can arise in bacteria, and this work supports the concept that several resistance genes and plasmids may have animal and environmental reservoirs.

The I-complex plasmid family includes incompatibility groups IncI1, IncIγ, IncB, IncZ and IncK (Praszkier and Pittard, 2005). In recent years, plasmids of the IncI1 and IncIγ families have been recognized in clinically relevant bacteria from human, animal and environmental sources (Carattoli, 2009)(Carattoli, 2011). Comparative analysis of fully sequenced plasmids has shown that contemporary IncI1 and IncIγ plasmids have structures consistent with those of historical reference plasmids, including conserved replication, stability, leading and transfer regions (Takahashi et al., 2011)(Smith et al., 2015)(Johnson et al., 2011). Beyond the conserved backbone, most IncI1 and IncIγ plasmids also contain variable regions such as AMR. Contemporary IncI1 and IncIγ plasmids have been recognized as major vehicles for the dissemination of Extended Spectrum Beta-Lactamase (ESBL) and plasmid AmpC genes (Carattoli, 2008). Public health concerns emerged in early 2000's in the USA, when CMY-2 producing *Salmonella* spp. and *Escherichia coli* of animal origin were identified and shown to be transmitted to humans (Fey et al., 2000)(Winokur et al., 2000). The bla_{CMY} genes were found on two major plasmid types of the IncC (formerly IncA/C_2) and IncI1 groups (Hopkins et al., 2006). A few years later, *E. coli* that produce ESBLs (CTX-M-15, CTX-M-1, TEM-52) were isolated from food-producing animals in several European countries.

Emergence of these resistant bacteria was attributed to selective pressure imposed by the indiscriminate use of third generation cephalosporins in veterinary medicine (Liebana et al., 2013). IncI1 was recognized as one of the most pervasive plasmid type in ESBL producers of animal origin (Smith et al., 2015).

This review aims to update information about prevalence and epidemiology of IncI1 and IncIγ plasmids and provides new data on the phylogeny of these plasmid families.

IncI1 structure and functions

The prototypes of the IncI1 group are plasmids R64 and ColIb-P9. Plasmid R64 was initially isolated from *Salmonella enterica* (*S.*) serovar Typhimurium in 1966 and completely sequenced in 2010 (AP005147) (Sampei et al., 2010), and ColIb-P9 was isolated from *Shigella sonnei* P9 and completely sequenced in 2014 (AB021078). These plasmids were important models for understanding plasmid conjugative transfer, replication control and incompatibility behaviour. The complete genome sequences of R64 and ColIb-P9 are still the benchmarks for annotation of contemporary fully sequenced IncI1 plasmids.

The IncI1 backbone is organized into four major conserved regions encoding replication, stability, leading and conjugative transfer (Figure 1). A negative control circuit that maintains a constant number of plasmid copies in the cell regulates the replication of IncI1 plasmids. Complete information regarding the mechanisms and control of replication in I-complex plasmids can be found in the review by Praszkier and Pittard (Praszkier and Pittard, 2005). Briefly, the most relevant genes governing replication in these plasmids are *inc*, *repY*, and *repZ*. The *inc* gene encodes *inc* RNA, which is involved in controlling replication, copy number and incompatibility behavior of the plasmid. The *repY* and *repZ* genes are equivalent to *repB* and *repA*, respectively, in other I-complex plasmids. These genes encode the regulator of *repZ* and the replicase, respectively. The origin of replication (*ori*) and the CIS–ter spacer between *repZ* and the *ori* are essential for initiation and termination of replication (Hama et al., 1990)(Mori et al., 1995)(Shiba and Mizobuchi, 1990). The

replication region is followed by a variable region whose content differs depending on the specific IncI1 plasmid. R64 and ColIb-P9 are equipped with variable regions that differ substantially from those identified in clinically relevant resistance plasmids. R64 confers resistance to tetracyclines, due to the presence of Tn*10* that is itself integrated into Tn*5393*, a transposon that confers resistance to streptomycin. The arsenic resistance cluster of R64 is interrupted by insertion of IS*2* into the *arsA1* gene (Sampei et al., 2010). ColIb-P9 does not carry resistance determinants, but instead encodes colicin Ib production and immunity to colicin Ib (Sampei et al., 2010).

The stability region includes many genes responsible for maintenance of the plasmid during cell division. These include partitioning genes *parA* and *parB*, the products of which drive the replicated plasmid DNA to the poles of the daughter cells before division is completed. These genes contribute to the incompatibility of IncI1 and IncIγ plasmids (Gerdes et al., 2004) (Sampei et al., 2010)(Takahashi et al., 2011).

The leading region that resides between the *impB* and *ygfB* genes is the first DNA segment to enter the recipient cell during the transfer reaction. This region is conserved among many IncI1 plasmids (Sampei et al., 2010). This region encodes important factors that help the plasmid counteract the defense response of the recipient cell upon entry of the plasmid in the conjugative process. These leading region factors include ArdA, an antirestriction protein that alleviates the activity of the EcoK1 restriction endonuclease encoded by the host (Nekrasov et al., 2007); and PsiAB, proteins that inhibit the recipient cell's SOS response (Loh et al., 1990). The genes that encode these factors are not only present in prototypic IncI1 plasmids, but also in clinically relevant IncI1 plasmids isolated in recent years.

The conjugative transfer of IncI1 plasmids is encoded by a region of 54 kb consisting of genes that are clustered functionally: *traA-D*, *pilJ-V*, *tra/trb*, *nikAB* and *excA-traY* (Sampei et al., 2010)(Komano et al., 2000)(Furuya and Komano, 1994). Conjugative transfer starts at the *oriT* site. The *nikA* and *nikB* genes, encoded in an operon adjacent to *oriT*, are essential for the initiation of DNA transfer by relaxosome formation at *oriT* (Furuya and Komano, 2003). The *trbA-C* and the

106 *traB-C* genes have been described as indispensable for R64 transfer (Kim et al., 1993)(Furuya and
107 Komano, 1996). The *pilJ-V* cluster consists of 14 genes responsible for the biogenesis of a Type IV
108 thin pilus (Kim and Komano, 1997). There is a shufflon at the 3' end of the *pilV* gene. The shufflon
109 is a site-specific recombination system that consists up to four DNA segments, randomly rearranged
110 by the activity of a site-specific recombinase of the tyrosine family (Rci). Shufflon-mediated
111 recombination generates variants of the *pilV* gene (Komano, 1999)(Komano et al., 1987)(Brouwer
112 et al., 2015)(Gyohda et al., 2006). IncI1 plasmids also encode the ExcA-TraY exclusion system.
113 This system prevents conjugation initiation with other IncI1-containing recipient cells by surface
114 exclusion (Furuya and Komano, 1994). The *pndCA* genes that are adjacent to the *traY* gene encode
115 a toxin-antitoxin system that promotes post-segregational killing of daughter cells that do not
116 receive the plasmid when the cell division is completed (Furuya and Komano, 1994). When found
117 on resistance plasmids, addiction systems work synergistically with the positive selection exerted
118 by antimicrobials, thus promoting persistence of these plasmids in bacterial populations.

119 ## IncI1 and IncIγ plasmid evolution

120 Within the I-complex plasmid family there are two compatible groups of plasmids named IncI1
121 (also known as IncIα) and IncIγ. The R621a plasmid isolated from *S.* Typhimurium in the 1970's is
122 the prototype of the IncIγ group; it was completely sequenced in 2011 (AP011954) (Takahashi et
123 al., 2011). More than 80% of the R621a backbone (including replication, leading, and transfer
124 regions) exhibits 96–98% identity with R64 or ColIb-P9. However, R621a was found to stably
125 transfer into *E. coli* harbouring an IncI1 plasmid, demonstrating that IncI1 and IncIγ were
126 compatible with each other (Hedges and Datta, 1973). Sequence comparison showed three major
127 differences in the replication (*inc*), partition (*parAB*) and entry exclusion (*traY-excA*) systems that
128 distinguish the IncI1 and IncIγ groups (Takahashi et al., 2011). R621a evolution has been described
129 as a series of sequence replacement events occurring most likely from a prototype plasmid similar
130 to ColIb-P9. These events involved deletion of the colicin Ib gene cluster, acquisition of a novel
131 *parAB* segment and replacement of a 1.5 kb segment between the 3'-end of the *traY* gene and the

5'-end of the *excA* gene. The 163 amino acids at the C terminus of the R621a ExcA show 56% sequence identity with the R64 ExcA protein (Takahashi et al., 2011). Exchanges in the exclusion properties allowed the R621a ancestor to succeed in entering a cell harbouring an IncI1 plasmid. Nucleotide variations in the *inc* gene allowed replication to occur, thus establishing the discrete variant now known as the IncIγ incompatibility group. The Inc-RNA encoded by the *inc* gene of I-complex plasmids folds into a single stem-loop structure (Asano et al., 1991) (Praszkier and Pittard, 2005). There are important differences in the Inc-RNA sequence encoded by IncI1 and IncIγ that contribute to the compatible behaviour expressed by the two plasmids (Figure 2). However, the *repZ* and *repY* genes, located immediately upstream of the *inc* gene, are identical in the two plasmid types (Takahashi et al., 2011). Interestingly, several contemporary plasmids show intermediate levels of evolution from IncI1 to IncIγ groups. For instance, ST7, ST31, ST37 plasmids show the *traY-excA* genes of R621a but the *inc* sequences are 100% identical to that of R64. ST7 has the *parAB* genes of R64; ST37 has the *parAB* of R621a; and ST31, instead of *parAB* genes, has the *soj-yfhA* genes, previously described as a partitioning system (Smith et al., 2015)(Smet et al., 2010).

Ten years of IncI1 plasmid typing and subtyping

The PCR-Based Replicon Typing (PBRT) method was proposed in 2005 to detect plasmid content in Enterobacteriaceae (Carattoli et al., 2005). It was devised based on replicons that expressed incompatibility towards reference plasmids used for the Inc-classification scheme based on conjugation (Datta and Hedges, 1971)(Couturier et al., 1988). In the decade following the introduction of the PBRT method, thousands of enterobacterial strains were classified on the basis of their replicon content using PBRT. Where possible, plasmids typed by PBRT maintained the same nomenclature conventions established for the conjugation-based. For example, PCR detection of the *inc* gene of R64 resulted in assignment of those contemporary plasmids to the IncI1 family. The Inc prefix continues to be used to name plasmid families, even when the Inc-phenotype has not been formally confirmed by conjugation with reference plasmids.

PBRT detected IncI1 plasmids by repI PCR; specifically one primer was designed inside the *inc* gene of the R64 plasmid and the other was designed downstream of that gene (Carattoli et al., 2005). This PCR correctly detected IncI1 plasmids, but in some cases also gave positive results for IncIγ plasmids. Recently, new primers were devised in the variable region of the *inc* gene to allow for discrete amplification of I1 and Iγ replicons (Figure 2)(Carloni et al., 2017).

The concept that plasmids are, in essence, *genomes* within the bacterial genome, suggested that a Multilocus Sequence Typing (MLST) approach could be used to further discriminate plasmids within each Inc-group (García-Fernández et al., 2008). In 2008, only three complete IncI1 plasmid sequences were available in the GenBank sequence database (R64-AP005147, ColIb-P9-AB021078 and pNF1358-DQ017661). These plasmids were compared and five loci were selected within conserved regions as candidates for the MLST scheme. These included *pilL* of the cluster for type IV pilus biogenesis; *sogS*, a primase that acts in discontinuous DNA plasmid replication; *ardA*, encoding a type I antirestriction enzyme; *trbA*, involved in plasmid transfer; and the same repI PCR target for the *inc* gene used in the PBRT method (García-Fernández et al., 2008). The IncI1 plasmid Multilocus Sequence Typing (pMLST) scheme was established at the PubMLST web site hosted by the University of Oxford (https://pubmlst.org/plasmid/). This website uses two linked databases powered by the BIGSdb genomics platform. The sequence definition database contains allele sequences and MLST profile definitions, whereas the isolate database contains epidemiological information on the strains from which the plasmids are obtained. In the isolate database, a field is dedicated to beta-lactamase genes identified on plasmids. After identifying the alleles for all the pMLST loci, a plasmid sequence type (ST) is assigned based on the combination of the alleles. A Clonal Complex (CC) is assigned when several STs are grouped by their similarity to a central allelic profile.

Since 2008, more than 2000 complete Enterobacteriaceae plasmids have been added to the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/) (Orlek et al., 2017). The PlasmidFinder web-tool for *in silico* detection of plasmids in WGS data has been built on replicon

sequences of more than 550 fully sequenced plasmids available at NCBI (Carattoli et al., 2014). On April 2018, 145 putative complete IncI1 and IncIγ plasmid sequences were identified by BLASTN against the NCBI nucleotide database, using the IncI_1_Alpha_AP005147 PlasmidFinder probe (cut off >90% nucleotide identity) that were not included in the pMLST database. These were downloaded from the NCBI nucleotide database and imported into the pMLST database. Incomplete plasmid sequences were removed. Beta-lactamase genes were identified by ResFinder (Zankari et al., 2012) and were added to the records along with available epidemiological data. Complete plasmid sequences are now available for 177 of the IncI1 and IncIγ plasmids in the pMLST database. Among them, 158 present *inc* genes that are 100% identical to those of the reference R64 or ColIb-P9, and four have one single point mutation within the *inc* gene, with unknown effects on functional compatibility. The remaining 15 fully sequenced plasmids show *inc* genes identical to the IncIγ reference plasmid R621a (data not shown).

A total of 774 records (597 tested by pMLST and 177 with pMLST alleles identified from whole genome sequences) are currently included in the pMLST database, and 291 STs have been assigned to the IncI1 and IncIγ family (April 2018). The main bacterial species included are *E. coli* (70%), *Salmonella* spp. (28%), *Shigella* (2%), *Klebsiella pneumoniae* (0.8%) and *Enterobacter aerogenes* (0.1%). Sources of the strains include humans (48%), poultry (32%), pigs (6%), cattle (5%), dogs (4%), horses (0.6%) and others (5%). The main countries that have contributed to the dataset are The Netherlands, USA, UK, and Germany.

The phylogenetic GrapeTree (Zhou et al., 2017) of IncI1 and IncIγ plasmids was obtained at the pMLST website using ST and country of isolation datasets. The most represented STs are labelled, and slices are coloured by country of isolation (Figure 3). This tree illustrates the current content of the pMLST database, the representation of STs, and their geographical origin.

IncI1 and IncIγ phylogeny update

The conspicuous allelic diversities found in the five loci of pMLST suggest that this scheme is highly discriminatory for distinguishing IncI1 and IncIγ plasmid variants. Previous phylogenetic

studies assigned several STs to CCs. These CCs fit well with epidemiological features of their bacterial hosts, supporting the association of specific CCs with specific resistance gene variants, and bringing new insights to the hypothesis that diverse populations of human or animal bacteria were hosting *epidemic* IncI1 and IncIγ plasmids (Leverstein-van Hall et al., 2011)(Folster et al., 2010). *Epidemic* plasmids are defined as indistinguishable plasmids identified in different periods, sources and bacterial species in distant geographical areas (Carattoli, 2009)(Figure 3).

We also provide an additional revision to the IncI1 and IncIγ phylogeny here. Concatenated FASTA files of the five loci sequences (1380 bp), derived from the 291 STs, were downloaded from the pMLST website. The concatenated FASTA sequences were aligned with MAFFT (Katoh and Standley, 2013) and a ST-phylogenetic tree was constructed. The ST-phylogenetic tree showed two major branches defined as Clusters I and II, respectively. ST236 generated a single branch from the root that was defined as Cluster III. Two main groups of branches within Cluster I were defined as Clades 1.1 and 1.2, and four major Clades, named 2.1, 2.2, 2.3 and 2.4, respectively, were defined in Cluster II. The ST-phylogeny was used to assign plasmids in the pMLST database to Clusters, Clades and genetic types. Among the 774 IncI1 and IncIγ records, 217 belonged to Cluster I, 556 to Cluster II and 1 to Cluster III. The STs previously grouped as CC-2, CC-3, CC-5, CC-7, CC-12, CC-31, respectively were identified as separate Clades in the ST-phylogenetic tree (Figure 4).

Plasmids belonging to the IncIγ subtype were found in Clade 1.1 and in separate small clades (1.7, 1.8, 2.5 and 2.6) within both Clusters I and II (indicated with "γ" and grey lines in Figure 4). Using the *repI* allele sequences, the MAFFT phylogenetic tree distinguished two different branches for the I1 and Iγ replicon variants, the latter associated with STs that usually contain the *repI*-4 and *repI*-14 alleles (Supplementary Figure 1, Panel A).

The separation between the two major Clusters in the ST-phylogeny was due to the *pilL* locus: nucleotide identity among *pilL* alleles within the same cluster was very high (>98% for Cluster I and >97% for Cluster II). However, *pilL* nucleotide identity between Clusters I and II, dropped to 90% (https://pubmlst.org/plasmid). Specific *pilL* alleles were exclusively segregated in Cluster I

and Cluster II, respectively, which generated the dichotomy of the ST-phylogenetic tree: *pilL-1* and *pilL-2* (sharing 98% nucleotide identity) were the most frequent *pilL* alleles in Cluster II, while *pilL-3* and *pilL-10* (99% nucleotide identity) were the most frequent in Cluster I.

A phylogenetic tree of the entire *pil* cluster was obtained by BLAST2N sequence analysis. This was performed using the 11.9 kb *pil* region, including genes from *pilV* to *pilI* of plasmid ColIb-P9 (between nt. positions 77762-89660, Acc. No. AB021078). The *pil* cluster that was used as query sequence was compared with 52 fully sequenced IncI1 and IncIγ plasmids representing most of the Clades and genotypes of the whole ST-phylogeny. This analysis reproduced a two-branched phylogenetic tree (average 92% nucleotide identity; Supplementary Figure 1, Panel B) and suggested that two main ancestral *pil* clusters exist in contemporary IncI1 and IncIγ plasmids. To our knowledge, the evolution and differences distinguishing these *pil* clusters have not been investigated yet.

The impact of IncI1-IncIγ on beta-lactamase epidemiology

In the last decade, several ESBL and AmpC producing Enterobacteriaceae have been described worldwide. In most of the literature published on this topic, pMLST and WGS analysis have been used to identify plasmids associated with the most relevant ESBL and AmpC genes.

In the following sections, the epidemiology of widely disseminated IncI1 and IncIγ resistance plasmids is discussed.

- **The spread of *bla*$_{\text{CTX-M-15}}$-IncI1 plasmids**

  CTX-M-15 is one of the most dominant ESBLs worldwide. Association between *bla*$_{\text{CTX-M-15}}$ and IncF-type plasmids carrying FII, FII-FIA or FII-FIA-FIB replicons in the high-risk clone *E. coli* ST131, has been described extensively (Mathers et al., 2015). However, in other *E. coli* clones and enterobacterial species, especially those of animal origin, *bla*$_{\text{CTX-M-15}}$ has been described frequently in association with IncI1 plasmids (Carattoli, 2011). Sixty-one records of *bla*$_{\text{CTX-M-15}}$-IncI1 plasmids belonging to 25 different STs are present in the pMLST database. These were primarily isolated from cattle and humans in UK, France, The

Netherlands, USA, Sweden and Germany. The most frequent plasmid types were ST31, ST16 and ST37 (Supplementary Table 1).

The epidemiology of these major STs, their worldwide spread, and the association with *bla*$_{CTX-M-15}$ have been reported extensively. The *bla*$_{CTX-M-15}$-IncI1 ST31 plasmid has been associated with *E. coli* from cattle and humans in UK, The Netherlands, Pakistan and Honduras, and with *S.* Anatum, *S.* Infantis, *S.* Ohio and *S.* Typhimurium in the UK (Smith et al., 2015)(Hopkins et al., 2006). These studies demonstrated that, in addition to poultry and pigs, cattle could serve as an animal reservoir for relevant ESBLs with potential transmission to humans (Madec et al., 2012). The complete sequence of a *bla*$_{CTX-M-15}$-IncI1 ST31 plasmid was also obtained from an isolate of ceftriaxone-resistant *S.* Typhi from the blood of a child in Bangladesh (Djeghout et al., 2018). The *bla*$_{CTX-M-15}$-IncI1 ST31 plasmid from a high-risk clone *E. coli* O104:H4 that caused an important outbreak in Germany has also been described (Buchholz et al., 2011)(Yamaichi et al., 2015).

The *bla*$_{CTX-M-15}$ gene identified during an outbreak of *S. sonnei* in South Korea was found by sequencing to be located on a IncI1 ST16 plasmid (Kim et al., 2014). This was the first time this gene had been reported on a plasmid of this sequence type. A *S.* Enteritidis isolate containing the *bla*$_{CTX-M-15}$-IncI1 ST16 plasmid was also described in China by the National Institute for Communicable Disease Control and Prevention (ICDC); the isolate was part of a surveillance program conducted from 2005-2010 (Wong et al., 2016). The *bla*$_{CTX-M-15}$-IncI1 ST16 plasmid has also been described in *E. coli* isolates from Ireland and UK, from human and cattle sources, respectively (https://pubmlst.org/plasmid/).

To gain an insight into the evolution of the IncI1 family, the *bla*$_{CTX-M-15}$ gene environment were investigated in plasmids assigned to different Clusters within the ST-phylogenetic tree (Tsafnat et al., 2009). Six fully sequenced ST31 and 3 ST16 plasmids, from different origin and sources, carry an 2971bp IS*Ecp1*-*bla*$_{CTX-M-15}$ transposition unit inserted in the *tnpA* gene of a *bla*$_{TEM-1}$-carrying Tn*2*-like transposon (Holt et al., 2013)(Kim et al., 2014). The six

ST31 plasmids, that were fully sequenced, carry an 2971bp transposition unit inserted between the *yagA* and *impB* genes flanked by 5bp DR (TTATC). This is in contrast with the three ST16 plasmids, in which the integration occurred in the region upstream the *cib* gene and the transposition unit was flanked by the 5bp DR, GAAAA. This suggests independent integration of IS*Ecp1*-*bla*$_{\text{CTX-M-15}}$ transposition unit in the two STs (Smet et al., 2010)(Ahmed et al., 2012). In ST37 plasmids the IS*Ecp1*-*bla*$_{\text{CTX-M-15}}$ environment was different from those of ST31 and ST16, and the transposition unit was integrated within the *yagA* gene (Zong et al., 2015)(Brouwer et al., 2014). From this analysis, we can conclude that the IS*Ecp1*-*bla*$_{\text{CTX-M-15}}$ transposition unit was independently acquired by the different plasmid types, but for each ST there was consistency in the transposition unit and integration site. These data suggest that well-conserved IncI1 plasmids existed across huge geographical distances and occurred in different bacterial strains from animal and human sources.

- **Animal reservoirs of the *bla*$_{\text{CTX-M-1}}$-IncI1 plasmids**

The *bla*$_{\text{CTX-M-1}}$ ESBL gene variant has been described in many IncI1 plasmids. The pMLST database contains 179 *bla*$_{\text{CTX-M-1}}$ positive isolates, including143 from *E. coli*, 35 from *Salmonella* (mainly serovars Typhimurium and Paratyphi B) and one from an undetermined species of bacteria. Thirty-nine were identified in isolates from humans and 122 were from animals. The *bla*$_{\text{CTX-M-1}}$ gene was identified in 33 different STs, with a large proportion from ST7 (80/179; 45%) and ST3 (53/179; 30%, Supplementary Table 2) plasmids. In the ST-phylogenetic tree, 169 of the *bla*$_{\text{CTX-M-1}}$ plasmids were in Cluster II, mostly in Clade 2.1 (141/169; 83 %), which included both ST7 and ST3 genotypes (Figure 4). The *bla*$_{\text{CTX-M-1}}$ gene environments were investigated in 12 fully sequenced ST3 and one ST7 plasmid. In all of them, an IS*Ecp1* was found at the 5'-end of the *bla*$_{\text{CTX-M-1}}$ gene. The ST3 plasmids carry a 3003bp IS*Ecp1*-*bla*$_{\text{CTX-M-1}}$ transposition unit inserted in the shufflon region flanked by 6bp DR (TAAAAA) (Zurfluh et al., 2014)(Baron et al., 2016) . In one ST3 plasmid (KJ484629),

IS*Ecp1* is interrupted by IS*Kpn26* integration (Wang et al., 2014). The ST7 plasmid carries a 2964bp IS*Ecp1*-*bla*$_{\text{CTX-M-1}}$-*orf477Δ* insert interrupted the *yacB* gene, no DR was present (Brouwer et al., 2014). From this analysis, we can conclude that *bla*$_{\text{CTX-M-1}}$ has been acquired by independent events of integration of different transposition units in ST3 and ST7 plasmids, and that this occurred after the divergence of different sub-lineages within the Clade 2.1 (Figure 4).

The epidemiological relevance of the ST7 plasmids is apparent based on the conspicuous number of records in the pMLST database corresponding to *E. coli* and *Salmonella* spp., mostly from poultry sources mainly isolated in The Netherlands, Germany and Ireland (Figure 3; https://pubmlst.org/plasmid/).

ST3 plasmids were also widespread in several countries. The pMLST database contains records mainly from UK, France and The Netherlands, from *E. coli* and *Salmonella* spp., from both poultry and human sources (Figure 3; Supplementary Table 2).

ST3 is the most commonly reported *bla*$_{\text{CTX-M-1}}$-IncI1 plasmid in Europe; it has been described in different *E. coli* lineages isolated in many countries (Leverstein-van Hall et al., 2011) (Touzain et al., 2018)(Madec et al., 2016) (Niero et al., 2018)(Accogli et al., 2013)(Smith et al., 2015)(Wang et al., 2014). In France, ST3 plasmids were described in pathogenic *E. coli* (APEC) strains obtained at slaughterhouses or from diseased broilers (Touzain et al., 2018), in drinking water (Madec et al., 2016), and in human *E. coli* isolates (Madec et al., 2015). In Italy, the UK, Poland and Switzerland *bla*$_{\text{CTX-M-1}}$-IncI1 ST3 plasmids have been described in *E. coli* and *Salmonella* spp. from food producing animals (Niero et al., 2018)(Accogli et al., 2013)(Smith et al., 2015)(Wang et al., 2014). In two studies, *bla*$_{\text{CTX-M-1}}$-IncI1 plasmids were, for the first time, clearly shown to move between *E. coli* strains from humans and animals residing in the same geographical region. The same plasmid was not only identified within the same bacterial clone as expected, but more importantly, found in unrelated *E. coli* lineages (Fischer et al., 2014)(Dierikx et al., 2010).

The current literature on plasmid types and their epidemiology highlights the importance of identifying AMR in commensal bacteria as well as pathogenic bacteria. This work has also highlighted the relevance of horizontal gene transfer of some successful plasmids in the gut of colonized animals.

- **Worldwide spread of *bla*$_{CMY-2}$-IncI1 and IncIγ plasmids**

The plasmid AmpC beta-lactamase CMY-2 is currently the most common mechanism conferring resistance to cefoxitin in Enterobacteriaceae (Philippon et al., 2002). The *bla*$_{CMY-2}$ gene has been found worldwide in *Salmonella* spp. and *E. coli* (Jacoby, 2009)(Mataseje et al., 2010)(Denisuik et al., 2013), and generally resides on transferable plasmids belonging to different Inc-groups, including IncI1 and IncIγ, IncA/C, IncF, and IncK (Naseer et al., 2010)(Carattoli, 2009).

The pMLST database contains 205 *bla*$_{CMY-2}$ records, 90 of which are plasmids from *E. coli*, and 111 of which are plasmids from *Salmonella* spp. (mostly serovars Heidelberg and Typhimurium). These records also include one plasmid from *Klebsiella pneumoniae*, and 3 plasmids for which the bacterial species was not reported. Sources included humans (112 strains) and animals (62 strains, 61% of which were poultry). Most of the records were submitted by USA, Canada, Taiwan and the UK (Supplementary Table 3).

The *bla*$_{CMY-2}$ gene has been identified in association with 77 different IncI1 and/or IncIγ STs, including ST12 (38%) and ST66 (5%), both members of CC-12. The *bla*$_{CMY-2}$ gene was also found in members of CC-2, including ST2 (11%), ST23 (3%) and ST17 (0,5%). Based on the ST-phylogenetic tree, of 199 *bla*$_{CMY-2}$-IncI1 and 9 *bla*$_{CMY-2}$-IncIγ plasmids, 177 (86%) were in Cluster II and 28 (14%) in Cluster I (Figure 4, Supplementary Table 3).

Analysis of 19 fully sequenced ST2 and ST12 plasmids demonstrated that *bla*$_{CMY-2}$ gene was mobilized by an IS*Ecp1* or IS*1294*/IS*1294b*. In 8/9 ST12 plasmids the 3910 bp IS*Ecp1*-*bla*$_{CMY-2}$-*blc*-*sugE* transposition unit inserted in the *yagA* gene , flanked by the 5bp DR (TGGGT) (Smith et al., 2015). (da Silva et al., 2017). In one ST12 plasmid (CP012929) the

3078 bp IS*Ecp1*-*bla*$_{CMY-2}$ transposition unit is inserted in the *ybaA* gene, flanked by the 5bp DR (TGGTT) (Labbé et al., 2016).

In 5 of 10 ST2 plasmids sequenced, a 4827bp IS*1294*-ΔIS*Ecp1*-*bla*$_{CMY-2}$-*blc*-*sugE* insert interrupted the *cia* gene (Tagg et al., 2014). In four other ST2 plasmids, a 5063bp IS*1294b*-ΔIS*Ecp1*-*bla*$_{CMY-2}$-*blc*-*sugE* insert interrupted the *yafA* gene. In only one ST2 plasmid a smaller 4624 bp IS*1294b*-ΔIS*Ecp1*-*bla*$_{CMY-2}$-*blc*-*sugE* insert, due to a deletion upstream the *sugE* gene, interrupted the *yafA* gene.. In these latter plasmids, other major rearrangements occurred in the plasmid scaffold in the region downstream of the replication termination site *ter*. The identification of two different mobile elements integrated in two different sites in ST2 plasmids suggests that a more discriminatory plasmid typing method may be needed to fully understand the epidemiology of this ST, and whole plasmid sequencing may help support a deeper analysis of these plasmids for epidemiological purposes (Tagg et al., 2014). Despite the fact that the pMLST has already assigned 291 different STs for the 774 plasmids of the IncI1 and IncIγ group, it is possible that the discriminatory power of this method is still too low to adequately represent the diversity of this plasmid type.

Strains and plasmid relatedness were investigated for 93 CMY-2-producing clinical and commensal *E. coli* isolates collected from 2006 to 2012 from humans, retail poultry meat, broilers, and dogs in Denmark (Hansen et al., 2016). In this study, ST12 was identified in 52% of the *E. coli* isolates from all sources except healthy humans and healthy dogs. However, ST2 was found in 30% of the *E. coli* isolated from human patients in 2009 and several dogs in Denmark from 2011 to 2012. (Hansen et al., 2016). Complete sequencing of ST2 plasmids from a human and a canine isolate showed that the plasmids were identical except for 36 additional nucleotides in the shufflon region of the human isolate, and a nonsynonymous SNP in both *ychA* and *repA4* genes. This study demonstrated that pMLST was discriminatory enough to recognize almost identical ST2 plasmids despite their

different source and time of isolation. IncI1 ST2 plasmids have also been described as predominant in *E. coli* isolates from healthy dogs in France (Haenni et al., 2014).

Examples of *bla*$_{CMY-2}$-IncI1 ST12 plasmids found to be indistinguishable by Restriction Fragment Length Polymorphism (RFLP) have been identified in *Salmonella* spp. of human origin isolated in USA and in *E. coli* of avian origin from Italy. There was not any apparent epidemiological link, such as the period of isolation, history of international travel, or importation of food, that could explain the spread of the same plasmid in the two countries. Strains were collected for independent national surveillance studies focused on cephalosporin resistance in humans and animals, performed at the Centers for Disease Control and Prevention in Atlanta (USA) and at the Istituto Superiore di Sanità in Rome (Italy). The identification of the same plasmid in unrelated bacterial isolates suggested that the *bla*$_{CMY-2}$-IncI1 ST12 plasmid has been described in several continents and in different years, hosted by both zoonotic pathogens and commensal bacterial species, circulating in both human and animal sources (Folster et al., 2011) (Accogli et al., 2013).

Examples of *bla*$_{CMY-2}$-IncI1 ST12 plasmids were also identified in *E. coli* found to colonize both Dutch farmers and broilers on their farms, suggesting that there is some professional risk for farmers of exposure to and colonization by AMR bacteria. The success of *bla*$_{CMY-2}$-IncI1 ST12 plasmids was also shown by similar colonization studies in animals and farm workers in the Netherlands (Dierikx et al., 2013). This pervasiveness was also supported in another study conducted in The Netherlands and Germany that showed high prevalence of ESBL/AmpC-producing *Salmonella* spp. and *E. coli* from poultry. The study showed that 81% of the plasmids from both countries were *bla*$_{CMY-2}$-IncI1 ST12 (Smith et al., 2015).

In Canada, a collection of 113 CMY-2-producing *S.* Heidelberg from humans, abattoir poultry and retail poultry was investigated in 2012. Whole genome sequencing of unassembled plasmids demonstrated that 57 strains carried *bla*$_{CMY-2}$-IncI1 ST12 plasmids, and 28 of the strains were isolated from human stool samples (Edirmanasinghe et al., 2017).

The *bla*$_{CMY-2}$-IncI1 ST12 plasmid was widely identified in samples from broiler farms, broilers at slaughter, and raw chicken from retail establishments in Colombia (Castellanos et al., 2017).

From these data, we can conclude that ST12 has spread worldwide and exhibits all the characteristics of an *epidemic* plasmid.

- **The evolution of *bla*$_{TEM-20}$/*bla*$_{TEM-52}$-IncI1 plasmids**

TEM-52 β-lactamase is one of the most common ESBL types (Bielak et al., 2011). Only one amino acid, K104E, distinguishes TEM-52 from TEM-20 (Arlet et al., 1999). Tn*2* and Tn*3* transposons have been associated with both *bla*$_{TEM-20}$ and *bla*$_{TEM-52}$ variants (Wang et al., 2015)(Cloeckaert et al., 2007). The pMLST database contains 2 *bla*$_{TEM-20}$ and 39 *bla*$_{TEM-52}$-IncI1 records. Except for one *bla*$_{TEM-52}$ plasmid that belongs to ST60, all belong to ST5, ST10, ST21 or ST36, which are part of CC-5. In the ST-phylogenetic tree, 40/41 TEM-52 plasmids clustered within Cluster I, Clade 1.1, genotype 1.1.1 (Figure 4, Supplementary Table 4). The 4 fully sequenced genotype 1.1.1 plasmids available for analysis, the 4949 bp *bla*$_{TEM-52}$-Tn2c transposon was inserted in the *yagA* gene. These plasmids have also been found in *E. coli*, *S.* Infantis, *S.* Paratyphi B and *S.* Virchow from human and poultry sources from 2005 to 2018 in The Netherlands, Belgium, Norway, Switzerland, France, Denmark and Germany (https://pubmlst.org/plasmid/). The potential poultry reservoir for this ESBL was independently described in the last decade in many European countries (Saliu et al., 2017). It was demonstrated that the epidemic *bla*$_{TEM-52}$-IncI1 CC-5 plasmid circulated in Europe in diverse *E. coli* strains (phylotypes A, B1 and D) (Bielak et al., 2011). Strains were isolated from humans and poultry, indicating possible transmission of the *bla*$_{TEM-52c}$-IncI1 CC-5 plasmid between these two sources (Cloeckaert et al., 2007)(Sunde et al., 2009). Poultry was not the unique source for TEM-52 producing *E. coli*. In France, the *bla*$_{TEM-52}$-IncI1 ST36 (CC-5) plasmid was found in cattle (Haenni et al., 2012). A *bla*$_{TEM-52c}$-IncI1

plasmid was also described in Belgium in a verocytotoxin-producing *E. coli* belonging to the serogroup O26 isolated from a human patient (Buvens et al., 2010).

The $bla_{\text{TEM-20}}$ gene variant was also found on an IncI1 ST10 (CC-5) plasmid in an *E. coli* strain isolated from a broiler in Norway in 2009. The fact that identical transposons carrying the two $bla_{\text{TEM-20}}$ and $bla_{\text{TEM-52}}$ gene variants were found on the same ST plasmid suggests that the mutation that distinguishes $bla_{\text{TEM-20}}$ from $bla_{\text{TEM-52}}$ occurred after the acquisition of the transposon, within the IncI1 plasmid (Sunde et al., 2009).

Conclusion

Bacterial typing, plasmid Inc-group classification, pMLST and epidemiological data all offer information relevant to understanding the dynamics of plasmid-mediated transmission of AMR genes. The pMLST-based phylogeny is a simple and useful tool for identifying homogeneous groups within the IncI1 and IncIγ family of plasmids, thus increasing our understanding of plasmid structure and evolution, and classifying lineages and sub-lineages. Country, source, and year of isolation captured in the pMLST database complement peer reviewed literature studies support the hypothesis that highly related IncI1 plasmids have spread worldwide as successful vehicles of relevant resistance genes, including $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$. IncI1 *epidemic* plasmids have been found, and it is not yet clear what is favoring the success of IncI1 plasmids. The worldwide spread of some IncI1 plasmids cannot be explained simply by positive selection exerted by antimicrobial drugs. Success is more likely attributable to particular plasmid features that provide resistance, persistence and consequently adaptive success in their bacterial hosts.

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Figure Legends

Figure 1. Schematic maps of R64 and ColIb-P9, prototypes of the IncI1 plasmid family

Coloured arrows indicate plasmid genes and clusters and their direction of transcription. Colours and their meaning are as follows: green, the transfer locus containing the *trb/tra* gene clusters; pale blue, the type IV *pil* cluster; dark blue, the replicon; red, resistance genes; yellow, transposon-related genes and insertion sequences; brown, stabilization genes; orange, shufflon; pink, colicin gene; white, other genes. Black bold lines indicate Direct Repeats. Short black lines below the map indicate the genes used as pMLST targets. The diagram is not to scale.

Figure 2. Alignment of nucleotide sequences of the *inc* region of R64 (AP005147) and R621a (AP011954). Nucleotide differences between IncI1 and IncIγ plasmids are indicated in boldface. Gaps are marked by dashes. The predicted stem-and-loop Inc RNA sequence is indicated by black lines. The positions of the reverse primers used to distinguish I1 and Iγ replicons in the PBRT-KIT 2.0 (Diatheva srl, Cartoceto, Italy) (Carloni et al., 2017) are indicated by dot arrows above and below the target sequences, respectively.

Figure 3. GrapeTree of IncI1 and IncIγ plasmid STs and country of isolation.

A minimum spanning tree based on allelic profiles of 774 IncI1 and IncIγ plasmids was downloaded from the pMLST website (https://pubmlst.org/bigsdb?page=plugin&name=GrapeTree&db=pubmlst_plasmid_isolates).

Pie size corresponds to number of isolates. The most represented Sequence types (ST) are labelled close to their respective pie. Colours indicate country of isolation, and the legend shows the number of isolates from each country.

Figure 4. ST-phylogenetic tree based of the five pMLST loci.

The concatenated FASTA sequences files, for the five pMLST loci derived from the 291 IncI1 and IncIγ STs, were aligned with MAFFT and a ST-phylogenetic tree was constructed and visualized as

a polar view tree by the FigTreeV.1.4.3 software. Branches are coloured by primary Cluster I, II, III (red, blue and black, respectively). Clusters I and II are further divided into Clades, which are shaded and labelled. Genotype numbers are reported in the outer ring. The branches corresponding to IncIγ plasmids are indicated in grey, with genotypes in brown. Previously assigned clonal complexes (CC) associated with $bla_{\text{TEM-52}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$ are shaded in pale blue within their respective clades.

Legend to Supplementary Figure 1

Supplementary Figure 1. Phylogenetic trees of the entire *pil* cluster and the *repI* pMLST locus.

Panel A: The FASTA sequences of the *repI* alleles were aligned with MAFFT, and a phylogenetic tree was constructed and visualized as a polar view tree by the FigTreeV.1.4.3 software. Branches corresponding to the IncI1 and IncIγ subgroups are coloured blue and red, respectively. The IncIγ subgroup is shaded.

Panel B: The Neighbor-Joining phylogenetic tree of the entire *pil* cluster obtained by BLAST2N using the *pil* region of plasmid ColIb-P9 as query sequence (between nt. positions 77762-89660, Acc. No. AB021078) on 54 representative IncI1 and IncIγ plasmids, visualized by the FigTreeV.1.4.3 software. Branches are coloured and shaded by primary Cluster I and II, in red and blue, respectively.

Figure 1

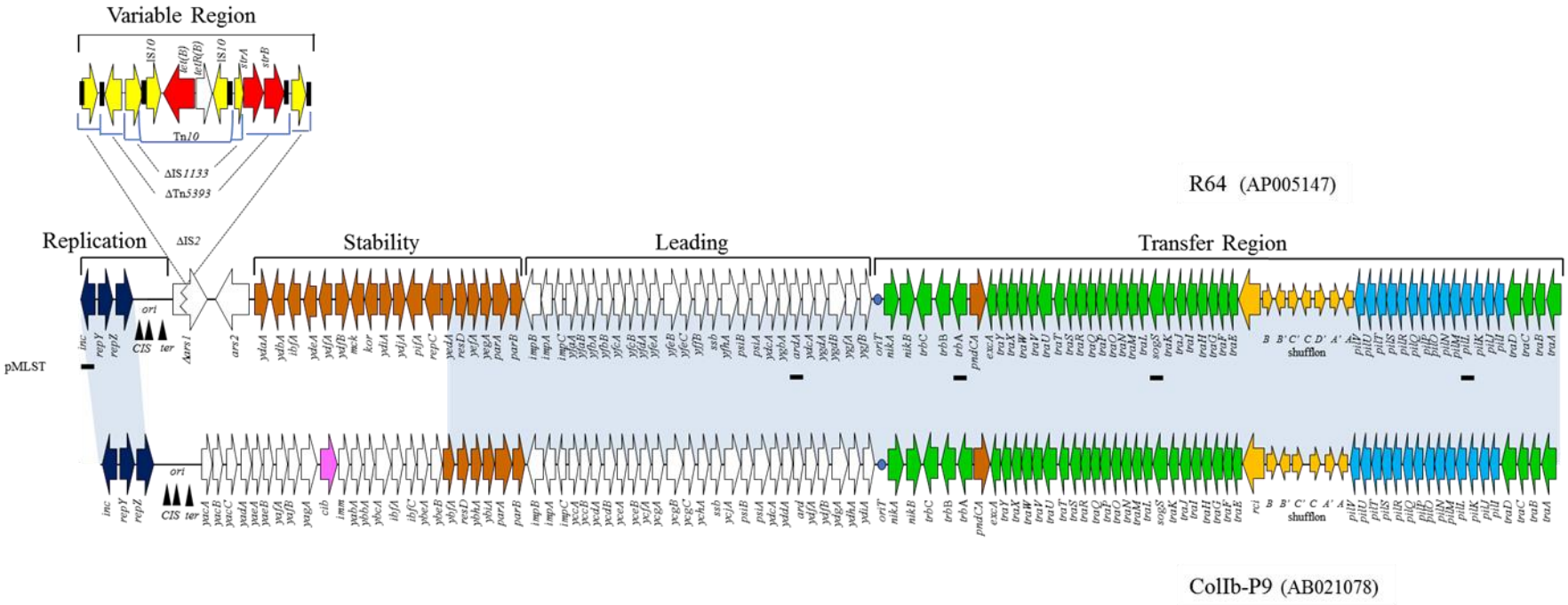

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Figure 2

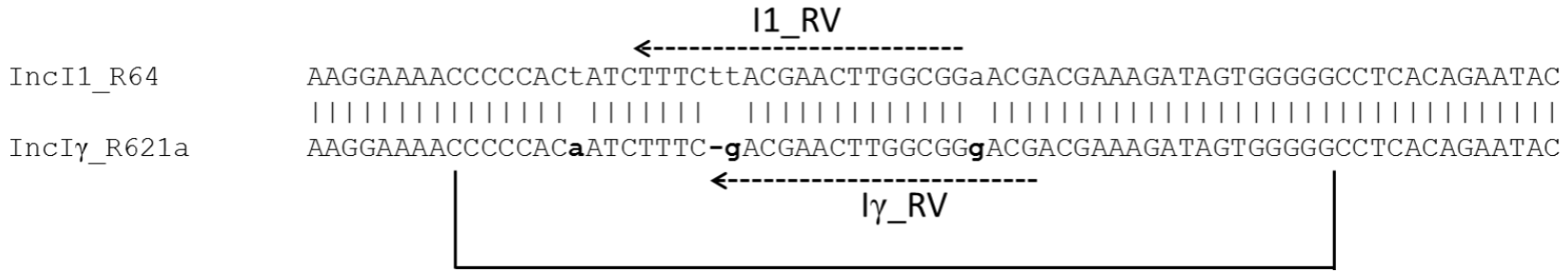
I1_RV
IncI1_R64 AAGGAAAACCCCCACtATCTTTCttACGAACTTGGCGGaACGACGAAAGATAGTGGGGGCCTCACAGAATAC
IncIγ_R621a AAGGAAAACCCCCACaATCTTTC-gACGAACTTGGCGGgACGACGAAAGATAGTGGGGGCCTCACAGAATAC
Iγ_RV

Figure 3

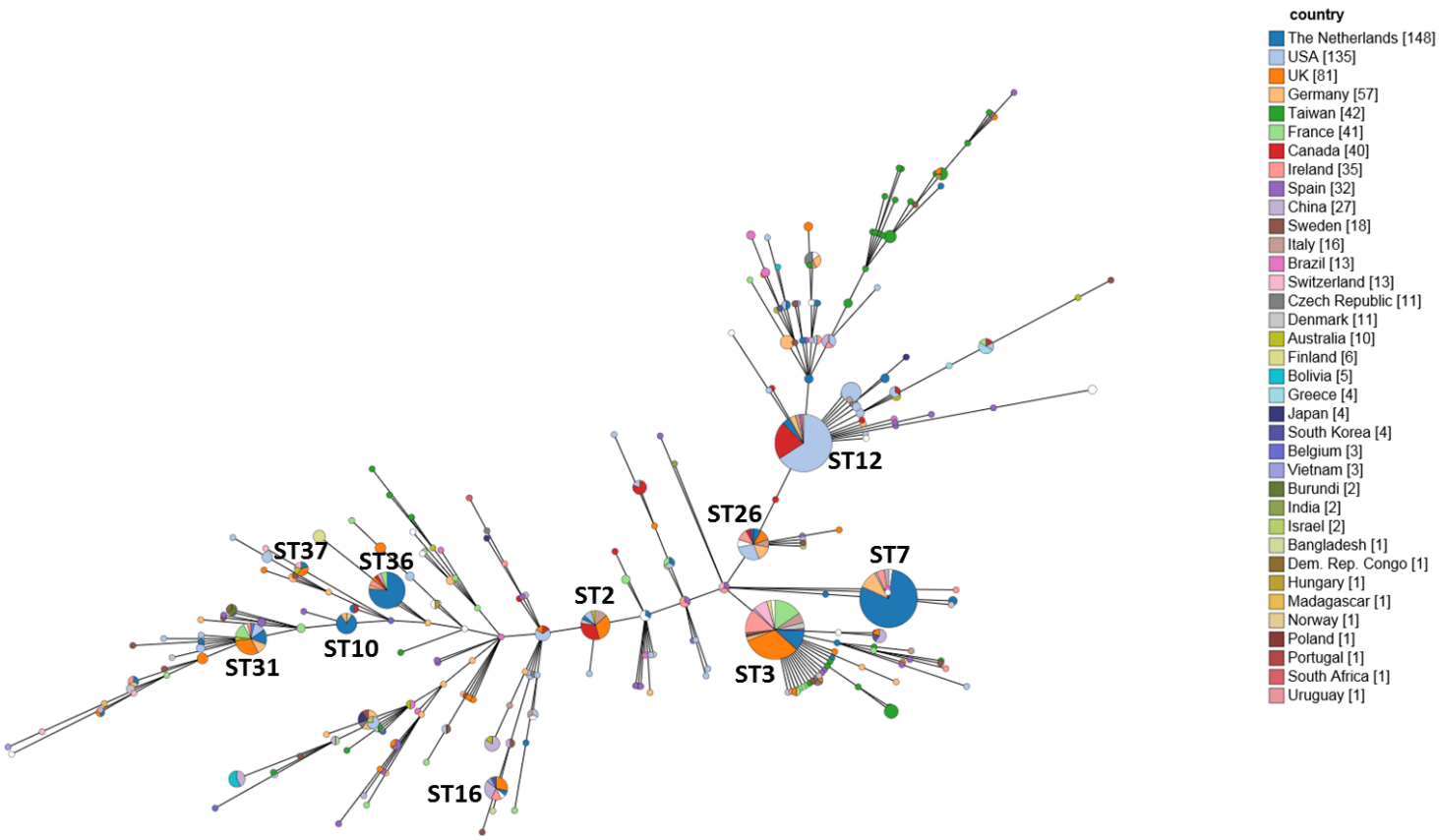
country
The Netherlands [148]
USA [135]
UK [81]
Germany [57]
Taiwan [42]
France [41]
Canada [40]
Ireland [35]
Spain [32]
China [27]
Sweden [18]
Italy [16]
Brazil [13]
Switzerland [13]
Czech Republic [11]
Denmark [11]
Australia [10]
Finland [6]
Bolivia [5]
Greece [4]
Japan [4]
South Korea [4]
Belgium [3]
Vietnam [3]
Burundi [2]
India [2]
Israel [2]
Bangladesh [1]
Dem. Rep. Congo [1]
Hungary [1]
Madagascar [1]
Norway [1]
Poland [1]
Portugal [1]
South Africa [1]
Uruguay [1]
ST12
ST26
ST7
ST2
ST3
ST37
ST36
ST10
ST31
ST16

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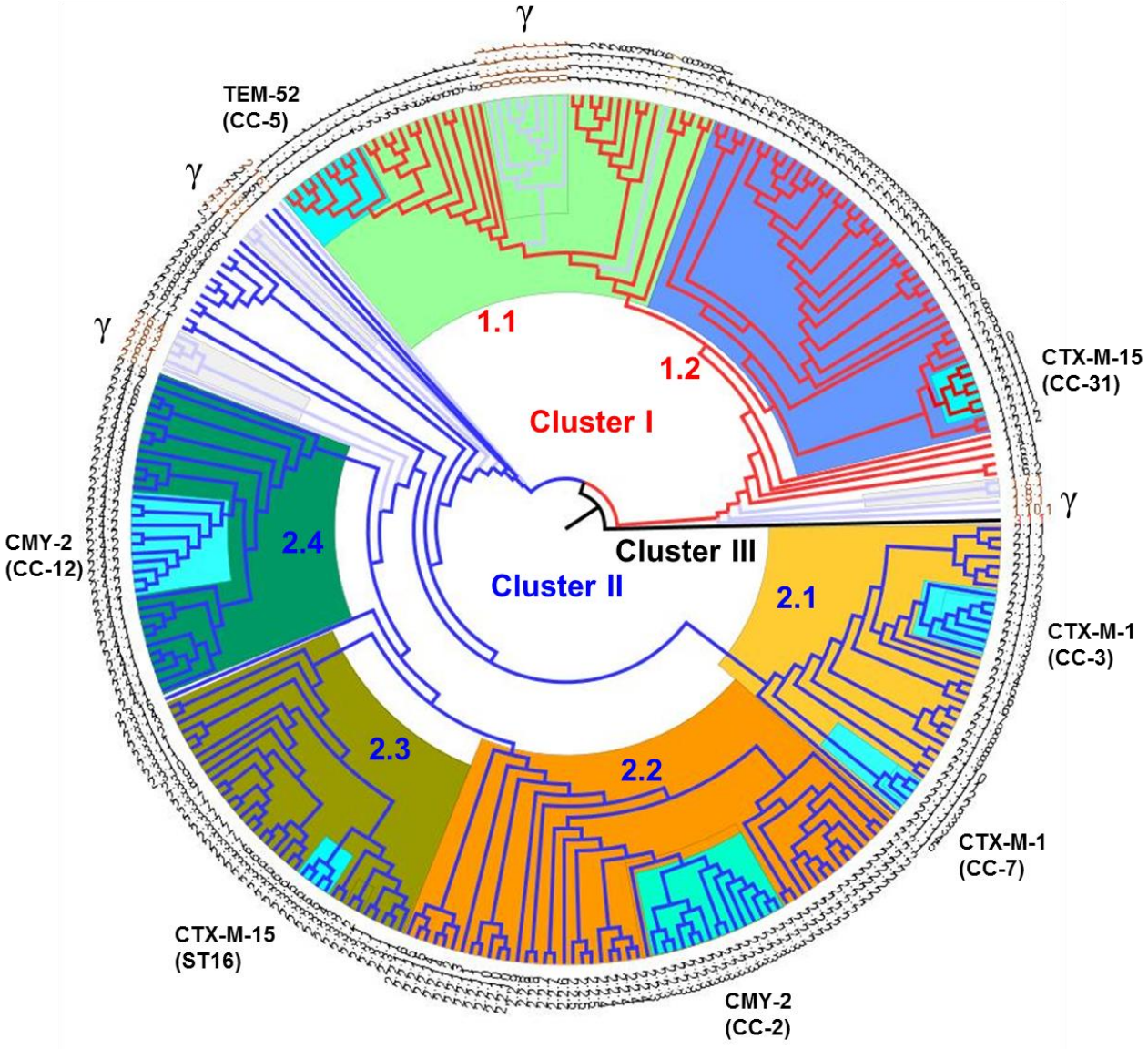
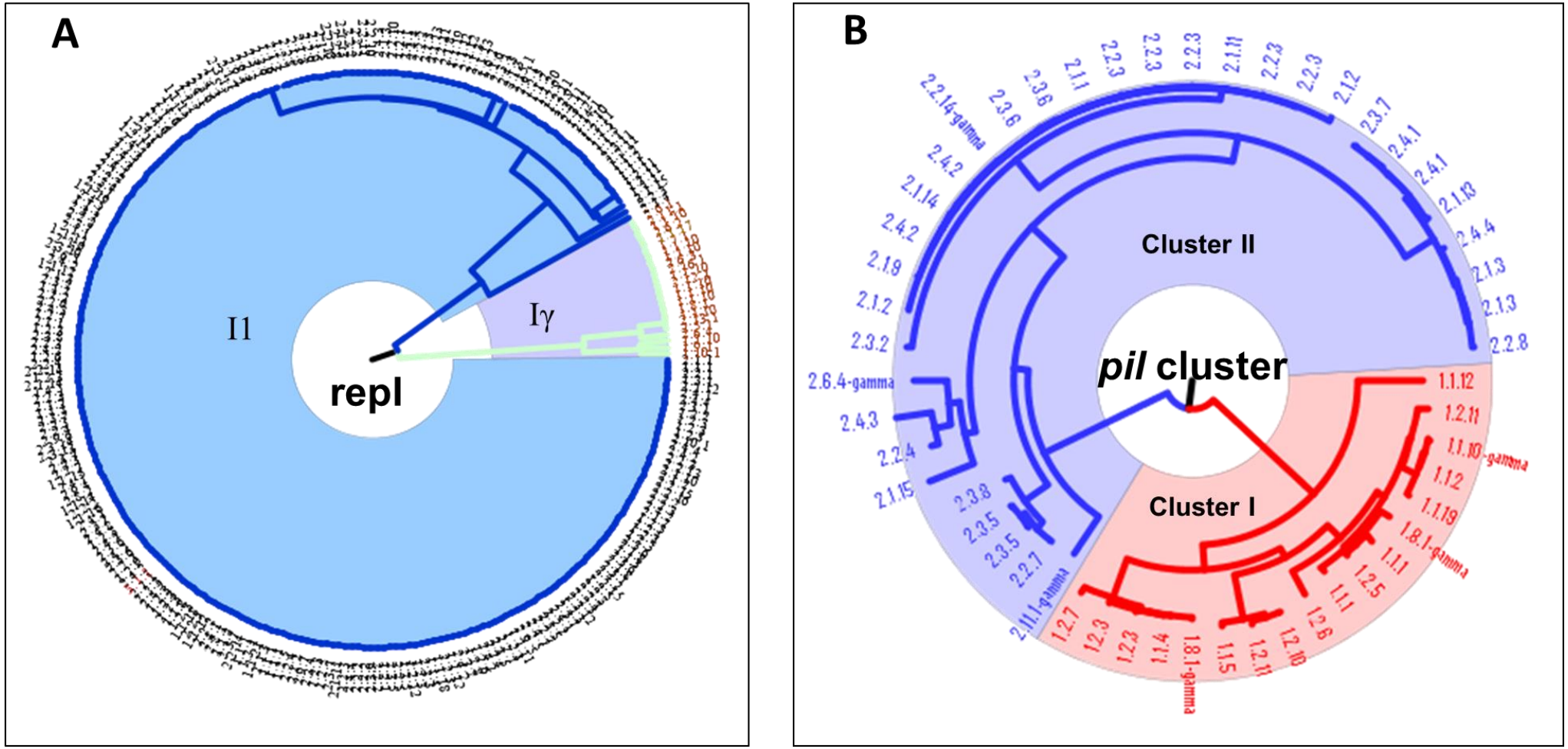
Figure 4


γ
TEM-52
(CC-5)
γ
γ
1.1
1.2
Cluster I
CTX-M-15
(CC-31)
γ
CMY-2
(CC-12)
2.4
Cluster III
Cluster II
2.1
CTX-M-1
(CC-3)
2.3
2.2
CTX-M-1
(CC-7)
CTX-M-15
(ST16)
CMY-2
(CC-2)

Supplementary Figure 1



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Supplementary Table 1. Origin and phylogeny of CTX-M-15-producers in pMLST database

No[1] (61)	Species[2]	Country	Year	Sources	ST[3] (CC)	Clade	Cluster
2	*Shigella sonnei*	USA	2005	human	32	1.1	I
1	*E. coli*	Taiwan	2013	human	180	1.1	I
1	*E. coli*	Taiwan	2013	human	181	1.1	I
1	*E. coli*	The Netherlands		human	188	1.1	I
25	*E. coli, Shigella sonnei*	Australia, Belgium, France, Germany, The Netherlands, UK, USA	2001-2011	human, horse, cattle	31 (CC-31)	1.2	I
3	*S.* Typhimurium, *S.* Anatum	UK	2001-2003	human	8 (CC-31)	1.2	I
2	*E. coli*	France	2009	cattle	68 (CC-31)	1.2	I
1	*E. coli*	Ireland	2010	human	57	1.2	I
1	*E. coli*	Sweden		human	170	1.2	I
1	*E. coli*	Sweden		human	171	1.2	I
1	*E. coli*	Sweden		other	175	1.2	I
1	*E. coli*	France	2012	human	192	1.2	I
1	*E. coli*	Australia	2006	human	88	1.5	I
1	*S.* Typhimurium	Germany	2005	horse	61 (CC-61)	2.1	II
1	*E. coli*	Czech Republic	2012	cattle	127	2.2	II
1	*E. coli*	Sweden		human	172	2.2	II
1	*E. coli*	France	2012	human	193	2.2	II
1	*E. coli*	France	2012	human	197	2.2	II
5	*E. coli, Shigella sonnei*	Ireland, South Korea, UK	2009	human, cattle	16	2.3	II
5	*E. coli*	Australia, Switzerland, The Netherlands, UK	2006-2009	human, cattle	37	2.3	II
1	*E. coli*	Switzerland	2012	other	176	2.3	II
1	*E. coli*	Switzerland	2010	human	177	2.3	II
1	*E. coli*	The Netherlands	2012	other	191	2.3	II
1	*E. coli*	France	2012	human	196	2.3	II
1	*E. coli*	Sweden		human	161	2.4	II

[1]No: Number of isolates per type (total number). [2]*E. coli*: *Escherichia coli, S.*: *Salmonella enterica.* [3]CC: Clonal Complex; ST: Sequence Type. (Source: https://pubmlst.org/plasmid/ April 2018).

825 **Supplementary Table 2. Origin and phylogeny of CTX-M-1-producers in pMLST database**

No[1] (179)	Species[2]	Country	Year	Sources	ST[3] (CC)	Clade	Cluster
1	*E. coli*	Germany	2009	poultry	10 (CC-5)	1.1	I
1	*E. coli*	France	2005	poultry	9 (CC-9)	1.1	I
2	*E. coli*	Denmark			49 (CC-9)	1.1	I
2	*E. coli*	The Netherlands, UK	2009	human	35	1.1	I
1	*E. coli*	Switzerland	2013	human	145	1.1	I
1	*E. coli*	France			179	1.1	I
1	*E. coli*	Czech Republic	2012	pig	113	1.2	I
1	*E. coli*	USA		human	154	1.2	I
2	*S.* Typhimurium	Germany	2009	horse, other	1	2.1	II
53	*E. coli, S.* Newport*, S.* Typhimurium*, S.* Llandoff*, S.* London*, S.* Agona	Czech Republic, Denmark, France, Germany, Ireland, Italy, Poland, The Netherlands, UK	2005-2010	human, poultry, pig, cattle, dog, other	3 (CC-3)	2.1	II
2	*E. coli*	Czech Republic	2012	cattle, pig	38 (CC-3)	2.1	II
1	*E. coli*	The Netherlands	2008	pig	64 (CC-3)	2.1	II
80	*E. coli, S.* Paratyphi B*, S.* Infantis*, S.* Agona	Germany, Ireland, The Netherlands	2006-2009	human, poultry, pig, cattle	7 (CC-7)	2.1	II
1	*E. coli*	The Netherlands	2006	poultry	30 (CC-7)	2.1	II
1	*E. coli*	Sweden	2012	cattle	135	2.1	II
5	*E. coli*	Germany, Italy	2007	poultry, pig	26 (CC-26)	2.2	II
2	*E. coli*	Germany	2009	human, cattle	58 (CC-58)	2.2	II
1	*E. coli*			cattle	59 (CC-58)	2.2	II
1	*S.* Typhimurium	Germany	2005	horse	62 (CC-61)	2.1	II
1	*E. coli*	UK		pig	108	2.2	II
2	*E. coli*	Czech Republic	2012	pig	128	2.2	II
1	*E. coli*	France	2011	human	157	2.2	II
1	*E. coli*	Sweden		cattle	160	2.2	II
1	*E. coli*	Sweden		human	169	2.2	II
1	*E. coli*	The Netherlands		human	190	2.2	II
1	*E. coli*	France	2012	human	195	2.2	II
1	*E. coli*	France	2012	human	198	2.2	II
2	*E. coli*	Germany	2009	cattle	84	2.3	II
1	*E. coli*	Italy	2009	human	97	2.3	II
1	*E. coli*	Italy	2009	poultry	11	2.4	II

5	*E. coli*	Germany	2009	cattle	63	2.4	II
1	*E. coli*	Czech Republic	2012	pig	129	2.4	II
1	*E. coli*	France	2010	other	106 Iγ	2.6	II

826 [1]No: Number of isolates per type (total number). [2]*E. coli*: *Escherichia coli, S.*: *Salmonella enterica.* [3]CC:
827 Clonal Complex; ST: Sequence Type. Iγ indicates a plasmid belonging to the IncIγ group, the others are
828 IncI1. (Source: https://pubmlst.org/plasmid/ April 2018).

Supplementary Table 3. Origin and phylogeny of CMY-2-producers in pMLST database

No[1] (206)	Species[2]	Country	Year	Sources	ST[3] (CC)	Clade	Cluster
1	*S.* Heidelberg	Canada	2006		21 (CC-5)	1.1	I
1	*E. coli*	UK	2007	human	39	1.1	I
1	*E. coli*	UK	2007	human	44	1.1	I
1	*S.* Choleraesuis	Taiwan	2007	human	51	1.1	I
5	*S.* Agona*, S.* Enteritidis*, E. coli*	Taiwan	2009-2010	human	56	1.1	I
1	*S. enterica*	Taiwan	2011	human	109	1.1	I
1	*E. coli*	Taiwan	2005		139	1.1	I
1	*S.* Typhimurium	Taiwan	2011	pig	217	1.1	I
1	*E. coli*	Brazil	2012	dog	225	1.1	I
1	*E. coli*	Taiwan	2006	human	137 Iγ	1.1	I
1	*E. coli*	Sweden		other	168 Iγ	1.1	I
1	*E. coli*	Spain	2014	human	239 Iγ	1.1	I
2	*S.* Typhimurium*, E. coli*	Taiwan, UK	2002-2010	human, dog	55 Iγ	1.1	I
1	*E. coli*	Canada	2006	other	19	1.2	I
4	*S.* Heidelberg*, E. coli*	Greece, USA	2009-2012	Poultry, dog	65	1.2	I
1	*E. coli*	USA	2007	human	104	1.2	I
1	*E. coli*	Greece	2011	dog	126	1.2	I
2	*E. coli*	Spain	2010-2011	human	134	1.2	I
1	*E. coli*	UK	2007	human	40	1.6	I
1	*E. coli*	Taiwan	2003	human	141 Iγ	1.7	I
4	*E. coli*	Canada	2004	human	20	2.1	II
1	*E. coli*	UK	2006	human	38 (CC-3)	2.1	II
2	*E. coli*	UK	2002-2007	human, dog	43	2.1	II
1	*E. coli*	UK	2007	human	45	2.1	II
1	*S.* Typhimurium	Taiwan	2010	human	53	2.1	II
1	*E. coli*	Taiwan	2009	human	83	2.1	II
1	*E. coli*	Taiwan	2009	human	87	2.1	II
1	*E. coli*	Taiwan	2009	human	92	2.1	II
1	*S. enterica*	Taiwan	2012	human	110	2.1	II
1	*E. coli*	Denmark	2009	human	116	2.1	II
1	*E. coli*	USA	2010	other	122	2.1	II
1	*E. coli*	USA	2010	dog	123	2.1	II
1	*E. coli*	Taiwan	2006	human	138	2.1	II
1	*S. enterica*	Taiwan	2013	human	142	2.1	II
1	*S. enterica*	Taiwan	2013	human	143	2.1	II
1	*E. coli*	The Netherlands	2014	other	206	2.1	II

1	*E. coli*	Spain	2014	human	258 Iγ	2.1	II
22	*E. coli, S.* Heidelberg*, S.* 4,5,12:i:-*, S.* Typhimurium	Australia, Canada, Denmark, Italy, The Netherlands, UK, USA	2005-2009	human, poultry, dog	2 (CC-2)	2.2	II
6	*E. coli, S.* Heidelberg*, S.* Newport	Canada, USA	2005-2009	human, dog	23 (CC-2)	2.2	II
3	*E. coli, S. enterica*	UK, USA	2007-2008	human	26 (CC-26)	2.2	II
1	*E. coli*	Canada	2006	other	18	2.2	II
1	*E. coli*	UK	2007	human	42	2.2	II
1	*E. coli*	UK	2008	human	47	2.2	II
1	*E. coli*	USA	2010	dog	120	2.2	II
1	*E. coli*	USA	2010	dog	121	2.2	II
1	*E. coli*	USA	2010	other	124	2.2	II
1	*E. coli*	USA	2010	dog	125	2.2	II
1	*Klebsiella pneumoniae*	Taiwan	2004	human	140	2.2	II
1	*E. coli*	Brazil	2011	dog	226	2.2	II
1	*E. coli*	Canada	2012	other	234	2.2	II
1	*S. enterica*	China	2006	other	265	2.2	II
1	*E. coli*	UK	2007	human	41	2.3	II
1	*S.* Choleraesuis	Taiwan	2008	human	52	2.3	II
2	*S.* Typhimurium	Taiwan	2010	human	54	2.3	II
1	*E. coli*	Italy	2009	human	85	2.3	II
1	*E. coli*	Taiwan	2009	human	89	2.3	II
1	*S. enterica*	Taiwan	2010	human	96	2.3	II
1	*S. enterica*	Taiwan	2012	human	111	2.3	II
1	*S. enterica*	Taiwan	2013	human	144	2.3	II
1	*S.* Typhimurium	Taiwan	2013	human	182	2.3	II
1	*E. coli*	Germany	2013	human	240	2.3	II
1	*E. coli*	Germany	2014	human	242	2.3	II
1	*S.* Thompson	USA	1996	human	4	2.4	II
1	*S.* Thompson	USA	2005	human	11	2.4	II
79	*S.* Heidelberg*, S.* Typhimurium*, S.* Litchfield*, E. coli, S.* Paratyphi B*, S.* Newport	Brazil, Canada, Germany, The Netherlands, Uruguay, USA	2001-2013	human, poultry, turkey	12 (CC-12)	2.4	II
10	*S.* Heidelberg	USA	2009	human, poultry	66 (CC-12)	2.4	II
1	*E. coli*	USA	2006	human	17 (CC-2)	2.4	II
1	*E. coli*	Italy	2009	poultry	86	2.4	II
1	*E. coli*	Canada	2012	other	233	2.4	II
1	*E. coli*	Spain	2014		237	2.4	II
1	*E. coli*	Germany	2011	poultry	241	2.4	II
1	*E. coli*	Taiwan	2009	human	90 Iγ	2.5	II

1	*E. coli*	France		dog	133 Iγ	2.6	II
1	*E. coli*	The Netherlands		human	189 Iγ	2.6	II
1	*E. coli, S.* Heidelberg	Canada, USA	2005-2006	human, poultry	22	2.8	II
1	*E. coli*	Taiwan	2009	human	93	2.9	II
1	*E. coli*	Taiwan	2009	human	94	2.9	II

830 [1]No: Number of isolates per type (total number). [2]*E. coli*: *Escherichia coli, S.*: *Salmonella enterica.* [3]CC:
831 Clonal Complex; ST: Sequence Type. Iγ indicates a plasmid belonging to the IncIγ group, the others are
832 IncI1. (Source: https://pubmlst.org/plasmid/ April 2018).

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Supplementary Table 4. Origin and phylogeny of TEM-20- and TEM-52-producers in pMLST database

No[1] (41)	Species[2]	Country	Year	Sources	TEM variant	ST[3] (CC)	Clade	Cluster
1	*S.* Infantis	Belgium	2005	human	TEM-52	5 (CC-5)	1.1	I
2	*E. coli, S.* Paratyphi B	Norway, The Netherlands	2006	poultry	TEM-20	10 (CC-5)	1.1	I
8	*E. coli, S.* Infantis*, S.* Paratyphi B	The Netherlands	2006-2009	human, poultry	TEM-52	10 (CC-5)	1.1	I
1	*E. coli*	The Netherlands	2007	poultry	TEM-52	21 (CC-5)	1.1	I
28	*E. coli, S.* Infantis*, S. enterica, S.* Paratyphi B	France, Germany, Switzerland, The Netherlands	2005-2018	human, poultry, cattle, pig	TEM-52	36 (CC-5)	1.1	I
1	*S.* Virchow	Germany	2007	other	TEM-52	60	1.2	I

[1]No: Number of isolates per type (total number). [2]*E. coli*: *Escherichia coli, S.*: *Salmonella enterica.* [3]CC: Clonal Complex; ST: Sequence Type. (Source: https://pubmlst.org/plasmid/ April 2018).

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