Global phylogenetic analysis of *Escherichia coli* and plasmids carrying the *mcr-1* gene indicates bacterial diversity but plasmid restriction

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Running title: Phylogenetic analysis of *mcr-1*-carrying *E. coli* isolates

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Abstract

To understand the dynamics behind the worldwide geographical spread of the *mcr-1* gene, we investigated the population structure and genetic background of 410 Enterobacteriaceae, isolated from human, animal and environmental samples, and their mobile genetic elements carrying the *mcr-1* gene.

All available whole genome sequences and MLST profiles of *mcr-1*-carrying *E. coli* isolates, along with 6 newly sequenced travel related isolates, were analysed for their phylogenetic relatedness. We show the presence of two clusters of *mcr-1*-carrying *E. coli* isolates that are possible reservoirs for the gene that, despite a high overall diversity of the population. One cluster is linked to ST10, a sequence type known for its ubiquity in human- and food-related environments. No clustering by geographical origin or isolation source (humans, animal or environment) could be identified.

The IncI2, IncX4 and IncHI2 plasmid incompatibility types accounted for more than 90% of 219 mobile genetic elements carrying the *mcr-1* gene. We found significant geographical clustering consistent with regional spread of IncHI2 plasmids in Europe and IncI2 in Asia.

These findings point towards promiscuous spread of the *mcr-1* gene by efficient horizontal gene transfer dominated by a limited number of plasmid incompatibility types.

Keywords

mcr-1; *Escherichia coli*; antimicrobial resistance; multilocus sequence typing; whole genome sequencing; plasmid; phylogeny; colistin; extended spectrum beta-lactamase (ESBL); international travel; ISA*pl1* transposon element.

Introduction

Antimicrobial resistance (AMR) represents a growing threat to global health [1]. With almost no new antimicrobial drugs in development [2], limiting the spread of AMR is key in order to maintain current treatment options [3].

Colistin is an antibiotic of the polymyxin class, discovered in 1950 and effective against Gram-negative bacteria [4]. The emergence of multidrug-resistant Gram-negative bacteria, especially those producing carbapenemases, has reintroduced colistin as a last resort antibiotic for the treatment of severe infections [5]. In contrast to its limited use in humans, colistin is widely used in food-producing animals [6]. While colistin resistance was long thought to be caused by chromosomal mutations only [7], the emergence of plasmid-mediated resistance, conferred by the *mcr-1* gene, was recently reported [8]. Since its discovery in China, this gene has been described in several bacterial species that were isolated from animals, animal food products, humans and environmental samples from around the world [9–13]. Our previous study in travellers indicated acquisition of *mcr-1* carrying bacteria by healthy individuals during travel to destinations around the world, potentially related to food exposure, as well as rapid clearance after return [14]. It has been suggested that *mcr-1* has spread from food animals to humans [8,15–17], but there is a lack of comparison of *mcr-1* carrying isolates on a global level to support this hypothesis.

We studied the global population structure as well as the geographic and host distribution of *mcr-1*-carrying *Escherichia coli,* and mobile genetic elements (MGEs), to establish the population structure and to assess whether the spread of the *mcr-1* gene is linked to clonal dissemination or transmission of MGEs from animal, human, or environmental sources within geographic regions.

Results

Literature search

A systematic review of the literature on *mcr-1*, published until 1 January 2017 resulted in the inclusion of 95 articles, representing a total of 410 entries (whole genome sequences, MLST profiles, and/or plasmid types) for analysis (See detailed methods and results in Supplementary data, Supplementary Figure 1 and Supplementary Table 1).

Population structure

Whole genome sequencing (WGS)

The genomes of 65 *mcr-1*-carrying *E. coli* were analysed, including 6 genomes obtained from *E. coli* isolated from travellers that were sequenced for the purpose of the present study. Isolates originated from Asia (n=36; 55.4%), Europe (n=20; 30.8%), North-America (n=4; 6.2%), South-America (n=4; 6.2%) and Africa (n=1; 1.5%). 45 were of animal origin (69.2%), 19 of human origin (29.2%) and one strain (1.5%) was isolated from water (Supplementary Table 1).

The average size of the genomes of these 65 isolates was 4.9 Mbp, with a median number of genes identified of 4785 (ranging from 4266 to 7083), representing a pangenome of 23248 genes and a core genome (defined by genes present in at least 99% of the isolates) of 2216 genes. Bayesian analysis of the nucleotide alignment of the core genome using the BAPS software [18] revealed the presence of 5 distinct phylogenetic clusters (Figure 1; Supplementary Figure 2; Supplementary Table 2). The largest cluster (cluster 1) consisted of 26 isolates from 16 different STs (26/65; 40.0%) and the second cluster of 24 isolates from 15 different STs (36.9%). Clustering was independent of geographical origin or isolation source (Figure 1) except that all 5 isolates that belong to BAPS cluster 3 are from Europe. Twenty isolates showed less than 10 SNPs/Mbp difference and were considered clonally related (Supplementary Tables 3 and 4).

Multilocus sequence typing (MLST)

For 312 *E. coli* isolates originating from 69 studies a MLST profile was published or could be deduced from the corresponding WGS. Of these, 206 were isolated from animals or animal products (66.0%), 101 were isolated from humans (32.4%), including the 6 travel acquired isolates, and 5 from the environment (1.6%). 141 Isolates from 25 studies (141/312; 45.2%) originated from Asia and 125 isolates from 25 studies (40.1%) from Europe together accounting for 85.3% of all included isolates. The isolates represented 112 unique sequence types (STs) with ST10 being most common, comprising 40/312 (12.8%) isolates originating from Africa, Asia, Europe and South-America.

eBURST analysis identified three main clusters that clustered around ST10, ST1114 and ST410. The largest cluster contained all 40 ST10 isolates and an additional 46 isolates in 21 STs that were single (SLV) or double locus variants (DLV) of ST10 (86/312; 27.6%) (Supplementary Figure 3). The predicted founder of the second largest cluster was ST1114, a SLV of ST165 and ST100, and included 19 isolates belonging to 7 different STs (5.4%), while the third cluster was centred on ST410 and included 14 isolates from 3 different STs (4.5%).

A maximum-likelihood tree based on concatenated MLST gene sequences showed a main clade of 128 isolates, including most, but not all, isolates from the eBURST clusters of ST10 and ST1114 (Supplementary Figure 4A). All isolates from these two eBURST clusters for which a WGS was available were grouped in BAPS cluster 2. Similarly, all the isolates from the eBURST cluster ST410 grouped into BAPS cluster 1, along with 6 isolates from ST155. Seven isolates belonged to the globally successful extra-intestinal pathogenic *E. coli* clone ST131 (Supplementary Figure 4A).
As observed in the WGS analysis, animal isolates were interspersed with isolates from humans and the environment throughout the tree, as were isolates from different continents indicating a lack of clustering by isolation source or geographical origin (Figure 2). Similarly, no clustering by health status of the host was observed (Supplementary Figure 4B).

Mobile genetic elements

The plasmid incompatibility group of the *mcr-1*-carrying plasmids could be determined for 217 Enterobacteriaceae isolates from 7 different genera (*Escherichia sp., Salmonella* sp., *Klebsiella* sp., *Cronobacter* sp., *Enterobacter* sp., *Kluyvera* sp. and *Shigella* sp.), representing a total of 219 plasmids since 2 isolates carried 2 different plasmids carrying the *mcr-1* gene (Table 1). These plasmids were described in 71 studies (1 to 33 plasmids per study, average = 3.1). In addition, the gene was integrated in the chromosome of 6 isolates. The incompatibility group could not be determined for 27 of the 65 isolates for which WGS was available. Similarly the plasmid type was not available for 182 of the 312 isolates included in the MLST analysis. A total of 14 different plasmid incompatibility groups were identified. 198/219 (90.4%) of the identified plasmids belonged to one of 3 incompatibility groups: IncX4 (77/219 plasmids, 35.2%), IncI2 (76/219 plasmids, 34.7%) and IncHI2 (45/219 plasmids, 20.5%). 50/76 IncI2 plasmids (65.8%) originated from Asia and 33/45 IncHI2 plasmids (73.3%) from Europe. IncX4 plasmids were more evenly distributed: 44/77 (57.1%) were recovered from Europe, 29 from Asia (37.7%) and 4 from other regions (5.2%). Observed proportions were significantly different from expected for IncI2 (χ^2-test, $p < 0.001$) and IncHI2 ($p < 0.001$) but not for IncX4. The distribution of these three plasmid types between animal, human and environmental sources were not significantly different from expected. Isolates from the BAPS groups 1 and 2 carried

plasmids from the 3 major types in similar proportions (Supplementary Figure 5A). Isolates from the eBURST clusters of ST10 carried plasmids belonging to 7 different incompatibility groups, including IncHI2, IncI2 and IncX4. No clustering of plasmid type with MLST phylogeny was observed either (Supplementary Figure 5B).

Figure 3 shows the alignment of the complete sequences or contigs from IncI2 (panel A), IncX4 (B) and IncHI2 (C) incompatibility group plasmids. IncHI2 plasmids had the largest size, with sequence lengths up to 267486 bp.

The ISA*pl1* transposon element situated upstream of the *mcr-1* gene was present in 7/9 (77.8%) IncHI2 plasmids, but only in 11/29 (37.9%) of IncI2 plasmids and completely missing in all of the 24 reported IncX4 plasmids (Figure 3). In the isolates from travellers the ISA*pl1* transposon was identified in 3 out of our 6 *mcr-1*-carrying contigs including one isolate from a traveler to Asia (ST101, IncI2 incompatibility group), one to Africa (ST744, IncHI2) and one to South-America (ST744, incompatibility group not identified).

Antimicrobial resistance genes

Multiple resistance genes were detected in most of the studied isolates (Supplementary Results). The florfenicol resistance gene *floR* was present in 32 (49.2%) isolates; in 22 of 45 isolates from animals (48.9%) and 10 of 19 isolates from humans (52.6%). The *baeR* and *baeS* genes, encoding novobiocin resistance, were found in 64 (98.5%) and 65 (100%) isolates, respectively.

Plasmid analysis from the WGS data showed that 4 of the 29 *mcr-1*-carrying IncI2 plasmids (13.8%) contained an additional ESBL gene. IncHI2 plasmids (n=9) carried between 0 and 12 additional AMR genes. In particular, 4 plasmids carried CTX-M ESBL genes and 2 carried the *floR* gene. In 4 out of the 9 IncHI2 plasmids analysed in this study, the *mcr-1* gene was shown to be integrated alongside a large multi-drug resistance (MDR) gene cassette (Figure 3C). None of the IncX4 plasmids carried additional AMR genes.

Discussion

Analysis of all reported WGS of *mcr-1*-carrying isolates shows that the population of *E. coli* is highly diverse, but is dominated by two large groups of related isolates. Most of the isolates from BAPS group 2 grouped into a MLST cluster centred on ST10. An overrepresentation of isolates related to ST10 and ST165 (a SLV of ST1114) in *mcr-1*-carrying *E. coli* isolates was previously reported at a smaller scale in isolates from European farm animals [19]. *E. coli* ST10 and closely related STs are frequently recovered from food and human intestinal samples and studies have shown a higher prevalence of plasmid-carried AMR genes in ST10, including CTX-M ESBL genes, compared to other STs [20–23].

The second BAPS group of interest in our study included isolates belonging to ST155. This ST has been described as a major vector of spread of ESBL genes from animals to humans [24]. It is thus possible that zoonotic transmission lead to the spread of the *mcr-1* gene, as has been suggested in studies from China and Vietnam [16,17], notably through the two main phylogenetic clusters identified in this study.

Additionally, we found clonally related isolates, including some belonging to ST744, a SLV of ST10, carrying the *mcr-1* gene on different plasmid backbones and recovered from different continents (see Supplementary Results). These results point towards a worldwide dissemination *of mcr-1* driven mainly by highly promiscuous plasmids rather than the worldwide spread of one or more *mcr-1*-carrying clones. We hypothesize that several populations of *E. coli* isolates, notably those related to ST10 or ST155, acquired the *mcr-1* gene due to their intrinsic ability of acquiring AMR genes and their high prevalence in humans and food animals. These populations of commensal isolates then may play a crucial role as a reservoir for this gene, which can explain their over-representation in the present study.

In the timeframe of our literature search, 3 *E. coli* strains carrying the *mcr-2* gene were isolated from animals in Belgium. These isolates belonged to ST10 (2 isolates) and ST167 which is a SLV of ST10 and carried the gene on an IncX4-type plasmid. No WGS data was available from these isolates [25].

More than 90% of published plasmid types carrying *mcr-1* genes belonged to either IncI2, IncX4 or IncHI2. Almost 75% of the isolates carrying an IncHI2 plasmid originated from Europe: 26 from animals and 7 from humans (Table 1 and Supplementary Table 1). In a traveller's isolate acquired in Tunisia, the *mcr-1*-carrying plasmid was identified as an IncHI2-type backbone of the ST4 pMLST subtype which co-carried a CTX-M-1 ESBL gene (Figure 3C). This traveller reported consumption of beef and chicken and eggs during travel to Tunisia which can potentially be the source for the acquisition of the *mcr-1* positive isolate. When investigating the presence of the *mcr-1* gene in cephalosporin resistant *E. coli* isolates from chicken farms in Tunisia, Grami *et al.* [26] found that all 37 *mcr-1*-carrying plasmids also belonged to the IncHI2-type, ST4 subtype and harboured CTX-M-1 genes. PFGE typing of the isolates harbouring this plasmid showed various bacterial genetic backgrounds. Interestingly, these chickens were all imported from France, either as adults or chicks. Other studies showed the presence of this IncHI2, CTX-M-1 and *mcr-1* combination in *Salmonella enterica* Typhimurium isolates from meat samples in Portugal from 2011 [27,28] and diarrhoeic veal calves in France [29]. The IncHI2 subtype ST4 was also detected in an *E. coli* isolate from retail chicken breast in Germany [30] and the faecal sample of a veal calve from the Netherlands [31], suggesting widespread dissemination of this particular plasmid in European farm animals and possible transmission to humans.

The high prevalence of novobiocin *baeR* and *baeS* and florfenicol *floR* resistance genes [32,33] in the genomes of isolates of human and animal origin together with the fact that florfenicol and novobiocin are used almost exclusively in veterinary medicine further supports the potential role of food animals as an important reservoir of *mcr-1* containing bacteria and MGEs [15].

In contrast with the IncHI2 plasmids, 65.8% of all IncI2 plasmid recovered so far originated from Asia, with a much lower prevalence in *mcr-1* carrying Enterobacteriaceae from other regions. Taken together, these elements point toward a more regional circulation and dissemination of the *mcr-1*-carrying plasmids IncI2 and IncHI2.

We found the ISA*pl1* transposon element associated with the *mcr-1* gene, as originally described by Liu *et al*. [8] to be present in a minority of studied plasmids and contigs. However, since some of the *mcr-1*-carrying contigs were obtained by assembly of Illumina short reads from WGS data, we cannot exclude that some of these gaps are explained by an incomplete assembly of (plasmid) sequences. The ISA*pl1* transposon element is considered to be the main driver of horizontal gene transfer of the *mcr-1* gene and has been shown to be highly unstable in IncI2 plasmids [34–36]. The absence of the ISA*pl1* transposon element in *mcr-1*-carrying IncX4 plasmids as described here has recently been proposed to be essential for the maintenance of the *mcr-1* gene in this particular backbone, but the exact mechanism still requires further investigation [37].

WGS analysis provided in-depth information about the *mcr-1*-carrying *E. coli* isolates and their phylogenetic relationship, but the number of available genomes was limited. On the other hand, whilst MLST data have a lower resolution, the higher number of available profiles allowed analysis of the isolates' origin (geographical, source of isolation, diseased status of the host, etc.).

A limitation of our study is the potential for bias. The overrepresentation of isolates originating from Asia and Europe could be explained by a higher prevalence of *mcr-1* genes on these continents, but the effect of publication bias cannot be excluded. Isolates from North-America only represented 2.2% of the collection. Noteworthy, colistin, except for ophthalmic ointment, has never been marketed for use in animals in the United States [38,39].

Sampling bias should also be considered when several isolates with an identical ST are presented from a single study, as is the case for ST100 and ST752. Additionally, in the absence of a control population of *mcr-1*-negative isolates obtained from similar sources as the *mcr-1* positive isolates, results of analysis of population structures should be interpreted with caution. Because many studies screened existing collections of (resistant) isolates for colistin resistance or presence of *mcr-1*, selection bias has probably been introduced.

The findings in this study suggests that the *mcr-1* gene has locally and globally disseminated through MGEs that are mainly IncHI2, IncI2 an IncX4 plasmids and provides additional support for the hypothesis of the animal reservoir, that is driven by the use of colistin in livestock, as a source of *mcr-1* in humans. A global ban of colistin use in animals to preserve colistin for use in human medicine seems therefore justified.

Material and Methods

Selection of isolates for whole genome sequencing

We subjected six *mcr-1* positive isolates that were collected as part of a prospective study (COMBAT study) aimed at studying acquisition of extended-spectrum β-lactamase (ESBL) -producing Enterobacteriaceae during travel to whole-genome sequencing [14,40]. Additionally, we included 22 whole genome sequences of isolates from Vietnamese chickens and humans that were still unpublished when performing our literature search [17].

Literature search

Relevant papers that published on *mcr-1* and *mcr-2* were identified in PubMed, Web of Science, Scopus, ScienceDirect and Google Scholar using the query 'mcr-1 OR mcr1 OR mcr-2 OR mcr2 OR (mcr AND colistin)' (see Supplementary Material for full search strategies).

Whole genome sequencing of *mcr-1*-positive *E. coli* isolates

Bacterial DNA was extracted from fresh pure cultures using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Library preparation was done according to manufacturer's instruction (Illumina, San Diego, CA, USA) and sequenced using Illumina MiSeq technology with 150 paired-end settings. Sequences have been deposited in the European Nucleotide Archive under the accession numbers ERS1694334 to ERS1694339.

Bio-informatic analysis

MLST analysis

For each *mcr-1*-carrying *E. coli* isolate for which the ST or the whole genome sequence was available, the sequences of the corresponding alleles were downloaded from the *E. coli* MLST genes repository of the University of Warwick (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/handlers/getFileData/home/cbailster/mlst/zope/Extensio

292 ns/gadfly/Ecoli/DB/) and concatenated. When STs of isolates were not described in literature, the ST
293 was determined from available whole genomes using the online service provided by the Center for
294 Genomic Epidemiology (https://cge.cbs.dtu.dk/services/MLST/) according to the Achtman MLST
295 scheme [41,42]. MLST clusters (STs and their single locus or double locus variants) were defined using e-
296 burst V3 (http://eburst.mlst.net/v3/enter_data/single/) [43] and goeBURST v1.2.1 [44] using only profiles
297 from this study.

298 ### *WGS and plasmid analysis*

299 Whole genome and plasmid sequences were downloaded from online databases for all available
300 isolates. Additional sequences not yet deposited in online databases were requested from their
301 respective authors. The quality of the sequence reads was checked using fastqc
302 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), quast [45] and KmerFinder 2.0
303 (https://cge.cbs.dtu.dk/services/KmerFinder/) (see Supplementary Methods for more details). Reads
304 were trimmed using Trimmomatic V0.33 [46]. *De-novo* genome assembly was performed with SPAdes
305 3.9 [47] for Illumina short reads and with Canu v1.3 for PacBio long reads [48]. Identification of open
306 reading frames (ORFs) and gene contents in the assembled genomes was performed using Prokka
307 v1.11 [49]. Core genome analysis was performed with Roary v3.6.8 [50]. Clustering of isolates was
308 performed using the hierBAPS module of the Bayesian Analysis of Population Structure (BAPS)
309 software v6.0 [18]. The core genome alignment output provided by Roary was used as input with 2
310 levels of hierarchy and a maximum number of cluster (K) of 10. The estimated number of clusters
311 was 5 for both levels of hierarchy.
312 Sequences (concatenated MLST loci or concatenated core genes) were aligned using mafft v6.864b
313 [51]. The resulting alignment was used as input for calculation of distances and tree building using
314 RAxML v8.1.6 [52]. TMLST and WGS trees were visualized using iTOL v3.3.1 (http://itol.embl.de/) [53].

315 Identification of plasmid incompatibility group and typing of IncHI2 plasmids were performed via the
316 CGE online services PlasmidFinder v1.3 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and pMLST
317 v1.4 (https://cge.cbs.dtu.dk/services/pMLST/) [54]. Alignment and visualization of plasmids was
318 performed with BRIG v0.95 [55].

319 Two different databases were used for identification of other antibiotic resistance genes: ResFinder
320 (https://cge.cbs.dtu.dk/services/ResFinder/ [56]) was used to detect acquired resistance genes
321 commonly located on mobile genetic elements (MGEs) and CARD Resistance Gene Identifier
322 (https://card.mcmaster.ca/analyze/rgi [57]) was used to detect chromosomal genes.

Statistics

The distribution of isolates from different geographical origins and isolation sources was determined by a chi-square test comparing the expected distribution (proportions of the total studied population) to the observed proportions using GraphPad Prism6 (La Jolla, CA, USA).

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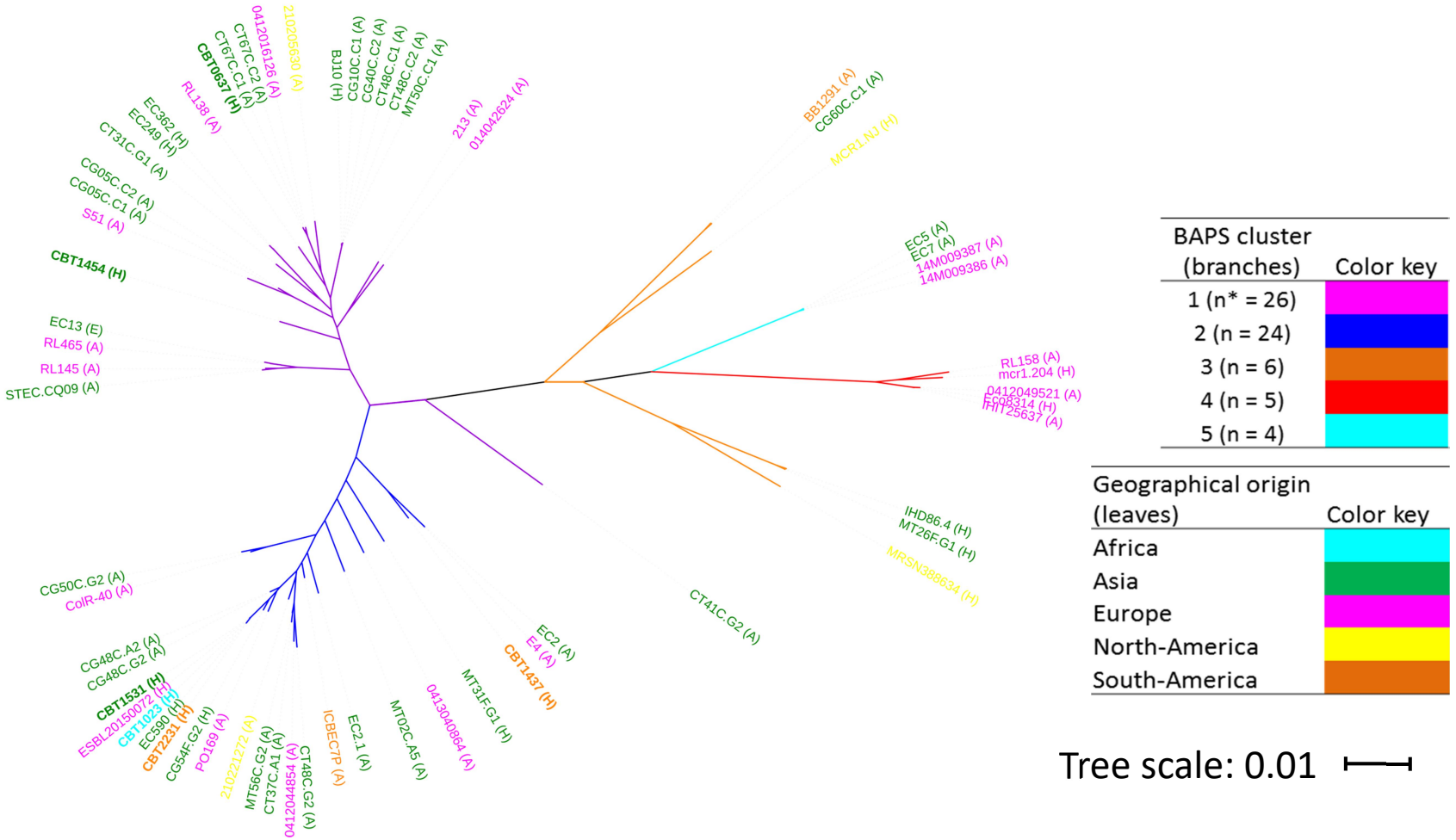


Figure 1: Maximum-likelihood tree based on concatenated core genome sequences of 65 *mcr-1*-carrying *E. coli* isolates.

Branch colours indicate phylogenetic clusters as determined by BAPS. Isolates from ST10, ST165 and closely related isolates are all grouped in the blue cluster. Leaves (isolates identifiers) colours indicate geographical region of origin. Isolation source is indicated in brackets: A = animal or meat; H = human; E = environment. Isolates from travellers are highlighted in bold. Tree scale in number of substitutions per site.

*number of isolates in the corresponding cluster.

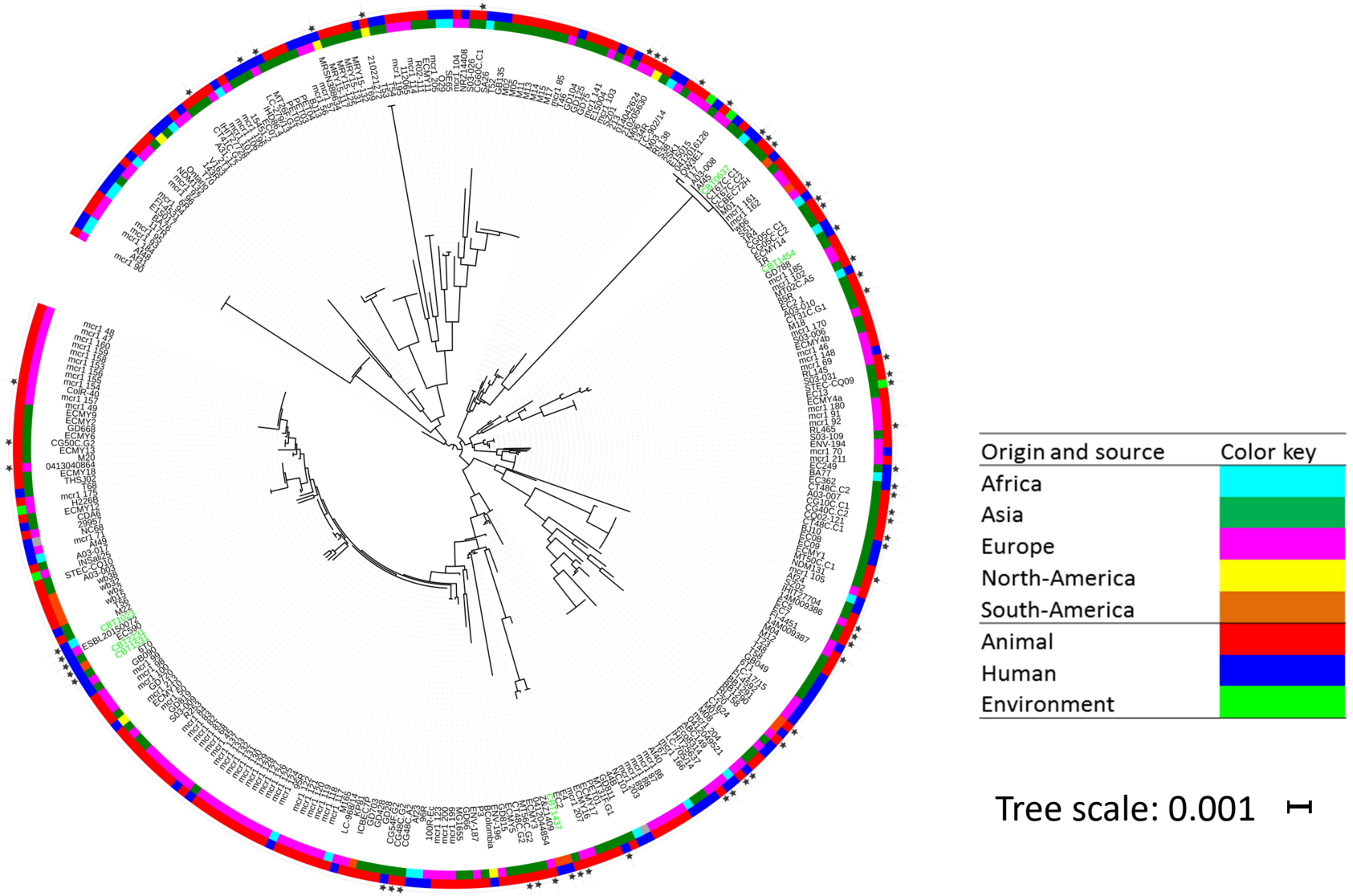


Figure 2: Phylogeny of the *mcr-1*-carrying *E. coli* isolates. Maximum-likelihood tree based on concatenated MLST gene sequences, mid-point rooted.

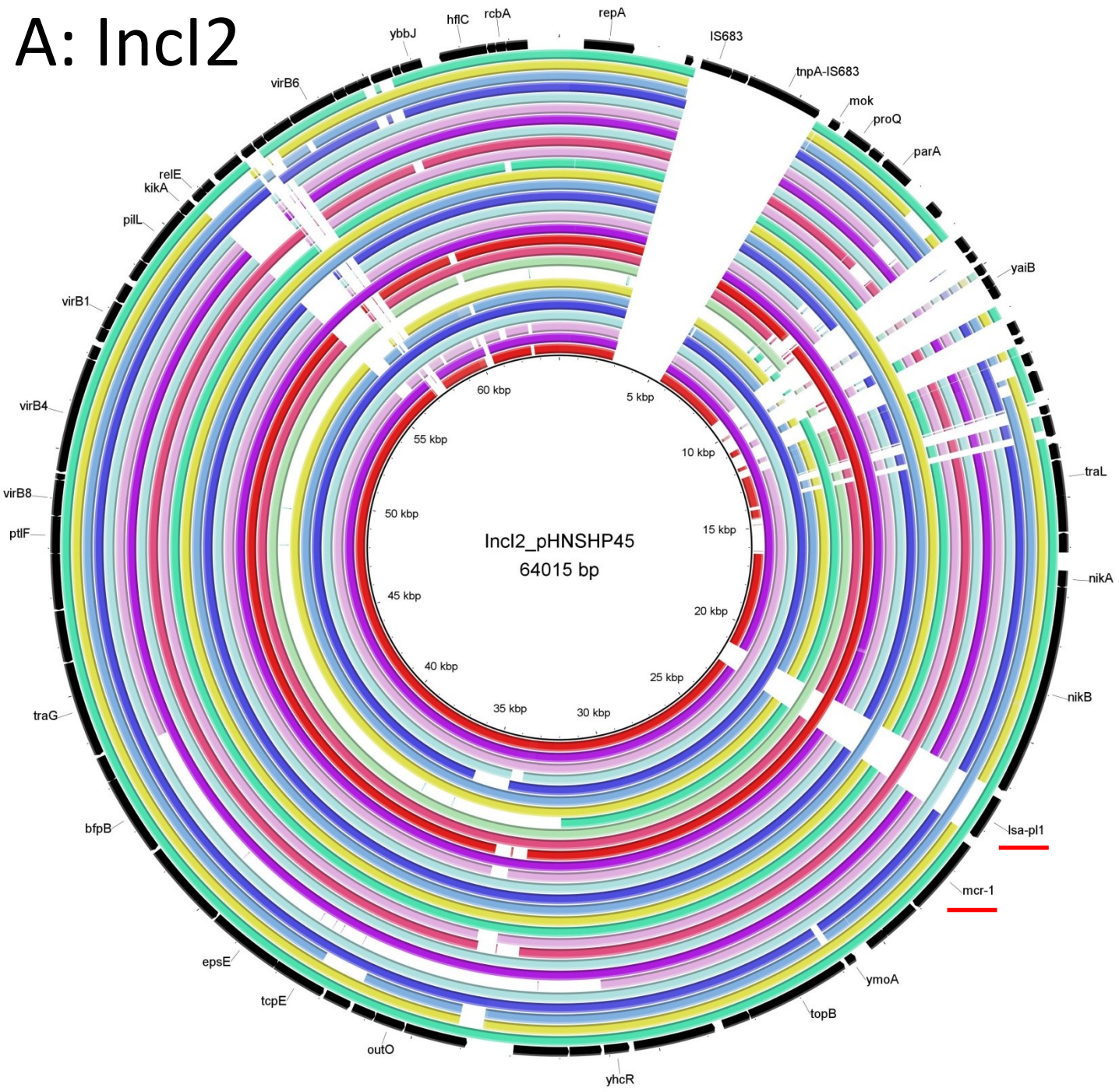
Inner coloured circle: region of origin; outer circle: isolation source. Stars indicate the isolates from which a whole genome sequence was available. Isolates from travellers are highlighted in green. Tree scale in number of substitutions per site.

See Supplementary Figure 4A for additional information on the relationship between STs, eBURST clustering and WGS BAPS clustering.

	IncF	IncFI	IncFIB	IncFII	IncHI1	IncHI1A/ IncHI1B/ IncFIA	IncHI2	IncI2	IncI2-IncX4	IncP	IncX3-X4	IncX4	IncY	repB (p0111)	**Total plasmids**		Chromosome
Africa	0	0	1	0	0	0	4	8	0	0	0	1	0	0	**14**	**6.4%**	0
Asia	1	3	0	0	0	2	7	50	0	1	1	29	2	0	**96**	**43.8%**	2
Europe	0	0	2	1	1	0	33	5	0	3	0	44	0	1	**90**	**41.1%**	4
North-America	1	0	0	0	0	0	1	4	0	0	0	1	0	0	**7**	**3.2%**	0
Oceania	0	0	0	0	0	0	0	2	0	0	0	0	0	0	**2**	**0.9%**	0
South-America	0	0	0	0	0	0	0	7	1	0	0	2	0	0	**10**	**4.6%**	0
Total (% of total)	**2 (0.9)**	**3 (1.4)**	**3 (1.4)**	**1 (0.5)**	**1 (0.5)**	**2 (0.9)**	**45 (20.5)**	**76 (34.7)**	**1 (0.5)**	**4 (1.8)**	**1 (0.5)**	**77 (35.2)**	**2 (0.9)**	**1 (0.5)**	**219 (100)**	**100.0%**	**6**

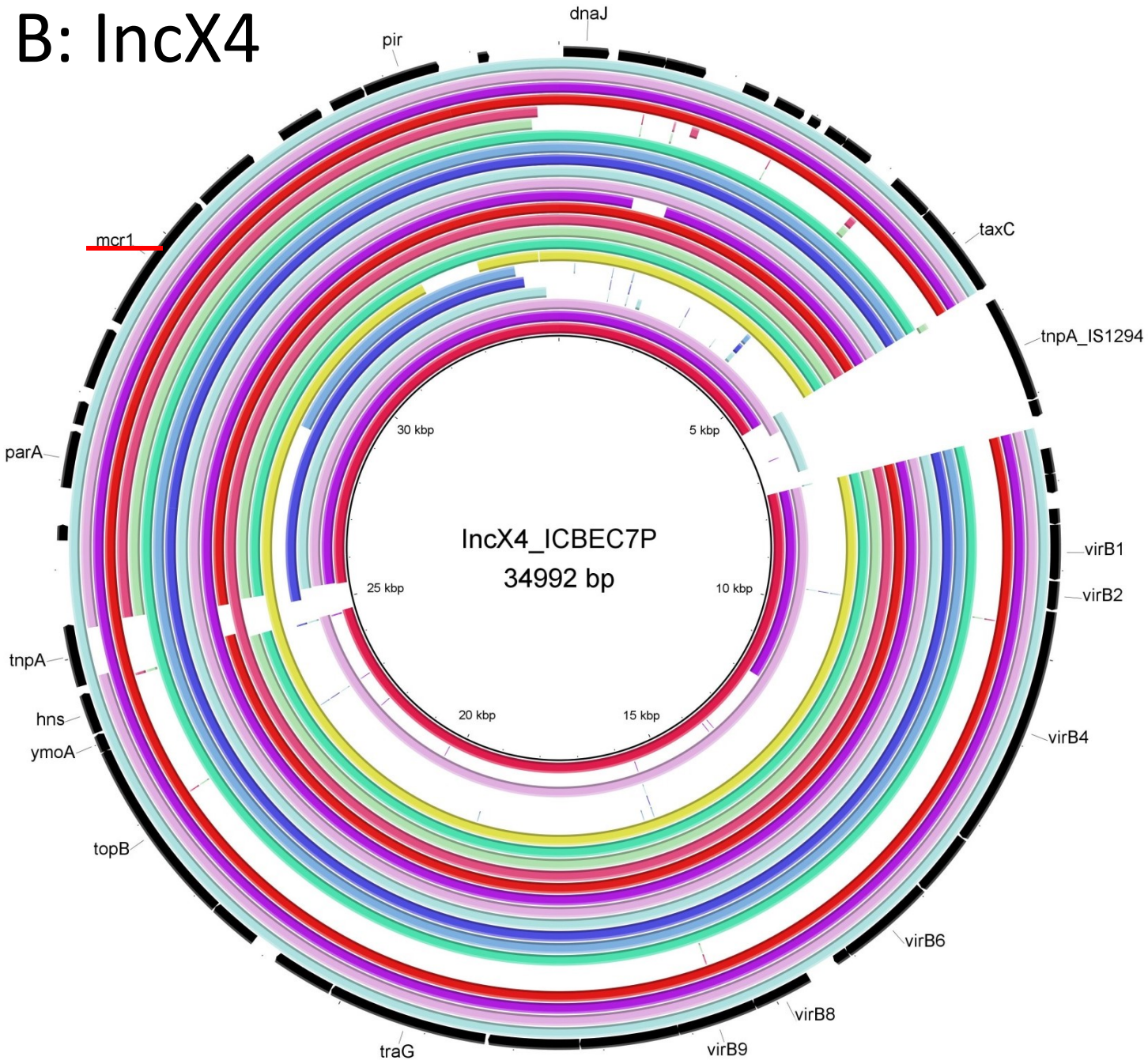
Table 1: Incompatibility types of mcr-1 carrying plasmids and repartition by geographical regions

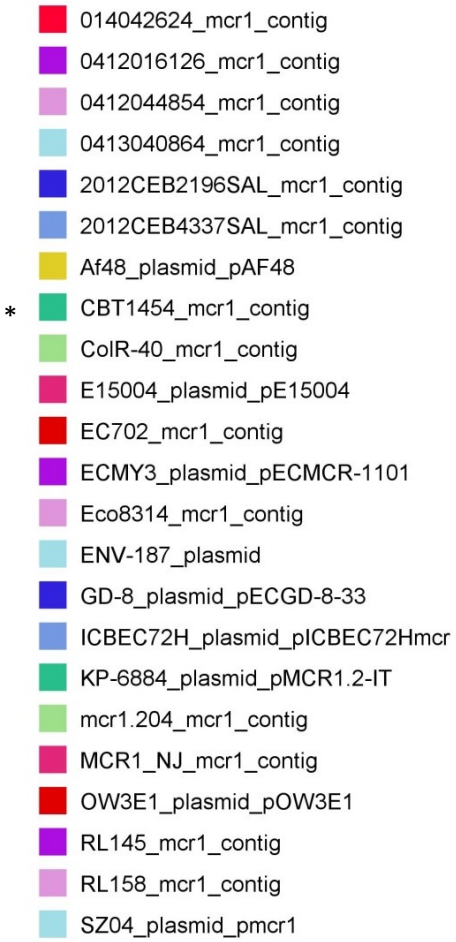
A: IncI2


IncI2_pHNSHP45
64015 bp
5 kbp
10 kbp
15 kbp
20 kbp
25 kbp
30 kbp
35 kbp
40 kbp
45 kbp
50 kbp
55 kbp
60 kbp
repA
IS683
tnpA-IS683
mok
proQ
parA
yaiB
traL
nikA
nikB
lsa-pl1
mcr-1
ymoA
topB
yhcR
outO
tcpE
epsE
bfpB
traG
ptlF
virB8
virB4
virB1
pilL
kikA
relE
virB6
ybbJ
hflC
rcbA

210205360_pSLy1
210221272_plasmid_pSLy21
A31-12_plasmid_pA31-12
ABC149_plasmid_pABC149
Af23_plasmid_pAF23
BA76_plasmid_pBA76
BA77_plasmid_pBA77
BB1291_mcr1_contig
* CBT0637_mcr1_contig
* CBT1531_mcr1_contig
CT31C.G1_mcr1_contig
E15017_plasmid_pE15017
EC13_plasmid_pEC13-1
EC249_mcr1_contig
EC5_plasmid_pEC5-1
EG0430_plasmid_pEG430-1
ESBL20150072_mcr1_contig
JS-61_plasmid_pECJS-61-63
mcr1_213_payne_mcr1_contig
MT02C.A5_mcr1_contig
PO169_mcr1_contig
S2.14_plasmid_pS2.14-2
S3_mcr1_contig
SC23_plasmid_pSCS23
SZ02_plasmid_pmcr1_IncI2
VT55363_plasmid_pVT553
WCH1410_plasmid_pMCR_1410
WF-5-19C_plasmid_pWF-5-19C_mcr-1

B: IncX4


dnaJ
pir
mcr1
taxC
tnpA_IS1294
parA
30 kbp
5 kbp
IncX4_ICBEC7P
34992 bp
25 kbp
10 kbp
virB1
virB2
tnpA
hns
ymoA
20 kbp
15 kbp
virB4
topB
virB6
virB8
traG
virB9


014042624_mcr1_contig
0412016126_mcr1_contig
0412044854_mcr1_contig
0413040864_mcr1_contig
2012CEB2196SAL_mcr1_contig
2012CEB4337SAL_mcr1_contig
Af48_plasmid_pAF48
* CBT1454_mcr1_contig
ColR-40_mcr1_contig
E15004_plasmid_pE15004
EC702_mcr1_contig
ECMY3_plasmid_pECMCR-1101
Eco8314_mcr1_contig
ENV-187_plasmid
GD-8_plasmid_pECGD-8-33
ICBEC72H_plasmid_pICBEC72Hmcr
KP-6884_plasmid_pMCR1.2-IT
mcr1.204_mcr1_contig
MCR1_NJ_mcr1_contig
OW3E1_plasmid_pOW3E1
RL145_mcr1_contig
RL158_mcr1_contig
SZ04_plasmid_pmcr1

C: IncHI2

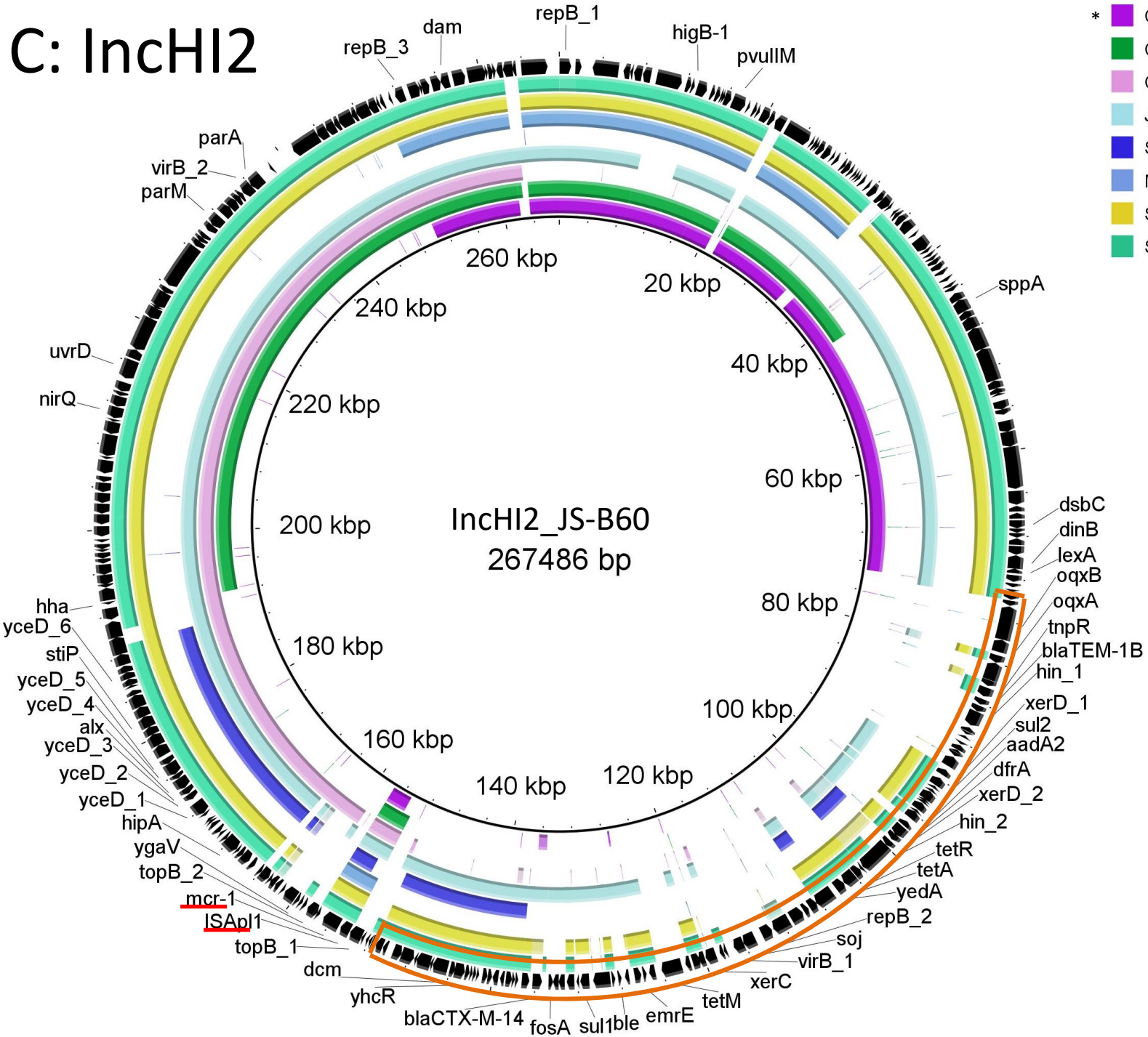

IncHI2_JS-B60
267486 bp
20 kbp
40 kbp
60 kbp
80 kbp
100 kbp
120 kbp
140 kbp
160 kbp
180 kbp
200 kbp
220 kbp
240 kbp
260 kbp
repB_1
higB-1
pvuIIM
sppA
dsbC
dinB
lexA
oqxB
oqxA
tnpR
blaTEM-1B
hin_1
xerD_1
sul2
aadA2
dfrA
xerD_2
hin_2
tetR
tetA
yedA
repB_2
soj
virB_1
xerC
tetM
emrE
ble
sul1
fosA
blaCTX-M-14
yhcR
dcm
topB_1
ISApl1
mcr-1
topB_2
ygaV
hipA
yceD_1
yceD_2
yceD_3
alx
yceD_4
yceD_5
stiP
yceD_6
hha
nirQ
uvrD
parM
virB_2
parA
dam
repB_3
* CBT1023_mcr1_contig
CG10C.C1_mcr1_contig
CT41C.G2_mcr1_contig
JS-59_plasmid_pECJS-59-244
Sal25_plasmid
MT31F.G1_mcr1_contig
S38_plasmid_pS38
S38_plasmid_pS38

Figure 3: Alignment of *mcr-1*-containing plasmids and contigs.

Panel A: IncI2 plasmids (n = 29); panel B: IncX4 (n = 24); panel C: IncHI2 (n = 9).

Black outer ring: plasmid used as reference for the alignment; name and size of the reference indicated in the middle of each panel. Plasmid names followed by the mention "_mcr1_contig" refer to assembled contigs from whole genome sequences. Other names refer to plasmid sequences deposited in online databases. The *mcr-1* gene and ISA-pl1 location are underlined in red.

Plasmids indicated with an asterisk are from the isolates newly sequenced for this study.

Panel C: Putative MDR cassette is highlighted in orange.