Science of the Total Environment 876 (2023) 162649

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

First reported detection of the mobile colistin resistance genes, *mcr*-8 and *mcr*-9, in the Irish environment

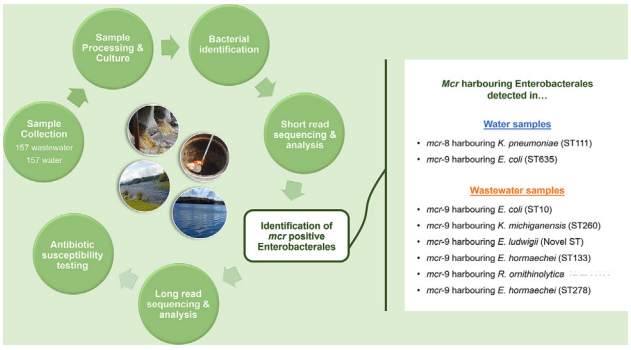
Niamh Cahill [a,b,*], Brigid Hooban [a,b], Kelly Fitzhenry [a,b], Aoife Joyce [a,b], Louise O'Connor [a,b], Georgios Miliotis [a,b], Francesca McDonagh [a,b], Liam Burke [a,b], Alexandra Chueiri [a,b], Maeve Louise Farrell [a,b], James E. Bray [c], Niall Delappe [d], Wendy Brennan [d], Deirdre Prendergast [e], Montserrat Gutierrez [e], Catherine Burgess [f], Martin Cormican [a,b,d], Dearbháile Morris [a,b]

[a] Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Galway, Ireland
[b] Centre for One Health, Ryan Institute, University of Galway, Galway, Ireland
[c] Department of Biology, University of Oxford, Oxford, United Kingdom
[d] National Carbapenemase-Producing Enterobacterales Reference Laboratory, National Salmonella, Shigella and Listeria Reference Laboratory, University Hospital Galway, Galway, Ireland
[e] Department of Agriculture, Food and the Marine, Celbridge, Co. Kildare, Ireland
[f] Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin, Ireland

HIGHLIGHTS

- *mcr* genes were detected in water (*mcr*-8 and *mcr*-9) and wastewater samples (*mcr*-9).
- Two *mcr*-9 positive Enterobacterales also harboured carbapenemase-encoding genes.
- Colistin resistance was only observed in the *mcr*-8 harbouring strains.
- Results highlight potential sources of *mcr* contamination in the environment.
- Further research investigating *mcr* genes in the environment is required.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damià Barceló

Keywords:
Antimicrobial resistance
Colistin
mcr-8
mcr-9
Enterobacterales
Environment

ABSTRACT

The emergence and dissemination of mobile colistin resistance (*mcr*) genes across the globe poses a significant threat to public health, as colistin remains one of the last line treatment options for multi-drug resistant infections. Environmental samples (157 water and 157 wastewater) were collected in Ireland between 2018 and 2020. Samples collected were assessed for the presence of antimicrobial resistant bacteria using Brilliance ESBL, Brilliance CRE, mSuperCARBA and McConkey agar containing a ciprofloxacin disc. All water and integrated constructed wetland influent and effluent samples were filtered and enriched in buffered peptone water prior to culture, while wastewater samples were cultured directly. Isolates collected were identified via MALDI-TOF, were tested for susceptibility to 16 antimicrobials, including colistin, and subsequently underwent whole genome sequencing. Overall, eight *mcr* positive Enterobacterales (one *mcr*-8 and seven *mcr*-9) were recovered from six samples (freshwater ($n = 2$), healthcare facility wastewater (n = 2), wastewater treatment plant influent ($n = 1$) and integrated constructed wetland influent (piggery farm waste) (n = 1)). While the *mcr*-8 positive *K. pneumoniae* displayed resistance to colistin, all seven *mcr*-9 harbouring Enterobacterales remained susceptible. All isolates demonstrated multi-drug resistance and through whole genome sequencing analysis, were found to harbour a wide variety of antimicrobial resistance genes i.e., 30 ± 4.1 (10–61), including the carbapenemases, bla_{OXA-48} ($n = 2$) and bla_{NDM-1} ($n = 1$), which were harboured by three of the isolates.

* Corresponding author at: Discipline of Bacteriology, Orbsen Building, University of Galway, Galway, Ireland.
E-mail address: niamh.cahill@universityofgalway.ie (N. Cahill).

http://dx.doi.org/10.1016/j.scitotenv.2023.162649
Received 25 November 2022; Received in revised form 1 March 2023; Accepted 1 March 2023
Available online 10 March 2023

The *mcr* genes were located on IncHI2, IncFIIK and IncI1-like plasmids. The findings of this study highlight potential sources and reservoirs of *mcr* genes in the environment and illustrate the need for further research to gain a better understanding of the role the environment plays in the persistence and dissemination of antimicrobial resistance.

1. Introduction

Antimicrobial resistance (AMR) is a global health concern. Carbapenem resistant bacteria, including carbapenemase-producing Enterobacterales (CPE), are one of the most concerning antibiotic resistant threats at present (WHO, 2017). The global spread of carbapenem resistant bacteria, including CPE, is compromising treatment options and, in cases where carbapenem antibiotics are no longer effective, tigecycline and colistin are among the last resort treatment options (Andrade et al., 2020; Hussein et al., 2021).

Colistin was used as a treatment option in humans from the 1950s up until the 1970s/1980s, when it was then largely replaced by alternative antibiotics such as aminoglycosides and beta-lactams due to its toxic side effects (Andrade et al., 2020; El-Sayed Ahmed et al., 2020). Unfortunately, due to the increase in infections associated with CPE and limited treatment options, it was reintroduced in the 1990s for use in humans as a last line antimicrobial (El-Sayed Ahmed et al., 2020). From 2011 to 2020, in the EU/EEA, an increase of 67 % in the consumption of polymyxin antibiotics, mainly colistin, was reported (OECD, 2022). In Ireland, based on the most recent data reported by the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) (ECDC, 2022), a slight decrease in polymyxin consumption was observed each year from 2011 to 2017, however in 2018 a slight increase was reported and remained the same in 2019 and 2020. In relation to polymyxins, in Ireland, the defined daily dose (DDD) per 1000 inhabitants per day in 2011 was 0.0279, while in 2017 this dropped to 0.0196, although in 2018, 2019 and 2020 it increased to 0.02 DDD per 1000 inhabitants per day (ECDC, 2022). In animals, colistin has been used for decades for the treatment and prevention of bacterial infections (Poirel et al., 2017). It has also been used as a feed additive for growth promotion in livestock across the world (Poirel et al., 2017). Due to an increase in multi-drug resistance, since 2006 the use of antibiotics, including colistin, is no longer permitted in animals for growth promotion across the European Union (European Commission, 2005). In addition, due to the rise in colistin resistance, its use as an animal feed additive has also been banned in other countries across the world in recent years, for example in China, Thailand, Argentina and Brazil (Olaitan et al., 2021). However, it is still being used for treatment or prophylactic purposes in many countries. In Ireland, in a bid to protect this last line antibiotic for human health, the cessation of colistin use in the animal sector was announced in April 2021 (DAFM, 2021).

Up until 2015, all reports relating to colistin resistance indicated that it was due to chromosomal mutations. However, in 2015 the first mobile colistin resistance (*mcr*) gene, *mcr*-1, was detected in pigs in China (Liu et al., 2016). These *mcr* genes have the potential to cause colistin resistance through target alteration as they encode phosphoethanolamine transferase, an enzyme that is able to alter the lipid A in the bacterial outer membrane when expressed (Aghapour et al., 2019). As this is the target site for colistin any modifications to the site may impact on colistin activity (Aghapour et al., 2019). Since the discovery of *mcr*-1, nine other *mcr* genes have been detected, namely *mcr*-2 to *mcr*-10 (Hussein et al., 2021). However, despite only being detected over the last seven years, it appears that *mcr* genes have been in circulation for some time prior to this, with reports of detection of *mcr*-1 in poultry samples dating back as far as the 1980s (Shen et al., 2016). The spread of plasmid mediated *mcr* genes and their acquisition by bacterial pathogens is hugely concerning due to the threat they pose to the treatment of multi-drug resistant (MDR) bacterial infections for which effective antimicrobial treatment options are limited.

The 'One Health' concept recognises that the health of humans, animals and the environment are all interlinked. It is increasingly acknowledged that the only effective way to tackle the global challenge of AMR is to take a One Health approach. In 2019, Elbediwi et al. (2019) highlighted the widespread dissemination of *mcr* genes across the globe, as they reported the detection of these genes in various bacterial species in human, animal and environmental sources across 47 countries. Elbediwi et al. (2019) reported that environmental samples, including wastewater, river, and sea water samples, had the highest prevalence of *mcr* harbouring strains, while human samples had the lowest. However, they also highlighted the lack of studies investigating the environment for *mcr* genes across the globe and the need for further studies in this area (Elbediwi et al., 2019).

In Ireland, as across the globe, there is no routine surveillance for, or reporting of, *mcr* genes in the human, animal and/or environmental sectors. While there has been reports of *mcr*-1 presence in both humans (NCPERLS, 2018) and in animals (Terveer et al., 2017) in Ireland, to the best of our knowledge this is the first report of *mcr* detection in the Irish environment.

2. Methods

2.1. Sample collection, processing and isolate recovery

Environmental samples, including water (n = 157) and wastewater (n = 157) samples, were collected between November 2018 and November 2020. Water samples collected consisted of seawater (n = 81), estuarine (n = 24), freshwater (n = 40) and drinking water treatment plant influent (untreated) (n = 12) samples. Wastewater samples included those from healthcare facilities (i.e., hospitals and long-term care facilities) (n = 33), airports (n = 4), wastewater treatment plant (WWTP) influents (n = 12) and effluents (n = 12), as well as integrated constructed wetland (ICW) influents (piggery farm waste) (n = 48) and effluents (n = 48).

All ICW samples discussed in this paper were collected and processed as previously described by Prendergast et al. (2022), while Hooban et al. (2021) and Hooban et al. (2022) outlined the collection and processing of all other wastewater samples, as well as water samples. As outlined, the samples were screened for extended-spectrum beta-lactamase (ESBL) producing Enterobacterales, carbapenemase-producing Enterobacterales and fluoroquinolone resistant Enterobacterales, with primary identification of Enterobacterales carried out using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (Bruker).

2.2. Whole genome sequencing (WGS)

2.2.1. Short read sequencing

Initially, a selection of 288 Enterobacterales recovered from water (n = 155) and wastewater (n = 133) samples underwent paired-end short read sequencing using Illumina (Illumina, USA) NovaSeq 6000 or MiSeq platforms. Prior to sequencing, DNA was extracted from ICW wastewater samples using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Diagnostics) as per the manufacturer's instructions, while DNA extraction for all remaining wastewater samples and water samples was carried out using the QIAamp® DNA Mini kit (Qiagen) or EZ1 DNA tissue kit (Qiagen) as previously described by Hooban et al. (2021) and Hooban et al. (2022). All extracted DNA was quantified using the Qubit fluorometer (Qubit dsDNA High Sensitivity Assay Kit). DNA purity was assessed using the NanoDrop ND-1000 spectrometer for DNA extracted from ICW samples, while the DeNovix DS-11 spectrophotometer was used for all other DNA extracted.

After sequencing, bacterial genome assembly of the short reads from ICW samples were assembled using the BioNumerics software platform

(Applied Maths, Sint-Martens-Latem, Belgium), while assembly of the short reads for all other genomes was carried out using SPAdes v3.15.3 (Prjibelski et al., 2020) or Velvet v1.2.10 (Zerbino and Birney, 2008). Prokka v1.12 (Seemann, 2014) was used for annotation of the assembled genomes.

Through ABRicate v1.0.1 (Seemann, n.d.-a, https://github.com/tseemann/abricate) (last updated on the 27th March 2020), antimicrobial resistance genes (ARGs) were identified using the ResFinder (9th March 2021 update) (Zankari et al., 2012) and the comprehensive antibiotic resistance database (CARD) v3.1.2 (Jia et al., 2017) databases. Only hits with both an identity and coverage >90 % were retained.

2.2.2. Long read sequencing

Long read sequencing was performed on all *mcr* positive Enterobacterales (n = 9) i.e., two *mcr*-8 harbouring *K. pneumoniae* (B19137; CMCR2021) as well as *mcr*-9 harbouring *E. coli* (B20339; B18161), *K. michiganensis* (B18164), *E. ludwigii* (B20086), *E. hormaechei* (B20311; GB19–003626) and *R. ornithinolytica* (B20308) (Table 1).

DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) or the QIAamp® DNA Mini kit (Qiagen) as per the manufacturer's instructions. All DNA was quantified using the Qubit fluorometer and Qubit dsDNA High Sensitivity Assay Kit and the DeNovix DS-11 spectrophotometer/fluorometer was used to assess purity.

DNA libraries were prepared using the Nanopore Rapid Sequencing Kit (SQK-RAD004) for B20311 and Rapid Barcoding Sequencing kit (SQK-RBK004) for all remaining isolates and subsequently sequenced using the MinION sequencer (Oxford Nanopore Technologies, Oxford, UK). Hybrid assembly of the Illumina short reads and Nanopore long reads was performed using Unicycler v.0.5.0 (Wick et al., 2017). Due to issues with the hybrid assembly, the genome of isolate B20086 was assembled using long reads only with the de-novo genome assembler Flye (v.2.9) (Kolmogorov et al., 2019).

The ribosomal MLST tool on PubMLST was used to confirm species identification (Jolley et al., 2012), while the sequence type (ST) of all isolates was determined by Multilocus Sequence Typing (MLST) v2.19.0 (Seemann, n.d.-b, https://github.com/tseemann/mlst) (Jolley et al., 2018). Assemblies for the strains, in FASTA format, were annotated using PROKKA v 1.14.5 (Seemann, 2014). The GFF3 format annotated assemblies were used as input for the generation of a nucleotide core genome alignment using MAFFT v 7.407 (Page et al., 2015) with default settings. MAFFT's core genome alignment was used as input for the inference of a phylogenetic tree using FastTree v 2.1.10 (Price et al., 2010). Results were visualised using GrapeTree v.1.5.0 (Zhou et al., 2018).

Using Bandage (Wick et al., 2015), all assembled genomes were visualised and eight of the nine *mcr* harbouring plasmids were fully recovered. Plasmid incompatibility type of the *mcr* harbouring plasmids was determined using the pMLST database (Jolley et al., 2018). The mobilisation and conjugation potential of the *mcr* harbouring plasmids was predicted using the Mobtyper tool from the MOB-suite software package (Robertson and Nash, 2018).

Proksee (https://proksee.ca/) was used to assess and visualise the genetic environment surrounding the *mcr* genes. ResFinder (9th March 2021 update) (Zankari et al., 2012) and CARD v3.1.2 (Jia et al., 2017) were used, through ABRicate v1.0.1 (Seemann, n.d.-a, https://github.com/tseemann/abricate) (last updated on the 27th March 2020), to assess the whole genomes and *mcr* carrying plasmids for the presence of ARGs. All hits with an identity and coverage of >90 % were kept.

To assess the genetic relatedness between all of the recovered *mcr* harbouring plasmids, an all-vs-all plasmid average nucleotide identity (ANI) clustermap was generated using ANIclustermap (v1.1.0) (Shimoyama, 2022a) based on the fastANI and seaborn algorithms. A BLASTn analysis of the *mcr* carrying plasmids recovered in this study (2 *mcr*-8 and 6 *mcr*-9) was carried out to compare these plasmid sequences to one another and also with those previously detected. The MGCplotter tool (Shimoyama, 2022b) was used to visually compare all *mcr*-8 and *mcr*-9 plasmids in this study. Genes were coloured based on their classification into COG (Cluster of Orthologous Genes) functional categories using COGclassifier (v.1.0.5) (Shimoyama, 2022c).

2.3. Antimicrobial susceptibility testing

Phenotypic resistance was determined by performing antimicrobial susceptibility testing (AST) on all *mcr* harbouring Enterobacterales using disc diffusion and broth microdilution methods in line with EUCAST (EUCAST version 11.0, 2021) and CLSI criteria (CLSI version 31, 2021). The disc diffusion method was used when testing for susceptibility to the following 15 antimicrobial agents: cefpodoxime (10 μg), cefotaxime (5 μg), cefoxitin (30 μg), ceftazidime (10 μg), ampicillin (10 μg), ertapenem (10 μg), meropenem (10 μg), streptomycin (10 μg), kanamycin (30 μg), gentamycin (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), trimethoprim (5 μg) and tetracycline (30 μg). *Klebsiella pneumoniae* strain ATCC 700603 and *Escherichia coli* strain ATCC 25922 were used for quality control. Susceptibility to colistin was tested for by broth microdilution using *E. coli* ATCC 25922 as a control strain. The results for all antimicrobials were interpreted using EUCAST breakpoints, except in instances where these were unavailable i.e., for nalidixic acid, streptomycin, tetracycline and kanamycin. In these cases, CLSI breakpoints were applied.

In addition to the environmental isolates, both phenotypic and genotypic testing, as outlined above, was also carried out on a *mcr*-8 harbouring *Klebsiella pneumoniae* (CMCR2021) recovered from a clinical sample in Ireland in 2021. This isolate was included in this study for comparison purposes as the *mcr*-8 genes from clinical and environmental samples were both detected in *K. pneumoniae* belonging to the same ST, ST111. Both the isolate and WGS short reads were provided by the National CPE Reference Laboratory Service, University Hospital Galway, Ireland.

Table 1
Sample, isolate and genome information for all mcr harbouring Enterobacterales in this study.

Sample type	Sampling date	*mcr* gene	Isolate ID	Species	MLST	Genome size (bp)	Genome coverage	ENA accession number
Freshwater (Lake)	Feb 2019	*mcr*-8	B19137	*Klebsiella pneumoniae*	ST111	5,561,511	196	GCA_947047335.1
Clinical	2021	*mcr*-8	CMCR2021	*Klebsiella pneumoniae*	ST111	5,730,698	123	GCA_947047375.1
Freshwater (River)	Oct 2020	*mcr*-9	B20339	*Escherichia coli*	ST635	5,871,554	229	GCA_947047345.1
Healthcare facility effluent (untreated)	Nov 2018	*mcr*-9	B18161	*Escherichia coli*	ST10	5,329,007	94	GCA_947044895.1
		mcr-9	B18164	*Klebsiella michiganensis*	ST260	6,009,282	119	GCA_947047385.1
Healthcare facility effluent (untreated)	Feb 2020	*mcr*-9	B20086	*Enterobacter ludwigii*	Novel ST	5,559,484	119	GCA_947047395.1
WWTP influent (untreated)	Sept 2020	*mcr*-9	B20311	*Enterobacter hormaechei*	ST133	5,325,113	287	GCA_947047405.1
		mcr-9	B20308	*Raoultella ornithinolytica*	No MLST scheme available	6,415,564	268	GCA_947047355.1
ICW influent (untreated farm waste)	Aug 2019	*mcr*-9	GB19–003626	*Enterobacter hormaechei*	ST278	5,146,001	107	GCA_947047365.1

Note: Species identification and MLST were determined through WGS analysis. All assembled genomes have been deposited in the European Nucleotide Archive (ENA) under project number PRJEB55578 (Secondary Study Accession: ERP140489). Abbreviations: WWTP – Wastewater treatment plant, ICW – Integrated constructed wetland.

3. Results

3.1. Detection of mcr positive isolates

Overall, eight isolates harbouring *mcr* genes were recovered from six individual environmental samples. A breakdown of the sample types, collection dates and *mcr* genes detected are outlined in Table 1, which also includes the clinical *mcr*-8 harbouring *Klebsiella pneumoniae*. One *mcr*-8 harbouring *Klebsiella pneumoniae* was detected in a freshwater lake sample. The seven *mcr*-9 harbouring Enterobacterales were recovered from one freshwater river ($n = 1$), two healthcare facility effluents ($n = 3$), one WWTP influent ($n = 2$) and one ICW influent (n = 1). With the exception of the two healthcare facility effluent samples, all samples were collected in different geographical locations, some within the same county, while others in different counties across Ireland. Despite the healthcare facility effluents being collected within the same vicinity, both samples were collected on different dates, one in November 2018 and the other in February 2020 and from different sewers, with one sample consisting of hospital waste only while the other consisted of waste from both the hospital, as well as a long-term care facility.

By MALDI-TOF, both *mcr*-8 harbouring isolates, B19137 and CMCR2021, were identified as *Klebsiella pneumoniae*, while the isolates containing *mcr*-9 genes were identified as *Escherichia coli* ($n = 2$), *Klebsiella oxytoca* ($n = 1$), *Enterobacter cloacae* ($n = 3$) and *Raoultella ornithinolytica* (n = 1). Based on WGS analysis, the isolate previously identified as *Klebsiella oxytoca* (n = 1) was identified as *Klebsiella michiganensis* and the isolates identified as *Enterobacter cloacae* (n = 3) were identified as *Enterobacter ludwigii* (n = 1) and *Enterobacter hormaechei* (n = 2) (Table 1).

MLST analysis revealed that both *mcr*-8 *K. pneumoniae* were ST111. Their calculated OrthoANIu value, which represents the average nucleotide identity between both, is 99.93 %. The *mcr*-9 isolates were linked to a variety of different STs including ST635 (*E. coli*), ST10 (*E. coli*), ST260 (*K. michiganensis*), ST133 (*E. hormaechei*) and ST278 (*E. hormaechei*), while the *E. ludwigii* was found to belong to a novel ST within the *E. cloacae* complex MLST scheme (Table 1). As no MLST scheme is currently available for *R. ornithinolytica,* no ST was obtained for this isolate. A phylogenetic tree based on core-genome phylogeny for all *mcr* harbouring Enterobacterales is provided in the supplementary information (Fig. S1).

3.2. Phenotypic analysis

All *mcr* harbouring Enterobacterales (n = 9) were MDR (Fig. 1). Overall, five of the nine isolates, one *mcr*-8 (CMCR2021) and four *mcr*-9 (B20339, B20086, B20311, B20308), demonstrated phenotypic resistance to ertapenem.

With regards to colistin resistance, both *mcr*-8 harbouring *K. pneumoniae* were resistant with MICs of 8 mg/L (B19137) and 4 mg/L (CMCR2021), while colistin MICs of ≤1 mg/L were noted for all *mcr*-9 positive isolates.

3.3. Antimicrobial resistance genes

Through ResFinder and CARD, 24 different ARGs were detected in the environmental *mcr*-8 *K. pneumoniae* (B19137), while the clinical *mcr*-8 *K. pneumoniae* (CMCR2021) contained 20, with an overlap of 18 ARGs (Fig. 2). In addition to the *mcr* genes, both harboured genes associated with resistance to aminoglycosides, beta-lactams, fosfomycin, phenicols, fluoroquinolones, sulphonamides and tetracyclines. Both isolates also contained a range of genes linked with intrinsic drug resistance, including the efflux genes *KpnE*, *KpnF*, *KpnG*, *oqxA, oqxB* and *acrA*, and the bacterial porin *OmpK37*. Further investigations would be required to determine whether these genes actually confer additional resistance in these isolates.

B19137 also had genes linked to macrolide and trimethoprim resistance. While both *mcr*-8 positive *K. pneumoniae* harboured the ESBL gene $bla_{\text{CTX-M-15}}$, CMCR2021 (ertapenem resistant) also contained the carbapenemase-encoding gene $bla_{\text{OXA-48}}$.

WGS analysis revealed that the *mcr*-9 positive Enterobacterales harboured a wide variety of ARGs i.e., 30 ± 4.1 (10–61) (Fig. 2). In addition to *mcr*-9, genes associated with resistance to beta-lactams (7 isolates), aminoglycosides (6 isolates), trimethoprim (6 isolates), sulphonamides (6 isolates), tetracycline (5 isolates), fosfomycin (3 isolates), fluoroquinolones (3 isolates), macrolides (3 isolates), phenicols (1 isolate), glycopeptides (1 isolate) and other polypeptides (2 isolates) were identified among these isolates. The two *E. coli* isolates (B18161 and B20339) also contained a wide range of other genes associated with MDR efflux and regulatory systems, with both harbouring 36 of the same genes (Fig. 2).

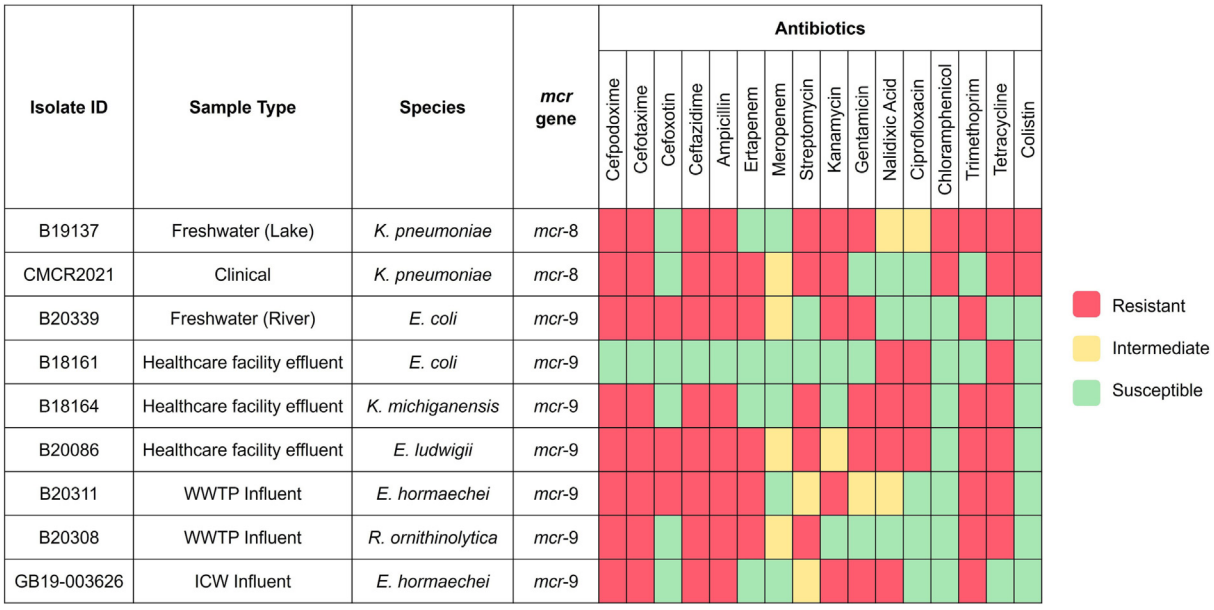


Fig. 1. Antimicrobial susceptibility profiles of *mcr* harbouring Enterobacterales. Antimicrobial susceptibility testing was carried out using the disc diffusion method for all antimicrobials, with the exception of colistin for which the broth microdilution method was used. WWTP = Wastewater treatment plant, ICW = Integrated constructed wetland.

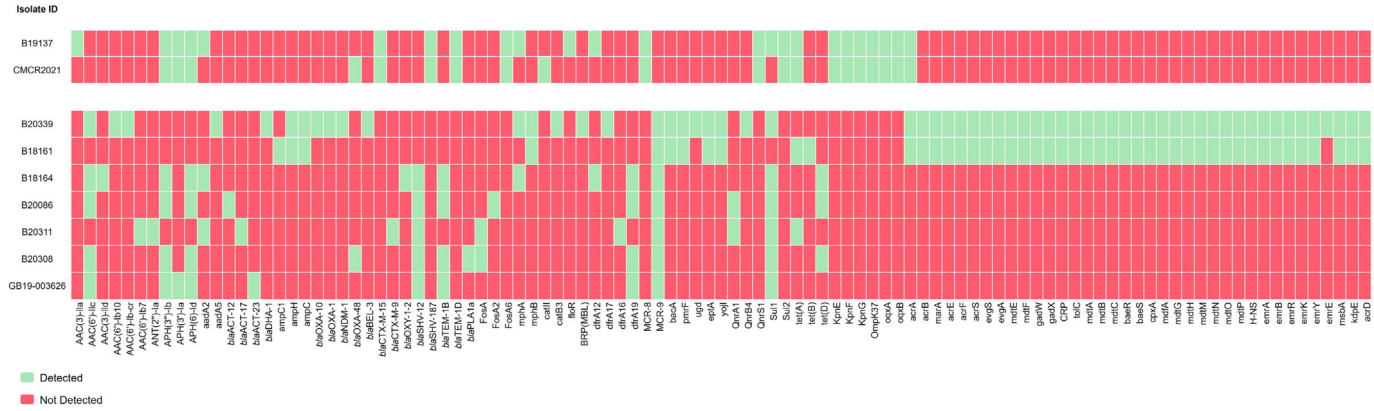


Fig. 2. Antimicrobial resistance genes in the *mcr* harbouring Enterobacterales as determined by WGS analysis.

Other than *mcr*-9, the most prevalent gene detected was the sulphonamide resistance gene *sul*1, identified in 6 out of the 7 *mcr*-9 positive isolates. This was closely followed by *AAC(6′)-Ilc* and bla_{SHV-12} which were both detected in 5/7 isolates and are associated with aminoglycoside and beta-lactam resistance, respectively.

With regards to beta-lactamases, all 7 of the *mcr*-9 positive Enterobacterales harboured at least 3 genes (Fig. 2). Overall, 4 different ESBL genes were detected, bla_{SHV-12} (5 isolates), bla_{BEL-3} (1 isolate), $bla_{CTX-M-9}$ (1 isolate) and $bla_{OXY-1-2}$ (1 isolate). Despite 4 of the *mcr*-9 harbouring isolates displaying resistance to ertapenem, only 2 harboured a carbapenemase-encoding gene, the *E. coli* (B20339) isolated from river water which was found to contain bla_{NDM-1}, while the *R. ornithinolytica* (B20308) recovered from WWTP influent harboured bla_{OXA-48}.

3.4. mcr location and genetic environment

Analysis of the assembled genomes using Bandage (Wick et al., 2015) revealed that all *mcr* genes were located on plasmids. With the exception of the *mcr* harbouring plasmid in GB19–003626, all *mcr* carrying plasmids ($n = 8$) were fully recovered (2 *mcr*-8 and 6 *mcr*-9). Further analysis using mobtyper (Robertson and Nash, 2018) predicted all 8 plasmids to be conjugative. While a relaxase, a mating pair formation (mpf) system and an origin of transfer (oriT) were identified in the 6 *mcr*-9 harbouring plasmids, no oriT was identified in those carrying *mcr*-8. However, despite this, they are still predicted to be conjugative, as both of these plasmids had a relaxase and mpf system.

The *mcr*-8 gene in CMCR2021 was located on an IncFIIK plasmid, pCMCR2021 (100,578 bp), while in B19137, although no direct match was found for the *mcr*-8 harbouring plasmid pB19137 (101,201 bp), the pMLST tool (Jolley et al., 2018) indicated the closest match was plasmid incompatibility type IncI1.

Although through BLASTn analysis these plasmids do not appear to be closely related, they do share some regions of 100 % similarity (30 % coverage). Fig. 3A displays the circular comparison of both *mcr*-8 harbouring plasmids. When compared to previously detected plasmids, through BLASTn analysis, pB19137 was found to be 99.70 % identical to part of the *mcr*-8 carrying plasmid pKP32 (37 % coverage, Genbank accession no. OL804391) detected in a *K. pneumoniae* strain (ST37) isolated from a chicken cloacae sample in China in 2017 (Wu et al., 2020). With regards to pCMCR2021, the most closely related plasmid was p2018C01–046-1_MCR8 (92 % coverage, 98.46 % identity, Genbank accession no. CP044369.1), which had been isolated previously from a *K. pneumoniae* strain recovered from a human sample in Taiwan in 2018.

Analysis of the genetic environments revealed that the *mcr*-8 gene on pB19137 was flanked upstream by the insertion sequence (IS) IS*26* (IS*6* family transposase) and downstream by IS*903* (IS*5* family transposase) (Fig. 4). Within this flanking region, *copR* (copper homeostasis transcription factor), *sasA* (sensory-kinase), *dgkA* (diacylglycerol kinase) and bla_{TEM-1D} (beta-lactamase) genes were present upstream of the *mcr* genes, while *inhA* (enoyl reductase) and *ymoA* (modulating protein) were downstream. The *mcr*-8 gene in pCMCR2021 was flanked by the IS*6* family transposase IS*26* downstream and, while no IS was detected upstream of this gene, the *copR*, *sasA* and *dgkA* genes were also present in this plasmid (Fig. 4).

In addition to *mcr*-8, ARGs associated with resistance to other antimicrobial agents were also detected in these plasmids (Fig. 5). While the beta-lactamase gene bla_{TEM-1D} was detected in both plasmids, pB19137 also harboured *AAC(3)-IIa* and *floR* genes which are linked with resistance to aminoglycosides and phenicol antimicrobial agents, respectively.

All of the *mcr*-9 harbouring plasmids identified as IncHI2 plasmids, namely pB18161 (279,086 bp), pB20311 (286,905 bp), pB20308 (295,770 bp), pB20086 (308,679 bp), pB20339 (342,066 bp) and pB18164 (374,334 bp), and while 5 of these plasmids belonged to the plasmid MLST ST1, one (pB18161) belonged to ST2. Genetic analysis revealed that all *mcr*-9 genes were flanked upstream by the IS*5* family transposase IS*903* (Fig. 4). In pB20339, pB18164 and pB20308, the *mcr*-9 genes were flanked downstream by IS*6* family transposase IS*26*, while the remaining 3 were bracketed by IS*481* family transposase IS*Azs36* (pB18161), IS*6* family transposase IS*15DII* (pB20086) and IS*1* family transposase IS*1R* (pB20311). In pB18161, the two-component regulatory system genes *qse*B and *qse*C genes were also present in the same flanking region, downstream of the *mcr* gene.

BLASTn analysis revealed that all of the *mcr*-9 plasmids recovered in this study were closely related to one another, with their percentage identity ranging from 98.25 % to 100 %, and coverage from 69 % to 97 %. The circular comparison of these *mcr*-9 harbouring plasmids is displayed in Fig. 3B. BLASTn analysis also revealed their close relation to the previously detected IncHI2 plasmid pCTXM9_020038 (Genbank accession no. NZ_CP031724.1; identity range 98.42 % to 99.9 %; coverage range 70 % to 97 %) in an *E. hormaechei* strain isolated from a human sample in China in 2016.

The *mcr*-9 carrying plasmids harboured between 2 and 13 different ARGs (Fig. 5). In addition to *mcr-9,* these included genes associated with resistance to aminoglycoside (*AAC(6′)-IIc*; *AAC(3)-IId*; *AAC(6′)-Ib-cr*; *AAC (6′)-Ib7*; *APH(3″)-Ib*; *APH(6)-Id*; *aadA2*; *aadA5*; *ANT(2″)-Ia*), macrolide (*mphA*), phenicol (*catB3*), glycopeptides (BRP_{MBL}), fluoroquinolone (*QnrA1*; *QnrB4*), tetracycline (*tet(A)*; *tet(D)*), sulphonamide (*sul1*), trimethoprim (*dfrA12*; *dfrA16*; *dfrA17*; *dfrA19*) and beta-lactam (bla_{DHA-1}; bla_{OXA-1}; bla_{NDM-1}; $bla_{CTX-M-9}$; bla_{SHV-12}; bla_{TEM-1B}) antimicrobials, as well as other genes linked to MDR efflux systems (*mdtE*; *mdtF*; *gadW*).

Following analysis of the ANI values, which were determined to assess genomic similarity, a range of values from 97.1 % to 99.8 % were observed for all recovered *mcr*-9 harbouring plasmids, indicating a high genetic similarity between all (Fig. 6). In relation to the *mcr*-8 carrying plasmids,

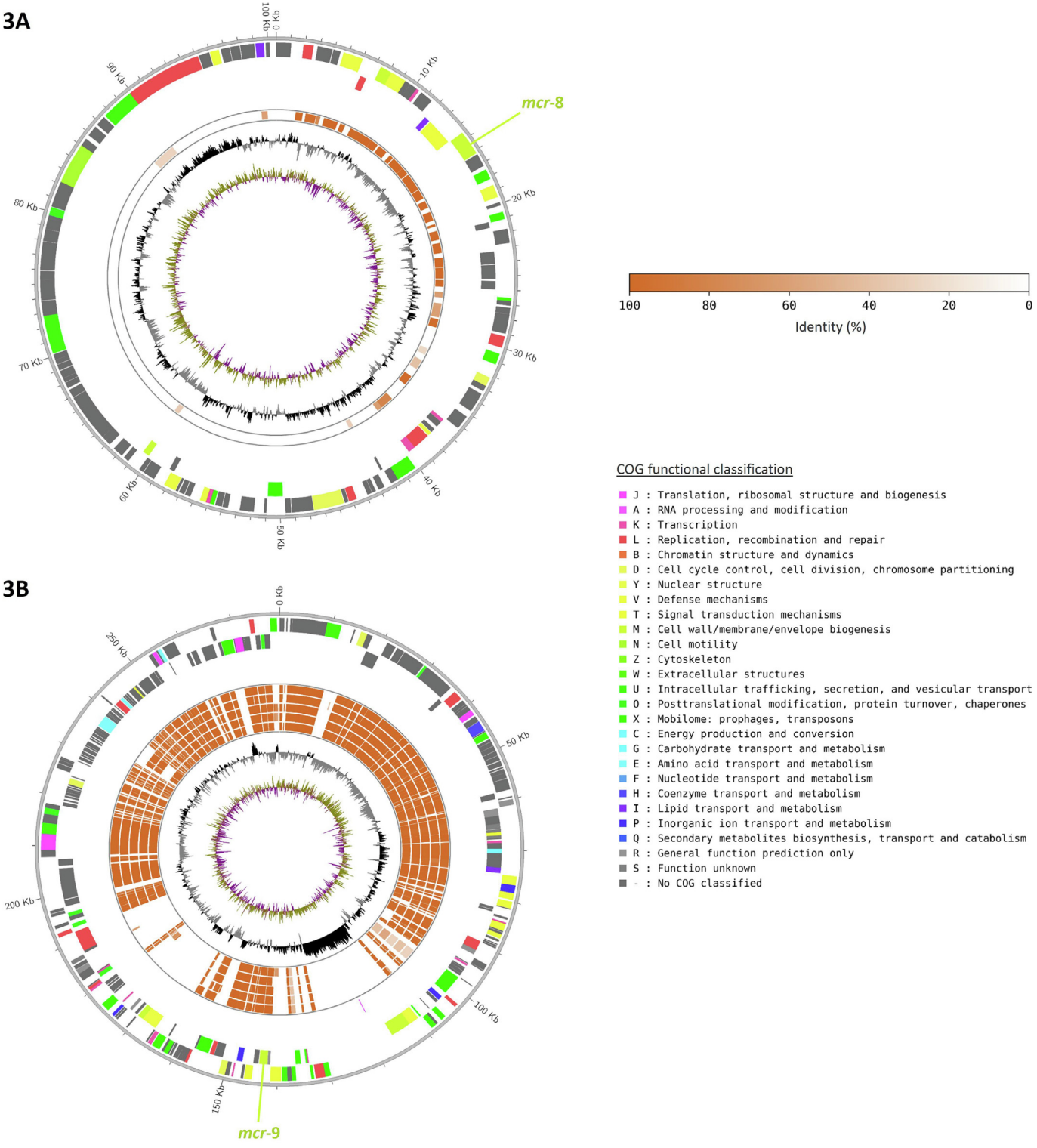


Fig. 3. Genetic comparison and functional information for all recovered *mcr* harbouring plasmids. (A) Circular comparison of *mcr*-8 harbouring plasmids. The two outermost tracks in this figure represent the forward (outer track) and reverse (inner track) strands of plasmid pCMCR2021, which was used as the reference genome sequence. The *mcr*-8 gene, located on the forward strand, is indicated. The third track in represents regions of pB19137 that show similarity to pCMCR2021. (B) Circular comparison of the *mcr*-9 harbouring plasmids. The two outermost tracks in this figure display the forward (outer track) and reverse (inner track) strands of plasmid pB18161, which was used as the reference genome sequence. The *mcr*-9 gene, located on the reverse strand, is indicated. From the third track inwards regions of similarity between pB18161 and the other *mcr*-9 harbouring plasmids are displayed as follows: pB18161, pB18164, pB20308, pB20311, pB20339 and pB20086. Figure generated using MGCplotter (Shimoyama, 2022b).

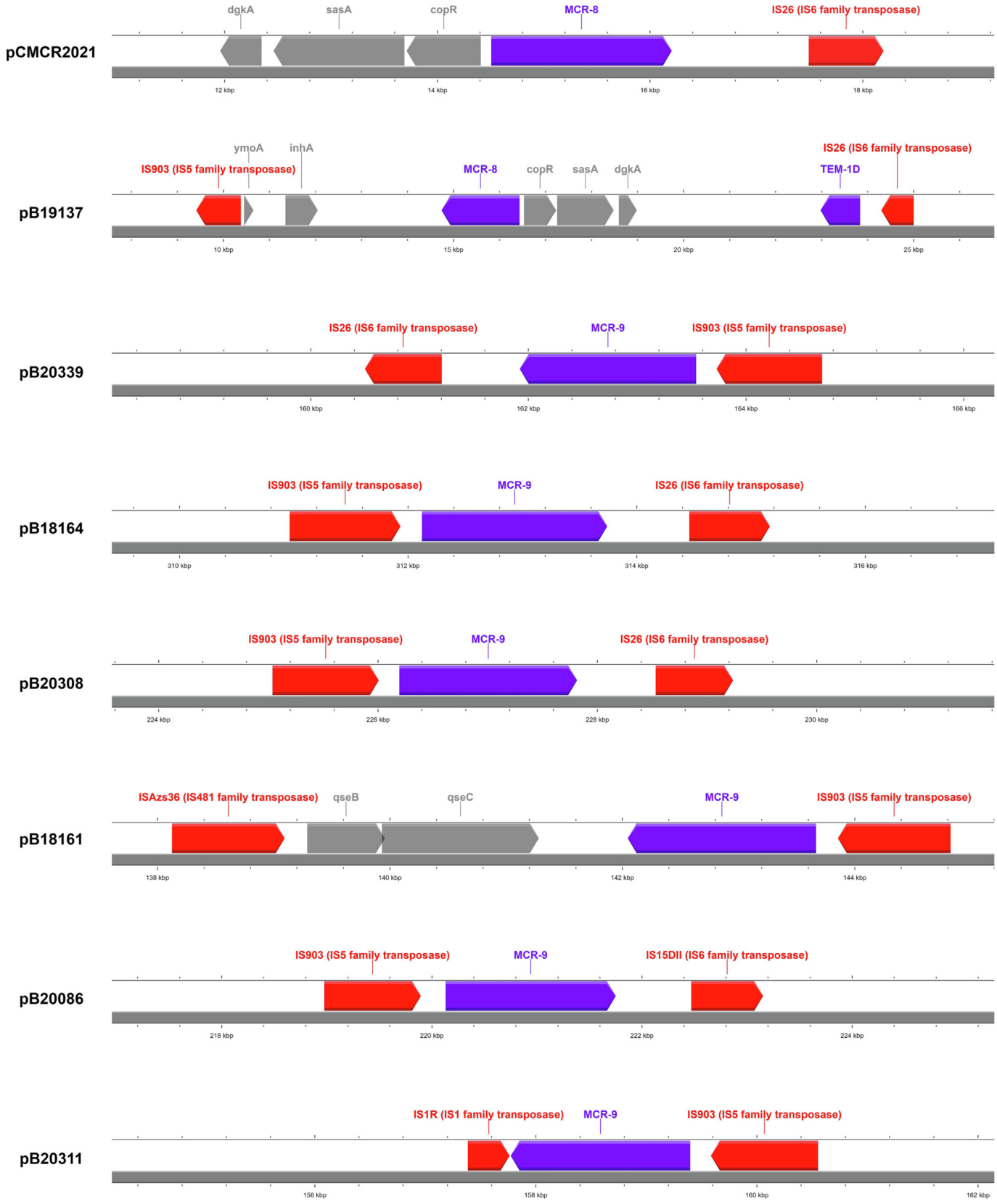


Fig. 4. Genetic context of *mcr*-8 and *mcr*-9 genes. The *mcr* genes and their surrounding environments are shown. Antimicrobial resistance genes, including the *mcr* genes, are shown in purple, insertion sequences flanking the *mcr* genes are indicated in red, while all other genes present within the flanking regions are in grey.

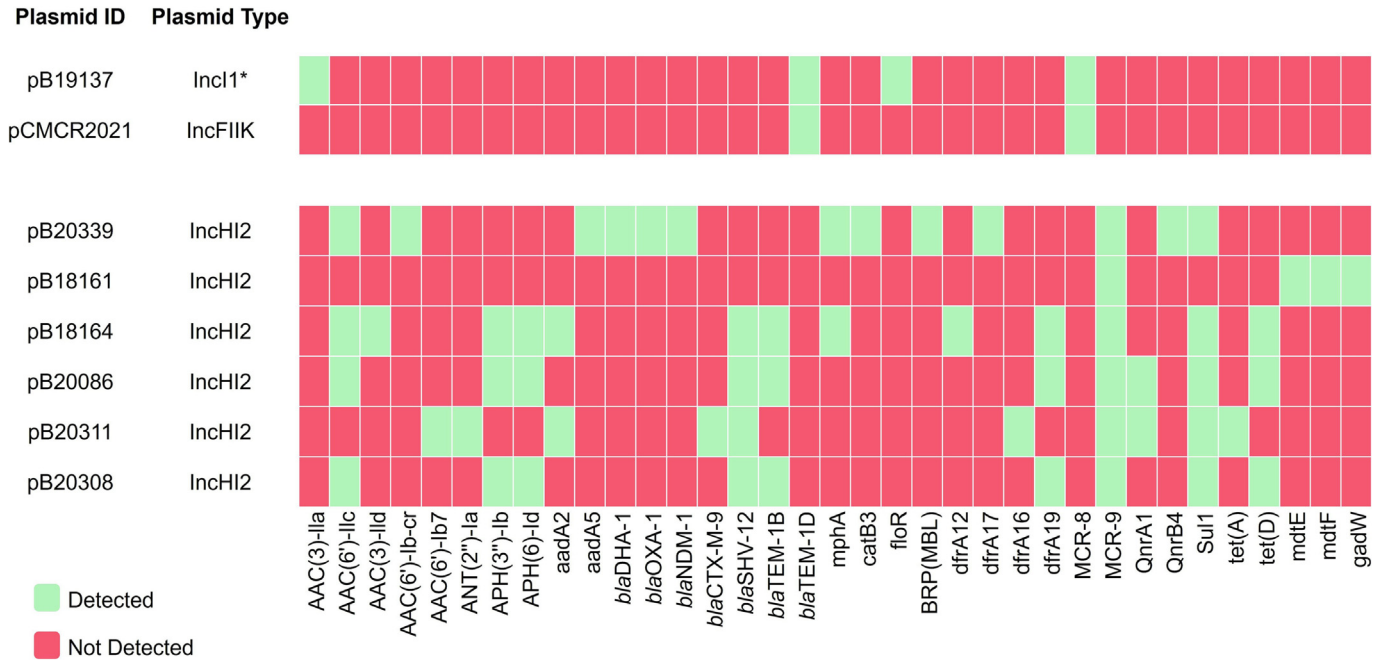


Fig. 5. Antimicrobial resistance genes in the *mcr* harbouring plasmids as determined by WGS analysis. *Closest match determined by pMLST.

despite the ANI value being lower (94.5 %), there still appears to be a high genetic relatedness between them. In addition, the *mcr*-8 harbouring plasmid pB19137 also shared ANI values of >90 % with pB18164 (93.6 %) and pB20086 (91.1 %), potentially indicating the presence of many similar insertion sequences, transposons or other mobile genetic elements in these plasmids.

4. Discussion

The detection of *mcr* genes associated with transferrable colistin resistance across the globe is concerning due to the threat they pose to public health. Since their initial discovery, many of the reports published worldwide on *mcr*-8 and *mcr*-9 have highlighted their presence in humans and animals, while reports of their detection in the environment remain scarce. Our finding of *mcr*-8 and *mcr*-9 genes in different environmental samples highlights potential sources and reservoirs of these genes in the environment in Ireland. However, these findings are just indicative of the presence of *mcr* genes in the MDR strains sequenced in our study. As we were not actively looking for colistin resistance or the presence of *mcr* genes, we believe *mcr* prevalence in the Irish environment is likely to be much higher than observed. Although this study only contained samples from Ireland, this situation is likely mirrored in other countries as routine surveillance of the environment for ARGs, including *mcr*, is lacking.

The release of inadequately treated or untreated wastewaters into the environment may contribute to the dissemination of *mcr* genes within the environment and subsequently have the potential to spread to humans or animals. While in the majority of regions, wastewaters are treated prior to discharge into the environment there is no guarantee that current processes can eliminate these ARGs, as is evident following previous studies, which demonstrated the persistence of resistant bacteria and genes following wastewater treatment (Morris et al., 2016; Smyth et al., 2020). The detection of *mcr* harbouring isolates in the environment, particularly in areas used for recreational or drinking water purposes, is a concern regarding potential impact on public health.

4.1. mcr-8

In 2018, the first discovery of *mcr*-8 was reported by Wang et al. (2018), following its detection in strains of *K. pneumoniae* isolated from human and animal samples in China. However, according to Martiny et al. (2022), *mcr*-8 genes have in fact been circulating for some time prior to their initial finding, as following analysis of over 214,000 publicly available metagenomic data sets uploaded over the last 10 years, these genes were detected in metagenomes dating back to 2006.

In this study, we detected *mcr*-8 in a freshwater lake sample. Similarly, Tereza et al. (2020) reported its presence in lake water, in addition to other freshwater bodies (ponds and rivers) in the Czech Republic. The *mcr*-8 gene has also been detected following investigation of water supply sources and the influents and effluents of drinking water treatment plants (DWTP) in China by Khan et al. (2021), in poultry farm wastewater in China by Li et al. (2019), as well as in marine and wastewater metagenomes analysed retrospectively by Martiny et al. (2022).

While *mcr*-8 has primarily been found in *K. pneumoniae* to date (Farzana et al., 2020; Hadjadj et al., 2019; Wu et al., 2020), there are some reports of its detection in *K. oxytoca*, *K. quasipneumoniae*, *R. ornithinolytica*, *E. coli* and *C. werkmanii* (Ngbede et al., 2020; Phetburom et al., 2021; Tereza et al., 2020; Wang et al., 2019). Both clinical and environmental *mcr*-8 harbouring isolates reported in this paper were identified as *K. pneumoniae* belonging to ST111. While *K. pneumoniae* ST111 has been previously linked to the carriage of other clinically significant ARGs including ESBL and carbapenemase-encoding genes (Eilertson et al., 2017; Lester et al., 2011; Simões et al., 2022; Uz Zaman et al., 2014), we believe this is the first report of this sequence type harbouring the *mcr*-8 gene.

With regards to colistin susceptibility, consistent with our findings, reports published to date have indicated that *mcr*-8 has primarily been found to mediate resistance (Li et al., 2019; Liu et al., 2021a; Salloum et al., 2020; Wang et al., 2018). Phenotypic testing and analysis also revealed that both *mcr*-8 harbouring *K. pneumoniae* were resistant to a range of other clinically important antimicrobials including those used for the treatment of MDR gram-negative bacterial infections, while through genotypic testing a range of ARGs were detected. Among these were ESBL ($bla_{\text{CTX-M-15}}$) and carbapenemase-encoding genes ($bla_{\text{OXA-48}}$), as well as a gene linked with resistance to fosfomycin (*Fos*A6), a reserve antibiotic for the treatment of carbapenem resistant infections. The co-carriage of such genes poses a significant threat to the treatment of gram-negative bacterial infections, particularly in cases where carbapenemase-encoding genes co-exist with *mcr* genes, as was the case in the clinical isolate CMCR2021, as both have the potential to confer resistance to last resort antibiotics.

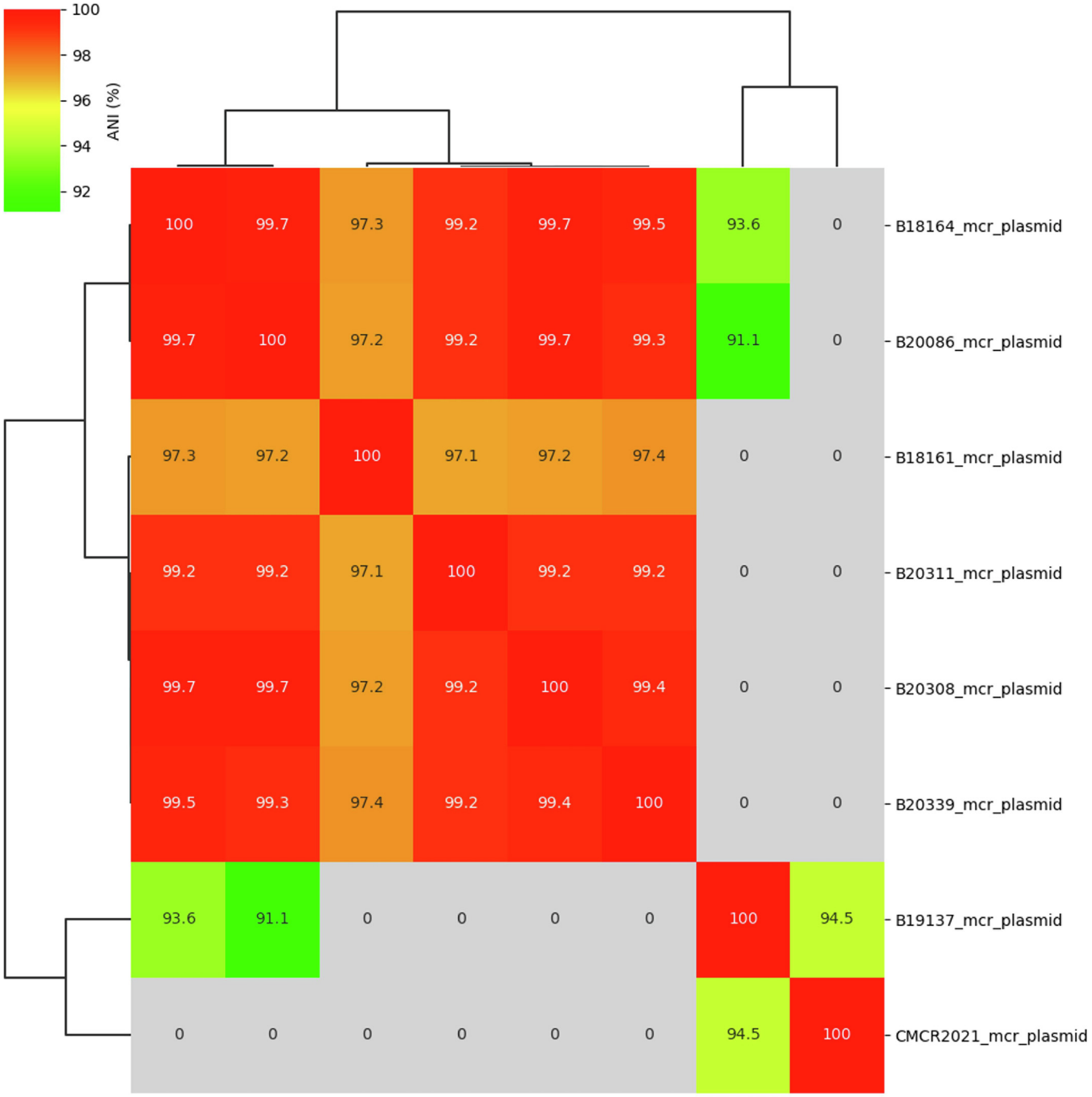


Fig. 6. Average nucleotide identity (ANI) between the recovered *mcr* harbouring plasmids.

As the *mcr*-8 genes are located on conjugative IncI1-like and IncFIIK plasmids, they have the ability to mobilise among bacterial species in different environments. While IncFIIK plasmids have been previously found to carry *mcr*-8 (Eltai et al., 2020; Wu et al., 2020) in addition to other important ARGs including $bla_{\text{CTX-M-15}}$ (Coelho et al., 2010) and carbapenemase-encoding genes bla_{KPC}, bla_{NDM} and bla_{IMP} (Mataseje et al., 2014; Mavroidi et al., 2012; Yao et al., 2020), this is the first reported detection of *mcr*-8 on IncI1 type plasmids to date. However, other *mcr* variants including *mcr*-1 and *mcr*-3 have been associated with IncI1 plasmids (Brouwer et al., 2020; Hadjadj et al., 2019), as well as other clinically relevant ARGs including the ESBL genes $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$ (Dahmen et al., 2012; Sukmawinata et al., 2020).

In addition to their location on conjugative plasmids, insertion sequence elements, which further enable the spread of ARGs, were identified within the *mcr*-8 gene cassettes. While IS*903* has been associated with *mcr*-8 previously, in addition to IS*Kpn26*, IS*Kpn21* and IS*Ecl1* (Farzana et al., 2020; Li et al., 2021; Wu et al., 2020), this is the first report to describe IS*26* bracketing the *mcr*-8 gene to date. The IS*26* element is known for its involvement in the acquisition and transmission of clinically relevant ARGs in strains of Enterobacterales (Varani et al., 2021).

4.2. mcr-9

Carroll et al. (2019) reported the first detection of the *mcr*-9 gene in 2019, after its isolation from a *Salmonella enterica* serovar Typhimurium strain recovered from a human sample which had been collected in the U.S.A in 2010. However, through retrospective screening of publicly available metagenomes, Martiny et al. (2022) identified these genes in samples dating back to 2007. Through the analysis of these metagenomes, *mcr*-9 was also described as the most abundant *mcr* variant to date (Martiny et al., 2022).

With regards to *mcr*-9 in the environment, there have been reports of its detection in WWTPs in the U.S.A and China (Hassan et al., 2022; Shi et al., 2022), hospital effluent in China (Xu et al., 2021), river water in South Africa (Mbanga et al., 2021), lake water in Switzerland (Biggel et al., 2022), sands and seawaters in Brazil (Furlan et al., 2021), as well as in the source water, influents and effluents of DWTPs in China (Khan et al., 2021). In addition, following a retrospective analysis, Martiny et al. (2022) identified *mcr*-9 genes in different environmental metagenomes, including those derived from wastewater, air, salt marsh, sludge, subsurface, marine and freshwater environments, which had been uploaded to public databases over the last 10 years. While we also detected this gene in river water, healthcare facility effluents and WWTPs, to our knowledge, it is the first to report the presence of *mcr*-9 in ICW influent/waste from a piggery farm. The detection of *mcr*-9, in addition to other ARGs, in the ICW influent in this study is concerning, as on farms where no ICW treatment is available this waste may lead to AMR contamination of surrounding surface or ground waters. However, with regards to ICW treatment, while previous studies have reported on the efficacy of this treatment in the reduction of antibiotics, antimicrobial resistant organisms and genes, some have been found to persist in the final effluents (Chen et al., 2015; Prendergast et al., 2022). Although *mcr*-9 was only detected in the influent in this study, there is potential for these genes to persist following treatment. To avoid both contamination of the surrounding environment and spread of these genes, further investigations into the effective removal of ARGs, including *mcr*, from farm wastes should be considered going forward.

To date, the *mcr*-9 gene has been described in *Salmonella* spp., as well as many clinically relevant Enterobacterales species including *Enterobacter*, *Morganella*, *Klebsiella*, *Citrobacter*, *Cronobacter*, *Escherichia, Kluyvera*, *Raoultella, Phytobacter* and *Leclercia* spp. (Hassan et al., 2022; Kim et al., 2021; Li et al., 2020). In this study, *mcr*-9 was detected in a range of different Enterobacterales belonging to different MLST genotypes, some of which have been found previously in humans, animals and the healthcare environment. Previous reports have indicated the detection of *mcr*-9 in *E. coli* ST10 isolated from pigs in the U.S.A (Hayer et al., 2020), *E. coli* ST635 recovered from hospital sinks in the UK (Constantinides et al., 2020) and *E. hormaechei* ST133 in humans in Egypt (Soliman et al., 2020). However, this report is the first to describe the *mcr*-9 gene in *E. hormaechei* ST278 and *K. michiganensis* ST260.

In contrast to other *mcr* genes, susceptibility to colistin has been observed in the majority of studies relating to *mcr*-9 to date (Carroll et al., 2019; Kieffer et al., 2019; Mbanga et al., 2021; Tyson et al., 2020). However, investigations carried out previously have indicated that expression of the *mcr*-9 gene can confer colistin resistance (Carroll et al., 2019; Kieffer et al., 2019). In addition, Kieffer et al. (2019) highlighted the potential link between the two-component system *qse*BC and the induction of *mcr*-9 gene expression. However, while the *qse*B and *qse*C genes have been detected in colistin resistant isolates previously (Cha et al., 2020; Ding et al., 2021; Yuan et al., 2019), in our study, despite the presence of these genes in plasmid pB18161 they were not associated with phenotypic colistin resistance. Similarly, others have also reported on isolates remaining susceptible to colistin despite the presence of this system, leading to the belief that there may be other unidentified factors associated with the expression of *mcr*-9 and induction of colistin resistance (Hendrickx et al., 2021; Kananizadeh et al., 2020; Ribeiro et al., 2021). Overall, although the *mcr*-9 gene is a phosphoethanolamine transferase and is similar in ways to other *mcr* genes, it does not seem to be as concerning as, in general, it does not appear to mediate phenotypic resistance to colistin. However, despite this, it should not be disregarded as uncertainty still remains around both its physiological function and clinical significance and therefore, further research is required in these areas.

Similar to the *mcr*-8 positive isolates, all *mcr*-9 harbouring Enterobacterales were also MDR and contained a wide range of ARGs, including ESBL ($bla_{\text{BEL-3}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{OXY-1-2}}$, $bla_{\text{SHV-12}}$), and carbapenemase-encoding genes ($bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$), in addition to fosfomycin resistance genes (*Fos*A, *Fos*A2). Of particular concern is the carriage of the ESBL ($bla_{\text{CTX-M-9}}$, $bla_{\text{SHV-12}}$) and carbapenemase ($bla_{\text{NDM-1}}$) encoding genes on *mcr*-9 harbouring plasmids, especially as these plasmids are predicted to be conjugative which may therefore facilitate the extensive spread of these genes together to different bacterial species in different environments. IncHI2 plasmids, on which all the *mcr*-9 genes were located, have been reported previously by Li et al. (2020) as the predominant type linked to the carriage of *mcr*-9. Similar to our findings, other studies have also reported the co-occurrence of both ESBL and carbapenemase-encoding genes with *mcr*-9 on IncHI2 plasmids (Ai et al., 2021; Faccone et al., 2020; Ha et al., 2021; Haenni et al., 2020; Liu et al., 2021b).

With regards to the genetic environment, similar to our findings, many reports to date have indicated the presence of the IS*5* family element IS*903* upstream of the *mcr*-9 gene, while downstream, they have been generally flanked by IS*1* (IS*1R*) or IS*6* (IS*26* and IS*15DII*) elements (Ai et al., 2021; Biggel et al., 2022; Diaconu et al., 2021; Hendrickx et al., 2021; Kamathewatta et al., 2020; Ribeiro et al., 2021; Tyson et al., 2020). This highlights the potential involvement of IS*1*, IS*5* and IS*6* family elements in the mobilisation of the *mcr*-9 gene. Although Marchetti et al. (2021) reported the presence of a truncated IS*481* element downstream of a *mcr*-9 gene previously, to our knowledge, the IS*481* family transposase IS*Azs36* found in this study has not been reported to flank *mcr*-9 to date. Overall, our findings highlight the variety of potential vehicles involved in the transfer of *mcr*-9 genes between plasmids and chromosomes. Despite the diverse genetic environments, their presence on conjugative plasmids alone facilitates the mobilisation and dissemination of these ARGs among bacterial species.

5. Conclusion

Our findings provide evidence that *mcr* genes, including *mcr*-8 genes which are clearly linked to phenotypic colistin resistance, are circulating in the environment. Although in this study the *mcr*-9 gene did not mediate phenotypic resistance to colistin, the presence of these genes in the environment should not be overlooked. Overall, more investigations into the prevalence, persistence and dissemination of AMR, including *mcr*, in the environment are required. Further research is also needed to gain a better understanding of the role the environment plays in the persistence and dissemination of ARGs, including *mcr* genes. Further environmental research may also highlight any unidentified dissemination of ARGs in humans and animals and subsequently enable us to better control the spread of AMR.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162649.

CRediT authorship contribution statement

Niamh Cahill: Investigation, Formal analysis, Visualization, Writing – original draft. **Brigid Hooban:** Investigation, Formal analysis, Writing – review & editing. **Kelly Fitzhenry:** Investigation. **Aoife Joyce:** Investigation. **Louise O'Connor:** Conceptualization, Investigation, Resources, Writing – review & editing. **Georgios Miliotis:** Software, Formal analysis, Visualization, Writing – review & editing. **Francesca McDonagh:** Formal analysis, Visualization. **Liam Burke:** Investigation, Writing – review & editing. **Alexandra Chueiri:** Investigation, Writing – review & editing. **Maeve Louise Farrell:** Investigation, Writing – review & editing. **James E. Bray:** Software, Writing – review & editing. **Niall Delappe:** Investigation.

Wendy Brennan: Investigation. **Deirdre Prendergast:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Montserrat Gutierrez:** Conceptualization, Writing – review & editing. **Catherine Burgess:** Conceptualization, Writing – review & editing. **Martin Cormican:** Conceptualization, Writing – review & editing. **Dearbháile Morris:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

All assembled genomes have been deposited in the European Nucleotide Archive (ENA) under project number PRJEB55578 (Secondary Study Accession: ERP140489). The accession numbers are outlined in Table 1

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This project is jointly funded by the Environmental Protection Agency, under the EPA Research Programme 2014–2020, and the Health Service Executive (2017-HW-LS-1). The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action and Environment. It is administered by the Environmental Protection Agency, which has the statutory function of co-ordinating and promoting environmental research. We would like to acknowledge all personnel involved in the collection, processing, testing and analysis of samples, including the National CPE Reference Laboratory Service, University Hospital Galway who provided us with a clinical *mcr*-8 isolate for inclusion in our study for comparison purposes.

References

Aghapour, Z., Gholizadeh, P., Ganbarov, K., Bialvaei, A.Z., Mahmood, S.S., Tanomand, A., Yousefi, M., Asgharzadeh, M., Yousefi, B., Kafil, H.S., 2019. Molecular mechanisms related to colistin resistance in Enterobacteriaceae. Infect. Drug Resist. 12, 965–975. https://doi.org/10.2147/IDR.S199844.

Ai, W., Zhou, Y., Wang, B., Zhan, Q., Hu, L., Xu, Y., Guo, Y., Wang, L., Yu, F., Li, X., 2021. First report of coexistence of blaSFO–1 and blaNDM–1 β-lactamase genes as well as colistin resistance gene mcr-9 in a transferrable plasmid of a clinical isolate of Enterobacter hormaechei. Front. Microbiol. 12 (June), 1–11. https://doi.org/10.3389/fmicb.2021.676113.

Andrade, F.F., Silva, D., Rodrigues, A., Pina-Vaz, C., 2020. Colistin update on its mechanism of action and resistance, present and future challenges. Microorganisms 8 (11), 1–12. https://doi.org/10.3390/microorganisms8111716.

Biggel, M., Zurfluh, K., Hoehn, S., Schmitt, K., Frei, A., Jans, C., Stephan, R., 2022. Complete genome sequence of Hafnia paralvei isolate AVS0177, harboring mcr-9 on a plasmid. Microbiol.Resour.Announc. 11 (1), 9–10. https://doi.org/10.1128/mra.00966-21.

Brouwer, M.S.M., Goodman, R.N., Kant, A., Mevius, D., Newire, E., Roberts, A.P., Veldman, K.T., 2020. Mobile colistin resistance gene mcr-1 detected on an IncI1 plasmid in Escherichia coli from meat. J.Glob.Antimicrob.Resist. 23, 145–148. https://doi.org/10.1016/j.jgar.2020.08.018.

Carroll, L.M., Gaballa, A., Guldimann, C., Sullivan, G., Henderson, L.O., Wiedmanna, M., 2019. Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible Salmonella enterica serotype typhimurium isolate. Clin. Sci.Epidemiol. 10 (3), 1–6. https://doi.org/10.1128/mBio.00853-19.

Cha, M.H., Woo, G.J., Lee, W., Kim, S.H., Woo, J.H., Kim, J., Ryu, J.G., Kwak, H.S., Chi, Y.M., 2020. Emergence of transferable mcr-9 gene-carrying colistin-resistant Salmonella enterica dessau ST14 isolated from retail chicken meat in Korea. Foodborne Pathog. Dis. 17 (11), 720–727. https://doi.org/10.1089/fpd.2020.2810.

Chen, J., Liu, Y.S., Su, H.C., Ying, G.G., Liu, F., Liu, S.S., He, L.Y., Chen, Z.F., Yang, Y.Q., Chen, F.R., 2015. Removal of antibiotics and antibiotic resistance genes in rural wastewater by an integrated constructed wetland. Environ. Sci. Pollut. Res. Int. 22 (3), 1794–1803. https://doi.org/10.1007/s11356-014-2800-4.

CLSI, 2021. CLSI M100-ED31:2021 Performance Standards for Antimicrobial Susceptibility Testing. 31st edition. .

Coelho, A., González-López, J.J., Miró, E., Alonso-Tarrés, C., Mirelis, B., Larrosa, M.N., Bartolomé, R.M., Andreu, A., Navarro, F., Johnson, J.R., Prats, G., 2010. Characterisation of the CTX-M-15-encoding gene in Klebsiella pneumoniae strains from the Barcelona metropolitan area: plasmid diversity and chromosomal integration. Int. J. Antimicrob. Agents 36 (1), 73–78. https://doi.org/10.1016/j.ijantimicag.2010.03.005.

Constantinides, B., Chau, K.K., Quan, T.P., Rodger, G., Andersson, M.I., Jeffery, K., Lipworth, S., Gweon, H.S., Peniket, A., Pike, G., Millo, J., Byukusenge, M., Holdaway, M., Gibbons, C., Mathers, A.J., Crook, D.W., Peto, T.E.A., Walker, S.A., Stoesser, N., 2020. Genomic surveillance of Escherichia coli and Klebsiella spp. in hospital sink drains and patients. Microbial Genomics 6 (7), 4–16. https://doi.org/10.1099/mgen.0.000391.

DAFM - Department of Agriculture, Food and the Marinecollab, 2021. Minister McConalogue welcomes the announcement of a Statement of Intent around the Voluntary Cessation of Use of Colistin in the Animal Health sector in Ireland. available https://www.gov.ie/en/press-release/1480e-minister-mcconalogue-welcomes-the-announcement-of-a-statement-of-intent-around-the-voluntary-cessation-of-use-of-colistin-in-the-animal-health-sector-in-ireland/.

Dahmen, S., Haenni, M., Madec, J.Y., 2012. IncI1/ST3 plasmids contribute to the dissemination of the blaCTX-M-1 gene in Escherichia coli from several animal species in France. J. Antimicrob. Chemother. 67 (12), 3011–3012. https://doi.org/10.1093/jac/dks308.

Diaconu, E.L., Alba, P., Feltrin, F., Di Matteo, P., Iurescia, M., Chelli, E., Donati, V., Marani, I., Giacomi, A., Franco, A., Carfora, V., 2021. Emergence of IncHI2 plasmids with mobilized colistin resistance (mcr)-9 gene in ESBL-producing, multidrug-resistant Salmonella typhimurium and its monophasic variant ST34 from food-producing animals in Italy. Front. Microbiol. 12 (July), 1–11. https://doi.org/10.3389/fmicb.2021.705230.

Ding, M., Shi, J., Ud Din, A., Liu, Y., Zhang, F., Yan, X., Li, Q., Bai, J., Chen, W., Zhou, Y., 2021. Co-infections of two carbapenemase-producing Enterobacter hormaechei clinical strains isolated from the same diabetes individual in China. J. Med. Microbiol. 70 (3). https://doi.org/10.1099/jmm.0.001316.

ECDC - European Centre for Disease Prevention and Control, 2022. Antimicrobial consumption database (ESAC-Net) - Distribution of antimicrobial consumption by antimicrobial group. available https://www.ecdc.europa.eu/en/antimicrobial-consumption/database/distribution-by-antimicrobial-group.

Eilertson, B., Chen, L., Chavda, K.D., Kreiswirth, B.N., 2017. Genomic characterization of two KPC-producing Klebsiella isolates collected in 1997 in New York City. Antimicrob. Agents Chemother. 61 (4), 1–6. https://doi.org/10.1128/AAC.02458-16.

Elbediwi, M., Li, Y., Paudyal, N., Pan, H., Li, X., Xie, S., Rajkovic, A., Feng, Y., Fang, W., Rankin, S.C., Yue, M., 2019. Global burden of colistin-resistant bacteria: mobilized colistin resistance genes study (1980–2018). Microorganisms 7 (10), 1–18. https://doi.org/10.3390/microorganisms7100461.

El-Sayed Ahmed, M.A.E., Zhong, L.L., Shen, C., Yang, Y., Doi, Y., Tian, G.B., 2020. Colistin and its role in the era of antibiotic resistance: an extended review. Emerg.MicrobesInfect. 9 (1), 868–885. https://doi.org/10.1080/22221751.2020.1754133.

Eltai, N.O., Kelly, B., Al-Mana, H.A., Ibrahim, E.B., Yassine, H.M., Al Thani, A., Al Maslmani, M., Lammens, C., Xavier, B.B., Malhotra-Kumar, S., 2020. Identification of mcr-8 in clinical isolates from Qatar and evaluation of their antimicrobial profiles. Front. Microbiol. 11 (August), 1–8. https://doi.org/10.3389/fmicb.2020.01954.

EUCAST, 2021. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, valid from 2021-01-01. available https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.xlsx.

European Commission, 2005. Ban on antibiotics as growth promoters in animal feed enters into effect. available https://ec.europa.eu/commission/presscorner/detail/en/IP_05_1687.

Faccone, D., Martino, F., Albornoz, E., Gomez, S., Corso, A., Petroni, A., 2020. Plasmid carrying mcr-9 from an extensively drug-resistant NDM-1-producing Klebsiella quasipneumoniae subsp. quasipneumoniae clinical isolate. Infect. Genet. Evol. 81, 104273. https://doi.org/10.1016/j.meegid.2020.104273.

Farzana, R., Jones, L.S., Barratt, A., Rahman, A., Sands, K., Portal, E., Boostrom, I., Espina, L., Pervin, M., Uddin, A.K.M.N., Walsh, T.R., 2020. Emergence of mobile colistin resistance (mcr-8) in a highly successful Klebsiella pneumoniae sequence type 15 clone from clinical infections in Bangladesh. mSphere 5 (2), 1–6. https://doi.org/10.1128/msphere.00023-20.

Furlan, J.P.R., Ramos, M.S., dos Santos, L.D.R., Gallo, I.F.L., Lopes, R., Stehling, E.G., 2021. Appearance of mcr-9, blaKPC, cfr and other clinically relevant antimicrobial resistance genes in recreation waters and sands from urban beaches,Brazil. Mar. Pollut. Bull. 167, 1–8. https://doi.org/10.1016/j.marpolbul.2021.112334.

Ha, V.T.T., Tran, L.D., Mai, N.T.T., Hirabayashi, A., Nguyen, S.T., Tran, H.H., Shibayama, K., Suzuki, M., 2021. Potential spread of mcr-9-carrying IncHI2 plasmids in Enterobacter hormaechei in Vietnam. J.Glob.Antimicrob.Resist. 27, 332–334. https://doi.org/10.1016/j.jgar.2021.09.012.

Hadjadj, L., Baron, S.A., Olaitan, A.O., Morand, S., Rolain, J.M., 2019. Co-occurrence of variants of mcr-3 and mcr-8 genes in a Klebsiella pneumoniae isolate from Laos. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.02720.

Haenni, M., Métayer, V., Jarry, R., Drapeau, A., Puech, M.P., Madec, J.Y., Keck, N., 2020. Wide spread of blaCTX–M–9/mcr-9 IncHI2/ST1 plasmids and CTX-M-9-producing Escherichia coli and Enterobacter cloacae in rescued wild animals. Front. Microbiol. 11, 1–8. https://doi.org/10.3389/fmicb.2020.601317.

Hassan, J., Mann, D., Li, S., Deng, X., Kassem, I.I., 2022. First report of the mobile colistin resistance gene mcr-9.1 in Morganella morganii isolated from sewage in Georgia, USA. J. Glob.Antimicrob.Resist. 29, 540–541. https://doi.org/10.1016/j.jgar.2021.11.013.

Hayer, S.S., Lim, S., Hong, S., Elnekave, E., Johnson, T., Rovira, A., Vannucci, F., Clayton, J.B., Perez, A., Alvarez, J., 2020. Genetic determinants of resistance to extended-spectrum cephalosporin and fluoroquinolone in Escherichia coli isolated from diseased pigs in the United States. mSphere 5 (5), 1–24. https://doi.org/10.1128/msphere.00990-20.

Hendrickx, A., Debast, S., Pérez-Vázquez, M., Schoffelen, A., Notermans, D., Landman, F., Wielders, C., Cañada Garcia, J., Flipse, J., de Haan, A., Witteveen, S., van Santen-Verheuvel, M., de Greeff, S., Kuijper, E., Schouls, L., Dutch CPE surveillance study group, 2021. A genetic cluster of MDR Enterobacter cloacae complex ST78 harbouring a plasmid containing bla VIM-1 and mcr-9 in the Netherlands. JAC Antimicrob. Resist. 3 (2), 1–6. https://doi.org/10.1093/jacamr/dlab046.

Hooban, B., Fitzhenry, K., Cahill, N., Joyce, A., O'Connor, L., Bray, J.E., Brisse, S., Passet, V., Abbas Syed, R., Cormican, M., Morris, D., 2021. A point prevalence survey of antibiotic resistance in the Irish environment, 2018–2019. Environ.Int. 152, 106466. https://doi.org/10.1016/j.envint.2021.106466.

Hooban, B., Fitzhenry, K., O'Connor, L., Miliotis, G., Joyce, A., Chueiri, A., Farrell, M.L., DeLappe, N., Tuohy, A., Cormican, M., Morris, D., 2022. A longitudinal survey of antibiotic-resistant Enterobacterales in the Irish environment, 2019–2020. Sci. Total Environ. 828, 154488. https://doi.org/10.1016/j.scitotenv.2022.154488.

Hussein, N.H., AL-Kadmy, I.M.S., Taha, B.M., Hussein, J.D., 2021. Mobilized colistin resistance (mcr) genes from 1 to 10: a comprehensive review. Mol. Biol. Rep. 48 (3), 2897–2907. https://doi.org/10.1007/s11033-021-06307-y.

Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A., Brinkman, F.S., Wright, G.D., McArthur, A., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 45 (D1), D566–D573. https://doi.org/10.1093/nar/gkw1004.

Jolley, K.A., Bliss, C.M., Bennett, J.S., Bratcher, H.B., Brehony, C., Colles, F.M., Wimalarathna, H., Harrison, O.B., Sheppard, S.K., Cody, A.J., Maiden, M.C.J., 2012. Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. Microbiology 158 (4), 1005–1015. https://doi.org/10.1099/mic.0.055459-0.

Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.Org website and their applications. Wellcome Open Res. 3, 1–20. https://doi.org/10.12688/wellcomeopenres.14826.1.

Kamathewatta, K., Bushell, R., Rafa, F., Browning, G., Billman-Jacobe, H., Marenda, M., 2020. Colonization of a hand washing sink in a veterinary hospital by an Enterobacter hormaechei strain carrying multiple resistances to high importance antimicrobials. Antimicrob. Resist. Infect. Control 9 (1), 1–16. https://doi.org/10.1186/s13756-020-00828-0.

Kananizadeh, P., Oshiro, S., Watanabe, S., Iwata, S., Kuwahara-Arai, K., Shimojima, M., Ogawa, M., Tada, T., Kirikae, T., 2020. Emergence of carbapenem-resistant and colistin-susceptible Enterobacter cloacae complex co-harboring Bla IMP-1 and mcr-9 in Japan. BMC Infect. Dis. 20 (1), 1–8. https://doi.org/10.1186/s12879-020-05021-7.

Khan, H., Liu, M., Kayani, M.ur.R, Ahmad, S., Liang, J., Bai, X., 2021. DNA phosphorothioate modification facilitates the dissemination of mcr-1 and blaNDM-1 in drinking water supply systems. Environ. Pollut. 268. https://doi.org/10.1016/j.envpol.2020.115799.

Kieffer, N., Royer, G., Decousser, J.W., Bourrel, A.S., Palmieri, M., Ortiz De La Rosa, J.M., Jacquier, H., Denamur, E., Nordmann, P., Poirel, L., 2019. Mcr-9, an inducible gene encoding an acquired phosphoethanolamine transferase in Escherichia coli, and its origin. Antimicrob. Agents Chemother. 63 (9). https://doi.org/10.1128/AAC.00965-19.

Kim, J.S., Yu, J.K., Jeon, S.J., Park, S.H., Han, S., Park, S.H., Kang, M., Jang, J.I., Shin, E.K., Kim, J., Hong, C.K., Lee, J.H., Hwang, Y.O., Oh, Y.H., 2021. Distribution of mcr genes among carbapenem-resistant Enterobacterales clinical isolates: high prevalence of mcr-positive Enterobacter cloacae complex in Seoul, Republic of Korea. Int. J. Antimicrob. Agents 58 (5), 106418. https://doi.org/10.1016/j.ijantimicag.2021.106418.

Kolmogorov, M., Yuan, J., Lin, Y., Pevzner, P.A., 2019. Assembly of long, error-prone reads using repeat graphs. Nat. Biotechnol. 37 (5), 540–546. https://doi.org/10.1038/s41587-019-0072-8.

Lester, C.H., Olsen, S.S., Jakobsen, L., Arpi, M., Fuursted, K., Hansen, D.S., Heltberg, O., Holm, A., Højbjerg, T., Jensen, K.T., Johansen, H.K., Justesen, U.S., Kemp, M., Knudsen, J.D., Røder, B., Frimodt-Møller, N., Hammerum, A.M., 2011. Emergence of extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae in Danish hospitals; this is in part explained by spread of two CTX-M-15 clones with multilocus sequence types 15 and 16 in Zealand. Int. J. Antimicrob. Agents 38 (2), 180–182. https://doi.org/10.1016/j.ijantimicag.2011.03.018.

Li, J., Liu, S., Fu, J., Yin, J., Zhao, J., Zhong, C., Cao, G., 2019. Co-occurrence of colistin and meropenem resistance determinants in a Stenotrophomonas strain isolated from sewage water. Microb. Drug Resist. 25 (3), 317–325. https://doi.org/10.1089/mdr.2018.0418.

Li, T., Wang, L., Ko, W., Lv, Y., 2021. Genetic and phenotypic characteristics of mcr-8 positive extended-spectrum β-lactamase-producing Klebsiella pneumoniae isolated from patient in China. Eur. J. Clin. Microbiol. Infect. Dis. 40 (4), 901–904. https://doi.org/10.1007/s10096-020-04081-6.

Li, Y., Dai, X., Zeng, J., Gao, Y., Zhang, Z., Zhang, L., 2020. Characterization of the global distribution and diversified plasmid reservoirs of the colistin resistance gene mcr-9. Sci. Rep. 10 (1), 1–10. https://doi.org/10.1038/s41598-020-65106-w.

Liu, Y., Lin, Y., Wang, Z., Hu, N., Liu, Q., Zhou, W., Li, X., Hu, L., Guo, J., Huang, X., Zeng, L., 2021a. Molecular mechanisms of colistin resistance in Klebsiella pneumoniae in a tertiary care teaching hospital. Front. Cell. Infect. Microbiol. 11, 1–10. https://doi.org/10.3389/fcimb.2021.673503.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. 16 (2), 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7.

Liu, Z., Hang, X., Xiao, X., Chu, W., Li, X., Liu, Y., Li, X., Zhou, Q., Li, J., 2021b. Co-occurrence of blaNDM–1 and mcr-9 in a conjugative IncHI2/HI2A plasmid from a bloodstream infection-causing carbapenem-resistant Klebsiella pneumoniae. Front. Microbiol. 12, 1–8. https://doi.org/10.3389/fmicb.2021.756201.

Marchetti, V.M., Bitar, I., Sarti, M., Fogato, E., Scaltriti, E., Bracchi, C., Hrabak, J., Pongolini, S., Migliavacca, R., 2021. Genomic characterization of VIM and MCR co-producers: the first two clinical cases, in Italy. Diagnostics 11 (1). https://doi.org/10.3390/diagnostics11010079.

Martiny, H.-M., Munk, P., Brinch, C., Szarvas, J., Aarestrup, F.M., Petersen, T.N., 2022. Global distribution of mcr gene variants in 214K metagenomic samples. mSystems 7 (2), 1–15. https://doi.org/10.1128/msystems.00105-22.

Mataseje, L.F., Boyd, D.A., Lefebvre, B., Bryce, E., Embree, J., Gravel, D., Katz, K., Kibsey, P., Kuhn, M., Langley, J., Mitchell, R., Roscoe, D., Simor, A., Taylor, G., Thomas, E., Turgeon, N., Mulvey, M.R., Canadian Nosocomial Infection Surveillance Program, 2014. Complete sequences of a novel blandm-1-harbouring plasmid from providencia rettgeri and an FII-type plasmid from Klebsiella pneumoniae identified in Canada. J. Antimicrob. Chemother. 69 (3), 637–642. https://doi.org/10.1093/jac/dkt445.

Mavroidi, A., Miriagou, V., Malli, E., Stefos, A., Dalekos, G.N., Tzouvelekis, L.S., Petinaki, E., 2012. Emergence of Escherichia coli sequence type 410 (ST410) with KPC-2 β-lactamase. Int. J. Antimicrob. Agents 39 (3), 247–250. https://doi.org/10.1016/j.ijantimicag.2011.11.003.

Mbanga, J., Amoako, D.G., Abia, A.L.K., Allam, M., Ismail, A., Essack, S.Y., 2021. Genomic insights of multidrug-resistant Escherichia coli from wastewater sources and their association with clinical pathogens in South Africa. Front.Vet.Sci. 8 (February), 1–13. https://doi.org/10.3389/fvets.2021.636715.

Morris, D., Harris, S., Morris, C., Commins, E., Cormican, M., 2016. Report No. 162 Hospital Effluent: Impact on the Microbial Environment And Risk to Human Health.

NCPERLS - National Carbapenemase Producing Enterobacteriaceae Reference Laboratory of Ireland, 2018. NCPERLS ANNUAL REPORT 2017. available https://saolta.ie/documents/ncperls-annual-report-2017.

Ngbede, E.O., Poudel, A., Kalalah, A., Yang, Y., Adekanmbi, F., Adikwu, A.A., Adamu, A.M., Mamfe, L.M., Daniel, S.T., Useh, N.M., Kwaga, J.K.P., Adah, M.I., Kelly, P., Butaye, P., Wang, C., 2020. Identification of mobile colistin resistance genes (mcr-1.1, mcr-5 and mcr-8.1) in Enterobacteriaceae and Alcaligenes faecalis of human and animal origin, Nigeria. Int. J. Antimicrob. Agents 56 (3), 106108. https://doi.org/10.1016/j.ijantimicag.2020.106108.

OECD, 2022. Antimicrobial resistance in the EU/EEA: a one health response. available https://www.ecdc.europa.eu/sites/default/files/documents/antimicrobial-resistance-policy-brief-2022.pdf.

Olaitan, A.O., Dandachi, I., Baron, S.A., Daoud, Z., Morand, S., Rolain, J.M., 2021. Banning colistin in feed additives: a small step in the right direction. Lancet Infect. Dis. 21 (1), 29–30. https://doi.org/10.1016/S1473-3099(20)30915-4.

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A., Parkhill, J., 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31 (22), 3691–3693. https://doi.org/10.1093/bioinformatics/btv421.

Phetburom, N., Boueroy, P., Chopjitt, P., Hatrongjit, R., Akeda, Y., Hamada, S., Nuanualsuwan, S., Kerdsin, A., 2021. Klebsiella pneumoniae complex harboring mcr-1, mcr-7, and mcr-8 isolates from slaughtered pigs in Thailand. Microorganisms 9 (12), 1–12. https://doi.org/10.3390/microorganisms9122436.

Poirel, L., Jayol, A., Nordmann, P., 2017. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin. Microbiol. Rev. 30 (2), 557–596. https://doi.org/10.1128/CMR.00064-16.

Prendergast, D.M., O'Doherty, Á., Burgess, C.M., Howe, N., McMahon, F., Murphy, D., Leonard, F., Morris, D., Harrington, C., Carty, A., Moriarty, J., Gutierrez, M., 2022. Critically important antimicrobial resistant Enterobacteriaceae in irish farm effluent and their removal in integrated constructed wetlands. Sci. Total Environ. 806 (3). https://doi.org/10.1016/j.scitotenv.2021.151269.

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLOS ONE 5 (3), e9490. https://doi.org/10.1371/journal.pone.0009490.

Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., Korobeynikov, A., 2020. Using SPAdes de novo assembler. Curr. Protoc. Bioinformatics https://doi.org/10.1002/cpbi.102.

Ribeiro, T.G., Izdebski, R., Urbanowicz, P., Carmeli, Y., Gniadkowski, M., Peixe, L., 2021. Citrobacter telavivum sp. nov. with chromosomal mcr-9 from hospitalized patients. Eur. J. Clin. Microbiol. Infect. Dis. 41 (1), 123–131. https://doi.org/10.1007/s10096-020-04003-6.

Robertson, J., Nash, J.H.E., 2018. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microbial Genomics 4 (8). https://doi.org/10.1099/mgen.0.000206.

Salloum, T., Panossian, B., Bitar, I., Hrabak, J., Araj, G.F., Tokajian, S., 2020. First report of plasmid-mediated colistin resistance mcr-8.1 gene from a clinical Klebsiella pneumoniae isolate from Lebanon. Antimicrob. Resist. Infect. Control 9 (1), 4–10. https://doi.org/10.1186/s13756-020-00759-w.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30 (14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

Seemann, T., Abricate, Github. available https://github.com/tseemann/abricate.

Seemann, T., MLST, Github. available https://github.com/tseemann/mlst.

Shen, Z., Wang, Y., Shen, Y., Shen, Ji, Wu, C., 2016. Early emergence of mcr-1 in Escherichia coli from food-producing animals. Lancet Infect. Dis. 16 (3), 293. https://doi.org/10.1016/S1473-3099(16)00061-X.

Shi, L., Zhang, J., Lu, T., Zhang, K., 2022. Metagenomics revealed the mobility and hosts of antibiotic resistance genes in typical pesticide wastewater treatment plants. Sci. Total Environ. 817, 153033. https://doi.org/10.1016/j.scitotenv.2022.153033.

Shimoyama, Y., 2022a. ANIclustermap: A tool for drawing ANI clustermap between all-vs-all microbial genomes [Computer software]. available https://github.com/moshi4/ANIclustermap.

Shimoyama, Y., 2022b. MGCplotter: microbial genome circular plotting tool. Available at: https://github.com/moshi4/MGCplotter.

Shimoyama, Y., 2022c. COGclassifier: A tool for classifying prokaryote protein sequences into COG functional category [Computer software]. available. https://github.com/moshi4/COGclassifier.

Simões, A.S., Touret, T., Faria, N.Al., Ladeiro, S.P., Costa, J., Bispo, S., Serrano, M., Palos, C., Miragaia, M., Leite, R.B., Sá-leão, R., 2022. Using whole genome sequencing to investigate a mock-outbreak of carbapenem-resistant Klebsiella pneumoniae in real-time. Acta Medica Port. 35 (1), 36–41. https://doi.org/10.20344/amp.15174.

Smyth, C., O'Flaherty, A., Walsh, F., Do, T.T., 2020. Antibiotic resistant and extended-spectrum β-lactamase producing faecal coliforms in wastewater treatment plant effluent. Environ. Pollut. 262, 114244. https://doi.org/10.1016/j.envpol.2020.114244.

Soliman, A.M., Maruyama, F., Zarad, H.O., Ota, A., Nariya, H., Shimamoto, T., Shimamoto, T., 2020. Emergence of a multidrug-resistant Enterobacter hormaechei clinical isolate from Egypt co-harboring mcr-9 and blaVIM-4. Microorganisms 8 (4), 1–9. https://doi.org/10.3390/microorganisms8040595.

Sukmawinata, E., Uemura, R., Sato, W., Mitoma, S., Kanda, T., Sueyoshi, M., 2020. IncI1 plasmid associated with blactx-m-2 transmission in ESBL-producing escherichia coli isolated from healthy thoroughbred racehorse, Japan. Antibiotics 9 (2), 1–7. https://doi.org/10.3390/antibiotics9020070.

Tereza, G., Alžběta, B., Renáta, K., 2020. 'Variability of mcr genes encoding colistin resistance from the natural environment of the Czech Republic', OHEJP ASM 2020. available https://www.anses.fr/fr/system/files/OHEJPASM2020AbstractbookFINAL.pdf.

Terveer, E., Stein, K., Fenelon, L., Drudy, D., Lynch, H., Sanders, I., Nijhuis, R., Kuijper, E.J., 2017. Prevalence of Clostridium difficile and colistin resistance gene (mcr-1) in faeces of piglets and sows in Ireland', ECCMID Conference April 2017 available: file:///C:/Users/Administrator/Downloads/Papers/Terveer et al.pdf.

Tyson, G.H., Li, C., Hsu, C.H., Ayers, S., Borenstein, S., Mukherjee, S., Tran, T.T., McDermot, P.F., Zhao, S., 2020. The mcr-9 gene of Salmonella and Escherichia coli is not associated with colistin resistance in the United States. Antimicrob. Agents Chemother. 64 (8). https://doi.org/10.1128/AAC.00573-20.

Uz Zaman, T., Aldrees, M., Al Johani, S.M., Alrodayyan, M., Aldughashem, F.A., Balkhy, H.H., 2014. Multi-drug carbapenem-resistant Klebsiella pneumoniae infection carrying the OXA-48 gene and showing variations in outer membrane protein 36 causing an outbreak in a tertiary care hospital in Riyadh,Saudi Arabia. Int. J. Infect. Dis. 28, 186–192. https://doi.org/10.1016/j.ijid.2014.05.021.

Varani, A., He, S., Siguier, P., Ross, K., Chandler, B., 2021. The IS*6* family, a clinically important group of insertion sequences including IS*26*. Mob. DNA 12 (11), 1–18. https://doi.org/10.1186/s13100-021-00239-x.

Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z., Wang, Y., 2018. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg.MicrobesInfect. 7 (1), 1–9. https://doi.org/10.1038/s41426-018-0124-z.

Wang, X., Wang, Y., Zhou, Y., Wang, Z., Wang, Y., Zhang, S., Shen, Z., 2019. Emergence of colistin resistance gene mcr-8 and its variant in Raoultella ornithinolytica. Front. Microbiol. 10, 1–5. https://doi.org/10.3389/fmicb.2019.00228.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13 (6), 1–22. https://doi.org/10.1371/journal.pcbi.1005595.

Wick, R.R., Schultz, M.B., Zobel, J., Holt, K.E., 2015. Bandage: interactive visualization of de novo genome assemblies. Bioinformatics 31 (20), 3350–3352. https://doi.org/10.1093/bioinformatics/btv383.

WHO - World Health Organization, 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. available https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

Wu, B., Wang, Y., Ling, Z., Yu, Z., Shen, Z., Zhang, S., Wang, X., 2020. Heterogeneity and diversity of mcr-8 genetic context in chicken-associated Klebsiella pneumoniae. Antimicrob. Agents Chemother. 65 (1), 1–8. https://doi.org/10.1128/AAC.01872-20.

Xu, T., Zhang, C., Ji, Y., Song, J., Liu, Y., Guo, Y., Zhou, K., 2021. Identification of mcr-10 carried by self-transmissible plasmids and chromosome in Enterobacter roggenkampii strains isolated from hospital sewage water. Environ. Pollut. 268, 115706. https://doi.org/10.1016/j.envpol.2020.115706.

Yao, H., Cheng, J., Li, A., Yu, R., Zhao, W., Qin, S., Du, X.D., 2020. Molecular characterization of an IncFIIk plasmid co-harboring blaIMP–26 and tet(A) variant in a clinical Klebsiella pneumoniae isolate. Front. Microbiol. 11 (July), 1–6. https://doi.org/10.3389/fmicb.2020.01610.

Yuan, Y., Li, Y., Wang, G., Li, C., Xiang, L., She, J., Yang, Y., Zhong, F., Zhang, L., 2019. Coproduction of MCR-9 and NDM-1 by colistin-resistant Enterobacter hormaechei isolated from bloodstream infection. Infect.Drug Resist. 12, 2979–2985. https://doi.org/10.2147/IDR.S217168.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67 (11), 2640–2644. https://doi.org/10.1093/jac/dks261.

Zerbino, D.R., Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18 (5), 821–829. https://doi.org/10.1101/gr.074492.107.

Zhou, Z., Alikhan, N.F., Sergeant, M.J., Luhmann, N., Vaz, C., Francisco, A.P., Carriço, J.A., Achtman, M., 2018. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res. 28 (9), 1395–1404. https://doi.org/10.1101/gr.232397.117.