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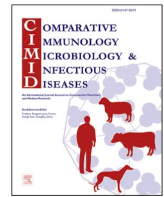

Full Length Article

Clonal and plasmid-mediated dissemination of *mcr-1* in *Escherichia coli* strains at the human–companion animal interface: Genomic characterisation of colistin resistance plasmids

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ABSTRACT

The global emergence of plasmid-mediated colistin resistance (*mcr-1*) gene poses a critical threat to human and animal health due to its ability for horizontal dissemination. While the role of food-producing animals is well recognised, the contribution of companion animals and household environments to the persistence and circulation of *mcr-1* remains poorly understood. In this study, we investigated the genetic relatedness of *mcr-1*-positive *Escherichia coli* strains and their associated plasmids from dogs and their cohabiting humans in Portugal (2018–2020). Whole-genome sequencing, was performed on 17 strains, including repeated sampling from the same hosts over time. Core genome SNP analysis revealed clonal relatedness among several strains from the same host and between household members (≤6 SNPs). A total of 14 *mcr-1*-harbouring plasmids were identified and classified into three major incompatibility groups: IncX4 (n = 2), IncHI2 (n = 5), and IncI2 (n = 7). IncX4 plasmids were detected in clonally related strains from the same human host and were identical, indicating maintenance within a persistent lineage. A subset of IncI2 plasmids formed a closely related cluster (1–6 SNPs) across genetically distinct hosts, supporting the possibility of horizontal dissemination. IncHI2 plasmids displayed greater structural diversity and carried multiple antimicrobial and metal resistance determinants. Notably, chromosomal integration of *mcr-1* was identified in three strains, suggesting a potential pathway for stabilisation of colistin resistance. Overall, these findings highlight the combined role of clonal expansion and plasmid circulation in shaping the epidemiology of *mcr-1* genes in community settings, reinforcing the importance of genomic surveillance within a One Health framework.

1. Introduction

Colistin has long been considered a last-resort antimicrobial for the treatment of infections caused by multidrug-resistant Gram-negative bacteria, particularly carbapenem-resistant Enterobacterales [1]. For decades, resistance to colistin was thought to arise exclusively from chromosomal mutations, limiting its horizontal dissemination [2]. This paradigm changed in 2015 with the identification of the plasmid-mediated colistin resistance gene *mcr-1*, which revealed an unprecedented potential for transferable resistance [3]. Since then, *mcr* variants have been detected worldwide in a broad range of bacterial species, hosts and ecological compartments, raising major concerns for

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public health [1,4].

While the role of food-producing animals and the food chain in the dissemination of *mcr-1* has been extensively documented, considerably less attention has been given to companion animals [5,6]. Dogs and cats live in close contact with humans, share domestic environments and are frequently exposed to antimicrobial agents in veterinary medicine, creating favourable conditions for the maintenance and exchange of resistant bacteria [7]. Increasing evidence suggests that companion animals may act as important reservoirs and potential bridging hosts for antimicrobial resistance transmission between animals and humans [8]. However, detailed genomic investigations addressing the diversity, persistence and relatedness of *mcr-1*-carrying plasmids within household and close-contact settings remain limited [5,9].

Recent studies have demonstrated the wide ecological distribution of *mcr*-harbouring plasmids across human, animal, food and environmental reservoirs [10–14]. However, it remains unclear whether identical or highly similar plasmids circulate among unrelated households within the same urban environment, and to what extent companion animals contribute to the broader community-level dissemination of plasmid-mediated colistin resistance. In particular, longitudinal data exploring plasmid persistence within households and potential inter-host transmission events are still scarce.

To address this knowledge gap, the present study builds upon previous findings from the PET-Risk Consortium, a One Health surveillance initiative that investigated antimicrobial-resistant Enterobacterales in companion animals and their household members [15]. In this follow-up work, we compiled all *mcr*-positive Enterobacterales identified during the PET-Risk project and performed whole-genome sequencing with detailed plasmid reconstruction. The aim of this study was to characterise the diversity, genetic relatedness and dissemination patterns of *mcr-1*-carrying plasmids recovered from dogs and humans living in the community and household settings in Portugal between 2018 and 2020.

2. Materials and methods

2.1. Bacterial isolates

All *mcr*-positive strains included in this study were selected from the strain collection generated within the PET-Risk project, an international One Health consortium involving Portugal, the United Kingdom, Germany, Switzerland and Canada, aimed to investigate antimicrobial-resistant Enterobacterales in companion animals and their household members [15]. The present analysis focuses exclusively on isolates collected in Portugal.

Briefly, this prospective longitudinal study was conducted at the small animal veterinary teaching hospital of the Faculty of Veterinary Medicine, University of Lisbon, Portugal, between January 2018 and December 2020. Faecal samples were collected from companion animals (dogs and cats) and their cohabiting human household members. Animals included both clinically healthy individuals and animals diagnosed with skin and soft tissue infection or urinary tract infection. Sampling was performed at multiple timepoints within households, including at initial recruitment (T0) and during follow-up visits (T1, T2 and T3), corresponding to approximately 1 week, 1 month and 2 months after recruitment, respectively.

A total of 125 humans and 102 cohabiting animals from 80 households were recruited, as previously described [15]. From this collection, *mcr-1*-positive *Escherichia coli* isolates recovered from dogs and humans at different sampling timepoints were selected for further analysis.

In this follow-up study, complete nucleotide sequence characterisation was performed for all *mcr-1*-carrying plasmids recovered (n = 14), encompassing IncHI2 (n = 5), IncI2 (n = 7), and IncX4 (n = 2) incompatibility groups.

2.2. Whole-genome sequencing

Genomic DNA from colistin-resistant strains (n = 17) was extracted using the NZY Tissue gDNA Isolation kit (NZYTech, Portugal). Library preparation was performed with the TruSeq DNA PCR-Free preparation kit and sequenced using the Illumina NovaSeq 6000 system with 2 × 150 bp paired-end reads (Illumina, US) at a commercial company (Macrogen, Seoul, Republic of Korea).

Raw reads were quality-checked with FastQC v0.11.9 [16], and filtered using PRINSEQ v0.20.4, retaining reads with an average Phred score ≥ 20 and a minimum length of 90 nucleotides [17]. After filtering, an average of 7.75×10^6 high-quality reads *per* sample were obtained. De novo genome assemblies were generated using SPAdes v3.14.1 [18], followed by two runs of polishing with Pilon v1.24 [19]. Assembly qualities were determined using QUAST v5.0.2. The resulting genome assemblies showed a mean L50 value of 12.11 (range 7–15), an average N50 of 1.5×10^5 bp (range 6.86×10^4–2.70×10^5 bp), and an average sequencing depth of 214 × (Supplementary Table S1).

For strains in which plasmid reconstruction could not be resolved using short-read sequencing (n = 3), long-read sequencing was performed using Oxford Nanopore Technology (UK). Genomic DNA was extracted with the Wizard® Genomic DNA Purification Kit (Promega, US). Libraries were prepared with the Rapid Sequencing Kit (SQK-RAD004) and sequenced on R9 flow cells using MinION Mk1C device. Assembly and polishing were carried out with EPI2ME (v5.2.5) using the wf-bacterial-genomes workflow (v1.4.2).

Gene annotation was performed with Prokka v1.14.6 [20]. Antimicrobial resistance genes were assessed using ResFinder [21], while plasmid replicons and plasmid multilocus sequence typing (ST) were obtained using PlasmidFinder and pMLST 2.0, respectively [22]. Virulence-associated genes were obtained using VirulenceFinder 2.0 [23], and *E. coli* strains were classified as extraintestinal pathogenic (ExPEC), uropathogenic *E. coli* (UPEC) or avian pathogenic *E. coli* (APEC) according to established molecular criteria [24].

Core genome single nucleotide polymorphism (SNP) alignments were generated with Parsnp v1.2 [25] using *E. coli* K-12 MG1655 (GenBank accession: GCA_000005845.2) as the reference genome. Recombinant regions were filtered using Gubbins [26] and maximum likelihood phylogenies were inferred with RaxML-NG [27] were used to provide a repeats based on core genome alignment, with 100 bootstrap replicates. Pairwise SNP distances were calculated using snp-dists v.0.8.2 [28]. A threshold of ≤ 10 core-genome SNPs was considered indicative of close genetic relatedness, as previously proposed for *E. coli* strains [29]. Phylogenetic trees were visualised and annotated alongside antimicrobial resistance profiles using the Microreact platform [30]. Plasmid phylogenetic analysis employed the same mapping and SNP calling methods as described for chromosomal phylogeny. The plasmid pS38 (GenBank accession no. KX129782.1) served as the reference plasmid genome for IncHI2 analysis, and pHNSHP45 (GenBank accession no. KP347127.1) for IncI2 plasmids.

Comparative analyses of plasmid sequences were performed using BRIG v0.95 to align reconstructed plasmids against closely related reference sequences retrieved from NCBI.

All sequencing data strains are available in public repositories under the BioProject accession numbers PRJEB45751 and PRJNA1321126.

3. Results

3.1. Phylogenetic relationships between colistin-resistant strains

A total of 17 colistin-resistant *mcr-1*-positive *E. coli* isolates recovered from eight dogs and four humans across eight households were included in this study. These isolates were obtained at different sampling time points and therefore include repeated sampling of the same host over time.

Core genome phylogenetic analysis revealed the presence of distinct

E. coli lineages, with clustering primarily driven by host or household origin (Fig. 1). Bacterial strains recovered from a human and his cohabiting dog in household PT102 differed by ≤ 6 SNPs (Supplementary table S2), indicating the presence of a shared clone across hosts. Similarly, two strains (PT219/1-H2F7E1 and PT219/2-H2F7E1) obtained from the same human host at different sampling timepoints in household PT219, differed by only 2 SNPs, consistent with within-host persistence. These strains were classified as APEC due to the presence of *hlyF, iroN, iss, ompT* virulence genes (Supplementary table S3). The same level of persistence was observed for the dog from household PT115, with no SNP differences between strains from different timepoints (Supplementary table S2).

In contrast, isolates from different households generally exhibited high SNP distances (>20,000 SNPs), indicating the presence of unrelated lineages. Notably, isolates from two dogs within household PT051 differed by > 40 SNPs (Supplementary table S2), despite clustering closely in the phylogenetic tree, confirming that they represent distinct clones.

3.2. Distribution and diversity of mcr-1 plasmid backbones

Fourteen strains carrying *mcr-1*–positive plasmids were identified, originating from ten dogs and four human hosts across seven households. These plasmids were classified into three major incompatibility groups: IncX4 (n = 2), IncHI2 (n = 5) and IncI2 (n = 7) (Supplementary table S4).

In three additional *mcr-1*-positive *E. coli* strains belonging to family PT118, plasmid reconstruction was unsuccessful even after long-read sequencing, as the *mcr-1* gene was not associated with any identifiable plasmid replicon. Instead, *mcr-1* was consistently located within a chromosomal contig (~3.75 Mb), embedded in a conserved genomic region enriched in housekeeping and metabolic genes, including hydrogenase operon components (HybABCDEFG), regulatory systems (QseBC), and core genes such as *dnaG* and *rpoD*. The genetic environment of *mcr-1* comprised the canonical *mcr-1–pap2* cassette flanked by multiple mobile genetic elements, including transposases belonging to ISL30, IS2, and IS3 families, as well as IS21-like elements, suggesting acquisition via mobile genetic elements. However, no complete IS*Apl1* elements were detected flanking the cassette.

3.3. Comparative analysis of IncX4 mcr-1 plasmids

The two IncX4 plasmids (~33 kb), pPT219/1-H2F7E1 and pPT219/2-H2F7E1, were identical, sharing 100% nucleotide coverage and 100% sequence identity (Fig. 2). Both were carried by *E. coli* trains obtained from the same human host at different sampling times.

These plasmids showed an almost complete nucleotide identity (>99.99%) with pMFDS2258.1, an IncX4 plasmid reported from *E. coli* isolated from chicken meat in Brazil, and very high similarity (98% coverage, 100% identity) to the pHNSHP49 plasmid originally described in a porcine *E. coli* strain from China (Fig. 2).

In both PT219H2 plasmids, the *mcr-1.1* gene was located within the characteristic pHNSHP49-like IncX4 backbone, flanked by a hypothetical protein–encoding gene and a PAP2 family transmembrane protein (Fig. 2). No additional antimicrobial resistance genes, beyond *mcr-1.1*, was observed.

3.4. Comparative analysis of IncHI2 mcr-1 plasmids

The five IncHI2 plasmids ranged from approximately 247–266 kb. Plasmid multilocus sequence typing revealed that four plasmids belonged to ST4 (pPT102/1-H3F7E1, pPT102/1-D1F7E1.1, pPT102/2-D1F7E2 and pPT214/3-D1F7E1), whereas a single plasmid, pPT124/1-D1F3E1, was assigned to ST2. This difference in plasmid sequence type was consistent with the pronounced structural heterogeneity observed within the IncHI2 group (Fig. 3).

SNP-based analysis of IncHI2 plasmids revealed limited genetic variation among the sequences included in this study. Plasmids from household PT102 showed no SNP differences. The plasmid pPT214/3-D1F7E1 differed by only 3 SNPs from the PT102 plasmids, while

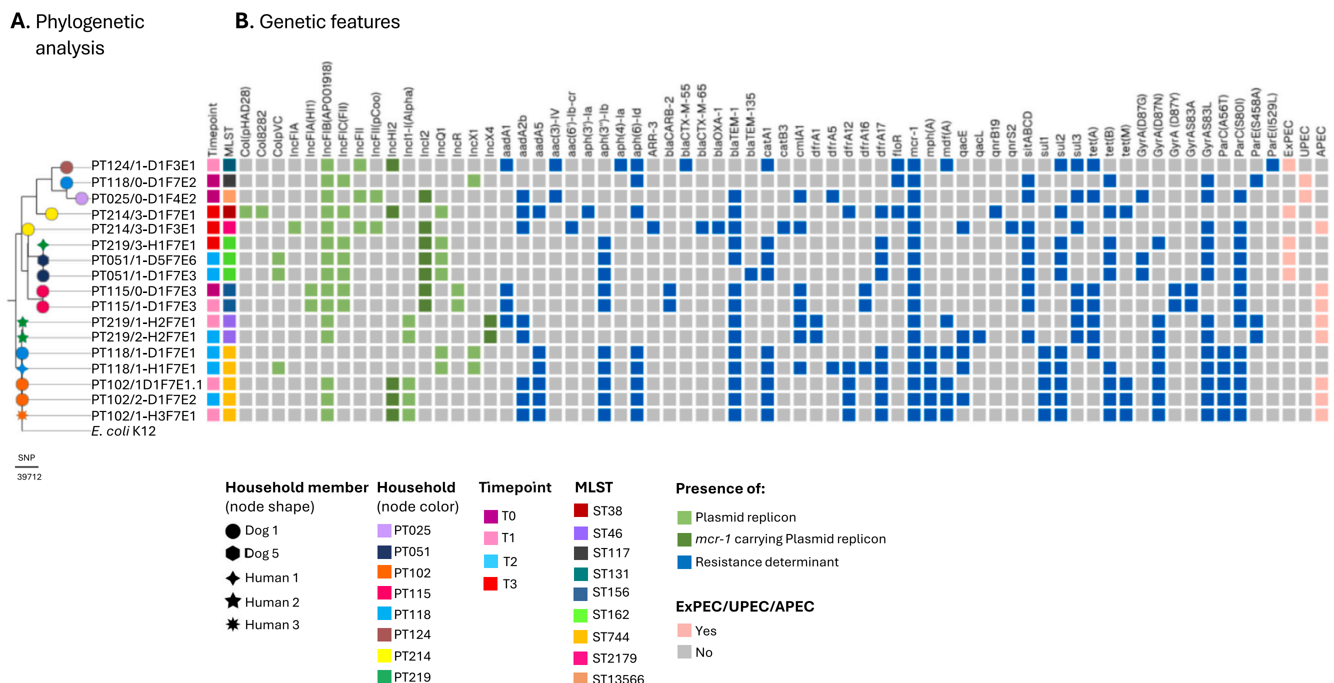

Fig. 1. Core genome SNP analysis and genetic features of *mcr-1*-positive *Escherichia coli* strains from companion animals and their cohabitant humans. (A) Maximum likelihood phylogeny of the core genome of 17 *E. coli* strains from humans ad dogs and the *E. coli* K-12 MG1655 strain. (B) Heatmap shows the *E. coli* sequence types, plasmid replicons, antimicrobial resistance determinants and pathotypes based on a repertoire of virulence factors for each strain (see colour key on the bottom of the figure). Sampling timepoints are defined as follows: T0, at recruitment T1, one week after recruitment; T2, one month after recruitment; and T3, two months after recruitment.

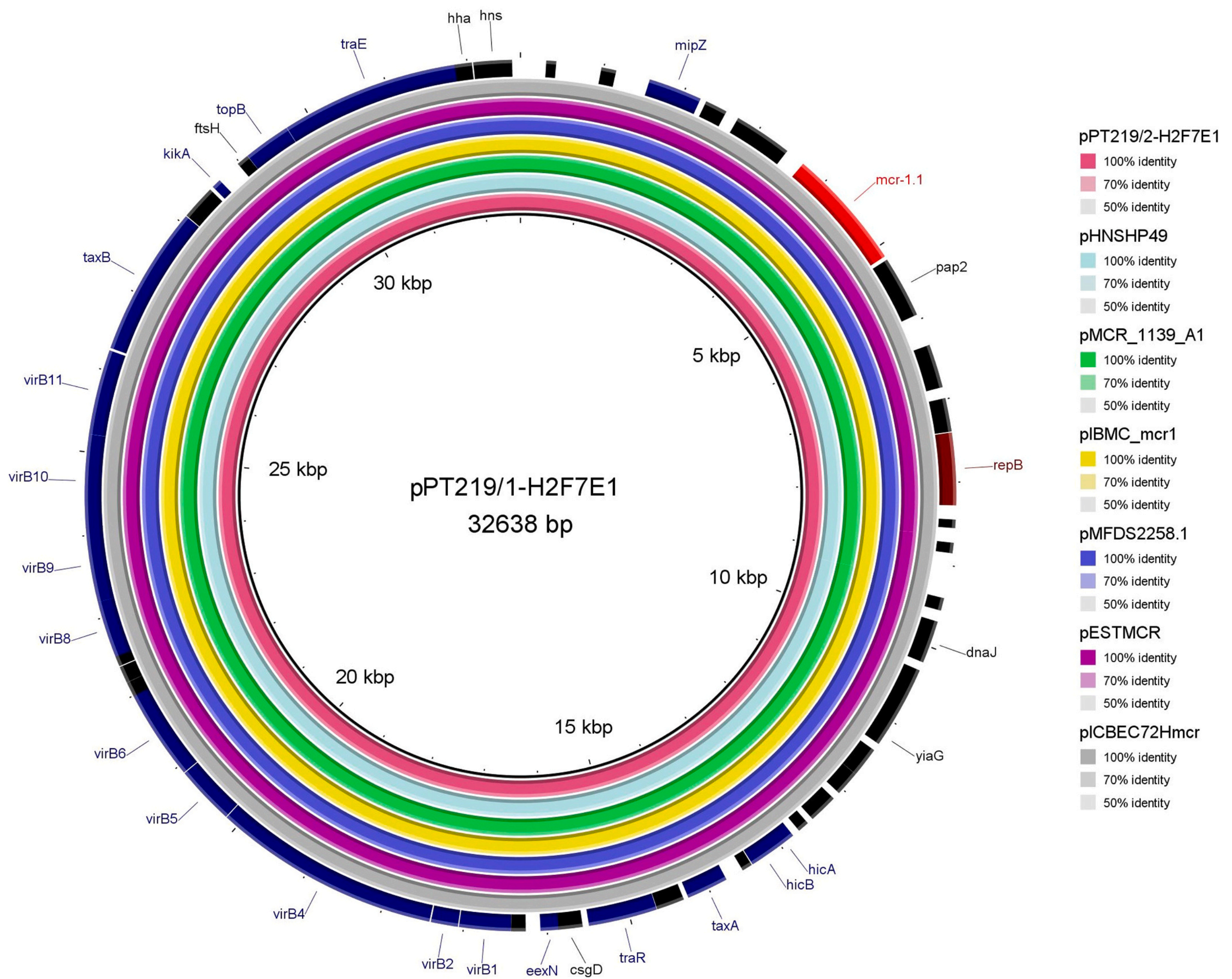

Fig. 2. Comparison of *mcr-1*-positive IncX4 plasmids identified in this study and closely related plasmids from human, animal, food and environmental sources reported worldwide. Rings are arranged from inside out starting with the backbone plasmid reference (black inner ring) pPT219/1-H2F7E1, followed by pPT219/2-H2F7E1, both from human faecal samples from this work. These are compared with pHNSHP49 from a pig in China (GenBank accession no. NZ_MF774188.1), pMCR_1139_A1 from raw turkey meat from Poland (MT929277.1), pIBMC_mcr1 from river water in Italy (NZ_MF449287.1), pMFDS2258.1 from chicken meat from Brazil (MK869757.1), pESTMCR from pig slurry in Estonia (NZ_KU743383.1), and pICBEC72Hmcr from a human clinical sample from Brazil (NZ_CP015977.1). Genes are represented by coloured blocks in the outer circle: red, antibiotic resistance genes; orange, transposase genes, transposons and insertion sequences; dark blue, genes associated with partition, modification and stability systems; brown, replication genes; black, other genes.

comparisons with the reference plasmid pS38 showed ≤ 12 SNP differences (Supplementary figure S1).

The IncHI2 plasmids characterised here shared a conserved backbone typical of the IncHI2 family, encompassing regions involved in replication (*repHI2*), conjugative transfer (the *tra* operon), and plasmid maintenance (*par* genes). Structural comparisons revealed that the PT102 plasmids were similar to other ST4 IncHI2 plasmids (pMCR_915_E1, pSE08–00436–1 and pMCR_1085_C1), reported from different sources worldwide (Fig. 3).

The IncHI2 ST2 plasmid pPT124/1-D1F3E1 also exhibited a backbone similar to IncHI2 ST4 plasmids previously reported from raw turkey meat in Poland (pMCR_915_C1 and pMCR_170_D1), as well as to pPT214/3-D1F7E1 recovered in this study from a canine isolate (Fig. 3). Differences were observed in the accessory regions among these plasmids.

In all IncHI2 plasmids the *mcr-1.1* gene was consistently associated with a IS*Apl1* upstream and followed by a *pap2* gene encoding a PAP2 family transmembrane protein. These plasmids carried tellurium resistance genes (*terZABCDEF*), together with *terY1*, *terY2* and *terW* genes. In addition, plasmids pPT124/1-D1F3E1 and pPT214/3-D1F7E1 harboured the *sil* and *pco* operons, conferring resistance to silver and copper, respectively.

Additional antimicrobial resistance genes were identified within transposon-associated regions. Plasmid pPT124/1-D1F3E1 carried genes conferring resistance to tetracyclines (*tet(A)*), aminoglycosides (*aph(4)-Ia*, *aac(3)-IV*, *aadA1*), macrolides (*mef(B)*) and chloramphenicol (*catA1*). PT102 plasmids (pPT102/1-H3F7E1, pPT102/1-D1F7E1.1 and pPT102/2-D1F7E2) harboured *aadA2* (streptomycin/spectinomycin resistance) and *dfrA1* (trimethoprim resistance) genes, located downstream of the *mcr-1.1* region [15]. In plasmid pPT214/3-D1F7E1, *aadA1* gene was the only additional resistance determinant detected.

3.5. Comparative analysis of IncI2 mcr-1 plasmids

Seven IncI2 elements of approximately 60 kb were detected and represented the most frequently observed plasmid type in this dataset.

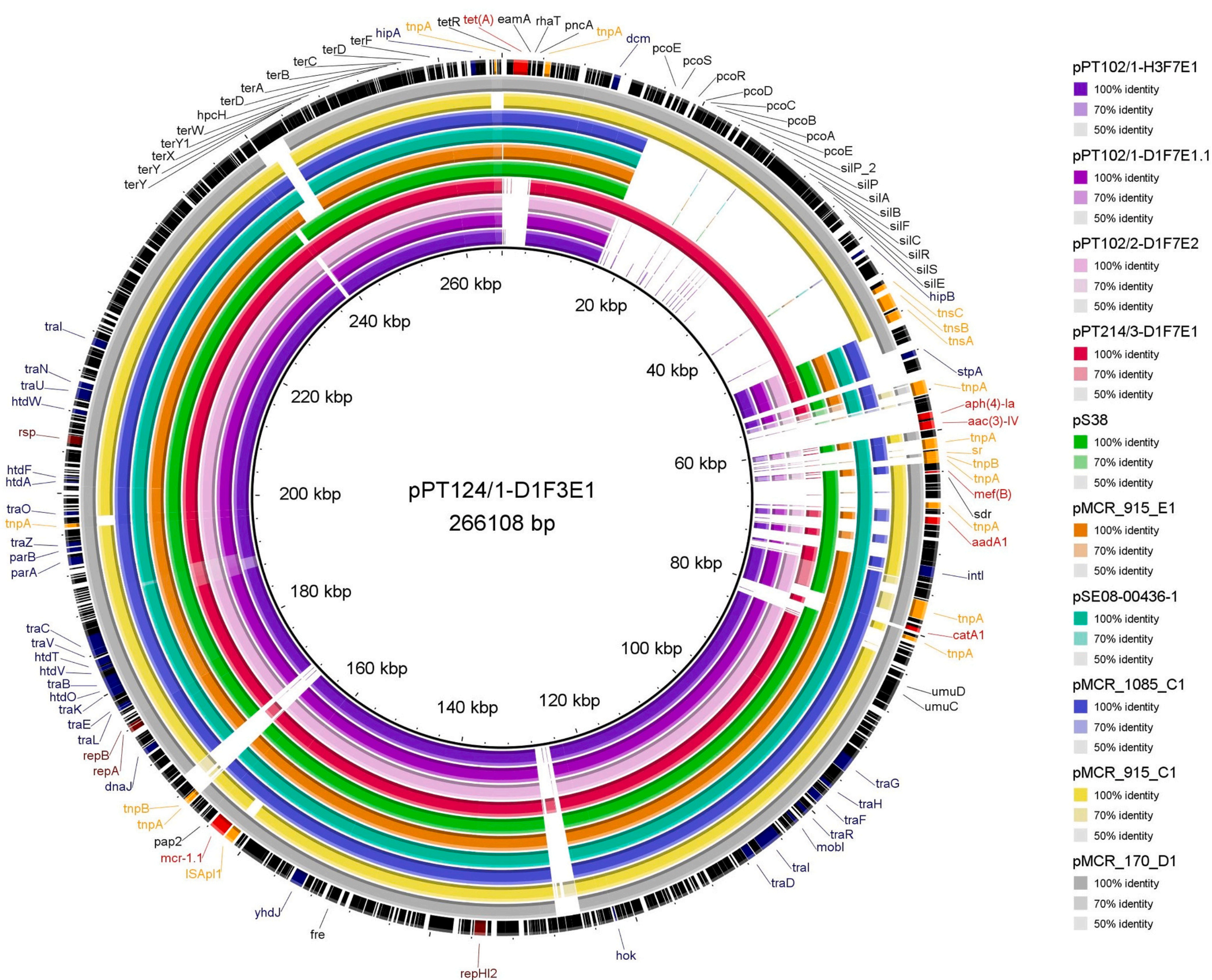

Fig. 3. Comparison of *mcr-1*-carrying IncHI2 plasmids detected in this study and globally distributed IncHI2 plasmids. Rings are arranged from the inside out starting with the backbone plasmid reference (black inner ring) pPT124/1-D1F3E1, recovered from a dog faecal sample in Portugal, followed by pPT102/1-H3F7E1 from a human faecal sample in Portugal, pPT102/1-D1F7E1.1, pPT102/2-D1F7E2 and pPT214/3-D1F7E1, all obtained from dog faecal samples in Portugal in this study. These are compared with pS38 from poultry meat in Italy (GenBank accession no. KX129782.1), pMCR_915_E1 from raw turkey meat in Poland (NZ_MT929285.1), pSE08-00436–1 from chicken skin in Germany (NZ_CP020493.1), pMCR_1085_C1 from raw turkey meat in Poland (NZ_MT929286.1), pMCR_915_C1 from raw turkey meat in Poland (NZ_MT929284.1), and pMCR_170_D1 from raw turkey meat in Poland (NZ_MT929288.1). Genes are represented by coloured blocks in the outer circle: red, antibiotic resistance genes; orange, transposase genes, transposons and insertion sequences; dark blue, genes associated with partition, modification and stability systems; brown, replication genes; black, other genes.

Among the IncI2 plasmids, pPT219/3-H1F7E1 from a human and pPT025/0-D1F4E1 from a dog, recovered from different households, displayed an almost identical genetic structure, sharing 99% sequence coverage and 100% nucleotide identity and only 1 SNP difference. These plasmids also exhibited similarity to previously described *mcr-1.1*–positive IncI2 plasmids present in public databases (Fig. 4).

Plasmids recovered from clonally related strains (pPT115/0-D1F7E3 and pPT115/1-D1F7E3) showed no SNP differences. Notably, plasmids from household PT115 and PT051, together with pPT214/3-D1F3E1, formed a closely related cluster, differing by only 1–6 SNPs, despite being identified in genetically distinct bacterial hosts (Supplementary figure S1).

The two similar IncI2 plasmids from household PT051 dogs, pPT051/1-D1F7E3 and pPT051/1-D5F7E6, showed a high degree of similarity to pHNSHP45 (91% coverage, 98.99% identity), originally described in swine in China [3]. However, in contrast to pHNSHP45, none of the IncI2 plasmids characterised in this study carried the IS*Apl1* insertion sequence upstream of the *mcr-1.1* gene. The *mcr-1.1*–*pap2* element was consistently located downstream of the *nikB* gene within the IncI2 backbone (Fig. 4).

None of the IncI2 plasmids carried additional antimicrobial resistance or virulence-associated genes beyond *mcr-1.1*.

4. Discussion

The emergence and global dissemination of plasmid-mediated colistin resistance represents a major threat for both human and veterinary medicine [5,31]. In this study, whole-genome sequencing enabled a comprehensive characterisation of *mcr-1*-harbouring plasmids recovered from *E. coli* strains obtained from humans and companion animal faecal samples, providing insights into maintenance and spread of colistin resistance within household and community settings.

Three major incompatibility groups were identified among the *mcr-1*-positive plasmids, namely IncX4, IncHI2 and IncI2, which have been

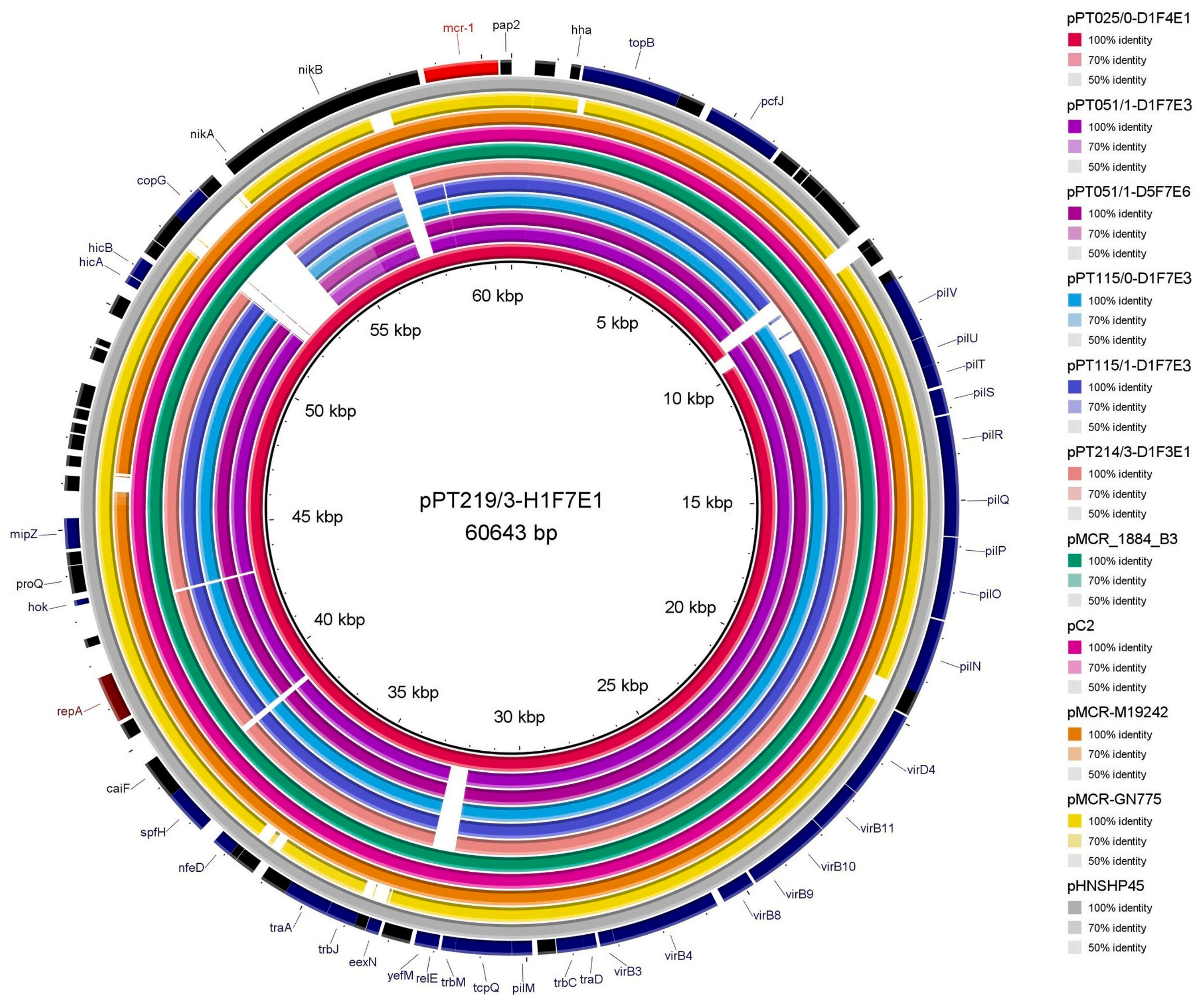

Fig. 4. Comparison of *mcr-1*–carrying IncI2 plasmids identified in this study and closely related IncI2 plasmids reported worldwide. Rings are arranged from the inside out starting with the backbone plasmid reference (black inner ring) pPT219/3-H1F7E1, recovered from a human faecal sample in Portugal, followed by pPT025/0-D1F4E1 from dog faeces in Portugal, pPT051/1-D1F7E3 and pPT051_T1_D5F7E6 from dog faecal samples in Portugal, pPT115/0-D1F7E3 and pPT115/1-D1F7E3 recovered from the same dog at different faecal sampling time points in Portugal, and pPT214/3-D1F3E1 from a dog faecal sample in Portugal in this study. These are compared with pMCR_1884_B3 from raw rabbit meat in China (GenBank accession no. MT929283.1), pC2 from chicken meat in Algeria (CP042471.2), pMCR-M19242 from a human clinical sample in Argentina (KY471312.1), pMCR-GN775 from a human clinical sample in Canada (KY471307.1), and pHNSHP45 from swine in China (KP347127.1). Genes are represented by coloured blocks in the outer circle: red, antibiotic resistance genes; orange, transposase genes, transposons and insertion sequences; dark blue, genes associated with partition, modification and stability systems; brown, replication genes; black, other genes.

most frequently reported in association with the *mcr-1* gene worldwide [10,12,31].

IncX4 plasmids displayed near-identical sequences. While this high level of conservation is consistent with previous reports describing IncX4 plasmids as highly stable and structurally conserved [31,32], the genomic similarity observed in this study is best explained by persistence of the clonally related *E. coli* strain rather than independent plasmid transfer events. Indeed, these plasmids were identified in clonally related strains obtained from the same human host (PT219H2) at different timepoints differed by ≤ 2 SNPs, indicating within-host persistence. This distinction is important, as it suggests that carriage of *mcr-1* may be driven not only by plasmid stability but also by bacterial host persistence within the gut microbiota. Furthermore, IncX4 plasmids showed high sequence identity and coverage with plasmids previously reported worldwide from diverse hosts and ecological niches, a feature likely associated with their compact structure and limited accessory gene content, which may reduce fitness costs and favour maintenance and dissemination even in the absence of antimicrobial selective pressure [32–34].

In contrast, IncHI2 plasmids exhibited marked structural heterogeneity, in line with previous reports describing these large plasmids as genetically plastic elements with mosaic accessory regions [11,31,35]. In this study, five IncHI2 plasmids were reconstructed and assigned to two plasmid sequence types (ST2 and ST4), with pronounced variability in accessory regions but a conserved backbone comprising replication, conjugation and maintenance functions. All IncHI2 plasmids carried tellurium resistance genes, in agreement with their frequent association with this plasmid family [11].

Importantly, the IncHI2 plasmids characterised here carried multiple antimicrobial resistance determinants in addition to *mcr-1.1*, reinforcing their role as efficient platforms for the accumulation and dissemination of multidrug resistance. The coexistence of antibiotic and heavy metal

resistance determinants further suggests that these plasmids may be maintained under diverse selective pressures. In particular, exposure to metals such as zinc and copper, widely used in animal husbandry, may contribute to co-selection and persistence, while additional anthropogenic pressures may further shape their maintenance in complex ecological settings [36].

Within household PT102, IncHI2 plasmids showed no SNP differences, consistent with their occurrence in clonally related bacterial hosts (≤6 SNPs differences). Therefore, as observed for IncX4 plasmids, the high level of plasmid similarity is more likely explained by vertical inheritance within bacterial lineages rather than independent plasmid transfer. Interestingly, these IncHI2 ST4 plasmids also exhibited a high degree of structural similarity with previously described plasmids from diverse sources, including pS38 originally described in an *E. coli* strain from imported poultry meat from Italy [36], pMCR_915_E1 and pMCR_1085_C1 from raw turkey meat in Poland [11], and pSE08–00436–1 from a *Salmonella enterica* strain from chicken in Germany [37]. Such observations reinforce the importance of domestic settings as overlooked reservoirs for multidrug-resistant plasmids with broader dissemination potential and emphasise the relevance of a One Health perspective when addressing the epidemiology of colistin resistance.

IncI2 plasmids constituted the most frequently identified group in this study and were characterised by a high degree of structural conservation. As observed for other plasmid groups, identical IncI2 plasmids were frequently associated with clonally related strains, including strains recovered from the same dog (household PT115) at different timepoints, again supporting vertical inheritance within bacterial lineages. In contrast, evidence of horizontal plasmid transfer was observed within different dogs from household PT051, where closely related plasmids (≤5 SNPs) were identified in genetically distinct *E. coli* strains (>40 SNPs). A similar pattern was observed when comparing plasmids from household PT115 and PT051 together with pPT214/3-D1F3E1 (1–6 SNP differences), further supporting horizontal dissemination. These findings highlight the coexistence of clonal spread and horizontal gene transfer as complementary mechanisms shaping the dissemination of the *mcr-1* gene.

These IncI2 plasmids closely resembled previously described *mcr-1.1*–positive plasmids from diverse sources, including *E. coli* isolated from raw rabbit meat in China [11], chicken meat in Algeria [38], and human clinical sample from Argentina and Canada [12]. A conserved genetic architecture was observed, including the characteristic *mcr-1.1–pap2* module located downstream of *nikB* gene, a configuration widely recognised as a hallmark of pHNSHP45-like plasmids [12, 39–41]. Notably, IS*Apl1* was absent in all IncI2 plasmids, further supporting stabilisation of the resistance determinant within the plasmid backbone, given that the loss of IS*Apl1* is associated with reduced genetic instability while preserving resistance [9,42,43]. Together, these features likely contribute to the successful maintenance and dissemination of IncI2 plasmids across different hosts and ecological settings.

All *mcr-1.1*–harbouring plasmids identified in this study encoded conserved conjugative transfer regions. IncX4 and IncI2 plasmids exhibited complete sets of type IV secretion system–associated genes, including *virB/virD4* and *pil/tra* operons, consistent with the well-described architecture of self-transmissible plasmids [44,45]. Similarly, the IncHI2 plasmids contained extensive conjugative transfer modules, including *tra* gene clusters, consistent with their classification as self-transmissible elements [43,45]. The conservation of these conjugative systems supports their potential for horizontal dissemination, although our findings indicate that, in the studied setting, clonal expansion often plays a dominant role. Nevertheless, no conjugation assays were performed in this study, and conclusions regarding plasmid transferability are based solely on *in silico* detection of conjugative transfer genes.

Interestingly, none of the plasmids characterised in this study carried known virulence-associated genes, and additional antimicrobial resistance determinants were unevenly distributed, being mainly confined to IncHI2 plasmids. This finding suggests that plasmid-mediated colistin resistance in these strains is not necessarily linked to enhanced virulence but rather reflects the independent spread of resistance elements capable of persisting in commensal bacterial populations. Such reservoirs may remain clinically silent while serving as a continuous source for resistance gene dissemination.

Notably, in PT118 strains, the *mcr-1* gene was located on the chromosome rather than on a plasmid. This chromosomal integration, in the absence of detectable IS*Apl1* elements, is consistent with previously described evolutionary pathways of *mcr-1*. Mobilisation of this gene is thought to be mediated by the IS*Apl1,* followed by stabilisation through partial or complete loss of the insertion sequence [9]. Chromosomal integration may favour maintenance of colistin resistance by reducing dependence on plasmid transfer while ensuring stable inheritance during bacterial replication. This phenomena has been previously reported in diverse *Enterobacteriaceae*, including *E. coli, Klebsiella pneumoniae,* and *Enterobacter cloacae,* often in the absence of flanking IS*Apl1* elements, supporting the hypothesis of post-insertion stabilisation [46,47].

This study has some limitations. Bacterial isolates were obtained through colony selection from faecal samples, which may underestimate within-sample diversity, as multiple strains or plasmid variants may coexist but remain undetected. In addition, the relatively small number of subjects limited the ability to capture the full diversity and transmission dynamics of the *mcr-1* gene within the community. Participation in the longitudinal component was voluntary and dependent on owner willingness, which may have introduced self-selection bias, leading to a non-random study population and potentially limiting generalisability.

Taken together, our findings demonstrate that the spread of *mcr-1* among *E. coli* from humans and companion animals is driven by a complex interplay of plasmid stability, horizontal transfer and, importantly, persistence and dissemination of bacterial clones within households. The frequent observation of identical plasmids in clonally related strains indicates that plasmid similarity alone should not be interpreted as evidence of horizontal transfer. These results highlight the need for integrated genomic approaches that consider both plasmid and host bacterial phylogeny to accurately infer transmission pathways. Nonetheless, the identification of identical or highly similar plasmids across different hosts within the household, as well as their resemblance to plasmids reported from diverse ecological and geographical settings, highlights the potential for sustained circulation of resistance determinants in close-contact environments. From a One Health perspective, households represent important interfaces for the maintenance and spread of antimicrobial resistance. The results presented here reinforce the importance of coordinated surveillance across human and animal populations to better understand and mitigate the dissemination of last-resort antimicrobial resistance genes.

Author contributions

Study conception and design were performed by Juliana Menezes, and Constança Pomba. All authors contributed to the material preparation and data collection and interpretation. The first draft of the manuscript was written by Juliana Menezes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Juliana Menezes: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joana Moreira da Silva:** Writing – review & editing, Investigation. **Laura Fernandes:** Writing – review & editing, Investigation. **Andreia J. Amaral:** Writing – review & editing, Supervision, Formal analysis, Data curation. **Constança Pomba:** Writing – review & editing, Supervision, Resources, Project administration, Funding

acquisition, Conceptualization.

Research data

The sequencing data generated during the current study is available NCBI repository, under the BioProjects PRJEB45751 and PRJNA1321126. High-resolution phylogenetic tree of *E. coli* strains linked to molecular data is available at Microreact platform, https://microreact.org/project/hhQduFU3xwHygnCzQMrijQ-mcr-1-positive-e-coli-strains

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juliana Menezes reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cimid.2026.102477.

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